

Organ admissibility and donor safety:
Functional metrics to improve donor
selection for a safe expansion of living donor
kidney transplantation

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To the memory of my parents.

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Original Articles

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<https://www.ncbi.nlm.nih.gov/pubmed/36397892>

- Almeida, M., Pereira, PR., Ramos, M., Carneiro, D., Madaleno, M., Silva, F., Pedroso, S., França, M., Martins, LS. and Malheiro, J. *CT volumetry performs better than nuclear renography in predicting estimated renal function one year after living donation*. *International Urology and Nephrology* (2022): <https://doi.org/10.1007/s11255-022-03441-9>

- Almeida M.; Calheiros Cruz, G; Sousa, C.; Figueiredo, C., Ventura, S., Silvano, J., Pedroso, S.; Martins, LS., Ramos, M. and Malheiro, J., *External Validation of the Toulouse-Rangueil Predictive Model to Estimate Donor Renal Function After Living Donor Nephrectomy*. *Transplant International* 36 (2023): <http://dx.doi.org/10.3389/ti.2023.11151>.

- Almeida, M.; Ribeiro, C.; Silvano, J.; Pedroso, S.; Tafulo, S.; Martins, LS., Ramos, MS.; and Malheiro, J. *Living Donors' Age Modifies the Impact of Pre-Donation Estimated Glomerular Filtration Rate on Graft Survival*. *Journal of Clinical Medicine* (2023): 12(21):6777. <https://doi.org/10.3390/jcm12216777>

- Almeida, M., Pereira, PR.; Silvano, J.; Ribeiro, C.; Pedroso, S.; Tafulo, S; Martins, LS; Ramos MS., and Malheiro, J., *Longitudinal Trajectories of Estimated Glomerular Filtration Rate in a European Population of Living Kidney Donors*. *Transplant International* (2024): <http://dx.doi.org/10.3389/ti.2024.13356>

- Almeida, M.; Ribeiro, C.; Silvano, J.; Pedroso, S.; Tafulo, S.; Martins, LS., Ramos, MS.; and Malheiro, J. *Clinical performance of the iPREDICTLIVING tool for the prediction of the post-transplant recipient and living donor outcomes in a European cohort*. Clinical Transplantation (2024): <https://doi.org/10.1111/ctr.15283>. 10.1111/ctr.15283

- Almeida, M. and Machado, D., *Exploring the Complex Ethical Challenges Associated with Living Donor Kidney Transplantation*, Portuguese Kidney Journal, (2025). ;39(1):AOP77. doi:10.71749/pkj.77

- Almeida, M., Frade, IC., Sousa, A., Martins, LS., Ramos, MS., Malheiro, J., Fonseca, I. and Lopes, A. *Psychological Outcomes in a Cohort of Portuguese Living Kidney Donors*. Transplant Proc, 2025, Jun 16:S0041-1345(25)00274-X. doi: 10.1016/j.transproceed.2025.05.006.

- Almeida, M., Frade, IC., Sousa, A., Martins, LS., Ramos, MS., Malheiro, J., Fonseca, I. and Lopes, A. *Living Kidney Donor Perceptions About Organ Donation: Who are They, and How Do They Feel About the Process?* Transplant Proc, 2025, Jul 8:S0041-1345(25)00317-3. doi: 10.1016/j.transproceed.2025.05.036.

- Cunha A., Almeida, M., Braga, B., Sousa, S., Silvano, J., Ribeiro, C., Pedroso, S., Martins, LS., and Malheiro, J., *Prevalence of Obesity after Living Kidney Donation and its Association with Cardiovascular Risk Factors and Renal Function*, Journal Of Clinical Medicine 2025, 2025, 14, 6411. <https://doi.org/10.3390/jcm14186411>

- Fernandes, J.; Silvano, J., Pedroso, S.; Martins, LS., Malheiro, J. and Almeida, M., *Hypertension after Living Kidney Donation: Incidence, Predictors, and Consequences*, BMC Nephrology 2025, doi: 10.1186/s12882-025-04513-5

- Almeida M., Braga, P., Carvalho, B, Carrageta, D., Pereira, P., Ribeiro, C., Silvano, J., Bezerra, J., Malheiro, J., Martins, LS, Alves, M; and Rodrigues, *Urinary metabolomics reveals early renal metabolic adaptation following living kidney donation: a longitudinal pilot study*. Submitted for publication.

Abstract

Introduction

Living donor kidney transplantation (LDKT) is widely recognized as the optimal treatment for patients with end-stage renal disease, offering superior survival and quality of life compared with dialysis or deceased donor transplantation. Nevertheless, LDKT remains underutilized in Portugal. In 2024, only 6.96 procedures per million population were performed, a figure lower than that observed in other European countries. This disparity reflects structural and cultural barriers and underscores the urgent need to reinforce donor safety as the central principle guiding the responsible expansion of LDKT.

This thesis was grounded in the central hypothesis that living donor evaluation can be significantly enhanced through the development and integration of more precise risk assessment tools, designed to balance the imperative of organ admissibility with the paramount obligation to safeguard donor health. To test this hypothesis, clinical, imaging, psychosocial, and metabolomic perspectives were systematically integrated across four domains: renal functional adaptation, cardiometabolic vulnerability, psychosocial resilience, and donor–recipient matching, with the overarching goal of advancing a personalized, evidence-based, and ethically responsible approach to LDKT.

Materials and Methods

The research was structured around four interrelated hypotheses. Renal health was assessed through the analysis of long-term post-donation trajectories of estimated glomerular filtration rate (eGFR) and their determinants, external validation of the Toulouse–Ranguel score, and comparison of computed tomography (CT) volumetry with nuclear renography in predicting remaining renal function. Cardiovascular–kidney–metabolic (CKM) health was probed by examining the impact of obesity and the incidence, predictors, and consequences of post-donation hypertension, complemented by a pilot urinary metabolomics study. Psychosocial health was explored through quantitative assessment of quality of life, anxiety, and depression using validated instruments, combined with a survey on donor perceptions and satisfaction. Recipient outcomes were studied by validating the iPREDICTLIVING predictive model, analyzing the interaction between donor age and baseline eGFR, and investigating the influence of genetic relatedness and HLA-DR mismatch on outcomes. Finally, a complementary ethical reflection addressed autonomy, justice, and the imperative of long-term donor follow-up.

Results

Renal outcomes were favorable overall, with most donors exhibiting early compensatory hyperfiltration and long-term stability, although variability was greater in donors with obesity, lower baseline eGFR, and those with greater early recovery. The Toulouse–Ranguel score was validated in the Portuguese context, and CT volumetry was more accurate than nuclear renography in predicting remaining renal function, providing a superior tool for surgical planning and donor counseling.

In the CKM domain, obesity identified donors with greater cardiometabolic vulnerability, underscoring the need for targeted lifestyle and risk management strategies. At long-term follow-up, 30% of donors developed *de novo* hypertension, a clinically significant complication independently linked to accelerated eGFR decline. A pilot metabolomics study demonstrated feasibility and revealed early biochemical signatures of CKM stress, supporting future biomarker-guided surveillance approaches.

The psychosocial analyses demonstrated high satisfaction and little regret overall, yet ambivalence, anxiety, and depression were observed in specific subgroups. These findings revealed heterogeneity in donor adaptation and exposed the absence of structured psychosocial follow-up in Portugal.

Recipient outcome analysis validated the iPREDICTLIVING model, demonstrated that donor age and baseline eGFR interacted to influence graft survival, and confirmed that HLA-DR mismatch was the primary immunological determinant of rejection, while genetic relatedness alone was not independently predictive.

Discussion and Conclusions

These findings underscore the need for a multidimensional approach to donor evaluation and follow-up in which renal, cardiometabolic, psychosocial, and donor-recipient matching dimensions are integrated under the overarching principle of donor safety. Detailed renal trajectories and their determinants, validated predictive tools, and CT volumetry improve accuracy in anticipating post-donation renal outcomes. The identification of modifiable CKM risk factors and the preliminary feasibility of biomarker discovery through urinary metabolomics provide a foundation for precision medicine strategies in long-term donor surveillance. Psychosocial analyses revealed high satisfaction overall but also heterogeneity in adaptation, highlighting the urgent need for structured, donor-specific psychosocial follow-up. Recipient outcome studies offered practical insights into donor–recipient matching, demonstrating that safety and benefit can be balanced through refined

risk stratification. Ethically, the thesis reinforces the principles of autonomy, beneficence, and justice, identifying a national donor registry as a strategic priority to guarantee surveillance and equity. Ultimately, it advances LDKT as a practice that must evolve under the highest scientific and ethical standards, with donor safety as the absolute and non-negotiable foundation upon which recipient benefit can be responsibly maximized.

Resumo

Introdução

O transplante renal de dador vivo (TRDV) é amplamente reconhecido como o melhor tratamento para a doença renal terminal, proporcionando melhor sobrevida e qualidade de vida, comparado tanto com a diálise como com o transplante de dador cadáver. Apesar desta evidência, o TRDV continua a ser subutilizado em Portugal.

Em 2024 realizaram-se apenas 6,96 procedimentos por milhão de habitantes, um valor inferior ao observado noutros países europeus. Esta disparidade reflete barreiras estruturais e culturais e evidencia a necessidade urgente de reforçar a segurança do dador como princípio central que deve orientar a expansão responsável do TRDV.

Esta tese assenta na hipótese central de que a avaliação do dador vivo pode ser significativamente melhorada através do desenvolvimento e da integração de ferramentas de estratificação de risco mais precisas, concebidas para equilibrar o imperativo de admissibilidade do órgão com a obrigação primordial de proteger a saúde do dador. Para testar esta hipótese, foram sistematicamente integradas perspetivas clínicas, imagiológicas, psicossociais e metabólicas em quatro domínios: adaptação funcional renal, vulnerabilidade cardiometabólica, resiliência psicossocial e compatibilidade dador–recetor, com o objetivo de promover uma abordagem personalizada, baseada na evidência e eticamente responsável ao TRDV.

Materiais e Métodos

A investigação foi estruturada em torno de quatro hipóteses inter-relacionadas. A saúde renal foi avaliada através da análise das trajetórias de filtração glomerular estimada (eGFR) a longo prazo após a doação e seus determinantes, da validação externa do score de Toulouse–Rangueil e da comparação entre a volumetria por tomografia computadorizada (TC) e a cintigrafia renal na previsão da função renal residual. A saúde cardiovascular–renal–metabólica (CKM) foi estudada através da análise do impacto da obesidade e da incidência, preditores e consequências da hipertensão pós-doação, complementada por um estudo-piloto de metabólica urinária. A saúde psicossocial foi explorada com recurso à avaliação quantitativa da qualidade de vida, ansiedade e depressão com instrumentos psicométricos validados, complementada por um inquérito sobre perceções e satisfação dos dadores. Os resultados nos recetores foram estudados mediante a validação do modelo preditivo iPREDICTLIVING, a análise da interação entre idade do dador e eGFR basal, e a investigação da influência da relação genética e do mismatch HLA-DR nos

resultados. Uma reflexão ética complementar abordou a autonomia, a justiça e o imperativo do seguimento a longo prazo do dador.

Resultados

Os resultados renais foram globalmente favoráveis, com a maioria dos dadores a apresentar hiperfiltração compensatória precoce e estabilidade a longo prazo, embora com maior variabilidade nos indivíduos com eGFR basal mais baixo, obesidade ou maior recuperação precoce. O score de Toulouse–Rangueil foi validado no contexto português e a volumetria por TC demonstrou ser mais precisa do que a cintigrafia renal na predição da função renal residual, constituindo uma ferramenta superior para o planeamento cirúrgico e o aconselhamento do dador.

No domínio CKM, a obesidade identificou dadores com maior vulnerabilidade cardiometabólica, sublinhando a necessidade de estratégias de estilo de vida e de gestão do risco dirigidas. No seguimento a longo prazo, 30% dos dadores desenvolveram hipertensão *de novo*, uma complicação clinicamente significativa, independentemente associada a declínio acelerado da eGFR. Um estudo-piloto de metabolómica confirmou a viabilidade metodológica e revelou assinaturas bioquímicas precoces de stress CKM, abrindo caminho para futuras estratégias de vigilância baseadas em biomarcadores.

As análises psicossociais demonstraram elevada satisfação global e reduzido arrependimento, mas também ambivalência, ansiedade e depressão em subgrupos específicos, revelando heterogeneidade na adaptação e expondo a ausência de um seguimento psicossocial estruturado pós-doação em Portugal.

Os estudos sobre os recetores validaram o modelo iPREDICTLIVING, demonstraram que a idade do dador e a eGFR basal interagem para influenciar a sobrevivência do enxerto e confirmaram que o desajuste HLA-DR é o principal determinante imunológico da rejeição, enquanto a relação genética, por si só, não foi preditiva de forma independente.

Discussão e Conclusões

Estes resultados evidenciam a necessidade de uma abordagem multidimensional na avaliação e no seguimento dos dadores, integrando dimensões renais, cardiometabólicas, psicossociais e de match dadores-recetores sob o princípio orientador da segurança do dador.

Trajetórias renais detalhadas e seus determinantes, ferramentas preditivas validadas e volumetria por TC melhoram a precisão na previsão dos resultados pós-doação. A

identificação de fatores de risco CKM modificáveis e a viabilidade preliminar da descoberta de biomarcadores através da metabolómica fornecem bases para estratégias futuras de medicina de precisão no acompanhamento a longo prazo dos dadores.

As análises psicossociais revelaram elevada satisfação, mas também heterogeneidade na adaptação, salientando a necessidade urgente de um seguimento psicossocial estruturado e específico para dadores. Os estudos nos recetores forneceram informações práticas sobre a compatibilidade dador–recetor, demonstrando que a segurança e o benefício podem ser equilibrados por meio de uma estratificação de risco refinada.

Do ponto de vista ético, a tese reforça os princípios de autonomia, beneficência e justiça, identificando a criação de um registo nacional de dadores como prioridade estratégica para garantir vigilância e equidade. Em última análise, este trabalho promove o TRDV como uma prática que deve evoluir sob os mais elevados padrões científicos e éticos, tendo a segurança do dador como fundamento absoluto e inegociável sobre o qual o benefício do recetor pode ser responsavelmente maximizado.

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Keywords

Cardiovascular–Kidney–Metabolic (CKM) Health
Computed Tomography (CT) Volumetry
Donor Registry
Donor Safety
End-Stage Renal Disease
Estimated Glomerular Filtration Rate (eGFR)
Ethical Responsibility in Living Donation
HLA-DR Mismatch
Living Donor Kidney Transplant
Living Kidney Donation
Metabolomics / Urinary Biomarkers
Obesity and Hypertension in Donors
Portugal / Transplant Policy
Predictive Models (Toulouse-Rangueil, iPREDICTLIVING)
Psychosocial Outcomes
Renal Functional Trajectories

Palavras-chave

Aspetos Éticos da Doação Renal
Doação Renal de Dador Vivo
Transplante Renal de Dador Vivo
Índice de Filtração Glomerular Estimado (eGFR)
Insuficiência Renal Crónica Terminal
Metabolómica / Biomarcadores Urinários
Modelos Preditivos (Toulouse-Rangueil, iPREDICTLIVING)
Mismatch HLA-DR
Obesidade e Hipertensão nos Dadores
Perfil Cardiovascular–Renal–Metabólico (CKM)
Política de Transplante em Portugal
Registo de Dadores
Resultados Psicossociais
Segurança do Dador
Trajetórias Funcionais da Função Renal
Transplante Renal de Dador Vivo
Volumetria por Tomografia Computorizada

List of Abbreviations

ABMR	Antibody-Mediated Rejection
ABO	ABO Blood Group
ACE	Angiotensin-Converting Enzyme
ACR	Acute Cellular Rejection
AHA	American Heart Association
ANOVA	Analysis of Variance
ANZDATA	Australia and New Zealand Dialysis and Transplant Registry
ATG	Anti-Thymocyte Globulin
AUC	Area Under the Curve
AUROC	Area Under the Receiver Operating Characteristic Curve
BMI	Body Mass Index
BMJ	British Medical Journal
BP	Blood Pressure
BPAR	Biopsy-Proven Acute Rejection
BSA	Body Surface Area
BTS	British Transplantation Society
CDC	Complement-Dependent Cytotoxicity
CG	Cockcroft-Gault
CG-BSA	Cockcroft-Gault Adjusted for Body Surface Area
CGF	Censored Graft Failure
CGN	Chronic Glomerulonephritis
CI	Confidence Interval
CITL	Calibration-in-the-Large
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CKM	Cardiovascular-Kidney-Metabolic
CN	Calcineurin Inhibitor
cPRA	Calculated Pannel Reactive Antibody
CT	Computed Tomography
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DD	Deceased Donor
DDKT	Deceased Donor Kidney Transplantation

DGF	Delayed Graft Function
DM	Diabetes Mellitus
DOI	Digital Object Identifier
DR	HLA-DR Antigen
DTPA	Diethylenetriaminepentaacetic Acid (used in nuclear renography)
EAU-YAU	European Association of Urology - Young Academic Urologists
EFLM	European Federation of Clinical Chemistry and Laboratory Medicine
eGFR	Estimated Glomerular Filtration Rate
ELIPSY	European Living Donor Psychosocial Follow-Up
ELPAT	European Platform on Ethical, Legal and Psychosocial Aspects of Organ Transplantation
EPAT	European Psychosocial Assessment Tool
EPTS	Estimated Post-Transplant Survival Score
ERA-	European Renal Association - European Dialysis and Transplant
EDTA	Association
ESOT	European Society for Organ Transplantation
ESRD	End-Stage Renal Disease
EQ-5D	EuroQol-5 Dimensions
GF	Graft Failure
GS	Graft Survival
GFR	Glomerular Filtration Rate
HADS	Hospital Anxiety and Depression Scale
HLA	Human Leukocyte Antigen
HLAi	HLA Incompatible
HR	Hazard Ratio
HRQoL	Health-Related Quality of Life
HT	Hypertension
HUNT	Trøndelag Health Study
IL-2	Interleukin-2
IPST	Instituto Português do Sangue e Transplantação
IQR	Interquartile Range
KDIGO	Kidney Disease: Improving Global Outcomes
KDPI	Kidney Donor Profile Index
KFRR	Kidney Function Reduction Rate
KPE	Kidney Paired Exchange
KT	Kidney Transplant

LDC	Living Donor Collective
LDKT	Living Donor Kidney Transplantation
LKD	Living Kidney Donor
LKDPI	Living Kidney Donor Profile Index
LRD	Living Related Donor
LURD	Living Unrelated Donor
MARKD	Minnesota Attributable Risk of Kidney Donation study
mGFR	Measured Glomerular Filtration Rate
MDRD	Modification of Diet in Renal Disease
MM	Mismatch
NKR	National Kidney Registry
NHANES	National Health and Nutrition Examination Survey
NMR	Nuclear Magnetic Resonance
NR	Nuclear Renography
NUTSpro	NMR Data Processing Software
ONT	Organización Nacional de Transplantes (Spanish National Transplant Organization)
PCA	Principal Component Analysis
PCR	Protein-to-Creatinine Ratio
PIDRC	Programa Internacional de Doação Renal Cruzada
PLS-DA	Partial Least Squares-Discriminant Analysis
pmp	per million population
ProtU	Proteinuria
RKV/W	Remaining Kidney Volume per Weight
RMSE	Root Mean Square Error
ROC	Receiver Operating Characteristic Curve
RR	Relative Risk
RRT	Renal Replacement Therapy
SCr	Serum Creatinine
SD	Standard Deviation
SER	Standard Event Rate
SF-36	Short Form Health Survey (36 Items)
SRF	Split Renal Function
SRTR	Scientific Registry of Transplant Recipients
TCaScore	Total Calcification Score
TR	Toulouse-Rangueil (Prediction Model)

TRDV	Transplante Renal de Dador Vivo (Portuguese for LDKT)
ULSdSA	Unidade Local de Saúde de Santo António
UMIB	Unit for Multidisciplinary Research in Biomedicine
USA	United States of America
WHA	World Health Assembly
WHO	World Health Organization

CHAPTER I

INTRODUCTION

1.1. Thesis motivation

Living donor kidney transplantation (LDKT) remains the most effective treatment for end-stage renal disease (ESRD), providing superior patient and graft survival, improved quality of life, and lower costs compared with dialysis or deceased donor transplantation [1, 2]. Despite these advantages, LDKT is still underutilized in many healthcare systems, including Portugal, where living donations account for less than 15% of kidney transplants.

This thesis is motivated by the need to address this disparity while ensuring that the expansion of LDKT does not compromise donor safety. As acceptance criteria broaden and strategies such as ABO- or HLA-incompatible transplantation and kidney paired exchange (KPE) are adopted, the responsibility to protect donors over the long term becomes even more urgent. Portugal has developed a strong deceased donor program, but living donation remains limited. Increasing LDKT rates without compromising donor well-being is therefore both a clinical priority and an ethical obligation.

Our Center has performed nearly half of the country's LDKTs over the past decade, creating a unique opportunity to analyze real-world donor outcomes. The researcher's longstanding lead engagement with both the clinical and academic domains of LDKT, spanning over 15 years, offers a unique perspective on evolving donor selection practices, institutional performance, and systemic challenges associated with expanding access to living donation. This experience has reinforced the conviction that donor safety must remain the cornerstone of any effort to expand LDKT.

Recent shifts in the LDKT field have redirected attention from short-term surgical success to long-term donor outcomes [3, 4]. Growing awareness of the physiological, psychological, and social consequences of donation underscores the need for a more comprehensive strategy to assess, predict, and support donor health. This thesis addresses that need by proposing an integrated framework informed by institutional data and grounded in current scientific evidence.

To achieve this, the thesis adopts a multidimensional model of donor safety, that combines renal physiology, cardiovascular-kidney-metabolic (CKM) health, and psychosocial health, while incorporating predictive tools and ethical reflection. The structure of this work mirrors the approach: it begins with an analysis of renal function trajectories and predictive models (Toulouse-Rangueil, CT volumetry), then extends beyond renal health to systemic CKM risk factors, including hypertension, elevated BMI, and metabolomic profiling. Psychosocial outcomes are examined in detail, addressing donor satisfaction, mental health, and long-term well-being. The final sections address recipient outcomes in the context of donor complexity and relatedness and conclude with a dedicated ethical analysis that examines

the responsibilities of transplant programs in evaluating medically complex donors, integrating predictive tools, and ensuring long-term stewardship. Ultimately, this thesis seeks to provide evidence-based insights and practical strategies to ensure that the expansion of LDKT in Portugal remains aligned with the principle of donor safety.

1.2. Thesis hypothesis

This thesis is grounded in the hypothesis that donor safety and organ admissibility can be more effectively balanced through the integration of functional metrics into living donor evaluation. By refining risk assessment, these metrics aim to improve donor selection while ensuring that the expansion of living donor kidney transplantation remains safe and ethically responsible.

The research presented herein tests this hypothesis through four interconnected domains, centered on donor safety: renal functional adaptation, cardiometabolic vulnerability, psychosocial resilience, and donor–recipient matching as it influences transplant outcomes. Collectively, these analyses aim to advance a more personalized, evidence-based, and ethically responsible approach to living donor kidney transplantation.

Hypothesis One

Renal Health: Personalized renal risk assessment in living kidney donors can be improved by integrating eGFR trajectories, predictive models, and imaging-based volumetric assessments.

We hypothesize that renal health after living kidney donation follows distinct trajectories shaped by individual baseline characteristics, and that these trajectories can be anticipated using predictive models and imaging-based assessments. Specifically, the integration of determinants of eGFR longitudinal trajectories, the Toulouse-Rangueil score, and CT-based renal volumetry will improve preoperative risk stratification and inform long-term donor monitoring strategies.

Hypothesis Two

Cardiovascular-Kidney-Metabolic Health: Systemic vulnerability beyond renal outcomes can be probed through the combined evaluation of BMI, blood pressure, and metabolomic profiling.

We hypothesize that cardiovascular–kidney–metabolic (CKM) health represents a critical determinant of long-term safety in living kidney donors, extending beyond renal function alone. Modifiable risk factors such as elevated BMI, borderline hypertension, and dyslipidemia may impair compensatory adaptation and predispose donors to post-donation hypertension and functional decline. Furthermore, we propose that urinary metabolomic profiling can reveal early biochemical signatures of CKM stress, enabling earlier identification of systemic vulnerability and supporting more personalized donor evaluation and follow-up.

Hypothesis Three

Psychosocial Health: Psychosocial health in living kidney donors can be comprehensively assessed through integrated qualitative and quantitative methods.

We hypothesize that psychosocial outcomes after living kidney donation are shaped more by individual and contextual factors than by the donation itself. Prior vulnerability, donor–recipient dynamics, medical complications, and stressful life events are expected to explain variability in long-term well-being. While most donors report high satisfaction, a subset may experience anxiety, depressive symptoms, or reduced quality of life. Identifying these risk factors through structured assessments can inform more personalized and ethically grounded psychosocial care.

Hypothesis Four

Donor–Recipient Matching: Adequate matching can be achieved by integrating predictive tools, donor characteristics, and immunologic compatibility.

We hypothesize that predictive models such as iPREDICTLIVING can refine prediction of donor–recipient outcomes in our Portuguese cohort. Donor characteristics such as baseline eGFR and age are expected to influence graft function and survival. Furthermore, we hypothesize that, when immunologic compatibility is ensured, outcomes in recipients of unrelated living donors are comparable to those of related donors.

1.3. Specific aims

Our aims, concerning **Hypothesis One**, were:

- To evaluate the association of post-donation eGFR trajectories with donor baseline characteristics and early recovery patterns (Kidney Function Reduction Rate (KFRR) at 6 months post-donation) and their value for renal risk stratification
- To validate the Toulouse-Rangueil model in predicting 1-year post-donation eGFR among Portuguese donors.
- To compare the predictive accuracy of CT-based renal volumetry and nuclear renography in estimating remaining renal function.

Our aims, with respect to **Hypothesis Two**, were:

- To evaluate the prevalence, predictors, and clinical associations of obesity ($\text{BMI} \geq 30 \text{Kg/m}^2$) after living kidney donation
- To evaluate the incidence, predictors, and correlates of *de novo* hypertension post-donation.
- To investigate whether urinary metabolomic profiles can reveal early biochemical signatures of CKM stress in living kidney donors.

Our aims, with respect to **Hypothesis Three**, were:

- To identify psychosocial risk factors associated with lower health-related quality of life, anxiety, and depressive symptoms after donation
- To assess donor perceptions and satisfaction following living kidney donation using dedicated questionnaires and structured psychometric tools

Our aims, with respect to **Hypothesis Four**, were:

- To validate the iPREDICTLIVING model in a Portuguese living donor cohort and evaluate its clinical utility for pre-transplant counseling.
- To assess the association of baseline donor characteristics, such as age and eGFR, with long-term graft function and graft survival.
- To compare transplant outcomes between recipients of related and unrelated living donors, controlling for immunologic compatibility

1.4. Thesis outline

This thesis is organized into five chapters, each addressing a specific component of the research:

Chapter I presents the thesis motivation, hypotheses, and specific aims, setting the central theme of optimizing the evaluation of living kidney donors. Donor safety is paramount, while organ admissibility remains a necessary condition for proceeding with donation.

Chapter II presents a comprehensive review of the current state of the field, focusing on the status of living kidney donation in Portugal and internationally. It discusses existing guidelines for donor evaluation, highlights persistent knowledge gaps, and addresses challenges related to acceptance criteria and long-term donor safety.

Chapter III presents the methodology used in the studies presented in this thesis, including study design, participant selection, data collection, and statistical analysis.

Chapter IV presents the consolidated results section that synthesizes the findings of peer-reviewed publications, accepted manuscripts, and submitted papers. Outcomes are summarized in a uniform format to facilitate coherent interpretation across the different hypotheses. The chapter concludes with a dedicated ethical analysis, informed by these results, which examines the responsibilities of transplant programs in evaluating medically complex donors, integrating predictive tools, and ensuring long-term stewardship.

Chapter V synthesizes the main findings and discusses their clinical and scientific implications. It also reflects on the limitations of the current work and proposes directions for future research and policy development.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1. Living Kidney Donation in Portugal: Strategic Need and Ethical Imperative

End-stage renal disease (ESRD) constitutes a growing global health burden, driven by aging populations, rising rates of diabetes and hypertension, and insufficient preventive strategies [5]. More than two million people currently receive renal replacement therapy worldwide; however, the true burden of ESRD is likely much higher, as many patients remain without access to dialysis or transplantation [6]. Kidney transplantation is widely recognized as the optimal treatment for most patients with ESRD, offering superior survival, enhanced quality of life, and reduced healthcare costs compared to dialysis [7-10]. Within transplantation modalities, living donor kidney transplantation (LDKT) provides the most favorable long-term outcomes, including improved graft survival and earlier access to transplantation [1, 2, 11].

Despite these well-established benefits, LDKT remains underutilized in many countries, including Portugal. In 2024, Portugal reported one of the highest ESRD prevalence rates in Europe (2018.3 per million population (pmp)) and an incidence of 237.2 pmp of renal replacement therapy [2, 12, 13]. In the same year, Portugal ranked sixth globally in the rate of deceased donor kidney transplantation (DDKT) [13, 14]. However, living donation accounted for only 13.1% of kidney transplants in Portugal ($n = 71$; 6.96 pmp LDKT), ranking the country 35th globally in LDKT activity [12-14].

The limited use of LDKT in Portugal has important clinical implications, as it may contribute to prolonged waiting times and reduced access to transplantation, particularly for highly sensitized patients who represent over 30% of the national waiting list (cPRA >98% for HLA-ABDR loci). By the end of 2023, nearly 1,900 individuals remained on the national waiting list, 36 of whom died without receiving a transplant, while another 161 were delisted [13].

The Unidade Local de Saúde de Santo António (ULSdSA), where this study was conducted, has been a national leader in living donor kidney transplantation (LDKT), performing over 30% of all living donor transplants in Portugal in 2024 and maintaining LDKT as approximately 30% of its total transplant activity [12, 13]. Over the past decade, the center has performed 275 of the country's 589 LDKTs, of which 20% were preemptive. These data demonstrate that the expansion of LDKT in Portugal is achievable, provided that key barriers are addressed, and donor safety remains the foremost priority.

Recent innovations, including immunologically complex transplants (ABO-/HLA-incompatible), kidney paired exchange (KPE), and the selective acceptance of medically complex donors, have further broadened access to LDKT. However, these strategies also introduce ethical and clinical challenges. Expansion should therefore be balanced with

rigorous safeguards, with donor welfare remaining central and living donation positioned as a complement, rather than a substitute, to the strong national deceased donor program.

This thesis is grounded in that balance. It examines how modern donor evaluation, encompassing renal, cardiovascular, metabolic, psychosocial, and ethical domains, can support a personalized, risk-adapted model of LDKT. Above all, donor safety is the paramount consideration, and the central aim is to improve living donor evaluation through a more precise definition of metrics that account for both organ admissibility and donor protection. The thesis follows a classical structure: introduction, materials and methods, results, discussion, and conclusions, guiding the reader from the clinical and scientific context through the evidence generated to the broader implications for donor safety.

2.2. Living Donation: Clinical Evolution and the Expanding Scope of Donor Safety

Living kidney donation has evolved substantially since the first successful transplant between the Herrick twins in 1954, a milestone in transplantation history [15]. Initially restricted to young, biologically related donors with no comorbidities, LDKT has expanded to include older individuals, emotionally related or unrelated donors, and carefully selected candidates with well-controlled medical conditions such as hypertension, obesity, or nephrolithiasis [18–21]. This expansion reflects advances in surgery and immunology, together with a progressive redefinition of acceptable donor profiles.

Despite this clinical expansion, access to LDKT remains highly variable worldwide shaped by cultural, legal, religious, and infrastructural contexts [16]. In low-resource settings, where deceased donor programs are limited or absent, living donation often constitutes the primary or only viable option for kidney transplantation [16].

The concept of donor safety has evolved in parallel, shifting from an early focus on minimizing perioperative risk to a multidimensional framework encompassing lifelong medical, psychosocial, reproductive, and financial outcomes [16]. This perspective has been progressively formalized in international guidelines: the 2004 Amsterdam Forum established the foundational principles of comprehensive assessment and informed consent [17]; the 2017 Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines, advanced individualized, risk-based assessments and transparent risk communication [18]; and similar approaches are reflected in the British Transplantation Society (BTS) guidelines [19], and the Spanish National Transplant Organization (ONT) recommendations [20].

More recently, the 2024 World Health Organization resolution (WHA77.4) explicitly recognized living donors as a population requiring structured surveillance and long-term support [21]. These developments mark a global shift from viewing donor protection as a perioperative concern to acknowledging it as a lifelong ethical responsibility, demanding systematic follow-up and personalized care.

Safeguarding living donors must remain the cornerstone of any sustainable transplant strategy. As donor profiles broaden and clinical innovation advances, robust tools for risk stratification and personalized assessment are essential to ensure that long-term safety remains central to LDKT practice.

2.3. Clinical Evaluation of the Living Kidney Donor: From Renal Function to Expanded Risk Profiles

2.3.1. Renal Function Assessment

Accurate assessment of renal function is central to donor evaluation, serving both to screen for kidney disease and to predict both graft function and post-donation remaining function. For living donors, kidney health remains the foremost concern, with the risk of future kidney failure consistently ranked as the primary outcome of interest [22].

Measured glomerular filtration rate (mGFR) using exogenous filtration markers, such as inulin or iothalamate, remains the gold standard, particularly in borderline cases [23]. However, logistical and cost constraints limit its routine use, and most centers rely on estimated GFR (eGFR) derived from creatinine or cystatin C [24, 25]. Among available equations, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation performs better than the older Modification of Diet in Renal Disease (MDRD) equation, with greater accuracy in healthy populations [24]. In Europe, the 2009 CKD-EPI equation remains standard, while the new race-free 2021 CKD-EPI version has not been recommended by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) because of accuracy concerns in European cohorts and the lack of global consensus [26]. Combined creatinine-cystatin C equations may enhance precision, particularly in donors with atypical body composition [27]. However, cystatin C assays remain costly and not universally available. When GFR measurement is not feasible, combining 24-hour creatinine clearance with eGFR may offer improved accuracy [28].

Current guidelines set different GFR thresholds for donor acceptance. KDIGO suggests routine acceptance at ≥ 90 mL/min/1.73 m², exclusion at < 60 mL/min/1.73 m², and individualized consideration in the intermediate range [18]. The BTS instead proposes age-calibrated thresholds, acknowledging the physiological decline in GFR with age [19]. Fixed

cut-offs risk exclude older donors unnecessarily or underestimate lifetime risk in younger candidates, highlighting the need to interpret GFR dynamically within risk models.

Beyond absolute GFR, markers of glomerular injury such as albuminuria remain essential [18, 19]. While isolated microalbuminuria is not an absolute contraindication, it may reflect subclinical renal or systemic endothelial injury [29]. Persistent albuminuria, particularly when combined with borderline GFR, should prompt caution and may preclude donation [18]. Consistent with the KDIGO 2024 CKD guidelines, albuminuria should be measured and repeated to confirm chronicity, and donor risk stratification should reflect the combined eGFR and albuminuria category rather than eGFR alone [30].

Emerging strategies aim to refine donor selection, beyond static GFR thresholds. These include evaluation of renal functional reserve [31, 32], lifetime-standardized GFR [33], and probabilistic models estimating mGFR from clinical variables [34, 35]. Biomarker- and histology-based approaches are also under investigation but remain experimental and are not yet integrated into routine practice [36-39].

At our center, renal assessment follows a multimodal approach, incorporating CKD-EPI creatinine-based equations, cystatin C measurement, 24-hour urinary creatinine clearance, and nuclear medicine studies (99mTc-DTPA). This layered approach balances feasibility and precision, enabling individualized donor risk estimation while keeping safety as the guiding principle.

2.3.2 Renal Imaging and CT Volumetry: Surrogate of Nephron Mass

Renal imaging complements biochemical assessment by providing detailed information on anatomy, vascularization, and split function, all of which are critical in living donor evaluation. Beyond its surgical applications, imaging, particularly CT-based renal volumetry, has emerged as a surrogate for nephron mass and a promising predictor of remaining renal function after donation [40].

Traditionally, nuclear renography (NR) has been the reference method to assess split renal function (SRF). However, increasing evidence supports CT volumetry as a more accessible and accurate method, particularly in anatomically normal kidneys [19]. Meta-analyses demonstrate a strong correlation between CT-derived renal volume and SRF, with volumetry effectively replacing NR in many settings [41]. This is especially relevant in borderline cases or when results between modalities are discordant [42].

Our center has contributed original research supporting the clinical value of CT-based renal volumetry in the evaluation of living donors. We demonstrated that the remaining kidney volume indexed to body weight (RKV/W) is a strong predictor of short- and mid-term post-

donation renal function. Donors with $RKV/W \leq 2.13 \text{ cm}^3/\text{kg}$ showed progressive decline in renal function over four years, highlighting the prognostic value of nephron mass quantification [43]. Furthermore, we found that the volume of the donated kidney was independently associated with recipient graft function and risk of acute rejection, reinforcing the role of volumetric analysis in optimizing donor–recipient matching [44].

Despite growing evidence, implementation of CT volumetry remains inconsistent across centers, and no consensus guidelines currently exist. Nevertheless, volumetric analysis offers a non-invasive, objective, and reproducible tool that can meaningfully complement or, in selected cases, substitute traditional assessments. In this thesis, we will evaluate the feasibility of CT volumetry as a replacement for NR, focusing on predictive accuracy, clinical concordance, and operational advantages, and positioning volumetry as a core element of individualized donor evaluation.

2.3.3. Medically Complex Donors: Individualized Risk Assessment

As the landscape of LDKT evolves, donor eligibility criteria have expanded to include individuals with conditions once considered contraindications [45, 46]. Driven by ongoing organ shortages, improved surgical outcomes, and accumulating long-term data, this shift requires an explicitly safety-first, individualized approach to evaluation. In this setting, individualized risk stratification becomes critical, not only to protect donor health but also to support ethically and clinically sound decision-making [46, 47]. Current international guidelines endorse considering medically complex donors, when risks are clearly communicated, and long-term follow-up is assured [18-20].

Older donor age is not an absolute contraindication but requires an age-adjusted assessment. Renal function should be interpreted with age-specific GFR thresholds, and comprehensive evaluation must include cardiovascular risk, oncologic screening, and frailty assessment [48]. Although recipient outcomes with kidneys from older living donors often exceed those from deceased donors, careful selection remains essential [49]. Importantly, the lifetime ESRD risk is generally lower for older than for younger donors [50],

Obesity is increasingly assessed along a continuum of risk. Many programs consider candidates with a BMI up to 35 kg/m^2 when other renal and metabolic risk factors are absent. Although long-term safety data are limited [48, 51], obesity has been associated with modestly increased risks of ESRD and mortality post-donation [50, 52]. Structured lifestyle counseling before and after donation may help mitigate long-term risk.

Well-controlled hypertension, typically on single-agent or dual therapy, without albuminuria or end-organ damage, may be acceptable with individualized assessment. Studies suggest

that such donors do not experience significantly accelerated renal decline, though long-term cardiovascular follow-up remains essential [51]. In contrast, the inclusion of individuals with prediabetes or impaired glucose tolerance remains controversial. Many programs, including ours, are cautious due to uncertain long-term renal, metabolic, and cardiovascular risks, and these candidates are generally deferred from donation [53].

Other conditions, such as nephrolithiasis [54, 55], inherited kidney diseases, selected low-recurrence-risk prior malignancies after oncology review [56], and vascular or ureteric anatomical variants [48, 57], require individualized multidisciplinary assessment. Risk stratification should include metabolic evaluation, imaging, and, in some cases, genetic testing.

The broadening of eligibility underscores a transition from rigid exclusion criteria to individualized, risk-based evaluation. While this approach can expand access to transplantation, it reinforces the need for better predictive models, robust registries, and structured lifelong follow-up, which are central priorities of this thesis.

2.4. Living Donor Safety — Clinical Outcomes and Risk Stratification

Donor safety is the cornerstone of living kidney transplantation. While donation is generally safe, concerns about long-term medical, psychosocial, and ethical consequences remain central. This section reviews the main outcome domains relevant to donor protection, beginning with mortality and ESRD.

2.4.1. Mortality and ESRD Risks in Living Kidney Donors

Living kidney donation is generally considered safe, with excellent short- and long-term outcomes in carefully selected individuals [16, 58-60]. However, the risks of mortality and progression to ESRD remain the most critical concerns for both ethical justification and clinical follow-up. These risks have been clarified by large-scale cohort studies [3, 4] and predictive models [61-63], which have transformed donor selection into an individualized, risk-based process [18].

2.4.1.1. Mortality: Perioperative and Long-Term Outcomes

The perioperative mortality associated with donor nephrectomy is exceptionally low, consistently below 0.03%, with major surgical complications reported in fewer than 3% of cases [64-66]. More recent registry data suggest mortality rates may be as low as <1 in 10,000 among normotensive, low-risk donors [67]. Nonetheless, perioperative risks are slightly higher in male donors, in those with pre-existing hypertension, and among donors

of African ancestry, who experience increased complication rates and longer hospital stays [65, 66].

Long-term survival has traditionally been considered comparable, or even superior, to that of the general population [68], likely reflecting the stringent selection of healthy individuals. This assumption was challenged by the landmark Norwegian cohort study by Mjoen et al. [4]. In 1,901 donors compared with 32,000 matched non-donors, and with a median follow-up exceeding 15 years, 25-year cumulative mortality was higher among donors (18% vs. 13%), with hazard ratios of 1.3 for all-cause and 1.4 for cardiovascular mortality [4]. Although donors with low GFR or significant comorbidities were excluded, renal function data were unavailable for controls, raising concerns about residual confounding. The findings nevertheless suggested that modest excess mortality may become evident with extended follow-up, even in carefully selected donors [69].

Subsequent registry studies [60] and a meta-analysis [70] have not consistently reproduced an increased risk of death in donors. Overall, the evidence suggests that any excess mortality among donors is modest, may emerge only after decades of follow-up, and must be interpreted considering the exceptionally healthy baseline profile of most living donors.

2.4.1.2. Risk of ESRD and Related Predictors

Donation was long assumed not to increase the lifetime risk of ESRD, based on comparisons with the general population [59]. More recent evidence has shown a small but statistically significant increase in risk compared to matched healthy non-donors [3, 4], prompting a fundamental shift toward individualized evaluation and long-term surveillance.

The pivotal US study by Muzaale et al. [3], which analyzed 96,217 living kidney donors and 20,024 matched controls, reported a higher cumulative incidence of ESRD in donors (30.8 vs. 3.9 per 10,000 at 15 years; adjusted HR 8.0). Although the absolute risk remained low (<0.5%), the relative increase changed risk perception. The estimated lifetime ESRD risk was 90 per 10,000 in donors, compared with 14 in non-donors and 326 in the general U.S. population. Donors of African ancestry had a particularly elevated risk (74.7 vs. 22.7 per 10,000). Most ESRD cases occurred in biologically related donors, but the association with relatedness was not statistically significant. These findings underscore that even healthy, screened donors are not entirely free from long-term risks [3].

The Norwegian study by Mjoen et al. [4], reached similar conclusions regarding ESRD, reporting an incidence of 0.47% in donors compared to 0.07% in controls (HR 11.3; 95% CI: 4.4–29.6). Although based on only nine donor events, primarily immune-mediated glomerular disease, the study emphasized that donation is not risk-neutral [69]. Notably, all

affected donors were first-degree relatives of their recipients, suggesting possible genetic susceptibility. This same study, uniquely among major cohorts, also reported increased long-term mortality, as discussed in the preceding section.

Meta-analyses have synthesized these findings, providing pooled estimates that reinforce the observed increase in ESRD risk while also highlighting important limitations, such as heterogeneity across cohorts. A 2023 review by Park et al. [71] confirmed an increased risk of ESRD (pooled HR 3.29), though heterogeneity was high ($I^2 = 81\%$), limiting certainty. By contrast, the risk of CKD (eGFR <60 mL/min/1.73 m²) was consistently increased (pooled HR 13.59; 95% CI 9.42–19.61) with no heterogeneity ($I^2 = 0\%$), indicating that reduced GFR is a robust and reproducible post-donation outcome.

Because ESRD is rare, many studies have focused on intermediate outcomes such as reduced GFR and proteinuria. In a long-term cohort, Ibrahim et al. [72] showed that severe post-donation renal impairment was uncommon and strongly associated with post-donation diabetes and hypertension. Other longitudinal studies from Japan [73] and the U.S. [74] have shown that most donors maintain stable GFR trajectories until a new systemic or renal disease develops. These findings support the "multi-hit" hypothesis proposed by Cheng et al. [75], in which kidney failure arises only when subsequent insults like hypertension, diabetes, or glomerulonephritis compound donor nephron mass reduction. These results highlight the need to identify donor subgroups at high risk, rather than assuming uniform risk for all donor's post-donation. Moreover, preventing and effectively managing a "second hit" following donation is crucial.

Time-dependent changes in ESRD etiology were highlighted by Anjum et al. [53], who found that glomerulonephritis predominated in early post-donation ESRD (<10 years), while diabetes and hypertension became more common by 25 years. The cumulative incidence of ESRD increased from 10 per 10,000 at 10 years to 85 per 10,000 at 25 years, illustrating the importance of monitoring late-onset systemic disease.

Genetic predisposition is increasingly recognized as an important determinant of post-donation ESRD, particularly in related donors where familial risk factors may be shared. The most studied example is the APOL1 high-risk genotype, prevalent among individuals of African ancestry, which has been associated with lower baseline GFR and an increased lifetime risk of ESRD [76]. Among living donors, carriers demonstrate higher ESRD incidence, although their post-donation eGFR decline appears comparable to APOL1-matched non-donors, suggesting that donation amplifies an underlying genetic vulnerability rather than initiating disease [76]. However, the predictive value of APOL1 testing remains uncertain, and its implementation raises ethical challenges: universal testing could

stigmatize individuals of African ancestry or reduce equitable access to transplantation [77, 78]. Current recommendations, therefore, emphasize that testing, when offered, should be embedded in structured counseling frameworks that address both clinical limitations and psychosocial and ethical implications [77, 78]. Beyond APOL1, other inherited nephropathies (e.g., COL4A-related disease, cystic kidney disorders) and familial clustering are also clinically relevant. In our cohort, some genetic evaluations were performed, but these were not applied systematically or by protocol, and therefore were not formally addressed in this thesis. Their potential impact on donor safety must nonetheless be acknowledged. Systematic genetic evaluation, whether implemented prospectively or through retrospective reevaluation of donor–recipient pairs, could reveal distinct risk profiles. Moreover, the frequent detection of variants of uncertain significance underscores the importance of embedding any testing strategy within structured genetic counseling frameworks [77, 78].

In summary, while the absolute risk of ESRD after donation is low, the relative risk compared with matched healthy non-donors is consistently higher. Most cases occur in the presence of a “second hit,” such as diabetes, hypertension, or glomerulonephritis, emphasizing the need for lifelong follow-up and proactive management of acquired risk factors. These findings highlight the importance of transparent counseling and the development of individualized risk prediction tools, priorities that are central to this thesis. In the future, the integration of genetic predisposition into such models may further refine donor risk stratification.

2.4.1.3 Predictive Tools and Risk Stratification in Living Donors

Although the absolute risk of ESRD after donation is low, it varies substantially between individuals. To account for this heterogeneity, predictive models have been developed to estimate long-term renal outcomes, shifting donor assessment from fixed thresholds to probabilistic, individualized risk estimates. These models typically integrate demographic, clinical, and laboratory parameters to project lifetime risks of ESRD or reduced kidney function, thereby supporting transparent counseling and informed decision-making.

The most widely cited model is the ESRD Risk Tool developed by Grams et al. [18, 63]. Derived from pooled data on over 4.9 million individuals from general population cohorts, the model estimates 15-year and lifetime ESRD risk in the absence of donation, based on nine variables, including age, sex, race, eGFR, BMI, blood pressure, and albuminuria. Compared to matched non-donors, kidney donors showed a 3.5–5.3-fold increased ESRD risk at 15 years, with the most significant excess risk observed in young male donors of African American descent [63]. While it introduced the concept of quantifying donation-

attributable risk by comparing predicted outcomes in donors versus matched non-donors, its derivation from population rather than donor cohorts means it may misestimate risk in carefully selected populations.

A complementary approach, derived from OPTN and U.S. ESRD data [79], identified African American ancestry, male sex, biological relation to the recipient, and elevated BMI as independent risk factors for ESRD. While the median 20-year predicted ESRD risk was low (34 per 10,000), a high-risk subgroup (1% of donors) exhibited substantially higher risk, reaching up to 256 per 10,000 [79]. This stratification highlights the heterogeneity of outcomes among donors who all initially meet eligibility criteria, reinforcing the importance of individualized assessment.

Beyond ESRD-focused models, some efforts have sought to predict intermediate outcomes. Building on their long-term cohort, Ibrahim et al. [72] proposed a calculator for proteinuria and reduced GFR, emphasizing functional decline as an early endpoint. Although useful conceptually, its uptake has been limited because it has not been externally validated and focuses on surrogate rather than definitive outcomes.

In Europe, the Toulouse-Rangueil score offers a pragmatic tool to predict post-donation eGFR using only age and baseline eGFR [80]. This simple tool has shown strong performance in external validations [81-83] and provides a reliable way to anticipate renal functional trajectories. Although it does not directly predict ESRD, it complements ESRD-focused models by informing expectations about remaining kidney function after donation.

A critical limitation of all current models is their reduced accuracy in younger donors, who face the longest exposure to potential “second hits” such as diabetes or hypertension [84]. Most calculators are derived from cohorts with fewer than 20 years of follow-up, inevitably underestimating lifetime risk in donors in their 20s or 30s. [84]. Data from the U.S. Renal Data System suggest that half of lifetime ESRD risk occurs before the age of 64 [2], underscoring the challenge of predicting outcomes in younger donors who still face decades of potential exposure. This limitation complicates counseling and highlights the need for dynamic, longitudinal approaches to reclassify risk over time. Furthermore, most of these models have not been externally validated in non-U.S. populations, including Portuguese donors.

More recently, the iPREDICTLIVING model, developed in Europe, represents a new generation of integrated tools that combine both donor and recipient variables [85]. Its primary strength lies in forecasting recipient outcomes, though it also provides estimates of donor mortality. Although not designated as a dedicated donor safety tool, its development

reflects the growing movement toward multidimensional modeling that balances donor protection with transplant benefit in a single framework.

Finally, prospective initiatives such as the Minnesota Attributable Risk of Donation (MARD) study [86] are designed to follow donors and matched controls for up to 50 years. By incorporating refined baseline variables and monitoring post-donation health trajectories, MARD is expected to provide the most robust estimates of lifetime donation-attributable risk to date and could serve as the foundation for future predictive models.

In summary, current risk calculators are valuable aids to counseling but have notable limitations, particularly in younger donors and outside the U.S. context. While U.S.-derived models such as the ESRD Risk Tool and OPTN analyses may not fully apply to European populations, tools like the Toulouse-Ranguel score and iPREDICTLIVING illustrate alternative approaches. These instruments should be used as supportive aids rather than rigid criteria, always within frameworks that prioritize individualized evaluation, transparent counseling, and long-term donor safety.

2.4.2. Cardiovascular and Metabolic Outcomes

2.4.2.1. Cardiovascular-Kidney-Metabolic Health as a Framework for Donor Safety

While renal function remains the primary focus of living donor evaluation, the interconnectedness of renal, cardiovascular, and metabolic health has become increasingly evident. Following nephrectomy, this systemic interplay may unmask latent vulnerabilities, particularly in individuals with borderline cardiometabolic traits. In this thesis, the recently proposed Cardiovascular-Kidney-Metabolic (CKM) health framework is used not as proven donor evidence, but as a conceptual lens to integrate these dimensions into donor safety.

The CKM model, introduced by the American Heart Association (AHA), views cardiovascular disease, chronic kidney disease, and metabolic dysfunction as components of a single continuum [29, 87]. It emphasizes that shared mechanisms such as excess adiposity, insulin resistance, chronic inflammation, and neurohormonal activation can simultaneously affect the heart, vessels, and kidneys [29, 87, 88]. In living donors, these processes may remain silent at baseline but become clinically relevant after nephrectomy, particularly in those with obesity, hypertension, or dyslipidemia [88-90].

Observational studies highlight the importance of this perspective in living donation. In Latin American [81] and European [89] screening studies, over one-third of potential donors presented one or more components of metabolic syndrome, often leading to exclusion from donation [80, 82]. Early histopathologic changes, such as glomerulosclerosis and interstitial fibrosis, have been observed even in individuals with preserved eGFR [91]. Moreover,

obesity has been associated with a modest but significant increase in long-term ESRD and mortality risk in U.S. donor registries, independent of baseline renal function [52, 92]. These findings illustrate how cardiometabolic traits can shape donor outcomes, reinforcing the value of a multidimensional assessment.

CKM syndrome carries a heavy burden of morbidity and mortality in the general population, primarily through cardiovascular events and CKD progression [29, 87, 88]. To operationalize this concept, the AHA has proposed a five-stage CKM classification, ranging from Stage 0 (no risk factors) to Stage 4 (established cardiovascular disease) [29, 87]. This staging supports dynamic, risk-stratified prevention, from lifestyle modification at earlier stages to pharmacologic therapy in advanced stages [29]. Emerging therapeutic agents, such as sodium-glucose cotransporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and non-steroidal mineralocorticoid receptor antagonists (e.g., finerenone) have demonstrated renal and cardiovascular benefit across diverse CKM phenotypes, including non-diabetic patients [87]. While their role in living kidney donors remains to be defined, these agents highlight new avenues for preventive strategies in donors with cardiometabolic risk.

The CKM framework also highlights the influence of non-biological determinants, including socioeconomic status, healthcare access, and sex-specific risks such as adverse pregnancy outcomes [29, 87, 88]. These factors must be considered during donor selection and follow-up to ensure equity and safety [16].

In summary, CKM health provides a unifying framework that expands donor safety beyond renal outcomes alone. By using it as a lens for integration, rather than a validated evidence base, this thesis frames cardiovascular, metabolic, and adaptive responses to nephrectomy as interconnected processes, and paves the way for the following sections on hypertension, cardiovascular outcomes, metabolic adaptations, and metabolomics.

2.4.2.2. Hypertension and Kidney Donation

Hypertension represents a key intersection of renal, cardiovascular, and metabolic health, and thus fits naturally within the CKM framework. In living donors, it is relevant both as a pre-existing trait that may influence selection and as a potential consequence of nephron mass reduction. Within this perspective, hypertension can be unmasked by donation and, in turn, drive long-term cardiovascular and renal risk.

In the general population, reduced nephron mass is strongly associated with incident hypertension [93, 94]. In living donors, however, the evidence is more nuanced [95-99]. Several cohort studies and meta-analyses suggest modest post-donation increases in blood

pressure, but findings are inconsistent due to differences in study design, definitions, and follow-up intensity [90, 95, 96]. Early studies reported small rises in systolic and diastolic pressure, but heterogeneity limited generalizability [95]. More recent evidence is more reassuring: a meta-analysis by O'Keeffe et al. [70] found no significant difference in clinically diagnosed hypertension compared with matched controls, and large prospective studies by Garg et al. [100] and Kasiske et al. [101] also reported no differences in incident hypertension or albuminuria over 7–9 years of follow-up.

Despite these findings, uncertainty persists regarding long-term trajectories. Donors with borderline blood pressure, higher BMI, or a family history of hypertension may face increased risk over time. To improve risk prediction, a model developed at the University of Minnesota and validated in Norway incorporated baseline characteristics such as age, sex, BMI, blood pressure, eGFR, glucose, smoking, dyslipidemia, and donor–recipient relationship [102]. While promising, it requires validation in more diverse populations.

In summary, the short- to mid-term risk of hypertension in standard-risk donors appears low, but the long-term outlook is less certain, especially in younger or higher-risk individuals. In line with KDIGO recommendations [18], our center is actively assessing the incidence and predictors of post-donation hypertension in Portuguese donors. This will help develop risk-adapted surveillance strategies, supporting personalized treatment and strengthening long-term donor safety.

2.4.2.3. Cardiovascular Outcomes in Living Donors

While hypertension represents a measurable intermediate phenotype, the broader concern is whether living kidney donation contributes to long-term cardiovascular morbidity or mortality. Cardiovascular disease (CVD) is a leading cause of morbidity and mortality in CKD [103], but its implications for LKDs are less clear. Although nephrectomy may reduce GFR into the CKD range, donors typically lack the systemic inflammation, endothelial dysfunction, and comorbidity burden that characterize medical CKD [104].

Overall, the evidence is largely reassuring. Large registry studies and meta-analyses generally report no excess risk of cardiovascular events or mortality among donors compared with matched healthy non-donors [65, 70, 105-109]. The notable exception is the Norwegian study by Mjoen et al. [4], which found higher cardiovascular and all-cause mortality after more than 15 years of follow-up. However, this finding has not been consistently replicated and may reflect residual confounding, including genetic susceptibility, socioeconomic differences, or baseline comorbidities [69].

Emerging data suggest that donors with baseline CKM traits, such as obesity, borderline hypertension, or dyslipidemia, may follow less favorable long-term trajectories, though robust evidence on clinical events remains limited [29, 79, 110]. These observations highlight the importance of careful baseline risk stratification, particularly in younger donors who face longer exposure to potential risk. Consistent with current guidelines [18-20], candidates with cardiovascular risk factors should undergo individualized evaluation, and after donation, they benefit from structured follow-up that prioritizes risk factor control, lifestyle counseling, and timely pharmacological intervention when appropriate.

In summary, current evidence does not support a uniform increase in cardiovascular events or mortality after living donation. Instead, outcomes appear to be shaped by baseline risk profile and length of follow-up, underscoring the need for individualized donor assessment and structured surveillance that extend beyond renal endpoints.

2.4.2.4. Metabolic adaptations

Beyond renal and cardiovascular outcomes, kidney donation may influence metabolic pathways, although the clinical impact appears modest. Prospective studies found small increases in uric acid, parathyroid hormone, and homocysteine levels post-donation [101, 111]. These biochemical changes are usually mild and rarely translate into overt disease, but they suggest subtle shifts in solute handling and endothelial stress.

Clinical consequences have been less evident. A Canadian population study observed a modest but statistically significant increase in the incidence of gout (6.8% vs. 4.9%) over 20 years in LKDs compared to matched controls [112]. Reduced small artery elasticity has also been detected 6–9 years post-donation, potentially signaling early vascular remodeling [101].

Although these metabolic changes have not been associated with increased incidence of diabetes, CKD, or cardiovascular disease in the general donor population, they may represent early signs of CKM imbalance in donors with predisposing traits, such as obesity, prediabetes, or a family history of metabolic disease.

These findings underscore the importance of structured, risk-adapted surveillance strategies. Novel approaches such as metabolomic profiling (Section 2.4.2.6) may help distinguish adaptive from maladaptive responses, offering a pathway to personalized prevention in living donors. Reassuringly, large population-based studies from the Donor Nephrectomy Outcomes Research (DONOR) Network in Ontario, Canada, further support the overall safety of donation, reporting no excess of non-renal complications when compared with matched healthy non-donors [112-116].

2.4.2.5. Compensatory Physiology in Living Donors

Following unilateral nephrectomy, the remaining kidney undergoes compensatory hypertrophy and hyperfiltration, allowing partial restoration of function despite the loss of approximately half of the nephron mass. Clinically, the immediate fall in GFR is usually around 30%, reflecting physiological adaptation rather than irreversible injury [117]. Long-term follow-up studies in carefully selected donors generally support a benign compensatory response [118]. Hyperfiltration in this context appears to result from glomerular hypertrophy and increased renal plasma flow, and in most donors it is not accompanied by persistent hypertension or significant albuminuria, suggesting that the process is largely adaptive [117].

Nevertheless, not all compensatory responses may be benign. Sustained hyperfiltration can increase intraglomerular pressure, potentially accelerating subclinical injury in susceptible individuals [117]. Donors with a low nephron endowment due to impaired nephrogenesis, or with comorbidities such as obesity, hypertension, or advanced age, may have a lower functional reserve. The multi-hit hypothesis proposed by Cheng et al. [75] supports this theoretical risk: kidney donation may act as a “second hit” in individuals already predisposed by genetic or developmental factors (“first hit”); with subsequent exposures, such as diabetes, uncontrolled hypertension, or obesity, compounding injury and accelerating progression toward CKD or ESRD [75]. This model underscores the importance of both baseline donor characteristics and post-donation exposures in shaping long-term outcomes.

Beyond glomerular adaptations, emerging data suggest that tubular and interstitial processes also play a role in maladaptation [119]. The kidney’s high metabolic demands render it vulnerable to mitochondrial dysfunction and oxidative stress, which may trigger early fibrotic changes long before GFR decline or proteinuria becomes apparent [119]. Such subclinical alterations remain difficult to detect with current clinical tools, highlighting a major gap in donor surveillance.

In summary, most donors exhibit durable adaptive compensation, but a subset may follow maladaptive trajectories, particularly in the presence of additional risk factors. Distinguishing between these pathways remains a significant challenge, reinforcing the need for innovative approaches, such as metabolomic profiling, to characterize the biology of compensation and enhance long-term donor risk stratification.

2.4.2.6. Metabolomics in Living Kidney Donors

Traditional tools for evaluating donor outcomes, such as GFR, albuminuria, and blood pressure, capture overt functional changes but are often insensitive to early biological stress.

In contrast, metabolomics provides a systems-level snapshot of intermediary metabolism, integrating renal, cardiovascular, and metabolic pathways [120]. This approach offers the potential to identify biochemical signatures of adaptive versus maladaptive compensation after nephrectomy, before conventional clinical markers become abnormal.

Within the framework of CKM health (Section 2.4.2.1), metabolomic studies have already characterized pathways relevant to hypertension [121], diabetes [122], cardiovascular events [123], CKD progression [124, 125], and graft dysfunction [126-129]. Alterations in amino acid, fatty acid, and purine metabolism have been linked to early endothelial dysfunction and tubulointerstitial fibrosis [130], both hallmarks of CKM syndrome and potential harbingers of maladaptive post-donation trajectories. Although promising, these insights remain underexplored in living donors [126, 131].

In this thesis, metabolomics is therefore introduced not as a validated predictive tool, but as a proof-of-concept approach. Building on prior evidence from cardiometabolic and kidney disease, we applied a pilot urine metabolomics study in Portuguese living kidney donors to explore the feasibility of sample collection, processing, and analysis, and to search for early biochemical signatures of stress and adaptation. This exploratory analysis is intended to complement standard assessments and to inform the development of more precise, personalized strategies for long-term donor monitoring.

Together, these dimensions illustrate why donor safety cannot be assessed by renal outcomes alone. By situating hypertension, cardiovascular disease, metabolic adaptations, compensatory physiology, and metabolomic profiling within the CKM framework, this thesis aims to provide a multidimensional unifying view of donor health.

2.4.3. Pregnancy-Related Risks in Living Kidney Donors

Beyond the shared pathways of CKM health, sex-specific risks must also be considered in the evaluation of donor safety. Pregnancy is a unique physiological context, where the reduced nephron mass after donation may interact with the hemodynamic and metabolic adaptations of gestation [132]. The main concern is whether living kidney donors face an increased risk of gestational complications, particularly hypertensive disorders of pregnancy.

Early studies suggested no significant differences between pre- and post-donation pregnancies. However, more recent matched cohort studies and meta-analyses have shown a two- to three-fold increased risk of hypertensive disorders in post-donation pregnancies [133-137]. In a population-based matched cohort study by Garg et al. [136], gestational hypertension or preeclampsia occurred in 11% of post-donation pregnancies compared to 5% in controls (OR 2.4, 95% CI 1.2–5.0, $p = 0.01$). Importantly, while the

relative risk is elevated, the absolute risk remains modest, and most pregnancies proceed without complications. Neonatal outcomes appear unaffected, with no consistent increase in preterm birth, low birth weight, or perinatal mortality [137].

Available evidence suggests that risk is not uniform across all donors. Donors with pre-existing metabolic traits (e.g., obesity, hypertension, prediabetes), lower baseline eGFR, or a family history of CKD may be more vulnerable [135]. Other potential risk modifiers, including donor age and parity, should be considered in this stratification [47]. These findings align with both the CKM [29] and multi-hit [75] frameworks, where pregnancy acts as a “second hit” in donors with latent vascular or metabolic predisposition.

Our center has also reported outcomes from a small cohort of Portuguese donors, which are generally consistent with these international observations [138]. However, the limited sample size and retrospective design preclude firm conclusions. Given that over 60% of donors in Portugal are women, often of childbearing age, these risks warrant explicit consideration during evaluation and counseling.

International guidelines, including those of KDIGO and the Amsterdam Forum, recommend structured counseling of all female candidates about the potential for increased pregnancy-related risk [18, 19]. A minimum 12-month interval between nephrectomy and conception is advised to allow compensatory adaptation of the remaining kidney [137]. Post-donation pregnancies warrant close monitoring, particularly in women with cardiometabolic traits [19].

In summary, fertility and live birth rates after living kidney donation appear preserved, although the risk of hypertensive complications in pregnancy is consistently higher. For women considering donation, these risks must be explicitly addressed during evaluation, with emphasis on individualized counseling and long-term follow-up. Pregnancy-related risks, therefore, represent a distinct but integral aspect of donor safety, complementing the broader CKM framework addressed in this thesis.

2.4.4. Psychosocial Outcomes in Living Kidney Donors

The psychosocial dimension of living kidney donation is a core component of donor safety, complementing medical outcomes. Most donors report high satisfaction and stable or improved quality of life, often linked to altruistic motivation and the perceived benefit for their recipient [139-141]. Nevertheless, 5–15% experience difficulties such as anxiety, depression, or regret, particularly when confronted with surgical complications, financial strain, or unfavorable recipient outcomes [139-141]. These findings highlight that donation, while often positive, carries potential emotional costs that require attention.

Longitudinal studies show that psychosocial outcomes are not static but evolve over time. While many donors regain baseline well-being within the first year, others encounter late difficulties triggered by factors such as adverse recipient outcomes, graft loss, or the onset of their own comorbidities [142]. This dynamic course underscores the importance of structured long-term follow-up beyond the perioperative period.

Ensuring autonomy and informed consent are central to psychosocial safety [16]. In familial donations, subtle relational pressures or feelings of moral obligation may compromise voluntariness, particularly in parent-child or sibling settings [140]. International guidelines recommend a comprehensive psychosocial evaluation to ensure that donors make autonomous, informed decisions and are emotionally prepared for potential consequences [18-20, 143]. However, standardized, validated instruments for assessing psychosocial health remain limited and inconsistently applied. Tools such as the ELPAT Living Organ Donor Psychosocial Assessment Tool (EPAT) offer structured assessment of motivation, mental health, and support, but they remain underutilized and require further validation [143].

At our center, psychosocial evaluation is systematically incorporated before and after donation. Earlier studies from our center also reported generally stable donor well-being, though greater emotional burden was observed in those experiencing graft failure or with pre-existing mental health issues [144, 145]. Portuguese guidelines mandate pre-donation psychological assessment and a structured one-year follow-up [146], yet there is no formal protocol for long-term psychosocial monitoring, which risks overlooking delayed psychological effects.

The absence of a national donor registry (see Section 4.6) further limits the ability to track psychosocial outcomes. Integrating mental health screening into registries would enable the early detection of distress, support research, and reinforce the principle that donor care must encompass not only medical outcomes but also emotional and social well-being. Moreover, chronic psychosocial stress may contribute to long-term cardiovascular and metabolic vulnerability through mechanisms such as neurohormonal activation and systemic inflammation [147].

2.4.5. Financial burden and donor protection

Financial well-being is an important but often overlooked component of donor safety. While living kidney donation is altruistic and non-remunerated, donors may face indirect costs such as travel, accommodation, lost wages, and caregiving responsibilities, as well as potential long-term effects on employment or insurance coverage [7, 148, 149]. These burdens can generate psychological stress and, in some cases, influence both the decision

to donate and post-donation recovery [150]. Ethical guidelines emphasize that donation should never result in financial harm, as this undermines donor autonomy and fairness [16, 151].

International data indicate that up to 40% of donors experience financial hardship [151] disproportionately affecting those with lower socioeconomic status or precarious employment [152, 153]. In response, several countries, including Canada and the United States, have introduced reimbursement and wage-protection programs to mitigate these inequities [151, 153].

In Portugal, the National Health Service (SNS) covers all medical costs related to donor evaluation, surgery, and follow-up care, and national legislation provides for reimbursement of direct non-medical expenses [154]. However, implementation is often delayed or incomplete. Donors may still experience wage loss, particularly those who are self-employed or in insecure jobs, and reimbursement procedures are frequently bureaucratic. In practice, this can create financial vulnerability and discourage potential donors with fewer resources, despite a strong legislative framework.

These inconsistencies risk widening inequities in living kidney donation and may exacerbate psychosocial distress, particularly when combined with medical complications or adverse recipient outcomes [150]. Addressing this gap requires a transparent and timely reimbursement system, ideally integrated within a future national donor registry (see Section 4.6). Ensuring true financial neutrality is therefore essential to align Portuguese practice with the broader principle of donor protection that underpins this thesis.

2.4.6. Donor Registries and Long-Term Follow-Up

Ensuring long-term donor safety requires structured follow-up systems capable of monitoring outcomes over decades. Living donor registries are indispensable for this purpose, providing longitudinal data on renal function, cardiovascular events, metabolic complications, psychosocial well-being, and pregnancy outcomes, dimensions that single-center or short-term studies cannot reliably capture [155]. Without such registries, the true spectrum of post-donation outcomes remains underestimated, and opportunities for timely intervention are lost.

Several countries, including Switzerland, Norway, and Australia/New Zealand, have established national donor registries integrated into their healthcare systems, demonstrating both feasibility and value [155-157]. These initiatives provide lifelong surveillance, ensure systematic data collection, and have shaped international understanding of long-term donor outcomes.

European legislation also supports this approach. Directive 2010/53/EU, which establishes standards of quality and safety for human organs intended for transplantation, explicitly advocates for the establishment of national living donor registries [158]. Portuguese law (Law No. 36/2013 of 12 June) transposed these requirements [159]. Nevertheless, in practice, monitoring remains center-based and fragmented, with national reporting focused on transplant activity rather than donor outcomes. As a result, delayed complications such as hypertension or metabolic disorders are not systematically tracked, and psychosocial and financial issues (see Sections 2.4.4 and 2.4.5) remain under-assessed at the national level. The 2024 World Health Assembly resolution (WHA77.4) further reinforced the global call to provide equitable access to transplantation and lifelong oversight for living donors [160].

Establishing a national living donor registry in Portugal is therefore a strategic priority. Ideally, it should be integrated with digital health platforms to enable centralized and standardized follow-up. Telemedicine tools could facilitate participation for donors living far from transplant centers, while digital applications could streamline data collection and ensure continuity of care. Such an infrastructure would not only enhance safety and transparency but also demonstrate a sustained commitment to donor welfare, reinforcing public trust in the living donor program.

2.5. Expanding Living Kidney Donation: Challenges and Innovations

The expansion of living kidney donation is widely recognized as a key strategy to reduce waiting times and improve transplant outcomes. In Portugal, as in many countries, the proportion of LKD remains modest, reflecting persistent barriers as well as the challenges of integrating innovative strategies into clinical practice [12, 13]. These challenges and innovations provide the necessary context for understanding contemporary approaches to donor–recipient matching [161].

2.5.1. Barriers to Living Kidney Donation

Barriers to LKD are multifactorial and operate at individual, social, and systemic levels. Educational and informational gaps remain frequent. Both patients and healthcare providers often underestimate the survival and quality-of-life benefits of LDKT compared with DDKT [162, 163]. Evidence shows that targeted educational strategies, particularly when past donors act as advocates, can increase donation rates.[164, 165]. Portuguese centers may benefit from broader use of digital tools to enhance outreach, and our center has piloted a health-literacy platform to address this gap [165].

Psychosocial barriers include fear of surgery, concerns about long-term health, and family or social pressure, particularly among women and younger potential donors [166, 167]. Structured psychosocial assessment, as routinely practiced in our center, can mitigate these risks and support informed, autonomous decision-making [143, 146, 168].

Medical ineligibility also excludes many candidates. In our cohort, only 35.4% of 395 assessed candidates proceeded to donation, with 37.2% withdrawing voluntarily and 34.3% excluded due to medical contraindications such as cardiovascular disease, impaired renal function, or cancer [169]. International data are comparable, with up to 38% of candidates deemed ineligible [170]. Importantly, donor evaluations frequently uncover previously unrecognized conditions, creating opportunities for preventive health management [171, 172]. Increasingly, candidates present with complex profiles, including obesity, hypertension, requiring nuanced individualized risk stratification rather than automatic exclusion [45, 46].

Systemic and logistical barriers further constrain efficiency. In our Center, the median evaluation-to-transplant time is 9.5 months, consistent with international experience [172]. Prolonged pathways, lack of dedicated donor programs, and the absence of a national registry limit both selection and follow-up. Models like the one-day evaluation pathway used in Northern Ireland demonstrate how streamlined processes can reduce delays and increase LDKT rates [173], offering potential lessons for Portugal.

Socioeconomic and cultural disparities add further constraints. While Portuguese law provides for reimbursement of non-medical expenses, implementation remains inconsistent [154]. Broader inequities related to education, income, and cultural background are underexplored, despite the increasing diversity of the Portuguese population [165]. Internationally, sociodemographic disparities are well documented. The U.S. Living Donor Collective (LDC) pilot showed that only half of candidates ultimately proceeded to donation, with medical contraindications and voluntary withdrawal as the leading causes [170]. Importantly, the LDC also highlighted the influence of factors such as education, marital status, and income, underscoring the role of structural social determinants of health [170]. Beyond these findings, the LDC is pioneering systematic long-term follow-up of both accepted and declined donors, providing a model that could be reproduced in Portugal to strengthen surveillance, equity, and policy development [170].

Together, these barriers highlight the need for integrated strategies that combine better education, psychosocial support, streamlined evaluation, and equity-focused policies with robust registry-based follow-up. Only within such frameworks can the expansion of LDKT proceed while safeguarding donor welfare.

2.5.2. Innovative strategies to expand LDKT: donor safety and ethical considerations

The persistent gap between organ demand and availability has driven the development of innovative strategies to expand LDKT. Approaches such as ABO- and HLA-incompatible transplantation and kidney paired exchange (KPE) have increased transplant opportunities, particularly for highly sensitized recipients. These advances, however, must always be framed within a commitment to donor safety, voluntariness, and autonomy [16].

ABO- and HLA-incompatible transplantation has become increasingly feasible through desensitization protocols (e.g., plasmapheresis, intravenous immunoglobulin, rituximab) and optimized immunosuppressive regimens. Outcomes for ABO-incompatible transplants, are now comparable to those of ABO-compatible LDKT in experienced centers [174, 175]. In contrast, HLA-incompatible transplantation remains associated with higher rates of rejection and infection, but long-term survival is generally superior, or non-inferior to remaining on dialysis [176, 177]. From the donor perspective, the critical issue is ensuring transparent counseling so that participation is based on an informed and voluntary decision, free from therapeutic misconception [174-177].

Kidney Paired Exchange (KPE), first proposed by Rapaport in 1986 [178], is an effective strategy to overcome immunological barriers by matching incompatible donor–recipient pairs with others. KPE programs range from simple two-way exchanges to multi-way chains, domino donations, and non-directed donor-initiated chains [179, 180]. Internationally, models such as voucher systems, which allow donation in advance for future recipients, trans-organ exchange, and global exchanges (e.g., Global Kidney Exchange, GKE) further enhance access, though they raise significant ethical and logistical concerns [181-183].

Beyond resolving incompatibility, KPE increasingly involves compatible pairs to improve match quality [184] and expand transplant opportunities for hard-to-match recipients [185]. In Portugal, compatible pairs have been formally included in the KPE program since 2022 [186]. While this broadens recipient access, KPE may also introduce additional burdens for donors, including prolonged waiting, logistical complexity, and a reduced sense of direct altruism [187, 188]. These risks must be mitigated by transparent processes, independent donor advocacy, and the possibility to withdraw freely at any stage. Respecting donor autonomy remains particularly crucial, as some individuals may view incompatibility as a natural endpoint to donation.

Beyond these established approaches, eplet-based HLA matching has gained increasing clinical relevance as a more granular tool for immunological risk assessment. Studies over the past decade suggest that lower class II eplet mismatches are associated with reduced risk of *de novo* donor-specific antibodies [184]. While promising, rigid application could

exacerbate inequities in access, underscoring the need for careful, context-sensitive implementation.

In conclusion, innovative strategies such as ABO-/HLA-incompatible transplantation and KPE have expanded access to LDKT, while refined approaches such as eplet-based matching may further improve recipient outcomes. Their adoption, however, must never come at the expense of donor protection. These innovations should complement, not override, the foundational principle of donor safety, and must be integrated into broader frameworks of individualized risk stratification and long-term follow-up.

2.6. Recipient Outcomes as Ethical and Clinical Justification for Living Kidney Donation

While this thesis focuses on donor safety, the ethical justification for living kidney donation (LKD) ultimately rests on the benefit to the recipient. Donors accept surgical risk without direct medical gain; therefore, recipient outcomes must clearly outweigh these risks [16, 45].

LDKT is widely recognized as the optimal treatment for ESRD, offering superior graft and patient survival, improved quality of life, shorter waiting times, and reduced healthcare costs compared to DDKT [1, 7, 8, 16]. These advantages are particularly evident in younger recipients [8] and those transplanted preemptively or shortly after starting dialysis [1, 189], and they are observed regardless of genetic relatedness [190] or prior transplants [191]. Large registries data reinforce these findings. ERA-EDTA data show 5-year adjusted graft survival of 87.7% for LDKT vs. 80.9% for DDKT, with patient survival rates of 95.3% and 91.1%, respectively [192]. Comparable outcomes are consistently reported in the U.S., with benefit evident across age groups, including recipients ≥ 65 years (80.8% vs. 67.8%) [193].

These favorable outcomes reflect multiple factors, including healthier donors, shorter ischemia times, and more frequent preemptive transplantation, which avoids the complications of prolonged dialysis, which is independently associated with poorer graft and patient survival [1, 189, 194, 195]. Remarkably favorable outcomes extend to recipients of kidneys from medically or immunologically complex donors, including older donors, those with controlled comorbidities, or with ABO/HLA incompatibility, [48, 49, 51, 56, 57].

Recognition that donor characteristics influence outcomes [196, 197] has stimulated the development of predictive tools such as the Living Kidney Donor Profile Index (LKDPI) [198], the Ashby model [199], and the iPREDICTLIVING tool [85]. While these instruments currently show only modest accuracy and limited external validation, they represent an important step toward individualized risk prediction and are further addressed in this thesis [85, 198-200].

In conclusion, recipient outcomes provide the ethical foundation for LDKT, consistently demonstrating superiority over DDKT even when donors fall outside the “ideal” profile. Yet these benefits can only justify the practice when donor safety is safeguarded without compromise, ensuring that recipient benefit never outweighs the primacy of donor welfare.

2.7. Conclusions

Living donor kidney transplantation (LDKT) represents the best treatment for ESRD, offering superior graft survival, improved quality of life, and cost-effectiveness compared with dialysis and DDKT. However, its ethical justification depends on ensuring that recipient benefit never comes at the expense of donor safety.

As reviewed in this chapter, the concept of donor safety has broadened beyond perioperative risk to encompass long-term renal trajectories, cardiovascular–kidney–metabolic health, pregnancy-related risks, psychosocial well-being, and financial neutrality. Alongside these dimensions, systemic issues such as evaluation pathways, social inequities, and the absence of robust registries remain critical determinants of both access and protection.

Despite important progress, challenges remain. Current predictive tools are imperfect, particularly for younger or medically complex donors, and follow-up structures do not reliably capture late complications. In Portugal, the absence of a national registry remains a major limitation, impeding systematic monitoring and quality improvement.

This thesis is anchored in addressing those gaps. By integrating clinical data, CT-based renal volumetry, assessment of CKM risk, and exploratory metabolomic profiling, it aims to refine donor selection and identify early biological signals of stress or maladaptation. In doing so, it seeks to advance a predictive, individualized framework for donor care, reinforcing that the long-term welfare of donors must remain the foundation of any sustainable transplant strategy.

CHAPTER III

MATERIALS AND METHODS

3.1. Study population

This research was conducted in the Nephrology and Kidney Transplantation Unit of the Unidade Local de Saúde de Santo António (ULSdSA) with support from the Centre of Blood and Transplantation of Porto, Portuguese Institute of Blood and Transplantation (IPST), and the Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute for Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal.

This thesis employed a mixed design. Most studies were retrospective, complemented by two cross-sectional psychosocial studies and one exploratory prospective cohort study of living kidney donors undergoing paired pre- and post-donation urine metabolomic profiling.

The study population includes living kidney donors (LKDs) and their corresponding recipients, followed at ULSdSA. Recipients were excluded from studies exclusively focused on donor outcomes. Specific populations were defined according to each sub-study's aims, design, and timeframe. Detailed cohort descriptions are provided in the corresponding sections of the Results chapter.

3.2. Data collection

3.2.1. Clinical data

Demographic, clinical, and immunological data were obtained retrospectively from institutional databases and medical records for both donors and recipients.

Donor variables

Pre-donation donor variables included demographic characteristics (age, sex, donor–recipient relationship, anthropometry), smoking habits, comorbidities, medication use, renal parameters (serum creatinine, cystatin C where available, eGFR by CKD-EPI 2009, proteinuria, albuminuria when available), lipid profile, and imaging data. Split renal function was assessed via nuclear renography, and anatomy by computed tomography (CT). Surgical data comprised nephrectomy laterality, technique, and peri- and postoperative outcomes.

Post-donation follow-up variables included renal function, proteinuria/albuminuria, and major clinical events (new-onset hypertension, diabetes, cardiovascular/cerebrovascular disease, malignancy, and mortality).

Psychosocial outcomes were evaluated through two cross-sectional observational studies combining quantitative and qualitative approaches. Donors completed pre- and post-

donation assessments with the SF-36 Health Survey (quality of life, 0–100 scale) and the Hospital Anxiety and Depression Scale (HADS; scores ≥ 8 indicating possible disorder). Baseline data were collected during routine evaluation of donor–recipient pairs in 2018–2019. Post-donation assessments were performed in May 2021 during dedicated consultation days. In addition, a structured questionnaire explored donor perceptions of decision-making, concerns, emotions, and post-donation impacts.

Recipient and Kidney Transplant Variables

Recipient data included demographics, comorbidities, CKD etiology, and analytical results. Renal function was estimated with CKD-EPI. Renal biopsies were performed based on clinical indication, with acute rejection diagnosed according to established histopathological criteria (last updated Banff classification). Follow-up extended until death, graft failure (GF), or 15 years. Graft survival (GS) was defined as time from transplantation to GF (dialysis, retransplantation, or death with functioning graft).

Transplant variables included era (1998–2009 vs. 2010–2019), peak calculated panel reactive antibody (cPRA), and HLA A, B, and DR mismatches. Induction therapy involved anti-IL-2 receptor monoclonal antibody or polyclonal anti-thymocyte globulin (ATG), with ATG reserved for HLA-incompatible transplants, sensitized patients, and retransplants. Maintenance immunosuppression comprised a calcineurin inhibitor, mycophenolate mofetil, and corticosteroids. HLA- or ABO-incompatible transplants underwent desensitization (rituximab, plasmapheresis, and intravenous immunoglobulin).

3.2.2. Laboratory data (Metabolomics)

This was the only prospective component of the thesis, designed as an exploratory paired study with pre- and post-donation urine samples from the same donors. Urine metabolomics were analyzed by untargeted nuclear magnetic resonance (NMR) spectroscopy to detect amino acids, lipids, and carbohydrate metabolites. Data processing (using NUTSpro software) employed best-fit curve algorithms. Multivariate analyses included Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) to identify class separation and relevant metabolites. Quantitative analysis of metabolite variation was performed through spectral integration. Identified metabolites were mapped to biological pathways using MetaboAnalyst software, with pathway enrichment and topology analyses performed to identify affected metabolic networks.

3.2.3. Image Data

Split renal function was assessed by technetium-labeled diethylenetriamine-pentaacetate (Tc99m-DTPA) nuclear renography, following standardized protocols with dynamic acquisition in the supine position. GFR was determined by radionuclide clearance, with individual kidney contributions calculated to assess split function.

In the retrospective cohort, all donors also underwent contrast-enhanced multidetector CT imaging (GE VCT LightSpeed 64-detector or GE BrightSpeed 16-detector). Nephrographic and excretory phases were acquired using standardized protocols. Kidney volumes were measured on 2.5-mm nephrographic slices by voxel counting, excluding the renal sinus, using OsiriX software. Split renal volume (SRV) was expressed as the proportion of each kidney relative to total renal volume. Volumetric assessment was performed with a semiautomatic segmentation technique, with manual correction when required, and all measurements were carried out by a single surgeon.

In the prospective cohort, CT angiography was performed using more recent multidetector scanners (Siemens Somatom Go Top 64, GE Revolution Evo 64, Philips Incisive, and GE Revolution 128). Volumetric measurements were obtained on a dedicated workstation (AW 4.4 VolumeShare 2, GE Healthcare, Milwaukee, USA), also using a semiautomatic segmentation technique with manual adjustment when required. Measurements were performed by a trained radiology technician. This methodology was consistent with the retrospective analysis, while reflecting the evolution of scanner technology and software platforms in our Unit.

3.3. Ethical analysis- narrative review

In addition to clinical and laboratory data, an independent narrative review was conducted to identify and synthesize key ethical considerations in living donor kidney transplantation. Sources included peer-reviewed articles, policy documents, and international guidelines from transplantation societies and bioethics organizations. The review examined the application of core bioethical principles—autonomy, beneficence, non-maleficence, and justice—to donor safety, informed consent, risk–benefit balance, donor selection, and equitable access to transplantation. Gender disparities and social or familial expectations were given particular attention. The expanding panorama of living donation, including strategies such as ABO- or HLA-incompatible transplantation, Kidney Paired Exchange, and altruistic donation, was acknowledged as raising specific ethical dilemmas. However, these aspects were not developed in depth, as the review remained focused on the broader ethical principles and donor safety in the Portuguese context.

3.4. Statistical analysis

Continuous data were described using mean and standard deviation (SD) or median (interquartile range [IQR]), and categorical data were expressed as numbers (and percentages) as appropriate. Categorical data were compared using the Pearson chi-square test or Fisher exact test. Continuous variables with parametric distribution were compared with the Student's t-test, or analysis of variance (ANOVA) test, as appropriate. In the case of continuous variables with asymmetrical distribution, a comparison was performed with the Mann–Whitney U-test.

Overall and death-censored graft survival were estimated using Kaplan–Meier curves and compared with the log-rank test. In the case of death with a functioning allograft, allograft was censored at the time of death. Risk factors for time-independent binary outcomes were determined by univariate and multivariate logistic regression models. Potential predictors of time-to-event outcomes were explored by univariate and multivariable Cox proportional hazards models. The proportional hazards assumption was verified using log–log plots and Schoenfeld residuals.

Predictive model performance was evaluated by discrimination and calibration metrics. Discrimination was assessed using Harrell's C-statistic and time-dependent or standard AUC. Calibration was evaluated by the calibration slope.

Longitudinal eGFR changes were analyzed using linear mixed-effects models with random intercepts and slopes. Time was modeled as a continuous variable, and also as linear splines with knots at 2, 5, and 10 years to identify nonlinear trends. Bonferroni correction was applied for multiple comparisons.

Given the heterogeneous study designs (retrospective cohorts, cross-sectional studies, and one prospective exploratory cohort), statistical methods were adapted to each analysis as specified in the Results chapter.

A two-sided p-value < 0.05 was considered statistically significant. Analyses were performed using STATA/MP v15.1-17 (StataCorp, College Station, TX, USA). Study-specific statistical methods are detailed in the Results chapter.

3.5. Ethical and Institutional approval

The study was reviewed and authorized by the Departamento de Ensino, Formação e Investigação/ Gabinete Coordenador da Investigação and Comissão de Ética of Centro Hospitalar do Porto “Organ admissibility and donor safety: functional metrics to improve donor selection for a safe expansion of living donor kidney transplantation” 147-21 (119-

DEFI/122-CE)] in May 2022. All procedures adhered to the principles of the Declaration of Helsinki and institutional regulations. Anonymity and confidentiality were strictly maintained throughout all stages, and all databases were de-identified before analysis.

CHAPTER IV

RESULTS

RESULTS

4. Results

This chapter presents the findings of a series of interconnected studies designed to test the overarching hypothesis that long-term safety for living kidney donors can be enhanced through comprehensive evaluation and personalized risk assessment. Using both retrospective and prospective cohorts of living donors, and in selected analyses their recipients, the results are organized around four key hypotheses concerning renal health, cardiovascular–kidney–metabolic (CKM) health, psychosocial health, and Donor-Recipient Matching (Table 1).

All studies have been published or submitted to peer-reviewed journals, ensuring methodological rigor and external validation. Data were derived from structured clinical assessments, advanced imaging, biochemical and metabolomic profiling, psychometric evaluation, and immunological analyses. Selected study-specific details are included to enhance the interpretation of results, complementing the general descriptions provided in the Materials and Methods (Chapter III). Baseline donor characteristics are reported selectively, limited to variables directly relevant to the outcomes under study.

Results are presented using descriptive and inferential statistics, with selected figures and tables adapted from peer-reviewed publications or manuscripts under review. Complete datasets, extended baseline characteristics, and sensitivity analyses are available in the respective Appendices. Beyond the hypothesis-driven studies, the chapter concludes with a structured ethical reflection on the principles, dilemmas, and responsibilities inherent in living kidney donation, providing a broader societal and professional context for the empirical findings.

Table 1 -Thesis publications according to the studied hypotheses and objectives

Hypotheses	Aims	Publications
1 <u>Renal Health:</u> Personalized renal risk assessment in living kidney donors can be improved by integrating eGFR trajectories, predictive models, and imaging-based volumetric assessments.	To evaluate the association of post-donation eGFR trajectories with donor baseline characteristics and early recovery patterns and their value for renal risk stratification.	1. Almeida M et al., Transpl Int 2024, 37, doi: 10.3389/ti.2024.13356
	To validate the Toulouse-Ranguel model in predicting 1-year post-donation eGFR among Portuguese donors.	2. Almeida M. et al, Transpl Int 2023,36, doi: 10.3389/ti.2023.11151.
	To compare the predictive accuracy of CT-based renal volumetry and nuclear renography in estimating remaining renal function.	3. Almeida M et al., Int Urol Nephrol 2023, 55, doi: 10.1007/s11255-022-03441-9
2 <u>Cardiovascular-Kidney-Metabolic (CKM) Health:</u> Systemic vulnerability beyond renal outcomes can be identified through the combined evaluation of BMI, blood pressure, and metabolomic profiling.	To evaluate the prevalence, predictors and clinical associations of obesity (BMI$\geq$30Kg/m²) after living kidney donation.	1. Cunha A, et al, J Clin Med 2025 14, 6411.doi.org/10.3390/jcm14186411
	To evaluate the incidence, predictors, and correlates of <i>de novo</i> hypertension post-donation.	2. Fernandes J et al, BMC Nephrology, 2025
	To investigate whether urinary metabolomic profiles can reveal early biochemical signatures of CKM stress in living kidney donors.	3. Almeida M et al. 2025, submitted October 2025
3 <u>Psychosocial Health:</u> Psychosocial health in living kidney donors can be comprehensively assessed through integrated qualitative and quantitative methods.	To identify psychosocial risk factors associated with lower health-related quality of life, anxiety, and depressive symptoms after donation.	1. Almeida M. et al., Transplant Proc. 2025; doi: 10.1016/j.transproceed.2025.05.006.
	To assess donor perceptions and satisfaction following living kidney donation using dedicated questionnaires and structured psychometric tools.	2. Almeida M. et al. Transplant Proc. 2025, doi:10.1016/j.transproceed.2025.05.036.
4 <u>Donor–Recipient Matching:</u> Adequate matching can be achieved by integrating predictive tools, donor characteristics, and immunologic compatibility	To validate the iPREDICTLIVING model in a Portuguese living donor cohort and evaluate its clinical utility for pre-transplant counseling.	1. Almeida M. et al., Clin Transplant. 2024 doi: 10.1111/ctr.15283
	To assess the association of baseline donor characteristics, such as age and eGFR, with long-term graft function and graft survival.	2. Almeida M. et al., J Clin Med 2023, doi: 10.3390/jcm12216777
	To compare transplant outcomes between recipients of related and unrelated living donors, controlling for immunologic compatibility.	3. Reis Pereira P et al., Cureus 2022, doi 10.7759/cureus.30189
<u>Ethics in living kidney donation – Narrative review (not hypothesis driven)</u>	To Identify and analyze key ethical challenges in living donor kidney transplantation, including autonomy, informed consent, risk–benefit balance, justice, and gender disparities.	1.Almeida M. et al; Portuguese Kidney Journal, 2025, doi. 10.71749/pkj.77

BMI, Body Mass Index; CKM; Cardio Kidney Metabolic; CT, Computed Tomography; eGFR, estimated Glomerular Filtration Rate;

4.1. Hypothesis One: Renal Health

Personalized renal risk assessment in living kidney donors can be improved by integrating eGFR trajectories, predictive models, and imaging-based volumetric assessments.

Preservation of long-term renal function is central to safeguarding the health of living kidney donors. Accurate pre-donation risk assessment requires not only baseline kidney function but also tools that can anticipate post-donation trajectories. In this hypothesis, three approaches were evaluated: (1) longitudinal analysis of estimated glomerular filtration rate (eGFR) and their determinants after donation, (2) external validation of the Toulouse–Rangueil predictive model, and (3) computed tomography (CT)-based kidney volumetry.

Study 1:

Longitudinal Trajectories of Estimated Glomerular Filtration Rate and their determinants after donation

Adapted from: Almeida M. et al, *Longitudinal Trajectories of Estimated Glomerular Filtration Rate in a European Population of Living Kidney Donors*. Transplant International. 2024; 37:13356. doi: 10.3389/ti.2024.13356.

APPENDIX 1

This section is based on published work by the author. Content has been adapted and reformatted for coherence within this thesis. Figures and Tables are adapted from the above article, distributed under the terms of the Creative Commons Attribution License (CC BY 4.0).

Study-specific material and methods

We retrospectively analyzed 320 LKDs (1998–2020) with a serum creatinine measurement at 6 months and at least three subsequent measurements during follow-up. eGFR was estimated using the CKD-EPI equation. Long-term trajectories (6 months–15 years) were modeled using linear mixed-effects models with random intercepts and slopes, supplemented by linear-spline models with knots at 2, 5, and 10 years. Subgroup analyses included age, sex, BMI, hypertension, smoking, dyslipidemia, pre-donation eGFR, and proteinuria, with adjustment for baseline covariates. Early kidney function reduction rate (KFRR) at 6 months was calculated and categorized into three groups: <26.2%, 26.2–36.1%, and >36.1%.

RESULTS

Results

Baseline characteristics are summarized in Table 2. Donors had a mean age of 47.3 ± 10.5 years, 71% were female, and the mean baseline eGFR was 100.4 ± 14.6 mL/min/1.73 m². The mean BMI was 25.3 ± 3.3 kg/m²; 16% had hypertension, and 15% were active smokers. Donors were distributed across the KFRR tertiles, with a median KFRR of 31.9%.

Table 2 - Baseline characteristics of the study cohort (n=320)

Characteristic	Value
Age, years (mean $\pm$ SD)	47.3 $\pm$ 10.5
Female, n (%)	227 (71%)
BMI, kg/m ² (mean $\pm$ SD)	25.3 $\pm$ 3.3
BMI, kg/m ² (n, %)	
<25	155 (48)
25–30	132 (41)
≥ 30	33 (10)
Hypertension, n (%)	51 (16%)
Dyslipidemia, n (%)	44 (14)
ProtU 0.15–0.5 g/g, n (%)	96 (30)
Active smoker, n (%)	48 (15%)
Pre-donation eGFR, mL/min/1.73 m ² (mean $\pm$ SD)	100.4 $\pm$ 14.6
KFRR group, n (%)	<26.2%: 106 (33%)
	26.2–36.1%: 107 (33%)
	>36.1%: 107 (33%)

*KFRR post-donation = $[-(eGFR_{6M} - eGFR_{pre-donation}) / eGFR_{pre-donation} * 100]$.

BMI, body mass index; eGFR, estimated glomerular filtration rate; IQR, interquartile range; KFRR, kidney function reduction rate; n, number; ProtU, protein/creatinine ratio in the urine; ProtU 0.15–0.5 g/g, a protein/creatinine ratio of 0.15–0.5 g/g in a urinary sample; SCr, serum creatinine; SD, standard deviation.

From six months post-donation, the mean overall eGFR slope was $+0.35$ mL/min/1.73 m²/year (95% CI $+0.20$ to $+0.50$). Recovery was steepest in the first two years ($+0.85$ mL/min/1.73 m²/year), slower between 2 and 10 years, and modestly negative thereafter (-0.24 mL/min/1.73 m²/year) (Table 3, Figure 1).

Table 3 - Estimated annual eGFR change (mL/min/1.73 m²/year) in 320 donors from 6 months onward, overall and by spline periods.

Period	Slope (mL/min/1.73 m ² /year)	95% CI
Overall (6M–15Y)	+0.35	+0.20 to +0.50
6M–2Y	+0.85	+0.53 to +1.16
2–5Y	+0.45	+0.18 to +0.72
5–10Y	+0.24	–0.06 to +0.54
10–15Y	–0.24	–0.71 to +0.23

CI, confidence interval; M, months; Y, years

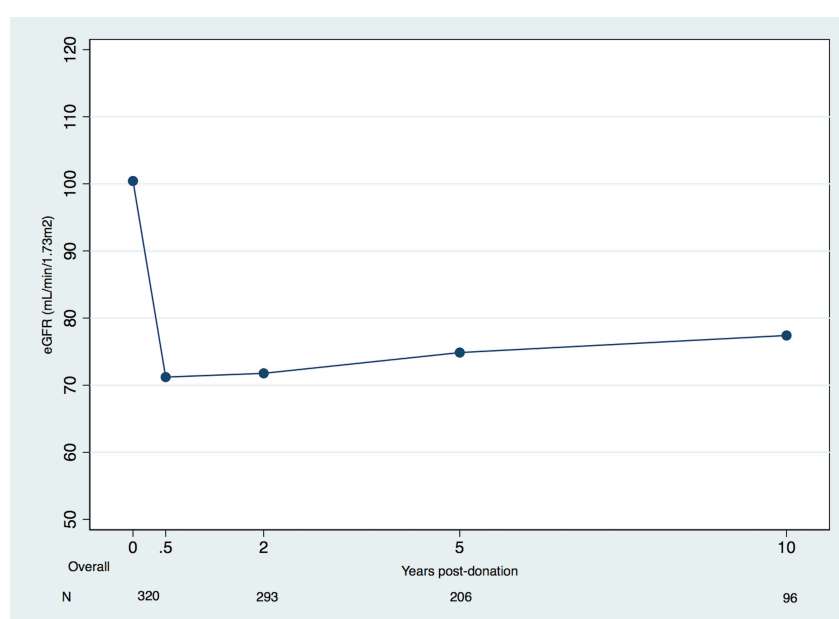


Figure 1. Mean eGFR (mL/min per 1.73 m²) in living kidney donors pre-donation and during post-donation follow-up (eGFR- estimated glomerular filtration rate).

Subgroup analysis showed that obesity (BMI ≥ 30 kg/m²) and lower pre-donation eGFR were associated with less favorable slopes (Table 4, Appendix 1). In the overall period, normal-weight donors improved (+0.59 mL/min per 1.73 m²/year), whereas donors with obesity declined eGFR (–0.18 mL/min per 1.73 m²/year, $p=0.020$). Donors with pre-donation eGFR <80 mL/min per 1.73 m² /year showed a late decline at 5–10 years (–1.16/year, $p=0.049$). Only variables with statistically significant associations are shown in Table 4; complete analyses, including non-significant covariates, are available in Appendix 1.

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Table 4 - Changes in eGFR (mL/min/1.73 m²/year) in living kidney donors (n=320) by subgroup over different periods during follow-up from 6 months onward (multivariable analysis)

Linear spline model					
	Overall	6M-2y	2y-5y	5y-10y	10-15y
BMI*					
<25	+0.59 (+0.37, +0.80) ^B	+0.67 (-0.44, +1.77) ^{AB}	+0.97 (+0.36, +1.57) ^B	+0.32 (-0.17, +0.81) ^A	-0.11 (-1.14, +0.91) ^A
25-30	+0.35 (-0.14, +0.56) ^{AB}	+2.17 (+0.98, +3.36) ^B	-0.17 (-0.81, +0.46) ^A	+0.49 (+0.02, +0.95) ^A	-0.26 (-1.01, +0.48)
>=30	-0.18 (-0.68, +0.31) ^A	-2.47 (-4.92, -0.03) ^A	+1.18 (-0.17, +2.53) ^{AB}	-0.64 (-1.82, +0.54) ^A	-0.62 (-2.39, +1.16) ^A
p	0.02	0.002	0.021	0.214	0.897
Pre-donation eGFR*					
<80	-0.09 (-0.63, +0.44) ^A	+2.85 (+0.24, +5.46) ^A	+0.14 (-1.41, +1.69) ^A	-1.16 (-2.39, +0.06) ^B	-0.44 (-2.03, +1.15) ^{AB}
80-90	+0.52 (+0.16, +0.88) ^A	+2.32 (+0.34, +4.29) ^A	-0.33 (-1.41, +0.75) ^A	+0.51 (-0.31, +1.32) ^A	+1.62 (+0.23, +3.01) ^B
>=90	+0.43 (+0.27, +0.60) ^A	+0.54 (-0.34, +1.41) ^A	+0.70 (+0.23, +1.17) ^A	+0.40 (+0.04, +0.77) ^A	-0.58 (-1.33, +0.18) ^A
p	0.141	0.105	0.217	0.049	0.028

* In variables with three or more groups, each box will present letters A to C (superscript). It should be concluded that subgroups that share the same letters in the same box are non-significantly different.

BMI, body mass index (kg/m²); eGFR, estimated glomerular filtration rate (mL/min/1.73m²); M, months; Y, years.

The KFRR classification also stratified early and long-term trajectories (Table 5, Figure 2). Donors with KFRR <26.2% had negative slopes (-0.21/year), while those with higher KFRR showed positive slopes (+0.53 and +0.65/year; p<0.001).

Table 5 - Annual eGFR change (mL/min/1.73 m²/year) by KFRR group over follow-up (multivariable analysis, n=320)

Linear spline model					
	Overall	6M-2y	2y-5y	5y-10y	10-15y
%KFRR post-donation					
<26.2	-0.21 (-0.42, +0.01) ^{B*}	-2.71 (-4.04, -1.39) ^A	+0.35 (-0.34, +1.03) ^A	+0.03 (-0.47, +0.53) ^A	-0.36 (-1.14, +0.42) ^A
26.2-36.1	+0.53 (+0.28, +0.78) ^A	+1.50 (+0.20, +2.80) ^B	+0.79 (+0.08, +1.51) ^A	+0.09 (-0.48, +0.66) ^A	0.49 (-1.77, +0.80) ^A
>36.1	+0.65 (+0.39, +0.92) ^A	+3.66 (+2.38, +4.94) ^C	+0.04 (-0.67, +0.76) ^A	+0.43 (-0.19, +1.05) ^A	-0.61 (-1.84, +0.61) ^A
p	<0.001	<0.001	0.339	0.595	0.941

Models adjusted for baseline covariates (age, sex, BMI, hypertension, smoking, dyslipidemia, proteinuria, and pre-donation eGFR)

* In variables with 3 or more groups, each box will present letters A to C (superscript). It should be concluded that subgroups that share the same letters in the same box are not significantly different.

y- years; M- months BMI- body mass index; eGFR -estimated glomerular filtration rate; KFRR: kidney function reduction rate post-donation.

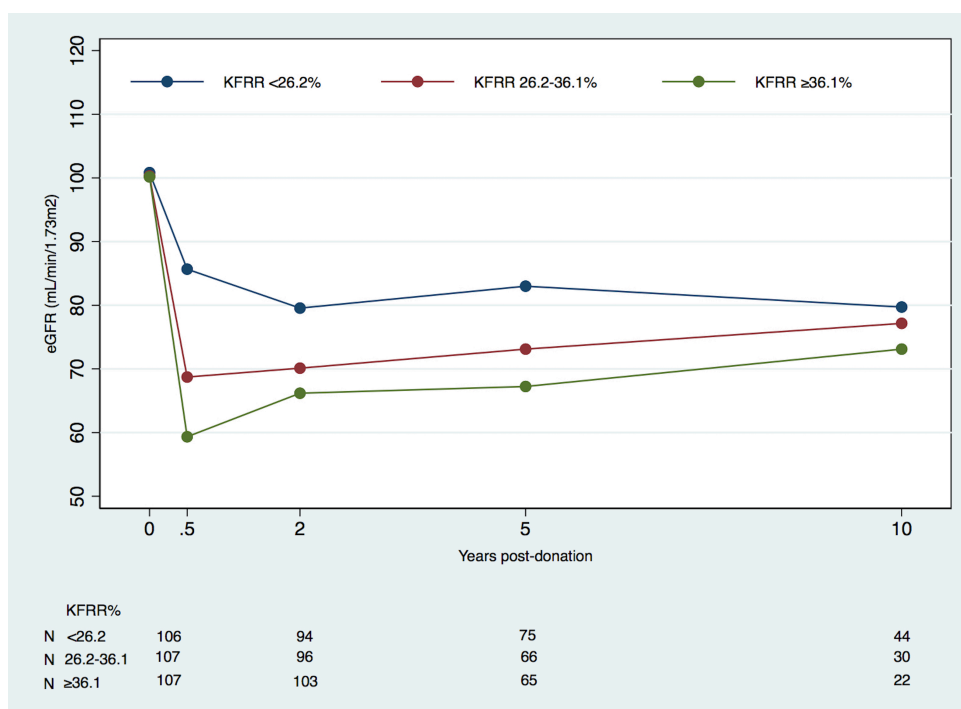


Figure 2. Mean eGFR (mL/min/1.73 m²) in living kidney donors by KFRR subgroup at 6 months, from pre-donation through long-term follow-up

At the last follow-up, no donor reached eGFR <15 mL/min/1.73 m², and only 6 (2%) progressed to CKD stages 3b and 4 (Table 6).

Table 6 - eGFR (mL/min/1.73 m²) category for donors based on the last available SCr measurement, using the CKD-EPI equation.

eGFR (mL/min/1.73 m ²)	n=320 n (%)
<15	0
15-30	2 (1)
30-45	4 (1)
45-60	55 (17)
60-90	205 (64)
≥90	54 (17)

Scr.- serum creatinine; eGFR- estimated glomerular filtration rate; CKD-EPI - Chronic Kidney Disease-Epidemiology Collaboration equation

The prevalence of eGFR <60 mL/min/1.73 m² decreased from 25% at 6 months to 13% at 10 years. More severe reductions (<45 mL/min/1.73 m²) remained infrequent (<5%). Early risk was higher in older donors (≥55 years) and those with lower pre-donation eGFR or high KFRR, but differences disappeared beyond 5 years. Proteinuria also declined, from 30%

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pre-donation to 10% after 6 months, and remained stable long term, with a median of 0.07 (0.06-0.10) g/g at 10 years of follow-up.

In summary, from six months post-donation, eGFR trajectories showed an overall positive slope, with the steepest recovery in the first two years, slower gains until 10 years, and a modest decline thereafter. Baseline BMI, pre-donation eGFR, and early KFRR significantly influenced these patterns. The long-term prevalence of low eGFR and proteinuria remained low in this cohort.

Study 2:

External Validation of the Toulouse-Rangueil Predictive Model

Adapted from: Almeida M, et al. *External Validation of the Toulouse-Rangueil Predictive Model Estimate Donor Renal Function After Living Donor Nephrectomy*, Transplant International. 2023; 36:11151. <https://doi.org/10.3389/ti.2023.11151> .

APPENDIX 2

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Given the variability observed in long-term eGFR trajectories among living kidney donors, we next examined whether pre-donation models could predict post-donation renal function. Specifically, we evaluated the performance of the Toulouse–Rangueil score [80] in our cohort.

Study-specific material and methods

We retrospectively analyzed 333 living kidney donors who underwent nephrectomy between 1998 and 2020 and had a serum creatinine measurement at 1-year post-donation. eGFR was estimated using the CKD-EPI equation. The Toulouse–Rangueil score, based on age and baseline eGFR, was calculated for each donor ($\text{Predicted eGFR} = 31.71 + (0.521 \times \text{preoperative eGFR}) - (0.314 \times \text{age})$). Model performance was assessed by comparing predicted and observed eGFR at 1 year using correlation and Bland–Altman analysis. Discrimination for post-donation CKD ($<60 \text{ mL/min/1.73 m}^2$) was evaluated with receiver-operating characteristic (ROC) curves, and calibration was evaluated with calibration plot, calibration slope, and calibration-in-the-large.

Results

The donors had a mean age of 47.3 ± 10.6 years, 71% were female (71%), and the mean pre-donation eGFR was 100.3 ± 14.7 mL/min/1.73m. At 1 year, the mean observed eGFR was 71.4 ± 16.2 mL/min/1.73m², close to the model-predicted value of 69.1 ± 10.0 mL/min/1.73m². A total of 85 donors (25.5%) met the CKD definition (eGFR <60 mL/min/1.73m²) at 1 year (Table 7).

Table 7 - Baseline characteristics of the donor cohort included in the Toulouse-Rangueil model validation study (n = 333).

Variable	Value
Age, years (mean $\pm$ SD; range)	47.3 ± 10.6 (20.7–76.2)
Female sex, n (%)	236 (71%)
Body mass index, kg/m ² (mean $\pm$ SD)	25.3 ± 3.4
Hypertension pre-donation, n (%)	50 (15%)
Smoking habits, n (%)	51 (15%)
Pre-donation eGFR, mL/min/1.73m ² (mean $\pm$ SD)	100.3 ± 14.7
Observed eGFR at 1 year, mL/min/1.73m ² (mean $\pm$ SD)	71.4 ± 16.2
Predicted eGFR at 1 year, mL/min/1.73m ² (mean $\pm$ SD)	69.1 ± 10.0
CKD at 1 year (eGFR <60 mL/min/1.73m ²), n (%)	85 (25.5%)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; n, number; SD, standard deviation.

Predicted and observed values were strongly correlated ($r = 0.67$, $p < 0.001$), with good agreement on Bland–Altman analysis and no systematic bias (Figure 3).

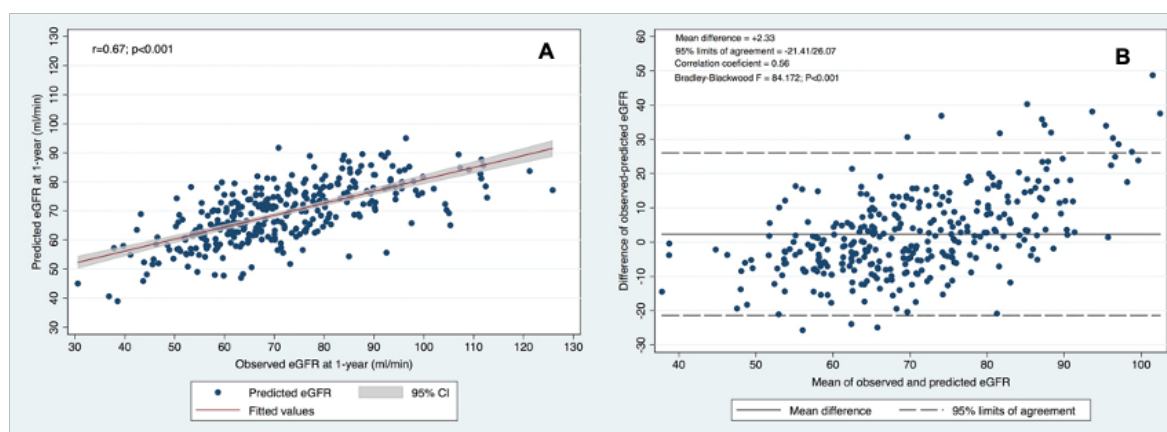


Figure 3. Agreement between predicted and observed eGFR at 1-year post-donation.

- A- Scatterplot showing strong correlation ($r = 0.67$, $p < 0.001$), with greater dispersion at higher observed values but no evidence of systematic bias,
- B- Bland–Altman plot confirming good agreement, with differences symmetrically distributed around zero and wider variability at higher eGFR values but no evidence of proportional bias.

RESULTS

Discrimination for post-donation CKD was high (AUC 0.83, 95% CI 0.78–0.88), with an optimal cutoff of 65 mL/min/1.73 m² (sensitivity 77%, specificity 75%). The calibration analysis confirmed close alignment between predicted and observed values, with a slope near 1 and no evidence of systematic miscalibration. (Figure 4) Secondary analyses showed a slightly lower optimal cutoff in female donors (62.2 mL/min/1.73 m²).

Detailed calibration outputs are provided in Appendix 2.

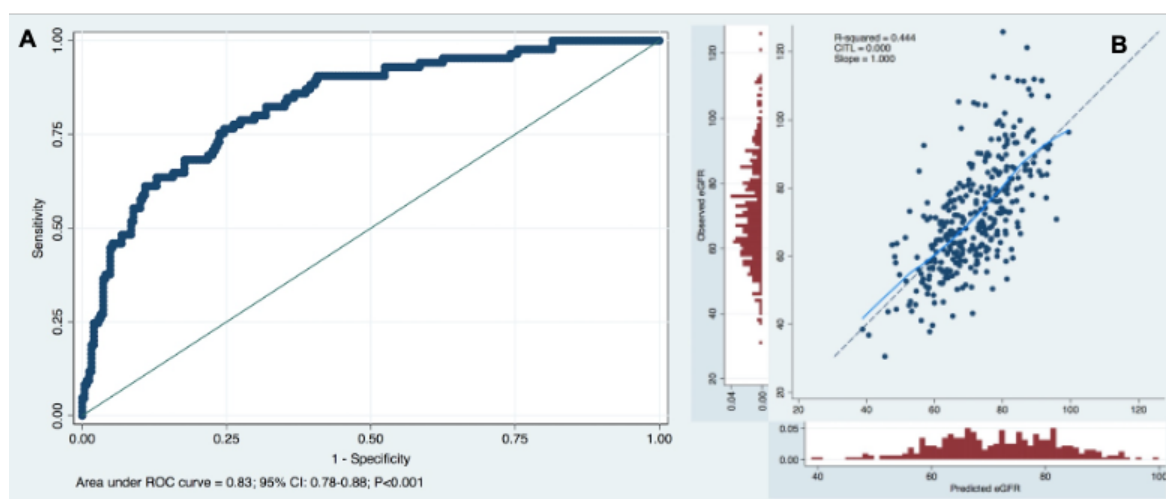


Figure 4. Discrimination and Calibration of the Toulouse–Rangueil model for predicting CKD at 1-year post-donation.

- A-** Receiver operating characteristic (ROC) curve showing high discrimination (AUC 0.83, 95% CI 0.78–0.88);
- B-** Calibration plot demonstrating close agreement between predicted and observed values, with slope~1 and CIL =0.

In summary, the Toulouse–Rangueil score, calculated from two routinely available clinical variables (age and baseline eGFR), demonstrated strong correlation with observed post-donation eGFR, accurate calibration across the prediction range, and high discriminative ability for identifying donors with eGFR <60 mL/min/1.73 m² at one year.

Study 3:

CT Volumetry and Post-Donation Renal Function

Adapted from Almeida M, et al. *CT volumetry performs better than nuclear renography in predicting estimated renal function one year after living donation*, International Urology and Nephrology. 2023; 55:553-562. <https://doi.org/10.1007/s11255-022-03441-9>.

APPENDIX 3

This section is based on published work by the author. Content has been adapted and reformatted for coherence within this thesis. Figures are adapted from the above article, distributed under the terms of the Creative Commons Attribution License (CC BY 4.0).

To complement longitudinal trajectories and predictive modeling, we next examined whether incorporating anatomical measurements from pre-donation imaging could further refine the prediction of post-donation renal function. Given that kidney size has been linked to nephron mass and functional reserve, we evaluated the association between remaining kidney volume measured by computed tomography and subsequent renal outcomes.

Study-specific material and methods

We retrospectively analyzed 193 living donors (2008-2018) who underwent both contrast-enhanced CT volumetry and technetium-labeled DTPA nuclear renography (NR) performed during preoperative assessment. Agreement between split renal volume (SRV) and split renal function (SRF) was evaluated using Pearson's correlation coefficient, Bland–Altman analysis, and weighted Cohen's kappa statistics. Predictive capacity for 1-year post-donation observed eGFR was assessed using multivariable linear regression models adjusted for age, sex, baseline eGFR, and nephrectomy laterality, applying both CKD-EPI and Cockcroft–Gault body surface area-adjusted (CG-BSA) equations. Model performance was compared using adjusted R^2 and root mean square error (RMSE) as measures of fit and prediction error. Non-nested modeling was then used to compare the ability of each imaging technique to predict the remnant donor kidney function.

Results

Donors had a median age of 48-9 years (IQR, 40.6–56.2), 74% were female, and the mean pre-donation eGFR was 100.4 ± 13.9 ml/min/1.73 m² by CKD-EPI, and 106.7 ± 21.9 ml/min/1.73 m² by CG BSA adjusted. The left kidney was donated in 82% of cases. The median SRV was 49.9 ± 3.3 % by CT and 51.1 ± 3.4 % by NR. Predicted and estimated post-donation renal function at 12 months after donation are depicted in Table 8, according to the imaging technique and the formula used to evaluate eGFR.

RESULTS

Table 8 - Baseline characteristics of living kidney donors included in the CT volumetry study (n=193)

Characteristic	Value
Age, years	48.9 (40.6–56.2)
Female sex, n (%)	142 (74%)
Body mass index, kg/m ²	25.2 (22.7–28.0)
Pre-donation eGFR, CKD-EPI, ml/min/1.73 m ²	100.4 ± 13.9
Pre-donation eGFR, CG (BSA-adjusted), ml/min/1.73 m ²	106.7 ± 21.9
Pre-donation creatinine clearance (24 h), ml/min/1.73 m ²	128.3 ± 29.5
Split function of remaining kidney by CT, %	49.9 ± 3.3
Split function of remaining kidney by nuclear renography (NR), %	51.1 ± 3.4
Predicted post-donation eGFR, CKD-EPI (CT), ml/min/1.73 m ²	50.0 ± 7.1
Predicted post-donation eGFR, CKD-EPI (NR), ml/min/1.73 m ²	51.2 ± 7.5
Predicted post-donation eGFR, CG (CT), ml/min/1.73 m ²	53.2 ± 11.3
Predicted post-donation eGFR, CG (NR), ml/min/1.73 m ²	54.4 ± 11.0
Left kidney donated, n (%)	158 (82%)
Observed donor eGFR at 12 months, CKD-EPI, ml/min/1.73 m ²	71.2 ± 14.5
Observed donor eGFR at 12 months, CG (BSA-adjusted), ml/min/1.73 m ²	74.7 ± 15.3

BSA: Body Surface Area; CG, Cockcroft-Gault; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation; CT, Computed Tomography; eGFR, estimated glomerular filtration rate; SRV, split renal volume; NR, nuclear renography.

CT volumetry showed a weak correlation with nuclear renography for SRF estimation ($R^2 = 0.15$; Figure 5A). Bland–Altman analysis indicated a mean difference of -1.2% (Nuclear Renography higher), with 95% limits of agreement between -8.51% and $+6.11\%$ (Figure 5B). Complete agreement within $\pm 5\%$ occurred in 55% of donors, and weighted agreement was 75% ($\kappa = 0.26$).

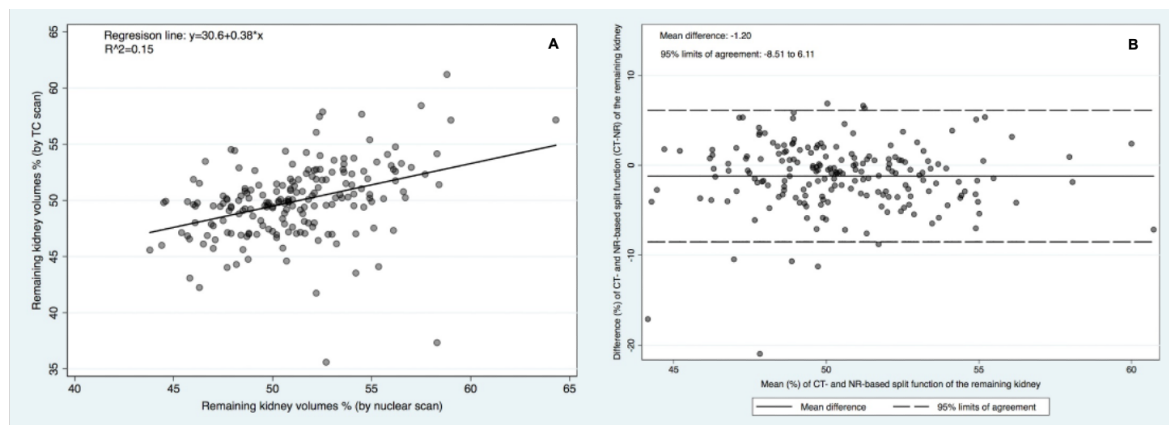


Figure 5. Agreement between CT volumetry and nuclear renography for the determination of the remaining kidney volume.

- A- Scatterplot showing weak correlation ($R^2 = 0.15$).
- B- Bland–Altman plot with mean difference 1.2% (NR higher) and 95% limits of agreement 8.51% to +6.11%.

Predicted and observed eGFR at 1-year post-donation were calculated using the CKD-EPI and CG-BSA equations. CT volumetry showed higher predictive accuracy than nuclear renography for both formulas (adjusted $R^2 = 0.42$ vs 0.37 for CKD-EPI, and 0.61 vs 0.60 for CG-BSA) (Table 9). Prediction error was slightly lower with CT volumetry (RMSE 11.09 vs 11.52 for CKD-EPI; 9.51 vs 9.71 for CG-BSA). The best correlation was observed with the CG-BSA formula (Table 9, Figure 6). Non-nested model testing (J-test and Cox–Pesaran test) confirmed the superiority of CT volumetry for both equations.

Table 9 - Multivariable linear regression predicting 1-year post-donation mGFR according to imaging modality (Models adjusted for age, sex, laterality of remaining kidney, and BMI)

Formula used	Imaging modality	Coefficient of renal function for every 10 ml/min predicted (95% CI)	P-value	Adjusted R^2	rMSE
CKD-EPI	NR	9.56 (7.08–12.05)	<0.001	0.37	11.52
CKD-EPI	CT	10.98 (8.51–13.45)	<0.001	0.42	11.09
CG-BSA	NR	7.52 (6.01–9.02)	<0.001	0.60	9.71
CG-BSA	CT	7.71 (6.25–9.17)	<0.001	0.61	9.51

BSA, Body Surface Area; CG, Cockcroft-Gault; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation; CT, Computed Tomography; NR: Nuclear Renography; rMSE, Root Mean Square Error.

Adjusted R^2 represents the proportion of variability in the outcome explained by the regression model, adjusted for the number of predictors; higher values reflect better model fit; Root Mean Square Error (RMSE) quantifies the average magnitude of prediction errors, expressed in the same units as the outcome. Lower values indicate greater accuracy, with RMSE corresponding to the typical distance between predicted and observed values.

RESULTS

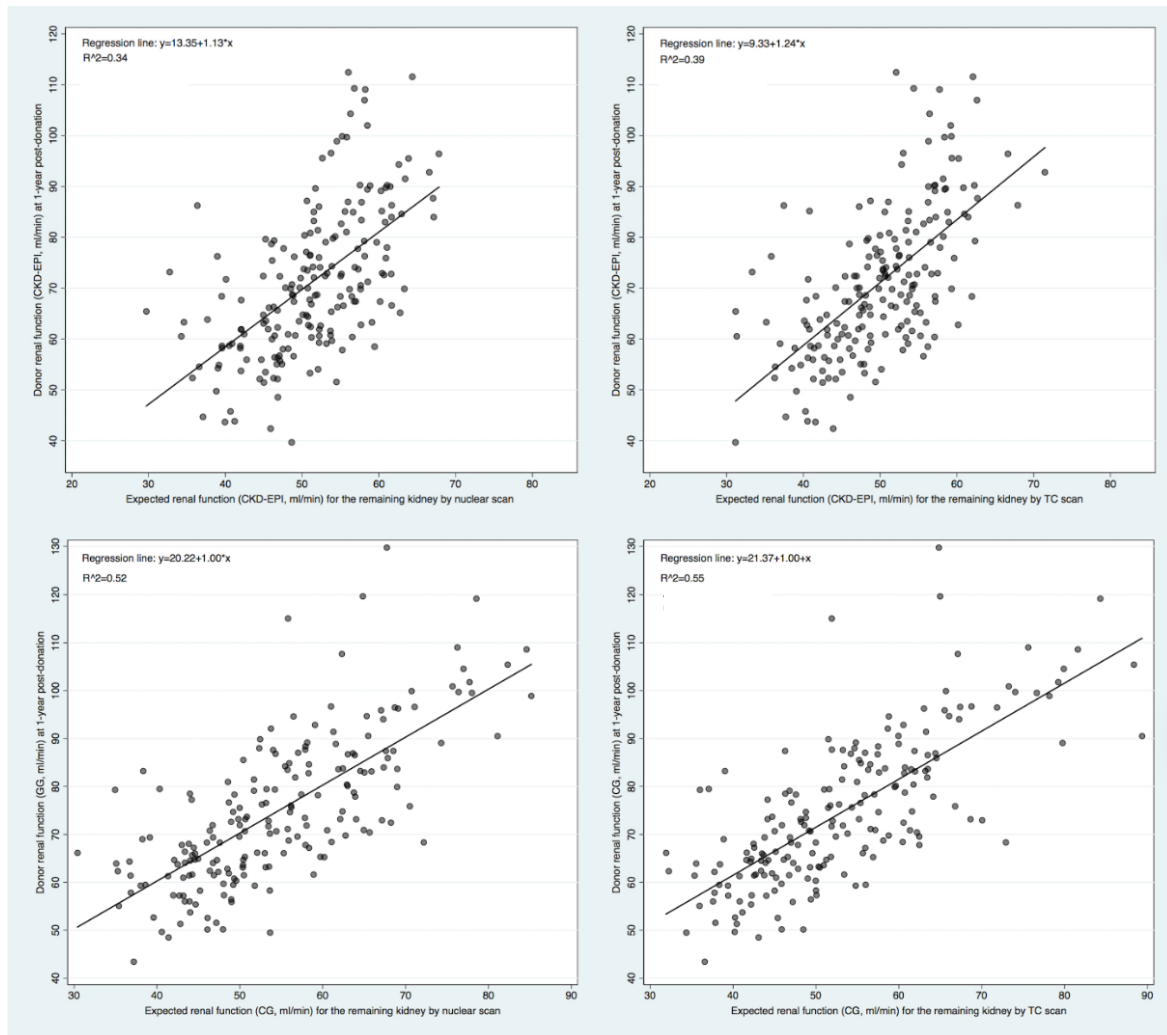


Figure 6. Scatter plots of predicted renal function for the remaining kidney (x-axis) versus observed eGFR at 1-year (y-axis), using CKD-EPI (upper panels) and Cockcroft–Gault BSA- adjusted (lower panels) equations. NR (left) is compared with CT volumetry (right).

Regression lines (solid black).

In the sensitivity analysis, cases where NR predicted higher SRF were associated with significantly lower observed eGFR at 1 year, whereas discordance in favor of CT volumetry was not. Full results are provided in the Appendix 3 (Table 4).

In summary, CT volumetry provided reproducible and non-invasive SRF estimates, with good agreement with nuclear renography despite weak correlation. It achieved higher adjusted R^2 , lower RMSE, and superior performance in non-nested model comparisons for predicting 1-year renal function, while also offering anatomical information relevant for surgical planning.

Together, these three studies demonstrate that longitudinal eGFR trajectories and their determinants, simple clinical prediction models, and imaging-based volumetry provide complementary perspectives on donor renal risk. Long-term eGFR follow-up captures patterns of post-donation adaptation; the Toulouse–Rangueil model offers a rapid, low-cost estimate of early function; and CT volumetry refines prediction by incorporating anatomical data. Integrated into a multidimensional assessment, these approaches can support more individualized donor selection and long-term safety monitoring.

4.2. Hypothesis Two: Cardiovascular-Kidney-Metabolic Health

Systemic vulnerability beyond renal outcomes can be identified through the combined evaluation of BMI, blood pressure, and metabolomic profiling.

While Hypothesis one primarily addressed renal outcomes and predictors of post-donation kidney function, Hypothesis Two focused on the broader cardiovascular–kidney–metabolic (CKM) profile of living kidney donors. This hypothesis probed selected risk factors that extend beyond renal reserve, specifically obesity ($\text{BMI} \geq 30 \text{ Kg/m}^2$), hypertension, and early metabolic alterations detectable through urinary metabolomic profiling. The overarching aim was to determine how these CKM risk factors evolve after donation, how they influence long-term donor safety, and whether they provide opportunities for early risk stratification and intervention.

The analysis comprised three complementary studies: 1) An obesity study, retrospective longitudinal evaluation of BMI trajectories and prevalence of obesity after donation, stratified by pre-donation BMI, age, and sex; 2) a hypertension study with a retrospective assessment of incident hypertension after donation, including risk factor evaluation and its impact on renal functional outcomes and 3) a Metabolomics study, a prospective pilot analysis of urinary metabolic profiles before and after donation to identify early biochemical signatures of CKM stress.

Study1:

Obesity after Living Kidney Donation: Prevalence, Predictors, and Associations with Cardiovascular and Renal Parameters

Adapted from: Cunha A., et al. *Prevalence of Obesity After Living Kidney Donation and Associated Risk Factors: Cardiovascular and Renal Implications*; published at Journal of Clinical Medicine 2025, 14, 6411. <https://doi.org/10.3390/jcm14186411>

APPENDIX 4

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Given the established relationship between body weight and CKM risk, we examined the prevalence, evolution, and determinants of obesity ($\text{BMI} \geq 30 \text{ Kg/m}^2$) in living kidney donors after nephrectomy, together with its associations with cardiovascular and renal outcomes.

Study-specific material and methods

This retrospective longitudinal study included 306 living kidney donors (LKD) with at least two BMI measurements during follow-up (baseline and one-year post-donation). Donors were stratified by pre-donation BMI category (normal weight- BMI < 25 kg/m², overweight- BMI 25-29.9 kg/m², and donors with obesity- BMI ≥30 kg/m²).

The primary outcome was BMI change over time and incidence of obesity up to 15 years post-donation. Secondary outcomes included associations between post-donation obesity and cardiovascular (hypertension, dyslipidemia) and renal (eGFR, proteinuria) parameters.

Statistical analyses used mixed-effects models for BMI trajectories and multivariable regression for predictors of post-donation obesity. Longitudinal trends in renal and cardiovascular parameters according to obesity status were examined using paired and stratified approaches.

Results

Among donors, 49% were classified as normal weight, 41% as overweight, and 10% as having obesity. Baseline cardiovascular and metabolic parameters differed significantly between BMI categories. Donors with obesity had a higher prevalence of hypertension (41% vs. 7% in normal weight, $p < 0.001$), dyslipidemia (41% vs. 9%, $p < 0.001$), and a modestly lower mean eGFR (96.3 vs. 102.4 mL/min/1.73 m², $p = 0.038$), as detailed in Table 10.

Table 10 - Baseline Characteristics of Kidney Donors Stratified by Pre-Donation Body Mass Index Status (full characteristics are available at Annex 4)

	Total	Normal weight (BMI<25 kg/m ²)	Overweight (BMI:25- 29.9kg/m ²)	Obesity (BMI≥30 kg/m ²)	p
N (%)	306 (100%)	150 (49%)	124 (41%)	32 (10%)	-
Age, mean ±SD	47.2±10.7	45.4±11.3	48.4±10.2	50.8±8.8	0.009
Sex F, n (%)	217 (71)	114 (76)	83 (67)	20 (63)	0.140
BMI Kg/m², mean ±SD	25.3±3.4	22.5±1.7	27.3±1.4	31.2±1.1	-
Smoking habits, n (%)	49 (16)	32 (21)	13 (10)	4 (13)	0.045
Dyslipidemia, n (%)	44 (14)	13 (9)	18 (15)	13 (41)	<0.001
HT, n (%)	48 (16)	11 (7)	24 (19)	13 (41)	<0.001
TAS, mean ±SD	122.5±13.4	118.8±12.0	125.0±13.3	130.0±14.3	<0.001
TAD, mean ±SD	73.2±8.7	71.6±8.2	74.2±8.9	76.8±8.8	0.002
Pre-donation eGFR ml/min/1.73m², mean ± SD	100.3±14.6	102.4±15.0	98.9±13.9	96.3±14.5	0.038

BMI, body mass index; eGFR, estimated glomerular filtration rate; F, female; HT, hypertension; SD, standard deviation; TAD, diastolic blood pressure; TAS, systolic blood pressure.

RESULTS

During the first 10 years post-donation, the prevalence of obesity remained relatively stable (8.8–17.4%). Beyond 10 years, prevalence exceeded 20%, although interpretation is limited by a smaller sample size at extended follow-up.

In multivariable logistic regression model three baseline factors emerged as significant independent predictors of post-donation obesity over 15 years: younger age at donation (OR 0.882; 95% CI 0.808–0.963; $p = 0.005$), higher pre-donation BMI (OR 5.324; 95% CI 3.471–8.168; $p < 0.001$), and baseline dyslipidemia (OR 6.048; 95% CI 1.065–34.348; $p = 0.042$). The complete regression model, including non-significant variables, is provided in Annex 4, Table 2.

Consistent with these predictors, BMI trajectories differed according to baseline BMI and age. Among normal-weight donors, 6.8% developed obesity by 10 years ($p = 0.083$). Overweight donors showed steady progression, with 11.3% obese at year 1 ($p < 0.001$), 14% at year 3 ($p = 0.001$), and 12.5% at year 10 ($p = 0.014$). Donors with obesity showed an early decline in prevalence (65.6% at year 1, 56.3% at year 3, $p < 0.001$), followed by a rebound to 83.3% at year 10 ($p = 0.317$). Donors younger than 40 years showed a higher risk of obesity after donation, with prevalence increasing from 11.5% at year 1 ($p = 0.014$) to 21.7% at year 10 ($p = 0.046$). (Figure 7)

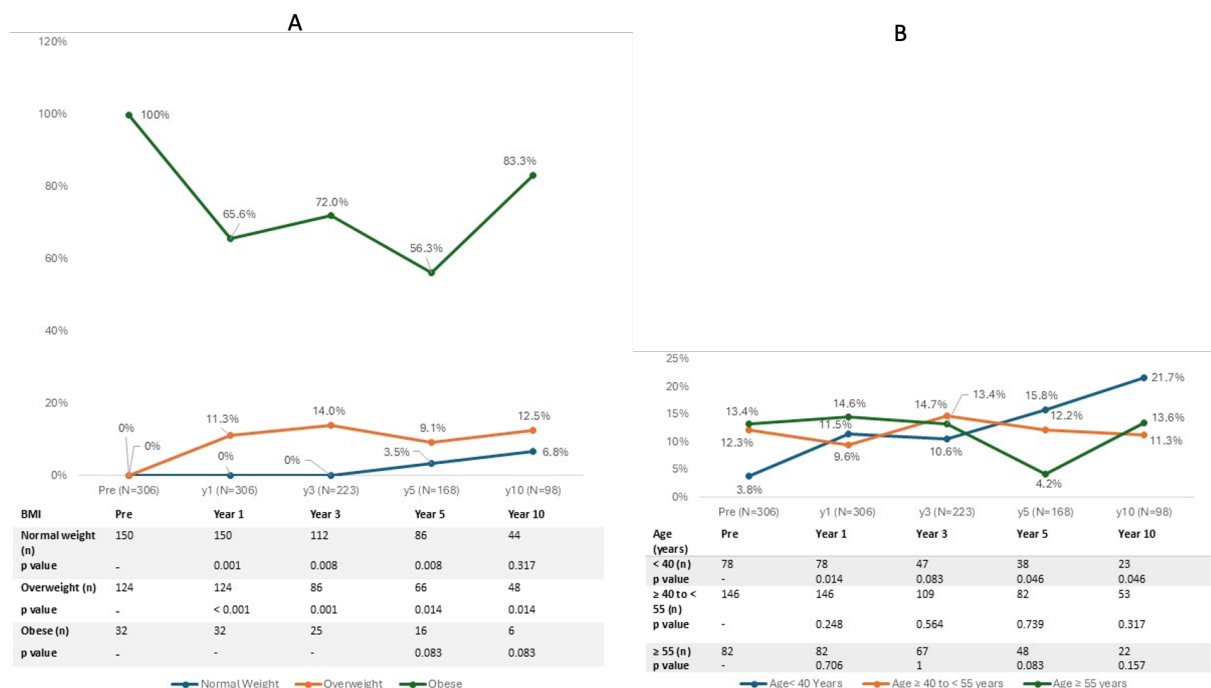


Figure 7. Longitudinal trajectories of obesity prevalence in living kidney donors.

- A-** Evolution according to baseline BMI category (normal weight, overweight, obesity)
- B-** Evolution according to age at donation (<40, ≥40 to <55 and ≥55 years) using McNemar's paired analysis.

Hypertension prevalence increased in all groups over time (Figure 8A) but was consistently higher in donors with obesity (42.9% vs. 21.9% at year 1, $p = 0.010$; and 61% vs. 30.4% at year 5, $p = 0.011$). No significant differences were observed between groups for proteinuria, dyslipidemia, or eGFR decline. Incident diabetes was rare ($n = 4$), precluding meaningful statistical comparisons. (Figure 8).

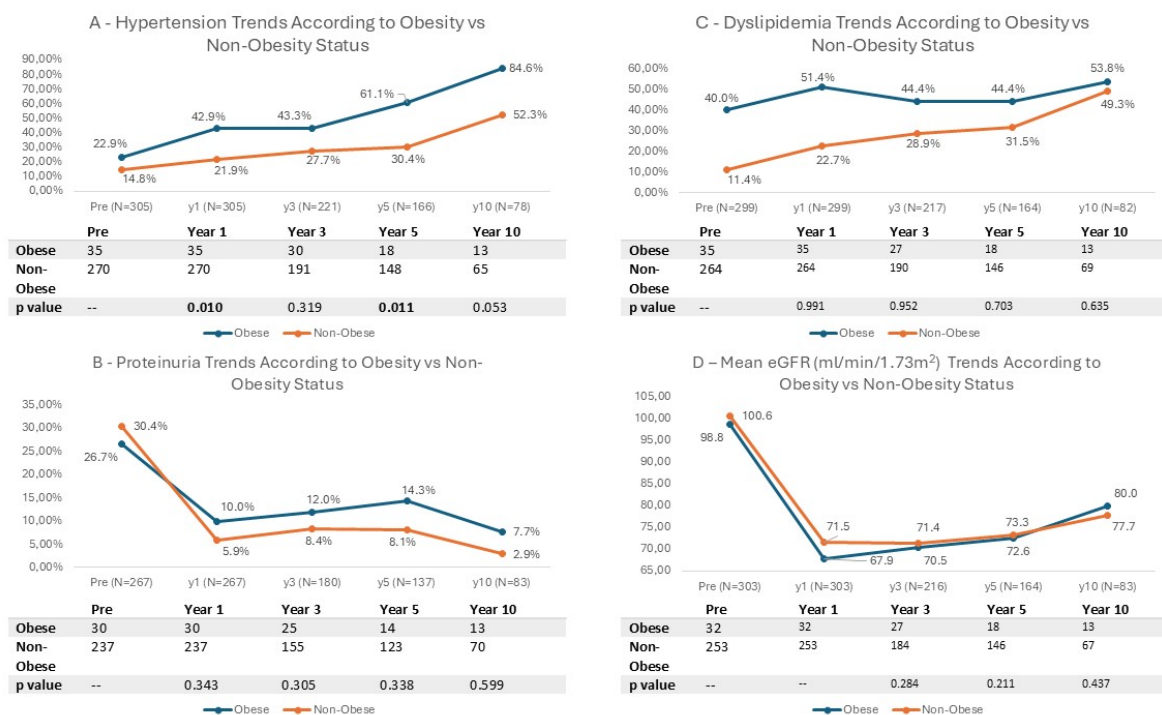


Figure 8. Clinical outcomes stratified by obesity status (obesity vs non-obesity).

- A- Prevalence of hypertension.
- B- Prevalence of proteinuria.
- C- Prevalence of dyslipidemia.
- D- Mean estimated glomerular filtration rate (eGFR) during follow-up.

In summary, across up to 15 years of follow-up, obesity prevalence after kidney donation differed according to baseline BMI and age. Younger donors and those with higher pre-donation BMI (especially those pre-obese at donation) were more likely to develop obesity, while donors with obesity consistently exhibited higher rates of hypertension. No significant differences were observed in renal function decline between groups.

Study 2:

Hypertension after Living Kidney Donation: Incidence, Predictors, and Renal Consequences

Adapted from: Fernandes J., et al., *Hypertension after Living Kidney Donation: Incidence, Predictors, and Consequences*, published at BMC Nephrology 2025,

APPENDIX 5

This section is based on submitted work by the author. Content has been adapted and reformatted for coherence within this thesis.

Following the analysis of BMI trajectories and their associations with cardiovascular risk, the following study evaluated the incidence and determinants of *de novo* hypertension in living kidney donors, together with their relationship to renal and metabolic outcomes.

Study-specific details

This retrospective cohort study included 300 living kidney donors who underwent nephrectomy between 1998 and 2020. Donors with pre-existing hypertension at baseline (n = 55), or incomplete follow-up data (n = 10) were excluded.

The primary outcome was the incidence of new-onset hypertension (HT) after donation, defined as systolic blood pressure >140 mmHg, diastolic blood pressure > 90 mmHg, or initiation of antihypertensive therapy during follow-up. Secondary outcomes included identification of baseline predictors and evaluation of associations with renal function and cardiovascular parameters.

Cumulative incidence was estimated using Kaplan–Meier methods. Event rates were compared with an age- and sex-matched general population cohort from Northern Portugal using standardized event ratios (SER). Cox proportional hazards regression was applied to identify independent predictors of *de novo* HT. Longitudinal renal outcomes were analyzed using mixed-effects models.

Results

Baseline characteristics are summarized in Table 11. Compared to donors who remained normotensive, those who developed hypertension (n = 89; 30%) had significantly higher baseline BMI (26.0 ± 3.0 vs. 24.5 ± 3.2 kg/m², $p < 0.001$), systolic blood pressure (125.4 ± 10.2 vs. 118.3 ± 11.0 mmHg, $p < 0.001$) and diastolic blood pressure (76.3 ± 7.6 vs. 71.2 ± 8.4 mmHg, $p < 0.001$), and were more likely to have a family history of hypertension (58%

vs. 35%, $p < 0.001$). Overweight and obesity were also more prevalent at baseline in the hypertensive group. No significant differences were observed in sex distribution, baseline eGFR, or proteinuria.

Table 11 - Baseline demographic, anthropometric, cardiovascular, and renal characteristics of donors stratified by the development of *de novo* hypertension during follow-up (full data is available at Annex 4, Table 1)

Variable	Total n=300	No HT n=211 (70%)	HT n=89 (30%)	P
Demographic and anthropometric				
Age, mean $\pm$ SD	45.6 $\pm$ 10.5	45.2 $\pm$ 10.7	46.6 $\pm$ 10.5	0.287
Sex F, n (%)	213 (71)	152 (72)	61 (69)	0.542
BMI Kg/m ² , mean $\pm$ SD	24.9 $\pm$ 3.2	24.5 $\pm$ 3.2	26.0 $\pm$ 3.0	<0.001
BMI, n (%)				0.001
<25	162 (54)	127 (60)	35 (39)	
25-30	117 (39)	68 (32)	49 (55)	
≥ 30	21 (7)	16 (8)	5 (6)	
Smoking habits, n (%)	52 (17)	44 (21)	8 (9)	0.013
Related donor, n (%)	200 (67)	132 (63)	68 (76)	0.020
Family history HT, n (%)	105 (35)	53 (25)	52 (58)	<0.001
Clinical and metabolic				
Dyslipidemia, n (%)	30 (10)	17 (8)	13 (15)	0.084
ProtU 0.15-0.5 g/g, n (%)	83 (28)	64 (30)	19 (21)	0.112
Pre-donation eGFR ml/min/1.73m ² , mean $\pm$ SD	101.8 $\pm$ 14.7	102.1 $\pm$ 15.0	101.1 $\pm$ 14.0	0.570
Blood pressure				
SBP, mean $\pm$ SD	120.4 $\pm$ 11.2	118.3 $\pm$ 11.0	125.4 $\pm$ 10.2	<0.001
DBP, mean $\pm$ SD	72.7 $\pm$ 8.5	71.2 $\pm$ 8.4	76.3 $\pm$ 7.6	<0.001

Values are mean $\pm$ standard deviation or number (%). p-values from independent t-test or χ^2 test as appropriate. BMI - Body Mass Index; DBP- diastolic blood pressure; eGFR - estimated glomerular filtration rate F - female; HT – hypertension; HR – hazard ratio; n – number; ProtU - protein/creatinine ratio in the urine; SBP- systolic blood pressure; SD - standard deviation.

During a mean follow-up of 6.4 ± 4.5 years, 89 donors (30%) developed *de novo* hypertension. The cumulative incidence at 1-, 5-, 10-, and 15-years post-donation was 11.1% (95% CI 8.0–15.3), 21.1% (16.6–26.6), 41.1% (33.8–49.5), and 52.2% (41.9–63.4), respectively (Figure 9). The median time to onset was 14.6 years.

RESULTS

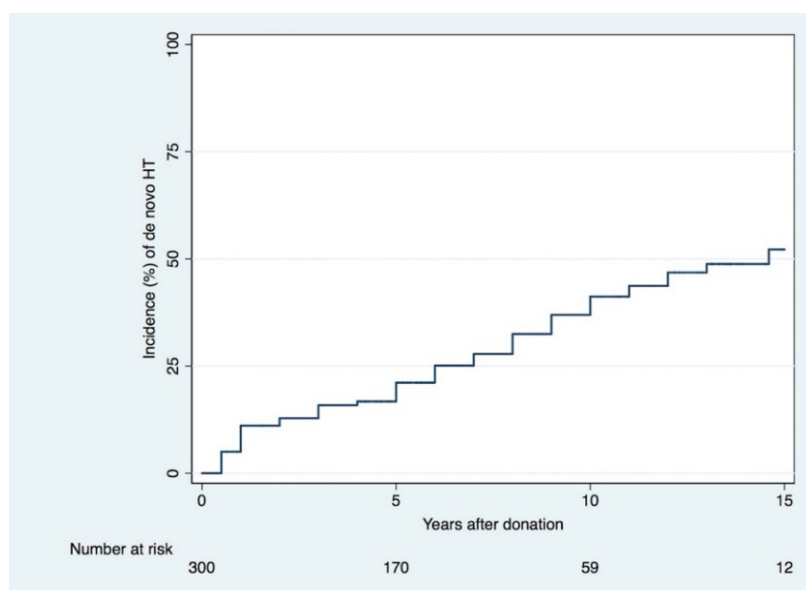


Figure 9. Kaplan-Meier curve depicting the cumulative incidence of *de novo* hypertension in the living kidney donor cohort during follow-up.

Compared with an age- and sex-matched general population cohort [201], donors had higher standardized event ratios (SER) for HT: 3.7-fold higher in the first 5 years (95% CI 2.94–4.86), 3.3-fold higher between 5 and 10 years (95% CI 2.12–4.76), and 2.3-fold higher between 10 and 15 years (SER 2.28; 95% CI 0.84–4.97), although the latter was not statistically significant (Figure 10).

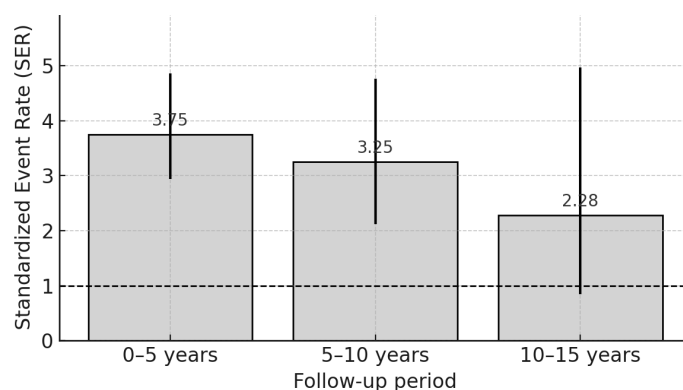


Figure 10. Standardized event rates (SER) for hypertension in living kidney donors compared with a matched general population cohort from Northern Portugal, stratified by follow-up period.

Bars show SER values with 95% confidence intervals.

Dashed line indicates parity with the general population (SER = 1).

Data derived from the institutional donor cohort; figure prepared for the present thesis

Multivariable Cox regression identified BMI (HR 1.074 per kg/m², $p = 0.039$), dyslipidemia (HR 1.959, $p = 0.035$), systolic blood pressure (HR 1.030 per mmHg, $p = 0.009$), and diastolic blood pressure (HR 1.036 per mmHg, $p = 0.039$) as independent predictors of

incident HT (Table 12). Age, sex, baseline eGFR, and proteinuria were not significant predictors.

Table 12 - Independent baseline predictors of *de novo* hypertension after kidney donation (multivariable Cox regression)

Variable	HR	95% CI	p-value
BMI, per kg/m²	1.074	1.004–1.149	0.039
Dyslipidemia	1.959	1.050–3.656	0.035
Systolic BP, per mmHg	1.030	1.007–1.053	0.009
Diastolic BP, per mmHg	1.036	1.002–1.067	0.039

BMI – body mass index; BP – blood pressure. CI – confidence interval; HR – hazard ratio.

Donors who developed HT had a greater annual decline in eGFR (–0.03 vs. +0.40 mL/min/1.73 m²/year, difference –0.43, *p* = 0.026) and a greater annual increase in systolic blood pressure (+0.65 vs. +0.18 mmHg/year, difference +0.48, *p* = 0.024) compared with those who remained normotensive. Stratified analysis confirmed that overweight and obesity remained significantly more prevalent among hypertensive donors throughout follow-up. Dyslipidemia was more frequent in hypertensive donors at 10 years (53% vs. 24%, *p* = 0.002). No significant differences were found for proteinuria or incident diabetes. No donor progressed to end-stage kidney disease during follow-up, and cardiovascular events were rare.

In summary, *de novo* hypertension developed in nearly one-third of living kidney donors during follow-up, with cumulative incidence exceeding 50% at 15 years. The standardized event rate was approximately threefold higher than that observed in a contemporaneous, matched regional cohort. Higher baseline BMI, dyslipidemia, and elevated pre-donation systolic and diastolic blood pressure were independent predictors of incident hypertension. No donor progressed to end-stage kidney disease, and the number of major cardiovascular events was insufficient for analysis.

RESULTS

Study 3

Metabolomic signatures of adaptation after living kidney donation: associations with eGFR and remaining kidney volume

Adapted from: Almeida, M. et al, Urinary metabolomics reveals early renal metabolic adaptation following living kidney donation: a longitudinal pilot study. *Submitted October 2025*.

APPENDIX 6

This section is based on submitted work by the author. Content has been adapted and reformatted for coherence within this thesis.

Given the observed variability in post-donation obesity and hypertension trajectories, we next explored whether early biochemical signals of cardiovascular–kidney–metabolic (CKM) stress could be detected using urinary metabolomics.

Study-specific details

This exploratory feasibility study included eighteen living kidney donors who were analyzed at baseline (T0) and six months after nephrectomy (T6). Samples were processed and analyzed using untargeted ^1H -NMR spectroscopy, following the standardized handling, biobanking, and analytical workflows described in the Materials and Methods chapter.

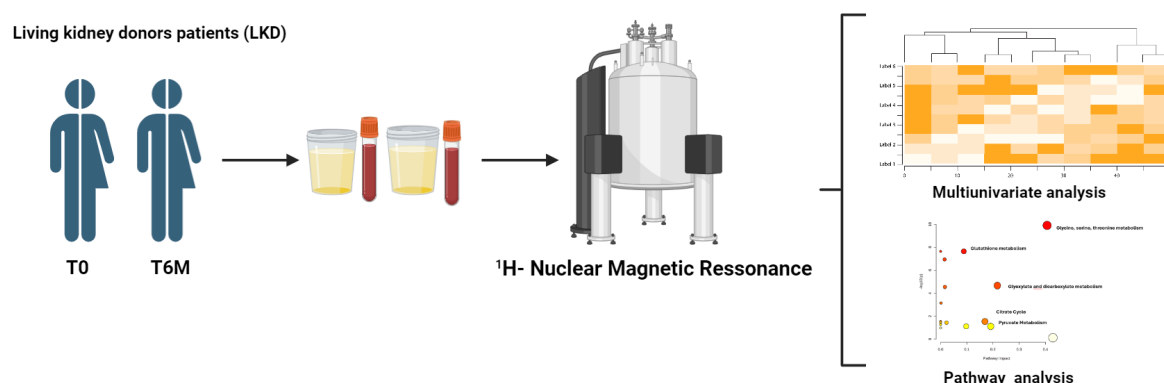


Figure 11. Illustration of the experimental design of the work.

First-morning urine and blood samples were collected from healthy individuals recruited as living kidney donors before (T0) and six months after donation (T6). The study included 18 donors (4 men and 14 women). Urine samples were analyzed using untargeted ^1H -NMR spectroscopy, and metabolite data were correlated with clinical and biochemical parameters obtained from paired blood and urine analyses.

RESULTS

Results

Eighteen living kidney donors were analyzed at baseline (T0) and six months after nephrectomy (T6). Mean age was 51.4 ± 7.9 years, with 77.8% female. After donation, serum creatinine, cystatin C, urea, and uric acid increased significantly, while eGFR declined from 103.6 ± 7.7 to 69.1 ± 11.7 mL/min/1.73 m² ($p < 0.0001$). CT-based volumetry demonstrated the expected halving of kidney volume (346.9 ± 58.5 vs. 175.4 ± 32.2 cm³, $p < 0.0001$), with kidney volume indexed to weight decreasing from 5.42 ± 1.06 to 2.69 ± 0.58 cm³/kg ($p < 0.0001$). Albuminuria and lipid profile remained stable. (Table 13).

Table 13 - Clinical and biochemical parameters of kidney donors' patients before (T0) and after 6 months of kidney donation (T6).

	T0	T6	p-value
Gender (% Female)	77.8		-
Age (years)	51.44 ± 7.92		-
BMI (Kg/m ²)	24.54 ± 3.71	25.14 ± 4.04	0.65
Uric acid (mg/dl)	3.94 ± 0.81	4.86 ± 0.32	0.02
Serum Creatinine (mg/dl)	0.68 ± 0.11	1.06 ± 0.17	<0.0001
GFR (CKD-EPI) (ml/min)	103.6 ± 7.73	69.11 ± 11.71	<0.0001
Urea (mg/dl)	32.67 ± 9.49	41.67 ± 9.51	0.0075
Lipid Profile			
Total Cholesterol (mg/dl)	183.4 ± 34.76	189.0 ± 34.91	0.64
Triglycerides (mg/dl)	91.61 ± 33.00	100.3 ± 37.36	0.46
HDL (mg/dl)	57.83 ± 13.48	56.40 ± 12.24	0.75
LDL (mg/dl)	108.3 ± 26.80	114.3 ± 29.65	0.55
Proteins			
Cystatin C (mg/l)	0.83 ± 0.13	1.14 ± 0.19	<0.0001
Albumin (g/dl)	4.38 ± 0.42	4.42 ± 0.31	0.95
Hormones			
PTH (pg/ml)	54.56 ± 17.71	72.61 ± 44.91	0.21
TSH (μUI/ml)	1.77 ± 0.86	2.11 ± 1.24	0.53
T3 (ng/ml)	2.30 ± 1.00	2.30 ± 1.00	>0.99
T4 (μg/ml)	3.54 ± 3.54	6.04 ± 3.67	0.04
Urine			
Albumin (mg/mg creat)	8.91 ± 12.35	9.06 ± 12.91	0.25
Proteins (g/g creat)	0.09 ± 0.07	0.07 ± 0.03	0.06
Volumetry			
KV (Total, cm ³)	346.9 ± 58.45	175.4 ± 32.19	<0.0001
KV/W (cm ³ /Kg)	5.42 ± 1.06	2.69 ± 0.58	<0.0001

BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; T4, total thyroxine; T3, total triiodothyronine; KV, kidney volume; KV/W, kidney volume indexed to body weight.

Urinary metabolomic profile pre and post-donation

Principal Component Analysis (PCA) and supervised Partial Least Squares Discriminant Analysis (PLS-DA) showed only partial separation between T0 and T6, but variable importance of projection identified betaine and formate as the most altered metabolites (VIP > 1.5) (Figure 12).

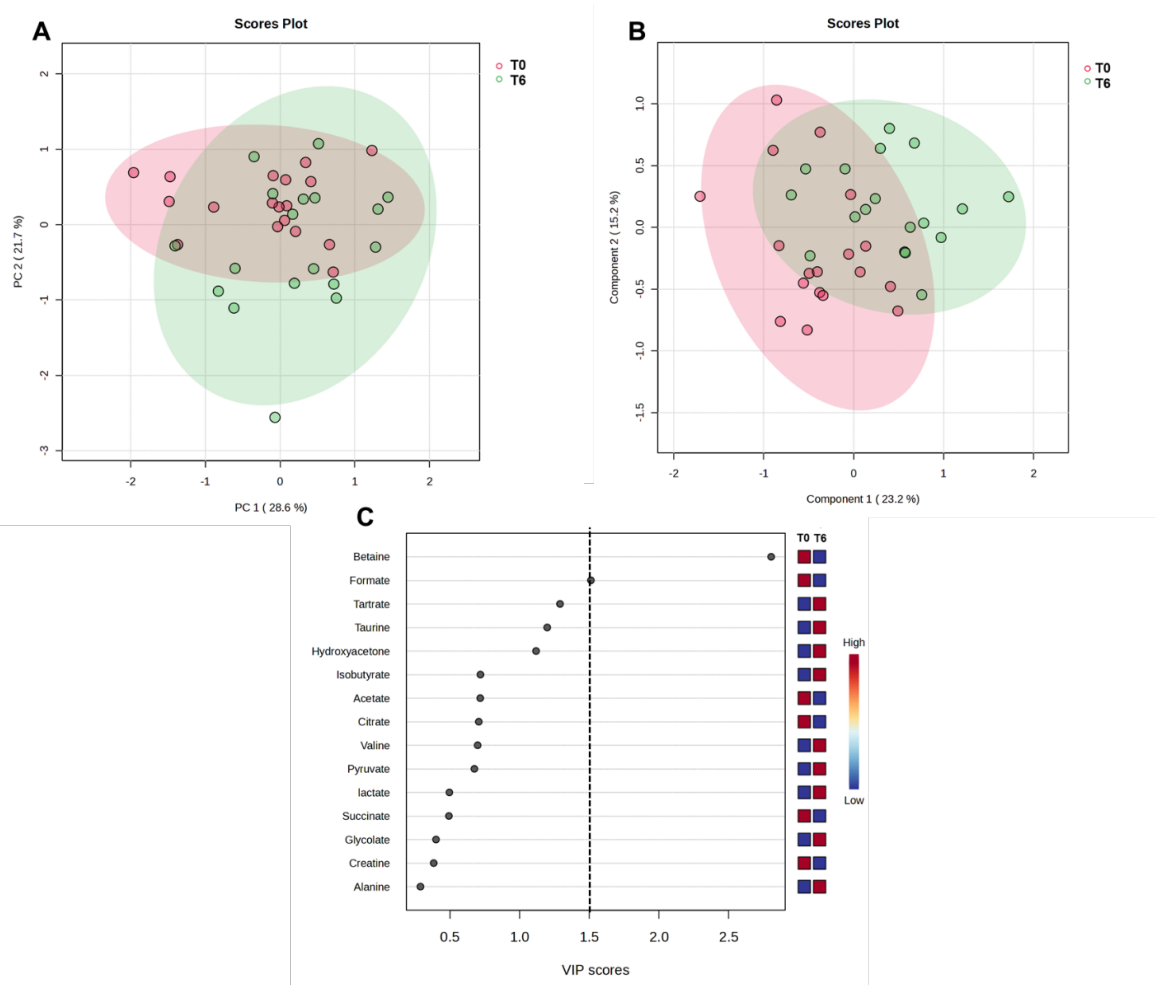


Figure 12. Multivariate analysis of the integrated urinary metabolome of living kidney donors before (T0) and after kidney donation (T6).

Score scatterplots resulting from:

- (A) Principal Component Analysis (PCA).
 - (B) Supervised Partial Least Square Discriminant Analysis.
 - (C) Obtained Variable Importance in Projection (VIP) scores of the 15 most relevant metabolite.
- A VIP > 1.5 (dotted line) was considered relevant for group separation.

Overall, 19 urinary metabolites were quantified (Appendix 6), of which seven decreased significantly post-donation: citrate, hypoxanthine, betaine, creatine, acetate, formate, and glycine (Figure 13). Alanine and succinate showed near-significant reductions ($p = 0.052$ and $p = 0.055$, respectively).

RESULTS

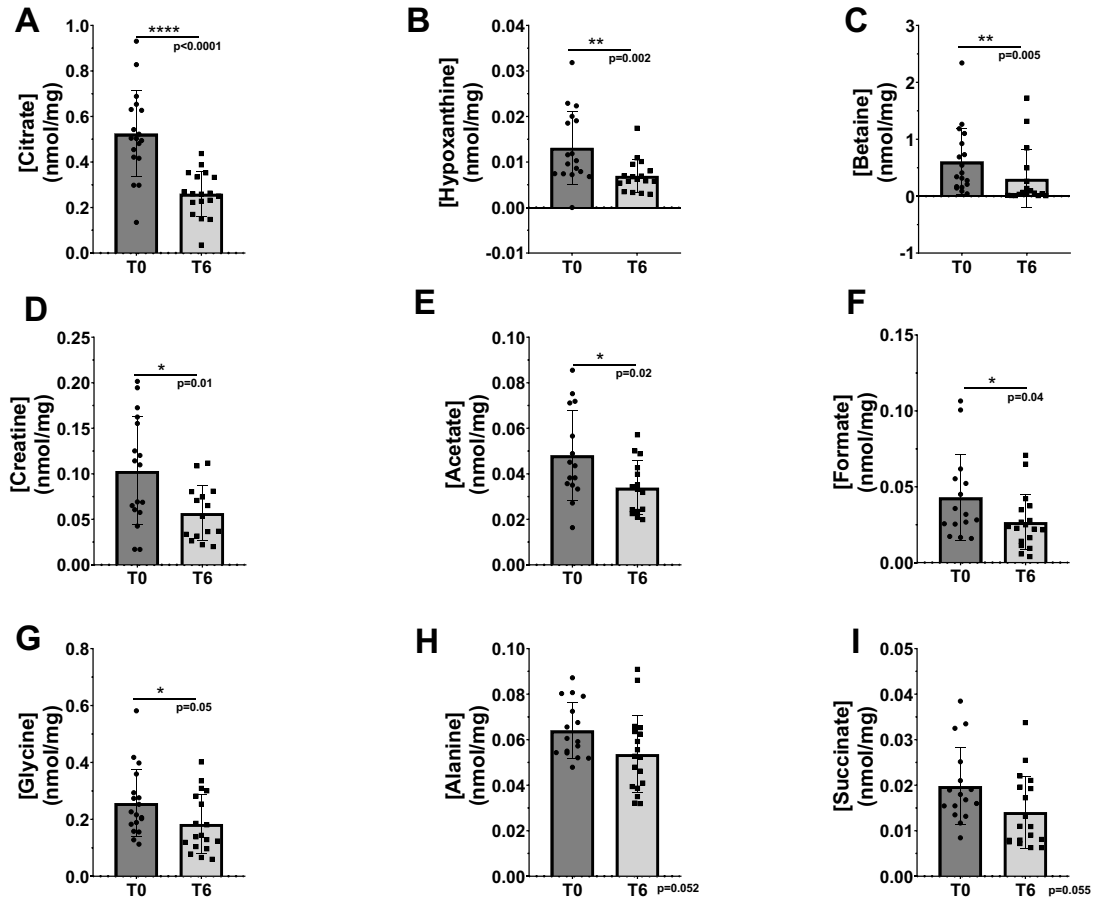


Figure 13. Metabolites altered in urinary metabolome of healthy individuals recruited for living kidney donation before (T0) and after surgery (T6).

Results are expressed as mean $\pm$ SD. Data were tested with the T-test, the Mann-Whitney Test, or the unpaired T-test, depending on the normality test of the Shapiro-Wilk test. Significance was considered at $P < 0.05$. * $P < 0.05$, ** $P < 0.005$ and *** $P < 0.0005$.

Functional correlations

Correlation analyses reinforced the biological relevance of metabolite shifts. Post-donation eGFR correlated strongly with remnant kidney volume, both absolute ($r = 0.83$, $p < 0.01$) and weight-indexed ($r = 0.79$, $p < 0.01$). Among urinary metabolites, citrate ($r = 0.59$, $p < 0.01$), hypoxanthine ($r = 0.51$, $p < 0.01$) showed significant associations with eGFR. Additional moderate correlations were observed with betaine, creatine, glycine, succinate, and dimethylamine ($r = 0.39$ – 0.47 , all $p < 0.05$). Together, these findings highlight that both structural reserve and metabolic efficiency contribute to functional recovery after nephrectomy (Figure 14).

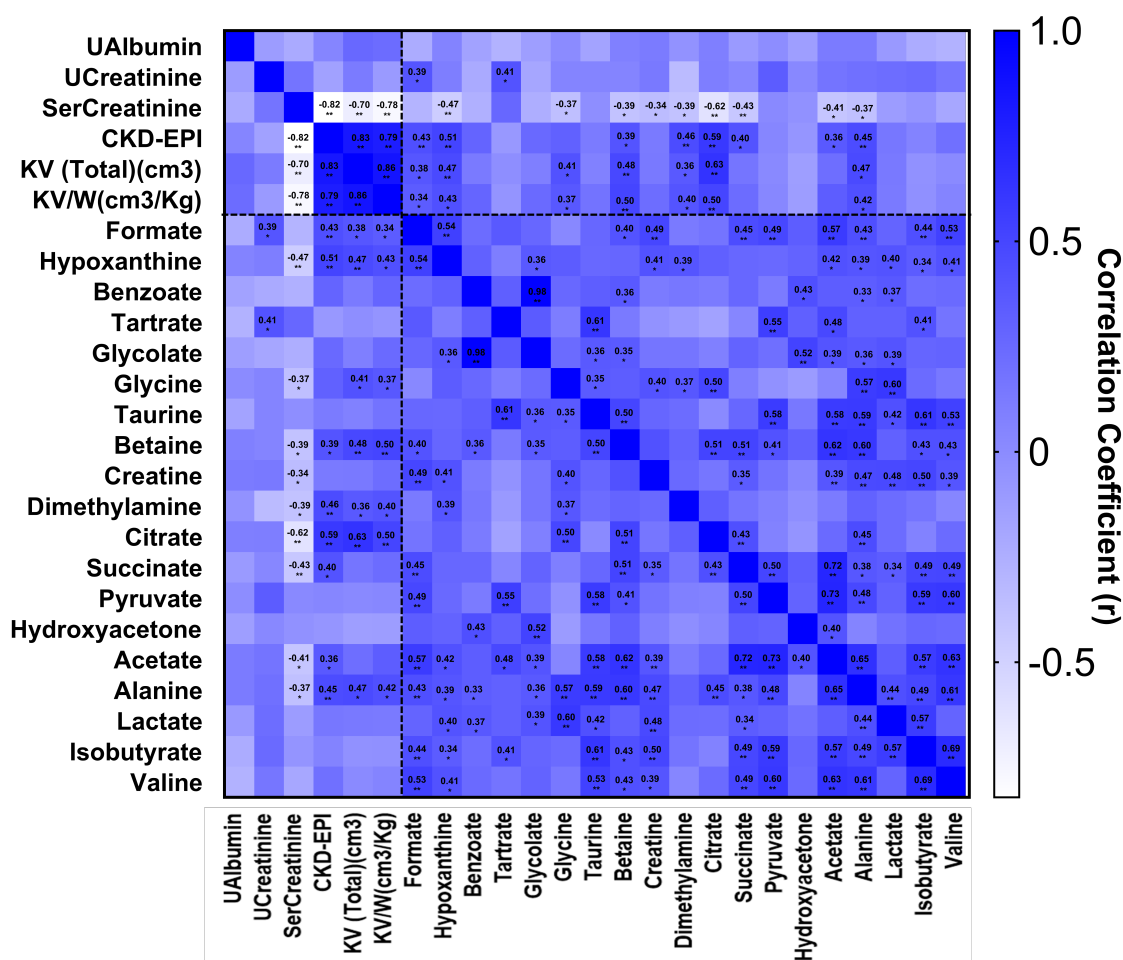


Figure 14. Correlation analysis heatmap for the combined data of metabolite and biomarkers of kidney disease from living kidney donors before (T0, n=18) and after (T6, N=18), surgery.

Corresponding Pearson or Spearman correlation coefficient (r) were represented when $p < 0.05$ (*) and $p < 0.01$ (**). CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; KV, kidney volume; KV/W, kidney volume indexed to body weight; UAlbumin, urinary albumin; UCreatinine, urinary creatinine.

Biomarker performance for metabolic alterations post-donation

Receiver operating characteristic (ROC) curve analysis demonstrated discriminatory value for Citrate (AUC = 0.90, $p < 0.001$), hypoxanthine (AUC = 0.81, $p = 0.002$), and betaine (AUC = 0.77, $p = 0.006$). The optimal cut-off value for citrate was < 0.402 nmol/mg (sensitivity=94.4%, specificity=83.33%), hypoxanthine < 0.007 nmol/mg (sensitivity=70.6%, specificity=82.35%) and betaine 0.139 nmol/mg (sensitivity=70.6%, specificity=88.89%).

These results suggest that, despite the subtlety of global metabolomic shifts, targeted metabolites may serve as candidate urinary biomarkers of donor adaptation.

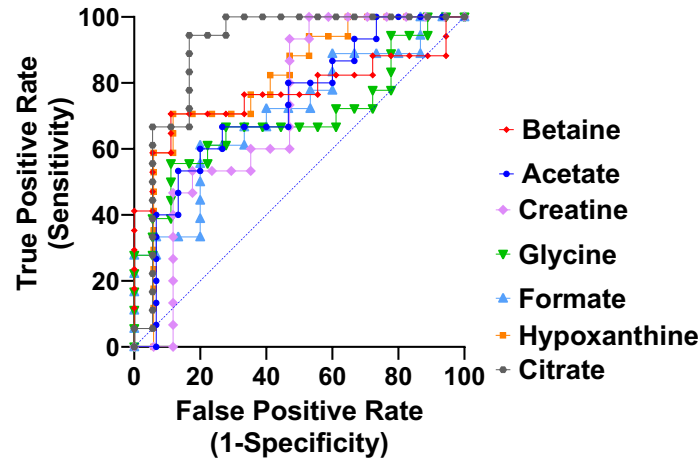


Figure 15. Receiver Operating Characteristic (ROC) curve analysis for assessing the potential of using metabolic fingerprint as a biomarker of alteration after kidney donation.

The obtained AUC curves for living kidney donors.

Betaine (♦), Acetate (■), Creatine (●), Glycine (▲), Formate (▲), Hypoxanthine (■) and Citrate (●). ROC curves of the main metabolites identified with p value ≤ 0.05

Metabolic interpretation of key findings

Although formal pathway enrichment analysis was not performed, the pattern of metabolite changes provides insight into renal metabolic adaptation following nephrectomy. Perturbations in mitochondrial energy metabolism were suggested by the reduction in citrate and succinate, consistent with early bioenergetic stress in the remnant kidney. Redox and purine metabolism also appeared affected, with lower levels of hypoxanthine and formate indicating altered purine turnover and antioxidant balance during compensatory hyperfiltration. In parallel, decreases in glycine, betaine, and creatine reflected shifts in osmoregulatory and one-carbon metabolism, pathways that play a key role in tubular adaptation, solute handling, and methyl-donor balance. Together, these findings suggest that nephrectomy induces coordinated metabolic reprogramming across energy, redox, and osmoregulatory axes, even in donors who remain clinically stable.

This pilot study demonstrates that urinary metabolomics can reveal subtle yet biologically meaningful adaptations in living kidney donors, with citrate, hypoxanthine, and betaine emerging as candidate biomarkers linked to eGFR and remnant kidney volume. When integrated with analyses of BMI trajectories and incident hypertension, metabolomics may add a complementary biochemical dimension to the evaluation of CKM health. Together, these approaches provide a multidimensional framework for donor risk stratification and long-term safety monitoring, extending beyond traditional renal outcomes.

4.3. Hypothesis Three: Psychosocial Health

Psychosocial health in living kidney donors can be comprehensively assessed through integrated qualitative and quantitative methods.

Having examined donor safety from the perspective of renal function alone, and then within the broader framework of cardiovascular–kidney–metabolic health, the next step is to address the psychological and social dimensions of recovery. Hypothesis Three integrates quantitative psychometric tools with qualitative donor perceptions to capture the full spectrum of psychosocial well-being after living kidney donation.

This hypothesis was tested through two complementary studies in the same donor cohort: (1) a quantitative analysis of pre- and post-donation changes in health-related quality of life (SF-36) and psychological symptoms (HADS), and (2) a qualitative survey using a structured EULID-based questionnaire to explore donor motivations, concerns, and post-donation reflections.

Study 1.

Health-Related Quality of Life, Anxiety, and Depression in Living Kidney Donors

Adapted from Almeida, M., et al., *Psychological Outcomes in a Cohort of Portuguese Living Kidney Donors*. *Transplant Proc*, 2025, Jun 16:S0041-1345(25)00274-X, DOI 10.1016/j.transproceed.2025.05.006.

APPENDIX 7

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Study-specific material and methods

Forty-four living kidney donors who underwent nephrectomy between January 2018 and December 2019 were evaluated before surgery and at a median of 3.35 years post-donation (IQR 2.72–3.73). Psychosocial outcomes were assessed using the Short Form-36 Health Survey (SF-36), which covers eight domains of health-related quality of life (HRQoL), and the Hospital Anxiety and Depression Scale (HADS), with separate subscales for anxiety and depression. Clinical and sociodemographic data were obtained from structured questionnaires and medical records.

Results

Participants had a mean age of 47.3 ± 10.3 years, and two-thirds were female (68.2%). The majority were married or in a stable partnership (81.8%). Before donation, 70.5% of donors were employed, and the proportion remained stable at follow-up. Over half had completed only primary school education (52.4%). Donation most frequently occurred to a partner (40.9%) or sibling (29.5%), with 63.6% performed directly to compatible recipients, 25.0% through kidney-paired exchange, and 11.4% via ABO-incompatible protocols. (Table 14).

Table 14 - Demographic, clinical, and perioperative characteristics of the study cohort (n=44)

Variable	n (%) or Mean $\pm$ SD
Age (years)	47.3 $\pm$ 10.3
Female sex	30 (68.2)
Relationship with recipient	Partners: 18 (40.9); Siblings: 13 (29.5) Parents: 7 (15.8); Others* 6 (12.8%)
Educational level	Primary school: 23 (52.4); Secondary school: 9 (20.3) ; University degree: 12 (27.3)
Marital status pre-donation	Married/ partnership: 36 (81.8); Single: 5 (11.4); Divorced: 3 (6.8)
Employment status pre-donation	Active: 31 (70.5); Unemployed: 5 (11.4) ; Retired: 6 (13.6); Medical leave: 1 (2.3)
Type of donation	Compatible: 28 (63.6) Kidney-paired exchange: 11 (25.0) ABO-incompatible: 5 (11.4)
Post-operative complications	Any: 15 (34.1); Pain: 6 (13.6)
Stressful life events	Any: 19 (43.2); Medical: 11 (25.0); Psychosocial: 3 (6.8); Both: 5 (11.4)
Median time pre-donation evaluation	5.80 months (IQR: 3.22–13.51)
Median time post-donation evaluation	3.35 years (IQR: 2.72–3.73)

*Other includes nephews (n=2), sons (n=1), brothers-in-law (n=1), cousins (n=1), and friends (n=1); *Adapted from Table 1, Almeida et al, Transplant Proceedings 2025*

Post-operative complications were documented in 15 donors (34.1%), most frequently surgical pain (n = 6, 13.6%). Stressful life events unrelated to donation were reported by 19 donors (43.2%), including medical events (n = 11), psychosocial events (n = 3), and combined causes (n = 5). Among recipients, one experienced early graft loss due to thrombosis, two had acute rejection episodes successfully treated, and one was readmitted for a urinary fistula.

Health Related Quality of Life (HRQoL) before and after kidney donation (SF-36)

In the SF-36 questionnaire (0–100 scale, higher scores indicating better health, Minimal Clinical Important Difference, MCID, 3-5 points), several domains declined significantly with small-to-moderate effect sizes (Cohen's $d=0.32$ – 0.44) (Table 15). Physical functioning decreased from 93.64 ± 8.78 to 86.45 ± 20.96 ($p = 0.037$, Cohen's $d=0.32$), bodily pain from 86.70 ± 17.92 to 74.52 ± 27.03 ($p = 0.003$, Cohen's $d=0.47$), vitality from 70.45 ± 12.38 to 61.61 ± 23.76 ($p = 0.023$, Cohen's $d=0.35$), social functioning from 91.48 ± 12.87 to 80.81 ± 26.77 ($p = 0.023$, Cohen's $d=0.36$), and mental health from 80.00 ± 14.28 to 67.64 ± 26.13 ($p = 0.006$, Cohen's $d = 0.44$). Although mean scores remained above Portuguese population norms [202], all changes exceeded the MCID, indicating that donors themselves would likely perceive these declines as clinically meaningful.

Subgroup analyses revealed more pronounced declines in donors who reported stressful life events after donation ($n=19$). In this subgroup, significant reductions were observed in Physical Functioning ($\Delta -13.9$, $p = 0.012$, Cohen's $d=0.64$), Physical Role ($\Delta -22.8$, $p = 0.028$, Cohen's $d=0.55$), Bodily Pain ($\Delta -18.4$, $p = 0.006$, Cohen's $d=0.71$), and Mental Health ($\Delta -18.8$, $p = 0.009$, Cohen's $d=0.67$). Donors with post-operative complications demonstrated a significant decrease in bodily pain ($\Delta -13.9$, $p=0.047$, Cohen's $d=0.77$) and Mental Health ($\Delta -14.9$, $p=0.034$, Cohen's $d=0.61$). These subgroup results should be interpreted with caution, given modest sample sizes, but suggest that psychosocial burden is concentrated in identifiable vulnerable groups.

Comparison by donation type revealed no significant differences across most domains. However, in the mental health domain, compatible donors had significantly lower post-donation scores compared with donors in paired exchange or ABO-incompatible protocols (77.81 ± 23.67 vs. 61.82 ± 26.07 ; $p = 0.05$, Cohen's $d=0.61$).

Table 15 - SF-36 Scores Before and After Donation

Domain	Pre mean ± SD	Post mean ± SD	Δ Mean	p-value	Cohen's d	MCID (3–5 pts)	Clinical interpretation
Physical Function	93.6 ± 8.8	86.5 ± 21.0	-7.2	0.037	0.32	Exceeds	Small–moderate decline, clinically meaningful
Role Physical	90.8 ± 25.1	80.0 ± 35.0	-10.8	0.055	0.30	Exceeds	Borderline significant, small effect
Pain	86.7 ± 17.9	74.5 ± 27.0	-12.2	0.003	0.47	Exceeds	Moderate decline, clinically meaningful
General Health	76.8 ± 18.8	70.2 ± 24.3	-6.6	0.146	0.22	Exceeds	Not significant, small effect
Vitality	70.5 ± 12.4	61.6 ± 23.8	-8.8	0.023	0.36	Exceeds	Moderate decline, clinically meaningful
Social Function	91.5 ± 12.9	80.8 ± 26.8	-10.7	0.018	0.37	Exceeds	Moderate decline, clinically meaningful
Role Emotional	88.3 ± 27.6	79.4 ± 36.1	-8.9	0.159	0.22	Exceeds	Small effect, not significant
Mental Health	80.0 ± 14.3	67.6 ± 26.1	-12.4	0.006	0.44	Exceeds	Moderate decline, clinically meaning

Mean values are presented with standard deviations (SD). Δ Mean indicates the mean change from baseline to the follow-up evaluation. Cohen's d represents effect size, where 0.2=small, 0.5=moderate, and 0.8=large. MCID (Minimal Clinically Important Difference) for SF-36 domains is 3–5 points; changes exceeding this threshold are considered clinically meaningful, even if not statistically significant.

Anxiety and Depression Scores (HADS) pre- and post-donation

In the Hospital Anxiety and Depression Scale (HADS; range 0–21 per domain, ≥8 indicating possible clinical disorder, and ≥11 probable clinical disorder; MCID=1.5-2), mean anxiety scores increased slightly without statistical significance. Depression scores increased significantly from 2.05 ± 2.10 to 3.37 ± 3.76 ($p=0.029$, Cohen's $d=0.38$). Despite these changes, post-donation mean scores for both subscales remained below the clinical threshold (Table 16).

For anxiety, most donors scored in the normal range before donation (90.9%), with three (6.8%) in the moderate range and one (2.3%) in the severe range. After donation, five donors (11.4%) were in the moderate range and two (4.5%) in the severe range.

For depression, nearly all donors scored in the normal range before donation, with only one (2.3%) reporting mild symptoms. After donation, eight donors (18.2%) reported mild symptoms, one (2.3%) moderate symptoms, and one (2.3%) severe symptoms.

Subgroup analysis showed that donors reporting stressful life events after donation ($n=19$) had a significant increase in depression scores (2.79 ± 2.46 to 5.79 ± 4.04 , $p = 0.015$,

Cohen's $d=0.61$). In contrast, donors with post-nephrectomy complications ($n=15$) had non-significant increases in both anxiety and depression.

Table 16 - HADS Anxiety and Depression Scores Before and After Donation

Domain	Pre-donation Mean $\pm$ SD	Post-donation Mean $\pm$ SD	<i>p</i> -value	Cohen's <i>d</i>	Post-donation categories <i>n</i> (%)
Anxiety	5.16 $\pm$ 3.59	5.79 $\pm$ 4.45	0.308	0.15	No symptoms: 26 (59.1); Mild: 10 (22.7); Moderate: 5 (11.4); Severe: 2 (4.5)
Depression	2.05 $\pm$ 2.10	3.37 $\pm$ 3.76	0.029	0.38	No symptoms: 34 (77.3); Mild: 8 (18.2); Moderate: 1 (2.3); Severe: 0 (0)

Higher HADS scores indicate more severe symptoms (0–21 per domain). A score between 0 and 7 is “normal,” between 8 and 10 is “mild,” between 11 and 14 is “moderate,” and between 15 and 21 is “severe. Scores ≥ 8 suggest possible clinical anxiety or depression; scores ≥ 11 indicate probable clinical disorder, Cohen's *d* represents effect size, where 0.2=small, 0.5=moderate, and 0.8=large. MCID (Minimal Clinically Important Difference) for HDAS domains is 1.5-2 points; Changes in anxiety and depression did not exceed the MCID

Correlation analysis demonstrated a significant positive association between pre-donation anxiety and pre-donation depression ($r = 0.602$, $p < 0.001$), post-donation anxiety ($r = 0.526$, $p < 0.001$), and post-donation depression ($r = 0.309$, $p = 0.049$). Post-donation anxiety showed a strong correlation with post-donation depression ($r = 0.788$, $p < 0.001$). Anxiety scores, both pre- and post-donation, were negatively correlated with the interval between evaluation and donation ($r = -0.367$, $p = 0.015$).

Study 2:

Donor perceptions of the Living Kidney Donation Process

Adapted from Almeida, M., et al., *Living Kidney Donor Perceptions About Organ Donation: Who are They, and How Do They Feel About the Process?* Transplant Proc, 2025, Jul 8:S0041-1345(25)00317-3. doi: 10.1016/j.transproceed.2025.05.036.

APPENDIX 8

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Study-specific material and methods

Donor perceptions were assessed in the same cohort of 44 living kidney donors using a structured questionnaire adapted from the EULID framework. The survey explored decision-

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making, adequacy of information, pre-donation concerns, the experience of the donation process, post-donation emotions, perceived impact on donor–recipient relationships, and social and financial implications. Responses were collected at a median of 3.35 years after donation (IQR: 2.72–3.73).

Data were analyzed descriptively. Given the sample size, subgroup comparisons were not performed systematically, and results are presented as counts and percentages.

Results

Qualitative survey responses revealed predominantly positive donor perceptions (Table 17). Most donors (86.4%) described the decision to donate as easy, with only one donor (2.3%) reporting feeling pressured to donate. All participants considered the information provided before donation adequate, although 34.1% noted insufficient information before the multidisciplinary evaluation.

Pre-donation concerns were common and often multiple, including surgical risk (61.4%), fear of adverse long-term health effects (50.0%), fear that the transplant would fail (52.3%), and worry about recipient adherence to medical care (36.3%). The donation process was perceived as physically and/or emotionally demanding by 62.0% of donors, but only 6.8% considered discontinuing the process.

Post-donation, the predominant emotions were joy (52.3%), pride (29.5%), and satisfaction (18.2%), with no negative emotions reported. Relationships with recipients remained unchanged in 79.5% of cases and improved in 20.5%. Nearly nine in ten donors (88.6%) perceived a significant improvement in recipient health. Most donors (72.7%) reported family support, 70.5% were not the primary caregiver, and only two (4.5%) described a significant financial burden.

Overall, 95.5% would donate again, and 77.3% would strongly recommend living kidney donation to others, while the remainder (22.7%) considered it a strictly individual decision.

Table 17 - Donor Perceptions of the Donation Process (n=44)

Theme	Variable	n (%)
Decision-making	Decision to donate: Easy / Challenging	38 (86.4) / 6 (13.6)
	Felt pressured to donate: Yes / No	1 (2.3) / 43 (97.7)
Information	Reported adequate pre-donation information	44 (100)
	Lack of information before multidisciplinary evaluation: Yes / No	15 (34.1) / 29 (65.9)
Pre-donation concerns	Surgical risk	27 (61.4)
	Fear of adverse long-term health effects	22 (50.0)
	Fear that the transplant would fail	23 (52.3)
	Recipient care concerns	16 (36.3)
Experience of donation	Perceived as physically and/or emotionally demanding: Agree / Disagree	27 (62.0) / 15 (34.1)
	Considered stopping the process: Yes / No	3 (6.8) / 41 (93.2)
Post-donation outcomes	Predominant emotions: Joy / Pride / Satisfaction / Negative	23 (52.3) / 13 (29.5) / 8 (18.2) / 0 (0)
	Relationship with recipient: Unchanged / Improved / Worsened	35 (79.5) / 9 (20.5) / 0 (0)
	Perceived improvement in recipient's health: Significant / No-minor	39 (88.6) / 5 (11.4)
Social/financial impact	Not primary caregiver for recipient	31 (70.5)
	Family support: Yes / Perceived lack of family support	32 (72.7) / 11 (26.0)
	Significant financial burden	2 (4.5)
Overall evaluation	Would donate again: Yes / No	42 (95.5) / 2 (4.5)
	Would strongly recommend living kidney donation to others: Yes / Individual decision	34 (77.3) / 10 (22.7)

Percentages are calculated from the total number of donors responding to each question. Examples of the questions provided to the donors are available in Table 2 from Appendix 8.

Together, these two studies provide a complementary view of psychosocial health in living kidney donors. Psychometric evaluation demonstrated that, while overall quality of life and psychological scores remained within favorable ranges, significant declines were observed in selected SF-36 domains and significant increases were seen in HADS depression scores, particularly among donors experiencing medical complications or stressful life events. Donor perception data were predominantly positive, with most describing the decision to donate as easy, reporting adequate pre-donation information, and expressing satisfaction, pride, or joy after donation. Regret was rare, and nearly all indicated willingness to donate again. At the same time, some donors reported ambivalence, perceived the process as demanding, or faced financial or emotional challenges. Taken together, these findings

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indicate that most donors maintained favorable psychosocial well-being, while a subgroup experienced measurable strain.

4.4. Hypothesis Four: Donor – Recipient Matching

Adequate matching can be achieved by integrating predictive tools, donor characteristics, and immunologic compatibility.

The psychosocial findings from Hypothesis Three highlighted that living kidney donor safety is shaped by both medical and non-medical factors. In addition to safeguarding donors, optimizing transplant success also requires attention to recipient outcomes, which are influenced by donor characteristics, immunological compatibility, and functional reserve of the donated kidney.

This hypothesis focuses on the impact of donor characteristics and complexity on post-transplant recipient outcomes. It integrates three complementary analyses: (1) the external validation of the iPREDICTLIVING prognostic model in a Portuguese living donor cohort; (2) an investigation, using our institutional dataset, of donor-related variables associated with long-term graft and recipient survival, aiming to identify factors that may enhance predictive accuracy beyond the application of an existing European-derived model; and (3) a comparative assessment of outcomes in recipients of genetically related versus unrelated donors, with attention to both clinical and immunological implications.

Study one

External validation of the iPREDICTLIVING tool

Adapted from Almeida M. et al, *Clinical performance of the iPREDICTLIVING tool for the prediction of the post-transplant recipient and living donor outcomes in a European cohort*, Clinical Transplantation 2024; 38:e15283. DOI: 10.1111/ctr.15283.

APPENDIX 9

This section is based on published work by the author. Content has been adapted and reformatted for coherence within this thesis. Figures and Tables are adapted from the above article, distributed under the terms of the Creative Commons Attribution License (CC BY 4.0).

Study-specific material and methods

We retrospectively analyzed all living donor kidney transplant (LDKT) pairs performed at our center between January 1998 and January 2020 (n = 365). Eleven cases of early graft failure (<48 h post-transplant) and two cases of early recipient death were excluded, leaving 352 pairs for analysis. Follow-up extended until death, graft failure, or 31 December 2021.

RESULTS

The iPREDICTLIVING tool was applied to estimate three different outcomes: donor mortality at 10- and 20-years post-donation; recipient mortality at 10 years post-transplant, and censored graft failure (CGF) at 10 years post-transplant. The models incorporate donor- and recipient-specific variables, including age, comorbidities, dialysis vintage, HLA mismatches, and transplant era. Detailed model specifications have been published previously [85] and were applied unchanged in this cohort.

For comparison, the more widely known Living Kidney Donor Profile Index (LKDPI) [198] was also calculated for the same cohort and evaluated for its ability to predict CGF at 10 years.

Model performance was assessed in terms of discrimination and calibration. Discrimination was measured using Harrell's C-statistic (time-independent) and time-dependent area under the curve (AUC), with higher values indicating better ability to separate outcomes. The Youden index was used to identify optimal cut-off values for risk classification (maximum combined sensitivity and specificity values). Calibration was evaluated by calibration slopes, with a slope of 1 representing perfect agreement between predicted and observed risk.

Results

Baseline donor, recipient, and transplant characteristics of interest for the iPREDICTLIVING and LKDPI tools are summarized at Table 18. Full baseline characteristics are available in Appendix 9. Donors had a mean age of 46.8 ± 10.7 years (69% female), median BMI 25.0 kg/m^2 (IQR 22.7–27.7), and mean eGFR $100.5 \pm 14.8 \text{ mL/min/1.73 m}^2$. Recipients had a mean age of 40.4 ± 13.6 years (67% male), median BMI 23.0 kg/m^2 (IQR 21.0–26.1), and glomerulonephritis was the leading cause of kidney disease (47%).

During follow-up, six donors (1.7%) and 17 recipients (5.0%) died; 44 recipients (13.0%) experienced censored graft failure. The observed cumulative incidence of donor mortality was 1.4% at 10 years ($n = 5$) and 1.7% at 20 years ($n = 6$). Recipient mortality at 10 years was 2.5% ($n = 9$). For censored graft failure, the observed cumulative incidence at 10 years was 7.9%. The predicted outcomes generated by iPREDICTLIVING models are depicted in Table 19 along with the observed cumulative incidences for each outcome. The LKDPI score was 7.9 ($-5.2 - 23.1$) for our cohort.

Table 18 – Baseline donor, recipient, and transplant characteristics (included only characteristics of interest for the iPREDICTLIVING and LKDPI models)

Category	Variable	Value in cohort
Donor variables	Age, years (mean $\pm$ SD)	46.8 $\pm$ 10.7
	Female sex – n (%)	243 (69.0)
	Weight, Kg - median (IQR)	68 (60-76)
	BMI, Kg/m ² - median (IQR)	25.0 (22.7–27.7)
	Systolic BP, mmHg- mean $\pm$ SD	126 $\pm$ 12
	Smoking history – n (%)	39 (11.1)
	SBP- median (IQR)	120 (113-130)
	Serum creatinine – median (IQR)	0.72 (0.64-0.88)
	eGFR, mL/min/1.73 m ² (mean $\pm$ SD)	100.5 $\pm$ 14.8
	Donor Follow-up – median (IQR)	7.1 (4.2–11.8)
Recipient variables	Age, years - mean $\pm$ SD	40.4 $\pm$ 13.6
	Female sex – n (%)	117 (33.2)
	Weight, Kg- median (IQR)	67 (59-75)
	BMI, Kg/m ² - median (IQR)	23.0 (21.0–26.1)
	Primary renal disease: Glomerulonephritis – n (%)	166 (47)
	Dialysis vintage, years – median (IQR)	2.5 (1.1–4.5)
	Cardiovascular disease – n (%)	26 (7)
	Cerebrovascular disease - n (%)	5 (1)
	Peripheral vascular Disease- n (%)	3 (1)
	Recipient Follow-up – median (IQR)	8.1 (5.1–12.7)
Transplant variables	Transplant Year	2013 (2009-2016)
	ABO incompatible – n (%)	15 (4)
	HLA-A MM – mean $\pm$ SD	0.9 $\pm$ 0.66
	HLA-B MM – mean $\pm$ SD	1.13 $\pm$ 0.69
	HLA-DR MM – mean $\pm$ SD	0.97 $\pm$ 0.68
	Graft Follow-up – median (IQR)	7.5 (4.9–11.4)

BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; GN, glomerulonephritis; IQR, interquartile range; MM-Mismatch; R, recipient; SBP, systolic blood pressure; SD, standard deviation.

RESULTS

Table 19 - Observed and predicted risks for donor mortality, recipient mortality, and censored graft failure (CGF) in the study cohort

Outcome	Observed risk	Predicted risk (median, IQR)
Donor mortality	1.4% (n = 5) at 10 Y 1.7% (n = 6) at 20 Y	0.2% (0.1–0.5) at 10 Y 1.4% (0.6–3.1) at 20 Y
Recipient mortality	2.5% (n = 9) at 10 Y	6.8% (3.5–13.9) at 10 Y
Censored graft failure	7.9% (n = 28) at 10 Y	12.0% (8.7–18.1) at 10 Y

D: Donor; IQR, inter quartile range; R, recipient; Y-years

For donor mortality, iPREDICTLIVING showed moderate discrimination (Harrell's C 0.699 at 10 years and 0.712 at 20 years) with good calibration (slopes 0.992 and 0.955). Recipient mortality prediction performed best, with high discrimination (Harrell's C 0.776, AUC 0.773) and excellent calibration (slope 1.003); at the optimal Youden threshold, sensitivity was 94% and specificity 62%. In contrast, censored graft failure prediction was modest (AUC 0.566, calibration slope 0.998). LKDPI performed slightly better for this outcome (AUC 0.617, calibration slope 1.000), but both models had limited sensitivity and specificity.

Complete results for discrimination and calibration across all evaluated outcomes are summarized in Table 20, while Figure 16 presents the calibration plots for donor mortality, recipient mortality, and censored graft failure

Table 20 – Discrimination and calibration metrics for iPREDICTLIVING and LKDPI models.

Model / Outcome	Harrell's C*	AUC	Optimal cutoff**	Sensitivity	Specificity	Calibration slope
iPREDICTLIVING Donor mortality (20 Y)	0.712	0.694	3.1	64	77	0.955
iPREDICTLIVING Donor mortality (10 Y)	0.699	0.636	0.1	100	30	0.992
iPREDICTLIVING Recipient mortality (10 Y)	0.776	0.773	8.3	94	62	1.003
iPREDICTLIVING CGF (10 Y)	0.665	0.566	10	41	79	0.998
LKDPI CGF (10 Y)	0.633	0.617	10	75	60	1.000

* Harrell's C time-independent analysis; all the other metrics are time dependent at the specified horizon time

** Youden threshold; CGF: Censored Graft Failure; LKDPI- Living Kidney Donor Profile Index; AUC: time-dependent area under the receiver operating characteristic curve; Y-years

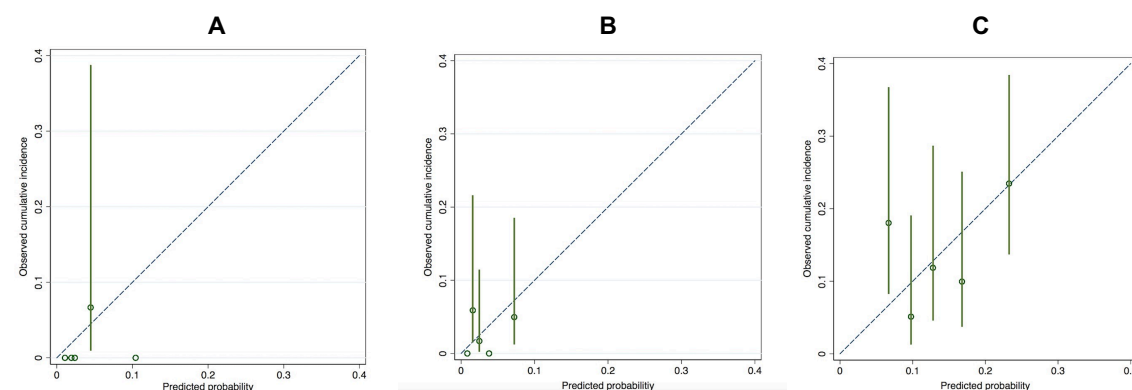


Figure 16. Calibration plots for the prediction models of the iPREDICTLIVING tool.

- A-** Calibration plot for the prediction of donor death at 20 years.
- B-** Calibration plot for the prediction of recipient death at 10 years.
- C-** Calibration plot for the prediction of censored graft failure at 10 years.

In summary, the iPREDICTLIVING tool demonstrated moderate-to-good discrimination for donor and recipient mortality, with strong calibration for both endpoints. Its best performance was observed in predicting recipient mortality, whereas prediction of censored graft failure was less accurate, with limited sensitivity and specificity.

Study 2:

Influence of Donor Age and Pre-Donation eGFR on Recipient Outcomes

Adapted from Almeida M. et al, *Living Donors' Age Modifies the Impact of Pre-Donation Estimated Glomerular Filtration Rate on Graft Survival*; Journal of Clinical Medicine, 2023 Oct 26;12(21):6777. Doi: 10.3390/jcm12216777.

APPENDIX 10

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In the previous section, the iPREDICTLIVING model was externally validated in our Portuguese living donor cohort, confirming its ability to estimate post-transplant outcomes from pre-donation donor and recipient characteristics. Building on this, we next examined a broader set of donor variables, adjusted for recipient and transplant-related covariates, to determine their association with long-term graft survival and function. Among the factors assessed, only baseline eGFR and donor age showed statistically significant associations, and these were therefore selected for detailed analysis.

Study-specific material and methods

This retrospective analysis included 352 donor–recipient pairs transplanted between January 1998 and January 2020, after exclusion of 13 cases of primary non-function or early recipient death. Follow-up was censored at 15 years post-transplant. The primary endpoints were overall and death-censored graft survival. The main exposures were pre-donation eGFR (<90 vs. ≥ 90 mL/min/1.73 m²), donor age (<50 vs. ≥ 50 years), and their interaction. Additional donor factors (hypertension, dyslipidemia, obesity, smoking, and mild proteinuria) were also examined, but were not significant predictors. Graft survival was analyzed using Kaplan–Meier methods with log-rank testing, and independent predictors were identified by multivariable Cox regression models adjusted for significant covariates. The interaction between donor age and pre-donation eGFR was tested a priori, with stratified analyses when significant.

Results

Summarized cohort characteristics are presented in Table 21, with the full dataset available in Appendix 10. Donors with pre-donation eGFR <90 mL/min/1.73 m² were older (52.8 ± 9.5 vs. 44.9 ± 10.4 years; $p < 0.001$) and more frequently had hypertension (23% vs. 13%; $p = 0.019$) and dyslipidemia (26% vs. 9%; $p < 0.001$) than those with eGFR ≥ 90 mL/min/1.73 m². Their recipients ($n = 82$, 23%) were also older (43.1 ± 14.6 vs. 39.6 ± 13.1 years; $p = 0.040$).

Table 21 – Baseline characteristics of the cohort, according to donor eGFR pre-donation

	Total <i>n</i> = 352	eGFR < 90 mL/min/1.73 m ² <i>n</i> = 82 (23%)	eGFR ≥ 90 mL/min/1.73 m ² <i>n</i> = 270 (77%)	<i>p</i>
<u>Recipient</u>				
Age, mean ± SD	40.4 ± 13.6	43.1 ± 14.6	39.6 ± 13.1	0.040
F sex, <i>n</i> (%)	117 (33)	24 (29)	93 (34)	0.384
BMI, mean ± SD	23.6 ± 3.7	23.4 ± 3.6	23.6 ± 3.8	0.581
DM, <i>n</i> (%)	20 (6)	7 (9)	13 (5)	0.202
Smoking habits, <i>n</i> (%)	80 (23)	17 (21)	63 (23)	0.622
Coronary heart disease, <i>n</i> (%)	26 (7)	11 (13)	15 (6)	0.017
Cerebrovascular disease <i>n</i> (%)	5 (1)	2 (2)	3 (1)	0.331
CKD etiology, GNF <i>n</i> (%)	166 (47)	40 (49)	126 (47)	0.614
Vintage (years), median (IQR)	1.17 (0.28–2.59)	1.19 (0.10–2.52)	1.17 (0.29–2.70)	0.656
Preemptive RRT, <i>n</i> (%)	73 (21)	20 (24)	53 (20)	0.642
<u>Donor</u>				
Age, mean ± SD	46.8 ± 10.7	52.8 ± 9.5	44.9 ± 10.4	<0.001
Age ≥50, <i>n</i> (%)	143 (41)	53 (65)	90 (33)	<0.001
F sex <i>n</i> (%)	244 (69)	55 (67)	189 (70)	0.615
BMI, mean ± SD	25.2 ± 3.3	25.8 ± 3.1	25.0 ± 3.4	0.067
Pre-donation SCr, median (IQR)	0.72 (0.64–0.88)	0.90 (0.80–1.02)	0.69 (0.61–0.78)	<0.001
Pre-donation eGFR, mean ± SD	100.5 ± 14.7	79.7 ± 8.4	106.8 ± 9.5	<0.001
Hypertension, <i>n</i> (%)	53 (15)	19 (23)	34 (13)	0.019
Dyslipidemia, <i>n</i> (%)	46 (13)	21 (26)	25 (9)	<0.001
Smoking habits <i>n</i> (%)	56 (16)	9 (11)	47 (17)	0.163
ProtU 0.15–0.5 g/g, <i>n</i> (%)	98 (28)	23 (28)	75 (28)	0.962
<u>Transplant</u>				
Previous transplant, <i>n</i> (%)	64 (18)	18 (22)	46 (17)	0.312
HLA DR MM, mean ± SD	0.97 ± 0.68	0.99 ± 0.69	0.96 ± 0.68	0.776
Acute rejection, <i>n</i> (%)	47 (13)	12 (15)	35 (13)	0.697
Censored graft failure <i>n</i> (%)	42 (12)	17 (21)	25 (9)	0.005
Overall graft failure <i>n</i> (%)	52 (15)	17 (21)	35 (13)	0.082
Years of follow-up, median (IQR)	7.4 (4.9–11.5)	6.6 (4.5–9.5)	7.8 (5.1–12.0)	0.041

ATG, anti-thymocyte thymoglobulin; BMI, body mass index; CDC, complement-dependent cytotoxicity; CGN, chronic glomerulonephritis; CKD, chronic kidney disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; F, female; IQR, interquartile range; PRA, panel-reactive antibody; ProtU, proteinuria; SD, standard deviation; statistically significant values are represented in bold.

RESULTS

Overall graft survival at 5, 10, and 15 years was higher in recipients from donors with pre-donation eGFR ≥ 90 mL/min/1.73 m² compared with those from donors with eGFR < 90 mL/min/1.73 m² (98%, 86%, and 67% vs. 97%, 72%, and 46%; $p=0.013$). Death-censored survival showed a similar pattern (98%, 90%, and 75% vs. 97%, 72%, and 46%; $p=0.0004$), with differences becoming most evident beyond 5 years post-transplant (Figure 17).

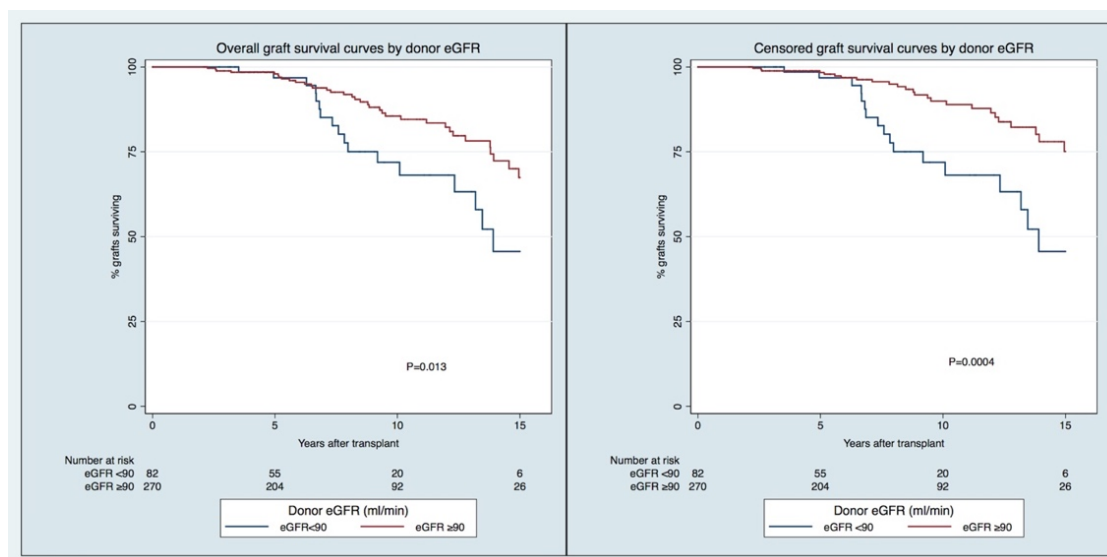


Figure 17. Overall (right) and censored (left) graft survival curves by donor eGFR pre-donation.

In multivariable analysis, a pre-donation eGFR < 90 mL/min/1.73 m² was independently associated with a higher risk of overall graft failure (HR 1.89, 95% CI 1.03–3.48, $p=0.040$), while donor age ≥ 50 years did not reach statistical significance. (Table 22). Among non-donor factors, acute rejection (HR 3.316, 95% CI 1.826–6.020, $p < 0.001$) and retransplantation (HR 2.643, 95% CI 1.453–4.810, $p = 0.001$) were significant predictors.

In the death-censored graft failure model, both pre-donation eGFR < 90 mL/min/1.73 m² (HR 2.378, 95% CI 1.224–4.622, $p = 0.011$) and donor age ≥ 50 years (HR 2.108, 95% CI 1.043–4.260, $p = 0.038$) were independently associated with poorer graft survival (Table 22). Additional independent predictors were acute rejection (HR 2.137, 95% CI 1.109–4.479, $p = 0.044$), retransplantation (HR 3.458, 95% CI 1.715–6.975, $p = 0.001$), younger recipient age (HR 0.945 per year, 95% CI 0.911–0.980, $p = 0.002$), and chronic glomerulonephritis as the cause of CKD (HR 2.529, 95% CI 1.202–5.323, $p = 0.015$).

Remarkably, none of the remaining donor variables evaluated, including hypertension, dyslipidemia, smoking, proteinuria, and obesity, were independent predictors of either overall or death-censored graft failure in these models

Table 22 - Donor-derived predictors of graft failure in multivariable Cox models

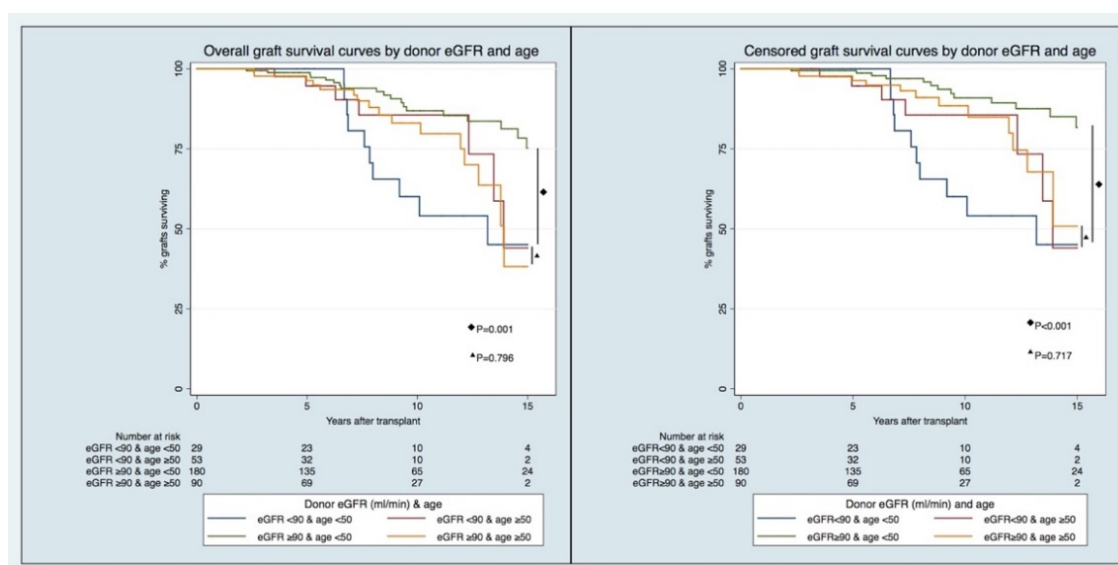
Predictor	Overall GF HR (95% CI)	p-value	Death-censored GF HR (95% CI)	p-value
Pre-donation eGFR <90	1.893 (1.030–3.478)	0.040	2.378 (1.224–4.622)	0.011
Donor age ≥50 years	1.730 (0.947–3.159)	0.075	2.108 (1.043–4.260)	0.038

CI, Confidence Interval; eGFR, estimated Glomerular filtration rate, in mL/min/1.73m²; GF, Graft Failure; HR, Hazard Ratio.

To assess whether the effect of lower pre-donation eGFR varied by donor age, multivariable Cox models were stratified into two age groups (<50 vs. ≥50 years). In younger donors, a pre-donation eGFR <90 mL/min/1.73 m² was significantly associated with increased risk of both overall graft failure (HR 2.578, 95% CI 1.120–5.795, $p = 0.026$) and death-censored graft failure (HR 3.216, 95% CI 1.300–7.959, $p = 0.012$). In older donors, no significant associations were observed for either endpoint.

Mean post-transplant recipient eGFR was consistently lower in those receiving kidneys from younger donors with pre-donation eGFR <90 mL/min/1.73 m², with differences evident from the first year and persisting throughout follow-up ($p < 0.05$ at multiple time points; detailed data in Appendix 10).

Kaplan–Meier survival curves stratified by donor age and pre-donation eGFR corroborated the Cox model findings (Figure 18). In younger donors, lower pre-donation eGFR was associated with significantly reduced overall and death-censored graft survival, whereas in older donors no significant differences were observed between eGFR categories.

**Figure 18.** Overall (right) and censored (left) graft survival curves by donor eGFR and age.

RESULTS

In summary, lower pre-donation eGFR was independently associated with poorer long-term graft outcomes, an effect that was limited to recipients of younger donors. Donor age ≥ 50 years alone was not a consistent predictor, and no other donor factors, including hypertension, dyslipidemia, smoking, proteinuria, or obesity, showed independent associations with graft failure.

Study 3

Biological Relatedness and Immunological Outcomes

Adapted from: Reis Pereira, P., et al. *Biologically Unrelated Living Donor for Kidney Transplantation Associated With Higher Acute Rejection Rate but With Similar Graft Survival of a Related Living Donor* Cureus. 2022;14(10): e30189. Doi: 10.7759/cureus.30189

APPENDIX 11

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After identifying baseline renal reserve and donor age as prognostic determinants, we next evaluated the impact of donor–recipient biological relatedness and immunological matching on graft outcomes.

Study-specific material and methods

We conducted a retrospective cohort study of all living donor kidney transplants performed at our center between January 2008 and December 2017. Donor–recipient pairs were categorized as living related donors (LRD) or living unrelated donors (LURD). ABO-incompatible transplants were excluded. The primary outcomes were biopsy-proven acute rejection (BPAR), graft survival, and patient survival. Predictors of BPAR were analyzed by multivariable cox regression. Survival was assessed by Kaplan–Meier analysis and log-rank testing.

Results

A total of 198 living donor kidney transplants (LDKTs) were analyzed, including 116 (59%) from LRD and 82 (41%) from LURD. Recipients of LURD were significantly older (48.5 ± 10.9 vs. 35.9 ± 12.2 years; $p < 0.001$) and had a higher degree of HLA mismatch at all loci

(HLA-A, -B, and -DR; all $p < 0.001$), while no significant differences were found for other donor, recipient, or transplant characteristics (Table 23).

Table 23 – Baseline characteristics of living donor pairs and transplants, based on donor-recipient relatedness.

	Total N=198	LRD N=116 (59%)	LURD N=82 (41%)	P
<u>Recipient</u>				
Age R, mean $\pm$SD	41.1 $\pm$ 13.2	35.9 $\pm$ 12.2	48.5 $\pm$ 10.9	<0.001
Sex R F, n (%)	56 (28)	36 (31)	20 (24)	0.307
Time on dialysis before KT (months), median (IQR)	13.9 (0-30.3)	12.6 (0-27.2)	16.3 (3.9-32.3)	0.125
Preemptive KT	50 (25)	33 (28)	17 (21)	0.061
<u>Donor</u>				
Age D, mean $\pm$ SD	48.1 $\pm$ 10.5	47.4 $\pm$ 11.4	49.1 $\pm$ 9.2	0.265
Sex D F, n (%)	143 (72)	80 (69)	63 (77)	0.224
eGFR (pre-D), mean $\pm$SD	100.2 $\pm$ 14.3	101.4 $\pm$ 14.0	98.4 $\pm$ 14.6	0.144
Left kidney donated, n (%)	156 (83)	89 (81)	67 (85)	0.486
<u>Transplant</u>				
Year of KTn (%)				0.903
2008-2012	71 (36)	42 (36)	29 (35)	
2013-2017	127 (64)	74 (64)	53 (65)	
Retransplant, n (%)	27 (14)	18 (16)	9 (11)	0.359
Calculated PRA >0%, n (%)	60 (30)	37 (32)	23 (28)	0.562
HLA-incompatible KT, n (%)	22 (11)	14 (12)	8 (10)	0.610
HLA-A MM, mean $\pm$SD	0.94 $\pm$ 0.68	0.63 $\pm$ 0.52	1.39 $\pm$ 0.62	<0.001
HLA-B MM, mean $\pm$SD	1.16 $\pm$ 0.72	0.80 $\pm$ 0.64	1.66 $\pm$ 0.50	<0.001
HLA-DR MM, mean $\pm$SD	0.97 $\pm$ 0.69	0.67 $\pm$ 0.59	1.40 $\pm$ 0.61	<0.001
IS Induction ATG n (%)	21 (11)	12 (10)	9 (11)	0.614
Maintenance IS, TAC/MMF/PRED n (%)	192 (97)	112 (97)	80 (98)	1
DGF, n (%)	8 (4)	5 (4)	3 (4)	1
Follow-up (years), median (IQR)	5.1 (3.3-7.2)	5.2 (3.4-8.3)	4.9 (3.0-6.9)	0.327

ATG, antithymocyte globulin; BMI, body mass index; D, donor; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; F, female; HD, hemodialysis; HLA, human leukocyte antigen; IQR, interquartile range; KT, kidney transplant; LRD, living related donor; LURD, living unrelated donor; MM, mismatch; MMF, mofetil mycophenolate; PD, peritoneal dialysis; PRA, panel-reactive antibodies; pred, prednisone; R, recipient; RRT, renal replacement therapy; SD, standard deviation; TAC, tacrolimus.

RESULTS

Biopsy-proven acute rejection (BPAR) occurred in 23 recipients (12%) and was more frequent in the LURD group (17%) than in the LRD group (8%; $p = 0.044$). Most episodes occurred within the first-year post-transplant. Time to acute rejection did not differ significantly between groups.

In univariate analysis, LURD status was associated with an increased risk of BPAR (HR 2.35, 95% CI 1.02–5.43, $p=0.046$), but this association was not independent in multivariable models adjusted for demographic, immunological, and transplant factors. Independent predictors of BPAR were HLA-DR mismatch (HR 2.26, 95% CI 1.21–4.22, $p=0.011$) and transplantation during 2008–2012 (HR 2.48, 95% CI 1.05–5.87, $p=0.039$) (Table 24).

When rejection subtypes were analyzed, higher BMI independently predicted acute cellular rejection (HR 1.18 per kg/m^2 , 95% CI 1.04–1.34, $p=0.013$). Antibody-mediated rejection was independently associated with HLA-DR mismatch (HR 2.89, 95% CI 1.02–8.17, $p=0.045$) and HLA incompatibility (HR 5.07, 95% CI 1.42–18.07, $p=0.012$).

Table 24 – Predictors of biopsy-proven acute rejection (Multivariable analysis)

	HR	95% CI	p-value
<i>BPAR predictors*</i>			
HLA-DR mismatch	2.256	1.205–4.223	0.011
KT in 2008–2012 vs 2013–2017	2.480	1.047–5.874	0.039
<i>ACR-specific predictors**</i>			
BMI (per kg/m^2)	1.179	1.035–1.344	0.013
<i>ABMR-specific predictors**</i>			
HLA-DR mismatch	2.892	1.024–8.167	0.045
HLA incompatibility	5.070	1.422–18.070	0.012

* adjusted to R age, D age, D sex, Induction IS, IS of maintenance, retransplant rate, months on RRT, type of dialysis/preemptive, D eGFR, HLA A MM, HLA B MM, KT HLAI, and R BMI.; ** Adjusted to R age, D age, R sex, D sex, Induction IS, IS of maintenance, retransplant rate, months on RRT, type of dialysis/preemptive, D eGFR, HLA A MM, HLA B MM, KT HLAI, and time period of KT.

ACR: Acute cellular rejection; ABMR: Antibody-mediated rejection; BPAR: Biopsy proved acute rejection; CI: Confidence interval; HR: Hazard ratio; IS: Immunosuppression; KT: Kidney transplant; LRD: Living related donor.

Death-censored graft survival was comparable between groups, with rates of 96.9% vs. 98.0% at 5 years and 87.8% vs. 79.4% at 10 years ($p=0.837$), and remained non-significant after multivariable adjustment (Figure 19). Independent predictors of graft loss were biopsy-proven acute rejection (HR 16.58, 95% CI 4.44–61.82, $p<0.001$) and

preformed donor-specific antibodies with HLA-incompatible transplantation (HR 3.39, 95% CI 1.01–11.36, $p < 0.05$).

Patient survival was excellent and similar in both groups, with one death (1%) in each ($p = 0.422$). Graft function was also comparable, with median eGFR values of approximately 60–65 mL/min/1.73 m² throughout follow-up ($p > 0.05$ for all time points). Full data are provided in Appendix 11.

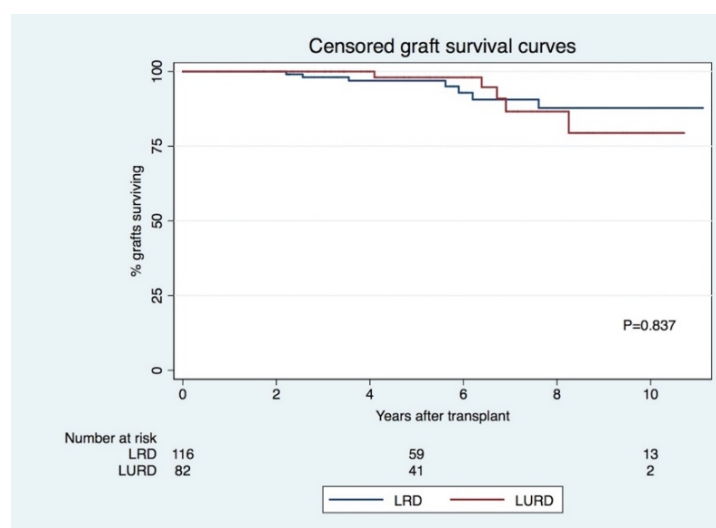


Figure 19. Censored graft survival for LRD and LURD recipients.

LRD: Living related donor; LURD: Living unrelated donor

In summary, LURD kidney transplants were associated with a higher crude rate of biopsy-proven acute rejection, but multivariable analyses identified immunological factors rather than biological relatedness as independent predictors. Long-term graft function, death-censored graft survival, and patient survival were comparable between LURD and LRD recipients.

These findings collectively support Hypothesis 4, showing that recipient outcomes after LDKT can be informed by the integrated analysis of predictive modeling, donor derived characteristics, and immunological compatibility. External validation of the iPREDICTLIVING tool confirmed the feasibility of prognostic modeling in our cohort, although prediction of censored graft failure was less accurate. Donor pre-donation and age were the only donor-derived variables independently associated with long-term graft survival, with the prognostic impact of eGFR limited to younger donors. Finally, while unrelated donation was associated with higher crude rates of acute rejection, independent predictors were immunological factors rather than biological relatedness, and long-term graft and patient survival remained comparable.

4.5. Ethical Considerations in Living Kidney Donation

Adapted from: Almeida M, Machado D. *Exploring the Complex Ethical Challenges Associated with Living Donor Kidney Transplantation*. Port Kidney J. 2025;39(1): ahead of print, 77. Doi:10.71749/pkj.77

APPENDIX 12

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Alongside the four hypothesis-driven studies discussed in this thesis, this chapter also presents the results of a structured ethical reflection on living kidney donation. Unlike the previous analyses, this work does not rely on original cohort data but offers a conceptual and normative review of key issues such as autonomy, uncertainty, long-term follow-up, and institutional responsibility. Published in a national journal, it provides a complementary framework for understanding the empirical findings explained earlier and placing them within wider ethical and policy debates.

This structured ethical reflection examined the practice of living kidney donation through the four fundamental principles of biomedical ethics: autonomy, beneficence, non-maleficence, and justice. The analysis emphasized the moral challenges that remain despite advances in surgical techniques and clinical outcomes, with particular focus on issues of uncertainty, donor complexity, and institutional responsibility. Living kidney donation involves a healthy person voluntarily accepting surgical and lifelong medical risks without direct physical benefit, solely to improve another person's life. This core characteristic highlights the ethical duty to keep donor protection as a primary and ongoing obligation.

Regarding autonomy, the analysis emphasized that informed consent in living kidney donation goes beyond just signing a document. It should be seen as a dynamic process that relies on clear, thorough, and timely communication of both known and uncertain risks. This is especially important for younger or medically complex donors, whose long-term outcomes are harder to predict. In these cases, the ethical responsibility of transplant teams is greater, ensuring that donor decisions truly reflect an understanding of risks and align with personal and social contexts.

The principles of beneficence and non-maleficence were discussed by recognizing that most donors experience positive psychosocial outcomes and do not face long-term medical harm. However, a small number may face psychological distress, regret, or medical complications, highlighting the need for clear communication and proper support during evaluation and follow-up. Ethical responsibility also applies to individuals considered ineligible to donate, who often experience exclusion as a significant personal loss. The lack

of structured psychosocial support for this group was identified as a gap. At the same time, donor evaluation was found to offer incidental health benefits, such as early detection of conditions like hypertension, diabetes, or cancer, emphasizing the protective value of comprehensive screening for all candidates.

The principle of justice was explored in relation to equitable access, gender imbalance, and long-term care. The ongoing overrepresentation of women among living donors, observed both globally and in Portugal, was identified as an ethical issue related to social expectations, caregiving norms, and subtle forms of coercion. These influences must be carefully recognized and excluded during donor evaluation to ensure that the decision to donate remains fully autonomous. Financial protection for donors was also highlighted as an issue of justice, along with the need for systematic, fair follow-up. In Portugal, the lack of a national living donor registry was acknowledged as a significant shortcoming, restricting long-term monitoring, evidence collection, and organized care.

These findings were further contextualized within the European legal framework, especially Directive 2010/53/EU [158], which requires systematic donor follow-up and reporting. Although Portugal provides lifelong medical care for living donors, the absence of centralized data collection and coordination remains a significant gap. The analysis concluded that ethical responsibilities in living donor transplantation must develop alongside clinical advancements, necessitating systems that go beyond surgical outcomes and formal consent to ensure continuity, accountability, and protection for all donors.

Taken together, the empirical results from Hypotheses 1–4 and the ethical analysis in Section 4.5 offer a comprehensive view of living kidney donation, covering donor safety, recipient outcomes, and normative responsibilities. These findings will be integrated and critically appraised in Chapter V, where their clinical, scientific, and ethical implications are discussed in detail.

CHAPTER V

DISCUSSION AND CONCLUSIONS

5.1. Discussion

The central aim of this thesis was to demonstrate that donor evaluation and safety can be improved by defining better functional and systemic metrics, thereby enabling the safe expansion of living donor kidney transplantation. As outlined in the Background chapter, donor safety must be understood as a multidimensional construct extending beyond perioperative events to encompass lifelong health trajectories, psychosocial well-being, and transplant outcomes. The individual studies interrogated these dimensions, while the Discussion integrates them into a coherent whole, showing how evaluation and follow-up can be reframed in clinical practice and policy-making.

Within this perspective, the work focused on four core domains that constitute the empirical foundation of the thesis. Renal health was examined through post-donation functional trajectories, external validation of predictive models, and comparison of imaging-based volumetry with nuclear renography as a surrogate of nephron mass. Cardiovascular–kidney–metabolic (CKM) health was addressed as a marker of systemic vulnerability, with attention to obesity, hypertension, and metabolomics. Psychosocial health was investigated through mixed-methods studies assessing donor quality of life, emotional well-being, and perceptions of the donation process. Finally, donor–recipient matching was evaluated clinically and immunologically, linking baseline donor characteristics, predictive tools, and compatibility to long-term outcomes.

Together, these domains support a more integrated approach to donor evaluation and follow-up, with ethics providing the normative foundation for decisions on living donation. The Discussion synthesizes findings across domains and illustrates how predictive tools, systemic profiling, and psychosocial insights can be integrated into a broader safety framework. This responds to the recognition that binary eligibility criteria are insufficient; instead, individualized risk stratification is needed to reconcile transplant expansion with donor protection.

Accordingly, the chapter is organized around the four domains, complemented by an ethical reflection situating the clinical findings within principles of autonomy, justice, and stewardship. It concludes with the New Perspectives section, where an integrative framework for donor safety is proposed and future directions outlined.

As a roadmap, Table 25 summarizes the aims and key results of each study by hypothesis. Abbreviated scopes are presented for clarity, while the complete statements remain in Table 1 of the Results chapter to avoid repetition.

Table 25 – Thesis Overview

Hypothesis number /Scope	Aims	Key Results & Conclusions
Hypothesis 1: Renal Health	To evaluate the association of post-donation eGFR trajectories with donor baseline characteristics and early recovery patterns and their value for renal risk stratification	Baseline eGFR, BMI, and 6-month Kidney Function Reduction Rate predicted long-term trajectories, supporting risk-adapted follow-up.
	To validate the Toulouse-Rangueil model in predicting 1-year post-donation eGFR among Portuguese donors	The score was externally validated accurately predicting 1-year eGFR and identifying donors at risk for CKD.
	To compare the predictive accuracy of CT-based renal volumetry and nuclear renography in estimating remaining renal function	CT volumetry outperformed nuclear renography in predicting post-donation renal function, improving surgical planning and donor counseling
Hypothesis 2: CKM Health	To evaluate the prevalence, predictors, and clinical associations of obesity (BMI>=30Kg/m ²) after living kidney donation	Obesity was associated with higher post-donation hypertension risk but not with faster eGFR decline. Overweight at baseline often progressed to obesity, underscoring the need for special oversight early post-donation and targeted lifestyle follow-up.
	To evaluate the incidence, predictors, and correlates of <i>de novo</i> hypertension post-donation,	<i>De novo</i> hypertension developed in 1/3 of donors (15-year incidence >50%), with a threefold higher risk than matched controls. Higher BMI, dyslipidemia, and elevated baseline BP independently predicted its onset. It was associated with faster eGFR decline emphasizing the need for proactive surveillance and management.
	To investigate whether urinary metabolomic profiles can reveal early biochemical signatures of CKM stress in living kidney donors.	Urinary metabolomics detected early biochemical shifts after donation, demonstrating feasibility for CKM risk detection
Hypothesis 3: Psychosocial Health	To identify psychosocial risk factors associated with lower health-related quality of life, anxiety, and depressive symptoms after donation	Overall quality of life was preserved, but some donors, particularly those with medical complications or stressful life events, experienced anxiety or depressive symptoms highlighting the need for structured psychosocial follow-up
	To assess donor perceptions and satisfaction following donation using structured questionnaires	Donors reported high satisfaction and altruistic motivation, but ambivalence was common, reinforcing the importance of psychosocial support and longitudinal monitoring.
Hypothesis 4: Donor-Recipient Matching	To validate the iPREDICTLIVING model in a Portuguese living donor cohort and evaluate its clinical utility for pre-transplant counseling	The iPREDICTLIVING model accurately predicted recipient survival supporting its use in pre-transplant counseling
	To assess the association of baseline donor characteristics, such as age and eGFR, with long-term graft function and graft survival.	Younger donor age with lower baseline eGFR were associated with higher risk of graft failure, reinforcing their role in donor–recipient matching
	To compare transplant outcomes between recipients of related and unrelated living donors, controlling for immunologic compatibility	Unrelated recipients had more rejection, but only HLA-DR mismatch predicted acute rejection, highlighting the importance of immunologic matching.
Ethical Reflection	To identify and analyze key ethical challenges in living donor kidney transplantation, including autonomy, informed consent, risk–benefit balance, justice, and gender disparities.	Long-term donor follow-up, the establishment of national donor registries, and evidence-based informed consent represent moral imperatives for safeguarding donor welfare and ensuring responsible expansion of living donation

BMI, Body Mass Index; BP, Blood Pressure; CKM, Cardio-Kidney-Metabolic; CT, Computed Tomography; eGFR, Estimated Glomerular Filtration Rate; LDs, Living Donors; NR, Nuclear Renography

5.1.1. Hypothesis One: Renal Health

Personalized renal risk assessment in living kidney donors can be improved by integrating eGFR trajectories, predictive models, and imaging-based volumetric assessments.

Renal safety remains the primary concern in living kidney donation, with the potential development of end-stage renal disease (ESRD) representing the most critical long-term risk for donors and the foremost challenge for clinicians. Landmark studies by Muzaale [3] and Mjøen [4] demonstrated that, although the absolute risk of ESRD is low, it is significantly higher than in matched non-donors. These pivotal findings reshaped the field, emphasizing the need for individualized and multidimensional risk assessment rather than rigid exclusion criteria, and underscoring the ethical obligation for structured long-term follow-up [18-20]. At the same time, applying safety thresholds derived from oncologic nephrectomy cohorts (e.g., eGFR >45 mL/min/1.73 m²) may underestimate the physiological reserve required for otherwise healthy donors, particularly younger individuals [203, 204]. As acceptance criteria broaden to include medically complex donors, refining risk stratification has become essential.

Our eGFR trajectories study provides the first long-term analysis of Portuguese living kidney donors, with follow-up extending beyond 15 years [205]. In our retrospective cohort of 320 living donors, overall renal outcomes were favorable. From six months post-nephrectomy, mean eGFR increased modestly (+0.35 mL/min/1.73 m²/year), with the steepest recovery in the first 2 years (+0.85 mL/min/1.73 m²/year), a plateau around 10 years, and a slight decline between 10–15 years (-0.24 mL/min/1.73 m²/year). Clinically significant functional loss was rare ($\leq 5\%$ of donors had an eGFR <50 mL/min/1.73 m² from 2 to 15 years post-donation), and no cases of ESRD were observed. These findings align with prior reports of early hyperfiltration followed by long-term stabilization, reinforcing that while registry studies have shown higher relative risk, the absolute risk of severe outcomes remains low in carefully selected donors [101, 206]. The stabilization of mean trajectories reflects compensatory adaptation in most individuals, yet ongoing surveillance is warranted given the persistent long-term risk signal. Importantly, these averages conceal interindividual variability, with some donors showing attenuated recovery or later decline.

Subgroup analyses revealed important modifiers of long-term outcomes. Donors with obesity (BMI ≥ 30 kg/m²) exhibited attenuated recovery and less favorable trajectories, particularly within the first five years, consistent with prior studies that have linked obesity to reduced renal reserve and increased glomerular workload [59, 79]. Lower baseline eGFR was associated with later decline, most evident between five and ten years. In contrast, no

significant differences were observed by age, sex, or well-controlled hypertension, suggesting that carefully selected older and hypertensive donors can be safely accepted.

In addition to baseline characteristics, early recovery dynamics provided additional prognostic information. A distinctive contribution of this work was the introduction of the Kidney Function Reduction Rate (KFRR), defined as the percentage decline in eGFR at six months relative to pre-donation values ($\text{KFRR} = [(\text{pre-donation eGFR} - 6\text{-month eGFR}) / \text{pre-donation eGFR}] \times 100$). Donors with a smaller early reduction ($\text{KFRR} < 26.2\%$) paradoxically experienced steeper subsequent decline, particularly between six months and two years. This contrasts with earlier reports suggesting that faster early recovery predicts better outcomes [207, 208]. The divergence may reflect methodological differences in the timing of assessment, or alternatively, may indicate that a smaller early reduction represents maladaptive hyperfiltration, a marker of long-term vulnerability rather than recovery. To our knowledge, no prior study has operationalized a standardized six-month percentage reduction as a prognostic marker [32, 206-208]. Although exploratory, KFRR is simple, easily derived from routine data, and merits replication in larger, prospective cohorts before consideration for clinical use.

Urinary markers may also provide further prognostic insight, complementing clinical characteristics. Proteinuria, assessed by spot protein-to-creatinine ratio (PCR), remained low and stable in most donors during follow-up, consistent with SRTR-based data suggesting that mild proteinuria post donation may not reliably predict long-term decline in otherwise healthy donors [209]. However, systematic albuminuria monitoring was not available in early cohorts, limiting our ability to detect subclinical glomerular stress. Since albuminuria is a more sensitive marker of early renal injury, its standardized assessment should be systematically incorporated into donor follow-up, a practice that has already been adopted in our center.

Clinically, these findings support a risk-informed rather than uniform follow-up strategy. Most donors maintain favorable renal outcomes, but those with obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$), lower baseline eGFR, or low KFRR represent subgroups at higher risk and may benefit from closer surveillance, particularly during the first decade when variability in adaptation is greatest. Counseling should therefore balance reassurance with transparency about differential trajectories of renal reserve.

The heterogeneity in post-donation trajectories underscores the need for tools that can anticipate early renal function, a parameter closely linked to long-term outcomes. In a large registry study, Massie et al. [210] reported that each $10 \text{ mL/min/1.73 m}^2$ reduction in six-month eGFR was associated with a 28% higher risk of ESRD, emphasizing the clinical

importance of prediction models. Against this background, we externally validated the Toulouse–Ranguel (TR) score in our cohort of 333 donors.

The TR score, based solely on age and baseline eGFR, predicts one-year renal function and has been adopted in several European centers following earlier validations [82, 83]. In our Portuguese cohort, it demonstrated strong discrimination (AUROC >0.80) and calibration, and a high negative predictive value for CKD stages 3–5, which supports its use in donor counseling [81]. Performance was consistent across sexes, with only minor threshold variations, suggesting broad applicability. Interpreting CKD staging in living donors requires nuance, as many technically meet criteria for stage 3a based on eGFR alone despite stable trajectories and minimal progression risk. In this setting, the TR score is best viewed not as a tool for assigning a CKD label but as a means of identifying donors at risk of clinically meaningful functional decline.

Other, more recent validations have reported mixed results. Patel et al. [211], in a large US registry analysis, reported systematic overestimation of eGFR in younger, male, and Black donors, highlighting potential limitations in its generalizability across diverse donor populations. By contrast, preliminary Tunisian data [212] suggest that the score may retain predictive value in non-European settings, though formal peer-reviewed validation is still required. Together, these findings highlight both the promise of the TR model and the need for calibration to diverse populations.

Viewed alongside our trajectory analysis, the TR score provides a pragmatic short-term (one-year eGFR) predictor linked to long-term outcomes but does not capture the full spectrum of adaptation. Its scope remains narrow, excluding other relevant donor factors. While validated in our cohort, calibration will be required for broader application. No single model can therefore suffice for comprehensive evaluation.

Computed tomography (CT)–based volumetry provides a structural dimension to donor evaluation by directly quantifying renal parenchymal volume. Although nuclear renography (NR) has long been considered the reference method, volumetry shows closer correlation with nephron mass and post-donation outcomes [40]. Several high-volume transplant centers now include volumetric analysis in their standard work-up [19], yet its wider adoption remains limited by protocol variability and the lack of universal guidelines.

In our cohort of 193 living donors (a subset of the overall population analyzed in Studies 1 and 2, restricted by availability of volumetric data and inclusion criteria), CT volumetry outperformed NR in predicting 1-year post-donation eGFR [213]. When the two modalities provided discordant results, NR consistently overestimated function, and this discrepancy was associated with poorer observed outcomes. These findings align with previous reports

[42, 214-217] and support our earlier research showing that the remnant kidney volume indexed to donor body weight is a reliable predictor of both short- and mid-term renal function after nephrectomy [43]. More recently, a meta-analysis that included our study [218], confirmed this signal: despite methodological heterogeneity, CT- and MRI-based volumetry consistently showed better predictive performance and reproducibility than scintigraphy, with favorable correlation and agreement. Within the Portuguese context, volumetric assessment has so far been investigated only in our center, reflecting a stepwise effort to introduce and validate this approach locally.

Because CT angiography is already performed routinely for anatomical and vascular mapping in donor evaluation, volumetric analysis can be extracted without additional radiation exposure or major cost, offering added prognostic value without increasing procedural burden. In our current practice, when NR and volumetry provide discordant results, surgical decisions are guided by volumetry, reflecting its stronger predictive value and clinical relevance. Although NR remains part of standard donor evaluation, its continued use reflects current guideline practice rather than clear evidence of superiority.

Viewed alongside our trajectory and predictive modeling studies, CT volumetry contributes the anatomical substrate to donor risk stratification. Functional trajectories and KFRR capture the dynamics of adaptation, while the TR score predicts short-term function and volumetry estimates nephron mass. Together, these tools support a multidimensional renal safety framework that recognizes variability in post-donation adaptation and enables individualized counseling, surgical decision-making, and risk-adapted follow-up [18, 19]. Because donors consistently identify renal function as their foremost concern [22], such integrated assessment strengthens both clinical care and the ethical foundation of informed consent.

5.1.1.1. Hypothesis One: Limitations

This work has several limitations that must be acknowledged. Its retrospective, single-center design restricts generalizability, and renal function was estimated with eGFR rather than measured directly. Albuminuria was inconsistently captured in early cohorts, limiting the detection of subclinical injury. The KFRR signal, while physiologically plausible, remains exploratory and requires prospective validation. The TR score validation was performed in a relatively homogeneous Portuguese population, which may reduce applicability in more diverse settings. Volumetry relied on a semi-automatic technique performed by a single operator and was limited to total renal volume rather than cortical assessment, constraining reproducibility and accuracy. Finally, although the three studies drew on the same overall

donor population, each applied specific exclusion criteria, resulting in partially overlapping cohorts. This sequential approach allowed incremental evaluation of functional, predictive, and anatomical markers but limited direct within-individual comparison.

Although broader future perspectives are discussed in the final chapter, the limitations of this work suggest several specific next steps. Prospective multicenter studies should validate KFRR as an early recovery marker, recalibrate the Toulouse–Rangueil score in diverse populations, and advance automatic cortical volumetry as a reproducible surrogate of nephron mass [214, 215]. Integrating these tools into composite predictive models will strengthen the continuum of renal safety assessment outlined in this thesis.

In summary, Hypothesis One demonstrates that post-donation renal outcomes are best understood through dynamic assessment of functional reserve rather than static thresholds. Predictive models and volumetric imaging add complementary value for individualized donor selection, and when integrated with longitudinal trajectories, they support a multidimensional approach to risk stratification. These findings underscore that safeguarding donor safety requires moving beyond single cut-off values toward comprehensive, layered assessment strategies that can be refined through future validation.

Table 26 - Key Messages – Hypothesis One: Renal Health

Personalized renal risk assessment in living kidney donors can be improved by integrating eGFR trajectories, predictive models, and imaging-based volumetric assessments.

Domain	Key Messages
Main findings	• Long-term follow-up showed stable renal outcomes overall, though with marked interindividual variability. • Obesity, lower baseline eGFR, and low KFRR emerged as key predictors of donors at risk for maladaptive renal trajectories • The Toulouse–Ranguel (TR) score was externally validated, predicting one-year eGFR with good accuracy in our cohort. • Semi-automated CT volumetry outperformed nuclear renography in predicting one-year post-donation renal function.
Integrated framework	• Continuum of risk assessment: trajectories → TR score → CT volumetry. • Renal safety is best understood dynamically, not by static thresholds. • Functional, predictive, and anatomical tools capture complementary risk dimensions. • The integration supports multidimensional stratification for counseling and tailored follow-up.
Clinical & ethical implications	• Donor selection should move beyond single thresholds towards risk-informed models. • TR score and CT volumetry can inform counseling and surgical planning, but should be applied alongside other donor factors • KFRR at 6 months may offer early identification of at-risk donors, if prospectively validated. • Transparent communication of risk reinforces the ethical duty to safeguard donor safety.
Limitations	• Retrospective, single-center data. • Reliance on eGFR, with incomplete albuminuria assessment. • Volumetry limited to total rather than cortical volume • Relatively homogeneous cohort; generalizability limited. • Each tool was tested separately, limiting combined evaluation.

CT, Computed Tomography; eGFR, estimated Glomerular Filtration Rate; KFRR, Kidney Function Reduction Rate; TR, Toulouse Ranguel score.

5.1.2. Hypothesis Two: Cardiovascular-Kidney-Metabolic Health

Systemic vulnerability beyond renal outcomes can be identified through the combined evaluation of BMI, blood pressure, and metabolomic profiling.

Living kidney donation involves not only a reduction in nephron mass but also a systemic adaptation that engages multiple physiological domains. While Hypothesis One examined the nuances of renal functional reserve, donor safety also depends on broader systemic factors that extend beyond kidney-specific parameters.

The recently proposed cardiovascular–kidney–metabolic (CKM) framework offers such a perspective, emphasizing how disturbances in one domain accelerate vulnerability in the others [29, 87]. Recognizing the integration of these domains, the American Heart Association's CKM Health Initiative 2024 is advancing integrated, whole-person care for cardiovascular, kidney, and metabolic conditions. For living donors, this paradigm is especially relevant: although nephrectomy alone confers only a modest absolute risk of ESRD, comorbidities such as obesity, hypertension, or dyslipidemia may magnify long-term risk [3, 4, 110]. Traditional donor evaluation has relied on binary thresholds (e.g., BMI <30 kg/m², controlled blood pressure) to ensure safety [17]. Yet, growing evidence shows that risk is more nuanced and shaped by baseline metabolic profile and compensatory capacity [81, 205, 213]. This shift from a single organ to a multidimensional perspective provides the rationale for Hypothesis Two, which examines how CKM traits influence donor outcomes and how novel biomarkers may capture early systemic stress.

Our second hypothesis therefore examines CKM donor vulnerability at three complementary levels: (1) a retrospective cohort study of donors with obesity (>30 Kg/m²), addressing a highly prevalent and potentially modifiable risk factor; (2) a targeted analysis of incident hypertension as a marker of systemic maladaptation; and (3) a pilot urinary metabolomics study as an exploratory biomarker approach to identify early biochemical signs of CKM stress. Together, these studies move from established clinical risks to novel biomarkers, underscoring the continuum of donor CKM health and its relevance for personalized surveillance.

Obesity represents one of the most pressing global health challenges and is increasingly encountered in candidates for living kidney donation. Its inclusion must be carefully weighed against the ethical imperative to ensure donor safety. While some transplant programs apply strict BMI cutoffs, others accept donors with obesity after a comprehensive evaluation [18, 19]. Despite its growing clinical relevance, robust data on the long-term impact of obesity and post-donation weight dynamics remain limited, particularly in European populations.

In our cohort of 306 Portuguese donors with follow-up up to 15 years, 10% had obesity and 41% were overweight at baseline. These proportions reflect national epidemiological patterns but also the selective nature of donor screening, which underrepresents individuals with obesity [219]. Donors with obesity were older, had higher baseline blood pressure, and more dyslipidemia, confirming a pre-existing metabolic burden. Higher pre-donation BMI, dyslipidemia, and younger age (<40 years) independently predicted post-donation obesity, highlighting both biological susceptibility and longer lifetime exposure. Although overall obesity prevalence remained stable, overweight donors often transitioned to obesity, underscoring the need for special oversight early after donation in those who were overweight at baseline, and emphasizing dynamic weight trajectories.

Obesity was associated with higher rates of post-donation hypertension at one and five years, while no significant differences were observed in proteinuria or renal decline. These findings likely reflect stringent donor selection and the preserved renal reserve of otherwise healthy individuals. However, the association with hypertension is clinically meaningful, given its role as a modifiable risk factor with downstream renal and cardiovascular consequences. Although diabetes incidence was too low for analysis, its established association with obesity further supports long-term metabolic vigilance.

When compared with hypothesis one, a key distinction emerges. The trajectory-based analysis, which stratified donors by baseline obesity status, showed that donors with obesity had attenuated renal recovery after nephrectomy, suggesting impaired adaptive capacity [205]. By contrast, the present obesity-focused study followed longitudinal obesity trajectories and did not detect significant differences in conventional endpoints such as eGFR decline or proteinuria [220]. This apparent divergence likely reflects differences in methodology, statistical power, and follow-up duration: trajectory modeling captures subtle adaptive deficits, whereas conventional clinical endpoints may miss early or modest changes. Importantly, both analyses converge in highlighting obesity as a clinically relevant risk factor, through either impaired renal adaptation or increased hypertension, underscoring its role as a marker of systemic vulnerability.

Clinically, obesity is one of the few modifiable risk factors. It requires tailored counseling, weight management before donation, and structured follow-up with early screening for hypertension, dyslipidemia, and diabetes. Younger donors warrant special vigilance due to longer lifetime exposure. Overweight donors also deserve early, proactive intervention, as they frequently progress to obesity after donation. Current guidelines support careful evaluation rather than universal exclusion [18, 19], while recognizing that a pro-inflammatory and insulin-resistant milieu associated with excess adiposity [87] may compound the physiological demands of reduced nephron mass. Our findings support this

cautious but not prohibitive approach: as obesity prevalence rises, donor evaluation must balance the expansion of living donation with the duty to safeguard donor safety. Together with Hypothesis One, these results highlight the need for multidimensional frameworks that integrate renal reserve and systemic cardiometabolic health in donor counseling.

The impact of baseline cardiometabolic traits was further demonstrated in our hypertension analysis. Hypertension is one of the most debated long-term outcomes in LKDs, reflecting persistent uncertainties regarding the cardiovascular and renal effects of nephron loss. While large registries have provided valuable data, estimates vary widely due to differences in definitions, follow-up duration, and comparator groups [70, 95]. Our study adds one of the first extended, region-specific analyses in Southern European donors with matched controls.

Among 300 Portuguese donors without pre-donation hypertension, 30% developed hypertension over a mean follow-up of 6.4 years, and the cumulative incidence exceeded 50% at 15 years. This demonstrates that blood pressure elevation is both common and sustained after nephrectomy. Strong predictors of incident hypertension included higher baseline BMI, dyslipidemia, and pre-donation blood pressure, underscoring the long-term influence of modifiable traits. Remarkably, hypertension was not associated with pre-donation eGFR, suggesting that systemic metabolic and vascular factors outweigh renal reserve in driving this risk.

Donors who developed hypertension had a modest but statistically significant decline in renal function compared to normotensive peers (eGFR slope: -0.03 vs. $+0.40$ mL/min/year; $p = 0.026$), despite no progression to ESRD in the study period. Although causality cannot be proven, this association indicates that post-donation hypertension may signal early renal vulnerability and maladaptation.

Biologically, unilateral nephrectomy may induce hypertension via multiple pathways, including renin-angiotensin system activation, increased sympathetic activity, impaired vascular compliance, and altered sodium homeostasis [95, 105]. Our findings are consistent with this multifactorial pathophysiology and support hypertension as a sentinel marker of CKM stress in donors. Compared with a matched regional cohort [201], donors showed a 3–4-fold higher incidence of hypertension, reinforcing that this is a true biological effect rather than detection bias.

Notably, recent prospective studies from North America, including those by Garg et al. [100] and Kasiske et al. [101], did not report a higher incidence of hypertension or albuminuria among donors compared with matched controls. These divergent results may reflect differences in cohort demographics, baseline metabolic risk, follow-up intensity, and the

definitions applied for hypertension. In our study, standardized event rates (SER) were evaluated against a general population cohort [201]. While this provides an age- and sex-adjusted benchmark, it does not account for all baseline covariates. In the context of a Southern European population with high cardiometabolic burden and long follow-up, this methodological difference, combined with regional risk profile likely contributes to the higher incidence we observed and underscore the importance of regional context in donor counseling and risk prediction.

In contrast to Hypothesis One, which suggested that obesity compromises renal recovery, baseline blood pressure had no effect on eGFR trajectories. Instead, post-donation hypertension revealed itself as a marker of systemic vulnerability with modest renal consequences. These complementary findings illustrate that risk factors act through different pathways and timeframes, reinforcing the rationale for comprehensive, longitudinal surveillance.

The high incidence of post-donation hypertension, affecting nearly one-third of donors and exceeding 50% at 15 years, emerges as a major clinical finding of this work with direct implications for long-term donor care. Hypertension is not only a frequent CKM outcome but also a modifiable one, demanding a structured and proactive strategy. This should include systematic counseling, frequent monitoring with ambulatory blood pressure measurement (ABPM) to exclude white-coat or masked hypertension, and the integration of telemedicine platforms to facilitate surveillance. When confirmed, hypertension requires timely lifestyle modification and early pharmacologic intervention (e.g., ACE inhibitors, SGLT2 inhibitors) to mitigate renal and cardiovascular risk. As with obesity, post-donation hypertension reflects systemic vulnerability and must be integrated into donor-centric, long-term care pathways.

Beyond established risk factors such as obesity and hypertension, there is growing interest in metabolomic profiling to detect early biochemical signatures of CKM stress. Urinary metabolomics offers a non-invasive approach to capture systemic metabolic perturbations and may identify subclinical vulnerability well before overt clinical changes [29, 87, 122]. However, its application in the living donor context remains largely unexplored [126, 131, 221], making exploratory studies such as ours an important first step toward integrating omics-based tools into donor surveillance.

In this pilot study, untargeted urinary metabolomics was implemented in 18 living kidney donors with paired samples collected before nephrectomy and at 6 months post-donation. This approach demonstrated feasibility and revealed consistent downregulation of metabolites across energy, redox, and osmoregulatory pathways. Functional correlations

with eGFR and CT volumetry support the biological relevance of these early signals, even in donors who remained clinically stable.

Although multivariate analyses (PCA, PLS-DA) showed only modest separation of pre- and post-donation profiles, selected metabolites (citrate, hypoxanthine, betaine) displayed discriminatory value, suggesting their potential as candidate biomarkers of donor adaptation. These molecular changes contrasted with the stable clinical profile of the same cohort, underscoring the added value of metabolomics in detecting early stress not captured by conventional monitoring [222].

Limitations must be acknowledged: small cohort size, single-center design, and analysis at only one post-donation time point. Nevertheless, this proof-of-concept study demonstrates the feasibility and potential utility of urinary metabolomics in living donors. Larger multicenter studies with longitudinal follow-up are needed to validate these preliminary signals and to determine whether they reflect adaptive or maladaptive trajectories.

In summary, these findings suggest that metabolomics could complement conventional monitoring by detecting early biochemical stress, reinforcing the broader message of this thesis: donor safety depends on surveillance that goes beyond traditional risk factors.

Taken together, the three studies presented under Hypothesis Two emphasize the importance of embedding CKM health into the evaluation and long-term care of living kidney donors. Obesity and, most notably, hypertension emerged as clinically relevant and modifiable traits with major implications for long-term outcomes. Hypertension developed in a substantial proportion of donors and must be viewed as a clear call for action, requiring systematic screening, frequent blood pressure monitoring, and early intervention strategies. Obesity further contributes by increasing hypertension risk, and potentially amplifying psychosocial stress through effects on self-image and lifestyle [223]. In parallel, metabolomics provided proof-of-concept evidence that early biochemical shifts in CKM-related pathways can be detected even before overt clinical deterioration, supporting its future potential as a complementary monitoring tool. Collectively, these findings demonstrate that donor safety extends beyond renal outcomes to encompass cardiovascular and metabolic health, underscoring the need for integrated, proactive, and individualized long-term follow-up.

5.1.2.1. Hypothesis Two: Limitations

This work has several limitations that must be acknowledged. Both the obesity and hypertension analyses were retrospective, single-center studies, which may limit

generalizability and introduce selection and information bias. While follow-up extended up to 15 years, the median duration was shorter, potentially underestimating late-onset risks such as diabetes, CKD progression, or cardiovascular events. Blood pressure was recorded during routine visits rather than standardized ambulatory monitoring, likely under detecting masked or nocturnal hypertension. An additional methodological limitation is that event rates were standardized against the general population rather than based on individually matched controls. While this allowed robust age- and sex-adjusted estimates, it may not fully account for differences in baseline comorbidities, lifestyle, or socioeconomic factors, which could partly explain discrepancies with matched-control studies from North America. Details on antihypertensive treatment and adherence were not systematically captured, restricting interpretation of management effects. The inherent healthy donor effect, due to stringent selection, may have attenuated risk estimates, limiting extrapolation to higher-risk populations. Residual confounding from unmeasured lifestyle or genetic factors cannot be excluded.

The metabolomics analysis was exploratory and constrained by small sample size and short follow-up, precluding robust statistical inference. Only urinary metabolites were analyzed, without integration of plasma or multi-omic data, and potential influences of hydration, diet, and circadian rhythm could not be fully controlled. As such, mechanistic interpretations remain hypothesis-generating.

Despite these limitations, the strength of this hypothesis lies in its multidimensional approach, combining clinical outcomes with novel biomarker exploration. Together, these studies provide region-specific evidence that obesity and hypertension represent clinically relevant risks after donation, while metabolomics demonstrates feasibility for detecting early biochemical adaptation.

Looking forward, future work should embed CKM traits into donor evaluation more systematically. This includes testing structured lifestyle and pharmacologic interventions (e.g., weight management, ACE inhibitors, SGLT2 inhibitors) in at-risk donors, and expanding metabolomics from proof-of-concept toward multicenter, multi-omic validation. In parallel, the establishment of a dedicated clinic for donor health is foreseeable, providing a structured platform for long-term surveillance, early intervention, and integration of innovative tools. Such steps would operationalize CKM-informed donor care and help refine truly personalized follow-up for living kidney donors.

In summary, Hypothesis Two demonstrates that obesity and hypertension represent key, clinically relevant risks for living kidney donors. Obesity was linked to greater baseline metabolic burden and increased risk of post-donation hypertension, while hypertension

itself affected nearly one-third of donors at mid-term and more than half at long-term follow-up, particularly in those with additional metabolic risk factors. Hypertension correlated with modest eGFR decline, underscoring its impact on adaptation. Preliminary metabolomic profiling confirmed feasibility for detecting early CKM-related shifts, though validation in larger longitudinal studies is required. Collectively, these findings show that donor safety depends not only on renal reserve but also on integrating cardiovascular and metabolic health into individualized long-term monitoring.

Table 27 - Key Messages – Hypothesis Two: Cardiovascular-Kidney-Metabolic Health

Systemic vulnerability beyond renal outcomes can be identified through the combined evaluation of BMI, blood pressure, and metabolomic profiling.

Domain	Key Points
Main Findings	• Obesity and hypertension are prevalent among Portuguese donors and represent key modifiable CKM risks. • Obesity was linked to higher baseline metabolic burden and more post-donation hypertension, but not to faster renal decline. • Hypertension developed in ~30% of donors at mid-term and >50% at long-term, predicted by BMI, dyslipidemia, and baseline BP, and was associated with modestly faster eGFR decline. • Donors with post-donation HT showed modestly faster eGFR decline, though no ESRD occurred during follow-up. • Metabolomics was feasible, detecting exploratory CKM-related biochemical shifts
Integrative Framework	• Obesity and HT reflect systemic vulnerability rather than isolated renal risk. • Complementary to Hypothesis One: obesity was associated with less renal recovery capacity, whereas under Hypothesis Two it was linked to higher metabolic burden and greater hypertension risk • Together, these findings support a multidimensional view of donor adaptation, integrating renal reserve with systemic resilience.
Clinical & Ethical Implications	• Hypertension after donation should be considered a sentinel marker of maladaptation, calling for systematic surveillance and early intervention (e.g., ABPM, telemedicine, timely pharmacotherapy) • Donors with obesity or hypertension require tailored counseling, structured follow-up, and proactive risk reduction, especially younger donors. • Metabolomics, though exploratory, introduces innovation toward precision donor care. • Ethical responsibility includes transparent disclosure of CKM risk and equitable follow-up.
Limitations	• Retrospective, single-center design limits generalizability; follow-up duration was variable. • Hypertension assessed during routine visits, not standardized ABPM. • Structured interventions (weight management, ACE inhibitors, SGLT2 inhibitors) were not evaluated • Metabolomic was constrained by small sample size and limited longitudinal assessment (single 6-month post-donation time point).

ABPM. Ambulatory Blood Pressure Monitoring; ACEi, Angiotensin-Converting Enzyme inhibitors; BMI, Body Mass Index; BP, Blood Pressure; CKM, Cardio-Kidney-Metabolic; HT-hypertension, SGLT2i, Sodium–glucose cotransporter-2 inhibitors

5.1.3. Hypothesis Three: Psychosocial Health

Psychosocial health in living kidney donors can be comprehensively assessed through integrated qualitative and quantitative methods.

While Hypotheses One and Two demonstrated that donor adaptation after nephrectomy is shaped by renal functional reserve and cardiometabolic vulnerability, Hypothesis Three highlights the psychosocial domain as an equally critical pillar of donor safety. Together, these axes show that vulnerability after donation can manifest through physiological, metabolic, or emotional pathways, often in overlapping ways. A multidimensional perspective is therefore required to ensure that the ethical obligation of donor protection extends beyond clinical parameters to encompass long-term psychological well-being and quality of life.

In this context, two complementary studies were conducted in the same cohort of 44 living kidney donors, combining donor perceptions with standardized psychometric outcomes (SF-36 and HADS), providing novel insights into the Portuguese donor experience [224, 225]. While psychometric outcomes were presented first in the Results, the discussion begins with perceptions to situate standardized measures within the broader context of donor satisfaction and lived experience.

Our perceptions study examined how living kidney donors experienced the decision-making process and reflected on their post-donation journey [224]. The responses were overwhelmingly positive: most donors found the decision to donate easy (86%), felt adequately informed (100%), and perceived a clear health benefit for the recipient (89%). Only one donor expressed regret, and nearly all stated they would donate again (95.5%), with the majority also recommending donation to others (77.3%). These findings are consistent with international reports [140, 141] and confirm that altruism, satisfaction, and minimal regret characterize the majority of living donation experiences.

Several protective dimensions emerged that can be framed as resilience factors. Improvements in recipient health were frequently described as a source of psychological gratification, reinforcing donor well-being. Most donors (79.5%) reported stable relationships with the recipient, with no cases of deterioration, and over two-thirds (72.7%) affirmed active family support. These elements, relational stability, family approval, and psychological reward, likely function as buffers against distress, promoting adaptive coping after donation.

Nevertheless, beneath the prevailing positive narratives, latent vulnerabilities were evident, reflecting the coexistence of altruism with ambivalence and pre-donation fears, a dynamic

well recognized in psychosocial models of complex medical decision-making [226]. A minority (13.6%) described the decision as 'difficult' or accompanied by doubts, reflecting ambivalence. Pre-donation concerns were common and multifaceted: fear of surgical complications (61.4%), transplant failure (52.3%), long-term health consequences (50.0%), or recipient non-adherence to treatment (36.3%). Although only 6.8% considered withdrawing, these findings show that strong altruistic motivation does not preclude uncertainty or apprehension. Such ambivalence should be regarded as a normal aspect of complex decisions and openly acknowledged in counseling to uphold the ethical integrity of informed consent, while recognizing them as a key dimension of psychosocial vulnerability alongside renal and cardiometabolic risk [167].

Standardized psychometric assessments complemented the perception-based findings by revealing subtle but clinically relevant changes in donor well-being. Across the cohort, SF-36 scores showed significant declines in multiple domains, including physical functioning (Δ -7.1, Cohen's $d=0.32$), vitality (Δ -12.2, Cohen's $d=0.47$), social functioning (Δ -10.7, Cohen's $d=0.37$), and mental health (Δ -12.4, Cohen's $d=0.43$). Although scores remained within Portuguese population norms, all changes exceeded the Minimal Clinically Important Difference (MCID, 3–5 points), indicating that donors themselves would likely perceive them as meaningful.

Subgroup analyses indicated that psychosocial burden was concentrated in identifiable groups rather than uniformly distributed. Donors with medical complications showed medium-to-large effects in pain (Δ -13.9, Cohen's $d=0.77$) and mental health (Δ -14.9, Cohen's $d=0.61$). Those exposed to stressful life events displayed sharper declines across physical, pain, and mental health domains. In the compatible donation subgroup, mental health was significantly lower post-donation than in other donors (61.8 vs 77.8, $p=0.05$, Cohen's $d=0.62$). These findings indicate that vulnerability is amplified when clinical complications or life stressors are present, reinforcing the need for risk-adapted follow-up strategies instead of uniform approaches. In addition, the disproportionate representation of women among donors suggests potential gender-specific psychosocial pressures linked to family expectations. Although this dimension could not be assessed in our study, it remains an important avenue for future research.

The HADS results paralleled these findings. Depression scores rose significantly, albeit within subclinical thresholds, while anxiety showed an upward trend. Importantly, baseline depressive symptoms correlated with greater post-donation anxiety, underscoring the predictive value of pre-donation psychological screening.

Taken together, most donors reported stable psychosocial outcomes and high satisfaction, but a meaningful minority experienced clinically significant declines. Donor well-being was shaped by the interplay of baseline psychological traits, coping resources, medical complications, and life stressors. These results highlight that psychosocial adaptation is dynamic, and vulnerabilities can persist or emerge beyond the first year, reinforcing the need for longitudinal monitoring integrated into donor follow-up [139, 140].

In Portugal, psychosocial evaluation is mandatory before donation, and a one-year follow-up is formally recommended [146]. In practice, however, implementation remains inconsistent, and monitoring is often discontinued prematurely. Our results indicate that a single one-year assessment is insufficient: distress may persist or arise later, particularly in donors exposed to clinical complications or significant psychosocial stressors. This aligns with contemporary reviews showing generally preserved QoL with a substantive minority experiencing distress, and a notable lack of rigorously tested interventions to improve mid- to long-term psychosocial outcomes [140, 141]. A donor-centered model should therefore integrate systematic baseline screening for psychological vulnerabilities, structured exploration of relational and social context, and extended follow-up calibrated to evolving risk. Ensuring access to mental health professionals and organized peer support within transplant programs would facilitate early detection and timely intervention. Just as renal and cardiovascular outcomes are routinely monitored, psychosocial recovery deserves equivalent rigor. Safeguarding this dimension is integral to protecting donor autonomy, well-being, and the ethical integrity of living kidney donation. Importantly, donor well-being is also shaped by recipient outcomes, as satisfaction is often reinforced by graft success while complications may heighten distress [139, 141]. This interdependence sets the stage for Hypothesis Four, which examines how donor characteristics and matching strategies influence transplant results.

5.1.3.1. Hypothesis Three: Limitations

Both studies were single center with modest sample size, limiting statistical power and preventing robust subgroup analyses. Psychosocially vulnerable individuals may have self-excluded from donation, while qualitative narratives may over-represent positive experiences. Quantitative measures relied on generic psychometric tools (SF-36, HADS) rather than donor-specific scales, and the absence of a validated resilience instrument reduced granularity. The perceptions questionnaire was adapted from EULID [168] but was not externally validated, which may limit comparability with international studies. The follow-up duration was relatively short, restricting evaluation of long-term trajectories. Finally, as a

single center study in Portugal, generalizability may be limited, particularly in settings with different cultural or healthcare contexts.

Although a global perspective is provided later, these findings highlight the need for targeted psychosocial innovation. Future work should prioritize donor-specific psychometric tools, longitudinal follow-up embedded in national registries, and gender-sensitive approaches to identify vulnerable groups. These strategies would ensure that psychosocial safety is treated with the same rigor as renal and cardiometabolic outcomes.

In summary, Hypothesis Three demonstrates that donor safety must encompass psychosocial well-being alongside renal and cardiometabolic outcomes. Most donors report high satisfaction and preserved quality of life, but a vulnerable minority experience ambivalence, anxiety, or depressive symptoms, sometimes persisting beyond the first year. These findings establish psychosocial health as an essential dimension of donor safety, requiring structured evaluation and long-term monitoring.

Table 28 - Key Messages – Hypothesis Three: Psychosocial Health

Psychosocial health in living kidney donors can be comprehensively assessed through integrated qualitative and quantitative methods.

Domain	Key Points
Main Findings	• Donors reported high satisfaction, strong altruism, and minimal regret • Pre-donation concerns about surgery, transplant failure, and long-term health were frequent • SF-36 and HADS showed preserved overall QoL, but subgroup vulnerabilities emerged in donors with complications, stressful life events, • Baseline depressive symptoms predicted greater post-donation anxiety.
Integrative Framework	• Psychosocial outcomes interact with medical and social contexts. • Most donors remain resilient, but a relevant minority experience ambivalence, anxiety, or depressive symptoms • Psychosocial well-being is a core dimension of donor safety, alongside renal and CKM health.
Clinical & Ethical Implications	• Psychosocial assessment and follow-up should be systematic and extend beyond 1 year, along with renal and CKM monitoring • Counseling must openly address donor concerns and ambivalence. • Access to mental health professionals and peer support is essential for donor-centered care.
Limitations	• Single-center design with modest sample size limited statistical power • Short follow-up period limited assessment of long-term trajectories. • Use of generic psychometric tools (SF-36, HADS) did not capture donor-specific constructs (e.g., resilience, ambivalence). • Perceptions questionnaire was adapted from EULID but not externally validated, limiting comparability.

CKM, Cardio-Kidney-Metabolic; EULID, Euro Living Donor Project; HADS, Hospital Anxiety and Depression Scale; QoL, Quality of Life; SF-36 – Short Form Health Survey (36-item).

5.1.4. Hypothesis Four: Donor–Recipient Matching

Adequate matching can be achieved by integrating predictive tools, donor characteristics, and immunologic compatibility.

While the ethical justification for living kidney donation rests on recipient benefit, its long-term legitimacy depends fundamentally on safeguarding the donor. Recipient outcomes and donor safety are not competing but mutually reinforcing priorities. Achieving good recipient outcomes is not only essential for program viability but also strengthens donor reassurance and reduces the risk of regret or ambivalence, as shown in our psychosocial analyses.

As LDKT expands, increasingly heterogeneous donor profiles, including older individuals, those with borderline renal function, and genetically unrelated donors, have expanded access while introducing new clinical and ethical challenges [45, 46]. Addressing this complexity requires flexible frameworks that integrate predictive modeling, clinical donor characteristics, and immunologic compatibility.

In this context, Hypothesis Four examined donor–recipient matching through three complementary approaches: (1) external validation of the iPREDICTLIVING tool in a Portuguese cohort, (2) analysis of how donor age and baseline renal function interact in shaping graft survival, and (3) comparison of outcomes between related and unrelated donors. Together, these studies demonstrate that donor complexity cannot be reduced to rigid thresholds; rather, it requires individualized, ethically grounded assessment that balances donor protection with recipient benefit.

We first examined the role of predictive modeling through the external validation of the iPREDICTLIVING tool [227]. Developed in Norway, this European model uniquely estimates three outcomes in parallel: recipient mortality (10 years), graft loss (10 years), and donor mortality (10 and 20 years). By incorporating both donor (e.g., age, sex, BMI, blood pressure, baseline eGFR) and recipient variables (e.g., age, dialysis status, HLA mismatches, comorbidities), the model reflects the dual responsibility of safeguarding donor safety while maximizing recipient benefit.

Our Portuguese cohort, predominantly Caucasian and with low comorbidity, closely resembled the original derivation population, making it an appropriate setting for external validation [227].

In a cohort of 352 donor–recipient pairs, this was the first study to validate iPREDICTLIVING outside Norway, addressing a significant gap in the generalizability of predictive tools. The recipient mortality model showed strong discriminative ability (Harrell's $C = 0.776$) with excellent calibration, supporting its potential role in guiding long-term clinical decision-

making. The donor mortality model also performed moderately well (Harrell's $C = 0.712$), reinforcing its value for transparent communication of donor risks alongside recipient benefit, a balance central to ethically justified transplantation. By contrast, the graft loss model was less accurate (Harrell's $C = 0.665$), consistent with findings from the derivation cohort, and reflecting the fact that graft failure is strongly influenced by post-operative and immunological events not captured by baseline variables [85]. For counseling, these results mean that mortality predictions are reliable, but graft outcomes must be presented cautiously to avoid overstating prognostic certainty.

We also compared the performance of iPREDICTLIVING with the Living Kidney Donor Profile Index (LKDPI), a widely used US-derived score, and with several external validations. In our population, the LKDPI demonstrated similarly modest discrimination for death-censored graft failure, underscoring the inherent limitations of models based solely on pre-transplant characteristics [227]. However, unlike the LKDPI, iPREDICTLIVING integrates donor and recipient perspectives and was developed within a European context, offering a framework more aligned with national programs in Europe.

Importantly, predictive tools such as iPREDICTLIVING should be applied within their intended scope. The model was specifically developed to inform pre-transplant counseling by simultaneously estimating donor and recipient outcomes [85]. Although it was also proposed as a potential aid for KPE programs, its broader application in this setting has not yet been validated and should be approached with caution. The LKDPI has been applied to justify KPE participation when a more favorable donor is available [228]. Yet, a recent study by Irish et al. [229] using Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) and SRTR data showed that LKDPI does not reliably predict death-censored graft survival and should not be used to promote KPE among compatible pairs. By analogy, iPREDICTLIVING should remain primarily a counseling tool, and our study, as the first external validation in a Southern European population, supports its feasibility while reinforcing the need for careful, context-specific use.

To further explore how donor-related factors shape recipient prognosis, we analyzed baseline characteristics of 352 donor–recipient pairs in the same Portuguese cohort [230]. We evaluated baseline donor characteristics including age, pre-donation eGFR, and selected comorbidities. In our cohort, 23% of donors had eGFR <90 mL/min/1.73 m², and 41% were aged ≥ 50 years. Comorbidities such as hypertension (15%), dyslipidemia (13%), proteinuria (28%), smoking (16%), and obesity (9%) were also represented.

When assessed individually, most comorbidities, including hypertension, obesity, and proteinuria, were not found to be significantly associated with poorer recipient outcomes.

This finding likely reflects the stringent selection process and systematic exclusion of higher-risk donors.

By contrast, donor age and baseline kidney function emerged as the strongest determinants of graft survival. The relationship between eGFR and outcomes was strongly age-dependent: recipients of kidneys from younger donors with eGFR <90 mL/min/1.73 m² experienced more pronounced early declines in graft function, whereas similar kidney function values in older donors did not carry the same disadvantage. Longitudinal analyses reinforced this interaction, suggesting that “low-normal” kidney function has distinct prognostic significance in younger and older donors. In older individuals, it may reflect physiological decline rather than pathology.

Notably, this age-dependent interaction contrasts with our earlier analysis of donor renal trajectories [205], where younger donors with lower eGFR did not experience worse post-donation outcomes. This divergence underscores the asymmetry between donor and recipient perspectives: a kidney sufficient to preserve donor safety may nonetheless confer suboptimal prognosis for the recipient. Such findings highlight the need to evaluate donor complexity through a dual lens, balancing donor protection with recipient benefit.

These results carry important implications for donor evaluation and for nephrology more broadly. First, they question the rigid application of a fixed eGFR threshold (≥ 90 mL/min/1.73 m²), as currently recommended by KDIGO [18], and instead, support an age-adjusted interpretation of donor renal function, aligning more closely with British guidelines [19], which explicitly account for physiological aging. More generally, our findings underscore the limitations of static thresholds, not only in transplantation but also in CKD classification, where uniform cut-offs (<60 mL/min/1.73 m²) risk over diagnosing disease in older adults and underrecognizing early impairment in younger ones [231]. Living kidney donation thus provides a natural experiment exposing these limitations, while future integration of volumetric imaging [213], histology [36], and omics-based biomarkers [122] may help refine the biological meaning of “low-normal” eGFR across the age spectrum, ultimately improving both donor safety and graft outcome prediction.

Building on the interaction between donor age, baseline eGFR, and graft outcomes, we next examined whether another dimension of donor complexity, genetic relatedness to the recipient, shapes prognosis in living kidney transplantation. The question of whether related donors confer a biological advantage has long been debated: while living related donors (LRDs) may offer closer immunological compatibility, living unrelated donors (LURDs) expand access to transplantation but require robust evidence to confirm comparable

outcomes [197]. In Portugal, where unrelated donation has been legally permitted only since 2007 [232], this question holds particular relevance.

In our cohort of 198 donor–recipient pairs, we compared outcomes of LRD and LURD transplants over a ten-year period [233]. Baseline characteristics were broadly similar, though related pairs tended to have fewer HLA mismatches. Unadjusted analyses revealed higher acute rejection rates among LURD recipients (17% vs. 8%), but multivariable modeling demonstrated that HLA-DR mismatching, rather than genetic unrelatedness per se, was the main driver of rejection risk. Mismatches at HLA-A and HLA-B loci showed no significant effect. These findings align with international reports, confirming that unrelated donation can achieve excellent outcomes when immunological compatibility is adequately addressed [234–240].

Additional insights from this study further contextualize donor complexity. Recipients with obesity were at increased risk of rejection (HR = 1.179, $p = 0.013$), consistent with previous evidence linking obesity to heightened immunologic stress [241–243]. Higher rejection rates were also observed during earlier study years (2008–2012), reflecting evolving immunosuppressive strategies. Preemptive transplantation remained suboptimal, occurring in only 25% of cases, and at an even lower rate among LURDs (21%), underscoring a modifiable barrier to improved long-term outcomes [1].

Beyond classical antigen matching, molecular HLA assessment provides a more refined predictor of rejection risk. Our group has shown that class II eplet load better captures immunologic risk than antigen-level typing [184]. These insights support the expansion of unrelated donor programs, including KPE, when guided by advanced immunological tools. At our institution, KPE is now recommended for donor–recipient pairs with high HLA-DR mismatch or complete HLA mismatch as part of a proactive compatibility strategy.

The implications of relatedness also extend beyond the field of immunology. LRDs may carry unrecognized heritable risks, as familial clustering of CKD has been observed in 10–29% of cases across diverse etiologies [84, 244]. Such genetic predispositions may affect both long-term donor safety and graft durability [197]. This underscores the need for more precise genetic risk assessment to inform donor screening [78, 245]. To address this, our center has introduced a dedicated nephrogenetics consultation for families with clustering of CKD, ensuring ethical donor selection and tailored counseling. While LURDs may face distinct psychosocial or cultural challenges, they are not subject to these hereditary risks, highlighting the need for nuanced, individualized evaluation of both groups.

Together, the three studies presented under Hypothesis Four highlight that donor–recipient matching requires flexible, individualized, and ethically grounded strategies rather than rigid

thresholds. This principle provides the framework within which the following limitations should be interpreted.

5.1.4.1. Hypothesis Four, Limitations

While these studies provide novel insights, several limitations should be acknowledged. The retrospective, single-center design and predominantly Caucasian, low-risk donor–recipient population limit generalizability to more diverse or comorbid settings. The relatively small number of adverse events, especially donor mortality, constrains the precision of predictive modeling [246], and may overestimate performance in healthier cohorts. Reliance on creatinine-based eGFR (CKD-EPI) may have introduced misclassification in individuals with atypical body composition. In the age-eGFR study, donor health outcomes were assessed in separate analyses in the second study [205], which prevented simultaneous modeling. Most critically, the absence of systematic genetic evaluation among related donors with a family history of CKD limited the ability to fully assess hereditary risk. This represents a major limitation for donor safety and an urgent priority for integration into future screening protocols. These constraints call for cautious interpretation and underscore the need for larger, multicenter validations.

While the final chapter proposes an overarching framework, the limitations of this work also suggest clear avenues for future study. Validation of the iPREDICTLIVING model in broader, ethnically diverse cohorts, integration of donor-related variables into composite prediction tools, systematic incorporation of genetic data for donor risk stratification, and prospective comparison of related versus unrelated donation are essential. These steps would refine donor–recipient matching and strengthen evidence-based counseling.

In summary, Hypothesis Four demonstrates that donor–recipient matching can be optimized through integration of predictive tools, contextual evaluation of donor characteristics, and refined immunological assessment. The external validation of iPREDICTLIVING supports structured and transparent counseling, the age–eGFR interaction underscores the relevance of age-adapted criteria, and the analysis of related versus unrelated donors supports that immunological compatibility, rather than genetic relatedness, is the primary determinant of outcomes. Together, these findings reinforce that donor safety and recipient benefit are not competing priorities but complementary pillars of living donor kidney transplantation.

Table 29 - Key Messages – Hypothesis Four: Donor–Recipient Matching

Adequate matching can be achieved by integrating predictive tools, donor characteristics, and immunologic compatibility

Domain	Key Points
Main Findings	• iPREDICTLIVING was externally validated for the first time, showing strong predictive performance for recipient mortality and moderate utility for donor mortality and limited accuracy for graft loss. • Donor age and baseline eGFR interacted: “low-normal” eGFR conferred risk primarily in younger donors, while thresholds were less prognostic in older donors. • Genetic relatedness per se was not an independent determinant of outcomes; HLA-DR mismatching remained the critical immunological factor.
Integrative Framework	• Donor–recipient matching cannot be reduced to rigid thresholds but requires individualized, ethically grounded assessment. • Predictive models (iPREDICTLIVING) can provide structured, transparent counseling. • Age-adjusted interpretation of renal function refines donor selection beyond fixed eGFR cut-offs. • Immunological compatibility, not genetic relatedness, is the critical determinant of transplant outcomes, supporting safe expansion of LURD
Clinical & Ethical Implications	• Structured prediction models can improve transparency and counseling but should complement, not replace, clinical judgment. • Age- and context-adapted renal function assessment is preferable to rigid eGFR thresholds. • Expansion of LURD, including KPE, should be encouraged under robust immunological and ethical safeguards. • Systematic genetic screening should be considered for related donors when CKD clusters in families, as its absence remains a critical gap for donor safety.
Limitations	• Retrospective, single-center design and low number of adverse events limit generalizability and precision. • Cohort homogeneity (predominantly Caucasian, low comorbidity) may not reflect broader populations. • Absence of systematic genetic evaluation limited assessment of hereditary risk in related donors, representing a major gap in current donor safety protocols

eGFR – estimated Glomerular Filtration Rate; HLA – Human Leukocyte Antigen; KPE – Kidney Paired Exchange; LURD – Living Unrelated Donor

5.1.5. Ethical Reflections in Living Kidney Donation

While the preceding sections have discussed original empirical findings, the present section adopts a broader ethical perspective. Its aim is not to introduce new data but to interpret the implications of our results, considering internationally recognized ethical principles and guidelines. This reflection reinforces the thesis's central argument: living donor kidney transplantation (LDKT) must expand only in ethically defensible ways, guided by evidence-based metrics that protect donors while promoting access for recipients. As Joseph Murray observed in his 1990 Nobel lecture, "a normal healthy person was to be subjected to a major surgical operation, not for his own benefit" [15]. This paradox demands scrutiny beyond routine medical standards, placing donor safety at the center of principled evaluation.

Living kidney donation represents a deliberate tension with the principle *primum non nocere*. Transplant programs must therefore minimize harm while ensuring durable commitment to donor welfare. The findings of this thesis illustrate why this responsibility cannot be abstract: heterogeneity in renal adaptation, cardiometabolic vulnerability, and psychosocial outcomes demonstrate that risks are multifaceted and unevenly distributed. Ethical analysis must therefore move beyond generic assurances toward transparent, individualized counseling and structured long-term surveillance.

Autonomy requires that consent be informed by the best available evidence and interpreted within social contexts. Our data show that renal function, body mass index, and hypertension shape post-donation outcomes, and therefore must be integrated into counseling. Predictive tools may assist in this process, but they cannot replace individualized dialogue. Donor perceptions revealed both satisfaction and ambivalence, reflecting the influence of cultural and familial contexts [224]. In Portugal, where family ties strongly shape donation, ensuring voluntariness requires consistent, independent psychosocial assessments. Variability in how institutions conduct these assessments remains a concern, as it can undermine the robustness and equity of informed consent [247].

Beyond autonomy, the principles of non-maleficence and beneficence frame how risks and benefits must be balanced in LDKT. Donors face small but real perioperative and long-term risks, and responsibility also extends to those excluded from donation. Ineligible candidates may experience guilt or a sense of failure, underscoring that non-maleficence must encompass the entire evaluation process, not only the surgery itself [248]. At the same time, donor work-up can generate incidental benefits, such as early detection of silent diseases, which represents a form of beneficence that deserves recognition [172]. Tools such as iPREDICTLIVING may enhance transparency in risk communication, but their

limitations must be acknowledged to prevent fostering an illusion of certainty [227]. Transplant centers must also define clear boundaries for acceptable risk, beyond which donation should not proceed.

Justice demands equitable treatment and protection for all donors. Psychosocial findings in this thesis showed that vulnerability persists in a subset of donors, confirming the need for structured support systems. Gender disparities remain a particular concern: women are consistently more likely to donate yet less likely to receive a transplant, a trend also evident in Portugal [249, 250]. Addressing such imbalances requires clinician awareness, targeted education, and policy enforcement. Equitable access to psychosocial resources is essential not only for accepted donors but also for those who are declined.

Finally, beyond these principles, stewardship underscores the systemic duty to ensure that innovation in living donor transplantation remains anchored in donor safety and long-term sustainability and public trust. A major gap in Portugal is the absence of a national living donor registry, which limits transparency, long-term research, and robust counseling. Establishing such a registry is ethically imperative to strengthen donor protection and ensure lasting confidence in the system. These priorities are consistent with international frameworks, including the World Health Organization's 2024 Resolution (WHA77.4) [21] and the Forum of Amsterdam [17], which emphasize equity, transparency, and long-term donor care. Ethical vigilance is also required as innovative strategies such as kidney-paired exchange or ABO-incompatible transplantation expand. While these programs increase access, they may introduce subtle pressures or conditional altruism [251], underscoring the need for independent counseling and strong oversight.

In summary, the ethical legitimacy of living kidney donation rests on recipient benefit but is ultimately defined by donor protection. While good transplant outcomes may enhance meaning for donors, they can never justify compromising their safety. Evidence-based consent, equitable support, and sustained long-term surveillance are not optional: they are the foundation of the ethical future of LDKT.

Table 30 - Key Messages – *Ethical Reflections in Living Kidney Donation*

Ethical Principle	Key Issue Identified in Thesis	Practical Implication
Autonomy	Consent shaped by heterogeneous risks and family context;	Transparent, evidence-based informed consent
	Variability in psychosocial assessments.	Independent psychosocial evaluation. Recognition of relational influence
Beneficence / Non-maleficence	Small but real peri-operative and long-term risks;	Structured long-term medical and psychosocial follow-up;
	Exclusion may cause guilt or distress	Support for both accepted and declined donors;
	Evaluation may yield incidental health benefits;	Recognition of incidental health benefits;
	Predictive tools risk overstated certainty.	Cautious and transparent use of predictive models
Justice	Gender disparities (women donate more, receive less);	Policy enforcement and clinician awareness;
	Uneven access to psychosocial and follow-up resources	Equitable access to psychosocial and medical support.
Stewardship (systemic duty)	Absence of a national donor registry;	Establishment of a National Donor Registry
	Evolving innovations (KPE, ABO-incompatible transplantation) may create new pressures	Strong oversight of innovative programs;
	Alignment with WHO (WHA77.4) and Forum of Amsterdam standards.	

ABO, Blood group antigen system; KPE, Kidney paired exchange; WHA, World Health Assembly; WHO – World Health Organization

5.2. Conclusions

This thesis demonstrates that the safety of living kidney donors is best ensured through a multidimensional framework that integrates clinical, anatomical, cardiometabolic, psychosocial, and ethical dimensions, while accounting for the impact of donor characteristics on recipient outcomes.

Across four complementary hypotheses, this work illustrates how such a framework can be constructed. Renal functional trajectories, predictive modeling, and volumetry improve the precision of donor evaluation and help anticipate long-term reserve. Cardiovascular and metabolic risks, particularly hypertension and obesity, increasingly prevalent in contemporary donors, must be assessed dynamically, with exploratory metabolomic profiling offering early feasibility data that may yield insights into systemic vulnerability. Psychosocial findings demonstrate that donor well-being cannot be reduced to medical outcomes alone, but requires structured follow-up, validated tools, and recognition of both satisfaction and potential distress. Analyses of recipient outcomes, including the external validation of the iPREDICTLIVING tool, the influence of donor age and eGFR, and the finding that genetic relatedness exerts no independent effect once immunological factors are considered, highlight the interdependence of donor and recipient trajectories. Ultimately, while these results confirm that recipient benefit justifies living donor transplantation, its ethical legitimacy rests above all on the protection of donors and the assurance of their long-term safety.

Equally essential is the protection of donor autonomy through transparent, individualized communication of risks and ethically grounded informed consent. As the frontiers of living donation expand, such as the evolution of KPE with altruistic donation and the inclusion of compatible pairs, or through emerging approaches like biomarker-driven stratification, their implementation must be guided by oversight frameworks that safeguard voluntariness, prevent undue influence, and ensure sustained donor support.

This thesis advances a comprehensive, donor-centered model for safety and long-term care, integrating renal function metrics, anatomical imaging, CKM profiling, psychosocial risk, and predictive analytics into a coherent, evidence-based strategy. The retrospective design, single-center setting, and preliminary scope of omics analyses represent acknowledged limitations and constrain immediate generalizability. Nonetheless, the findings provide actionable insights to strengthen donor evaluation and follow-up, supporting safer, more transparent, and ethically grounded living donor programs in Portugal and beyond. The sustainability of living kidney donation will depend on advancing scientific precision, ethical responsibility, and donor-centered care together as one. Ultimately, the legitimacy of living

DISCUSSION AND CONCLUSIONS

kidney donation rests on a single principle: the hope it offers to recipients must always be matched by an uncompromising commitment to safeguard the well-being of those who give.

5.3. Future perspectives

Although each hypothesis suggested specific next steps, this chapter consolidates them into a unified framework for donor safety. The work presented in this thesis underscores the need for a multidimensional and forward-looking strategy to safeguard donors while enabling the responsible expansion of living kidney donation.

At the clinical and system level, the establishment of a national living donor registry should be prioritized. Integrated with the Portuguese Transplant Registry and aligned with European and WHO standards, it would fulfill both a scientific necessity and an ethical imperative. Such a registry would enable systematic long-term follow-up, risk reclassification, and equitable access to care. Donor surveillance should include standardized assessments of renal, cardiometabolic, and psychosocial health, supported by digital platforms to enhance engagement and continuity. Dedicated living donor or CKM clinics could provide the institutional framework for this surveillance, ensuring integrated follow-up that combines nephrology, cardiology, psychosocial, and genetic expertise. Sustainable funding, robust data integration, and long-term governance will be essential to ensure success, while policies must guarantee equity, financial neutrality, and universal access to psychosocial and medical follow-up.

A second priority is the advancement of predictive tools. Models such as the Toulouse–Ranguel score, CT volumetry, and the iPREDICTLIVING tool require validation in larger, more diverse cohorts. The Kidney Function Reduction Rate (KFRR) at six months, identified in this thesis as a potential marker of early renal adaptation, warrants further investigation before incorporation into predictive models. Over time, these parameters should be integrated into composite risk algorithms enabling dynamic reclassification of donor risk and informing tailored counseling, surgical planning, and follow-up.

A third priority is the systematic integration of nephrogenetics into donor evaluation. Given the current state of knowledge, donor safety in the next decade will only be adequately safeguarded if access to genetic counseling and diagnostic testing becomes standard practice. This must be embedded in structured protocols that systematically identify at-risk candidates and classify results into categories of eligibility, exclusion, or indeterminate risk to guide donor acceptance. Retrospective reanalysis of existing donor–recipient pairs, whenever ethically permissible—particularly in families with CKD clustering or early post-donation complications—could yield valuable insights and refine long-term surveillance. The principal challenge will be to ensure that genetic information is applied ethically and constructively, enhancing donor safety without introducing unjust barriers to donation, particularly in the context of variants of uncertain significance. Integrating genetics into

donor evaluation will therefore require not only technical capacity but also robust ethical frameworks, specialized counseling expertise, and supportive policy infrastructures.

A fourth area involves the management of cardiometabolic risk. Hypertension and obesity demand structured screening, tailored follow-up, and evaluation of targeted interventions. Dedicated CKM clinics or proactive surveillance programs would enable early detection and systematic management, combining lifestyle interventions, dietary counseling, and pharmacological strategies (such as ACE inhibitors and SGLT2 inhibitors). Embedding psychosocial support within these programs will ensure that long-term donor health is addressed holistically, reducing the burden of preventable complications.

Metabolomics and other biomarker approaches remain promising but exploratory. Pilot data from this thesis demonstrate feasibility, but larger prospective studies are required before these tools can mature into reliable clinical platforms. For now, metabolomics should be viewed as a research trajectory with potential to complement future predictive frameworks. Finally, ethical reflection and equity considerations must remain central. As living donation evolves through innovations such as paired exchange, altruistic-compatible donation, and genetic or biomarker-based stratification, policies must safeguard voluntariness, transparency, and fairness. Embedding psychosocial dimensions and gender-sensitive approaches within donor care will ensure that donor protection remains paramount. Future psychosocial research should rely on multicenter cohorts, the development of donor-specific psychometric tools, and longitudinal designs to clarify whether subclinical symptoms resolve or persist.

Taken together, these priorities define a coherent roadmap for the future of living kidney donation. Their implementation will depend on prospective, multicenter studies and sustained collaboration across nephrology, cardiology, surgery, genetics, and systems biology to ensure that innovation translates into practice. Maintaining a dynamic, donor-centered approach—where evaluation, follow-up, and policy evolve with emerging evidence—will be essential to preserve the sustainability of living donation while upholding the highest standards of care and ethical responsibility. Table 31 synthesizes these strategic priorities into an integrated framework encompassing clinical, predictive, genetic, preventive, and ethical dimensions, illustrating how donor protection depends on coordinated implementation across systems, technologies, and policy.

Table 31 - Key Messages – Future Perspectives in Living Kidney Donation

Domain	Strategic Priorities
Clinical & System Level	- Establish a national living donor registry integrated with the Portuguese Transplant Registry and EU and WHO platforms. - Create dedicated living donor or CKM clinics to provide structured, multidisciplinary long-term follow-up. - Implement systematic surveillance of renal, cardiometabolic, and psychosocial health, supported by digital platforms to enhance engagement and continuity. - Ensure equity and sustainability through financial neutrality, universal access to psychosocial and medical care, transparent governance, and stable funding mechanisms.
Predictive Tools	- Validate and refine models such as the Toulouse–Ranguel score, CT volumetry, and iPREDICTLIVING across diverse and multicenter cohorts. - Investigate the Kidney Function Reduction Rate as an early marker of adaptation before formal integration into predictive algorithms. - Develop composite, dynamic risk models that enable reclassification of donor profiles over time. - Incorporate predictive methods into clinical counseling, surgical planning, and long-term follow-up to improve precision and transparency.
Genetics	- Mandate access to genetic counseling and diagnostic testing as an essential component of donor evaluation - Integrate nephrogenetic assessment into standardized protocols that classify donor candidates into categories of eligibility, exclusion, or indeterminate risk, ensuring transparency and consistency in decision-making. - Reevaluate existing donor–recipient pairs retrospectively, when ethically permissible, to uncover hidden hereditary risks and guide surveillance. - Establish ethical and policy frameworks to ensure that genetic information strengthens donor safety without restricting equitable access.
Precision & Preventive Care	- Strengthen cardiometabolic surveillance with structured screening and early management of hypertension, obesity, and other modifiable risks. - Implement lifestyle, pharmacologic (ACE inhibitors, SGLT2 inhibitors), and psychosocial interventions to mitigate long-term complications. - Evaluate renal functional reserve testing with standardized challenges to better characterize adaptive capacity. - Advance biomarker research, including urinary metabolomics and other omics, through prospective validation before clinical application.
Ethics & Equity	- Safeguard voluntariness, transparency, and fairness as living donation evolves with paired exchange and genomic stratification. - Integrate psychosocial and gender-sensitive approaches throughout donor assessment and follow-up. - Develop and validate donor-specific psychometric tools in multicenter longitudinal studies. - Promote ethical disclosure and equitable application of CKM risk stratification in donor counseling and policy.

CKD, Chronic Kidney Disease; CT, Computed Tomography; EU, European; SGLT2 inhibitors, sodium–glucose cotransporter-2 inhibitors; WHO, World Health Organization

5.3.1.A Proposed Integrative Framework for Risk Stratification and Follow-up in Living Kidney Donors

The priorities outlined in this thesis converge within an Integrative Framework for Living Donor Safety, which synthesizes the clinical, metabolic, psychosocial, genetic, and ethical dimensions explored across the four hypotheses. Rather than a prescriptive algorithm, the framework provides a conceptual model to guide donor evaluation and long-term follow-up. It aims to improve individualized risk assessment, strengthen transparency in shared decision-making, adapt surveillance intensity to evolving risk, and support institutional and national policies aligned with international ethical standards.

The framework is organized around six interdependent domains. Renal Health represents the physiological foundation of donor safety, encompassing the assessment of renal function, reserve, and adaptive capacity. It integrates longitudinal eGFR trajectories, imaging-based volumetry as a surrogate of nephron mass, and predictive tools such as the Toulouse–Ranguel score to estimate post-donation function. Together, these approaches enable a more individualized evaluation of renal resilience and long-term reserve.

Cardiovascular–Kidney–Metabolic (CKM) Health addresses systemic vulnerability through the assessment and proactive management of obesity, hypertension, and metabolic factors. This domain underscores the importance of structured donor or CKM clinics to ensure coordinated screening, lifestyle guidance, and early treatment of post-donation hypertension or other modifiable risks.

Psychosocial Health encompasses donor well-being, resilience, and satisfaction, highlighting that emotional outcomes are integral to donor protection and require standardized, longitudinal follow-up supported by validated psychometric instruments.

The Immunological domain links donor–recipient compatibility with outcomes, emphasizing that immunologic matching remains a key determinant of transplant success. However, genetic relatedness, while often advantageous from an immunologic standpoint, may simultaneously confer a higher hereditary risk of kidney disease. This intersection highlights the need for integrated evaluation strategies in which immunologic and genetic assessments are interpreted together to ensure both donor safety and recipient benefit.

Genetics, as an emerging domain, represents a necessary evolution in donor evaluation. Growing evidence that inherited variants such as APOL1, COL4A, and cystic disease–related mutations may influence long-term outcomes underscores the need for systematic genetic counseling and targeted testing within structured protocols. When applied ethically and supported by expert interpretation, including careful management of variants of

uncertain significance, nephrogenetic assessment can strengthen donor safety without creating unjust barriers to donation.

Finally, Ethics functions as the cross-cutting foundation of the entire framework. It ensures that every clinical and scientific advancement is grounded in respect for autonomy, justice, and stewardship, guiding the responsible implementation of new technologies and the management of uncertainty.

Predictive models operate across all domains as integrative tools rather than separate components. Models such as the Toulouse–Ranguel score, the Kidney Function Reduction Rate, CT volumetry, and iPREDICTLIVING synthesize multidimensional data to inform personalized counseling, surgical planning, and post-donation surveillance. Their strength lies in facilitating structured, transparent decision-making that evolves alongside scientific evidence. However, these tools must be rigorously calibrated and validated in large, ethnically diverse, multicenter cohorts before they can be adopted as standardized components of donor evaluation.

Table 32 - Domains of the Integrative Framework for Living Kidney Donor Safety

Domain	Tools/Indicators	Purpose
Renal Health	eGFR trajectory, creatinine clearance, TR tool; <i>KFRR*</i> , <i>Renal reserve test* (investigational)</i> , CT volumetry, Grams ESRD risk calculator**	Predict renal reserve and adaptative capacity
Cardiovascular-Kidney-Metabolic Health	BMI, blood pressure, lipid profile, glucose, HgbA1c (<i>metabolomic profiling-investigational*</i>)	Detect systemic vulnerability and long-term metabolic stress;
Psychosocial Health	SF-36, HADS, donor perception, relational context; <i>Donor specific psychometric instruments*</i>	Detect mental health risk, resilience and emotional ambivalence
Immunological Risk***	HLA typing, epitope/epitope mismatch, <i>molecular mismatch algorithms</i>	Define compatibility, inform donor counseling, justify KPE
Emerging domain: Genetics****	Nephropathy gene panels APOL-1 (if applicable)	Assess hereditary CKD predisposition
Ethics (Cross-cutting)	Principles of autonomy, justice, informed consent, stewardship	Ensure ethical governance of donor selection, monitoring, and policy

Notes: Pregnancy-related and sex-specific risks were considered in the background as a distinct domain. They were not investigated in this thesis but remain integral to donor safety, particularly for younger female donors, and may be integrated alongside CKM and psychosocial domains in future applications of the framework.

** Investigational and requires validation before use*

***The Grams ESRD risk calculator for baseline non-donation risk [94], has not yet been validated for the Portuguese population.*

****Immunology is primarily recipient-oriented but impacts donor pathways*

*****Genetics was not studied here but is included as an emerging field.*

A central principle of this framework is that donor risk is dynamic, not static. It evolves with age, lifestyle, comorbidities, and emerging scientific knowledge. Continuous monitoring, supported by a national donor registry, periodic reassessment, multidisciplinary collaboration, and, ideally, facilitated through dedicated donor or CKM clinics, enables the reclassification of donor risk profiles over time. Novel biomarkers such as metabolomics and renal functional reserve testing remain promising but exploratory; their potential lies in complementing established methods once validated through larger studies.

In practice, donors can be conceptually grouped into lower-, intermediate-, and higher-risk categories based on the interplay of clinical, metabolic, psychosocial, and hereditary factors. Lower-risk donors, typically younger individuals with preserved renal reserve, optimal metabolic profiles, and no psychosocial vulnerabilities, may undergo standard surveillance. Intermediate-risk donors, such as those with overweight or mild obesity, controlled hypertension, or a family history of kidney disease, require tailored counseling, structured risk-factor management, and closer follow-up. Higher-risk donors, including those of advanced age, with reduced baseline eGFR, anatomical asymmetry, or psychosocial or hereditary vulnerabilities, warrant multidisciplinary evaluation, advanced testing, and more intensive monitoring. Pre-donation high-risk profiles may appropriately lead to exclusion to prevent harm, whereas post-donation high-risk profiles, such as the emergence of hypertension, metabolic syndrome, or accelerated GFR decline, should trigger reinforced surveillance and targeted interventions to preserve long-term health and well-being.

Figure 20 illustrates the principle of dynamic reclassification. The donor is centered and surrounded by six domains: renal, anatomical, CKM, psychosocial, immunological (indirect), and genetics (emerging). The exterior blue arrow represents dynamic reclassification. Predictive models act across all domains, synthesizing data for individualized risk assessment.

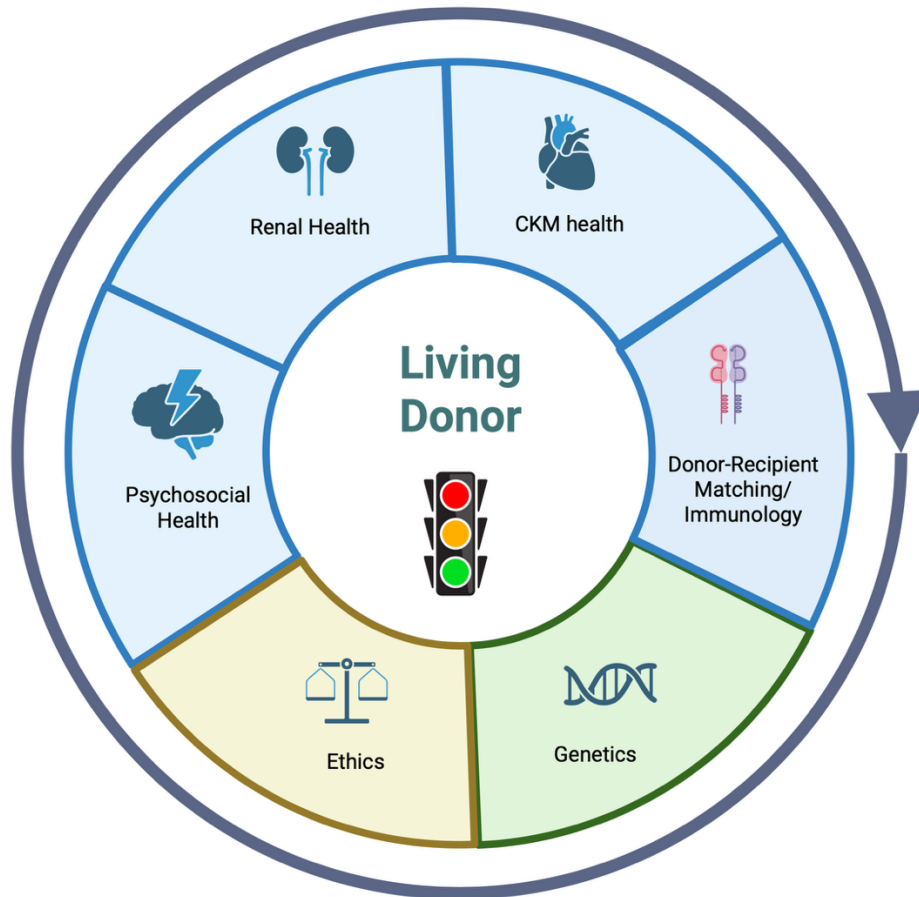


Figure 20. Integrative Framework for dynamic risk stratification and follow-up in living kidney donors.

Pregnancy-related and sex-specific risks, discussed in the background chapter, were not directly studied in this thesis but remain an important domain for comprehensive donor safety and should be incorporated into future adaptations of the framework.

This integrative framework embodies a dynamic and ethically grounded vision for living donor evaluation. By uniting renal, metabolic, psychosocial, immunological, and genetic insights within a continuous process of monitoring and ethical reflection, it provides a conceptual foundation for precision medicine in living kidney donation. Its ultimate value lies not in immediate implementation but in its potential to guide validation, multicenter collaboration, and policy development—advancing donor safety through multidimensional evaluation, continuous monitoring, and sustained ethical responsibility.

5.4. Final remarks

In an era when donor profiles are becoming increasingly complex, and eligibility boundaries continue to expand; the sustainability of living kidney donation cannot be secured by surgical success or recipient benefit alone. It requires a model rooted in personalized risk assessment, continuous monitoring, and unwavering ethical commitment to those who accept risk to benefit others.

This thesis demonstrates that donor safety can no longer be defined by static thresholds but requires a multidimensional framework that integrates renal functional trajectories, predictive modeling, anatomical imaging, cardiometabolic profiling, psychosocial well-being, and ethical responsibility. By generating the first long-term Portuguese data on donor trajectories, externally validating predictive tools, comparing CT volumetry with nuclear renography, exploring CKM vulnerability through obesity, hypertension and metabolomics, and documenting psychosocial experiences, this work advances the evidence base for both individualized counseling and system-level follow-up.

Its contribution lies not only in strengthening donor protection within Portugal but also in offering an integrative model with international relevance. Living donor kidney transplantation must remain grounded in the principle that the hope it provides to recipients is inseparable from the obligation to protect donors. This thesis redefines that obligation by embedding safety into every stage of donor evaluation and follow-up, providing a framework to guide programs nationally and globally toward a more ethical, transparent, and sustainable future. Only by making donor safety the foundation of practice can the generosity of living donors be fully honored, not only in the operating room, but across the entirety of their lives.

REFERENCES

REFERENCES

1. Meier-Kriesche, H.U. and B. Kaplan, *Waiting time on dialysis as the strongest modifiable risk factor for renal transplant outcomes: a paired donor kidney analysis*. Transplantation, 2002. **74**(10): p. 1377-81.
2. United States Renal Data System, *2023 USRDS Annual Data Report: Epidemiology of kidney disease in the United States*. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD.
3. Muzaale, A.D., et al., *Risk of end-stage renal disease following live kidney donation*. JAMA, 2014. **311**(6): p. 579-86.
4. Mjoen, G., et al., *Long-term risks for kidney donors*. Kidney Int, 2014. **86**(1): p. 162-7.
5. Liyanage, T., et al., *Worldwide access to treatment for end-stage kidney disease: a systematic review*. Lancet, 2015. **385**(9981): p. 1975-82.
6. Rafferty, Q., et al., *Global, regional, and national prevalence of kidney failure with replacement therapy and associated aetiologies, 1990-2023: a systematic analysis for the Global Burden of Disease Study 2023*. The Lancet Global Health, 2025. **13**(8): p. e1378-e1395.
7. Axelrod, D.A., et al., *An economic assessment of contemporary kidney transplant practice*. Am J Transplant, 2018. **18**(5): p. 1168-1176.
8. Wolfe, R.A., et al., *Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant*. N Engl J Med, 1999. **341**(23): p. 1725-30.
9. Rocha, M.J., et al., *Cost analysis of renal replacement therapy by transplant in a system of bundled payment of dialysis*. Clin Transplant, 2012. **26**(4): p. 529-31.
10. Park, Y.H., et al., *Comparison of survival probabilities for living-unrelated versus cadaveric renal transplant recipients*. Transplant Proc, 2004. **36**(7): p. 2020-2.
11. Gruessner, R.W.G. and A.C. Gruessner, *Solid-organ Transplants From Living Donors: Cumulative United States Experience on 140,156 Living Donor Transplants Over 28 Years*. Transplant Proc, 2018. **50**(10): p. 3025-3035.
12. Nefrologia, G.d.R.d.D.R.C.d.S.P.d. *Registo Nacional da Doença Renal Crónica - Gabinete de Registo da Doença Renal Crónica da Sociedade Portuguesa de Nefrologia*. 2023 [cited 2025 15th January 2025]; Available from: https://www.spnefro.pt/assets/relatorios/tratamento_doenca_terminal/er-2024---registo-atualizado.pdf.
13. Europe, E.C.o. *Newsletter Transplant - International figures on donation and transplantation* 2023. 2024.
14. Institute, D.a.T. *International Registry in Organ Donation and Transplantation*. 2024; Available from: https://www.irodat.org/img/database/pdf/IRODaT%20Newsletter%202024_preliminary%20aug%202025.pdf.
15. Organization, N.P. *The Nobel Prize in Physiology or Medicine 1990*. 1990; Available from: <https://www.nobelprize.org/prizes/medicine/1990/murray/25012-joseph-e-murray-nobel-lecture-1990/>.
16. Reese, P.P., N. Boudville, and A.X. Garg, *Living kidney donation: outcomes, ethics, and uncertainty*. Lancet, 2015. **385**(9981): p. 2003-13.
17. Delmonico, F. and S. Council of the Transplantation, *A Report of the Amsterdam Forum On the Care of the Live Kidney Donor: Data and Medical Guidelines*. Transplantation, 2005. **79**(6 Suppl): p. S53-66.

REFERENCES

18. Lentine, K.L., et al., *KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors*. Transplantation, 2017. **101**(8S Suppl 1): p. S1-S109.
19. Andrews, P.A. and L. Burnapp, *British Transplantation Society / Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: Summary of Updated Guidance*. Transplantation, 2018. **102**(7): p. e307.
20. Frutos, M.A., et al., *Living-donor kidney transplant: guidelines with updated evidence*. Nefrologia (Engl Ed), 2022. **42 Suppl 2**: p. 1-4.
21. *Increasing availability, ethical access and oversight of transplantation of human cells, tissues and organs*, in *January 24 2024*, W.H. Organization, Editor. 2024.
22. Hanson, C.S., et al., *Identifying Outcomes that Are Important to Living Kidney Donors: A Nominal Group Technique Study*. Clin J Am Soc Nephrol, 2018. **13**(6): p. 916-926.
23. Glasscock, R.J., *Evaluation of living donors: quo vadis for GFR criteria?* Kidney Int, 2019. **95**(4): p. 738-740.
24. Lujan, P.R., et al., *CKD-EPI instead of MDRD for candidates to kidney donation*. Transplantation, 2012. **94**(6): p. 637-41.
25. Gaillard, F., C. Legendre, and C.A. White, *GFR Assessment of Living Kidney Donors Candidates*. Transplantation, 2019. **103**(6): p. 1086-1093.
26. Delanaye, P., et al., *The new, race-free, Chronic Kidney Disease Epidemiology Consortium (CKD-EPI) equation to estimate glomerular filtration rate: is it applicable in Europe? A position statement by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)*. Clin Chem Lab Med, 2023. **61**(1): p. 44-47.
27. van der Weijden, J., et al., *Pre-donation assessment of cystatin C to improve prediction of pre- and post-donation GFR in potential living kidney donors*. Nephrol Dial Transplant, 2024. **39**(11): p. 1856-1866.
28. Garg, N., et al., *Performance of Creatinine Clearance and Estimated GFR in Assessing Kidney Function in Living Donor Candidates*. Transplantation, 2020. **104**(3): p. 575-582.
29. Ndumele, C.E., et al., *Cardiovascular-Kidney-Metabolic Health: A Presidential Advisory From the American Heart Association*. Circulation, 2023. **148**(20): p. 1606-1635.
30. Stevens, P.E., et al., *KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease*. Kidney International, 2024. **105**(4): p. S117-S314.
31. Figurek, A., V.A. Luyckx, and T.F. Mueller, *A Systematic Review of Renal Functional Reserve in Adult Living Kidney Donors*. Kidney Int Rep, 2020. **5**(4): p. 448-458.
32. van Londen, M., et al., *Renal functional reserve capacity before and after living kidney donation*. Am J Physiol Renal Physiol, 2018. **315**(6): p. F1550-F1554.
33. Gaillard, F., et al., *The age-calibrated measured glomerular filtration rate improves living kidney donation selection process*. Kidney Int, 2018. **94**(3): p. 616-624.
34. Huang, N., et al., *Estimated GFR for Living Kidney Donor Evaluation*. Am J Transplant, 2016. **16**(1): p. 171-80.
35. Gaillard, F., et al., *Estimated or Measured GFR in Living Kidney Donors Work-up?* Am J Transplant, 2016. **16**(10): p. 3024-3032.

36. Emmons, B.R., et al., *Association of Implantation Biopsy Findings in Living Donor Kidneys With Donor and Recipient Outcomes*. Am J Kidney Dis, 2024. **83**(3): p. 306-317.
37. Emmons, B.R., et al., *Age-related changes in nephrosclerosis in a multiethnic living kidney donor cohort*. Kidney Int, 2023. **104**(2): p. 394-397.
38. Issa, N., et al., *Kidney Structural Features from Living Donors Predict Graft Failure in the Recipient*. J Am Soc Nephrol, 2020. **31**(2): p. 415-423.
39. Yamauchi, J., I.E. Hall, and D. Raghavan, *Implantation Biopsy in Living-Donor Kidney Transplantation: Expectations, Utility, and Limitations*. Am J Kidney Dis, 2024. **83**(3): p. 291-292.
40. Luyckx, V.A. and B.M. Brenner, *The clinical importance of nephron mass*. J Am Soc Nephrol, 2010. **21**(6): p. 898-910.
41. Habbous, S., et al., *Can Split Renal Volume Assessment by Computed Tomography Replace Nuclear Split Renal Function in Living Kidney Donor Evaluations? A Systematic Review and Meta-Analysis*. Can J Kidney Health Dis, 2019. **6**: p. 2054358119875459.
42. Eum, S.H., et al., *Comparison of CT volumetry versus nuclear renography for predicting remaining kidney function after uninephrectomy in living kidney donors*. Sci Rep, 2022. **12**(1): p. 5144.
43. Nunes-Carneiro, D., et al., *Remaining kidney volume indexed to weight as a strong predictor of estimated glomerular filtration rate at 1 year and mid-term renal function after living-donor nephrectomy - a retrospective observational study*. Transpl Int, 2020. **33**(10): p. 1262-1273.
44. Silva, F., et al., *Lower donated kidney volume is associated with increased risk of lower graft function and acute rejection at 1 year after living donor kidney-a retrospective study*. Transpl Int, 2020. **33**(12): p. 1711-1722.
45. Reese, P.P., et al., *Creating a medical, ethical, and legal framework for complex living kidney donors*. Clin J Am Soc Nephrol, 2006. **1**(6): p. 1148-53.
46. Lafranca, J.A., et al., *Attitudes among transplant professionals regarding shifting paradigms in eligibility criteria for live kidney donation*. PLoS One, 2017. **12**(7): p. e0181846.
47. Ahmadi, A.R., et al., *Shifting paradigms in eligibility criteria for live kidney donation: a systematic review*. Kidney Int, 2015. **87**(1): p. 31-45.
48. Lentine, K.L., et al., *Summary of Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors*. Transplantation, 2017. **101**(8): p. 1783-1792.
49. Patel, K., et al., *Survival Advantage Comparing Older Living Donor Versus Standard Criteria Donor Kidney Transplants*. Transpl Int, 2024. **37**: p. 12559.
50. Gaillard, F., C. Fournier, and C. Legendre, *Lifetime ESKD risk stratification for living kidney donor studies*. Am J Transplant, 2019. **19**(9): p. 2658-2659.
51. Mohamed, M.M., et al., *Hypertension and obesity in living kidney donors*. World J Transplant, 2021. **11**(6): p. 180-186.
52. Locke, J.E., et al., *Obesity and long-term mortality risk among living kidney donors*. Surgery, 2019. **166**(2): p. 205-208.
53. Anjum, S., et al., *Patterns of End-Stage Renal Disease Caused by Diabetes, Hypertension, and Glomerulonephritis in Live Kidney Donors*. Am J Transplant, 2016. **16**(12): p. 3540-3547.

REFERENCES

54. Dropkin, B.M., et al., *The natural history of nonobstructing asymptomatic renal stones managed with active surveillance*. J Urol, 2015. **193**(4): p. 1265-9.
55. Burgher, A., et al., *Progression of nephrolithiasis: long-term outcomes with observation of asymptomatic calculi*. J Endourol, 2004. **18**(6): p. 534-9.
56. Sprott, P., et al., *Assessment of Restored Kidney Transplantation Including the Use of Wider Criteria for Accepting Renal Donors After Cancer Excision*. Transplant Direct, 2019. **5**(11): p. e498.
57. Lafranca, J.A., et al., *Vascular Multiplicity Should Not Be a Contra-Indication for Live Kidney Donation and Transplantation*. PLoS One, 2016. **11**(4): p. e0153460.
58. Fleetwood, V.A., N.N. Lam, and K.L. Lentine, *Long-Term Risks of Living Kidney Donation: State of the Evidence and Strategies to Resolve Knowledge Gaps*. Annu Rev Med, 2025. **76**(1): p. 357-372.
59. Ibrahim, H.N., et al., *Long-term consequences of kidney donation*. N Engl J Med, 2009. **360**(5): p. 459-69.
60. Matas, A.J. and A.D. Rule, *Long-term Medical Outcomes of Living Kidney Donors*. Mayo Clin Proc, 2022. **97**(11): p. 2107-2122.
61. Ibrahim, H.N., et al., *Intermediate Renal Outcomes, Kidney Failure, and Mortality in Obese Kidney Donors*. J Am Soc Nephrol, 2021. **32**(11): p. 2933-2947.
62. Grams, M.E., A.X. Garg, and K.L. Lentine, *Kidney-Failure Risk Projection for the Living Kidney-Donor Candidate*. N Engl J Med, 2016. **374**(21): p. 2094-5.
63. Grams, M.E., et al., *Kidney-Failure Risk Projection for the Living Kidney-Donor Candidate*. N Engl J Med, 2016. **374**(5): p. 411-21.
64. Mjoen, G., et al., *Morbidity and mortality in 1022 consecutive living donor nephrectomies: benefits of a living donor registry*. Transplantation, 2009. **88**(11): p. 1273-9.
65. Segev, D.L., et al., *Perioperative mortality and long-term survival following live kidney donation*. JAMA, 2010. **303**(10): p. 959-66.
66. Lentine, K.L., et al., *Perioperative Complications After Living Kidney Donation: A National Study*. Am J Transplant, 2016. **16**(6): p. 1848-57.
67. Massie, A.B., et al., *Thirty-Year Trends in Perioperative Mortality Risk for Living Kidney Donors*. JAMA, 2024. **332**(12): p. 1015-1017.
68. Fehrman-Ekholm, I., et al., *Kidney donors live longer*. Transplantation, 1997. **64**(7): p. 976-8.
69. Janki, S., et al., *Live kidney donation: are concerns about long-term safety justified?-A methodological review*. Eur J Epidemiol, 2017. **32**(2): p. 103-111.
70. O'Keefe, L.M., et al., *Mid- and Long-Term Health Risks in Living Kidney Donors: A Systematic Review and Meta-analysis*. Ann Intern Med, 2018. **168**(4): p. 276-284.
71. Park, J.Y., et al., *Long-term end-stage renal disease risks after living kidney donation: a systematic review and meta-analysis*. BMC Nephrol, 2023. **24**(1): p. 152.
72. Ibrahim, H.N., et al., *Renal Function Profile in White Kidney Donors: The First 4 Decades*. J Am Soc Nephrol, 2016. **27**(9): p. 2885-93.
73. Kido, R., et al., *How do living kidney donors develop end-stage renal disease?* Am J Transplant, 2009. **9**(11): p. 2514-9.
74. Matas, A.J., et al., *Causes and timing of end-stage renal disease after living kidney donation*. Am J Transplant, 2018. **18**(5): p. 1140-1150.

75. Cheng, X.S., et al., *Donation, Not Disease! A Multiple-Hit Hypothesis on Development of Post-Donation Kidney Disease*. *Curr Transplant Rep*, 2017. **4**(4): p. 320-326.
76. Doshi, M.D., et al., *APOL1 Genotype and Renal Function of Black Living Donors*. *J Am Soc Nephrol*, 2018. **29**(4): p. 1309-1316.
77. Mohan, S., et al., *APOL1 Genetic Testing in Living Kidney Transplant Donors*. *Am J Kidney Dis*, 2019. **74**(4): p. 538-543.
78. Thomas, C.P., et al., *Genetic evaluation of living kidney donor candidates: A review and recommendations for best practices*. *Am J Transplant*, 2023. **23**(5): p. 597-607.
79. Massie, A.B., et al., *Quantifying Postdonation Risk of ESRD in Living Kidney Donors*. *J Am Soc Nephrol*, 2017. **28**(9): p. 2749-2755.
80. Benoit, T., et al., *Predictive model of 1-year postoperative renal function after living donor nephrectomy*. *Int Urol Nephrol*, 2017. **49**(5): p. 793-801.
81. Almeida, M., et al., *External Validation of the Toulouse-Rangueil Predictive Model to Estimate Donor Renal Function After Living Donor Nephrectomy*. *Transpl Int*, 2023. **36**: p. 11151.
82. Kulik, U., et al., *External validation of a proposed prognostic model for the prediction of 1-year postoperative eGFR after living donor nephrectomy*. *Int Urol Nephrol*, 2017. **49**(11): p. 1937-1940.
83. Benoit, T., et al., *External Validation of a Predictive Model to Estimate Renal Function After Living Donor Nephrectomy*. *Transplantation*, 2021. **105**(11): p. 2445-2450.
84. Skrunes, R., et al., *Familial clustering of ESRD in the Norwegian population*. *Clin J Am Soc Nephrol*, 2014. **9**(10): p. 1692-700.
85. Haller, M.C., et al., *Predicting donor, recipient and graft survival in living donor kidney transplantation to inform pretransplant counselling: the donor and recipient linked iPREDICTLIVING tool - a retrospective study*. *Transpl Int*, 2020. **33**(7): p. 729-739.
86. Vock, D.M., et al., *The Minnesota attributable risk of kidney donation (MARKD) study: a retrospective cohort study of long-term (> 50 year) outcomes after kidney donation compared to well-matched healthy controls*. *BMC Nephrol*, 2023. **24**(1): p. 121.
87. Ndumele, C.E., et al., *A Synopsis of the Evidence for the Science and Clinical Management of Cardiovascular-Kidney-Metabolic (CKM) Syndrome: A Scientific Statement From the American Heart Association*. *Circulation*, 2023. **148**(20): p. 1636-1664.
88. Kanbay, M., et al., *Exploring the nexus: The place of kidney diseases within the cardiovascular-kidney-metabolic syndrome spectrum*. *Eur J Intern Med*, 2024. **127**: p. 1-14.
89. Joana Krättlia, D.B., Matthias Dieboldb, Caroline Wehmeierb, Daniel Sidlerc, Dela Golshayand, Tobias Zingge, Sophie de Seigneuxf, Fadi Haidarf, Isabelle Binetg, Alexander Ritterg, Kerstin Hübelhi, Fabian Rössleri, Jürg Steigerab, and P. Hirt-Minkowski, *Metabolic risk after living kidney donation: an analysis of the Swiss Organ Living-Donor Health Registry*. *Swiss Medical Weekly*, 2025.
90. Kanbay, M., et al., *Donor obesity and weight gain after transplantation: two still overlooked threats to long-term graft survival*. *Clin Kidney J*, 2023. **16**(2): p. 254-261.

REFERENCES

91. Ohashi, Y., et al., *Association of metabolic syndrome with kidney function and histology in living kidney donors*. Am J Transplant, 2013. **13**(9): p. 2342-51.
92. Locke, J.E., et al., *Obesity increases the risk of end-stage renal disease among living kidney donors*. Kidney Int, 2017. **91**(3): p. 699-703.
93. Bhathena, D.B., et al., *Focal sclerosis of hypertrophied glomeruli in solitary functioning kidneys of humans*. Am J Kidney Dis, 1985. **5**(5): p. 226-32.
94. Haylor, J., et al., *Renal function and morphometry in the dwarf rat following a reduction in renal mass*. Nephrol Dial Transplant, 1996. **11**(4): p. 643-50.
95. Boudville, N., et al., *Meta-analysis: risk for hypertension in living kidney donors*. Ann Intern Med, 2006. **145**(3): p. 185-96.
96. Lentine, K.L., et al., *Racial variation in medical outcomes among living kidney donors*. N Engl J Med, 2010. **363**(8): p. 724-32.
97. Lentine, K.L., et al., *Understanding antihypertensive medication use after living kidney donation through linked national registry and pharmacy claims data*. Am J Nephrol, 2014. **40**(2): p. 174-83.
98. Lentine, K.L., et al., *Postdonation eGFR and New-Onset Antihypertensive Medication Use After Living Kidney Donation*. Transplant Direct, 2019. **5**(8): p. e474.
99. Holscher, C.M., et al., *Self-Reported Incident Hypertension and Long-Term Kidney Function in Living Kidney Donors Compared with Healthy Nondonors*. Clin J Am Soc Nephrol, 2019. **14**(10): p. 1493-1499.
100. Garg, A.X., et al., *Hypertension and Kidney Function After Living Kidney Donation*. JAMA, 2024. **332**(4): p. 287-299.
101. Kasiske, B.L., et al., *A prospective controlled study of metabolic and physiologic effects of kidney donation suggests that donors retain stable kidney function over the first nine years*. Kidney Int, 2020. **98**(1): p. 168-175.
102. Helgeson, E.S., et al., *Development and Validation of a Hypertension Risk Calculator for Living Kidney Donors*. Transplantation, 2023. **107**(6): p. 1373-1379.
103. Go, A.S., et al., *Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization*. N Engl J Med, 2004. **351**(13): p. 1296-305.
104. Ferro, C.J. and J.N. Townsend, *Risk for subsequent hypertension and cardiovascular disease after living kidney donation: is it clinically relevant?* Clin Kidney J, 2022. **15**(4): p. 644-656.
105. Garg, A.X., et al., *Cardiovascular disease and hypertension risk in living kidney donors: an analysis of health administrative data in Ontario, Canada*. Transplantation, 2008. **86**(3): p. 399-406.
106. Garg, A.X., et al., *Cardiovascular disease in kidney donors: matched cohort study*. BMJ, 2012. **344**: p. e1203.
107. Janki, S., et al., *Long-term prognosis after kidney donation: a propensity score matched comparison of living donors and non-donors from two population cohorts*. Eur J Epidemiol, 2020. **35**(7): p. 699-707.
108. Chaudry, M., et al., *Hypertension, cardiovascular disease and cause of death in Danish living kidney donors: matched cohort study*. BMJ Open, 2020. **10**(11): p. e041122.
109. Grupper, A., et al., *Long term metabolic and renal outcomes of kidney donors compared to controls with excellent kidney function*. BMC Nephrol, 2019. **20**(1): p. 30.

110. Lentine, K.L., N.N. Lam, and D.L. Segev, *Risks of Living Kidney Donation: Current State of Knowledge on Outcomes Important to Donors*. Clin J Am Soc Nephrol, 2019. **14**(4): p. 597-608.
111. Kasiske, B.L., et al., *A prospective controlled study of living kidney donors: three-year follow-up*. Am J Kidney Dis, 2015. **66**(1): p. 114-24.
112. Lam, N.N., et al., *Gout after living kidney donation: a matched cohort study*. Am J Kidney Dis, 2015. **65**(6): p. 925-32.
113. Lam, N., et al., *Acute dialysis risk in living kidney donors*. Nephrol Dial Transplant, 2012. **27**(8): p. 3291-5.
114. Thomas, S.M., et al., *Risk of kidney stones with surgical intervention in living kidney donors*. Am J Transplant, 2013. **13**(11): p. 2935-44.
115. Thomas, S.M., et al., *Risk of serious gastrointestinal bleeding in living kidney donors*. Clin Transplant, 2014. **28**(5): p. 530-9.
116. Garg, A.X., et al., *Fracture risk in living kidney donors: a matched cohort study*. Am J Kidney Dis, 2012. **59**(6): p. 770-6.
117. Lenihan, C.R., et al., *Longitudinal study of living kidney donor glomerular dynamics after nephrectomy*. J Clin Invest, 2015. **125**(3): p. 1311-8.
118. Hostetter, T.H., et al., *Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation*. Am J Physiol, 1981. **241**(1): p. F85-93.
119. Braga, P.C., et al., *Mitochondrial Pathophysiology on Chronic Kidney Disease*. Int J Mol Sci, 2022. **23**(3).
120. Fiehn, O., *Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks*. Comp Funct Genomics, 2001. **2**(3): p. 155-68.
121. Graca, I.C.R., et al., *Understanding Hypertension: A Metabolomic Perspective*. Biology (Basel), 2025. **14**(4).
122. Pereira, P.R., et al., *Metabolomics as a tool for the early diagnosis and prognosis of diabetic kidney disease*. Med Res Rev, 2022. **42**(4): p. 1518-1544.
123. Zhang, B. and T. Schmidlin, *Recent advances in cardiovascular disease research driven by metabolomics technologies in the context of systems biology*. NPJ Metab Health Dis, 2024. **2**(1): p. 25.
124. Gagnebin, Y., et al., *Toward a better understanding of chronic kidney disease with complementary chromatographic methods hyphenated with mass spectrometry for improved polar metabolome coverage*. J Chromatogr B Analyt Technol Biomed Life Sci, 2019. **1116**: p. 9-18.
125. Grams, M.E., et al., *Metabolomic Alterations Associated with Cause of CKD*. Clin J Am Soc Nephrol, 2017. **12**(11): p. 1787-1794.
126. Gagnebin, Y., et al., *Combining the advantages of multilevel and orthogonal partial least squares data analysis for longitudinal metabolomics: Application to kidney transplantation*. Anal Chim Acta, 2020. **1099**: p. 26-38.
127. Stanimirova, I., et al., *Serum metabolomics approach to monitor the changes in metabolite profiles following renal transplantation*. Sci Rep, 2020. **10**(1): p. 17223.
128. Bohra, R., et al., *Proteomics and metabolomics in renal transplantation-quo vadis?* Transpl Int, 2013. **26**(3): p. 225-41.
129. Kuypers, D.R.J., et al., *Perspective: metabolomics has the potential to change the landscape of kidney transplantation diagnostics*. Biomark Med, 2024. **18**(17-18): p. 787-794.
130. Inoue, T., H. Maekawa, and R. Inagi, *Organelle crosstalk in the kidney*. Kidney Int, 2019. **95**(6): p. 1318-1325.

REFERENCES

131. Velenosi, T.J., et al., *Untargeted metabolomics reveals N, N, N-trimethyl-L-alanyl-L-proline betaine (TMAP) as a novel biomarker of kidney function*. Sci Rep, 2019. **9**(1): p. 6831.
132. Gonzalez-Martin, C., et al., *Podiatric Skin and Nail Involvement and Biomechanical Pathology in Renal Transplant Recipients: Assessment of the Foot as a Contributing Factor to Their Health*. Healthcare (Basel), 2023. **11**(23).
133. Reisaeter, A.V., et al., *Pregnancy and birth after kidney donation: the Norwegian experience*. Am J Transplant, 2009. **9**(4): p. 820-4.
134. Ibrahim, H.N., et al., *Pregnancy outcomes after kidney donation*. Am J Transplant, 2009. **9**(4): p. 825-34.
135. van Buren, M.C., et al., *Long-Term Kidney and Maternal Outcomes After Pregnancy in Living Kidney Donors*. Transpl Int, 2023. **36**: p. 11181.
136. Garg, A.X., et al., *Gestational hypertension and preeclampsia in living kidney donors*. N Engl J Med, 2015. **372**(15): p. 1469-70.
137. Pippias, M., et al., *Pregnancy after living kidney donation, a systematic review of the available evidence, and a review of the current guidance*. Am J Transplant, 2022. **22**(10): p. 2360-2380.
138. Francisco, J.T., et al., *Pregnancy After Kidney Donation: The Experience in a Cohort of Portuguese Living Donors*. Transplant Proc, 2023. **55**(6): p. 1373-1376.
139. Menjivar, A., et al., *Psychosocial risk factors for impaired health-related quality of life in living kidney donors: results from the ELIPSY prospective study*. Sci Rep, 2020. **10**(1): p. 21343.
140. Cazauvieilh, V., et al., *Psychological Impact of Living Kidney Donation: A Systematic Review by the EAU-YAU Kidney Transplant Working Group*. Transpl Int, 2023. **36**: p. 11827.
141. Massey, E.K., A.D. Rule, and A.J. Matas, *Living Kidney Donation: A Narrative Review of Mid- and Long-term Psychosocial Outcomes*. Transplantation, 2025. **109**(2): p. 259-272.
142. Massey, E.K., A.D. Rule, and A.J. Matas, *Living Kidney Donation: A Narrative Review of Mid- and Long-term Psychosocial Outcomes*. Transplantation, 2024.
143. Massey, E.K., et al., *The ELPAT living organ donor Psychosocial Assessment Tool (EPAT): from 'what' to 'how' of psychosocial screening - a pilot study*. Transpl Int, 2018. **31**(1): p. 56-70.
144. Lopes, A., et al., *Quality of life assessment in a living donor kidney transplantation program: evaluation of recipients and donors*. Transplant Proc, 2013. **45**(3): p. 1106-9.
145. Lopes, A., et al., *Depression and anxiety in living kidney donation: evaluation of donors and recipients*. Transplant Proc, 2011. **43**(1): p. 131-6.
146. Transplantação, I.P.d.S.e., *Protocolo de Avaliação Psicológica Pré e Pós Doação em Vida*, in 003/INF-IPSR, IP/18, IPST, Editor. 2018.
147. Armbrorst, D., et al., *Coping Strategies Influence Cardiometabolic Risk Factors in Chronic Psychological Stress: A Post Hoc Analysis of A Randomized Pilot Study*. Nutrients, 2021. **14**(1).
148. Fu, R., et al., *Economic Consequences of Adult Living Kidney Donation: A Systematic Review*. Value Health, 2021. **24**(4): p. 592-601.
149. Park, S., et al., *Economic Impact of Donating a Kidney on Living Donors: A Korean Cohort Study*. Am J Kidney Dis, 2022. **79**(2): p. 175-184 e1.

150. Barnieh, L., et al., *Living Kidney Donors' Financial Expenses and Mental Health. Transplantation*, 2021. **105**(6): p. 1356-1364.
151. Lentine, K.L., R.B. Mannon, and D. Mandelbrot, *Understanding and Overcoming Financial Risks for Living Organ Donors. Am J Kidney Dis*, 2022. **79**(2): p. 159-161.
152. Sieverdes, J.C., et al., *African American kidney transplant patients' perspectives on challenges in the living donation process. Prog Transplant*, 2015. **25**(2): p. 164-75.
153. Klarenbach, S., et al., *Economic consequences incurred by living kidney donors: a Canadian multi-center prospective study. Am J Transplant*, 2014. **14**(4): p. 916-22.
154. Saúde, M.d.S.-G.d.S.d.E.A.d.M.d., *Despacho n.º 2055/2015, in Despacho n.º 2055/2015, de 26 de fevereiro*, M.d.S.-G.d.S.d.E.A.d.M.d. Saúde, Editor. 2015: Diário da República.
155. Thiel, G.T., C. Nolte, and D. Tsinalis, *Prospective Swiss cohort study of living-kidney donors: study protocol. BMJ Open*, 2011. **1**(2): p. e000202.
156. Haugen, A.J., et al., *Increased risk of ischaemic heart disease after kidney donation. Nephrol Dial Transplant*, 2022. **37**(5): p. 928-936.
157. Clayton, P.A., et al., *Risk-Factor Profile of Living Kidney Donors: The Australia and New Zealand Dialysis and Transplant Living Kidney Donor Registry 2004-2012. Transplantation*, 2016. **100**(6): p. 1278-83.
158. UNION, T.E.P.A.T.C.O.T.E., *Directive 2010/53/EU of the European Parliament and of the Council of 7 July 2010 on standards of quality and safety of human organs intended for transplantation. 2010.*
159. Portuguesa, A.d.R., *Lei n.º 36/2013, de 12 de junho, REGIME DE GARANTIA DE QUALIDADE E SEGURANÇA DOS ÓRGÃOS DE ORIGEM HUMANA. Diário da República Portuguesa 2013.*
160. Pullen, L.C., *World Health Assembly resolution: A global path forward in transplantation. Am J Transplant*, 2024. **24**(9): p. 1521-1523.
161. Lentine, K.L., et al., *Expanding Opportunities for Living Donation: Recommendations From the 2023 Santander Summit to Ensure Donor Protections, Informed Decision Making, and Equitable Access. Transplantation*, 2025. **109**(1): p. 22-35.
162. Rodrigue, J.R., et al., *Evaluating living kidney donors: relationship types, psychosocial criteria, and consent processes at US transplant programs. Am J Transplant*, 2007. **7**(10): p. 2326-32.
163. Purnell, T.S., et al., *Association of Race and Ethnicity With Live Donor Kidney Transplantation in the United States From 1995 to 2014. JAMA*, 2018. **319**(1): p. 49-61.
164. Barnieh, L., et al., *A Scoping Review for Strategies to Increase Living Kidney Donation. Clin J Am Soc Nephrol*, 2017. **12**(9): p. 1518-1527.
165. Nogueira, D., M. Almeida, and A. Rodrigues, *Digital tools towards transplant first policy: Improving health literacy. Portuguese Journal of Nephrology & Hypertension*, 2022. **35**(4): p. 216-218.
166. Yoo, H.J., et al., *Attitudes and psychological characteristics of kidney donors toward organ donation. Transplant Proc*, 1996. **28**(3): p. 1932-3.
167. Tong, A., et al., *The motivations and experiences of living kidney donors: a thematic synthesis. Am J Kidney Dis*, 2012. **60**(1): p. 15-26.

REFERENCES

168. Manyalich, M., et al., *EULID project: European living donation and public health*. Transplant Proc, 2009. **41**(6): p. 2021-4.
169. Verissimo, R., et al., *Why Donors Do Not Donate: A Living Donation Program Experience*. Transplant Proc, 2022. **54**(5): p. 1197-1201.
170. Kasiske, B.L., et al., *Outcomes of Living Kidney Donor Candidate Evaluations in the Living Donor Collective Pilot Registry*. Transplant Direct, 2021. **7**(5): p. e689.
171. Muhlbacher, T., et al., *Living kidney donor evaluation is associated with early identification of life-changing diagnoses in potentially healthy donor candidates*. Clin Transplant, 2023. **37**(1): p. e14810.
172. Park, S.C., et al., *The living donor evaluation as a life-saving event*. Clin Transplant, 2023. **37**(1): p. e14885.
173. Graham, J.M. and A.E. Courtney, *The Adoption of a One-Day Donor Assessment Model in a Living Kidney Donor Transplant Program: A Quality Improvement Project*. Am J Kidney Dis, 2018. **71**(2): p. 209-215.
174. Scurt, F.G., et al., *Clinical outcomes after ABO-incompatible renal transplantation: a systematic review and meta-analysis*. Lancet, 2019. **393**(10185): p. 2059-2072.
175. de Weerd, A.E. and M.G.H. Betjes, *ABO-Incompatible Kidney Transplant Outcomes: A Meta-Analysis*. Clin J Am Soc Nephrol, 2018. **13**(8): p. 1234-1243.
176. Orandi, B.J., et al., *Survival Benefit with Kidney Transplants from HLA-Incompatible Live Donors*. N Engl J Med, 2016. **374**(10): p. 940-50.
177. Manook, M., et al., *Post-listing survival for highly sensitised patients on the UK kidney transplant waiting list: a matched cohort analysis*. Lancet, 2017. **389**(10070): p. 727-734.
178. Rapaport, F.T., *The case for a living emotionally related international kidney donor exchange registry*. Transplant Proc, 1986. **18**(3) Suppl. 2): p. 5-9.
179. Rees, M.A., et al., *A nonsimultaneous, extended, altruistic-donor chain*. N Engl J Med, 2009. **360**(11): p. 1096-101.
180. Kranenburg, L.W., et al., *Starting a crossover kidney transplantation program in the Netherlands: ethical and psychological considerations*. Transplantation, 2004. **78**(2): p. 194-7.
181. Rees, M.A., et al., *Kidney Exchange to Overcome Financial Barriers to Kidney Transplantation*. Am J Transplant, 2017. **17**(3): p. 782-790.
182. Roth, A.E., et al., *Global kidney exchange should expand wisely*. Transpl Int, 2020. **33**(9): p. 985-988.
183. Lo, A.L., E.M. Sonnenberg, and P.L. Abt, *Evolving swaps in transplantation: global exchange, vouchers, liver, and trans-organ paired exchange*. Curr Opin Organ Transplant, 2019. **24**(2): p. 161-166.
184. Tafulo, S., et al., *Degree of HLA class II eplet mismatch load improves prediction of antibody-mediated rejection in living donor kidney transplantation*. Hum Immunol, 2019. **80**(12): p. 966-975.
185. Gentry, S.E., et al., *Expanding kidney paired donation through participation by compatible pairs*. Am J Transplant, 2007. **7**(10): p. 2361-70.
186. Transplantação, I.P.d.S.e., *Programa Nacional de Doação Renal Cruzada (PNDRC) e Programa Internacional de Doação Renal Cruzada (PIDRC)*, in 001 / CN / 2022, I.P.d.S.e. Transplantação, Editor. 2022.
187. Garg, N., et al., *Practical and ethical considerations in kidney paired donation and emerging liver paired exchange*. Am J Transplant, 2025.

188. Martinelli, V., et al., *Ethical Issues in Living Donor Kidney Transplantation: An Update from a Psychosocial Perspective*. Healthcare (Basel), 2024. **12**(18).
189. Gill, J.S., et al., *Variation in Dialysis Exposure Prior to Nonpreemptive Living Donor Kidney Transplantation in the United States and Its Association With Allograft Outcomes*. Am J Kidney Dis, 2018. **71**(5): p. 636-647.
190. Matas, A.J., et al., *Nondirected donation of kidneys from living donors*. N Engl J Med, 2000. **343**(6): p. 433-6.
191. Matas, A.J., et al., *2202 kidney transplant recipients with 10 years of graft function: what happens next?* Am J Transplant, 2008. **8**(11): p. 2410-9.
192. Boenink, R., et al., *The ERA Registry Annual Report 2022: Epidemiology of Kidney Replacement Therapy in Europe, with a focus on sex comparisons*. Clin Kidney J, 2025. **18**(2): p. sf405.
193. Lentine, K.L., et al., *OPTN/SRTR 2022 Annual Data Report: Kidney*. Am J Transplant, 2024. **24**(2S1): p. S19-S118.
194. Legendre, C., G. Canaud, and F. Martinez, *Factors influencing long-term outcome after kidney transplantation*. Transpl Int, 2014. **27**(1): p. 19-27.
195. Yohanna, S., et al., *A Propensity Score-weighted Comparison of Outcomes Between Living and Standard Criteria Deceased Donor Kidney Transplant Recipients*. Transplantation, 2020. **104**(11): p. e317-e327.
196. Bellini, M.I., et al., *How good is a living donor? Systematic review and meta-analysis of the effect of donor demographics on post kidney transplant outcomes*. J Nephrol, 2022. **35**(3): p. 807-820.
197. Husain, S.A., et al., *Association Between Donor-Recipient Biological Relationship and Allograft Outcomes After Living Donor Kidney Transplant*. JAMA Netw Open, 2021. **4**(4): p. e215718.
198. Massie, A.B., et al., *A Risk Index for Living Donor Kidney Transplantation*. Am J Transplant, 2016. **16**(7): p. 2077-84.
199. Ashby, V.B., et al., *A Kidney Graft Survival Calculator that Accounts for Mismatches in Age, Sex, HLA, and Body Size*. Clin J Am Soc Nephrol, 2017. **12**(7): p. 1148-1160.
200. Haller, M.C., et al., *Prediction models for living organ transplantation are poorly developed, reported, and validated: a systematic review*. J Clin Epidemiol, 2022. **145**: p. 126-135.
201. Pereira, M., et al., *Incidence of hypertension in a prospective cohort study of adults from Porto, Portugal*. BMC Cardiovasc Disord, 2012. **12**: p. 114.
202. Ferreira, P.L., *[Development of the Portuguese version of MOS SF-36. Part I. Cultural and linguistic adaptation]*. Acta Med Port, 2000. **13**(1-2): p. 55-66.
203. Pecoraro, A., et al., *Estimating Postoperative Renal Function After Surgery for Nonmetastatic Renal Masses: A Systematic Review of Available Prediction Models*. Eur Urol Oncol, 2023. **6**(2): p. 137-147.
204. Capitanio, U., et al., *Hypertension and Cardiovascular Morbidity Following Surgery for Kidney Cancer*. Eur Urol Oncol, 2020. **3**(2): p. 209-215.
205. Almeida, M., et al., *Longitudinal Trajectories of Estimated Glomerular Filtration Rate in a European Population of Living Kidney Donors*. Transplant International, 2024. **37**.
206. Lam, N.N., et al., *Changes in kidney function follow living donor nephrectomy*. Kidney Int, 2020. **98**(1): p. 176-186.

REFERENCES

207. Kim, Y., et al., *Insufficient early renal recovery and progression to subsequent chronic kidney disease in living kidney donors*. Korean J Intern Med, 2022. **37**(5): p. 1021-1030.
208. van der Weijden, J., et al., *Early increase in single-kidney glomerular filtration rate after living kidney donation predicts long-term kidney function*. Kidney Int, 2022. **101**(6): p. 1251-1259.
209. Augustine, J.J., et al., *Factors Associated With Residual Kidney Function and Proteinuria After Living Kidney Donation in the United States*. Transplantation, 2021. **105**(2): p. 372-381.
210. Massie, A.B., et al., *Association of Early Postdonation Renal Function With Subsequent Risk of End-Stage Renal Disease in Living Kidney Donors*. JAMA Surg, 2020. **155**(3): p. e195472.
211. Patel, S.S., et al., *External Validation of Toulouse-Rangueil eGFR12 Prediction Model After Living Donor Nephrectomy*. Transpl Int, 2023. **36**: p. 11619.
212. Hiba Ghabi, R.E., Syrine Tlili et al., *External Validation of the Toulouse–Rangueil Model in Tunisian Living Kidney Donors*,. Research Square, 2025. **Preprint**.
213. Almeida, M., et al., *CT volumetry performs better than nuclear renography in predicting estimated renal function one year after living donation*. Int Urol Nephrol, 2023. **55**(3): p. 553-562.
214. Halleck, F., et al., *Volume matters: CT-based renal cortex volume measurement in the evaluation of living kidney donors*. Transpl Int, 2013. **26**(12): p. 1208-16.
215. Wahba, R., et al., *Computed Tomography Volumetry in Preoperative Living Kidney Donor Assessment for Prediction of Split Renal Function*. Transplantation, 2016. **100**(6): p. 1270-7.
216. Barbas, A.S., et al., *CT volumetry is superior to nuclear renography for prediction of residual kidney function in living donors*. Clin Transplant, 2016. **30**(9): p. 1028-35.
217. Mitsui, Y., et al., *The assessment of renal cortex and parenchymal volume using automated CT volumetry for predicting renal function after donor nephrectomy*. Clin Exp Nephrol, 2018. **22**(2): p. 453-458.
218. Lopez-Abad, A., et al., *Can CT or MRI volumetry substitute scintigraphy in living kidney donor evaluation? A systematic review*. World J Urol, 2024. **42**(1): p. 382.
219. Observatory, G.O. *World Obesity*. 2024 [cited 2024 26 April 2024]; Available from: <https://data.worldobesity.org/country/portugal-174/>.
220. Cunha, A., et al., *Prevalence of Obesity After Living Kidney Donation and Associated Risk Factors: Cardiovascular and Renal Implications*. Journal of Clinical Medicine, 2025. **14**(18): p. 6411.
221. Kielstein, J.T., et al., *SDMA is an early marker of change in GFR after living-related kidney donation*. Nephrol Dial Transplant, 2011. **26**(1): p. 324-8.
222. Cunha, A., et al., *Metabolic Profile of Living Kidney Donors: A One-Year Analysis*. Transplant Proc, 2025.
223. Gross, C.R., et al., *Health-related quality of life in kidney donors from the last five decades: results from the RELIVE study*. Am J Transplant, 2013. **13**(11): p. 2924-34.
224. Almeida, M., et al., *Living Kidney Donor Perceptions About Organ Donation: Who are They, and How Do They Feel About the Process?* Transplant Proc, 2025.
225. Almeida, M., et al., *Psychological Outcomes in a Cohort of Portuguese Living Kidney Donors*. Transplant Proc, 2025.

226. Kondrat, A., *One Patient, No Good Options: The Real Roots of Ambivalence in Medical Decision Making*. Am J Bioeth, 2022. **22**(6): p. 48-50.
227. Almeida, M., et al., *Clinical performance of the iPREDICTLIVING tool for the prediction of the post-transplant recipient and living donor outcomes in a European cohort*. Clin Transplant, 2024. **38**(3): p. e15283.
228. Basu, A., et al., *Ten Years of Kidney Paired Donation at Mayo Clinic: The Benefits of Incorporating ABO/HLA Compatible Pairs*. Transplantation, 2020. **104**(6): p. 1229-1238.
229. Irish, G.L., et al., *The living kidney donor profile index fails to discriminate allograft survival: implications for its use in kidney paired donation programs*. Am J Transplant, 2023. **23**(2): p. 232-238.
230. Almeida, M., et al., *Living Donors' Age Modifies the Impact of Pre-Donation Estimated Glomerular Filtration Rate on Graft Survival*. J Clin Med, 2023. **12**(21).
231. Levin, A. and P.E. Stevens, *Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward*. Kidney Int, 2014. **85**(1): p. 49-61.
232. República, A.d., *Colheita e transplante de órgãos ,tecidos e células de origem humana*, in *Lei n.º 22/2007, de 29 de junho*. 2007: Diário da República.
233. Reis Pereira, P., et al., *Biologically Unrelated Living Donor for Kidney Transplantation Associated With Higher Acute Rejection Rate but With Similar Graft Survival of a Related Living Donor*. Cureus, 2022. **14**(10): p. e30189.
234. Taylor, G.S., et al., *Living unrelated donor renal transplantation: a single center experience*. J Urol, 2005. **174**(1): p. 223-5.
235. Simforoosh, N., et al., *Living unrelated versus living related kidney transplantation: 20 years' experience with 2155 cases*. Transplant Proc, 2006. **38**(2): p. 422-5.
236. Simforoosh, N., et al., *Living Unrelated Versus Related Kidney Transplantation: A 25-Year Experience with 3716 Cases*. Urol J, 2016. **13**(1): p. 2546-51.
237. Ahmad, N., et al., *Living-unrelated donor renal transplantation: an alternative to living-related donor transplantation?* Ann R Coll Surg Engl, 2008. **90**(3): p. 247-50.
238. Kizilisik, A.T., et al., *Living donor kidney transplantation in a Veterans Administration medical center*. Am J Surg, 2004. **188**(5): p. 611-3.
239. Matter, Y.E., et al., *Impact of Donor Source on the Outcome of Live Donor Kidney Transplantation: A Single Center Experience*. Nephrourol Mon, 2016. **8**(3): p. e34770.
240. Santori, G., et al., *Kidney transplantation from living donors genetically related or unrelated to the recipients: a single-center analysis*. Transplant Proc, 2012. **44**(7): p. 1892-6.
241. Serna-Higueta, L.M., et al., *Impact of different immunosuppressive protocols on clinical outcomes in obese kidney transplant recipients: a propensity score-matched analysis*. Nephrol Dial Transplant, 2023. **38**(9): p. 2052-2066.
242. Scheuermann, U., et al., *Recipient obesity as a risk factor in kidney transplantation*. BMC Nephrol, 2022. **23**(1): p. 37.
243. Hill, C.J., et al., *Recipient obesity and outcomes after kidney transplantation: a systematic review and meta-analysis*. Nephrol Dial Transplant, 2015. **30**(8): p. 1403-11.
244. Connaughton, D.M., et al., *The Irish Kidney Gene Project--Prevalence of Family History in Patients with Kidney Disease in Ireland*. Nephron, 2015. **130**(4): p. 293-301.

REFERENCES

245. Caliskan, Y., et al., *Evaluation of Genetic Kidney Diseases in Living Donor Kidney Transplantation: Towards Precision Genomic Medicine in Donor Risk Assessment*. Curr Transplant Rep, 2022. **9**(2): p. 127-142.
246. Collins, G.S., E.O. Ogundimu, and D.G. Altman, *Sample size considerations for the external validation of a multivariable prognostic model: a resampling study*. Stat Med, 2016. **35**(2): p. 214-26.
247. Kortram, K., et al., *The need for a standardized informed consent procedure in live donor nephrectomy: a systematic review*. Transplantation, 2014. **98**(11): p. 1134-43.
248. Allen, M.B., P.L. Abt, and P.P. Reese, *What are the harms of refusing to allow living kidney donation? An expanded view of risks and benefits*. Am J Transplant, 2014. **14**(3): p. 531-7.
249. Katz-Greenberg, G. and S. Shah, *Sex and Gender Differences in Kidney Transplantation*. Semin Nephrol, 2022. **42**(2): p. 219-229.
250. Carrero, J.J., et al., *Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease*. Nat Rev Nephrol, 2018. **14**(3): p. 151-164.
251. IPST, *Programa Nacional de Doação Renal Cruzada (PNDRC) e Programa Internacional de Doação Renal Cruzada (PIDRC)*. in 001 / CN / 2022. 2022.

APPENDICES

APPENDIX 1 – Longitudinal Trajectories of Estimated Glomerular Filtration Rate in a European Population of Living Kidney Donors

Almeida, M., Pereira, PR.; Silvano, J.; Ribeiro, C.; Pedroso, S.; Tafulo, S; Martins, LS; Ramos MS., and Malheiro,J.

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Longitudinal Trajectories of Estimated Glomerular Filtration Rate in a European Population of Living Kidney Donors

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A living donor (LD) kidney transplant is the best treatment for kidney failure, but LDs safety is paramount. We sought to evaluate our LDs cohort's longitudinal changes in estimated glomerular filtration rate (eGFR). We retrospectively studied 320 LDs submitted to nephrectomy between 1998 and 2020. The primary outcome was the eGFR change until 15 years (y) post-donation. Subgroup analysis considered distinct donor characteristics and kidney function reduction rate (%KFRR) post-donation $[-(eGFR_{6\text{ months}(M)} - eGFR_{\text{pre-donation}}) / eGFR_{\text{pre-donation}} * 100]$. Donors had a mean age of 47.3 ± 10.5 years, 71% female. Overall, LDs presented an average eGFR change 6 M onward of $+0.35 \text{ mL/min/1.73 m}^2/\text{year}$. The period with the highest increase was 6 M–2 Y, with a mean eGFR change of $+0.85 \text{ mL/min/1.73 m}^2/\text{year}$. Recovery plateaued at 10 years. Normal weight donors presented significantly better recovery of eGFR $+0.59 \text{ mL/min/1.73 m}^2/\text{year}$, compared to obese donors $-0.18 \text{ mL/min/1.73 m}^2/\text{year}$ ($p = 0.020$). Noteworthy, these results only hold for the first 5 years. The subgroup with a lower KFRR ($<26.2\%$) had a significantly higher decrease in eGFR overall of $-0.21 \text{ mL/min/1.73 m}^2/\text{year}$ compared to the groups with higher KFRR ($p < 0.001$). These differences only hold for 6 M–2 Y. Moreover, an eGFR $<50 \text{ mL/min/1.73 m}^2$ was a rare event, with $\leq 5\%$ prevalence in the 2–15 Y span, correlating with eGFR pre-donation. Our data show that eGFR recovery is significant and may last until 10 years post-donation. However, some subgroups presented more ominous kidney function trajectories.

Keywords: kidney transplant, living donor, estimated glomerular filtration rate, living donor characteristics, estimated glomerular filtration rate trajectories

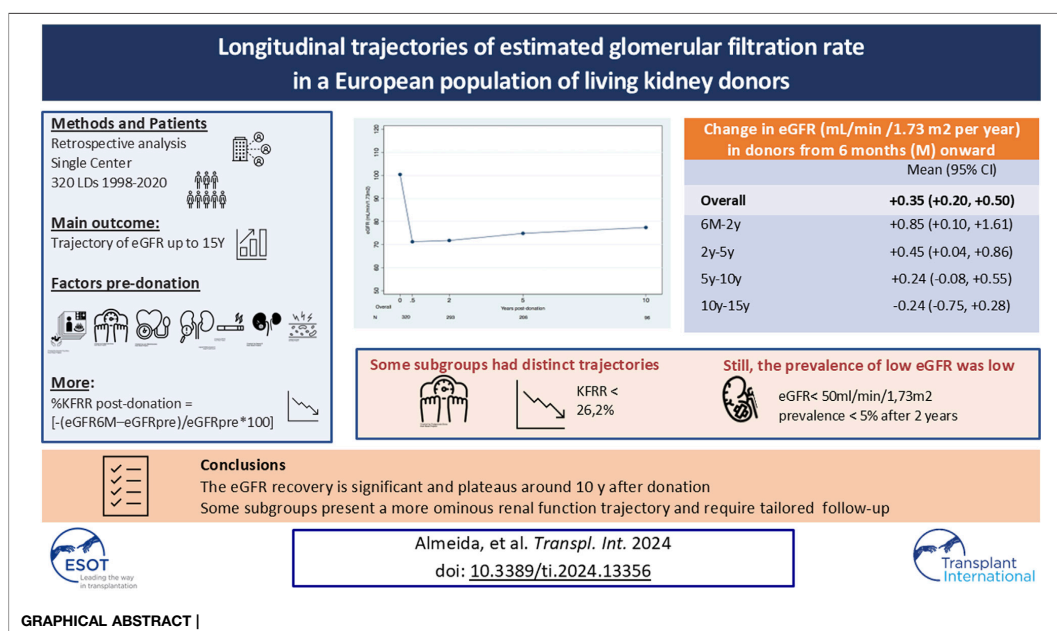
INTRODUCTION

A living donor (LD) kidney transplant (KT) is the best treatment for end-stage renal disease (ESRD) [1–3]. LDKT increases organ availability, decreases time on the waiting list, allows preemptive transplantation, and improves graft and patient survival with lower healthcare costs [1–5]. Although the perceived risks for the donors are considered low and ethically acceptable [3], two landmark studies showed an increased risk of ESRD in donors compared with matched

(kg/m²)

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Longitudinal Changes eGFR Living Donors



healthy non-donors, albeit the absolute risk was minimal [6, 7]. Subgroups with a higher risk of ESRD have been identified [8, 9], but post-donation kidney function evolution and the mechanisms involved in the hyperfiltration after donation are less well characterized [10–14]. Furthermore, due to organ scarcity, we are increasingly accepting donors with borderline abnormalities that were previously declined [15]. Long-term follow-up data are scarce.

For guiding clinical practice, it would be desirable to foresee the evolution of kidney function after nephrectomy in each LD and evaluate if it changes differently according to baseline donor characteristics and the kidney function reduction rate (KFRR) 6 months post-donation. We also investigated the prevalence of low eGFR (<50 mL/min/1.73 m²) in different donor subgroups and proteinuria after donation. We hypothesize that identifying different patterns of eGFR change after donation could signal

groups of LDs that would benefit from a better risk assessment and customized preventive care.

PATIENTS AND METHODS

We retrospectively reviewed the clinical data of all adult LDs submitted to nephrectomy at our center between January 1998 and January 2020 (n = 364). Inclusion criteria were serum creatinine (Scr) evaluation at 6 months and at least 3 Scr evaluations at follow-up (31 LKDs without Scr evaluations at 6 months and 13 without at least 3 evaluations at follow-up were excluded from the analysis, further details of non-included donors are available as **Supplementary Material**). The remaining 320 LKDs defined our studied cohort.

The Institutional Review Board at Unidade Local de Saúde de Santo António (ULSdSA) approved this retrospective observational study, which was conducted according to the Helsinki Declaration.

Donor Variables

Following international guidelines [16, 17], all donors were subjected to a standard evaluation protocol. Baseline demographic, anthropometric, analytical, and clinical data were collected from the LDs. Hypertension was defined by blood pressure in the consultation >140/90 mmHg, ABPM >135/85 mmHg, and past hypertension or antihypertensive medication. Uncontrolled hypertension or evidence of end-organ damage were criteria of exclusion.

TABLE 1 | Baseline characteristics of the study cohort.

	n = 320
Age (years), Mean ± SD	47.3 ± 10.5
Age (years), n (%)	
<40	81 (25)
40–55	154 (48)
≥55	85 (27)
Sex F:M, n (%)	227 (71):93(29)
BMI kg/m ² , Mean ± SD	25.3 ± 3.3
BMI kg/m ² , n (%)	
<25	155 (48)
25–30	132 (41)
≥30	33 (10)
Smoking habits, n (%)	48 (15)
Hypertension, n (%)	51 (16)
Dyslipidemia, n (%)	44 (14)
ProtU 0.15–0.5 g/g, n (%)	96 (30)
Pre-donation SCr mg/dL, Mean ± SD	0.75 ± 0.16
Pre-donation eGFR mL/min/1.73 m ² , Mean ± SD	100.4 ± 14.6
Pre-donation eGFR mL/min/1.73 m ² , n (%)	
<80	29 (9)
80–90	48 (15)
≥90	243 (76)
Number of SCr measurements, Median (IQR) [min. max.]	7 (5–11) [3,16]
% kidney function reduction rate (KFRR) post-donation*, Median (IQR)	31.9 (22.6–38.1)
% KFRR post-donation, n (%)	
<26.2	106 (33)
26.2–36.1	107 (33)
>36.1	107 (33)

*KFRR post-donation = $[-(eGFR_{6M} - eGFR_{pre-donation}) / eGFR_{pre-donation}] * 100$. SD, standard deviation; n, number; F, female; M, male; BMI, Body Mass Index; ProtU, protein/creatinine ratio in the urine; ProtU 0.15–0.5 g/g, a ratio protein/creatinine of 0.15–0.5 g/g in a urinary sample; SCr, Serum creatinine; eGFR, estimated glomerular filtration rate; IQR, interquartile range; KFRR, kidney function reduction rate.

TABLE 2 | Change in eGFR (mL/min/1.73 m²/year) in 320 donors from 6 months onward.

	Mean (95% CI)
Overall	+0.35 (+0.20, +0.50)
Linear spline model	
6M–2y	+0.85 (+0.10, +1.61)
2y–5y	+0.45 (+0.04, +0.86)
5y–10y	+0.24 (–0.08, +0.55)
10y–15y	–0.24 (–0.75, +0.28)

CI, confidence interval; M, months; y, years; eGFR, estimated glomerular filtration rate.

Potential donors with a history of malignancy, obesity, or diabetes were excluded. Serum creatinine-based CKD-Epidemiology Collaboration (CKD-EPI equation) [18] was used to predict eGFR. Although a lower limit of eGFR was not established by Unit protocol, potential donors with eGFR below 80 mL/min/1.73 m² were usually discarded. Upon urinary analysis, proteinuria was defined by a random urine protein/creatinine ratio of 0.15–0.5 g/g [19] and was confirmed by determination using a 24-h urinary sample. Donors with confirmed proteinuria over 300 mg/day were discarded [20, 21]. The final approval for kidney donation was reviewed in a

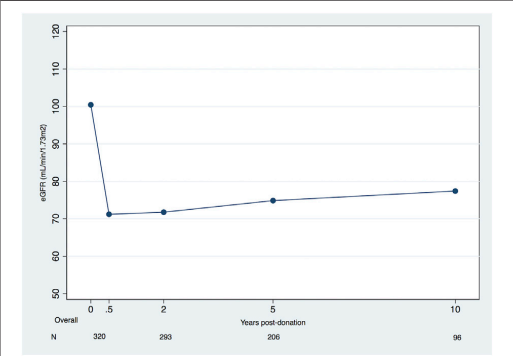


FIGURE 1 | Mean eGFR (mL/min/1.73 m²) in living kidney donors pre-donation and during post-donation follow-up (eGFR, estimated glomerular filtration rate).

multidisciplinary meeting, and ethical approval was mandatory.

The date of nephrectomy was defined as the beginning of follow-up. All donors have lifetime annual follow-up appointments.

Outcomes

The primary outcome was the change in eGFR until 15 years post-donation (mL/min/1.73m²/per year), using all available eGFR measurements from 6 months after the nephrectomy onward. Donors were followed from the nephrectomy date until one of the following occurred: death, ESRD, attaining 15-year follow-up, or end-of-study period (31 December 2022). We performed additional analyses to examine the effects of various characteristics on the progression of eGFR over time in living kidney donors presenting at the time of donation that could be associated with the recovery of kidney function after donation [22–25], including demographic and clinical data. Further, the kidney function reduction rate (%KFRR) post-donation $[-(eGFR_{6months(M)}_{post-donation} - eGFR_{pre-donation}) / eGFR_{pre-donation} * 100]$ in the remnant kidney in the first 6 months after donation was considered in the analysis, bearing in mind it was a variable available only after donation.

Statistical Analysis

Continuous data were described using mean and standard deviation (SD) or median (interquartile range [IQR]), and categorical data were expressed as numbers (and percentages). Categorical data were compared using Pearson chi-square test or Fisher exact test, and continuous variables were compared with Student's t-test or Mann–Whitney U-test.

Subgroup analysis considered the following donor characteristics: age, sex, obesity, diagnosis of hypertension, smoking habits, proteinuria, and pre-donation eGFR category; these same variables were included in the multivariable model. Differently, %KFRR post-donation as an independent predictor

TABLE 3 | Changes in eGFR (mL/min/1.73 m²/year) in living kidney donors (n = 320) by subgroup over different periods during follow-up from 6 weeks onward (univariate analysis).

	Overall	Linear spline model			
		6M–2y	2y–5y	5y–10y	10–15y
Age (years)					
<40	+0.39 (+0.11, +0.68) ^A	+0.09 (−1.44, +1.62) ^A	+0.97 (+0.14, +1.80) ^A	+0.10 (−0.51, 0.72) ^A	−0.04 (−0.94, +0.87) ^A
40–55	+0.34 (+0.13, +0.55) ^A	+1.03 (−0.05, +2.11) ^A	+0.36 (−0.22, +0.95) ^A	+0.30 (−0.14, +0.74) ^A	−0.41 (−1.14, +0.32) ^A
≥ 55	+0.52 (+0.21, +0.82) ^A	+1.39 (−0.07, +2.84) ^A	+0.33 (−0.46, +1.13) ^A	+0.47 (−0.20, +1.14) ^A	+0.12 (−1.08, +1.32) ^A
<i>p</i>	<i>0.642</i>	<i>0.457</i>	<i>0.445</i>	<i>0.73</i>	<i>0.698</i>
Sex					
Male	+0.54 (+0.25, +0.82)	+0.08 (−1.34, +1.50)	+0.79 (+0.00, +1.58)	+0.36 (−0.25, +0.98)	+0.86 (−0.14, +1.85)
Female	+0.28 (+0.10, +0.45)	+1.16 (+0.27, +2.04)	+0.34 (−0.14, +0.82)	+0.20 (−0.17, +0.56)	−0.65 (−1.24, −0.05)
<i>p</i>	<i>0.124</i>	<i>0.209</i>	<i>0.335</i>	<i>0.653</i>	0.011
BMI (kg/m ²) ^a					
<25	+0.50 (+0.28, +0.71) ^A	+0.46 (−0.62, +1.54) ^A	+0.96 (+0.37, +1.54) ^A	+0.17 (−0.30, +0.64) ^A	+0.01 (−0.85, +0.86) ^A
25–30	+0.32 (+0.10, +0.54) ^A	+2.23 (+1.06, +3.41) ^B	−0.29 (−0.92, +0.34) ^B	+0.50 (+0.05, +0.96) ^A	−0.49 (−1.20, +0.22) ^A
≥30	−0.01 (−0.50, +0.47) ^A	−2.52 (−4.82, −0.22) ^C	+1.28 (+0.02, +2.55) ^A	−0.51 (−1.60, +0.58) ^A	+0.08 (−1.44, +1.60) ^A
<i>p</i>	<i>0.143</i>	0.001	0.007	<i>0.205</i>	<i>0.616</i>
Hypertension					
No	+0.33 (+0.17, +0.49)	+0.95 (+0.12, +1.77)	+0.35 (−0.10, +0.79)	+0.30 (−0.04, +0.64)	−0.37 (−0.91, +0.18)
Yes	+0.53 (+0.13, +0.93)	+0.42 (−1.47, +2.31)	+1.05 (+0.03, +2.08)	−0.09 (−0.97, +0.79)	+0.83 (−0.69, +2.35)
<i>p</i>	<i>0.365</i>	<i>0.616</i>	<i>0.215</i>	<i>0.413</i>	<i>0.148</i>
Smoking habits					
No	+0.32 (+0.15, +0.48)	+1.26 (+0.45, +2.08)	+0.22 (−0.23, +0.66)	+0.28 (−0.05, +0.62)	−0.44 (−1.00, +0.13)
Yes	+0.58 (+0.19, +0.97)	−1.52 (−3.48, +0.43)	+1.92 (+0.84, +3.00)	+0.02 (−0.84, +0.87)	+0.65 (−0.57, +1.87)
<i>p</i>	<i>0.213</i>	0.01	0.004	<i>0.567</i>	<i>0.114</i>
Dyslipidemia					
No	+0.33 (+0.17, +0.49)	+0.82 (+0.00, +1.63)	+0.46 (+0.02, +0.91)	+0.22 (−0.12, +0.55)	−0.31 (−0.87, +0.24)
Yes	+0.50 (+0.08, +0.92)	+1.20 (−0.79, +3.20)	+0.41 (−0.70, +1.52)	+0.42 (−0.49, +1.32)	+0.21 (−1.22, +1.63)
<i>p</i>	<i>0.459</i>	<i>0.724</i>	<i>0.928</i>	<i>0.682</i>	<i>0.504</i>
ProtU 0.15–0.5 g/g					
No	+0.32 (+0.15, +0.49)	+1.20 (+0.30, +2.11)	+0.22 (−0.26, +0.71)	+0.30 (−0.05, +0.65)	−0.26 (−0.83, +0.30)
Yes	+0.44 (+0.14, 0.75)	+0.06 (−1.31, +1.42)	+1.01 (+0.25, +1.77)	+0.05 (−0.67, +0.77)	−0.17 (−1.42, +1.09)
<i>p</i>	<i>0.502</i>	<i>0.17</i>	<i>0.086</i>	<i>0.541</i>	<i>0.891</i>
Pre-donation eGFR (mL/min/1.73 m ²)					
<80	−0.06 (−0.61, +0.48) ^A	+2.84 (+0.31, +5.37) ^A	−0.06 (−1.58, +1.46) ^A	−0.88 (−2.08, +0.32) ^A	−0.75 (−2.23, +0.73) ^A
80–90	+0.47 (+0.10, +0.85) ^A	+2.15 (+0.24, +4.06) ^A	−0.43 (−1.48, +0.62) ^A	+0.48 (−0.32, +1.27) ^A	+1.38 (+0.15, +2.61) ^B
≥90	+0.38 (+0.21, +0.55) ^A	+0.37 (−0.49, +1.24) ^A	+0.70 (+0.24, +1.16) ^A	+0.32 (−0.03, +0.68) ^A	−0.54 (−1.15, +0.07) ^A
<i>p</i>	<i>0.256</i>	<i>0.07</i>	<i>0.122</i>	<i>0.146</i>	0.018
%KFR post-donation*, **					
<26.2	−0.12 (−0.34, +0.10) ^B	−2.89 (−4.19, −1.59) ^A	+0.59 (−0.08, +1.26) ^A	−0.01 (−0.48, +0.46) ^A	−0.24 (−0.90, +0.43) ^A
26.2–36.1	+0.62 (+0.36, +0.87) ^A	+1.54 (+0.24, +2.83) ^B	+0.88 (+0.17, +1.59) ^A	+0.25 (−0.30, +0.80) ^A	−0.29 (−1.36, +0.79) ^A
>36.1	+0.75 (+0.48, +1.02) ^A	+3.77 (+2.50, +5.04) ^C	+0.05 (−0.66, +0.76) ^A	+0.61 (+0.02, +1.20) ^A	−0.56 (−1.67, +0.55) ^A
<i>p</i>	<0.001	<0.001	<i>0.262</i>	<i>0.273</i>	<i>0.885</i>

^aIn variables with 3 or more groups, each box will present letters A to C (superscript). It should be concluded that subgroups that share the same letters in the same box are non-significantly different.

^{**}KFR post-donation = $[-(eGFR_{6M} - eGFR_{pre-donation})/eGFR_{pre-donation} \times 100]$.

y, years; M, months; BMI, body mass index; ProtU, protein/creatinine ratio in the urine; eGFR, estimated glomerular filtration rate; KFR, kidney function reduction rate. p values are depicted in italics; statistically significant p values (<.05) are shown in bold.

was evaluated separately, adjusted to the aforementioned donor characteristics pre-donation.

Donor eGFR change between 6 months and 15 years post-donation was assessed by univariate and multivariable linear mixed regression model that imputed subject-specific random effects (intercept and slope defined as eGFR at 6-month and time in years, respectively) on an unstructured covariance matrix. The Bonferroni test was used to correct multiple significance tests. The dependent variable was all eGFR measurements, and the independent variables were entered as 2-way interaction terms between them and the time (in years) variable. Additionally, distinct temporal trends of eGFR change were sought by imputing time as a linear spline with knots at 2, 5, and 10 years.

Statistical calculations were performed using STATA/MP, version 15.1 (Stata Corp, College Station, TX, United States). A 2-sided *P*-value <0.05 was considered as statistically significant.

RESULTS

Baseline Characteristics

The baseline characteristics for our study cohort are summarized in **Table 1**. The mean age of the population was 47.3 ± 10.5 years, and most were female (71%). The representation in the race of donors was nearly exclusively Caucasian. Most donors were either overweight

TABLE 4 | Changes in eGFR (mL/min/1.73 m²/year) in living kidney donors (n = 320) by subgroup over different periods during follow-up from 6 months onward (multivariable analysis).

	Overall	Linear spline model			
		6mo–2y	2y–5y	5y–10y	10–15y
Age* (years)					
<40	+0.36 (+0.07, +0.65) ^A	+0.66 (−0.94, 2.26) ^A	+0.69 (−0.18, +1.56) ^A	+0.05 (−0.61, +0.71) ^A	+0.08 (−1.03, +1.18) ^A
40–55	+0.41 (+0.21, +0.62) ^A	+1.18 (+0.08, +2.27) ^A	+0.42 (−0.17, +1.01) ^A	+0.36 (+0.08, +0.81) ^A	−0.47 (−1.31, +0.38) ^A
≥55	+0.44 (+0.12, +0.75) ^A	+0.96 (−0.59, +2.51) ^A	+0.45 (−0.39, +1.30) ^A	+0.40 (−0.34, +1.14) ^A	+0.07 (−1.51, +1.36) ^A
<i>p</i>	<i>0.932</i>	<i>0.87</i>	<i>0.879</i>	<i>0.706</i>	<i>0.727</i>
Sex					
Male	+0.61 (+0.31, +0.91)	+0.57 (−0.91, +2.05)	+0.60 (−0.23, +1.43)	+0.68 (−0.01, +1.37)	+0.53 (−0.93, +2.00)
Female	+0.33 (+0.16, +0.50)	+1.15 (+0.24, +2.06)	+0.46 (−0.03, +0.95)	+0.15 (−0.25, +0.55)	−0.52 (−1.24, +0.21)
<i>p</i>	<i>0.128</i>	<i>0.522</i>	<i>0.787</i>	<i>0.222</i>	<i>0.242</i>
BMI* (kg/m ²)					
<25	+0.59 (+0.37, +0.80) ^B	+0.67 (−0.44, +1.77) ^{AB}	+0.97 (+0.36, +1.57) ^B	+0.32 (−0.17, +0.81) ^A	−0.11 (−1.14, +0.91) ^A
25–30	+0.35 (−0.14, +0.56) ^{AB}	+2.17 (+0.98, +3.36) ^B	−0.17 (−0.81, +0.46) ^A	+0.49 (+0.02, +0.95) ^A	−0.26 (−1.01, +0.48) ^A
≥30	−0.18 (−0.68, +0.31) ^A	−2.47 (−4.92, −0.03) ^A	+1.18 (−0.17, +2.53) ^{AB}	−0.64 (−1.82, +0.54) ^A	−0.62 (−2.39, +1.16) ^A
<i>p</i>	0.02	0.002	0.021	<i>0.214</i>	<i>0.897</i>
Hypertension					
No	+0.37 (+0.21, +0.53)	+1.07 (+0.23, +1.90)	+0.34 (−0.11, +0.80)	+0.37 (+0.01, +0.73)	−0.22 (−0.88, +0.44)
Yes	+0.60 (+0.19, +1.01)	+0.58 (−1.48, +2.64)	+1.36 (+0.23, +2.48)	−0.12 (−1.08, +0.83)	−0.29 (−1.17, +1.59)
<i>p</i>	<i>0.313</i>	<i>0.672</i>	<i>0.11</i>	<i>0.361</i>	<i>0.946</i>
Smoking					
No	+0.41 (+0.25, +0.56)	+1.31 (+0.48, +2.14)	+0.30 (−0.15, +0.75)	+0.40 (+0.04, +0.75)	−0.30 (−1.01, +0.42)
Yes	+0.40 (−0.01, +0.82)	−0.86 (−2.93, +1.20)	+1.66 (+0.48, +2.83)	−0.31 (−1.32, +0.70)	+0.16 (−1.47, +1.78)
<i>p</i>	<i>0.985</i>	<i>0.059</i>	0.039	<i>0.211</i>	<i>0.648</i>
Dyslipidemia					
No	+0.37 (+0.22, +0.53)	+0.92 (+0.10, +1.75)	+0.51 (+0.06, +0.95)	+0.27 (−0.08, +0.62)	−0.30 (−0.96, +0.36)
Yes	+0.61 (+0.19, +1.04)	+1.45 (−0.72, +3.62)	+0.43 (−0.78, +1.64)	+0.46 (−0.52, +1.43)	+0.24 (−1.54, +2.03)
<i>p</i>	<i>0.311</i>	<i>0.661</i>	<i>0.904</i>	<i>0.725</i>	<i>0.585</i>
ProtU					
0.15–0.5 g/g	+0.39 (+0.23, +0.55)	+1.28 (+0.38, +2.20)	+0.32 (−0.16, +0.81)	+0.37 (+0.01, +0.72)	−0.24 (−0.88, +0.41)
No	+0.46 (+0.16, 0.76)	+0.19 (−1.19, +1.56)	+0.99 (+0.22, +1.75)	+0.08 (−0.65, +0.82)	−0.21 (−1.61, +1.18)
Yes					
<i>p</i>	<i>0.674</i>	<i>0.193</i>	<i>0.152</i>	<i>0.504</i>	<i>0.975</i>
Predonation eGFR* (mL/min/1.73m ²)					
<80	−0.09 (−0.63, +0.44) ^A	+2.85 (+0.24, +5.46) ^A	+0.14 (−1.41, +1.69) ^A	−1.16 (−2.39, +0.06) ^B	−0.44 (−2.03, +1.15) ^{AB}
80–90	+0.52 (+0.16, +0.88) ^A	+2.32 (+0.34, +4.29) ^A	−0.33 (−1.41, +0.75) ^A	+0.51 (−0.31, +1.32) ^A	+1.62 (+0.23, +3.01) ^B
≥90	+0.43 (+0.27, +0.60) ^A	+0.54 (−0.34, +1.41) ^A	+0.70 (+0.23, +1.17) ^A	+0.40 (+0.04, +0.77) ^A	−0.58 (−1.33, +0.18) ^A
<i>p</i>	<i>0.141</i>	<i>0.105</i>	<i>0.217</i>	0.049	0.028

*In variables with 3 or more groups, each box will present letters A to C (superscript). It should be concluded that subgroups that share the same letters in the same box are non-significantly different. y, years; M, months; BMI, body mass index; ProtU, protein/creatinine ratio in the urine; eGFR, estimated glomerular filtration rate. p values are depicted in italics; statistically significant, p values (<.05) are shown in bold.

(41%) or obese (10%). Fifteen percent had smoking habits, 14% had dyslipidemia, and 16% had hypertension. Pre-donation mean eGFR was 100.4 ± 14.6 mL/min/1.73 m². 76% of the cohort had eGFR >90 mL/min/1.73 m², and 29 donors (9%) had eGFR <80 mL/min/1.73 m². Ninety-six donors (30%) had proteinuria.

At follow-up, after discharge, the donors had a median number of SCr measurements of 7 (IQR 5–11), performed, per protocol, at 6 months, 1 year after donation, and then yearly.

Evolution of Renal Function After Donation

The median percentage of KFRR was 31.9 (IQR 22.6–38.1%). One-third of the cohort had a reduction rate of less than 26.2%, one-third between 26.2% and 36.1%, and one-third greater than 36.1% (Table 1).

Overall, after the first 6 months, our cohort's eGFR increased, on average, +0.35 mL/min/1.73 m²/year (95% confidence interval (CI), +0.20 to +0.50). Using the linear spline model results, the average changes of eGFR were,

respectively, from 6 months to 2 years, 2–5 years, 5–10 years, and 10–15 years, +0.85 mL/min/1.73 m²/year (95% CI, +0.10 to +1.61), +0.45 mL/min/1.73 m²/year (95% CI, +0.04 to +0.86), +0.24 mL/min/1.73 m²/year (95% CI, −0.08 to +0.55) and −0.24 mL/min/1.73 m²/year (95% CI, −0.75 to +0.28) (Table 2). A plateau was achieved around 10 years (Figure 1).

Donor Subgroup Analysis

Overall, there were no significant differences in the evolution of renal function after donation based on donor characteristics such as age, sex, hypertension, dyslipidemia, and presence of proteinuria (Tables 3, 4).

We found a non significant trend when comparing pre-donation eGFR subgroups. The donors with lower eGFR pre-donation (<80 mL/min/1.73 m²) presented a negative eGFR change overall of −0.09 (95% CI, −0.63 to +0.44) mL/min/1.73 m² vs. a positive shift in around 0.5 mL/min/1.73 m² in

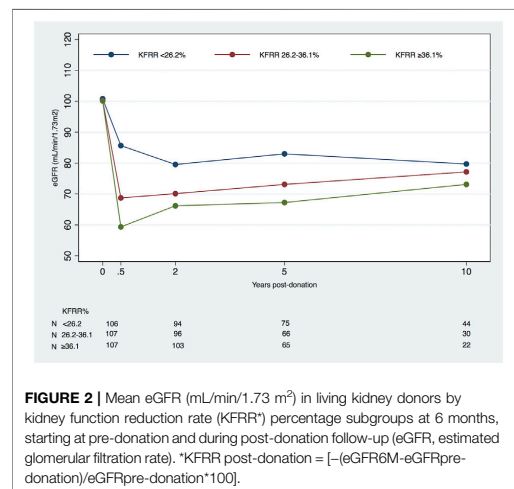
TABLE 5 | Changes in eGFR (mL/min/1.73 m²/year) in living kidney donors (n = 320) by % of KFRR post-donation donor group over different periods during follow-up from 6 months onward (multivariable analysis), adjusted to pre-donation variables previously analyzed: donor age, sex, BMI group: <25, 25–30, ≥30 kg/m², diagnosis of hypertension, smoking habits, dyslipidemia, presence of proteinuria and eGFR group <80, 80–90 and ≥90 mL/min/1.73 m².

	Overall	Linear spline model			
		6M–2y	2y–5y	5y–10y	10–15y
%KFRR post-donation*,**					
<26.2	–0.21 (–0.42, +0.01) ^B	–2.71 (–4.04, –1.39) ^A	+0.35 (–0.34, +1.03) ^A	+0.03 (–0.47, +0.53) ^A	–0.36 (–1.14, +0.42) ^A
26.2–36.1	+0.53 (+0.28, +0.78) ^A	+1.50 (+0.20, +2.80) ^B	+0.79 (+0.08, +1.51) ^A	+0.09 (–0.48, +0.66) ^A	–0.49 (–1.77, +0.80) ^A
>36.1	+0.65 (+0.39, +0.92) ^A	+3.66 (+2.38, +4.94) ^C	+0.04 (–0.67, +0.76) ^A	+0.43 (–0.19, +1.05) ^A	–0.61 (–1.84, +0.61) ^A
<i>p</i>	<0.001	<0.001	0.339	0.595	0.941

*In variables with 3 or more groups, each box will present letters A to C (superscript). It should be concluded that subgroups that share the same letters in the same box are non-significantly different.

**KFRR post-donation = $[-(eGFR_{6M} - eGFR_{pre-donation})/eGFR_{pre-donation} * 100]$.

y, years; M, months; BMI, body mass index; ProtU, protein/creatinine ratio in the urine; eGFR, estimated glomerular filtration rate; KFRR, kidney function reduction rate post-donation. *p* values are depicted in italics; statistically significant, *p* values (<.05) are shown in bold.



the subgroups with ≥80 mL/min/1.73 m² pre-donation (Table 4). Moreover, when analyzing eGFR variations by timespans, the subgroup with lower eGFR pre-donation presented a significant decline in eGFR in the period between 5 and 10 years of –1.16 (95% CI, –2.39 to +0.06) mL/min/1.73 m²/year, when compared to the other subgroups: +0.51 (95% CI, –0.31 to +1.32) mL/min/1.73 m²/year in the subgroup with pre-donation eGFR 80–90 mL/min/1.73 m², vs. +0.40 (95% CI, +0.04 to +0.77) mL/min/1.73 m²/year in those with ≥90 mL/min/1.73 m² pre-donation, *p* = 0.049. The lower function subgroup was associated with a more precocious increase in eGFR. The group with the highest eGFR pre-donation presented a more stable behavior, resembling the overall cohort, except for the last period of 10–15 years.

Pre-donation obesity was associated with a significantly greater decline of eGFR in the cohort over the entire period compared to normal weight donors (Table 4). Obese donors had a decrease of eGFR of –0.18 (95% CI –0.68 to +0.31) mL/min/

1.73 m²/year, while the second group had an increase of +0.59 (95% CI +0.37, +0.80) mL/min/1.73 m²/year (*p* = 0.02). This difference was more apparent in the earlier periods post-donation (6 months–5 years), with varying directions. Initially, at 6 months to 2 years, obese donors experienced significantly higher decline of eGFR of –2.47 (95% CI, –4.92 to –0.03) mL/min/1.73 m²/year, *p* = 0.002. However, in the 2–5 year period, they showed a temporary recovery of eGFR of +1.18 (95% CI, –0.17 to +2.53) mL/min/1.73 m²/year.

We carried out a separate analysis of %KFRR at 6 months post-donation, adjusted to the LD pre-donation factors (Table 5). The subgroup with a lower percentage of KFRR (<26.2%) had a significantly negative change in eGFR overall compared to the groups with higher loss rates of –0.21 (95% CI, –0.42 to +0.01) mL/min/1.73 m²/year vs. +0.53 (95% CI, +0.28 to +0.78) mL/min/1.73 m²/year in the group with KFRR >36.1%, *p* < 0.001. In the linear spline model, these differences only hold for 6 months to 2 years, where the three subgroups of kidney function recovery had significantly different eGFR changes (Figure 2).

Table 6 presents the observed eGFR values in our cohort, categorized by different subgroups, before and after donation at 6 months, 2, 5, 10, and 15 years.

Low eGFR and Proteinuria in LKD During Follow-Up

Table 7 depicts the eGFR category (mL/min/1.73 m²) for our cohort of LDs based on the last available SCr measurement. Notably, no donor reached eGFR <15 mL/min/1.73 m², and only six reached CKD stages 3b and 4.

We analyzed the prevalence of low eGFR by time frames after donation. We considered several cutoffs (Table 8). When we used eGFR <60 mL/min/1.73 m², according to KDIGO definition of CKD [26], the prevalence of low eGFR during follow-up diminished from 25% at 6 months to 13% at 10 years. This was not an unexpected finding. We have observed a steady increase in eGFR from 6 months post-donation up to 10 years after donation. No donor had eGFR <30 mL/min/1.73 m², and

TABLE 6 | Mean eGFR (mL/min per 1.73 m²) in living kidney donors by subgroup using the CKD-EPI equation, pre-donation, and post-donation at 6 months, 2 years, 5 and 10 years.

	Pre-donation	6M	2y	5y	10y
N	320	320	293	206	96
Overall	100.4 (14.6)	71.2 (16.3)	71.8 (14.9)	74.9 (16.1)	77.4 (14.8)
Age (years)					
<40	112.1 (12.8) ^A	81.6 (15.8) ^A	81.7 (14.7) ^A	86.7 (13.2) ^A	83.9 (11.4) ^A
40–55	99.1 (14.1) ^B	70.1 (15.7) ^B	70.7 (13.0) ^B	73.9 (14.8) ^B	77.7 (15.3) ^A
≥ 55	91.8 (10.3) ^C	63.3 (12.3) ^C	65.1 (13.8) ^C	64.9 (13.6) ^C	68.3 (13.4) ^B
<i>p</i>	<0.001	<0.001	<0.001	<0.001	0.001
Sex					
Male	99.3 (14.3)	70.3 (18.0)	69.4 (15.9)	73.5 (16.5)	75.2 (17.5)
Female	100.9 (14.8)	71.6 (15.5)	72.6 (14.4)	75.4 (16.0)	78.1 (13.9)
<i>p</i>	0.363	0.596	0.099	0.459	0.406
BMI (kg/m ²)					
<25	102.4 (15.0) ^A	73.5 (16.8) ^A	73.4 (15.7) ^A	78.4 (16.5) ^A	78.7 (13.9) ^A
25–30	99.0 (14.0) ^A	67.8 (13.8) ^B	70.5 (13.3) ^A	70.5 (13.4) ^B	76.0 (14.4) ^A
≥30	97.2 (14.6) ^A	74.2 (20.6) ^B	69.4 (16.2) ^A	75.0 (20.1) ^A	80.7 (22.8) ^A
<i>p</i>	0.060	0.007	0.187	0.004	0.583
Hypertension					
No	101.7 (14.6)	72.1 (16.4)	72.8 (14.9)	76.0 (16.2)	78.1 (14.6)
Yes	93.6 (12.6)	66.4 (15.0)	66.4 (13.9)	69.1 (14.1)	71.7 (15.7)
<i>p</i>	<0.001	0.022	0.007	0.025	0.176
Smoking habits					
No	99.3 (14.4)	70.3 (15.8)	71.4 (14.9)	74.0 (16.0)	77.0 (14.9)
Yes	106.5 (14.4)	76.6 (18.1)	74.1 (14.7)	79.8 (16.0)	79.7 (14.7)
<i>p</i>	0.002	0.013	0.256	0.069	0.552
Dyslipidemia					
No	102.0 (13.8)	72.6 (16.2)	73.1 (14.7)	76.3 (15.6)	77.8 (14.4)
Yes	90.8 (16.0)	62.7 (14.5)	64.0 (13.7)	65.1 (16.1)	74.7 (18.0)
<i>p</i>	<0.001	<0.001	<0.001	<0.001	0.523
ProtU 0.15–0.5 g/g					
No	100.0 (13.9)	70.9 (15.7)	71.5 (14.8)	73.4 (15.0)	77.0 (15.4)
Yes	101.5 (16.2)	72.0 (17.6)	72.2 (15.1)	78.2 (18.0)	79.5 (11.5)
<i>p</i>	0.375	0.573	0.714	0.051	0.546
Predonation eGFR*(mL/min/1.73m ²)					
<80	71.6 (6.7) ^A	52.7 (10.8) ^A	56.1 (12.2) ^A	59.3 (17.7) ^A	60.5 (9.4) ^A
80–90	85.7 (3.0) ^B	62.3 (12.1) ^B	63.6 (12.2) ^A	63.6 (12.5) ^A	71.8 (17.2) ^{AB}
≥90	106.8 (9.6) ^C	75.2 (15.3) ^C	75.3 (13.8) ^B	78.3 (14.8) ^B	80.1 (13.4) ^B
<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001
%KFRR post-donation*,**					
<26.2	100.8 (16.9) ^A	85.7 (16.1) ^A	79.6 (15.9) ^A	84.0 (16.8) ^A	79.7 (15.0) ^A
26.2–36.1	100.3 (14.1) ^A	68.7 (10.5) ^B	70.1 (12.8) ^B	73.1 (14.1) ^B	77.2 (14.9) ^A
>36.1	100.2 (12.7) ^A	59.3 (8.6) ^C	66.2 (12.6) ^B	67.2 (12.7) ^B	73.1 (14.0) ^A
<i>p</i>	0.941	<0.001	<0.001	<0.001	0.234

*In variables with 3 or more groups, each box will present letters A to C (superscript). It should be concluded that subgroups that share the same letters in the same box are non-significantly different.

**KFRR post-donation = [(eGFR6M-eGFRpre-donation)/eGFRpre-donation*100].

Y, years; M, months; BMI, body mass index; ProtU, protein/creatinine ratio in the urine; eGFR, estimated glomerular filtration rate; KFRR, kidney function reduction rate post-donation; CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration equation.

p values are depicted in italics; statistically significant, *p* values (<.05) are shown in bold.

only one percent had eGFR <40 mL/min/1.73 m². We selected the cutoff of 50 mL/min/1.73 m², as we believe that it defines a more meaningful CKD status, to analyze the prevalence of low eGFR by subgroup over different periods during follow-up (Table 9). The prevalence of low eGFR remained overall low after 6 months, ≤5%. Older LDs (≥55 years) had a significantly higher prevalence of low eGFR from 6 months until 5 years of follow-up (13% for those ≥55 years vs. 3% for those 40–55 years and none in the younger group, *p* = 0.005), not thereafter. The same holds for donors with lower eGFR pre-donation. Those with higher KFRR (>36.1%) had a higher prevalence of lower eGFR at 6 months of 19% vs. 6% in the intermedium group and 0% in the

group with KFRR <26.2%, *p* < 0.001. No difference in prevalence of lower eGFR was observed between KFRR subgroups thereafter.

These findings were accompanied by a non-significant rise in proteinuria after donation (Table 10). In fact, the prevalence of proteinuria decreased from 30% pre-donation to 10% 6 months after donation, and that prevalence remained stable afterward.

DISCUSSION

In this cohort of 320 LDs, we found reassuring results about the evolution of long-term eGFR in LDs. Overall, the donors

TABLE 7 | eGFR (mL/min/1.73 m²) category for donors based on the last available Scr measurement, using the CKD-EPI equation.

eGFR (mL/min/1.73 m ²)	n = 320 n (%)
<15	0
15–30	2 (1)
30–45	4 (1)
45–60	55 (17)
60–90	205 (64)
≥90	54 (17)

Scr, serum creatinine; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration equation.

TABLE 8 | Prevalence of low eGFR (mL/min/1.73 m²) using the CKD-EPI equation, in living kidney donors during follow-up n (%).

	Predonation	6 M	2y	5y	10y
N	320	320	293	206	96
<60	0	79 (25)	68 (23)	38 (18)	12 (13)
<55	0	44 (14)	31 (11)	16 (8)	5 (5)
<50	0	26 (8)	13 (4)	10 (5)	2 (2)
<45	0	8 (3)	3 (1)	4 (2)	2 (2)
<40	0	3 (1)	2 (1)	2 (1)	1 (1)
<35	0	0	2 (1)	0	0
<30	0	0	0	0	0

M, months; y, years; CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration equation.

Bold is used to indicate the cutoff for defining a low estimated glomerular filtration rate in the cohort.

presented an average change in eGFR 6 months onward of +0.35 (95% CI, +0.20 to +0.50) mL/min/1.73 m² per year. The period with the higher increase was from 6 months to 2 years with a mean increase of eGFR of +0.85 (95% CI +0.10 to +1.61) mL/min/1.73 m² per year, and the recovery after donation plateaued at 10 years, after which the calculated mean change in eGFR is −0.24 (95% CI, −0.75 to +0.28) mL/min/1.73 m² per year. To the best of our knowledge, this time span of 10–15 years post-donation trajectories have not been previously reported. As we hypothesized, when subgroups of donors were analyzed, we identified different kidney function recovery patterns. Obese LDs had a statistically significant overall worse recovery of eGFR compared to normal weight donors. The recovery trajectory of kidney function in obese donors showed a biphasic pattern at earlier timespans after donation, up to 5 years. The intermediate group of eGFR pre-donation has a better recovery than the extreme function groups. LDs with a lower %KFRR at 6 M (<26.2%) compared to eGFR pre-donation presented a significantly higher decrease of eGFR in the overall period compared to the other two groups. Still, the differences between groups only hold for the time frame of 6 M to 2 years. Moreover, an eGFR <50 mL/min/1.73 m² was a rare event, and the proteinuria prevalence did not increase during the follow-up.

A substantial nephron loss is expected in the aging kidneys [27]. Renal function decline was well-characterized in several general and healthy populations [28–30]. Studies in the

healthy Swedish population have demonstrated that the mean decline in GFR was 4 mL/min/1.73 m² per decade up to 50 years of age and then decreased annually by 1 mL/min [29, 30]. In a large series of healthy potential LDs, Fenton et al. [28] found the measured GFR (mGFR) had a linear decline after 35 years of 6.6–7.7 mL/min/1.73 m²/decade. In the long term (10–15Y), our cohort's mean change in eGFR stayed below these references.

Increases in GFR long-term after donation have been described for years [31, 32], but most studies lack detailed data about GFR trajectories. Matas et al. [32] found that the increase in eGFR continued until at least 20 years post-donation in their study of 2002 predominantly white donors.

Kasike et al. [14], in a prospective observational study, compared 205 living donor candidates and 203 healthy controls with serially measured iothexol GFR. Between 6 M and 9 years, the mean change in mGFR was significantly different among donors +0.02 (95% CI, −0.16 to −0.20) mL/min/1.73 m²/year vs. −1.26 (95% CI, −1.52 to −1.00) mL/min/1.73 m²/year in controls. Lam et al. [10], in a retrospective cohort study of LDs in Alberta, in 2002–2016, matched 604 donors to 2,414 healthy non-donors from the general population. Overall, from 6 weeks onwards, the eGFR increased by 0.35 mL/min/1.73 m² per year (95% CI, +0.21 to +0.48) in donors and significantly decreased by −0.85 mL/min/1.73 m² per year (95% CI, −0.94 to −0.75) in the matched non-donors [10]. Our data is largely in line with these observations.

After nephrectomy, there is compensatory hyperfiltration in the remaining kidney, such that while a donor immediately loses approximately 50% of the kidney mass, the net reduction in GFR early after the donation is only approximately 30% [16]. The mechanisms of compensatory hyperfiltration are not clear yet. In a remarkable long-term study of glomerular hemodynamics after kidney donation [12], it was noted that adaptive hyperfiltration after donor nephrectomy was attributable to hyperperfusion and hypertrophy of the remaining glomeruli, without glomerular hypertension in most donors, and these changes were sustained throughout the late post-donation period, without significant albuminuria [12]. Nevertheless, there is concern that adaptive hyperfiltration might result in faster progression of kidney disease in certain groups of donors with less functional reserve, such as those older, obese, or hypertensive [12].

Van de Weijden et al. [33], in a cohort of 1024 donors, found that individuals with a more pronounced increase in single-kidney GFR at 3 months after donation had better long-term kidney function, independent of pre-donation GFR and age. The authors hypothesized that an early increase in eGFR may reflect a more physiologic adaptation mechanism to an acute reduction in renal mass and a better renal functional reserve. These results could help personalize LD follow-up [33].

Our results were surprising when we evaluated the impact of the percentage of KFRR at 6M in the trajectories of eGFR over time. The individuals with less KFRR (<26.2%) in the first 6 months had a significantly higher decrease of eGFR in the overall period compared to the other subgroups. However, the significant differences between the three subgroups only held from 6 months to 2 years. Distinctly, it was not a predictive

TABLE 9 | Prevalence of eGFR <50 mL/min/1.73 m², in living kidney donors by subgroup over different time periods during follow-up from 6 months onward n (%), using the CKD-EPI equation.

	Predonation	6M	2y	5y	10y
n	320	320	293	206	96
Overall	0	26 (8)	13 (4)	10 (5)	2 (2)
Age (years)	—				
<40		1 (4)	1 (4)	0	0
40–55		14 (9)	4 (3)	3 (3)	1 (2)
≥ 55		11 (13)	8 (10)	7 (13)	1 (5)
<i>p</i>		0.009	0.025	0.005	0.446
Sex	—				
Male		9 (10)	6 (7)	3 (5)	2 (8)
Female		17 (7)	7 (3)	7 (5)	0
<i>p</i>		0.515	0.127	0.729	0.061
BMI (kg/m ²)	—				
<25		12 (8)	8 (6)	4 (4)	1 (3)
25–30		11 (8)	2 (2)	5 (6)	0
≥30		3 (9)	3 (10)	1 (5)	1 (14)
<i>p</i>		0.956	0.063	0.894	0.064
Hypertension	—				
No		18 (7)	9 (4)	6 (3)	1 (1)
Yes		8 (16)	4 (9)	4 (12)	1 (9)
<i>p</i>		0.031	0.138	0.057	0.217
Smoking	—				
No		24 (9)	13 (5)	10 (6)	2 (2)
Yes		2 (4)	0	0	0
<i>p</i>		0.394	0.227	0.364	1
Dyslipidemia	—				
No		17 (6)	7 (3)	6 (3)	1 (1)
Yes		9 (20)	6 (14)	4 (15)	1 (9)
<i>p</i>		0.001	0.001	0.025	0.217
ProtU 0.15–0.5 g/g	—				
No		19 (8)	9 (4)	9 (6)	2 (2)
Yes		7 (7)	4 (4)	1 (2)	0
<i>p</i>		0.721	1	0.287	1
Pre-donation eGFR (mL/min/1.73m ²)	—				
<80		14 (48)	8 (31)	5 (36)	1 (14)
80–90		9 (19)	3 (7)	3 (10)	1 (7)
≥90		3 (1)	2 (1)	2 (1)	0
<i>p</i>		<0.001	<0.001	<0.001	0.051
%KFR post-donation	—				
<26.2		0	2 (2)	2 (3)	1 (2)
26.2–36.1		6 (6)	3 (3)	3 (5)	0
>36.1		20 (19)	8 (8)	5 (8)	1 (5)
<i>p</i>		<0.001	0.162	0.395	0.711

*KFR post-onation = $[-(eGFR_{6M} - eGFR_{pre-donation}) / eGFR_{pre-donation} * 100]$.

y, years; M, months; BMI, body mass index; ProtU, protein/creatinine ratio in the urine; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration equation; KFR, kidney function reduction rate post-donation.

p values are depicted in *italics*; statistically significant, p values (<.05) are shown in **bold**.

factor of long-term renal function in our cohort, and we could not support the hypothesis of Weijden et al. [33].

Lower pre-donation eGFR has been associated with lower post-donation eGFR and a higher risk of ESRD [34, 35]. Tan et al. [11], in a retrospective cohort of 174 Southeast Asian LKDs, described that pre-nephrectomy eGFR was a good predictor of post-donation eGFR, especially in the short term (<6 M). Still, it was limited to <5 years and did not necessarily translate into a long-term (>5 Y) reduction in post-donation eGFR. In our cohort, we could not correlate better pre-donation eGFR with improved recovery of post-donation eGFR. We could hypothesize, as studied by

Chakkerla et al. [36], that adaptation reserves for increasing filtration after nephrectomy may be limited in donors with a high eGFR.

Several studies reported worse outcomes in obese LDs, including an increased risk of ESRD [8, 9, 34], although it is not a contraindication for donation [16]. Ibrahim et al. [35], in a white LDs population, showed that each increase of 1 unit in BMI pre-donation was associated with a 3%–10% higher risk of proteinuria and reduced GFR. In our study, obese donors at the time of donation experienced significantly worse overall eGFR change from 6 months onward compared to normal-weight donors. However, these differences only hold for the initial

TABLE 10 | Prevalence of proteinuria in living kidney donors pre-donation and over different periods during follow-up.

	Time				
	Pre-donation	6M	2y	5y	10y
n	302	283	251	172	78
Median (IQR)	0.11 (0.07–0.16)	0.08 (0.06–0.11)	0.07 (0.06–0.10)	0.08 (0.06–0.11)	0.07 (0.06–0.10)
≥0.15 g/g n (%)	96 (30)	30 (11)	19 (8)	18 (10)	6 (8)

n, number; %, percentage; IQR, interquartile range; g/g, protein g/creatinine g in a random urinary sample; M, months; Y, years.

time frames (6 M–5 Y). Some of these donors, with preexisting obesity-related hyperfiltration, may have a diminished capacity to undergo further adaptive hyperfiltration after nephrectomy compared to a normal-weight donor [37]. Our cohort results clearly red-flagged this population and deserve further investigation concerning the mechanisms involved and potential preventive primary or secondary measures that might be indicated [38].

In our cohort, we did not find significantly different trajectories of eGFR when considering donor age, sex, hypertension, dyslipidemia, and the presence of proteinuria pre-donation. The aging healthy kidney is associated with lower renal function and blunted adaptative capacity [30]. Our cohort of older donors has not been associated with worse outcomes for their recipients [21]. A comprehensive evaluation of an older LD could be a good strategy for many LDs pairs.

LDs diagnosed with hypertension pre-donation did not present a distinctive slope of eGFR after donation. Hypertension represents a leading cause of cardiovascular morbidity and mortality. It is associated with CKD and the risk of ESRD in the general population and is a frequently reported cause of ESRD in living donors [39]. Furthermore, it can reduce the renal reserve and limit the expected post-donation compensation [40]. Our results could be explained by our thorough practice in selecting these donors. Sanchez et al. [23], in a population of LDs, found that the risks for the different clinical outcomes, including eGFR < 60, 45, or 30 mL/min/1.72 m² or ESRD, between those with and without hypertension at the time of donation were not different. A different issue, the effect of hypertension after donation on the eGFR trajectories, is beyond the scope of this work.

The proportion of LDs with low eGFR, defined as eGFR <50 mL/min/1.73 m² was overall small, 5% or less after the 6 months, decreased with the follow-up time, which is expected with progressive improvement in kidney function, suggesting that the decline in eGFR was not progressive in the majority of LDs. The results on the prevalence of proteinuria in the follow-up period support this theory, pointing away from the hypothesis of hyperfiltration after a donation from the remnant nephron [38], which focused on the role of glomerular hypertension in the remaining nephrons as the main pathway for progressive renal damage and consequent glomerular leakage of proteins.

Our study has several limitations. First, donors were evaluated retrospectively, and unobserved confounders may

have introduced bias. Second, we have not assessed a non-donor control group, although we can compare our results with studies of the evolution of eGFR with aging in healthy European populations [28–30]. Third, our cohort consisted almost exclusively of Caucasian patients, limiting the generalization of our results. In addition, eGFR using estimation equations to assess kidney function has limitations, but it is the common practice in most transplant centers and agrees with the International Guidelines [16]. In addition, an added value of our study cohort is its larger size and the availability of serial SCr measurements. Nevertheless, longer follow-up studies must be required; prospective studies are necessary to allow a cause-effect analysis of the parameters studied. Furthermore, the influence of *de novo* comorbidities such as hypertension and diabetes were not evaluated as modifiers of the evolution of kidney function after donation.

CONCLUSION

Our data show that eGFR post-donation recovery is significant and may last until 10 years post-donation. Moreover, an eGFR <50 mL/min/1.73 m² was a rare observation, having a prevalence of 5% or less in the 2–15 years span. These observations confirm that in a carefully selected cohort of donors, the occurrence of a significant kidney function loss or accelerated decline is exceptional. However, some subgroups of donors presented a more ominous kidney function trajectory pattern, pointing to the necessity of tailored follow-up.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by Ref: 147-21 (119-DEFI/122-CE) ULSdSA. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MA, PR, and JM: Research design, data acquisition, data analysis, and paper writing. JS, CR, SP, and ST were engaged in the data acquisition and analysis. MS and LM were involved in the research design and data analysis. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Gruessner RWG, Gruessner AC. Solid-Organ Transplants From Living Donors: Cumulative United States Experience on 140,156 Living Donor Transplants Over 28 Years. *Transpl Proc* (2018) 50(10):3025–35. doi:10.1016/j.transproceed.2018.07.024
2. Park YH, Min SK, Lee JN, Lee HH, Jung WK, Lee JS, et al. Comparison of Survival Probabilities for Living-Unrelated Versus Cadaveric Renal Transplant Recipients. *Transpl Proc* (2004) 36(7):2020–2. doi:10.1016/j.transproceed.2004.08.122
3. Reese PP, Boudville N, Garg AX. Living Kidney Donation: Outcomes, Ethics, and Uncertainty. *Lancet* (2015) 385(9981):2003–13. doi:10.1016/S0140-6736(14)62484-3
4. Meier-Kriesche HU, Kaplan B. Waiting Time on Dialysis as the Strongest Modifiable Risk Factor for Renal Transplant Outcomes: A Paired Donor Kidney Analysis. *Transplantation* (2002) 74(10):1377–81. doi:10.1097/00007890-200211270-00005
5. Axelrod DA, Schnitzler MA, Xiao H, Irish W, Tuttle-Newhall E, Chang SH, et al. An Economic Assessment of Contemporary Kidney Transplant Practice. *Am J Transpl* (2018) 18(5):1168–76. doi:10.1111/ajt.14702
6. Muzaale AD, Massie AB, Wang MC, Montgomery RA, McBride MA, Wainright JL, et al. Risk of End-Stage Renal Disease Following Live Kidney Donation. *JAMA* (2014) 311(6):579–86. doi:10.1001/jama.2013.285141
7. Mjoen G, Hallan S, Hartmann A, Foss A, Midtvedt K, Øyen O, et al. Long-Term Risks for Kidney Donors. *Kidney Int* (2014) 86(1):162–7. doi:10.1038/ki.2013.460
8. Massie AB, Muzaale AD, Luo X, Chow EKH, Locke JE, Nguyen AQ, et al. Quantifying Postdonation Risk of ESRD in Living Kidney Donors. *J Am Soc Nephrol* (2017) 28(9):2749–55. doi:10.1681/ASN.2016101084
9. Grams ME, Garg AX, Lentine KL. Kidney-Failure Risk Projection for the Living Kidney-Donor Candidate. *N Engl J Med* (2016) 374(21):2094–5. doi:10.1056/NEJMc1603007
10. Lam NN, Lloyd A, Lentine KL, Quinn RR, Ravani P, Hemmelgarn BR, et al. Changes in Kidney Function Follow Living Donor Nephrectomy. *Kidney Int* (2020) 98(1):176–86. doi:10.1016/j.kint.2020.03.034
11. Tan RY, Allen JC, Kee T, Jafar TH. Predictors of Low Estimated Glomerular Filtration Rate After Living Kidney Donation in a Southeast Asian Population From Singapore. *Nephrology (Carlton)* (2017) 22(10):761–8. doi:10.1111/nep.12845
12. Lenihan CR, Busque S, Derby G, Blouch K, Myers BD, Tan JC. Longitudinal Study of Living Kidney Donor Glomerular Dynamics After Nephrectomy. *J Clin Invest* (2015) 125(3):1311–8. doi:10.1172/JCI78885
13. Kim Y, Kim MJ, Lee YH, Lee JP, Lee JH, Yang J, et al. Changes in Renal Function and Predictors Relating Renal Outcome of Living Kidney Donors: A Nationwide Prospective Cohort Study. *Nephrology (Carlton)* (2021) 26(6):541–8. doi:10.1111/nep.13880
14. Kasiske BL, Anderson-Haag TL, Duprez DA, Kalil RS, Kimmel PL, Pesavento TE, et al. A Prospective Controlled Study of Metabolic and Physiologic Effects of Kidney Donation Suggests That Donors Retain Stable Kidney Function Over the First Nine Years. *Kidney Int* (2020) 98(1):168–75. doi:10.1016/j.kint.2020.01.017
15. Ahmadi AR, Lafranca JA, Claessens LA, Imamdin RMS, Ijzermans JNM, Betjes MGH, et al. Shifting Paradigms in Eligibility Criteria for Live Kidney Donation: A Systematic Review. *Kidney Int* (2015) 87(1):31–45. doi:10.1038/ki.2014.118
16. Lentine KL, Kasiske BL, Levey AS, Adams PL, Alberú J, Bakr MA, et al. Summary of Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* (2017) 101(8):1783–92. doi:10.1097/TP.0000000000001770
17. Andrews PA, Burnapp L. British Transplantation Society/Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: Summary of Updated Guidance. *Transplantation* (2018) 102(7):e307. doi:10.1097/TP.0000000000002253
18. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* (2009) 150(9):604–12. doi:10.7326/0003-4819-150-9-200905050-00006
19. Lentine KL, Kasiske BL, Levey AS, Adams PL, Alberú J, Bakr MA, et al. KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* (2017) 101(8S Suppl. 1):S1–S109. doi:10.1097/TP.0000000000001769
20. Almeida M, Calheiros Cruz G, Sousa C, Figueiredo C, Ventura S, Silvano J, et al. External Validation of the Toulouse-Ranguel Predictive Model to Estimate Donor Renal Function After Living Donor Nephrectomy. *Transpl Int* (2023) 36:11151. doi:10.3389/ti.2023.11151
21. Almeida M, Ribeiro C, Silvano J, Pedrosa S, Tafalo S, Martins LS, et al. Living Donors' Age Modifies the Impact of Pre-Donation Estimated Glomerular Filtration Rate on Graft Survival. *J Clin Med* (2023) 12(21):6777. doi:10.3390/jcm12216777
22. Bellini MI, Nozdrin M, Pengel L, Knight S, Papalois V. Risks for Donors Associated With Living Kidney Donation: Meta-Analysis. *Br J Surg* (2022) 109(8):671–8. doi:10.1093/bjs/znac114
23. Sanchez OA, Ferrara LK, Rein S, Berglund D, Matas AJ, Ibrahim HN. Hypertension After Kidney Donation: Incidence, Predictors, and Correlates. *Am J Transpl* (2018) 18(10):2534–43. doi:10.1111/ajt.14713
24. Srivastava T, Hariharan S, Alon US, McCarthy ET, Sharma R, El-Meanawy A, et al. Hyperfiltration-Mediated Injury in the Remaining Kidney of a Transplant Donor. *Transplantation* (2018) 102(10):1624–35. doi:10.1097/TP.0000000000002304
25. Young A, Storsley L, Garg AX, Treleaven D, Ngan CY, Cuerden MS, et al. Health Outcomes for Living Kidney Donors With Isolated Medical Abnormalities: A Systematic Review. *Am J Transpl* (2008) 8(9):1878–90. doi:10.1111/j.1600-6143.2008.02339.x
26. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: Behind the Scenes, Need for Guidance, and a Framework for Moving Forward. *Kidney Int* (2014) 85(1):49–61. doi:10.1038/ki.2013.444
27. Delanaye P, Jager KJ, Bökenkamp A, Christensson A, Dubourg L, Eriksen BO, et al. CKD: A Call for an Age-Adapted Definition. *J Am Soc Nephrol* (2019) 30(10):1785–805. doi:10.1681/ASN.2019030238
28. Fenton A, Montgomery E, Nightingale P, Peters AM, Sheerin N, Wroe AC, et al. Glomerular Filtration Rate: New Age- and Gender-Specific Reference Ranges and Thresholds for Living Kidney Donation. *BMC Nephrol* (2018) 19(1):336. doi:10.1186/s12882-018-1126-8

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2024.13356/full#supplementary-material>

29. Granerus G, Aurell M. Reference Values for ⁵¹Cr-EDTA Clearance as a Measure of Glomerular Filtration Rate. *Scand J Clin Lab Invest* (1981) 41(6):611–6. doi:10.3109/00365518109090505
30. Fehrman-Ekholm I, Skeppholm L. Renal Function in the Elderly (>70 Years Old) Measured by Means of Iohexol Clearance, Serum Creatinine, Serum Urea and Estimated Clearance. *Scand J Urol Nephrol* (2004) 38(1):73–7. doi:10.1080/00365590310015750
31. Ibrahim HN, Foley R, Tan L, Rogers T, Bailey RF, Guo H, et al. Long-Term Consequences of Kidney Donation. *N Engl J Med* (2009) 360(5):459–69. doi:10.1056/NEJMoa0804883
32. Matas AJ, Vock DM, Ibrahim HN. GFR $\leq$ 25 Years Postdonation in Living Kidney Donors With (Vs. Without) a First-Degree Relative With ESRD. *Am J Transpl* (2018) 18(3):625–31. doi:10.1111/ajt.14525
33. van der Weijden J, Mahesh SVK, van Londen M, Bakker SJL, Sanders JS, Navis G, et al. Early Increase in Single-Kidney Glomerular Filtration Rate After Living Kidney Donation Predicts Long-Term Kidney Function. *Kidney Int* (2022) 101(6):1251–9. doi:10.1016/j.kint.2022.01.034
34. Locke JE, Reed RD, Massie A, MacLennan PA, Sawinski D, Kumar V, et al. Obesity Increases the Risk of End-Stage Renal Disease Among Living Kidney Donors. *Kidney Int* (2017) 91(3):699–703. doi:10.1016/j.kint.2016.10.014
35. Wainright JL, Robinson AM, Wilk AR, Klassen DK, Cherikh WS, Stewart DE. Risk of ESRD in Prior Living Kidney Donors. *Am J Transpl* (2018) 18(5):1129–39. doi:10.1111/ajt.14678
36. Chakkeri HA, Denic A, Kremers WK, Stegall MD, Larson JJ, Ravipati H, et al. Comparison of High Glomerular Filtration Rate Thresholds for Identifying Hyperfiltration. *Nephrol Dial Transpl* (2020) 35(6):1017–26. doi:10.1093/ndt/gfy332
37. Reese PP, Feldman HI, Asch DA, Thomasson A, Shults J, Bloom RD. Short-Term Outcomes for Obese Live Kidney Donors and Their Recipients. *Transplantation* (2009) 88(5):662–71. doi:10.1097/TP.0b013e3181b27a17
38. Garcia-Carro C, Vergara A, Bermejo S, Azancot MA, Sellarés J, Soler MJ. A Nephrologist Perspective on Obesity: From Kidney Injury to Clinical Management. *Front Med (Lausanne)* (2021) 8:655871. doi:10.3389/fmed.2021.655871
39. Anjum S, Muzaale AD, Massie AB, Bae S, Luo X, Grams ME, et al. Patterns of End-Stage Renal Disease Caused by Diabetes, Hypertension, and Glomerulonephritis in Live Kidney Donors. *Am J Transpl* (2016) 16(12):3540–7. doi:10.1111/ajt.13917
40. Okumura K, Yamanaga S, Tanaka K, Kinoshita K, Kaba A, Fujii M, et al. Prediction Model of Compensation for Contralateral Kidney After Living-Donor Donation. *BMC Nephrol* (2019) 20(1):283. doi:10.1186/s12882-019-1464-1

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APPENDIX 2 – External Validation of the Toulouse-Rangueil Predictive Model to Estimate Donor Renal Function After Living Donor Nephrectomy

Almeida M.; Calheiros Cruz, G; Sousa, C.; Figueiredo, C., Ventura, S., Silvano, J., Pedroso, S.; Martins, LS., Ramos, M. and Malheiro, J.

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External Validation of the Toulouse-Rangueil Predictive Model to Estimate Donor Renal Function After Living Donor Nephrectomy

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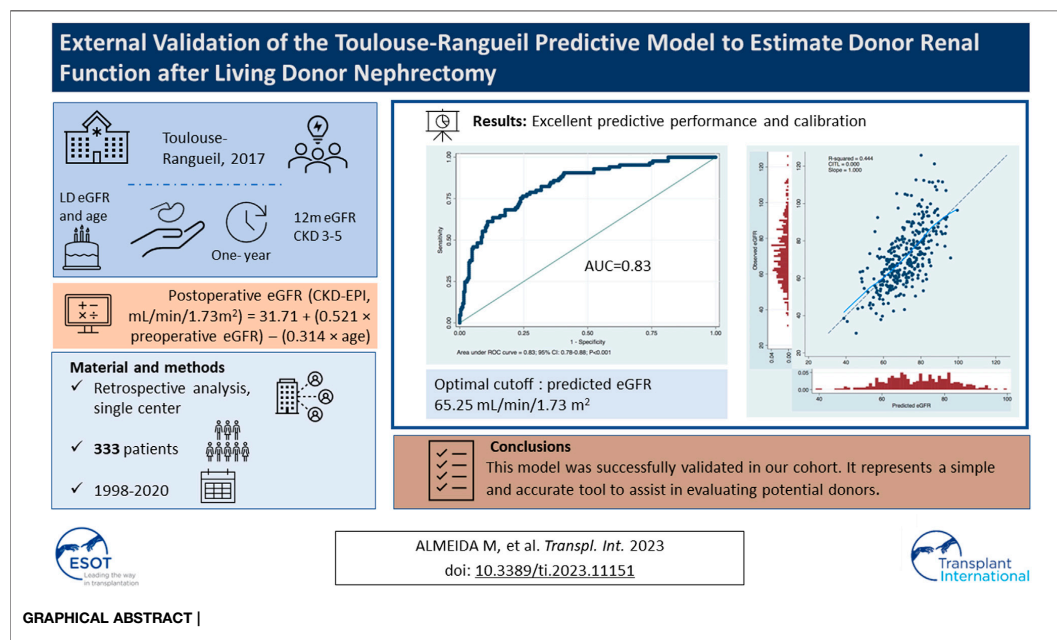
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A predictive model to estimate post-donation glomerular filtration rate (eGFR) and risk of CKD at 1-year was developed from a Toulouse-Rangueil cohort in 2017 and showed an excellent correlation to the observed 1-year post-donation eGFR. We retrospectively analyzed all living donor kidney transplants performed at a single center from 1998 to 2020. Observed eGFR using CKD-EPI formula at 1-year post-donation was compared to the predicted eGFR using the formula $eGFR (CKD-EPI, mL/min/1.73 m^2) = 31.71 + (0.521 \times \text{preoperative eGFR}) - (0.314 \times \text{age})$. 333 donors were evaluated. A good correlation (Pearson $r = 0.67$; $p < 0.001$) and concordance (Bland-Altman plot with 95% limits of agreement -21.41 – 26.47 mL/min/1.73 m²; $p < 0.001$) between predicted and observed 1-year post-donation eGFR were observed. The area under the ROC curve showed a good discriminative ability of the formula in predicting observed CKD at 1-year post-donation (AUC = 0.83; 95% CI: 0.78–0.88; $p < 0.001$) with optimal cutoff corresponding to a predicted eGFR of 65.25 mL/min/1.73 m² in which the sensibility and specificity to predict CKD were respectively 77% and 75%. The model was successfully validated in our cohort, a different European population. It represents a simple and accurate tool to assist in evaluating potential donors.

Keywords: external validation, predictive model, living donor renal function, kidney transplantation, chronic kidney disease

Abbreviations: AUC, area under the curve; BMI, body mass index; CI, confidence interval; CITL, calibration in the large; CKD, chronic kidney disease; CKD-EPI, chronic kidney disease epidemiology collaboration; ESRD, end-stage renal disease; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; LKD, living kidney donor; MDRD, modification of diet in renal disease; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; SD, standard deviation.



INTRODUCTION

Living donor kidney transplant is the best treatment for ESRD patients eligible for transplant (1, 2). Living donation increases organ availability, decreases time on the waiting list, allows pre-emptive transplantation, and improves graft and patient survival (1–3).

The evaluation of a living donor candidate is a multidisciplinary task to minimize the risk for the donor while ensuring the organ's suitability for the recipient (4, 5). Despite being the only surgical indication that grants no direct medical benefit to a healthy patient, a living nephrectomy is considered a safe procedure for the donor (5–7). Long-term follow-up data, however, have shown that donors are at an increased risk of CKD and, rarely, ESRD compared to healthy non-donors (6–9). As such, these patients would be subjected to the cardiovascular and global morbidity and mortality of CKD (10). Furthermore, the increasing acceptance of donors with increasing age or with minor medical changes that were previously declined (6), makes the issue of kidney donors' safety of utmost importance (6). Moreover, the scarcity of good-quality studies on their long-term follow-up must be acknowledged (6, 7).

Current Clinical practice guidelines on the evaluation and care of living kidney donors from Kidney Disease Improving Global Outcomes (KDIGO) recommend a comprehensive approach to risk assessment that should replace decisions based on assessments of single risk factors evaluation (4). Transplant programs should provide each donor candidate with individualized quantitative risks from donation and

communicate them clearly to donor candidates (4). Furthermore, each donor candidate's risk should be compared to predetermined thresholds for acceptance and declined if the risk exceeds the acceptable limit for the Transplant Unit (4). Nevertheless, precise tools to quantify individualized donor risks are lacking.

A predictive model to estimate the donor 1-year post-donation estimated glomerular filtration rate (eGFR) and risk of CKD was developed from a Toulouse-Rangueil cohort in 2017 (11). Benoit et al. retrospectively evaluated a single-center French cohort of 202 living donors and identified age and preoperative eGFR as independent predictors of postoperative eGFR. A formula using multiple linear regression was designed for clinical application and the authors described a good statistical performance (11). This model was then externally validated in a German center by Kullik et al. (12) and in a different French cohort (13) and was shown to have a good correlation to the observed 1-year post-donation eGFR.

We sought to externally validate this predictive tool in a different, large European cohort of patients who underwent a living donor kidney transplant at our center.

MATERIAL AND METHODS

This external validation study was conducted according to the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnostics (TRIPOD) guidelines (14).

TABLE 1 | Patients' characteristics of the 333 living donors.

	N = 333
Age, mean $\pm$ SD	47.3 $\pm$ 10.6
Sex F:M, n (%)	236 (71):97 (29)
BMI, mean $\pm$ SD (Kg/m ²)	25.3 $\pm$ 3.4
Smoking habits, n (%)	51 (15)
Hypertension, n (%)	50 (15)
Pre-donation SCr, mean $\pm$ SD (mg/dL)	0.75 $\pm$ 0.16
Pre-donation eGFR, mean $\pm$ SD	100.3 $\pm$ 14.7
1-year postdonation SCr, mean $\pm$ SD (mg/dL)	1.05 $\pm$ 0.23
1-year postdonation eGFR, mean $\pm$ SD	71.4 $\pm$ 16.2
Predicted 1-year postdonation eGFR, mean $\pm$ SD	69.1 $\pm$ 10.0

eGFR: mL/min/1.73 m².

We retrospectively reviewed the clinical data of all ($n = 366$) the donors who underwent nephrectomy for living donor kidney transplantation at our institution between 1998 and December 2019. After excluding 33 donors, in whom eGFR at 1 year was missing, the remaining 333 donors were included in this study.

Following international guidelines, all donors were subjected to a standard evaluation protocol. Baseline demographic, anthropomorphic, analytical, and clinical data were collected from the living kidney donors. Serum creatinine Serum creatinine-based CKD-EPI equation (15) was used to predict eGFR. Split renal function was evaluated by Nuclear Renography and renal anatomy by a Computed Tomography scan.

Hypertension was defined by blood pressure in the consultation $>140/90$ mmHg, ABPM $> 135/85$ mmHg, and past diagnosis of hypertension or antihypertensive medication. Uncontrolled hypertension or evidence of end-organ damage were criteria of exclusion. Potential donors with a history of malignancy, obesity, or diabetes were excluded. Although a lower limit of eGFR was not established by Unit protocol, potential donors with eGFR below 80 mL/min/1.73 m² were usually discarded. The final approval for kidney donation was reviewed in a multidisciplinary meeting and the ethical approval was mandatory.

Left-side procurement was preferred for anatomical reasons except for complex vessels anatomy or when a significant renal asymmetry was found, and the right kidney had the lower clearance. A transperitoneal laparoscopic approach was performed in most donors. Lifetime annual follow-up appointments are available for all donors.

For validation of the predictive model, eGFR was calculated using the CKD-EPI Chronic Kidney Disease Epidemiology pre-donation and 1 year (± 30 days) after donation.

Statistical Analysis

Data are presented as mean (and standard deviations for continuous variables and frequency (and percentages) for categorical variables.

Observed eGFR using CKD-EPI formula at 1-year post-donation was compared to the predicted eGFR using the formula developed in Toulouse-Rangueil: postoperative eGFR (CKD-EPI, mL/min/1.73 m²) = $31.71 + (0.521 \times \text{preoperative eGFR}) - (0.314 \times \text{age})$.

The ability of this formula to predict the observed GFR was analyzed by Pearson correlation, and agreement was explored by the Bland-Altman plot. The discriminative ability to predict CKD3-5 was evaluated by the area under the receiver operating characteristic (ROC) curve and using sensitivity, specificity, and positive, or negative predictive values (PPV or NPV). Furthermore, the accuracy of the predictive model was depicted by constructing a calibration plot and assessed through the calibration slope and the calibration in the large.

A 2-sided p -value < 0.05 was considered as statistically significant. Statistical calculations were performed using STATA/MP, version 15.1 (Stata Corp, College Station, TX, United States).

RESULTS

Baseline Characteristics

The baseline donors' characteristics for the cohort of 333 patients are presented in **Table 1**. The mean donor age was 47.3 $\pm$ 10.6 years old (age range 20.7–76.2 years old), and most were female (71%). The mean body mass index was 25.3 $\pm$ 3.4 Kg/m². Fifty donors (15%) were hypertensive pre-donation, and fifty-one (15%) had smoking habits. Pre-donation mean eGFR was 100.3 $\pm$ 14.7 mL/min/1.73 m², while the mean 1-year post-donation eGFR was 71.4 $\pm$ 16.2 mL/min/1.73 m². The mean predicted 1-year post-donation GFR was 69.1 $\pm$ 10.0 mL/min/1.73 m².

Eighty-five donors (25.5%) reached the definition of CKD at 1-year after donation as depicted in **Table 2**.

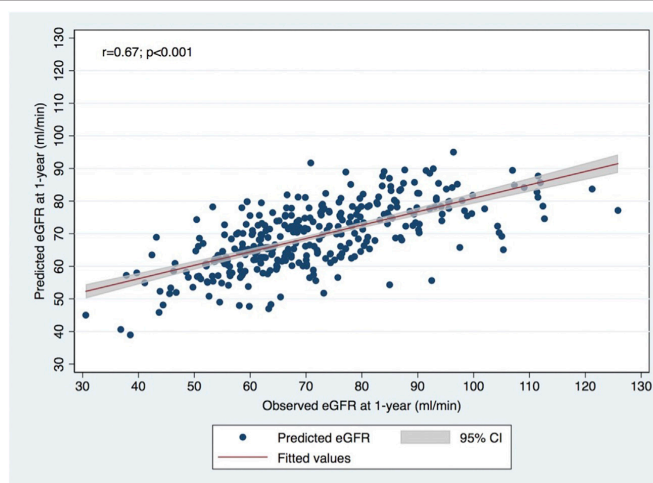
A significant correlation was observed between calculated and observed 1-year eGFR ($p < 0.001$; Pearson $R = 0.67$), as shown in **Figure 1**. The concordance is represented by the Bland-Altman plot with a mean difference of observed-predicted eGFR = $+2.33$ mL/min/1.73 m² (95% limits of agreement -21.41 – 26.47 mL/min/1.73 m²; $p < 0.001$) (**Figure 2**).

Furthermore, the model showed a good discriminative ability of the formula in predicting observed CKD at 1-year post-donation, with the area under the receiver operating characteristic (ROC) curve of 0.83 (95% CI: 0.78–0.88; $p < 0.001$), as shown in **Figure 3**, with optimal cutoff (by Youden criteria) corresponding to a predicted eGFR of 65.25 mL/min/1.73 m² (5.25 mL above the equality cutoff), for which the sensibility and specificity to predict CKD were respectively 77% and 75% (**Table 2**). Overall, the model performance was similar in females and males (data not shown), although the optimal cutoff for the female sex corresponded to 62.23 mL/min/1.73 m² (2.23 mL above the equality cutoff), for which the sensibility and specificity to predict CKD were respectively 66% and 85%. For the male sex, the optimal cutoff was similar to the global cohort, for which the sensibility and specificity to predict CKD were 77% and 82%, respectively.

The Calibration curves illustrated the model's accuracy in the prediction of eGFR < 60 mL/min/1.73 m² at 1 year. The calibration curve, shown in **Figure 4**, exhibited an excellent prediction with a slope = 1.000 and a Calibration In The Large (CITL) = 0.000.

TABLE 2 | ROC: McNemar's exact test for optimal cutoff and for CKD cutoff.

		Observed eGFR		Total
Predicted eGFR	<65.25	<60	≥60	
	≥65.25	65 (76)	61 (25)	
Total		20 (24)	187 (75)	207
		85	248	333
McNemar's exact test $p < 0.001$, Sensitivity 77%, Specificity 75%, PPV 52%, NPV 90%				
Predicted eGFR	<60	40 (47)	17 (7)	57
	≥60	45 (53)	231 (93)	276
Total		85	248	333
McNemar's exact test $p < 0.001$, Sensitivity 47%, Specificity 93%, PPV 70%, NPV 84%				

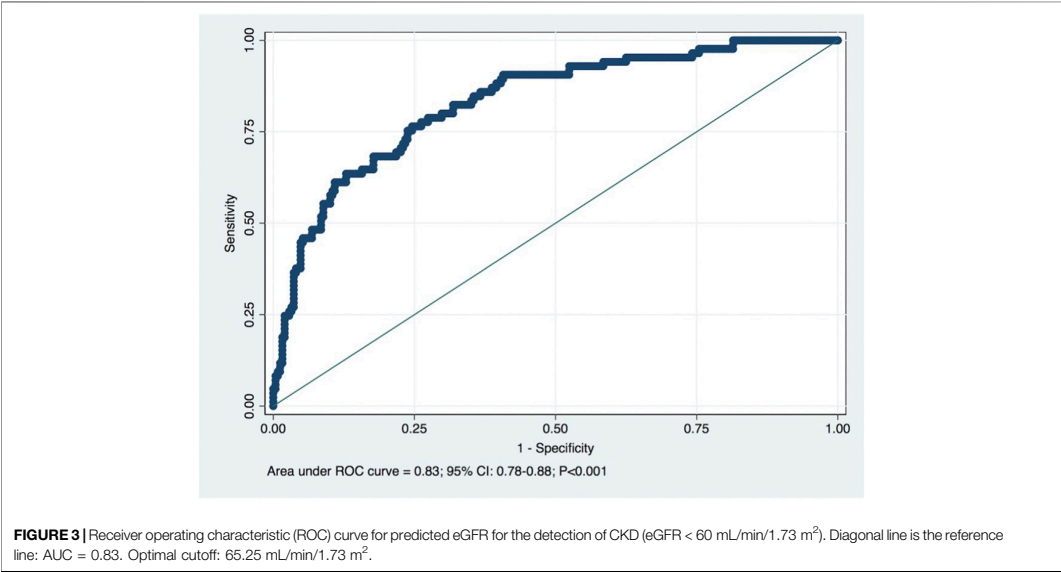
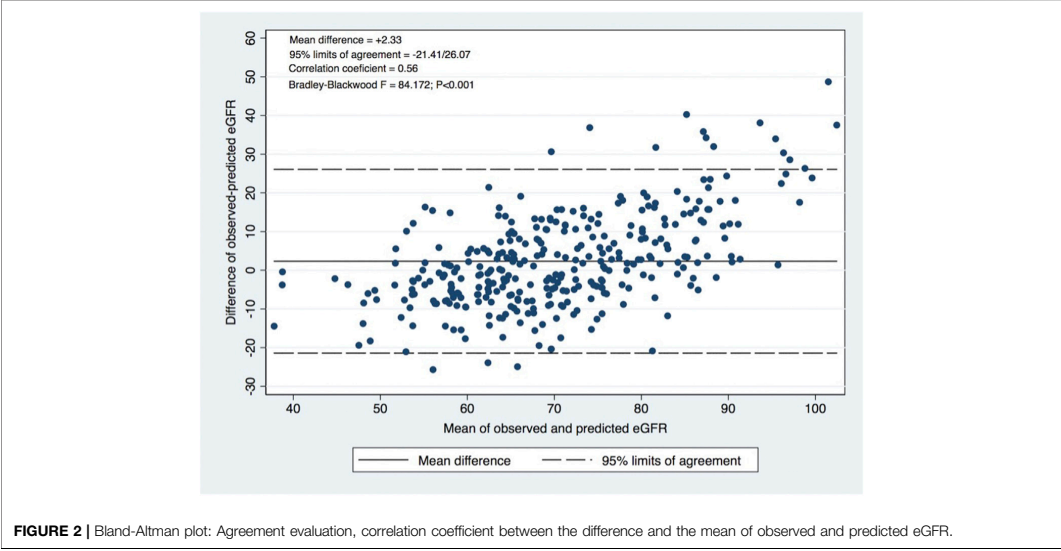
eGFR: mL/min/1.73 m².**FIGURE 1** | Correlation between observed eGFR using CKD-EPI formula at 1-year post-donation and predicted eGFR using the formula developed in Toulouse-Rangueil.

DISCUSSION

In this study, the predictive model developed at Toulouse-Rangueil (11) was validated externally in our cohort of living kidney donors in concordance with other external validation studies in different European populations (12, 13). A significant correlation was observed between calculated and observed 1-year eGFR (Pearson $r = 0.67$), and for the prediction of CKD (eGFR values < 60 mL/min/1.73 m²) at 1 year after donation, the model presented an AUROC of 0.83, which represents an excellent performance. Benoit et al. (13), in a population of 400 French living donors that performed nephrectomy at Necker Hospital, also described a significant correlation between predicted and observed 1-year eGFR (Pearson $r = 0.66$), and for the prediction of CKD at 1 year, the model presented an AUROC of 0.86. We must emphasize that the optimal value of predicted eGFR was around 5 mL/min higher than the equality cutoff for CKD detection at 1 year, an outcome that was

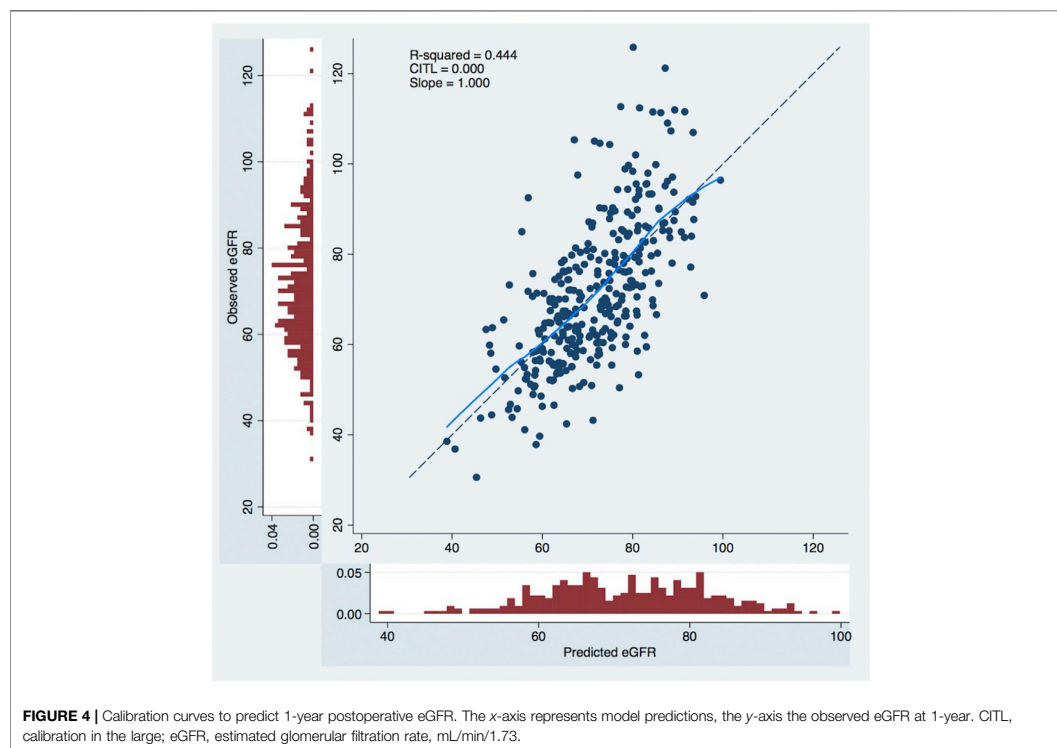
correctly predicted (both its presence and absence) in every 3 out of 4 donors. This tool represents a non-invasive, low cost and readily available tool that can be joined to the living donor evaluation routine consultation, improving the living donor risk estimation and the informed consent process. The predicted eGFR value ≥ 65.25 mL/min was associated with a very high NPV (90%), identifying donors that are clearly admissible concerning renal function (Table 2). Otherwise, a predicted eGFR < 60 mL/min was associated with a high PPV (70%), identifying donors that probably should not be accepted, concerning their renal function. Anyway, a global risk assessment is mandatory (4). An older donor will have a lower 1-year eGFR, and the lower expected lifespan will mitigate a higher chance of CKD, but the expected risk of ESRD compared to a younger donor.

LDKT is considered safe, but some donors will develop CKD. And, rarely, ESRD. Two landmark studies in the living kidney donation (8, 9) made this discussion more pertinent. Furthermore,



the characteristics of our living donors are changing. We are facing a population increasingly older in dialysis, and their potential donors are also older, with an increasing chance of having borderline preoperative eGFR. In this tool, age, and preoperative CKD-EPI eGFR were shown to be independent predictors of 1-year postoperative renal function.

The evaluation of the glomerular filtration rate is a crucial point in LKD. We used eGFR based on serum creatinine determinations because it is feasible and is the most common method worldwide (4). More reliable methods of isotopic evaluation are not routinely available (4). In a large retrospective study, Stevens et al. (16) demonstrated that



CKD-EPI estimates were more accurate than MDRD estimates considering the actual GFR measured by urinary or plasma clearance of exogenous filtration markers. It suggests that the CKD-EPI method must be preferred in the clinical practice (16). Most transplant centers use CKD-EPI equation eGFR in the initial assessment of renal function in potential living kidney donors (5), and it was the method used in the initial description of the model by Benoit et al. (11), although the external validation by Kulik et al. (12) used the MDRD formula to calculate the eGFR pre and after donation.

The risk of ESRD in living donors, although marginal, was evidenced in two studies in comparison with healthy controls (8, 9). As ESRD is a rare event, its surrogates have been pursued by several groups to improve living donor selection and donor safety. CKD, as defined by $\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$, was associated with an increased risk of death, cardiovascular events, and hospitalization in a large, community-based population (10). In a registry-based cohort study of 71,468 living kidney donors, reported an independent association of living kidney donor eGFR at postoperative 6 months and subsequent ESRD. A $10 \text{ mL/min/1.73 m}^2$ difference in early post-donation estimated glomerular filtration rate was significantly associated with a 28% higher risk of subsequent end-stage renal disease (17). However, no significant association has been found with the preoperative

eGFR (17), and no marker could be identified in pre donation evaluation. One-year post-donation eGFR was assessed in this study, and it can be assumed as a surrogate of long-term renal function in the donor. We hypothesize that donors with lower eGFR 1 year after donation would benefit from increased surveillance and further preventive measures for renal health. Considering the global performance of this formula, we can go further and hypothesize that at pre-donation consultation, potential donors with predicted lower 1-year eGFR could be considered unfit to donate, after a global risk assessment, considering donor age and expected lifespan.

Benoit et al. (11) developed a model to estimate the donor's 1-year post-donation eGFR. In this predictive model, Age and preoperative eGFR were shown to be independent predictors of 1-year postoperative renal function. Other donor characteristics like kidney size, gender, hypertension, obesity, dyslipidemia, and smoking were not found to influence the 1-year postoperative eGFR (11). In contrast, a recent retrospective study from Lam et al. (18) evaluated a Canadian cohort of living kidney donors and allowed a better understanding of kidney function over 5 years after living donor nephrectomy. In this study, changes in eGFR after donation varied by sex, percent decline in eGFR within the first 6 weeks after donation, and eGFR category at 1 year, but not by age category at donation, pre-

donation hypertension, pre-donation eGFR category, socioeconomic status, or distance to transplant center (18). Be it as it may, the predictive model developed from the Toulouse-Rangueil cohort unquestionably showed a good correlation between predicted and observed donor eGFR 1-year after donation in 3 different centers (11–13). These results, along with the fact that donor age was found to be a strong predictor of CKD after LDKT, may defer the wish to extend, without fair criticism, the age limit of donors, which has been advocated to expand the pool (19, 20). A global risk assessment must always guide the clinical decision.

At the original cohort (11), 22.4% of donors had CKD at 1-year after donation, meeting KDIGO criteria of CKD (21). Kullik et al. (12), in the external validation in a German cohort, found a surprisingly higher incidence of CKD in their LKD cohort: 70.8%. A careful interpretation is needed as eGFR was calculated using the MDRD formula and not CKD-EPI. Additionally, the authors refer that at least 30% of all living donors preferred external follow-up appointments and were not included in the study. In our population, 25.5% of donors (85 out of 333) reached the definition of CKD, although none had ESRD at long-term follow-up. These donors represent a population that deserves more careful long-term surveillance. Further studies are necessary to evaluate the different trajectories of the long-term evolution of kidney function in these donors. It is recognized that some groups of living donors have a higher long-term risk of ESRD than others. Massie et al. (22) used data from the Scientific Registry of Transplant Recipients of 133,824 living kidney donors in the United States between 1978 and 2015 to construct a risk calculator that includes sex, Age, race, BMI, and first-degree biological relationship. Male sex, black race, older Age in the non-black race, greater body mass index, and first-degree biological relationship to the recipient were associated with increased risk of ESRD (22). Although the predicted 20-year risk of ESRD for the median donor was only 34 cases per 10,000 donors, 1% of donors had predicted risk exceeding 256 cases per 10,000 donors (22). Ibrahim et al. (23) used data from the University of Minnesota from 3,956 White kidney donors between 1963 and 2013. Their calculator estimates ESRD risk in White donors using Age, BMI, and systolic blood pressure at the time of donation (23). ESRD was associated with older age, higher BMI, and higher systolic blood pressure in the donation (23).

Most of our living donors were females (71%). Women are more likely than men to become living kidney donors (24, 25). In a recently published review of country-specific sex disparities in living kidney donation (26), Kurnikowski et al., described a population size-weighted donor distribution consisting of 35.9% men and 64.1% women. This data cannot be explained by a comprehensive reason (24). Biological and sociocultural aspects must be considered. Biological reasons usually described include the sex distributions of some potential biological risk factors for disease, including smoking, and a higher incidence of hypertension and ischemic heart disease that can preclude the acceptance of male candidates more often. Although women have a higher prevalence of chronic kidney disease than men, end-stage renal disease incidence is higher in men (24). Socio-cultural aspects are very significant in most cultures. It is expected that increased altruism from women, is derived from the women's

more traditional role as the caregiver in the family (25–27). The family expectations frequently remain on her to be a living donor, whether it remains on the man to keep working and support the entire family. This is still very common in Portuguese society nowadays, mainly in the rural and less favored communities. The predictive model performance did not differ when both sexes were considered separately, although the optimal cutoff for the prediction of CKD was slightly lower in women.

We must recognize the limitations associated with this study, beginning with its retrospective and observational design. Thirty-one donors were excluded from the study because 1-year serum creatinine was unavailable to calculate eGFR. Still, later creatinine values were available and were not different from the rest of the cohort. We assume it would not compromise the results of our validation cohort. All patients were Caucasians, but they were representative of the Portuguese population. Other races and ethnic origins are not represented. We used CKD-EPI to calculate eGFR and not an isotopic method. However, we must point out the unsuitability of the latter in clinical practice, as it is not recommended as a standard of care by current guidelines (4), and the model itself was developed using the CKD-EPI formula. Although we must be aware of the potential risk of analysis bias judgment of the original model, it should not preclude the results of this and the other external validation results.

The primary goal in assessing a living donor candidate must ensure minimal risk to the donor. Hence, the prediction of postoperative renal function is a critical point in their evaluation and, in our population, can be achieved with this tool. Furthermore, the required variables are low-cost and easily assessed, so its potential as a counseling tool is undeniable. We recall, however, that validation out of Europe is lacking and that further studies are necessary to validate prognostic models for longer-term prediction of donor kidney function.

CONCLUSION

The formula developed in Toulouse-Rangueil was successfully validated in our cohort, a different European population than previously described. We must, anyway, emphasize that the optimal value of predicted eGFR was around 5 mL/min higher than the equality cutoff for CKD detection at 1 year. This model represents a simple and accurate tool that may be used to assist in the evaluation of potential donors, particularly in the current setting of increasing donor age, donors with minor comorbidities, or renal function close to the accepted threshold.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics board of Centro Hospitalar Universitário

do Porto (CHUPorto) [Ref.: 147-21 (119-DEFI/122-CE)]. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MA, CC, and JM: Research design, data acquisition, data analysis, and paper writing. CS, CF, SV, JS, SC, and SP were engaged in the data acquisition and analysis. MR and LM were involved in the research design and data analysis. All the authors approved the submitted version.

REFERENCES

1. Gruessner RWG, Gruessner AC. Solid-organ Transplants from Living Donors: Cumulative United States Experience on 140,156 Living Donor Transplants over 28 Years. *Transpl Proc* (2018) 50(10):3025–35. doi:10.1016/j.transproceed.2018.07.024
2. Park YH, Min SK, Lee JN, Lee HH, Jung WK, Lee JS, et al. Comparison of Survival Probabilities for Living-Unrelated versus Cadaveric Renal Transplant Recipients. *Transpl Proc* (2004) 36(7):2020–2. doi:10.1016/j.transproceed.2004.08.122
3. Meier-Kriesche HU, Kaplan B. Waiting Time on Dialysis as the Strongest Modifiable Risk Factor for Renal Transplant Outcomes: a Paired Donor Kidney Analysis. *Transplantation* (2002) 74(10):1377–81. doi:10.1097/00007890-200211270-00005
4. Lentine KL, Kasiske BL, Levey AS, Adams PL, Alberu J, Bakr MA, et al. Summary of Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* (2017) 101(8):1783–92. doi:10.1097/TP.0000000000001770
5. Andrews PA, Burnapp L. British Transplantation Society/Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: Summary of Updated Guidance. *Transplantation* (2018) 102(7):e307. doi:10.1097/TP.0000000000002253
6. Reese PP, Caplan AL, Kesselheim AS, Bloom RD. Creating a Medical, Ethical, and Legal Framework for Complex Living Kidney Donors. *Clin J Am Soc Nephrol* (2006) 1(6):1148–53. doi:10.2215/CJN.02180606
7. Reese PP, Boudville N, Garg AX. Living Kidney Donation: Outcomes, Ethics, and Uncertainty. *Lancet* (2015) 385(9981):2003–13. doi:10.1016/S0140-6736(14)62484-3
8. Muzaale AD, Massie AB, Wang MC, Montgomery RA, McBride MA, Wainright JL, et al. Risk of End-Stage Renal Disease Following Live Kidney Donation. *JAMA* (2014) 311(6):579–86. doi:10.1001/jama.2013.285141
9. Mjoen G, Hallan S, Hartmann A, Foss A, Midtvedt K, Oyen O, et al. Long-term Risks for Kidney Donors. *Kidney Int* (2014) 86(1):162–7. doi:10.1038/ki.2013.460
10. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic Kidney Disease and the Risks of Death, Cardiovascular Events, and Hospitalization. *N Engl J Med* (2004) 351(13):1296–305. doi:10.1056/NEJMoa041031
11. Benoit T, Game X, Roumiguie M, Sallusto F, Doumerc N, Beauval JB, et al. Predictive Model of 1-year Postoperative Renal Function after Living Donor Nephrectomy. *Int Urol Nephrol* (2017) 49(5):793–801. doi:10.1007/s11255-017-1559-1
12. Kulik U, Gwiada J, Oldhafer F, Kaltenborn A, Arelin V, Guelier F, et al. External Validation of a Proposed Prognostic Model for the Prediction of 1-year Postoperative eGFR after Living Donor Nephrectomy. *Int Urol Nephrol* (2017) 49(11):1937–40. doi:10.1007/s11255-017-1683-y
13. Benoit T, Prudhomme T, Adypagavane A, Malavaud B, Soulie M, Game X, et al. External Validation of a Predictive Model to Estimate Renal Function after Living Donor Nephrectomy. *Transplantation* (2021) 105(11):2445–50. doi:10.1097/TP.0000000000003643
14. Collins GS, Reitsma JB, Altman DG, Moons KGM. Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD). *Ann Intern Med* (2015) 162(10):735–6. doi:10.7326/L15-5093-2

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

15. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* (2009) 150(9):604–12. doi:10.7326/0003-4819-150-9-200905050-00006
16. Stevens LA, Schmid CH, Greene T, Zhang YL, Beck GJ, Froissart M, et al. Comparative Performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study Equations for Estimating GFR Levels above 60 mL/min/1.73 M2. *Am J Kidney Dis* (2010) 56(3):486–95. doi:10.1053/j.ajkd.2010.03.026
17. Massie AB, Holscher CM, Henderson ML, Fahmy LM, Thomas AG, Al Ammary F, et al. Association of Early Postdonation Renal Function with Subsequent Risk of End-Stage Renal Disease in Living Kidney Donors. *JAMA Surg* (2020) 155(3):e195472. doi:10.1001/jamasurg.2019.5472
18. Lam NN, Lloyd A, Lentine KL, Quinn RR, Ravani P, Hemmelgarn BR, et al. Changes in Kidney Function Follow Living Donor Nephrectomy. *Kidney Int* (2020) 98(1):176–86. doi:10.1016/j.kint.2020.03.034
19. Serrano OK, Yadav K, Bangdiwala A, Vock DM, Dunn TB, Finger EB, et al. Age Alone Is Not a Contraindication to Kidney Donation: Outcomes of Donor Nephrectomy in the Elderly. *Clin Transpl* (2018) 32(8):e13287. doi:10.1111/ctr.13287
20. Klop KW, Dols LFC, Weimar W, Dooper IM, Ijzermans JNM, Kok NFM. Quality of Life of Elderly Live Kidney Donors. *Transplantation* (2013) 96(7):644–8. doi:10.1097/TP.0b013e31829e6d9b
21. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the Scenes, Need for Guidance, and a Framework for Moving Forward. *Kidney Int* (2014) 85(1):49–61. doi:10.1038/ki.2013.444
22. Massie AB, Muzaale AD, Luo X, Chow EKH, Locke JE, Nguyen AQ, et al. Quantifying Postdonation Risk of ESRD in Living Kidney Donors. *J Am Soc Nephrol* (2017) 28(9):2749–55. doi:10.1681/ASN.2016101084
23. Ibrahim HN, Foley RN, Reule SA, Spong R, Kukla A, Issa N, et al. Renal Function Profile in White Kidney Donors: The First 4 Decades. *J Am Soc Nephrol* (2016) 27(9):2885–93. doi:10.1681/ASN.2015091018
24. Katz-Greenberg G, Shah S. Sex and Gender Differences in Kidney Transplantation. *Semin Nephrol* (2022) 42(2):219–29. doi:10.1016/j.semnephrol.2022.04.011
25. Carrero JJ, Hecking M, Chesnaye NC, Jager KJ. Sex and Gender Disparities in the Epidemiology and Outcomes of Chronic Kidney Disease. *Nat Rev Nephrol* (2018) 14(3):151–64. doi:10.1038/nrneph.2017.181
26. Kurnikowski A, Krenn S, Lewandowski MJ, Schwaiger E, Tong A, Jager KJ, et al. Country-specific Sex Disparities in Living Kidney Donation. *Nephrol Dial Transpl* (2022) 37(3):595–8. doi:10.1093/ndt/gfab305
27. Brar A, Markell M. Impact of Gender and Gender Disparities in Patients with Kidney Disease. *Curr Opin Nephrol Hypertens* (2019) 28(2):178–82. doi:10.1097/MNH.0000000000000482

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APPENDIX 3 – CT volumetry performs better than nuclear renography in predicting estimated renal function one year after living donation.

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CT volumetry performs better than nuclear renography in predicting estimated renal function one year after living donation

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Abstract

The evaluation of split renal function (SRF) is a critical issue in living kidney donations and can be evaluated using nuclear renography (NR) or computerized tomography (CT), with unclear comparative advantages. We conducted this retrospective study in 193 donors to examine the correlation of SRF assessed by NR and CT volumetry and compared their ability to predict remaining donor renal function at 1 year, through multiple approaches. A weak correlation between imaging techniques for evaluating the percentage of the remaining kidney volume was found in the global cohort, with an $R^2 = 0.15$. However, the Bland–Altman plot showed an acceptable agreement (95% of the difference between techniques falling within -8.51 to 6.11%). The predicted and observed eGFR one year after donation were calculated using the CKD-EPI, and CG/BSA equations. CT volume showed a better correlation than NR for both formulas (adjusted R^2 of 0.42, and 0.61 vs 0.37 and 0.61 for CKD-EPI and CG/BSA equations, respectively). In non-nested modeling tests, CT volumetry was significantly superior to NR for both equations. CT volumetry performed better than NR in predicting the estimated renal function of living donors at 1-year, independently from the eGFR equation.

Keywords Split renal function · Split renal volume · CT scan volumetry · Nuclear renography

Abbreviations

ACR Acute cellular rejection
BMI Body mass index
BSA Body surface area

GFR Glomerular filtration rate
CG Cockcroft-Gault
CKD-EPI Chronic kidney disease epidemiology collaboration
CT Computerized tomography
CrC Creatinine clearance
DTPA Diethylenetriamine-pentaacetate
eGFR Estimated glomerular filtration rate
ESRD End-stage renal disease
GFR Glomerular filtration rate
HR Hazard ratio
IQR Interquartile range
MAG-3 Mercapto-acetyltriglycine
MDRD Modification of diet in renal disease
RRT Renal replacement therapy
SD Standard deviation
SRF Split renal function

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Introduction

End-stage renal disease (ESRD) is a global health problem [1]. Living donor kidney transplants represent the best treatment for ESRD, with increased graft and patient survival compared to deceased kidney transplants [2]. Living donor nephrectomy is considered a safe procedure with low risk, and long-term follow-up data of donors are reassuring [3, 4]. However, two recent landmark studies showed an increased risk of ESRD in kidney donors compared with matched healthy non-donors [5, 6]. Additionally, the increasing shortage of organs for transplantation led to the acceptance of donors with minor abnormalities, usually referred to as “complex kidney donors” [7]. The absence of high-quality studies with long-term follow-up makes the safety of kidney donors an important issue. Defining precise metrics for accepting or discarding a given donor is currently a significant challenge for the transplant community.

Evaluation of living kidney donors is a complex and multidisciplinary task. Laboratory and imaging techniques complement medical and psychosocial assessments. This is a longstanding process [8], and most potential living donors end the process without becoming actual donors. Simplifying living donor evaluation and eliminating unnecessary examinations can increase its success if it does not compromise donor safety or the quality of the global process.

A particular focus of this process is the evaluation of renal function to predict the remaining renal function in the donor and estimated graft function in the recipient. Although the urinary clearance of an “ideal” filtration marker is considered the “gold standard” for the measurement of glomerular filtration rate (GFR), it is unsuitable for clinical practice [9]. Most Eurotransplant centers use creatinine-clearance (64%) to measure total renal function and radioisotope methods (82%) to assess the split renal function (SRF) [10]. In addition, radiological techniques, such as computed tomography (CT) and ultrasound, are used to analyze kidney anatomy. If there is a significant asymmetry, usually considered > 10% of SRF difference between both kidneys, the kidney with the lower function should be preferred for donation, even if it has anatomical variability [9].

Kidney volume has been proven to be a surrogate marker of nephron mass and renal function in living donors [11]. Evaluating renal volume using CT volumetry is routine in several transplant centers [2]. A recent meta-analysis suggested that split renal volume has the potential to replace split renal function in some candidates, eliminating this test from the evaluation process [12], and current evidence suggests [13] that CT volumetry should be

preferred mainly when discordance is found between the two imaging modalities. However, it is uncertain whether it can do so reliably and routinely across transplant centers, and global recommendations remain unclear.

We reviewed the practice at our center with the hypothesis that relative kidney volume determined by CT can be used as a substitute for SRF, as determined by nuclear renography, eliminating the need for additional tests in some potential donors. Hence, we assessed the correlation between imaging techniques in the evaluation of SRF and compared their ability to predict remaining kidney function following living donor nephrectomy.

Materials and methods

We retrospectively reviewed the clinical data of all the donors who underwent nephrectomy for living donor kidney transplantation at our institution between January 2008 and December 2017 ($n = 210$). After excluding 17 donors (10 without CT scan images available, one without nuclear renography evaluation, and another 6 in whom 1 year post-donation eGFR was lacking), the remaining 193 recipients defined our study cohort. The Institutional Review Board at Centro Hospitalar Universitário Porto (CHUPorto) approved this retrospective observational study, conducted according to the Helsinki Declaration.

Following international guidelines, all donors were subjected to a standard evaluation protocol. Baseline demographic, anthropomorphic, analytical, and clinical data were collected from the living kidney donors. Serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), and Cockcroft-Gault adjusted for body surface area (CG-BSA) equations were used to evaluate eGFR before and one year after donation. Creatinine clearance (CrCl) in a 24 h pre-donation urinary sample was available for all donors.

SRF was assessed by nuclear renography with technetium-labeled diethylenetriamine-pentaacetate (Tc99m-DTPA) using a simplified, standardized protocol with dynamic image acquisition in the supine position according to international guidelines [14]. Adequate hydration was ensured in all the donors. The clearance of radionuclides was used as a measure of GFR. Split renal function was evaluated based on each kidney's contribution to global renal function.

For anatomical evaluation, all living donors underwent one of two multidetector-row CT scans available at our institution (64-detector GE VCT LightSpeed™ or 16-detector GE BrightSpeed™). Images were obtained before and after contrast to evaluate the nephrographic and excretory phases of enhancement. Kidney volumes were retrospectively assessed using CT scans with the

same image-acquisition protocols. Volumes were measured using the voxel counting technique (the sum resulting from tracing the renal contours in sequential 2.5 mm transversal CT nephrographic images, excluding the renal sinus area) using the Osirix software (Pixmeo Sarl, Geneva, Switzerland). Both kidneys were evaluated separately. One surgeon performed all the evaluations. The SRF was defined as the percentage of the total renal volume for each kidney.

The preoperative eGFR from each equation was multiplied by the kidney split renal function percentage for each imaging technique to determine the predicted remaining renal function after nephrectomy. The estimated GFR at one year was also calculated using the CG-BSA, and CKD-EPI equations.

Procurement of the left kidney was preferred to balance the lengths of the renal vein and artery and facilitate anastomoses on the corresponding external iliac vessels, except in rare cases of anatomic variations or significant left kidney SRF.

Continuous data were described using mean and standard deviation (SD) or median (interquartile range [IQR]), and categorical data were expressed as numbers (and percentages) as appropriate.

First, we calculated the difference between the right and left kidneys using each imaging technique. Cohen's kappa test was then applied to measure agreement between the methods. Full agreement was defined as a difference between -5 to $+5\%$ in both techniques. We weighed the kappa test to deal with disagreement by assigning less weight to agreement categories that were further apart (i.e., giving more weight to the agreement, less weight to the partial agreement, and the smallest weight to no agreement) between techniques. A kappa score of 1 denotes perfect agreement, whereas a kappa score of 0 denotes chance agreement. The difference between the observed and chance agreements was considered significant if the p -value was less than 0.05.

We then analyzed the correlation (by linear regression) and agreement (by Bland–Altman plot) between the remaining kidneys' CT volumetry and nuclear renography-based SRF. Moreover, univariate and multivariable linear regression explored the correlation of predicted renal function according to each imaging modality and eGFR equation and observed renal donor function at 1 year post-donation. Multivariate analyses were adjusted for age, sex, laterality of the remaining kidney, and body mass index (BMI).

We subsequently performed non-nested modeling to compare the ability of each imaging technique to predict the residual donor kidney function. Non-nested tests assume that one model fits the observed data more closely. A significant P -value of a non-nested test indicates that the alternative model has a better fit. R^2 compared non-nested models:

root mean square error, J-test, and Cox–Pesaran test. The R^2 values were adjusted for degrees of freedom, in that they accounted for the number of explanatory terms in the model.

Finally, a sensitivity analysis was performed considering the agreement between the SRF techniques for the remaining kidneys, which allowed the definition of three groups (agreement, higher SRF of the remaining kidney by nuclear renography, and higher SRF of the remaining kidney by CT scan). These were included in three distinct multivariable linear regression models that determined their correlation with renal donor function at 1 year post-donation per eGFR equation evaluated.

A 2-sided P -value < 0.05 was considered as statistically significant. Statistical calculations were performed using STATA/MP, version 15.1 (Stata Corp, College Station, TX, USA).

Results

The baseline characteristics of the patients are shown in Table 1. The median age of donors was 48.9 (40.6–56.2) years, and most were female (74%). The median BMI was 25.2 (22.7–28.0). The mean pre-donation eGFR was 100.4 ± 13.9 ml/min/1.73 m² by CKD-EPI, and 106.7 ± 21.9 ml/min/1.73 m² by CG BSA adjusted. Pre-donation 24 h creatinine clearance was 128.3 ± 29.5 ml/min/1.73 m², higher than eGFR by any of the formulas, although the difference was lowest with eGFR by the CG BSA-adjusted equation. In most cases, the left kidney was donated ($n = 158$, 82%).

Split renal function of the remaining kidney by CT-scan was $49.9 \pm 3.3\%$ and by nuclear renography was $51.1 \pm 3.4\%$. Predicted and estimated post-donation renal function at 12 months after donation are depicted in Table 1, according to the imaging technique and the formula used to evaluate eGFR. The eGFR after donor nephrectomy was higher than the predicted renal function measured for every imaging technique and the eGFR equation.

The SRF agreement between CT volumetry and nuclear renography is presented in Table 2. Considering the difference in split function between right and left kidneys between -5 and $+5\%$, we observed complete agreement between techniques in 106 donors (55%). In the other donors, we found disagreement between methods with a higher split function (R-L) by nuclear renography in 57 donors and CT volumetry in 30 donors. The kappa test showed a weighted agreement of 75%, expected agreement of 68%, and kappa of 0.259.

When right (Supplemental Table 1) and left (Supplemental Table 2) remaining kidneys were assessed separately, the observed complete agreement was, respectively, 57% (kappa 0.272) and 46% (Kappa 0.127). The worst agreement was

Table 1 Baseline characteristics of living kidney donors

	N = 193
Age, median (IQR)	48.9 (40.6–56.2)
Sex F:M, n (%)	142 (74):51 (26)
BMI, median (IQR)	25.2 (22.7–28.0)
Estimated pre-donation renal function (ml/min/1.73 m ²), mean ± SD	
CKD-EPI	100.4 ± 13.9
CG (BSA adjusted)	106.7 ± 21.9
Split function of remaining kidney by CT (%), mean ± SD	49.9 ± 3.3
Split function of remaining kidney by nuclear renography: (%), mean ± SD	51.1 ± 3.4
Predicted post-donation renal function (ml/min/1.73 m ²), mean ± SD	
CKD-EPI CT	50.0 ± 7.1
CKD-EPI NR	51.2 ± 7.5
CG CT	53.2 ± 11.3
CG NR	54.4 ± 11.0
Pre-donation 24 h creatinine clearance (ml/min/1.73 m ²), mean ± SD	128.3 ± 29.5
Predicted post-donation 24 h creatinine clearance (ml/min/1.73 m ²), mean ± SD	
CT	64.0 ± 14.9
NR	65.4 ± 15.0
Left kidney donated, n (%)	158 (82)
Estimated donor renal function at 12-months (ml/min/1.73 m ²), mean ± SD	
CKD-EPI	71.2 ± 14.5
CG (BSA adjusted)	74.7 ± 15.3

Table 2 Agreement between techniques of SRF

Nuclear renography: difference in split function between right and left kidneys (R–L)	CT volumetry: difference in split function between right and left kidneys (R–L)			
	< – 5%	– 5 to 5%	> 5%	Total
< – 5%	16	19	2	37
– 5 to 5%	28	72	9	109
> 5%	8	21	18	47
Total	52	112	29	193

Observed full agreement: 106 (55%)

Reclassification for a more positive difference in split function (R–L) by CT volumetry: 30 (16%)

Reclassification of a more positive difference in split function (R–L) by nuclear renography: 57 (30%)

Weighted agreement: 75%, Expected agreement: 66%, Kappa: 0.259

found in the smaller group of donors with the remaining left kidney. Procurement of the left kidney was preferred, except in rare cases of anatomic variations or significant left kidney SRF. This represented a small proportion of our donors. Considering the influence of the depth of the kidneys on the variance of nuclear scintigraphy results and the fact that a lower number of cases implies more heterogeneity, we could assume those effects on the results.

The linear regression analysis has shown a weak correlation between imaging techniques for evaluating the percentage of remaining kidney volume with an $R^2 = 0.15$ for the global cohort (Fig. 1).

The Bland–Altman plot was used to assess the agreement between the two different clinical measures, assuming

neither was perfect: CT-based vs. nuclear renography-based determinations of SRF of the remaining kidney (Fig. 2). This analysis suggested a good clinical agreement, with 95% of the difference between techniques falling within – 8.51 to 6.11%. The mean difference was – 1.2%. On average, nuclear renography measures 1.2% more than CT scan for SRF of the remaining kidney, according to the use of nuclear renography to decide on the nephrectomy side.

The predicted and observed eGFR were calculated using the CKD-EPI, and CG-BSA equations. The univariate linear regressions of the predicted and observed eGFR according to the imaging technique and eGFR equation are shown in Fig. 3.

Fig. 1 Correlation between CT volumetry and nuclear renography for the determination of the remaining kidney volumes

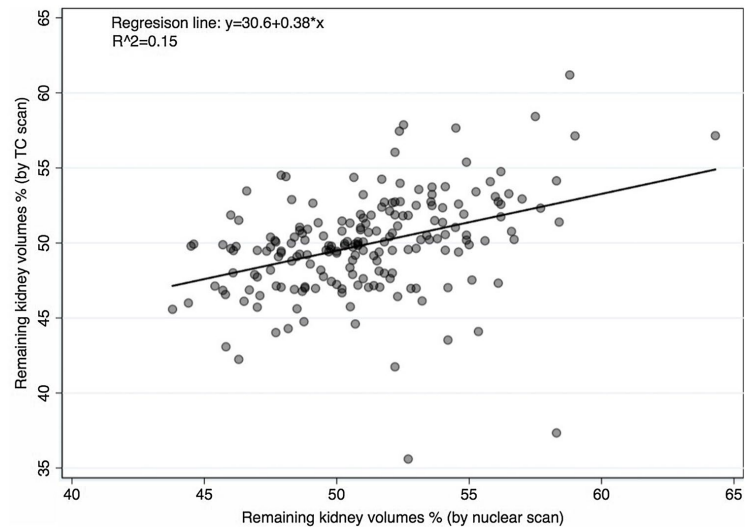
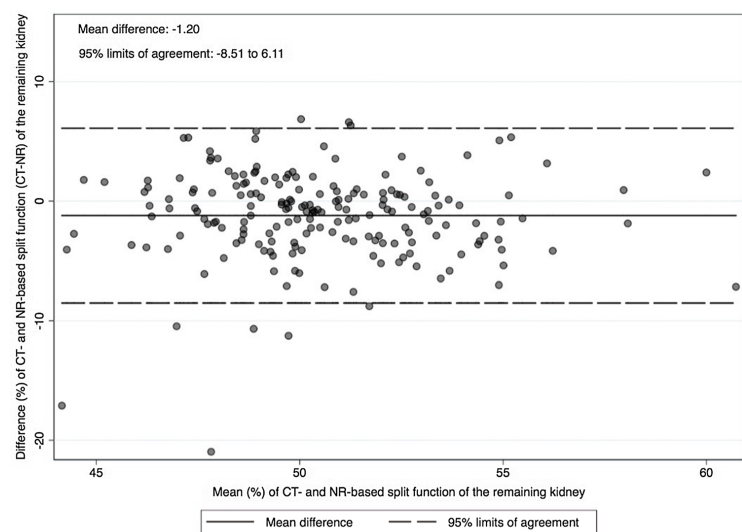


Fig. 2 The Bland–Altman plots with percentage SRF analysis between CT volumetry versus nuclear renography



Multivariate linear regression analysis of the predicted and observed eGFR was performed for both imaging techniques, adjusted for age, sex, laterality of the remaining kidney, and BMI (Table 3).

The results of both imaging techniques correlated with the eGFR at one year. However, CT volume SRF showed a better correlation than nuclear renography for both formulas

tested (adjusted R^2 of 0.42, and 0.61 vs. 0.37, and 0.61 for CKD-EPI, and CG-BSA equations). Interestingly, the best correlation was found with eGFR using the CG-BSA adjusted formula.

We then used non-nested modeling to compare imaging techniques to predict renal donor function one year after donation. Competing models: 1-nuclear renography and

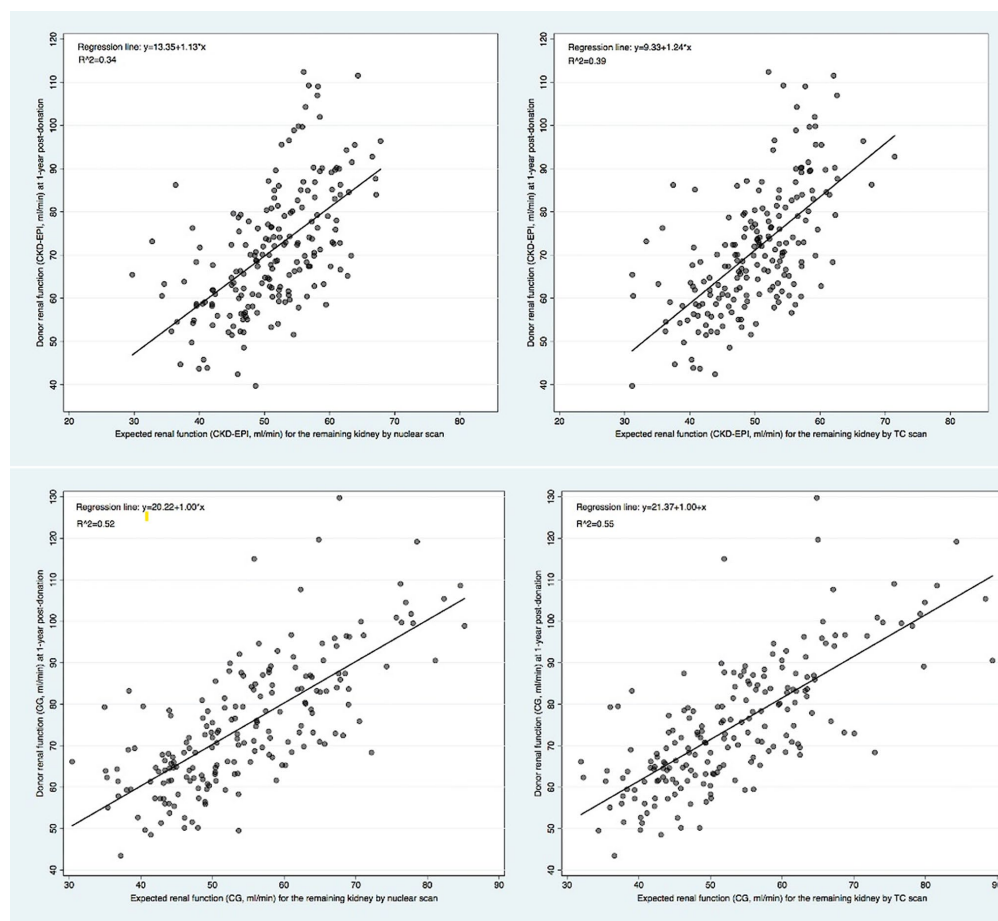


Fig. 3 Correlation between expected and observed eGFR one year after donation for nuclear renography (left column images) and CT volumetry

Table 3 Multivariable linear regression analysis of renal donor function at 1-year post-donation, according to each imaging modality prediction

Formula used	Imaging modality	Coefficient of renal function for every 10 ml/min predicted (95% CI)	<i>P</i>	Adjusted <i>R</i> ²	rMSE
CKD-EPI	Nuclear scan	9.56 (7.08–12.05)	<0.001	0.37	11.52
	CT volume	10.98 (8.51–13.45)	<0.001	0.42	11.09
CG	Nuclear scan	7.52(6.01–9.02)	<0.001	0.60	9.71
	CT volume	7.71 (6.25–9.17)	<0.001	0.61	9.51

Adjusted for age, sex, laterality of remaining kidney, and BMI

two CT-scan volumetry were considered (Supplemental Table 3). The null hypothesis of Model 1 was rejected in

all tests ($P < 0.05$), but we failed to reject the null hypothesis that Model 2 was superior ($p > 0.05$). We concluded

that CT volumetry was superior to nuclear renography in predicting donor renal function at 1-year post-donation for both equations (CKD-Epi equation: J-test P -value < 0.001 , Cox-Pesaran Test P value < 0.01 ; CG-BSA: J-test P value 0.002 , Cox-Pesaran Test P -value < 0.01).

Additionally, we performed a sensitivity multivariable linear regression analysis of renal donor function at one-year post-donation, according to the agreement between the SRF techniques of the remaining kidneys (Table 4). The group with complete agreement was considered as the reference in this analysis. Models 1 and 3 showed that a higher SRF predicted by nuclear renography was significantly associated with a lower one-year observed remaining function. In contrast, in Model 2, this observation was only significant for the CKD-EPI-based eGFR, and trended in the same direction for CG-BSA equation.

Discussion

The current protocol for renal imaging evaluation of potential living donors at our institution includes a CT scan for anatomical assessment and nuclear renography to assess SRF for all candidates. The decision of the kidney to donate and accept the donor depends on these results, with the primary goal of protecting and benefiting the donor. Thus, the correct identification of the SRF is critical. Our study supports using relative kidney volume determined by CT as a surrogate for SRF, as determined by nuclear renography, potentially eliminating the need for additional tests in some donors [10]. CT volumetry correlated better with one-year remaining kidney eGFR, mainly through the CG-BSA equation (Fig. 3). In multivariate analysis, CT volumetry performed better as a predictor of one-year donor eGFR (Table 3) and performed better in non-nested modeling, comparing both imaging techniques (Supplemental Table 3).

Table 4 Multivariable linear regression analysis of renal donor function at 1-year post-donation, according to split function agreement between CT volumetry and nuclear renography and remaining kidney—model 1, 2 and 3

Formula used	Group	Coefficient (95% CI)	P
<i>Model 1</i>			
CKD-EPI	Full agreement	Ref	
	Higher SF by CT	$-3.10 (-8.60 \text{ to } 2.39)$	0.266
	Higher SF NR	$-5.32 (-9.52 \text{ to } -1.12)$	0.013
CG	Full agreement	Ref	
	Higher SF by CT	$-2.78 (-7.76 \text{ to } 2.21)$	0.273
	Higher SF NR	$-4.86 (-8.67 \text{ to } -1.05)$	0.013
<i>Model 2</i>			
Formula used	Group	Coefficient (95% CI)	P
CKD-EPI	Full agreement	Ref	
	Higher SF by CT	$-0.06 (-4.66 \text{ to } 4.54)$	0.979
	Higher SF NR	$-4.25 (-7.74 \text{ to } -0.76)$	0.017
CG	Full agreement	Ref	
	Higher SF by CT	$-1.24 (-5.23 \text{ to } 2.75)$	0.539
	Higher SF NR	$-3.02 (-6.08 \text{ to } 0.04)$	0.053
<i>Model 3</i>			
Formula used	Group*estimated pre-donation renal function (1-way interaction)	Coefficient (95% CI)	P^*
CKD-EPI	Full agreement*eGFR pre-donation	0.63 (0.50–0.77)	Ref
	Higher SF by CT*eGFR pre-donation	0.63 (0.48–0.77)	0.710
	Higher SF NR*eGFR pre-donation	0.59 (0.45–0.73)	0.010
CG	Full agreement*eGFR pre-donation	0.40 (0.33–0.48)	Ref
	Higher SF by CT*eGFR pre-donation	0.38 (0.30–0.47)	0.284
	Higher SF NR*eGFR pre-donation	0.37 (0.29–0.45)	0.025

Model 1 covariates: age, sex, laterality of remaining kidney, and BMI

Model 2 covariates: age, sex, laterality of remaining kidney, BMI, and estimated pre-donation renal function

Model 3 covariates: age, sex, laterality of remaining kidney, BMI

* P value from the Wald test comparing the equality of each coefficient with the reference value

Furthermore, in the multivariate sensitivity analysis, our previous data were supported; in the groups without agreement between methods, a higher remaining kidney SRF by nuclear renography was associated with worse one-year observed remaining kidney function (Table 4). Our results are in line with recently published work from Eum et al. [13] that compared concordant and discordant subgroups according to CT volumetry and nuclear renography results and found a better predictive value of kidney function at several points after donation for CT volumetry. Donor safety is paramount during this major surgery.

Nuclear renography is a method for determining the SRF at most centers, although it is time-consuming and exposes the donor to radioisotopes. Moreover, renography results have been shown to have a relatively wide range of average values because of anatomical variations in kidney location, patient body type, state of diuresis, and operator-dependent variability [15, 16]. Geometric mean images from combined anterior and posterior views offer a more accurate and precise split renal function assessment [17], but these techniques are not widely used. Finally, the radionuclide trace used is variable; Tc-99 m-mercaptoacetyltyrosine (MAG3) and Tc-99 m-DTPA scans are often used based on in-center availability and experience.

The iodinated contrast material used for the CT scan is excreted by the kidneys, mainly by passive glomerular filtration. The plasma clearance of contrast has been used to evaluate renal function [18]. More recent studies evaluated the use of CT scan calculated split renal volume (SRV) as a measure of SRF, considering kidney volume as a surrogate marker of nephron mass and renal function in living donors. Most studies have shown a strong correlation between these two imaging techniques. Several methods have been described for volumetric evaluation. Total parenchymal and renal cortex volume evaluations have both been described. In some studies, only selected donors with asymmetric image evaluation by CT scan or renal ultrasonography were evaluated by nuclear renography [10, 12, 19–21]. Halleck et al. [10], in a retrospective evaluation of 167 consecutive living kidney donors, [10] found a strong correlation between CT-measured split cortex volume (3D reconstruction volume calculation) and MAG3-measured split renal function ($R=0.93$; $P<0.001$). The correlation between SRV and remaining renal function (CG) was also significant ($R=0.83$; $P<0.001$), as was the case for the recipient ($R=0.75$; $P<0.001$) [10].

Wahba et al. [19] evaluated three CT volumetry techniques (modified ellipsoid volume [MELV], smart region of interest [ROI] volume, and renal cortex volume [RCV]) in 101 LKD to calculate the SRF and compared the results with the MAG3 scan. RCV was determined to be the most accurate technique for pre-donation SRF and allowed reliable prediction of the postoperative renal function of the remaining kidney in the donor.

Mitsui et al. [20] assessed the renal cortex and parenchymal volume in 34 Japanese donors using automated CT volumetry and found a strong correlation with MAG3 measured SRF (cortex, $R=0.921$; parenchyma, $R=0.942$). Additionally, eGFR measured at any point after donation (3, 6, and 12 months) correlated with SRF measured by MAG3 and cortex or parenchymal volume. They suggested that parenchymal volumetry might sufficiently predict the eGFR after donation in healthy individuals. For technical reasons, we could not evaluate the cortical volume of our donors, but it should not preclude our results. Overall, these results agree with the data presented herein.

Our study found only a weak correlation of both imaging techniques for evaluating the percentage of remaining kidney volume with an $R^2=0.15$ for the global cohort (Fig. 1). We did not find an apparent reason for the low correlation, but we hypothesized that the technical aspects of imaging acquisition, processing, and interpretation could explain some differences. The volumes of the kidneys (all parenchyma) were evaluated retrospectively, through the voxel counting technique, semi-automatically, using volume analyzer software, and the same surgeon performed all volume measures. In contrast, different operators have conducted nuclear renography studies over a long period. Habbous et al. [12], in a retrospective review of 115 donors, also found a weak correlation between split renal volume (ellipsoid method) and SRF by nuclear renography ($R=0.22$ – 0.28), regardless of the technician who evaluated the volumes. Barbas et al. [22] used automated software to measure parenchyma volume through 3D reconstruction volume calculation, showing only a modest correlation compared to nuclear renography with Tc99m-DTPA (left kidney $R^2=26.2\%$, right kidney $R^2=26.7\%$). In addition, an acceptable clinical agreement between techniques was shown in the Bland–Altman analysis (Fig. 2), with 95% limits of agreement from -8.51 to 6.11% . The mean difference is -1.2% . On average, nuclear renography measures 1.2% more than CT scan for SRF of the remaining kidney, as expected in a population where nuclear renography was the main factor for the decision on the side of nephrectomy.

To evaluate the predictive ability of each imaging technique for remaining kidney function, we used eGFR based on serum creatinine levels because it is feasible and the most common method worldwide [9]. In epidemiological studies, the CKD-EPI equation has been shown to perform a more pertinent CKD diagnosis and staging [23], and most published studies have used it. We presented the results of eGFR using the CG-BSA-adjusted in addition to the CKD-EPI formula, as none was validated in a living donor population and CG is representative of 24 h creatinine clearance, which remains an important metric in the living donor evaluation [9], a not very heterogeneous population with median weights. In our study, both imaging techniques correlated with eGFR at one year, although CT volume showed a better

correlation than nuclear renography for both formulas (Fig. 3). The results were similar in the multivariable linear regression analysis of renal donor function at 1-year post-donation, according to each imaging modality prediction adjusted for age, sex, laterality of the remaining kidney, and BMI (Table 3). Surprisingly, the best correlation was found with eGFR using the modified CG-BSA formula for both imaging techniques. Although it is the only formula that considers donor weight, we adjusted it for BSA. In agreement with our results, Rule et al. [24] found a better correlation between eGFR using the CG equation and iodothalamate GFR ($R=0.35$) than between eGFR using the MDRD equation and iodothalamate GFR ($R=0.26$) in potential kidney donors. Halleck et al. [10] also described a better correlation between DTPA clearance and eGFR using the CG equation ($R=0.55$) than using the MDRD equation ($R=0.37$) or CKD-EPI equation ($R=0.30$) in a population of living donors, arguing that all their donors had an excellent GFR >80 ml/min. In contrast, both formulas have been validated in cohorts of patients with chronic kidney disease. Wahba et al. [19] also reported slightly higher correlations when CG was used instead of CKD-EPI.

The actual eGFR after donor nephrectomy was higher than the predicted renal function measured by each imaging technique. After nephrectomy, compensatory hypertrophy of the contralateral kidney is expected, and by three months, remaining kidney clearance increases to a mean GFR of approximately 65–75% of pre-donation renal function [9]. A recent study on glomerular hemodynamics after kidney donation noted that adaptive hyperfiltration after donor nephrectomy is attributable to hyperperfusion and hypertrophy of the remaining glomeruli without glomerular hypertension in most donors 6–8 years after donation [25]. Nevertheless, there is a discussion that adaptive hyperfiltration might result in faster progression of kidney disease, namely in certain groups of donors with a less functional reserve. Developing reproductive metrics to identify these patients is a significant challenge. The risk of ESRD in living donors is low, although an increased risk, compared to healthy controls, has been evidenced in two long-term studies [5, 6]. Because ESRD is rare, different groups have pursued several surrogates to improve living-donor selection and donor safety. Massie et al. [26] reported an independent association between the living kidney donor eGFR at post-operative six months and subsequent ESRD.

Nevertheless, no significant association was found with pre-operative eGFR. One year post-donation, eGFR was assessed in this study as a surrogate for long-term renal function in the donor instead of earlier values after donation, as it represents the time point at which the mechanisms of compensatory hyperfiltration have almost stabilized and renal function reaches a more stable value, [3, 27]. However, Lam et al. [28] recently described that the plateau in living kidney donors is reached by five years after donation, which is consistent with the observations in our cohort.

The CT scan SFR performed better in our population for this task, considering both univariate and multivariate analyses and non-nested modeling tests. Barbas et al. [22] also showed a better predictive value of volumetry than nuclear renography for postoperative remaining renal function at six months in the donor Eum et al. [13], in a very recent study, also found that CT volumetry outperformed nuclear renography for predicting kidney function at all time points after donation (1, 6 months, and >1 year), considering both concordant and discordant groups.

Other clinical implications of CT scan volumetry results have already been assessed by our group. The remaining kidney volume indexed to weight (RKV/W) was found to be a strong predictor of estimated glomerular filtration rate at 1 year and mid-term renal function after living-donor nephrectomy [29]. Considering recipients' perspectives, we showed that lower donated kidney volume was associated with an increased risk of lower graft function one year after living donor transplant and suggested it can be a tool for better selection of donors to improve graft outcomes, particularly in the setting of multiple potential living donors or kidney paired exchange programs [30].

Our study has some limitations. First, donors were evaluated retrospectively, with only selected living donor candidates being assessed, which does not allow inference of the results for the global population of potential living donors, as those with significant SRF differences from nuclear renography were excluded from donation. Second, our cohort consisted only of Caucasian patients. In addition, eGFR using estimation equations to assess kidney function has limitations. In addition, an added value of our study cohort is its larger size and that all donors have been evaluated by both imaging techniques and have eGFR at one year.

Hence, we conclude that CT scan volumetry has a better predictive performance for one year of remaining renal function than standard nuclear renography. When there was disagreement between techniques versus cases in agreement, a higher SRF by nuclear renography was significantly associated with worse eGFR in the donor one year after donation, while a higher SRF on CT scan was not. One can draw a hypothesis that renal volume is a surrogate of the renal reserve in healthy individuals, but it must be tested. This study supports the use of evaluation of SRF by CT volumetry at our center, avoiding the need for nuclear renography, which could also translate into an improved living-donor experience and reduced costs to the healthcare system.

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Authors contributions MA and JM: Research design, data acquisition, data analysis, and paper writing. PRP, DC, FS, MM, and SP were engaged in the data acquisition and analysis. MR, MF, and LSM were involved in the research design and data analysis. All the authors approved the submitted version.

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Data availability Individual-level data were de-identified and will be provided upon justified request.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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References

- Liyanage T et al (2015) Worldwide access to treatment for end-stage kidney disease: a systematic review. *Lancet* 385(9981):1975–1982
- Andrews PA, Burnapp L (2018) British transplantation society/renal association UK guidelines for living donor kidney transplantation 2018: summary of updated guidance. *Transplantation* 102(7):e307
- Ibrahim HN et al (2009) Long-term consequences of kidney donation. *N Engl J Med* 360(5):459–469
- Segev DL et al (2010) Perioperative mortality and long-term survival following live kidney donation. *JAMA* 303(10):959–966
- Muzaale AD et al (2014) Risk of end-stage renal disease following live kidney donation. *JAMA* 311(6):579–586
- Mjoen G et al (2014) Long-term risks for kidney donors. *Kidney Int* 86(1):162–167
- Reese PP et al (2006) Creating a medical, ethical, and legal framework for complex living kidney donors. *Clin J Am Soc Nephrol* 1(6):1148–1153
- Habbous S et al (2018) Duration of living kidney transplant donor evaluations: findings from 2 multicenter cohort studies. *Am J Kidney Dis* 72(4):483–498
- Lentine KL et al (2017) Summary of kidney disease: improving global outcomes (KDIGO) clinical practice guideline on the evaluation and care of living kidney donors. *Transplantation* 101(8):1783–1792
- Halleck F et al (2013) Volume matters: CT-based renal cortex volume measurement in the evaluation of living kidney donors. *Transpl Int* 26(12):1208–1216
- Luyckx VA, Brenner BM (2010) The clinical importance of nephron mass. *J Am Soc Nephrol* 21(6):898–910
- Habbous S et al (2019) Can split renal volume assessment by computed tomography replace nuclear split renal function in living kidney donor evaluations? A systematic review and meta-analysis. *Can J Kidney Health Dis* 6:2054358119875459
- Eum SH et al (2022) Comparison of CT volumetry versus nuclear renography for predicting remaining kidney function after uninephrectomy in living kidney donors. *Sci Rep* 12(1):5144
- Blaufox MD et al (2018) The SNMMI and EANM practice guideline for renal scintigraphy in adults. *Eur J Nucl Med Mol Imaging* 45(12):2218–2228
- Nilsson H et al (2004) Measuring split renal function in renal donors: can computed tomography replace renography? *Acta Radiol* 45(4):474–480
- Summerlin AL et al (2008) Determination of split renal function by 3D reconstruction of CT angiograms: a comparison with gamma camera renography. *AJR Am J Roentgenol* 191(5):1552–1558
- Weinberger S et al (2016) Optimizing scintigraphic evaluation of split renal function in living kidney donors using the geometric mean method: a preliminary retrospective study. *J Nephrol* 29(3):435–441
- Dawson P, Peters AM (1993) Functional imaging in computed tomography. The use of contrast-enhanced computed tomography for the study of renal function and physiology. *Invest Radiol* 28:S79–84
- Wahba R et al (2016) Computed tomography volumetry in preoperative living kidney donor assessment for prediction of split renal function. *Transplantation* 100(6):1270–1277
- Mitsui Y et al (2018) The assessment of renal cortex and parenchymal volume using automated CT volumetry for predicting renal function after donor nephrectomy. *Clin Exp Nephrol* 22(2):453–458
- Lal H et al (2021) Determination of split renal function in voluntary renal donors by multidetector computed tomography and nuclear renography: How well do they correlate? *SA J Radiol* 25(1):2009
- Barbas AS et al (2016) CT volumetry is superior to nuclear renography for prediction of residual kidney function in living donors. *Clin Transplant* 30(9):1028–1035
- Stevens LA et al (2010) Comparative performance of the CKD epidemiology collaboration (CKD-EPI) and the modification of diet in renal disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 m². *Am J Kidney Dis* 56(3):486–495
- Rule AD et al (2004) Measured and estimated GFR in healthy potential kidney donors. *Am J Kidney Dis* 43(1):112–119
- Lenihan CR et al (2015) Longitudinal study of living kidney donor glomerular dynamics after nephrectomy. *J Clin Invest* 125(3):1311–1318
- Massie AB et al (2020) Association of early postdonation renal function with subsequent risk of end-stage renal disease in living kidney donors. *JAMA Surg* 155(3):e195472
- Kasike BL et al (2015) A prospective controlled study of living kidney donors: three-year follow-up. *Am J Kidney Dis* 66(1):114–124
- Lam NN et al (2020) Changes in kidney function follow living donor nephrectomy. *Kidney Int* 98(1):176–186
- Nunes-Carneiro D et al (2020) Remaining kidney volume indexed to weight as a strong predictor of estimated glomerular filtration rate at 1 year and mid-term renal function after living-donor nephrectomy - a retrospective observational study. *Transpl Int* 33(10):1262–1273
- Silva F et al (2020) Lower donated kidney volume is associated with increased risk of lower graft function and acute rejection at 1 year after living donor kidney-a retrospective study. *Transpl Int* 33(12):1711–1722

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APPENDIX 4 – Prevalence of Obesity after Living Kidney Donation and its Association with Cardiovascular Risk Factors and Renal Function

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Article

Prevalence of Obesity After Living Kidney Donation and Associated Risk Factors: Cardiovascular and Renal Implications

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Abstract

Background: Living kidney donor (LKD) transplantation contributes to mitigating the organ shortage and some programs now accept donors with borderline criteria, such as obesity. However, the long-term impact of these criteria extension remains unclear. **Methods:** This study retrospectively analyzed 306 LKD from 1998 to 2020 to examine obesity trends, predictors, and impact on cardiovascular risk and kidney function. **Results:** Before donation, 49% of donors were normal weight, 41% were overweight, and 10% were obese. Obese donors were older (50.8 ± 8.8 years, $p = 0.009$) and had higher rates of dyslipidemia and hypertension (41%, $p < 0.001$ for both). Over 9 years, obesity rates were stable (8.8–14.8%). A mixed logistic regression model showed that dyslipidemia (OR 6.1, $p = 0.042$), age (OR 0.9, $p = 0.005$) and body mass index (OR 5.3, $p < 0.001$) were strong predictors of post-donation obesity. Overweight donors showed an increase in obesity rates over time in the McNemar's paired analysis [14% obesity by year 3 ($p = 0.001$); 12.5% at year 10 ($p = 0.014$)]. Post-donation hypertension was more prevalent in obese donors' (61.1% vs. 30.4%, $p = 0.011$), though proteinuria and estimated glomerular filtration rate (eGFR) did not differ significantly. **Conclusions:** These findings show that pre-donation overweight, younger age, and dyslipidemia predict post-donation obesity, with hypertension posing added risk for obese donors. There was no impact concerning proteinuria and eGFR. The study underscores the importance of careful donor selection and risk informed counseling.

Keywords: living kidney donor; obesity; cardiovascular risk



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1. Introduction

Chronic kidney disease (CKD) is a growing global health issue, affecting millions of people and posing challenges for healthcare systems worldwide [1]. For patients with end-stage kidney disease (ESKD), kidney transplantation remains the most effective treatment, significantly improving survival and quality of life compared to dialysis [2–4]. The demand for kidney transplants far exceeds the supply, leaving many patients on the waiting

list for extended periods. In Portugal, the median wait time for a kidney transplant is approximately five years, with an annual mortality rate on waiting list of over 5% [4].

The persistent imbalance between organ supply and demand has driven the expansion of living donor transplantation and, in this context, a growing acceptance of donors with borderline medical criteria—including those who are overweight or mildly obese but otherwise healthy [3,5,6]. This strategy is intended to shorten waiting times and to attenuate the deleterious effects of prolonged dialysis, such as elevated cardiovascular risk and increased mortality. This category of “medically complex living donors” now comprises around 25% of living donor’s kidney transplant (LDKT) programs, with data from the Organ Procurement and Transplantation Network [7] showing a 12% increase in overweight and a 20% increase in obese donors from 1999 to 2011. This trend is expected to continue as obesity is an escalating global pandemic. For example, according to Global Obesity Observatory [8], in 2019, 18% of the adult population in Portugal was obese and 38% was categorized as overweight.

Obesity poses unique challenges in kidney transplantation since it implies an increased risk of hypertension, impaired glucose tolerance, type 2 diabetes mellitus, stroke, coronary heart disease, and overall cardiovascular and general mortality [9,10]. It is also known to cause specific kidney alterations, including effacement of podocyte foot processes, focal segmental glomerulosclerosis, and glomerular hypertrophy, referred to as obesity-related glomerulopathy [11,12]. Furthermore, obesity leads to the progression of CKD irrespective of the underlying etiology [10] through different mechanisms like hyperfiltration, renin-angiotensin-aldosterone system activation, and oxidative stress [11,13–19].

The long-term safety data regarding living kidney donation among individuals with excessive weight is limited [20]. Existing studies raise concerns about potential risks, including an increased incidence of ESKD [21–24], cardiovascular complications [25] and overall mortality [25] for obese donors. Despite methodological limitations in those studies [26], these findings have highlighted the importance of informed consent [27] and the need for clear guidelines to protect this higher-risk donor population.

The impact of donor’s obesity on post-donation outcomes remains poorly understood in the existing literature, and even less is known about weight changes after kidney donation. As more transplant programs accept overweight and obese individuals as living kidney donors (LKD), understanding the long-term health implications for this group has become essential.

Hence, our study aims to address this knowledge gap by investigating the prevalence and predictors of obesity among LKD over a 15-year follow-up period; assessing changes in obesity status over time in relation to pre-donation body mass index (BMI); identifying risk factors for post-donation obesity; and examining how obesity influences long-term outcomes, particularly in terms of estimated glomerular filtration rate (eGFR) evolution and cardiovascular health.

2. Materials and Methods

2.1. Patients

We retrospectively reviewed the clinical data of all adult LKDs submitted to nephrectomy at our center between January 1998 and January 2020 ($n = 365$). Inclusion criteria were LKDs with documented body mass index (BMI) at the time of donation and at one-year post-donation. A total of 59 LKDs with missing weight measurements in the medical records were excluded, leaving a final cohort of 306 LKDs for analysis.

This retrospective observational study was approved by the Institutional Review Board of Unidade Local de Saúde de Santo António, under protocol Ref.: 147-21 (119-DEFI/122-CE, 21 September 2021). The study was conducted in compliance with the Declaration

of Helsinki and is reported following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

2.2. Donor Variables

Following international guidelines [28,29], all candidates underwent the standard living donor evaluation protocol, including eGFR using the Chronic Kidney Disease-Epidemiology Collaboration equation [30] (with measured GFR if borderline), urine protein-to-creatinine and albumin-to-creatinine ratios, renal ultrasound, computed tomography angiography, and cardiovascular assessment (electrocardiogram, with echocardiography if >40 years or in the presence of risk factors, and further work-up if indicated). Additional testing included chest radiography, comprehensive blood work and serologies, oral glucose tolerance testing, blood group typing, pregnancy testing in women of childbearing age, and full immunological assessment. Detailed evaluation and exclusion criteria have already been published [31,32]. Baseline demographic, anthropomorphic, analytical and clinical data were collected.

In our cohort, BMI was assessed at baseline and at follow-up. A BMI ≤ 30 kg/m² was strongly recommended, although carefully selected individuals with BMI > 30 and <35 kg/m² were accepted if otherwise healthy and after exclusion of major comorbidities such as hypertension and diabetes or pre-diabetes. For the classification, we considered normal weight a BMI lower than 25 kg/m², overweight a BMI between 25 and 29.9 kg/m² and obesity a BMI higher than 30 kg/m².

Upon urinary analysis, proteinuria (ProtU) was defined by a random urine protein measurement between 0.15 and 0.5 g/g [28], confirmed by a 24 h urine sample. Donors with confirmed proteinuria exceeding 300 mg/day were excluded from donation.

Hypertension during follow-up was defined as blood pressure $\geq 140/90$ mmHg or the initiation of antihypertensive therapy; de novo diabetes mellitus was defined as fasting glucose > 126 mg/dL [33] or by the prescription of antidiabetic agents; and dyslipidemia was defined as low-density lipoprotein cholesterol ≥ 115 mg/dL and/or triglycerides ≥ 150 mg/dL, or the use of lipid-lowering medication to manage these levels.

The earliest date of problem onset was considered in the analysis. The date of nephrectomy was considered the start of follow-up, and all donors were offered lifetime annual follow-up appointments. The median follow-up was 6.59 (3.7–10.2) years.

2.3. Outcomes

The primary outcome was the occurrence of obesity until 15 years post-donation, using all available weight measurements from donation evaluation onward. Donors were followed from the nephrectomy date until the first of either death, development of end-stage kidney disease (defined as the need of chronic dialysis or a kidney transplant), last follow-up, or end of study period (31 December 2022). An additional analysis with a mixed logistic model was performed to evaluate the influence of demographic and clinical factors at the time of donation on the development of obesity.

The remaining analyses were limited to a 10-year period due to the smaller number of patients with follow-up beyond 10 years post-donation. The association between obesity and other cardiovascular risk factors—such as hypertension, diabetes mellitus, and dyslipidemia—was assessed. The impact of obesity on eGFR trajectories and the occurrence of proteinuria was also examined.

Mortality was not accessed since only six donors died during long-term follow-up. The causes were neoplasia ($n = 4$), sudden unexplained death without autopsy ($n = 1$), and suicide ($n = 1$). None of these events could be directly attributed to donation. Given the small number of cases, meaningful subgroup or stratified analyses were not feasible.

2.4. Statistical Analysis

Continuous data were described using mean and standard deviation (SD) or median and interquartile range (IQR), and categorical data were expressed as numbers and percentages. Categorical data were compared using Pearson chi-square test or Fisher exact test, and continuous variables were compared with Student's *t*-test or Mann–Whitney U test.

In order to handle the high number of missing weight data in different time points, we first approached the analysis using a multivariate mixed logistic regression, which imputed subject-specific random effects (slope defined as time in years) on an unstructured covariance matrix. The dependent variable was all obesity prevalence time-point data, and the independent variables were those considered to have a potential clinical correlation with changes in obesity status over time.

Matched pair analysis using McNemar's test was performed to compare obesity prevalence in donors at defined time points: at donation, 1, 3, 5, and 10 years post-donation. Significance was sought comparing post-donation time points with pre-donation status. This type of analysis is more powerful than commonly utilized unpaired or independent tests it eliminates variation between samples that could be attributed to extraneous factors, given its intra-patient longitudinal comparison design. Cases with missing data were pairwise excluded. We then stratified this statistical approach considering relevant clinical factors: BMI, sex, and age. Moreover, cardiovascular risk factors prevalence (hypertension, proteinuria, dyslipidemia) was again compared at different time points, in a pairwise fashion, stratified by obesity status, and compared by McNemar's test within each group (obese and non-obese) and by unpaired proportion test between each group (obese vs. non-obese). Similarly, mean eGFR longitudinal trends were analyzed and significance was sought by paired and unpaired *t*-test.

Statistical calculations were performed using STATA/MP, version 15.1 (Stata Corp, College Station, TX, USA). A two-sided *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. Population Characteristics

The baseline characteristics of this cohort are summarized in Table 1.

Most donors (49%) had normal weight, 41% were overweight and 10% were obese. The mean age of the cohort was 47.2 ± 10.7 years, with obese donors being significantly older (50.8 ± 8.8 years, $p = 0.009$). Female donors represented 71% of the total sample.

The prevalence of dyslipidemia and hypertension increased with higher BMI, with both conditions being significantly more common in the obese group (41%, $p < 0.001$ for both). Overweight donors also had higher rates of dyslipidemia (15%, $p < 0.001$) and hypertension (19%, $p < 0.001$) compared to the normal weight group. Smoking habits varied across BMI categories, with the highest prevalence in the normal weight group (21%, $p = 0.045$). No cases of diabetes mellitus or other significant comorbidities were present, consistent with the rigorous donor selection process.

There were no significant differences between the groups regarding serum creatinine levels or urine protein–creatinine ratios. However, the pre-donation eGFR was higher in normal-weight donors (102.4 ± 15.0 mL/min/1.73 m²) compared to obese donors (96.3 ± 14.5 mL/min/1.73 m², $p = 0.038$).

In our center, procurement of the left kidney is generally preferred because of the longer renal vein, which facilitates implantation. The left kidney was procured in 82% of cases (251 donors), with no significant difference across subgroups. Right kidney procurement was performed only in cases of anatomical variations or relative differences in split renal function.

Table 1. Baseline Characteristics of Kidney Donors Stratified by Pre-Donation Body Mass Index Status.

	Total	Normal Weight (BMI < 25 kg/m ²)	Overweight (BMI 25–29.9 kg/m ²)	Obese (BMI ≥ 30 kg/m ²)	<i>p</i>
N (%)	306 (100%)	150 (49%)	124 (41%)	32 (10%)	-
Age (years), mean ± SD	47.2 ± 10.7	45.4 ± 11.3	48.4 ± 10.2	50.8 ± 8.8	0.009
Age (years), n (%)					
<40	78 (25)	50 (33)	25 (20)	3 (9)	0.021
40–55	146 (48)	65 (43)	63 (51)	18 (56)	
≥55	82 (27)	35 (23)	36 (29)	11 (34)	
Sex F, n (%)	217 (71)	114 (76)	83 (67)	20 (63)	0.140
BMI (kg/m ²), mean ± SD	25.3 ± 3.4	22.5 ± 1.7	27.3 ± 1.4	31.2 ± 1.1	-
Smoking habits, n (%)	49 (16)	32 (21)	13 (10)	4 (13)	0.045
Dyslipidemia, n (%)	44 (14)	13 (9)	18 (15)	13 (41)	<0.001
Total cholesterol (mg/dL), mean ± SD	194.3 ± 37.2	188.2 ± 36.0	196.5 ± 36.2	214.3 ± 40.2	0.001
HT, n (%)	48 (16)	11 (7)	24 (19)	13 (41)	<0.001
SBP (mmHg), mean ± SD	122.5 ± 13.4	118.8 ± 12.0	125.0 ± 13.3	130.0 ± 14.3	<0.001
DBP (mmHg), mean ± SD	73.2 ± 8.7	71.6 ± 8.2	74.2 ± 8.9	76.8 ± 8.8	0.002
ProtU 0.15–0.5 g/g, n (%)	87 (28)	43 (29)	33 (27)	11 (34)	0.683
Serum creatinine (mg/dL), mean ± SD	0.75 ± 0.16	0.73 ± 0.15	0.76 ± 0.17	0.78 ± 0.15	0.115
Pre- donation eGFR (mL/min/1.73 m ²), mean ± SD	100.3 ± 14.6	102.4 ± 15.0	98.9 ± 13.9	96.3 ± 14.5	0.038
Pre- donation eGFR (mL/min/1.73 m ²), n (%)					
<80	27 (9)	14 (9)	10 (8)	3 (9)	0.278
80–90	47 (15)	17 (11)	22 (18)	8 (25)	
≥90	232 (76)	119 (79)	92 (74)	21 (66)	
Related donor, n (%)	104 (34)	52 (35)	40 (32)	12 (38)	0.830
Donation of left kidney	251 (82)	115 (77)	108 (87)	28 (88)	0.624

BMI: Body Mass Index; SD: standard deviation; F: female; HT: Hypertension; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; ProtU: Proteinuria; eGFR: Estimated Glomerular Filtration Rate.

3.2. Evaluation of Obesity Prevalence and Predictors

We conducted a longitudinal analysis over a 15-year follow-up period after kidney donation to evaluate obesity prevalence. During the first 9 years of follow-up, obesity prevalence ranged from 8.8% to 14.8% (Figure 1, Supplementary Table S1). Although there is variability within this period, the overall trend did not show a sustained increase. In contrast, after year 10, prevalence rose more consistently, reaching over 24.4% by year 13, but the number of donors in analysis was progressively inferior. For precise percentages, according to the year of follow-up, the Supplementary Table S1 can be consulted.

A mixed logistic regression model identified several significant predictors of post-donation obesity over the 15-year period, as demonstrated in Table 2. Age at the time of donation was inversely associated with the risk of post-donation obesity (OR 0.882, 95% CI 0.808–0.963, $p = 0.005$), suggesting that younger donors are more likely to develop obesity post-donation. As anticipated, a higher pre-donation BMI was a strong independent

predictor of post-donation obesity (OR 5.324, 95% CI 3.471–8.168, $p < 0.001$), such as dyslipidemia at the time of donation (OR 6.048, 95% CI 1.065–34.348, $p = 0.042$).

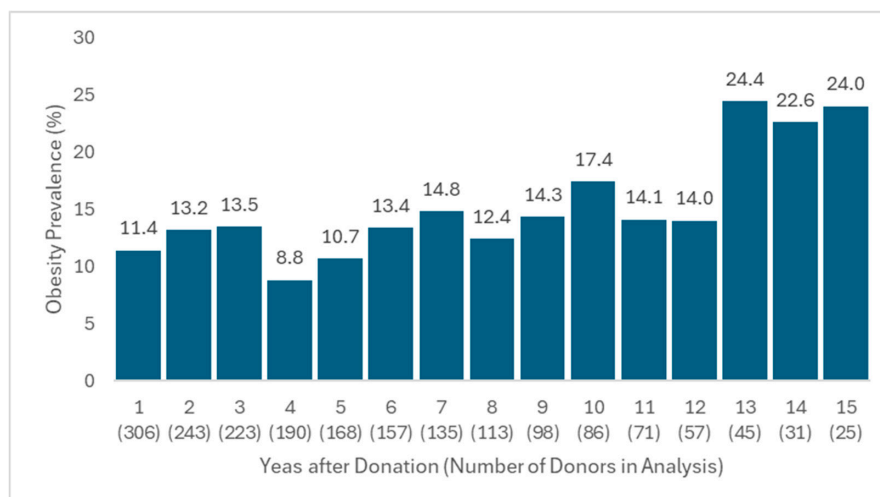


Figure 1. Longitudinal Obesity Prevalence in Percentage During 15 Years of Follow-up Post-Donation. Number of patients in analysis in parenthesis.

Table 2. Risk Factors at Donation for Post-Donation Obesity (Mixed Logistic Model).

	Multivariable OR (95% CI)	<i>p</i>
Time post-donation	0.985 (0.844–1.150)	0.848
Age	0.882 (0.808–0.963)	0.005
Female Sex	3.250 (0.731–14.453)	0.122
Pre-donation BMI kg/m ²	5.324 (3.471–8.168)	<0.001
Smoking habits	0.450 (0.055–3.647)	0.454
HT	0.182 (0.030–1.094)	0.063
Dyslipidemia	6.048 (1.065–34.348)	0.042
ProtU 0.15–0.5 g/g	0.361 (0.083–1.560)	0.172
Pre-donation eGFR mL/min/1.73 m ²	0.951 (0.899–1.006)	0.080
Related donor	0.745 (0.194–2.831)	0.668

OR: Odds Ratio; CI: Confidence Interval; BMI: Body Mass Index; HT: Hypertension; ProtU: Proteinuria (Protein–Creatinine Ratio); eGFR: Estimated Glomerular Filtration Rate.

3.3. Trends in BMI Categories

Figure 2 and Supplementary Table S2 represent McNemar’s paired analysis, which evaluates obesity evolution according with three different categories: pre-donation BMI category, donor sex, and pre-donation age.

Over a 10-year follow-up period, kidney donors exhibited varying weight trajectories depending on their initial weight status (Figure 2A). Patients classified as normal weight pre-donation demonstrated minimal progression toward obesity, with only 6.8% becoming obese by year 10 ($p = 0.083$), suggesting a relatively low risk of obesity development for

those starting in a normal weight range. In contrast, overweight donors showed a steady increase in obesity rates over time, with 11.3% classified as obese by year 1 ($p < 0.001$), 14% by year 3 ($p = 0.001$), and 12.5% at year 10 ($p = 0.014$), indicating a higher risk of long-term weight gain for this group. Interestingly, donors who were initially obese experienced an early decline in obesity prevalence, dropping to 65.6% by year 1 and further to 56.3% by year 5 ($p = 0.083$). However, by year 10, the obesity rate had rebounded to 83.3% ($p = 0.083$), indicating some weight regain in later years.

The difference between sexes (Figure 2B) did not appear to be clinically relevant in relation to obesity trends across most time points. However, a statistically significant difference was observed in females at the 10-year mark, with 14.9% ($p = 0.003$) being obese, an increase from 9.2% at donation.

The data on Figure 2C suggests that younger patients, particularly those under 40, are at an increased risk of becoming obese over time, an observation that confirms the result from the mixed logistic regression analysis. At 1 year, the obesity rate rose significantly to 11.5% ($p = 0.014$), indicating an early and notable increase in this age group. This trend continued, with further significant increases at 5 years (15.8%, $p = 0.046$) and 10 years (21.7%, $p = 0.046$).



Figure 2. Cont.

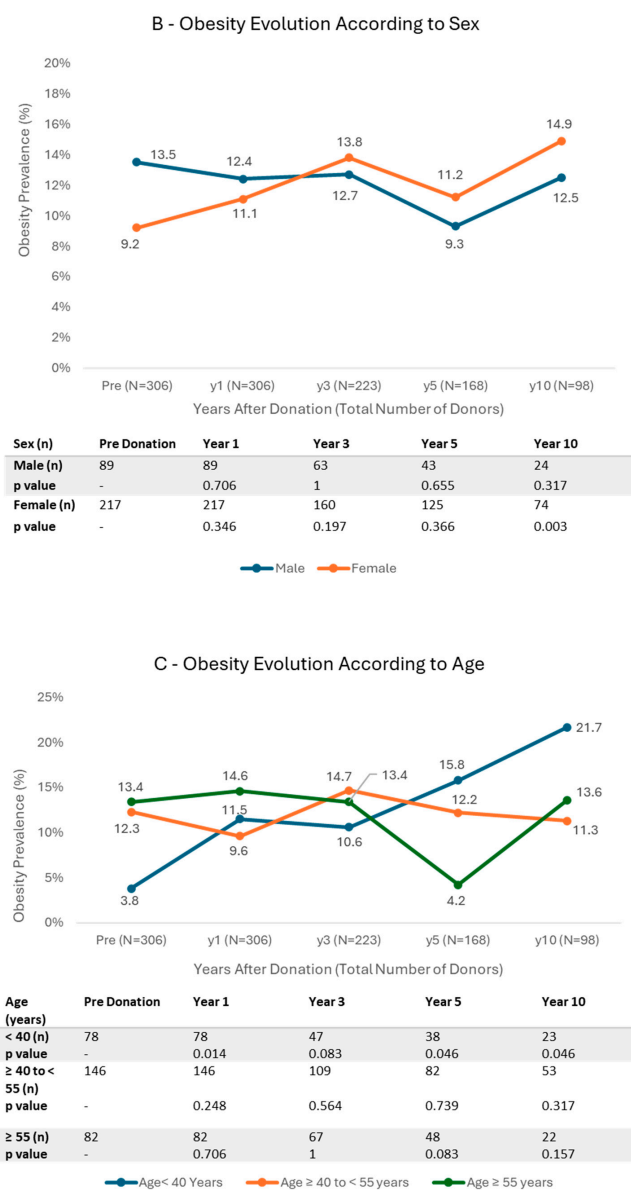


Figure 2. Evaluation of obesity percentage according with three different aspects: pre-donation BMI category (A), donor sex (B) and pre-donation age (C) using McNemar's paired analysis in 10 years of follow up. Overweight patients (A) had significantly higher obesity prevalence in the follow up. Donor sex (B) did not have impact in obesity prevalence in the majority of the follow-up. Younger age (C) was associated with increased obesity prevalence during all the follow-up.

3.4. Cardiovascular Risk Factors and Kidney Function Trends According to BMI

Hypertension prevalence increased across all groups following kidney donation (Figure 3A and Supplementary Table S3). However, a significant disparity is observed between obese and non-obese patients. At 1 year post-donation, hypertension was significantly more prevalent among obese patients (42.9%) compared to non-obese patients (21.9%, $p = 0.010$). This trend persisted at 5 years, where hypertension affected 61.1% of obese patients, in contrast to 30.4% of non-obese patients ($p = 0.011$).

No significant differences were observed between groups regarding proteinuria, dyslipidemia, or eGFR changes post-donation (Figure 3B–D and Supplementary Tables S4–S6).

The cohort consisted of a highly selected group of donors believed to be healthy, resulting in only 4 cases of diabetes, 3 in the non-obese cohort and 1 in the obese cohort. This limited the statistical power to detect significant differences for this variable. This information can be consulted in Table S7 of the Supplementary Material.

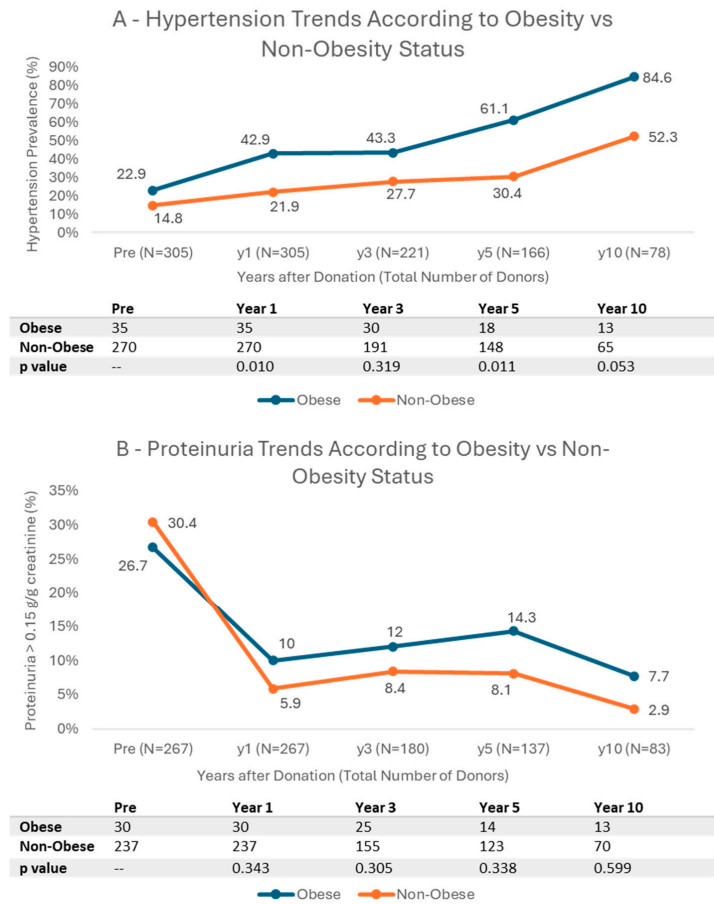


Figure 3. Cont.

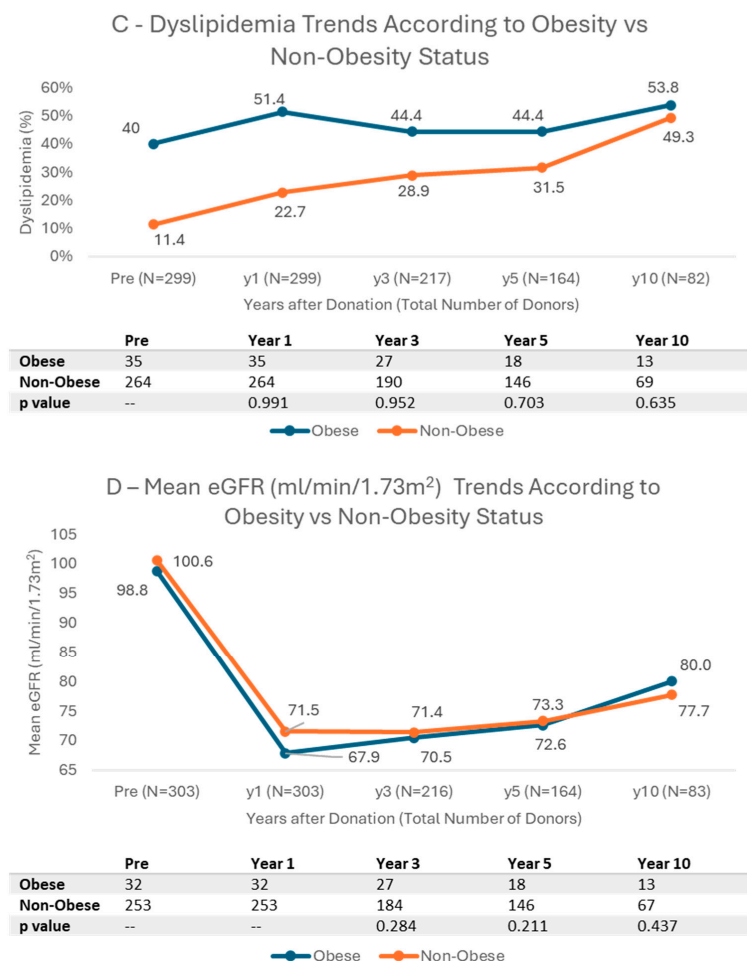


Figure 3. Evaluation of Hypertension (A), Proteinuria (B), Dyslipidemia (C), and Mean estimated glomerular filtration rate (D) trends according to Obese vs. Non-Obese Status in 10 years of follow-up. Hypertension (A) had significantly higher prevalence in the obese group. There were no differences between the obese and non-obese groups concerning proteinuria prevalence (B), dyslipidemia prevalence (C), and mean estimated glomerular filtration rate (D).

4. Discussion

In this study, most kidney donors (49%) had a normal weight, while 10% were classified as obese. Obese donors were generally older and more likely to have dyslipidemia and hypertension compared to those with normal weight. Before donation, normal-weight donors had better kidney function than obese donors (eGFR 102.4 ± 15.0 vs. 96.3 ± 14.5 mL/min/1.73 m², $p = 0.038$).

During the first nine years, obesity prevalence fluctuated between 8.8% and 14.8%, without evidence of a sustained upward trend. This observation is further supported by the lack of statistical significance for time after donation in the multivariable mixed logistic regression. The modest increase observed toward the end of the follow-up period may

reflect follow-up loss or surveillance bias, as donors with obesity or related complications may have been more likely to remain under closer medical monitoring.

The mixed logistic regression model identified three key predictors of post-donation obesity: younger age, higher BMI before donation, and dyslipidemia. These findings were reinforced by McNemar's paired analysis, which showed that overweight donors were the group most likely to become obese after donation, particularly those under 40 years old. Normal-weight donors generally maintained their weight, while obese donors initially reduced their obesity prevalence post-donation, but this trend reversed in later years. McNemar's paired analysis also showed no significant difference between sexes regarding obesity development.

Among comorbidities, hypertension was significantly more common in obese donors compared to non-obese donors. However, dyslipidemia, proteinuria and eGFR changes did not differ significantly between groups. Diabetes prevalence was low, with only a few cases in both groups.

4.1. Obesity and Age

The observed association between post-donation obesity and younger age (particularly under 40 years) is a novel finding, supported by both the mixed logistic regression model and McNemar's paired analysis. While this relationship has not been extensively described in the literature, it may be partly explained by older donors being more prone to sarcopenia, having a greater burden of comorbidities, or showing higher adherence to dietary counseling. Importantly, BMI is only a surrogate marker of body composition and carries notable limitations. A low BMI is often regarded as favorable; however, in older donors, it may instead reflect sarcopenia rather than a truly healthy metabolic profile [34]. Sarcopenia, characterized by reduced muscle mass and strength, can mask adverse health status and complicate the interpretation of BMI. In addition, reduced muscle mass in older donors may result in lower creatinine generation, potentially leading to an overestimation of eGFR when using creatinine-based equations. Future studies should therefore incorporate complementary assessments of body composition, such as bioimpedance analysis or imaging-based techniques, to more accurately differentiate healthy leanness from sarcopenia in living kidney donors.

4.2. Pre Donation Body Mass Index

The influence of higher pre-donation BMI on post-donation obesity was especially evident among overweight donors, consistent with prior studies. Bugeja et al. [35] conducted a single-center study involving 151 LKD who donated between 2009 and 2017, with a median follow-up of 392 days post-donation, showing that among overweight and obese donors ($\text{BMI} > 25 \text{ kg/m}^2$), weight increased significantly after donation, from $86.0 \pm 2.1 \text{ kg}$ to $88.8 \pm 2.7 \text{ kg}$ (mean difference $2.3 \pm 0.9 \text{ kg}$, $p < 0.0001$). Similarly, Punjala et al. [36] performed a retrospective analysis of 303 donors who proceeded to donate between 2012 and 2016. They reported that while some obese donors lost weight before donation (mean BMI difference of -1.32 kg/m^2 , $p < 0.001$), their weight returned to baseline levels one year after donation, with weight gain persisting at the two-year follow-up (mean BMI difference of $+1.47 \text{ kg/m}^2$, $p < 0.001$). These findings, in line with our results, emphasize the need for targeted and sustained weight control efforts in overweight and obese kidney donors.

4.3. Pre Donation Dyslipidemia

Pre-donation dyslipidemia also emerged as a significant predictor of post-donation obesity in our regression model. However, interpretation should be cautious, given the small number of donors with baseline dyslipidemia (14%), resulting in wide confidence intervals (OR 6.048, 95% CI 1.065–34.348, $p = 0.042$). Despite limited statistical power, this

finding is biologically plausible, as dyslipidemia often reflects an adverse metabolic profile and poor nutritional habits predisposing to later weight gain. Nonetheless, post-donation dyslipidemia prevalence did not differ significantly between obese and non-obese groups.

4.4. Cardiovascular Risk

Concerning cardiovascular risk, obese donors had significantly higher rates of hypertension compared to non-obese donors, indicating that obesity was strongly associated with hypertension following donation. This aligns with Issa et al. [37], who reported an increased risk of hypertension (RR 1.93, 95% CI 1.51–2.46, $p < 0.001$) associated with post-donation weight gain. Given the well-established link between obesity and hypertension, this relationship is likely exacerbated in LKDs, where the prevalence of hypertension is elevated due to reduced kidney mass [38]. It is important though to recognize that the studies that addressed the blood pressure changes post-donation showed that the differences in quantitative value are low. For example, Kim et al. [39] found that there was a statistically significant increase in BP post-donation, but the increase in BP was from 113/75 to 116/77 mm Hg. Thiel et al. [40] suggested that donors that develop hypertension post-donation were already pre-hypertensive before donation, which we know can be the case for obese patients. Preventive measures—including regular follow-up, early treatment initiation, and low-sodium diet—should therefore be prioritized.

Diabetes prevalence was low in our cohort, but prior studies (Issa et al. [37]) found a substantially higher risk of diabetes with post-donation weight gain (RR 4.18, 95% CI 2.05–8.5, $p < 0.0001$).

4.5. Kidney Function and Proteinuria

Our cohort did not have any significant differences between obese and non-obese groups in terms of proteinuria or post-donation eGFR changes. This finding should be interpreted cautiously, as obesity is well established as a risk factor for eGFR decline, proteinuria, and ESKD in the general population. Several possible explanations may account for the absence of significant differences in our study. First, the relatively limited number of obese donors and the length of follow-up (median 6.59 years) may have reduced the statistical power to detect differences that typically emerge after longer observation periods. Second, living kidney donors undergo rigorous medical evaluation before donation, which tends to exclude individuals at highest metabolic or renal risk. Such careful selection, as suggested by Issa et al. [37], may attenuate the impact of obesity in donor cohorts compared to the general population. Importantly, our previous study [41] examining 15-year trajectories showed that normal-weight donors had significantly better early post-donation eGFR recovery than overweight and obese donors (normal weight: $+0.59 \text{ mL/min/1.73 m}^2$, 95% CI: $+0.37$ to $+0.80$; overweight: $+0.35$, 95% CI: -0.14 to $+0.56$; and obese: -0.18 , 95% CI: -0.68 to $+0.31$, $p = 0.020$), but after the first five years of follow-up, those differences did not exist. This may reflect an initial reduced adaptive hyperfiltration capacity in overweight and obese individuals. Grams et al. [42,43] similarly showed that every 5 kg/m^2 increase in BMI above 30 increases ESKD risk by 16%, and Locke et al. [21] reported more than double ESKD risk for obese compared with non-obese donors (94 vs. 40 per 10,000 over 20 years). Praga et al. [44] also observed increased proteinuria and kidney insufficiency in obese donors, albeit typically emerging a decade or more after donation. In contrast, Serrano et al. [45] and Issa et al. [37] did not observe significant long-term differences. Overall, our findings likely reflect careful donor selection and shorter follow-up, but the broader literature strongly supports obesity as a long-term renal risk factor.

4.6. Limitations

Several limitations must be acknowledged. Although the study included follow-up periods of up to 15 years, the median follow-up was only 6.6 years, which may be insufficient to capture late complications such as ESKD that typically emerge beyond the first decade after donation. In addition, only a relatively small number of donors were followed beyond 10 years, reducing statistical power to detect long-term differences. Another important limitation is the absence of information on BMI trajectories before donation, which prevents us from knowing whether donors intentionally modified their weight in preparation for nephrectomy. Selection bias must also be considered, as the study population comprised exclusively medically suitable living donors, a group that is healthier than the general population. This careful selection process likely reduced the observed risks and limits the applicability of our findings to broader or less healthy populations.

Additional limitations relate to unmeasured variables. We did not collect data on inflammatory biomarkers such as C Reactive Protein, IL-6, or adipokines, despite increasing evidence that chronic low-grade inflammation plays a key role in the metabolic and cardiovascular risks associated with obesity [46,47]. The cohort was predominantly female, raising concerns about sex-related generalizability, as weight gain patterns and cardiometabolic risk may differ between men and women. Ethnicity variability was also scarce, which is relevant since cultural, lifestyle, and genetic factors strongly influence weight trajectories and cardiometabolic outcomes. Furthermore, several important potential confounders were not captured, including dietary habits, physical activity, socioeconomic status, and family history of metabolic disease—all of which could significantly affect obesity development and long-term health outcomes.

Finally, this was a single-center Portuguese study, which may limit the external generalizability of our findings. Donor populations, healthcare practices, and selection criteria vary across countries and centers, potentially influencing obesity prevalence and its predictors.

Nonetheless, the main risk factors identified in our cohort—higher pre-donation BMI, younger age, and dyslipidemia—are well-recognized metabolic determinants, suggesting that similar associations are likely to be observed in other settings.

4.7. Clinical Implications

The acceptance of overweight or obese donors remains controversial, with guideline variability [29,43,48–50]. The 2015 European Renal Best Practice guidelines recommend that individuals with a body mass index (BMI) greater than 35 kg/m² should not be considered for living kidney donation [49]. However, the British Transplantation Society and Kidney Disease: Improving Global Outcomes (KDIGO) guidelines suggest that each case should be evaluated individually for donors with a BMI greater than 30 kg/m², without specifying an absolute cut-off value [43,46].

Given the scarcity of donor organs and the favorable results from our study—showing no increased risk of proteinuria or eGFR decline in donors who became obese after donation—we argue that overweight and obese individuals should be included as potential living kidney donors. While these findings should be interpreted with caution, as they diverge from the broader literature linking obesity with long-term renal risk [21,42–44], they suggest that excluding such candidates outright may be unnecessarily restrictive. Instead, the focus should be on encouraging overweight and obese donors to achieve a healthier weight before donation when feasible, and on implementing structured, post-donation protocols centered on targeted weight control, nutritional counseling, and long-term follow-up. This approach balances organ availability with donor safety and provides a practical pathway for integrating higher-BMI individuals into the donor pool without disregarding their elevated metabolic risks.

Preventive strategies should focus on targeted counseling for high-risk individuals—particularly younger donors, those with pre-donation overweight, and those with dyslipidemia—combined with sustained weight management interventions both before and after donation, as well as closer long-term follow-up. Locke et al. [21] emphasized that BMI is the only donor factor that can be modified prior to donation to reduce long-term ESRD risk. In this context, integrating key predictors such as pre-donation BMI, younger age, and dyslipidemia into a practical “Obesity Risk Score” could be a valuable tool during donor evaluation. Such a score would allow stratification of candidates according to their risk of developing post-donation obesity, enabling more tailored counseling, preventive strategies, and individualized follow-up intensity.

In addition, novel therapeutic approaches—including glucagon-like peptide-1 receptor agonists (GLP-1a) and sodium–glucose cotransporter-2 inhibitors (SGLT2i)—should be considered as adjuncts to lifestyle modification, given their emerging roles in weight management and cardiometabolic protection. Future research should explore whether combining structured lifestyle interventions with pharmacologic therapy in high-risk donors, identified through an obesity risk stratification tool, can mitigate long-term obesity-related complications after kidney donation.

5. Conclusions

In this 15-year follow-up of living kidney donors, post-donation obesity remained stable during the first decade but increased thereafter, partly influenced by reduced follow-up. Higher pre-donation BMI, younger age, and dyslipidemia independently predicted post-donation obesity; obesity was strongly associated with hypertension but not with differences in proteinuria or eGFR decline over the available follow-up. Given organ scarcity—and despite these favorable findings diverging from prior literature—we support including overweight and obese candidates for donation, with preference for pre-donation weight optimization and structured, post-donation weight-control and monitoring protocols to safeguard outcomes. Multicenter studies with larger, more diverse cohorts and longer follow-up are needed to validate and extend these results.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/jcm14186411/s1>: Table S1: Longitudinal Obesity Prevalence During 15 Years of Follow-up of Living Kidney Donors Post-Donation; Table S2: Evaluation of body mass index evolution according with three different aspects: pre donation BMI category, donor sex, and pre-donation age (McNemar’s paired analysis); Tabel S3: Hypertension Trends For a 10 Years Follow-up According to Obese and Non-Obese Status; Table S4: Proteinuria Trends For a 10 Years Follow-up According to Obese and Non-Obese Status; Table S5: Dyslipidemia Trends For a 10 Years Follow-up According to Obese and Non-Obese Status; Table S6: Estimated Glomerular Filtration Rate (eGFR) Trends For a 10 Years Follow-up According to Obese and Non-Obese Status; Table S7: Diabetes Mellitus (DM) Trends For a 10 Years Follow-up According to Obese and Non-Obese Status.

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with the Declaration of Helsinki and is reported following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
CKD	Chronic Kidney Disease
eGFR	Estimated Glomerular Filtration Rate
ESKD	End-Stage Kidney Disease
HR	Hazard Ratio
IQR	Interquartile Range
KDIGO	Kidney Disease: Improving Global Outcomes
LKD	Living Kidney Donor
LKDT	Living Kidney Donor Transplantation
OR	Odds Ratio
SD	Standard Deviation

References

1. Kovesdy, C.P. Epidemiology of Chronic Kidney Disease: An Update 2022. *Kidney Int. Suppl.* **2022**, *12*, 7–11. [CrossRef]
2. Wolfe, R.A.; Ashby, V.B.; Milford, E.L.; Ojo, A.O.; Ettenger, R.E.; Agodoa, L.Y.C.; Held, P.J.; Port, F.K. Comparison of Mortality in All Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. *N. Engl. J. Med.* **1999**, *341*, 1725–1730. [CrossRef] [PubMed]
3. Ahmadi, A.R.; Lafranca, J.A.; Claessens, L.A.; Imamdi, R.M.S.; Ijzermans, J.N.M.; Betjes, M.G.H.; Dor, F.J.M.F. Shifting Paradigms in Eligibility Criteria for Live Kidney Donation: A Systematic Review. *Kidney Int.* **2015**, *87*, 31–45. [CrossRef]
4. Atividade Nacional Annual. 2021. Available online: https://www.ipst.pt/files/TRANSPLANTACAO/DOACAOETTRANSPLANTACAO/Dados_Anuais_Atividade_Doacao_Transplantacao2021.pdf (accessed on 6 September 2025).
5. McCormick, F.; Held, P.J.; Chertow, G.M. The Terrible Toll of the Kidney Shortage. *J. Am. Soc. Nephrol.* **2018**, *29*, 2775–2776. [CrossRef]
6. Rodrigues, S.; Escoli, R.; Eusébio, C.; Dias, L.; Almeida, M.; Martins, L.S.; Pedrosa, S.; Henriques, A.C.; Cabrita, A. A Survival Analysis of Living Donor Kidney Transplant. *Transplant. Proc.* **2019**, *51*, 1575–1578. [CrossRef] [PubMed]
7. Sachdeva, M. Weight Trends in United States Living Kidney Donors: Analysis of the UNOS Database. *World J. Transplant.* **2015**, *5*, 137. [CrossRef] [PubMed]
8. Sardinha, L.B.; Santos, D.A.; Silva, A.M.; Coelho-e-Silva, M.J.; Raimundo, A.M.; Moreira, H.; Santos, R.; Vale, S.; Baptista, F.; Mota, J. Prevalence of Overweight, Obesity, and Abdominal Obesity in a Representative Sample of Portuguese Adults. *PLoS ONE* **2012**, *7*, e47883. [CrossRef]
9. Yau, K.; Kuah, R.; Cherney, D.Z.I.; Lam, T.K.T. Obesity and the Kidney: Mechanistic Links and Therapeutic Advances. *Nat. Rev. Endocrinol.* **2024**, *20*, 321–335. [CrossRef]
10. Nam, K.H.; Chang, T.I.; Joo, Y.S.; Kim, J.; Lee, S.; Lee, C.; Yun, H.; Park, J.T.; Yoo, T.; Sung, S.A.; et al. Association Between Serum High-Density Lipoprotein Cholesterol Levels and Progression of Chronic Kidney Disease: Results from the KNOW-CKD. *J. Am. Heart Assoc.* **2019**, *8*, e011162. [CrossRef]
11. D'Agati, V.D.; Chagnac, A.; de Vries, A.P.J.; Levi, M.; Porrini, E.; Herman-Edelstein, M.; Praga, M. Obesity-Related Glomerulopathy: Clinical and Pathologic Characteristics and Pathogenesis. *Nat. Rev. Nephrol.* **2016**, *12*, 453–471. [CrossRef]
12. Bonnet, F.; Deprele, C.; Sassolas, A.; Moulin, P.; Alamartine, E.; Berthezène, F.; Berthouix, F. Excessive Body Weight as a New Independent Risk Factor for Clinical and Pathological Progression in Primary IgA Nephritis. *Am. J. Kidney Dis.* **2001**, *37*, 720–727. [CrossRef]

13. Griffin, K.A.; Kramer, H.; Bidani, A.K. Adverse Renal Consequences of Obesity. *Am. J. Physiol.-Ren. Physiol.* **2008**, *294*, F685–F696. [\[CrossRef\]](#)
14. Strazzullo, P.; Barba, G.; Cappuccio, F.P.; Siani, A.; Trevisan, M.; Farinaro, E.; Pagano, E.; Barbato, A.; Iacone, R.; Galletti, F. Altered Renal Sodium Handling in Men with Abdominal Adiposity: A Link to Hypertension. *J. Hypertens.* **2001**, *19*, 2157–2164. [\[CrossRef\]](#)
15. Engeli, S.; Böhnke, J.; Gorzelniak, K.; Janke, J.; Schling, P.; Bader, M.; Luft, F.C.; Sharma, A.M. Weight Loss and the Renin-Angiotensin-Aldosterone System. *Hypertension* **2005**, *45*, 356–362. [\[CrossRef\]](#)
16. Tsuboi, N.; Okabayashi, Y.; Shimizu, A.; Yokoo, T. The Renal Pathology of Obesity. *Kidney Int. Rep.* **2017**, *2*, 251–260. [\[CrossRef\]](#)
17. de Vries, A.P.J.; Ruggenti, P.; Ruan, X.Z.; Praga, M.; Cruzado, J.M.; Bajema, I.M.; D’Agati, V.D.; Lamb, H.J.; Barlovic, D.P.; Hojs, R.; et al. Fatty Kidney: Emerging Role of Ectopic Lipid in Obesity-Related Renal Disease. *Lancet Diabetes Endocrinol.* **2014**, *2*, 417–426. [\[CrossRef\]](#)
18. Kanbay, M.; Copur, S.; Ucku, D.; Zoccali, C. Donor Obesity and Weight Gain after Transplantation: Two Still Overlooked Threats to Long-Term Graft Survival. *Clin. Kidney J.* **2023**, *16*, 254–261. [\[CrossRef\]](#)
19. Sachdeva, M. Should Obesity Affect Suitability for Kidney Donation? *Semin. Dial.* **2018**, *31*, 353–356. [\[CrossRef\]](#)
20. Kamal, A.I.; Harraz, A.M.; Shokeir, A.A. Controversies related to living kidney donors. *Arab. J. Urol.* **2011**, *9*, 225–233. [\[CrossRef\]](#)
21. Locke, J.E.; Reed, R.D.; Massie, A.; MacLennan, P.A.; Sawinski, D.; Kumar, V.; Mehta, S.; Mannon, R.B.; Gaston, R.; Lewis, C.E.; et al. Obesity Increases the Risk of End-Stage Renal Disease among Living Kidney Donors. *Kidney Int.* **2017**, *91*, 699–703. [\[CrossRef\]](#)
22. Ibrahim, H.N.; Foley, R.N.; Reule, S.A.; Spong, R.; Kukla, A.; Issa, N.; Berglund, D.M.; Sieger, G.K.; Matas, A.J. Renal Function Profile in White Kidney Donors: The First 4 Decades. *J. Am. Soc. Nephrol.* **2016**, *27*, 2885–2893. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Massie, A.B.; Muzaale, A.D.; Luo, X.; Chow, E.K.H.; Locke, J.E.; Nguyen, A.Q.; Henderson, M.L.; Snyder, J.J.; Segev, D.L. Quantifying Postdonation Risk of ESRD in Living Kidney Donors. *J. Am. Soc. Nephrol.* **2017**, *28*, 2749–2755. [\[CrossRef\]](#)
24. Muzaale, A.D.; Massie, A.B.; Wang, M.-C.; Montgomery, R.A.; McBride, M.A.; Wainright, J.L.; Segev, D.L. Risk of End-Stage Renal Disease Following Live Kidney Donation. *JAMA* **2014**, *311*, 579. [\[CrossRef\]](#)
25. Mjøen, G.; Hallan, S.; Hartmann, A.; Foss, A.; Midtvedt, K.; Øyen, O.; Reisæter, A.; Pfeffer, P.; Jenssen, T.; Leivestad, T.; et al. Long-Term Risks for Kidney Donors. *Kidney Int.* **2014**, *86*, 162–167. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Lam, N.N.; Lentine, K.L.; Garg, A.X. End-Stage Renal Disease Risk in Live Kidney Donors. *Curr. Opin. Nephrol. Hypertens.* **2014**, *23*, 592–596. [\[CrossRef\]](#)
27. Reese, P.P.; Boudville, N.; Garg, A.X. Living Kidney Donation: Outcomes, Ethics, and Uncertainty. *Lancet* **2015**, *385*, 2003–2013. [\[CrossRef\]](#)
28. Lentine, K.L.; Kasiske, B.L.; Levey, A.S.; Adams, P.L.; Alberú, J.; Bakr, M.A.; Gallon, L.; Garvey, C.A.; Guleria, S.; Li, P.K.-T.; et al. Summary of Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* **2017**, *101*, 1783–1792. [\[CrossRef\]](#)
29. Andrews, P.A.; Burnapp, L. British Transplantation Society/Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: Summary of Updated Guidance. *Transplantation* **2018**, *102*, e307. [\[CrossRef\]](#)
30. Levey, A.S.; Stevens, L.A.; Schmid, C.H.; Zhang, Y.L.; Castro, A.F.; Feldman, H.I.; Kusek, J.W.; Eggers, P.; Van Lente, F.; Greene, T.; et al. A New Equation to Estimate Glomerular Filtration Rate. *Ann. Intern. Med.* **2009**, *150*, 604–612. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Almeida, M.; Calheiros Cruz, G.; Sousa, C.; Figueiredo, C.; Ventura, S.; Silvano, J.; Pedroso, S.; Martins, L.S.; Ramos, M.; Malheiro, J. External Validation of the Toulouse-Rangueil Predictive Model to Estimate Donor Renal Function After Living Donor Nephrectomy. *Transpl. Int.* **2023**, *36*, 11151. [\[CrossRef\]](#)
32. Almeida, M.; Ribeiro, C.; Silvano, J.; Pedroso, S.; Tafulo, S.; Martins, L.S.; Ramos, M.; Malheiro, J. Living Donors’ Age Modifies the Impact of Pre-Donation Estimated Glomerular Filtration Rate on Graft Survival. *J. Clin. Med.* **2023**, *12*, 6777. [\[CrossRef\]](#)
33. Sacks, D.B.; Arnold, M.; Bakris, G.L.; Bruns, D.E.; Horvath, A.R.; Kirkman, M.S.; Lernmark, A.; Metzger, B.E.; Nathan, D.M. Position Statement Executive Summary: Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. *Diabetes Care* **2011**, *34*, 1419–1423. [\[CrossRef\]](#)
34. Ge, Y.; Liu, T.; Deng, L.; Zhang, Q.; Liu, C.; Ruan, G.; Xie, H.; Song, M.; Lin, S.; Yao, Q.; et al. The Age-related Obesity Paradigm: Results from Two Large Prospective Cohort Studies. *J. Cachexia Sarcopenia Muscle* **2024**, *15*, 442–452. [\[CrossRef\]](#)
35. Bugeja, A.; Harris, S.; Ernst, J.; Burns, K.D.; Knoll, G.; Clark, E.G. Changes in Body Weight Before and After Kidney Donation. *Can. J. Kidney Health Dis.* **2019**, *6*, 2054358119847203. [\[CrossRef\]](#)
36. Punjala, S.R.; Adamjee, Q.; Silas, L.; Gökmen, R.; Karydis, N. Weight Trends in Living Kidney Donors Suggest Predonation Counselling Alone Lacks a Sustainable Effect on Weight Loss: A Single Centre Cohort Study. *Transpl. Int.* **2021**, *34*, 514–524. [\[CrossRef\]](#)
37. Issa, N.; Sánchez, O.A.; Kukla, A.; Riad, S.M.; Berglund, D.M.; Ibrahim, H.N.; Matas, A.J. Weight Gain after Kidney Donation: Association with Increased Risks of Type 2 Diabetes and Hypertension. *Clin. Transpl.* **2018**, *32*, e13360. [\[CrossRef\]](#)
38. Sanchez, O.A.; Ferrara, L.K.; Rein, S.; Berglund, D.; Matas, A.J.; Ibrahim, H.N. Hypertension after Kidney Donation: Incidence, Predictors, and Correlates. *Am. J. Transplant.* **2018**, *18*, 2534–2543. [\[CrossRef\]](#)

39. Kim, S.H.; Hwang, H.S.; Yoon, H.E.; Kim, Y.K.; Choi, B.S.; Moon, I.S.; Kim, J.C.; Hwang, T.K.; Kim, Y.S.; Yang, C.W. Long-Term Risk of Hypertension and Chronic Kidney Disease in Living Kidney Donors. *Transplant. Proc.* **2012**, *44*, 632–634. [\[CrossRef\]](#)
40. Thiel, G.T.; Nolte, C.; Tsinalis, D.; Steiger, J.; Bachmann, L.M. Investigating Kidney Donation as a Risk Factor for Hypertension and Microalbuminuria: Findings from the Swiss Prospective Follow-up of Living Kidney Donors. *BMJ Open* **2016**, *6*, e010869. [\[CrossRef\]](#)
41. Almeida, M.; Reis Pereira, P.; Silvano, J.; Ribeiro, C.; Pedrosa, S.; Tafulo, S.; Martins, L.S.; Silva Ramos, M.; Malheiro, J. Longitudinal Trajectories of Estimated Glomerular Filtration Rate in a European Population of Living Kidney Donors. *Transpl. Int.* **2024**, *37*, 13356. [\[CrossRef\]](#)
42. Grams, M.E.; Sang, Y.; Levey, A.S.; Matsushita, K.; Ballew, S.; Chang, A.R.; Chow, E.K.H.; Kasiske, B.L.; Kovesdy, C.P.; Nadkarni, G.N.; et al. Kidney-Failure Risk Projection for the Living Kidney-Donor Candidate. *N. Engl. J. Med.* **2016**, *374*, 411–421. [\[CrossRef\]](#)
43. Lentine, K.L.; Kasiske, B.L.; Levey, A.S.; Adams, P.L.; Alberú, J.; Bakr, M.A.; Gallon, L.; Garvey, C.A.; Guleria, S.; Li, P.K.-T.; et al. KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* **2017**, *101*, S7–S105. [\[CrossRef\]](#)
44. Praga, M.; Hernández, E.; Herrero, J.C.; Morales, E.; Revilla, Y.; Díaz-González, R.; Rodicio, J.L. Influence of Obesity on the Appearance of Proteinuria and Renal Insufficiency after Unilateral Nephrectomy. *Kidney Int.* **2000**, *58*, 2111–2118. [\[CrossRef\]](#)
45. Serrano, O.K.; Sengupta, B.; Bangdiwala, A.; Vock, D.M.; Dunn, T.B.; Finger, E.B.; Pruett, T.L.; Matas, A.J.; Kandaswamy, R. Implications of Excess Weight on Kidney Donation: Long-Term Consequences of Donor Nephrectomy in Obese Donors. *Surgery* **2018**, *164*, 1071–1076. [\[CrossRef\]](#)
46. Hotamisligil, G.S. Inflammation and metabolic disorders. *Nature* **2006**, *444*, 860–867. [\[CrossRef\]](#)
47. Ouchi, N.; Parker, J.L.; Lugus, J.J.; Walsh, K. Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* **2011**, *11*, 85–97. [\[CrossRef\]](#)
48. Frutos, M.Á.; Crespo, M.; de la Oliva Valentín, M.; Hernández, D.; de Sequera, P.; Domínguez-Gil, B.; Pascual, J. Living-Donor Kidney Transplant: Guidelines with Updated Evidence. *Nefrología* **2022**, *42*, 1–4. [\[CrossRef\]](#)
49. Pascual, J.; Cruzado, J.M.; Alonso, Á.; Diekman, F.; Gallego, R.J.; Gutiérrez-Dalmau, Á.; Hernández, D.; Morales, J.M.; Rodrigo, E.; Zárraga, S. Kidney Transplantation Group of the Spanish Society of Nephrology. *Nefrología* **2013**, *33*, 160–163. [\[CrossRef\]](#)
50. Lafranca, J.A.; Spoon, E.Q.W.; van de Wetering, J.; IJzermans, J.N.M.; Dor, F.J.M.F. Attitudes among Transplant Professionals Regarding Shifting Paradigms in Eligibility Criteria for Live Kidney Donation. *PLoS ONE* **2017**, *12*, e0181846. [\[CrossRef\]](#)

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APPENDIX 5 – Hypertension after Living Kidney Donation: Incidence, Predictors, and Consequences.

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RESEARCH

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Hypertension after living kidney donation: incidence, predictors, and consequences

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Abstract

Living donor (LD) kidney transplantation is the optimal treatment for elective end-stage kidney disease (ESKD) patients. However, concerns about the long-term health risks for donors, including hypertension, remain incompletely defined. We aimed to determine the incidence and predictors of hypertension after kidney donation and to assess its association with estimated glomerular filtration rate (eGFR) trajectories over time. We conducted a retrospective cohort study of 300 LD who underwent nephrectomy between 1998 and 2020, after excluding those with pre-donation hypertension. Donors were followed for a mean of 6.4 ± 4.5 years. The primary outcome was the development of de novo hypertension, defined by office blood pressure measurements exceeding 140/90 mmHg or initiation of antihypertensive therapy. Predictors of post-donation hypertension were evaluated using Cox regression analysis, and the association with longitudinal changes in eGFR was assessed using linear mixed-effects models. Hypertension developed in 30% of the cohort, with an incidence of 21% at 5 years and over 50% at 15 years post-donation. Higher body mass index (BMI) (HR 1.074 per Kg/m², $P=0.039$), dyslipidemia (HR 1.959, $P=0.035$), and elevated systolic (HR 1.030 per mmHg, $P=0.009$) and diastolic blood pressure (HR 1.036 per mmHg, $P=0.039$) were independently associated with an increased risk of hypertension post-donation. The development of hypertension was associated with a modest but statistically significant decline in eGFR over time (-0.03 vs. $+0.40$ ml/min/year, $P=0.026$). No donor progressed to ESKD during follow-up and cardiovascular events were rare in the cohort. In conclusion, hypertension was a frequent complication post-donation in this Southern European cohort and was associated with modifiable risk factors, as higher BMI and dyslipidemia. While the associated decline in renal function over time is modest, these findings highlight the importance of long-term monitoring and proactive management of cardiovascular risk factors in living kidney donors. These results should inform pre-donation counseling, post-donation follow-up strategies, and further research into interventions that may mitigate hypertension risk while preserving donor health.

Keywords Living kidney donor, Hypertension, Renal function, eGFR, Nephrectomy, Cardiovascular risk, Longitudinal study

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Introduction

Living donor (LD) kidney transplantation (KT) remains the best treatment for eligible patients with end-stage kidney disease (ESKD) [1–3]. Although living kidney donation does not provide direct medical benefits to the donor, the perceived risks are generally low and considered ethically acceptable [3]. However, emerging evidence suggests a small but measurable increase in risks such as hypertension, cardiovascular disease, ESKD, and mortality among donors [4–6]. As the global organ shortage persists, transplant programs are increasingly accepting donors with controlled hypertension or obesity, reflecting broader population trends [7]. Nevertheless, long-term data on these changing risks remain limited.

Reduction of renal mass has been linked to increased blood pressure and the development of hypertension in both animal models and humans with reduced nephron mass [8, 9]. Although a connection between nephron endowment, hypertension, and renal function has been proposed, these pathways are not well-defined in living donors [10, 11]. Kidney donation may increase the risk of developing hypertension through mechanisms such as hyperfiltration, altered vascular tone, and activation of the renin-angiotensin-aldosterone system [12–21]. However, it remains uncertain whether kidney donation independently increases the risk of hypertension or whether higher detection rates simply reflect closer medical follow-up of donors, a phenomenon known as ‘surveillance bias’ [12–19].

Hypertension is a major cause of cardiovascular and renal morbidity in the general population and is commonly reported as a cause of ESKD in living donors, particularly late after donation [22]. However, its underlying mechanisms, diagnosis, and management in donors are not well characterized [23]. Recognizing these uncertainties, the Kidney Disease Improving Global Kidney Outcomes (KDIGO) Clinical Practice guidelines highlight the need for robust studies to evaluate the incidence and consequences of hypertension in living donors [24].

In this context, our study investigates the incidence of hypertension after living kidney donation in a Portuguese cohort, identifies its risk factors, and examines its impact on renal function over time. To achieve this, we analyzed the incidence and predictors of hypertension following donation and evaluated its association with annual changes in estimated glomerular filtration rate (eGFR) in living donors.

Methods

We retrospectively reviewed the clinical data of all adult living kidney donors who underwent nephrectomy at our center between January 1998 and January 2020 ($n = 365$). Of these, 55 donors with pre-donation hypertension were excluded (detailed characteristics and comparisons with

the study cohort are provided in Table 1). An additional ten donors were excluded due to missing data on pre- and post-donation blood pressure. The final study cohort thus comprised 300 living kidney donors.

Medical records of the LDs were reviewed to extract relevant data, including baseline demographic, anthropometric, analytical, and clinical information. Hypertension was defined as an office blood pressure measurement exceeding 140/90 mmHg, a previous diagnosis of hypertension, or the use of antihypertensive medication. Ambulatory blood pressure monitoring (ABPM) was used to confirm or rule out hypertension in patients with high office blood pressure when diagnostic uncertainty existed, and in treated patients when there was doubt about blood pressure control. ABPM diagnostic thresholds followed ESC/ESH guidelines [25]: 135/85 mmHg for daytime average, 130/80 mmHg for 24-hour average, and 120/70 mmHg for nighttime average.

Dyslipidemia was defined as a total cholesterol ≥ 200 mg/dL, LDL cholesterol > 130 mg/dL, triglycerides > 150 mg/dL, HDL cholesterol < 40 mg/dL, or the use of lipid-lowering therapy. Obesity was defined as a Body Mass Index (BMI) ≥ 30 Kg/m², while overweight was defined as a BMI between 25 and 29.9 Kg/m². Estimated glomerular filtration rate (eGFR) was calculated using the CKD-Epidemiology Collaboration (CKD-EPI) creatinine-based equation, ignoring race. The date of nephrectomy was considered the start of follow-up and all donors are offered lifetime annual follow-up appointments at our center.

The primary outcome of this study was the development of hypertension after donation. Donors were followed from the date of nephrectomy until one of the following occurred: death, ESKD, attaining 15-year follow-up, or end-of-study period (December 31, 2022).

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Hospital de Santo António. Informed consent was obtained from all donors prior to donation, and data confidentiality was strictly maintained throughout the study.

Statistical analysis

Continuous data were described using mean and standard deviation (SD) or median (interquartile range [IQR]), and categorical data were expressed as numbers (and percentages) as appropriate. Categorical data including were compared using Pearson chi-square test or Fisher exact test, and continuous variables were compared with Student's *t*-test or Mann–Whitney *U*-test, as appropriate. The primary outcome was the incidence of de novo hypertension, analyzed as a time-to-event approach and visualized as a Kaplan–Meier curve. Predictors of this outcome were explored through Cox

Table 1 Baseline characteristics for the study cohort

	Total N=300	No HT N=211 (70%)	HT N=89 (30%)	P	Univariate HR (95% CI)	P
Age, mean ± SD	45.6 ± 10.5	45.2 ± 10.7	46.6 ± 10.5	0.287	1.019 (0.999–1.039)	0.064
Age, n (%)				0.787		
< 40	89 (30)	65 (31)	24 (27)		Ref	
40–55	148 (49)	103 (49)	45 (51)		1.224 (0.746–2.011)	0.424
≥ 55	63 (21)	43 (20)	20 (22)		1.476 (0.813–2.679)	0.200
Sex F, n (%)	213 (71)	152 (72)	61 (69)	0.542	0.864 (0.552–1.352)	0.521
BMI Kg/m ² , mean ± SD	24.9 ± 3.2	24.5 ± 3.2	26.0 ± 3.0	< 0.001	1.132 (1.061–1.208)	< 0.001
BMI, n (%)				0.001		
< 25	162 (54)	127 (60)	35 (39)		Ref	
25–30	117 (39)	68 (32)	49 (55)		1.997 (1.293–3.083)	0.002
≥ 30	21 (7)	16 (8)	5 (6)		1.216 (0.476–3.107)	0.682
Smoking habits, n (%)	52 (17)	44 (21)	8 (9)	0.013	0.461 (0.223–0.955)	0.037
Dyslipidemia, n (%)	30 (10)	17 (8)	13 (15)	0.084	1.997 (1.105–3.608)	0.022
ProtU 0.15–0.5 g/g, n (%)	83 (28)	64 (30)	19 (21)	0.112	0.831 (0.499–1.383)	0.476
Serum creatinine, mean ± SD	0.75 ± 0.16	0.74 ± 0.16	0.75 ± 0.16	0.805	1.338 (0.357–5.022)	0.666
Pre- donation eGFR ml/min/1.73m ² , mean ± SD	101.8 ± 14.7	102.1 ± 15.0	101.1 ± 14.0	0.570	0.993 (0.979–1.006)	0.294
Pre- donation eGFR ml/min/1.73m ² , n (%)				0.858		
< 80	25 (8)	18 (9)	7 (8)		1.033 (0.474–2.250)	0.936
80–90	39 (13)	26 (12)	13 (15)		1.337 (0.738–2.420)	0.338
≥ 90	236 (79)	167 (79)	69 (78)		Ref	
Related donor, n (%)	200 (67)	132 (63)	68 (76)	0.020	1.434 (0.876–2.348)	0.151
Family history HT, n (%)	105 (35)	53 (25)	52 (58)	< 0.001	1.850 (1.198–2.856)	0.006
SBP, mean ± SD	120.4 ± 11.2	118.3 ± 11.0	125.4 ± 10.2	< 0.001	1.048 (1.029–1.067)	< 0.001
DBP, mean ± SD	72.7 ± 8.5	71.2 ± 8.4	76.3 ± 7.6	< 0.001	1.057 (1.030–1.085)	< 0.001
Total cholesterol, mean ± SD	193.6 ± 36.2	191.5 ± 35.0	198.6 ± 38.6	0.119	1.003 (0.998–1.009)	0.257

n - number; HT - hypertension; HR - hazard ratio; SD - standard deviation; F - female; BMI - Body Mass Index; ProtU - protein/creatinine ratio in the urine; eGFR - estimated glomerular filtration rate, SBP- systolic blood pressure; DBP- diastolic blood pressure

regression models. Risk factors associated with pre-donation hypertension were analyzed by logistic regression models. Donor eGFR annual change between one-year to 15-years post-donation was assessed by univariate linear mixed regression model, that imputed subject-specific random effects (intercept and slope defined as eGFR at one-year and time in years, respectively) on an unstructured covariance matrix. The dependent variable was all eGFR measurements and the independent variable (de novo hypertension as a time-varying variable) was entered as 2-way interaction terms between them and the time (in years) variable and imputed as time-varying variable. A similar approach was used to analyze the annualized change in SBP and DBP.

To compare the incidence of hypertension in living kidney donors with that in the general population from Northern Portugal, we calculated standardized event rates (SERs) using the indirect standardization method, adjusted for age and sex. Specifically, the observed number of hypertension cases in the donor cohort was compared with the expected number of cases, calculated by applying the age- and sex-specific incidence rates from

the Portuguese population cohort to the age and sex distribution of the living kidney donor cohort.

Statistical calculations were performed using STATA/MP, version 17.1 (Stata Corp, College Station, TX, USA). A 2-sided P-value < 0.05 was considered as statistically significant.

Results

A total of 300 living kidney donors were included, with a mean follow-up of 6.4 ± 4.5 years. The baseline characteristics of our study cohort are summarized in Table 1.

The mean age of the population at the time of donation was 45.6 ± 10.5 years, and more than 20% of the individuals were 55 years or older. Most donors were female (71%), and all donors but one were caucasian. Overweight and obesity were present in 39% and 7% of donors, respectively, and 17% reported smoking habits. Two-thirds were related to the recipient, and 35% had a family history of hypertension.

During the follow-up, 89 patients (30%) developed hypertension. Donors who developed hypertension were comparable in age, sex, and pre-donation kidney function (creatinine and eGFR) to those who remained

Table 2 Cumulative incidence of hypertension post-donation

Timepoint	Incidence % (95% CI)
1 year	11.1 (8.0–15.3)
5 years	21.1 (16.6–26.6)
10 years	41.1 (33.8–49.5)
15 years	52.2 (41.9–63.4)

CI - confidence interval

normotensive but had higher pre-donation BMI and systolic blood pressure (SBP), were more likely to have dyslipidemia, to smoke, and to have donated to a first-degree relative.

The incidence of hypertension increased progressively, reaching 11% at 1 year, 21% at 5 years, 41% at 10 years, and over 50% at 15 years post-donation (Table 2). The median time to onset of hypertension was 14.6 years. The Kaplan-Meier curve (Fig. 1) illustrates the cumulative incidence of hypertension over time.

In the multivariable cox-regression analysis (Table 3), independent pre-donation predictors of de novo hypertension included higher BMI (HR 1.074 per 1 Kg/m² increase, 95% CI 1.004–1.149, $P=0.039$), dislipidemia

(HR 1.959, 95% CI 1.050–3.656, $P=0.035$), and higher baseline SBP (HR 1.030 per 1 mmHg increase, 95% CI 1.007–1.053, $P=0.009$), and diastolic blood pressure (DBP) (HR 1.036 per 1 mmHg increase, 95% CI 1.002–1.067, $P=0.039$). This indicates a 3% increase in risk for each one mmHg increase in pre-donation SBP and a 3.6% increase in risk for each one mmHg increase in pre-donation DBP.

De novo hypertension was associated with a modest yet significantly greater annual decline in eGFR compared to normotensive donors (-0.03 vs. $+0.40$ ml/min/1.73 m²/year; difference -0.43 ml/min/1.73 m²/year, $P=0.026$) (Table 4). No donor progressed to ESKD or CKD stage 5 during follow-up. At the last available assessment, two donors had reached CKD stage 4, four donors had CKD stage 3B, and 51 donors were classified as having CKD stage 3a, according to KDIGO CKD classification.

Donors with hypertension had a higher annual increase in systolic blood pressure ($+0.65$ vs. $+0.18$ mmHg/year; $P=0.024$), while diastolic blood pressure changes did not differ significantly. The mean ($\pm$ SD) SBP was 117.8 ± 0.3

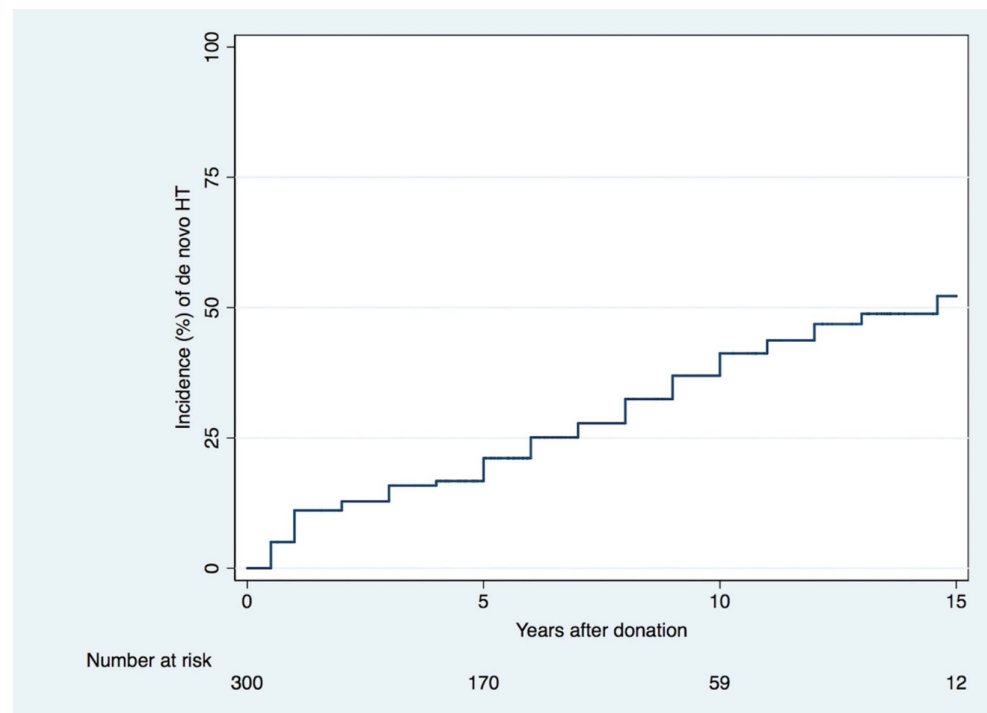
**Fig. 1** Kaplan-Meier curve depicting the cumulative incidence of de novo hypertension in the living kidney donor cohort during follow-up

Table 3 Independent predictors of de novo hypertension-multivariable analysis*

	Multivariable HR (95% CI)	P
Age (years)	-	ns
< 40	-	ns
40–55	-	
≥ 55	-	
Sex F	-	ns
BMI Kg/m ²	1.074 (1.004–1.149)	0.039
BMI	-	ns
<25	-	
25–30	-	
≥30	-	
Smoking habits	-	ns
Dyslipidemia	1.959 (1.050–3.656)	0.035
ProtU 0.15–0.5 g/g	-	ns
Serum creatinine	-	ns
Pre-donation eGFR ml/min/1.73m ²	-	ns
Pre-donation eGFR ml/min/1.73m ²	-	ns
<80	-	
80–90	-	
≥90	-	
Related donor	-	ns
Family history HTA	-	ns
SBP	1.030 (1.007–1.053)	0.009
DBP	1.036 (1.002–1.067)	0.039
Total cholesterol	-	ns

HR – hazard ratio; ns – non-significant; F – female; BMI – Body Mass Index; ProtU – protein/creatinine ratio in the urine; eGFR – estimated glomerular filtration rate, SBP – systolic blood pressure; DBP – diastolic blood pressure

*All variables in Table 3, including age and sex, were included in the multivariable model, regardless of univariate significance (Table 1). We did not report the non-significant ($P > 0.05$) HR (95% CI)

Table 4 Annual change in eGFR according to de novo hypertension status (time-varying) (data available in 264 donors)

De novo HT	Yearly change in eGFR, ml/min	P
No	+0.40 (-0.03; +0.84)	
Yes	-0.03 (-0.55; +0.50)	
Difference	-0.43 (-0.81; -0.05)	0.026

HT – hypertension; eGFR – estimated glomerular filtration rate

mmHg in the group without hypertension and 132.7 ± 2.5 in the group with de novo hypertension.

Overweight and obesity were significantly more prevalent among donors who developed hypertension throughout follow-up. Additionally, at 10 years post-donation, dyslipidemia was significantly more common among donors with hypertension compared to those without hypertension (53% vs. 24%, $P = 0.002$). In contrast, the prevalence of proteinuria and smoking did not differ significantly between the groups during the follow-up period.

Beyond these metabolic risk factors, we also evaluated post-donation cardiovascular events within the cohort. The very low number of events precluded meaningful statistical analysis. Within the main cohort, one donor without post-donation hypertension experienced an acute coronary syndrome with unremarkable coronary angiography. Another donor who developed hypertension post-donation suffered sudden death without a confirmatory autopsy.

To contextualize the incidence of hypertension in our donor cohort, we compared our findings with those of a contemporaneous, unselected cohort from northern Portugal [26] (Table 5). After adjusting for age and sex, the incidence of hypertension in our living donor cohort was markedly higher than expected based on population rates (Table 5). During the first five years post-donation, donors experienced a nearly fourfold higher incidence of hypertension (SER 3.75, 95% CI 2.94–4.86). Between 5 and 10 years post-donation, the incidence remained more than threefold higher (SER 3.25, 95% CI 2.12–4.76). At 10–15 years, the incidence was still approximately 2.3 times higher, although this difference did not reach statistical significance, likely due to the smaller number of donors at risk during this interval (SER 2.28, 95% CI 0.84–4.97).

Finally, as a sensitivity analysis, we characterized donors with pre-donation hypertension, who were excluded from the main analysis, and compared them with donors without hypertension (Supplemental Table S1). Moreover, we searched for pre-donation hypertension risk factors. Donors with pre-donation hypertension

Table 5 Incidence of hypertension in the cohort of LKD and in a cohort of the Portuguese population from the North of Portugal

Time after donation	LKD cohort (n)	Observed cases (n)	Crude incidence (%)	Expected cases (n)*	Standardized event ratio (SER) (95% CI)	Excess cases (Observed – Expected)
0–5 years	300	57	19%	15.2	3.75 (2.94–4.86)	+ 41.8
5–10 years	161	26	16%	8.0	3.25 (2.12–4.76)	+ 18.0
10–15 years	55	6	11%	2.6	2.28 (0.84–4.97)	+ 3.4

*Expected cases were calculated by applying age- and sex-specific incidence rates from a Northern Portuguese population cohort (Pereira et al., 2012 [26]) to the age/sex distribution of the donor cohort

Abbreviations: LKD = living kidney donor; SER = standardized event ratio (observed/expected cases, adjusted for age and sex); CI = confidence interval

were generally older, had a higher BMI, and were more likely to be smokers. Additionally, they had lower eGFR at the time of donation. In the multivariable analysis, age, BMI and SBP emerged as significant risk factors of pre-donation hypertension.

Discussion

In this Southern European cohort of 300 living kidney donors with a mean follow-up of over six years, we found that hypertension was common, affecting 30% of donors, with an incidence reaching 21.1% at five years, and over 50% at 15 years. Key pre-donation predictors of de novo hypertension included higher BMI, dyslipidemia, and elevated SBP, and DBP underscoring the importance of modifiable cardiovascular risk factors in this setting. Additionally, donors who developed hypertension experienced a modest but statistically significant decline in eGFR over time compared to those who remained normotensive. However, the clinical relevance of this decline is likely limited.

Prior studies have reported inconsistent findings regarding hypertension risk after donation, often due to variations in definitions, follow-up durations, and comparator groups. Studies such as those from Holshner et al. [12], Haugen et al. [27] and the seminal meta-analysis by Boudeville et al. [19] demonstrated a higher incidence of hypertension in donors compared to matched non-donors. In contrast, the meta-analysis by O'Keeffe [28], the work by Sanchez et al. [23], and two recent large prospective, matched control study by Garg et al. [29] and Kasiske [30] found no clear evidence of an increased risk for hypertension or cardiovascular disease in living kidney donors. Our findings differ from these latter studies. Whereas these North American cohorts were predominantly composed of standard-risk White donors and employed matched controls with a median follow-up of 7–9 years, our study excluded donors with pre-existing hypertension but no other comorbidities, extended follow-up to 15 years, and used regional population data for comparison [26]. Under these conditions, we observed a three- to fourfold higher incidence of hypertension among Portuguese donors. Moreover, Portuguese donors may carry a higher baseline cardiometabolic burden, which could amplify long-term hypertension risk. Taken together, these differences suggest that living donor outcomes are not uniform across populations, and that region-specific data are crucial to inform donor counseling and surveillance strategies. The observation of a higher incidence despite this selection highlights a potential role of kidney donation itself in developing hypertension over time.

We identified modifiable metabolic factors, including higher BMI and dyslipidemia, as significant predictors of post-donation hypertension, underscoring the

importance of these parameters in donor selection and follow-up. Additionally, higher pre-donation systolic and diastolic blood pressures were independent predictors of post-donation hypertension, with each 1 mmHg increase associated with a 3% and 3.6% higher risk, respectively. While these increments are modest per unit, they are clinically meaningful when evaluating donors with higher baseline blood pressures, highlighting the importance of thorough blood pressure assessment during donor evaluation. In contrast, although age was associated with pre-donation hypertension, it was not an independent predictor of post-donation hypertension in our cohort, emphasizing the prominent role of modifiable risk factors in the development of hypertension after nephrectomy. Post-donation hypertension also remained significantly associated with overweight and obesity throughout follow-up. Finally, we were unable to evaluate the relationship between post-donation hypertension and cardiovascular events due to the very low number of cardiovascular events observed in our cohort.

Regarding kidney function, donors who developed hypertension experienced a small but statistically significant decline in eGFR after diagnosing hypertension compared to those who remained normotensive. Although the clinical relevance of this decline is likely limited, the finding supports the need for continued monitoring of renal function in donors, particularly those who develop hypertension. Importantly, no donor progressed to end-stage kidney disease during follow-up, and only a small proportion ($n = 2$, 0.6%) developed stage 4 chronic kidney disease, consistent with literature indicating a low risk of severe kidney function decline in appropriately selected donors [31, 32].

While our study did not assess the pathophysiological mechanisms underlying the development of hypertension after kidney donation, our findings are consistent with proposed processes involving hyperfiltration, nephron hypertrophy, and neurohormonal activation [33, 34]. Although these compensatory adaptations are initially beneficial, they may contribute over time to detrimental effects, including disruption of the renin-angiotensin-aldosterone system, sodium balance, and progressive glomerulosclerosis [33, 34]. Notably, a long-term study of glomerular hemodynamics following kidney donation reported that glomerular hypertension did not emerge as a significant contributor [35]. Age-related physiological changes and the accumulation of additional cardiovascular risk factors may further amplify these effects, underscoring the importance of the adequacy of blood pressure monitoring and control in donors who develop hypertension. The association we observed between post-donation hypertension and modest eGFR decline warrants further investigation to clarify whether hypertension contributes to, or results from, reduced renal function. Although

hypertension was linked to a decline in eGFR over time in our cohort, causality cannot be established from these findings.

Several limitations should be acknowledged in our study. The retrospective design and the definition of hypertension based on either medication use or in-office blood pressure measurement may have introduced misclassification bias. The relatively modest sample size and the predominantly caucasian population in our cohort may limit the generalizability of the findings to more diverse populations and the ability to detect associations with less frequent outcomes, such as major cardiovascular events. Furthermore, the analysis did not account for the severity of hypertension, the specific anti-hypertensive regimens used, or the adequacy of blood pressure control over time, factors that could potentially influence renal outcomes and should be assessed in future studies.

Despite these limitations, our study adds important insights from a Southern European perspective, reinforcing the need for long-term, structured follow-up of living kidney donors with a focus on cardiovascular risk management. Future research should explore interventions aimed at mitigating post-donation hypertension and preserving donor kidney function, including the potential role of nephroprotective therapies, such as renin-angiotensin system inhibitors and SGLT2 inhibitors, in this population.

In conclusion, we found that hypertension is common after kidney donation in our cohort, with modifiable risk factors such as elevated BMI and dyslipidemia playing a significant role in its development. Post-donation hypertension is associated with a modest but measurable decline in kidney function over time, highlighting the importance of long-term monitoring and proactive management of metabolic risk factors in living kidney donors. These findings should guide pre-donation counseling and post-donation monitoring strategies to support donor health and inform shared decision-making in living kidney donation.

Abbreviations	
ABPM	Ambulatory Blood Pressure Monitoring
BMI	Body Mass Index
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CI	Confidence Interval
CV	Cardiovascular
DBP	Diastolic Blood Pressure
ESKD	End-Stage Kidney Disease
eGFR	Estimated Glomerular Filtration Rate
HR	Hazard Ratio
HT	Hypertension
IQR	Interquartile Range
KT	Kidney transplantation
LD	Living Donor
ProtU	Proteinuria
SBP	Systolic Blood Pressure
SD	Standard Deviation

Supplementary Information
The online version contains supplementary material available at <https://doi.org/10.1186/s12882-025-04513-5>.

Supplementary Material 1

Author contributions
LM, SP, and JS participated in data collection. JM contributed to data collection, article design, and statistical analysis. JF and MA contributed to data collection, article design, and writing.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Specific consent to participate was not obtained as it was not required under national regulations either for consent to participate or for data publication, but at time of transplant all patients consent data their clinical data might be collected, analyzed and published in a anonymously manner. Additionally, ethics approval was deemed unnecessary in accordance with Portuguese legislation.

Consent for publication
Specific consent for publication was not obtained as it was not required under national regulations either for consent to participate or for data publication, but at time of transplant all patients consent data their clinical data might be collected, analyzed and published in a anonymously manner.

Competing interests
The authors declare no competing interests.

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References

1. Gruessner RWG, Gruessner AC. Solid-organ transplants from living donors: cumulative united States experience on 140,156 living donor transplants over 28 years. *Transpl Proc.* 2018;50(10):3025–35.
2. Park YH, et al. Comparison of survival probabilities for living-unrelated versus cadaveric renal transplant recipients. *Transpl Proc.* 2004;36(7):2020–2.
3. Reese PP, Boudville N, Garg AX. Living kidney donation: outcomes, ethics, and uncertainty. *Lancet.* 2015;385(9981):2003–13.
4. Muzaale AD, et al. Risk of end-stage renal disease following live kidney donation. *JAMA.* 2014;311(6):579–86.
5. Mjoen G, et al. Long-term risks for kidney donors. *Kidney Int.* 2014;86(1):162–7.
6. Garg AX, et al. Gestational hypertension and preeclampsia in living kidney donors. *N Engl J Med.* 2015;372(15):1469–70.
7. Ahmadi AR, et al. Shifting paradigms in eligibility criteria for live kidney donation: a systematic review. *Kidney Int.* 2015;87(1):31–45.
8. Bhatena DB, et al. Focal sclerosis of hypertrophied glomeruli in solitary functioning kidneys of humans. *Am J Kidney Dis.* 1985;5(5):226–32.
9. Haylor J, et al. Renal function and morphometry in the Dwarf rat following a reduction in renal mass. *Nephrol Dial Transpl.* 1996;11(4):643–50.
10. Novick AC, et al. Long-term follow-up after partial removal of a solitary kidney. *N Engl J Med.* 1991;325(15):1058–62.
11. Al Ammary F, et al. Risk of ESKD in older live kidney donors with hypertension. *Clin J Am Soc Nephrol.* 2019;14(7):1048–55.

12. Holscher CM, et al. Self-Reported incident hypertension and Long-Term kidney function in living kidney donors compared with healthy nondonors. *Clin J Am Soc Nephrol*. 2019;14(10):1493–9.
13. Lentine KL, et al. Understanding antihypertensive medication use after living kidney donation through linked National registry and pharmacy claims data. *Am J Nephrol*. 2014;40(2):174–83.
14. Lentine KL, Segev DL. Understanding and communicating medical risks for living kidney donors: A matter of perspective. *J Am Soc Nephrol*. 2017;28(1):12–24.
15. Lentine KL, et al. Consistency of Racial variation in medical outcomes among publicly and privately insured living kidney donors. *Transplantation*. 2014;97(3):316–24.
16. Lentine KL, et al. Racial variation in medical outcomes among living kidney donors. *N Engl J Med*. 2010;363(8):724–32.
17. Lentine KL, et al. Postdonation eGFR and New-Onset antihypertensive medication use after living kidney donation. *Transpl Direct*. 2019;5(8):e474.
18. Garg AX, et al. Cardiovascular disease and hypertension risk in living kidney donors: an analysis of health administrative data in Ontario. *Can Transplantation*. 2008;86(3):399–406.
19. Boudville N, et al. Meta-analysis: risk for hypertension in living kidney donors. *Ann Intern Med*. 2006;145(3):185–96.
20. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? *Am J Hypertens*. 1988;1(4 Pt 1):335–47.
21. Torres VE, et al. Blood pressure determinants in living-related renal allograft donors and their recipients. *Kidney Int*. 1987;31(6):1383–90.
22. Anjum S, et al. Patterns of End-Stage renal disease caused by Diabetes, Hypertension, and glomerulonephritis in live kidney donors. *Am J Transpl*. 2016;16(12):3540–7.
23. Sanchez OA, et al. Hypertension after kidney donation: Incidence, predictors, and correlates. *Am J Transpl*. 2018;18(10):2534–43.
24. Lentine KL, et al. KDIGO clinical practice guideline on the evaluation and care of living kidney donors. *Transplantation*. 2017;101(8S Suppl 1):S1–109.
25. McEvoy JW, et al. 2024 ESC guidelines for the management of elevated blood pressure and hypertension. *Eur Heart J*. 2024;45(38):3912–4018.
26. Pereira M, et al. Incidence of hypertension in a prospective cohort study of adults from Porto, Portugal. *BMC Cardiovasc Disord*. 2012;12:114.
27. Haugen AJ, et al. Increased long-term risk for hypertension in kidney donors - a retrospective cohort study. *Transpl Int*. 2020;33(5):536–43.
28. O'Keefe LM, et al. Mid- and Long-Term health risks in living kidney donors: A systematic review and Meta-analysis. *Ann Intern Med*. 2018;168(4):276–84.
29. Garg AX, et al. Hypertension and kidney function after living kidney donation. *JAMA*. 2024;332(4):287–99.
30. Kasiske BL, et al. A prospective controlled study of metabolic and physiologic effects of kidney donation suggests that donors retain stable kidney function over the first nine years. *Kidney Int*. 2020;98(1):168–75.
31. Almeida M, et al. Longitudinal trajectories of estimated glomerular filtration rate in a European population of living kidney donors. *Transpl Int*. 2024;37:13356.
32. Textor SC, et al. Blood pressure and renal function after kidney donation from hypertensive living donors. *Transplantation*. 2004;78(2):276–82.
33. Choi SY, et al. Adaptive functional change of the contralateral kidney after partial nephrectomy. *Am J Physiol Ren Physiol*. 2017;313(2):F192–8.
34. Srivastava T, et al. Hyperfiltration-mediated injury in the remaining kidney of a transplant donor. *Transplantation*. 2018;102(10):1624–35.
35. Lenihan CR, et al. Longitudinal study of living kidney donor glomerular dynamics after nephrectomy. *J Clin Invest*. 2015;125(3):1311–8.

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APPENDIX 6 – Urinary metabolomics reveals early renal metabolic adaptation following living kidney donation: a longitudinal pilot study

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Urinary metabolomics reveals early renal metabolic adaptation following living kidney donation: a longitudinal pilot study

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The institutional review board at our institution approved this study (Ref.: 147-21 (119-DEFI/122-CE))

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Abstract

Background: Living donor kidney transplantation is the optimal treatment for selected patients with end-stage renal disease, but ensuring long-term donor safety remains essential. Routine clinical markers, including estimated glomerular filtration rate (eGFR) and albuminuria, capture only part of the adaptive response after nephrectomy, leaving early subclinical metabolic changes undetected.

Methods: In this exploratory pilot study, paired urine samples from 18 living kidney donors were collected before (T0) and six months after donation (T6). Untargeted proton nuclear magnetic resonance (¹H-NMR) metabolomics was performed, and integrated with clinical, biochemical, and CT volumetric data to characterize renal functional and metabolic adaptation.

Results: Consistent with the physiological response to nephrectomy, donors showed a decline in eGFR and increases in serum creatinine, urea, and cystatin C, while albuminuria remained stable. Urinary metabolomic profiling revealed significant decreases in citrate, hypoxanthine, betaine, creatine, acetate, formate, and glycine at T6. Higher eGFR correlated positively with urinary citrate and hypoxanthine, whereas serum creatinine correlated negatively. Both metabolites also showed positive associations with remnant kidney volume and the volume-to-weight ratio ($r = 0.43\text{--}0.63$), linking metabolic shifts to structural adaptation. Receiver-operating characteristic analyses identified citrate, hypoxanthine, and betaine as the top discriminatory metabolites between pre- and post-donation states (AUC 0.77–0.90).

Conclusions: This proof-of-concept study demonstrates that urinary metabolomics can reveal early biochemical adaptations in living kidney donors that are not captured by conventional measures, supporting a multidimensional framework for donor follow-up and personalized risk assessment.

Keywords:

Living kidney donation; urinary metabolomics; citrate; hypoxanthine; betaine; renal adaptation; NMR spectroscopy; biomarker; eGFR;

Introduction

Living donor (LD) kidney transplantation (KT) remains the best treatment for selected patients with end-stage renal disease (ESRD). Compared with deceased donor kidney transplantation, LDKT offers superior graft and patient survival, shorter waiting times, and lower overall costs [1-3]. While these recipient benefits are well documented, ensuring the long-term safety and well-being of living kidney donors (LKD) is both an ethical and clinical imperative.

After nephrectomy, the remaining kidney undergoes compensatory hypertrophy and adaptive hyperfiltration to preserve overall renal function [4-10]. Although kidney mass is reduced by approximately 50%, the glomerular filtration rate (GFR) usually falls by only 25–35%, reflecting effective physiological adaptation. This process is generally well tolerated, yet emerging evidence suggests that it may confer increased long-term susceptibility to hypertension, chronic kidney disease (CKD), and metabolic disturbances in some donors [11, 12]. These findings highlight the need for more sensitive biomarkers capable of detecting early, subclinical alterations and stratifying donors according to future risk [13, 14].

The recently introduced Cardiovascular–Kidney–Metabolic (CKM) health framework provides a multidimensional perspective on these processes, emphasizing the shared mechanisms that link renal, cardiovascular, and metabolic dysfunction [15, 16]. Although not yet applied to living donation, this concept is directly relevant: while the structural and functional compensations of the remnant kidney are well characterized, the accompanying systemic metabolic responses remain insufficiently understood.

Conventional clinical markers such as serum creatinine, estimated GFR, and albuminuria offer only partial insight into the biochemical dynamics of renal adaptation. In contrast, metabolomics, a systems biology approach that enables comprehensive profiling of small-molecule metabolites, can capture real-time metabolic perturbations that precede overt clinical changes [17]. Urinary metabolomics has previously identified disease-specific signatures in diabetic kidney disease [18], hypertensive nephrosclerosis [19], acute kidney injury [20], and graft dysfunction [21, 22]. However, while plasma metabolomics has been explored in donors, urinary metabolic responses remain largely unexplored and may better reflect renal-specific adaptation [22-24].

Given the kidney's pivotal role in metabolic regulation and excretion, urine represents an ideal biofluid for studying post-donation adaptation. We therefore performed an untargeted, longitudinal ¹H-NMR metabolomic analysis of paired pre- and post-donation urine samples from living kidney donors. We hypothesized that nephrectomy induces measurable changes in urinary metabolite profiles, particularly in pathways related to energy metabolism, redox balance, and osmolyte homeostasis, reflecting early adaptive responses with potential value for donor risk assessment and personalized follow-up.

Material and Methods

Study design and participants

This observational, longitudinal pilot study included 18 living kidney donors who underwent nephrectomy at our Institute between September 2022 and July 2023 and were evaluated before donation (T0) and again at 6 months after nephrectomy (T6) (Figure 1). Only donors who provided both pre- and post-donation urine samples were included to enable paired longitudinal analysis. All participants underwent standard clinical evaluations, following international recommendations [25, 26] and provided written informed consent. Potential donors with uncontrolled hypertension, evidence of end-organ damage, diabetes, malignancy, BMI > 35 kg/m², or proteinuria > 300 mg/day were excluded. Final donor approval was granted after multidisciplinary evaluation, and all procedures received institutional ethics committee approval.

As this was an exploratory pilot study, the sample size was determined by feasibility and resource availability rather than a priori power calculation. The goal was to identify within-subject metabolic changes and estimate effect sizes to guide the design of adequately powered future studies.

The study was approved by the Institutional Review Board of Our Center (anonymized for review) (Ref. 119-DEFI/122-CE) and conducted in accordance with the Declaration of Helsinki. The study is reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Sample collection and data collection

Demographic, anthropometric, and clinical data were recorded for all participants, including age, sex, weight, BMI, and comorbidities. Laboratory measurements comprised complete blood count, serum albumin, parathyroid hormone, calcium, phosphorus, thyroid-stimulating hormone (TSH), creatinine, urea, uric acid, fasting glucose, glycated hemoglobin, total proteins, and lipid profile (total, LDL, and HDL cholesterol, and triglycerides). Urinalysis included albuminuria, proteinuria, and albumin-to-creatinine ratio (ACR) from spot urine samples. The estimated glomerular filtration rate (eGFR) was calculated using the 2021 CKD-EPI creatinine equation [27].

To minimize dietary and circadian influences, fasting morning urine samples were collected before nephrectomy and at six months post-donation, immediately aliquoted, and stored at -80 °C until lyophilization. All samples were thawed only once prior to metabolomic analysis to prevent degradation.

CT volumetric assessment

CT angiography was performed using multidetector scanners (Siemens Somatom Go Top 64, Siemens Healthineers, Erlangen, Germany; GE Revolution Evo 64 and Revolution 128, GE Healthcare, Milwaukee, USA; Philips Incisive, Philips Healthcare, Best, The Netherlands). Renal volumetry was conducted on a dedicated workstation (AW 4.4 VolumeShare 2, GE Healthcare, Milwaukee, USA) using a semi-automatic segmentation method with manual correction as required. Each kidney was analyzed independently by the same trained radiology technician, who was blinded to the clinical and metabolomic data to minimize bias. Post-donation volume was defined as the parenchymal volume of the remaining kidney, excluding the collecting system and vessels.

Metabolomic workflow

Prior to ^1H -NMR analysis, proteins were precipitated from the urine samples. Specifically, 30 mg of lyophilized urine was dissolved in 105 μL of an ice-cold methanol/water solution (1:2 v/v), incubated at -20°C for 30 minutes, and then centrifuged at $13,000 \times g$ for 30 minutes. The supernatant was collected and dried using a SpeedVac concentrator. The dried samples were reconstituted in 225 μL of a sodium fumarate solution in 0.2 M phosphate buffer (pH 7.0) prepared in D_2O ([fumarate] = 2 mM). The samples were analyzed in 3-mm NMR tubes (Norell, Landisville, NJ, USA) with NOR5X3INSB optimizer inserts (Norell, Landisville, NJ, USA).

The metabolites in the urine samples were analyzed using ^1H -NMR spectroscopy and spectral analysis. One-dimensional ^1H -NMR spectra were recorded on a 500 MHz Bruker Avance III HD spectrometer equipped with a 5 mm TXI probe (Bruker Biospin, Karlsruhe, Germany) at 298 K. Solved-suppressed 1D- ^1H -NMR spectra were acquired using zgpr pulse sequence, with a spectral width of 7 kHz, relaxation delays of 7s, pulse angle of 30° , acquisition of 32 scans. A line broadening of 0.2 Hz and a Fourier transformation were applied to the spectra. Phase and baseline were manually corrected. Sodium fumarate served as an internal reference for chemical shift calibration and metabolite quantification (2 mM; 6.50 ppm). It was possible to identify the metabolites represented in Supplementary Table 1. The integration of selected metabolite peaks was performed using Bruker TopSpin 4.3 software (Bruker BioSpin, Rheinstetten, Germany). Metabolite concentrations were normalized to urinary creatinine and expressed as nmol/mg creatinine to correct for dilution effects.

Statistical analysis

All data analyses were performed in MetaboAnalyst 6.0, GraphPad Prism 8.0.1, and IBM SPSS Statistics 29.0. Metabolite intensities were log-transformed and auto-scaled before analysis. Principal component analysis (PCA) provided an unsupervised overview, followed by partial least squares–discriminant analysis (PLS-DA) to identify discriminant metabolites.

Variables with a variable importance in projection (VIP) score > 1.5 were considered relevant for group separation. Model quality was assessed using 5-fold cross-validation and reported R^2 (explained variance) and Q^2 (predictive ability) values.

Normality was assessed with the Shapiro–Wilk test. Because data were paired (T0 vs T6), comparisons were performed using paired t-tests or Wilcoxon signed-rank tests as appropriate. Correlations between metabolites and clinical or volumetric variables were assessed by Pearson (parametric) or Spearman (non-parametric) coefficients. Correlation matrices were visualized as heatmaps, and key associations illustrated by scatterplots.

Receiver operating characteristic (ROC) curve analyses were applied to evaluate the discriminatory performance of selected metabolites. Statistical significance was defined as $p < 0.05$.

Results

Donor population characteristics before and after kidney donation

A total of 18 living kidney donors were included in this study, of whom 14 (77.8%) were women and 4 (22.2%) were men, with a mean age of 51.4 ± 7.9 years. The clinical and biochemical characteristics of the donors before nephrectomy (T0) and at six months post-donation (T6) are summarized in Table 1. Anthropometric parameters, including body weight and BMI, showed no significant change over the six-month follow-up ($p = 0.65$).

Following nephrectomy, donors exhibited the characteristic decline in renal function. Serum creatinine increased from 0.68 ± 0.11 mg/dL to 1.06 ± 0.17 mg/dL ($p < 0.0001$), while eGFR (CKD-EPI 2021) decreased from 103.6 ± 7.7 to 69.1 ± 11.7 mL/min/1.73 m² ($p < 0.0001$). Serum urea and uric acid levels also rose significantly ($p = 0.0075$ and $p = 0.02$, respectively). Cystatin C increased from 0.83 ± 0.13 to 1.14 ± 0.19 mg/L ($p < 0.0001$), confirming reduced filtration capacity, whereas albumin and total protein concentrations remained unchanged. Albuminuria and proteinuria remained stable, confirming preserved glomerular integrity. No significant changes were observed in fasting glucose, HbA1c, lipid profile, or liver enzyme activities, indicating preserved systemic metabolic stability. Total thyroxine (T4) levels showed a mild but statistically significant increase at 6 months post-donation (3.54 ± 3.54 vs. 6.04 ± 3.67 µg/dL, $p = 0.04$), although all values remained within the physiological range. This variation likely reflects assay or short-term metabolic fluctuation rather than a clinically relevant endocrine change.

CT-based volumetry demonstrated the expected reduction in renal parenchymal volume, with total kidney volume decreasing from 346.9 ± 58.5 to 175.4 ± 32.2 cm³ ($p < 0.0001$) and kidney

volume indexed to body weight from 5.42 ± 1.06 to 2.69 ± 0.58 cm³/kg ($p < 0.0001$), consistent with loss of renal mass after unilateral nephrectomy.

Urinary metabolic profile pre-donation and post-donation

To compare the urinary metabolomic profiles of living kidney donors before (T0) and six months after donation (T6), principal component analysis (PCA) was first applied (Fig. 2A). The 95% confidence ellipses showed partial overlap between groups, indicating modest global metabolic variation. To enhance class discrimination and identify metabolites contributing most to group separation, a supervised partial least squares discriminant analysis (PLS-DA) was performed (Fig. 2B). Although the PLS-DA model did not achieve complete separation between pre- and post-donation samples, several metabolites exhibited strong discriminatory power. Betaine and formate were the most significantly altered metabolites, each presenting variable importance in projection (VIP) scores greater than 1.5 (Fig. 2C).

Urinary metabolomic signature after kidney donation

Using ¹H-NMR spectroscopy, a total of 19 urinary metabolites were identified in living kidney donors (Supplementary Table 1). Among these, seven showed significant decreases six months after donation compared with baseline (Fig. 3). Citrate demonstrated the most pronounced reduction (0.26 ± 0.10 vs. 0.52 ± 0.19 nmol/mg creatinine, $p < 0.0001$), followed by hypoxanthine (0.007 ± 0.004 vs. 0.013 ± 0.008 nmol/mg creatinine, $p = 0.002$), betaine (0.30 ± 0.51 vs. 0.61 ± 0.58 nmol/mg creatinine, $p = 0.005$), creatine (0.06 ± 0.03 vs. 0.10 ± 0.06 nmol/mg creatinine, $p = 0.01$), acetate (0.03 ± 0.01 vs. 0.05 ± 0.02 nmol/mg creatinine, $p = 0.02$), formate (0.027 ± 0.018 vs. 0.043 ± 0.028 nmol/mg creatinine, $p = 0.04$), and glycine (0.18 ± 0.10 vs. 0.26 ± 0.12 nmol/mg creatinine, $p = 0.05$). All values were normalized to urinary creatinine to account for concentration variability.

Alanine and succinate showed similar downward trends that approached significance ($p = 0.052$ and $p = 0.055$, respectively), suggesting consistent global reductions across key energy metabolism intermediates. These post-donation shifts predominantly involved metabolites linked to the tricarboxylic acid (TCA) cycle, purine degradation, and osmolyte balance, indicating a coordinated metabolic adaptation of the remnant kidney.

The remaining metabolites' tendencies are available in Supplementary Figure 1.

Correlations with renal function and volumetric parameters

Correlation analysis revealed that several urinary metabolites were significantly associated with renal functional and structural parameters. Both citrate and hypoxanthine showed positive correlations with eGFR (CKD-EPI) ($r = 0.59$ and $r = 0.51$, respectively, $p < 0.05$) and negative

correlations with serum creatinine ($r = -0.62$ and $r = -0.47$), $p < 0.05$. These associations were verified using Pearson or Spearman correlation tests, depending on data distribution.

Importantly, these metabolites also correlated positively with remnant kidney volume and kidney volume-to-weight ratio ($r = 0.43$ – 0.63), and kidney volumetric parameters correlated to eGFR ($r = 0.79$ – 0.83 , $p < 0.001$), linking metabolic shifts to compensatory renal hypertrophy and functional adaptation (Figure 4).

Urinary formate correlated positively with urinary creatinine ($r = 0.39$, $P = 0.022$), suggesting coordinated excretory dynamics and concentration-dependent metabolite handling.

Inter-metabolite correlation analysis revealed several strong associations consistent with shared biochemical pathways. Notably, formate correlated with hypoxanthine ($r = 0.54$, $p < 0.001$) and acetate ($r = 0.57$, $p < 0.001$), while succinate, pyruvate, and acetate were tightly linked ($r = 0.50$ – 0.73 , $p < 0.01$), reflecting coordinated changes in energy metabolism. Betaine showed positive correlations with acetate and alanine ($r = 0.6$ – 0.62 , $p < 0.001$), and glycine correlated with lactate and alanine ($r = 0.57$ – 0.60 , $p < 0.05$). Together, these inter-metabolite relationships illustrate a tightly interconnected metabolic network underlying post-donation renal adaptation.

Potential Biomarkers of post-donation adaptation

By our metabolite analysis, it was demonstrated that Citrate, Hypoxanthine, Betaine, Creatine, Acetate, Formate, and Glycine were differentiated in the patients before and after kidney donation. Receiver operating characteristic (ROC) curve analysis identified citrate, hypoxanthine, and betaine as the top discriminatory metabolites differentiating pre- and post-donation states (AUC = 0.90, 0.81, and 0.77, respectively; Figure 5). Optimal cut-off values were < 0.40 nmol/mg for citrate, < 0.007 nmol/mg for hypoxanthine, and < 0.14 nmol/mg for betaine. These metabolites, representing key nodes in organic acid, osmolyte, and purine metabolism, may serve as candidate biomarkers of metabolic adaptation following living kidney donation. Collectively, the results indicate a coordinated biochemical response paralleling structural and functional renal changes.

Discussion

This pilot study provides the first longitudinal assessment of urinary metabolomic changes after living kidney donation using untargeted ^1H -NMR spectroscopy. By integrating metabolomic, functional, and volumetric data, we identified early biochemical signatures that reflect the adaptive response of the remaining kidney to nephrectomy. Despite a physiological decline in eGFR, donors maintained stable albuminuria and normal metabolic profiles, confirming overall preserved renal integrity. In this cohort of eighteen paired donors, consistent

post-donation decreases were observed in urinary citrate, hypoxanthine, betaine, creatine, acetate, formate, and glycine. Among these, citrate, hypoxanthine, and betaine showed the strongest discriminatory ability between pre- and post-donation states and correlated with renal function and structural parameters, suggesting that they reflect integrated adaptive processes involving energy metabolism, osmotic regulation, and purine turnover.

After unilateral nephrectomy, the remnant kidney undergoes structural hypertrophy and hemodynamic hyperfiltration to restore total GFR [6]. These adaptations are largely beneficial but impose increased metabolic demand and oxidative stress on nephrons. The observed decline in urinary citrate aligns with its role as a key intermediate of the tricarboxylic acid (TCA) cycle and a regulator of acid–base homeostasis [28]. Reduced citrate excretion may result from enhanced proximal tubular reabsorption or altered mitochondrial metabolism within the remnant kidney [28–30], representing an adaptive mechanism to improve metabolic efficiency under increased nephron workload. In contrast to CKD, where low urinary citrate often reflects metabolic acidosis or mitochondrial dysfunction, this pattern likely indicates physiological conservation rather than pathology [29, 30], supporting its potential as a sensitive indicator of renal metabolic adaptation and early stress response.

Hypoxanthine, a purine degradation intermediate, plays a central role in cellular energy metabolism and redox regulation. It is metabolized by xanthine oxidoreductase to xanthine and uric acid, a process that generates reactive oxygen species (ROS) and contributes to oxidative stress, particularly during ischemia–reperfusion injury [31, 32]. The post-donation decline in urinary hypoxanthine may therefore reflect reduced oxidative activity and preferential recycling through the purine salvage pathway, enhancing energy conservation in the remnant kidney [33]. Unlike in pathological states characterized by hypoxia or tissue injury, where hypoxanthine accumulation signals metabolic distress, the reduction observed here likely represents restoration of redox homeostasis and metabolic stabilization during the adaptive recovery phase [32, 34, 35].

Betaine, an essential osmolyte and methyl donor essential for medullary osmoregulation [36, 37], showed a pronounced decline post-donation. The kidney plays a major role in betaine homeostasis, and its urinary excretion reflects both filtration and tubular reabsorption. Lower betaine levels likely represent increased tubular conservation to preserve medullary osmoprotection and methylation potential under altered hemodynamic and osmotic conditions [38]. Similar betaine depletion has been reported in patients with adaptive hyperfiltration and early chronic kidney disease [39, 40], supporting its sensitivity to renal workload.

Parallel decreases in formate, acetate, and glycine further suggest remodeling of one-carbon and mitochondrial energy pathways [41, 42]. Formate and acetate participate in intermediary

metabolism linking mitochondrial oxidation and methyl-group transfer, while glycine acts as a co-substrate in these cycles [34, 38]. The coherent directionality of these changes supports a coordinated energetic and redox adaptation of the remnant kidney.

Collectively, these findings fit within the recently proposed Cardiovascular–Kidney–Metabolic (CKM) framework, which conceptualizes renal adaptation as part of a broader systemic response linking energy metabolism, oxidative stress, and hemodynamic regulation [15, 16]. The observed metabolite patterns may thus represent early adaptive responses that preserve homeostasis, but which could also signal susceptibility to maladaptive remodeling in vulnerable individuals. Longitudinal monitoring of these metabolites could therefore offer a non-invasive means of assessing metabolic adaptation and stress resilience after living kidney donation.

While prior studies in donors have focused mainly on plasma metabolites or uremic toxins [22–24], this study emphasizes the utility of urinary metabolomics to capture real-time renal-specific metabolic activity. Plasma-based metabolomics has shown persistent changes in systemic uremic toxin levels post-nephrectomy (e.g., symmetric dimethylarginine (SDMA), trimethylamine N-oxide (TMAP), and indoxyl sulfate) and mitochondrial dysregulation [22–24]. However, plasma measurements provide limited information about intrarenal metabolic regulation. Our findings align with emerging literature showing persistent metabolic alterations in post-donation states, but our focus on the urine metabolome offers a more proximal lens on renal adaptation.

Moreover, the correlations between urinary citrate and hypoxanthine with both eGFR and kidney volume further reinforce the biological plausibility of these findings. Donors with higher remnant kidney volume indexed to weight exhibited higher eGFR and higher levels of these metabolites, supporting the concept that metabolic remodeling parallels structural and functional adaptation [43–45]. This observation is consistent with physiological studies demonstrating that compensatory hypertrophy after nephrectomy is accompanied by enhanced oxidative and anabolic activity within the remaining kidney parenchyma [46, 47].

The absence of microalbuminuria or other biochemical indicators of injury suggests that these metabolic alterations reflect adaptive rather than pathological hyperfiltration. Nevertheless, comparative profiling across other hyperfiltration states, such as pregnancy, obesity, or early diabetes, will be essential to distinguish physiological from maladaptive responses. Shared pathways between adaptive and pathological hyperfiltration, particularly in mitochondrial energy metabolism and osmotic regulation, may provide mechanistic insights into why some donors remain resilient while others develop long-term risk.

Despite its strengths, including standardized sample collection and integration of metabolomic, volumetric, and functional data, this study has several limitations. The sample

size was modest and derived from a single center, limiting generalizability and reducing statistical power. Furthermore, the study design included only one post-donation time point at six months, which prevents evaluation of temporal dynamics or long-term trajectories. Although fasting morning urine minimized dietary effects, residual influences from diet, gut microbiota, or circadian variation cannot be excluded. Untargeted ^1H -NMR metabolomics, though powerful for broad screening, remains semi-quantitative, and absolute concentrations of key metabolites should be confirmed using targeted LC–MS/MS approaches. Finally, the lack of a non-donor control group limits the ability to differentiate between donation-related changes and background variability.

Nevertheless, the consistency of the findings, their correlation with renal function and volume, and their convergence with prior reports underscore their robustness. The paired design, which controlled for interindividual variability, remains a key methodological advantage enabling the detection of subtle physiological adaptations.

Future studies should expand these findings through multicenter longitudinal cohorts, incorporating repeated urine and plasma metabolomics over extended follow-up, alongside volumetric imaging. Targeted quantification of citrate, hypoxanthine, and betaine, together with markers such as SDMA, TMAO, or choline, may refine metabolic panels for donor monitoring. Integration with imaging, genomics, and digital health data could enable dynamic risk reclassification and personalized long-term follow-up. Comparative studies across physiological and pathological hyperfiltration states will help delineate adaptive resilience from early maladaptation.

Conclusions

This pilot study demonstrates the feasibility and clinical potential of urinary metabolomic profiling in living kidney donors. Post-donation metabolite shifts reflect early renal and systemic adaptations that remain undetectable by conventional measures. The integration of metabolomic, volumetric, and functional data supports a multidimensional framework for donor surveillance and personalized risk stratification. These findings reinforce the ethical and clinical imperative of ensuring donor safety through sensitive, non-invasive, and advanced monitoring approaches in modern transplant care.

References

1. Axelrod, D.A., et al., *An economic assessment of contemporary kidney transplant practice*. Am J Transplant, 2018. **18**(5): p. 1168-1176.
2. Gruessner, R.W.G. and A.C. Gruessner, *Solid-organ Transplants From Living Donors: Cumulative United States Experience on 140,156 Living Donor Transplants Over 28 Years*. Transplant Proc, 2018. **50**(10): p. 3025-3035.

3. Reese, P.P., N. Boudville, and A.X. Garg, *Living kidney donation: outcomes, ethics, and uncertainty*. Lancet, 2015. **385**(9981): p. 2003-13.
4. Lam, N.N., et al., *Changes in kidney function follow living donor nephrectomy*. Kidney Int, 2020. **98**(1): p. 176-186.
5. Tan, R.Y., et al., *Predictors of low estimated glomerular filtration rate after living kidney donation in a Southeast Asian population from Singapore*. Nephrology (Carlton), 2017. **22**(10): p. 761-768.
6. Lenihan, C.R., et al., *Longitudinal study of living kidney donor glomerular dynamics after nephrectomy*. J Clin Invest, 2015. **125**(3): p. 1311-8.
7. Kim, Y., et al., *Changes in renal function and predictors affecting renal outcome of living kidney donors: a nationwide prospective cohort study*. Nephrology (Carlton), 2021. **26**(6): p. 541-548.
8. Kasiske, B.L., et al., *A prospective controlled study of metabolic and physiologic effects of kidney donation suggests that donors retain stable kidney function over the first nine years*. Kidney Int, 2020. **98**(1): p. 168-175.
9. Almeida, M., et al., *Longitudinal Trajectories of Estimated Glomerular Filtration Rate in a European Population of Living Kidney Donors*. Transplant International, 2024. **37**.
10. Srivastava, T., et al., *Hyperfiltration-mediated Injury in the Remaining Kidney of a Transplant Donor*. Transplantation, 2018. **102**(10): p. 1624-1635.
11. Muzaale, A.D., et al., *Risk of end-stage renal disease following live kidney donation*. JAMA, 2014. **311**(6): p. 579-86.
12. Mjoen, G., et al., *Long-term risks for kidney donors*. Kidney Int, 2014. **86**(1): p. 162-7.
13. Massie, A.B., et al., *Quantifying Postdonation Risk of ESRD in Living Kidney Donors*. J Am Soc Nephrol, 2017. **28**(9): p. 2749-2755.
14. Grams, M.E., A.X. Garg, and K.L. Lentine, *Kidney-Failure Risk Projection for the Living Kidney-Donor Candidate*. N Engl J Med, 2016. **374**(21): p. 2094-5.
15. Ndumele, C.E., et al., *Cardiovascular-Kidney-Metabolic Health: A Presidential Advisory From the American Heart Association*. Circulation, 2023. **148**(20): p. 1606-1635.
16. Ndumele, C.E., et al., *A Synopsis of the Evidence for the Science and Clinical Management of Cardiovascular-Kidney-Metabolic (CKM) Syndrome: A Scientific Statement From the American Heart Association*. Circulation, 2023. **148**(20): p. 1636-1664.
17. Fiehn, O., *Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks*. Comp Funct Genomics, 2001. **2**(3): p. 155-68.
18. Pereira, P.R., et al., *Metabolomics as a tool for the early diagnosis and prognosis of diabetic kidney disease*. Med Res Rev, 2022. **42**(4): p. 1518-1544.
19. Rinschen, M.M., et al., *Metabolic rewiring of the hypertensive kidney*. Sci Signal, 2019. **12**(611).
20. Patschan, D., et al., *Metabolomics in Acute Kidney Injury: The Clinical Perspective*. J Clin Med, 2023. **12**(12).
21. Bohra, R., et al., *Proteomics and metabolomics in renal transplantation-quo vadis?* Transpl Int, 2013. **26**(3): p. 225-41.
22. Gagnebin, Y., et al., *Combining the advantages of multilevel and orthogonal partial least squares data analysis for longitudinal metabolomics: Application to kidney transplantation*. Anal Chim Acta, 2020. **1099**: p. 26-38.

23. Kielstein, J.T., et al., *SDMA is an early marker of change in GFR after living-related kidney donation*. Nephrol Dial Transplant, 2011. **26**(1): p. 324-8.
24. Velenosi, T.J., et al., *Untargeted metabolomics reveals N, N, N-trimethyl-L-alanyl-L-proline betaine (TMAP) as a novel biomarker of kidney function*. Sci Rep, 2019. **9**(1): p. 6831.
25. Lentine, K.L., et al., *Summary of Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors*. Transplantation, 2017. **101**(8): p. 1783-1792.
26. Andrews, P.A. and L. Burnapp, *British Transplantation Society / Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: Summary of Updated Guidance*. Transplantation, 2018. **102**(7): p. e307.
27. Inker, L.A., et al., *New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race*. N Engl J Med, 2021. **385**(19): p. 1737-1749.
28. Hering-Smith, K.S. and L.L. Hamm, *Acidosis and citrate: provocative interactions*. Ann Transl Med, 2018. **6**(18): p. 374.
29. Baranovicova, E., et al., *Assessment of metabolites in urine in post-kidney transplant patients: insights into allograft function and creatinine clearance*. Metabolomics, 2025. **21**(2): p. 44.
30. Gianella, F.G., et al., *Spot urinary citrate-to-creatinine ratio is a marker for acid-base status in chronic kidney disease*. Kidney Int, 2021. **99**(1): p. 208-217.
31. Battelli, M.G., et al., *Xanthine Oxidoreductase-Derived Reactive Species: Physiological and Pathological Effects*. Oxid Med Cell Longev, 2016. **2016**: p. 3527579.
32. Granger, D.N. and P.R. Kvietys, *Reperfusion injury and reactive oxygen species: The evolution of a concept*. Redox Biol, 2015. **6**: p. 524-551.
33. Camici, M., et al., *Inborn Errors of Purine Salvage and Catabolism*. Metabolites, 2023. **13**(7).
34. Kim, Y.J., et al., *Hypoxanthine causes endothelial dysfunction through oxidative stress-induced apoptosis*. Biochem Biophys Res Commun, 2017. **482**(4): p. 821-827.
35. Vishwakarma, V.K., et al., *Pathophysiologic role of ischemia reperfusion injury: A review*. Journal of Indian College of Cardiology, 2017. **7**(3): p. 97-104.
36. Fan, C.Y., et al., *Betaine supplementation protects against high-fructose-induced renal injury in rats*. J Nutr Biochem, 2014. **25**(3): p. 353-62.
37. Garcia-Perez, A. and M.B. Burg, *Renal medullary organic osmolytes*. Physiol Rev, 1991. **71**(4): p. 1081-115.
38. Danilova, E.Y., et al., *CKD Urine Metabolomics: Modern Concepts and Approaches*. Pathophysiology, 2023. **30**(4): p. 443-466.
39. McGregor, D.O., et al., *Betaine supplementation decreases post-methionine hyperhomocysteinemia in chronic renal failure*. Kidney Int, 2002. **61**(3): p. 1040-6.
40. Alvarenga, L., et al., *The Role of Betaine in Patients With Chronic Kidney Disease: a Narrative Review*. Curr Nutr Rep, 2022. **11**(3): p. 395-406.
41. Richards, R.H., et al., *Acetate Metabolism in Normal Human Subjects*. American Journal of Kidney Diseases, 1982. **2**(1): p. 47-57.
42. Lamers, Y., et al., *Production of 1-Carbon Units from Glycine Is Extensive in Healthy Men and Women*. The Journal of Nutrition, 2009. **139**(4): p. 666-671.
43. Almeida, M., et al., *CT volumetry performs better than nuclear renography in predicting estimated renal function one year after living donation*. Int Urol Nephrol, 2023. **55**(3): p. 553-562.

44. Silva, F., et al., *Lower donated kidney volume is associated with increased risk of lower graft function and acute rejection at 1 year after living donor kidney-a retrospective study*. *Transpl Int*, 2020. **33**(12): p. 1711-1722.
45. Nunes-Carneiro, D., et al., *Remaining kidney volume indexed to weight as a strong predictor of estimated glomerular filtration rate at 1 year and mid-term renal function after living-donor nephrectomy - a retrospective observational study*. *Transpl Int*, 2020. **33**(10): p. 1262-1273.
46. Ozeki, M., et al., *Reactive oxygen species mediate compensatory glomerular hypertrophy in rat uninephrectomized kidney*. *J Physiol Sci*, 2009. **59**(5): p. 397-404.
47. Al-Awqati, Q., *Kidney growth and hypertrophy: the role of mTOR and vesicle trafficking*. *J Clin Invest*, 2015. **125**(6): p. 2267-70.

Table 1. Clinical and biochemical parameters of kidney donors' patients before (T0) and after 6 months of kidney donation (T6).

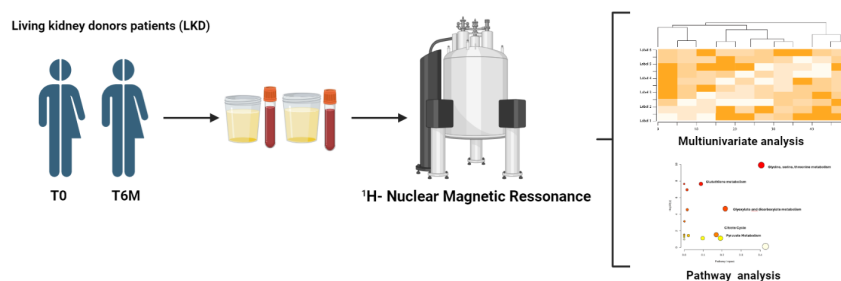
	T0	T6	p-value
Gender (% Female)	77.8		-
Age (Years)	51.44 ± 7.92		-
BMI (Kg/m ²)	24.54 ± 3.71	25.14 ± 4.04	0.65
Uric acid (mg/dL)	3.94 ± 0.81	4.86 ± 0.32	0.02
Calcium (mmol/L)	2.29 ± 0.14	2.32 ± 0.12	0.52
Phosphorous (mmol/L)	1.02 ± 0.24	1.06 ± 0.30	0.97
Glucose (mg/dL)	85.06 ± 12.70	89.00 ± 17.56	0.44
Serum Creatinine (mg/dL)	0.68 ± 0.11	1.06 ± 0.17	<0.0001
eGFR – CKD-EPI(ml/min)	103.6 ± 7.73	69.11 ± 11.71	<0.0001
Urea (mg/dL)	32.67 ± 9.49	41.67 ± 9.51	0.0075
Bilirubin mg/dL)	0.43 ± 0.21	0.37 ± 0.13	0.49
ALA/TGO (U/L)	16.33 ± 3.77	20.07 ± 8.73	0.17
ALA/TGP (U/L)	15.67 ± 9.13	18.87 ± 12.29	0.49
Alkaline Phosphatase (U/L)	60.11 ± 23.21	67.94 ± 19.05	0.29
Gama-glutamyl transferase (U/L)	16.78 ± 10.14	17.00 ± 11.63	0.84
HbA1c (%)	5.56 ± 0.41	5.64 ± 0.44	0.57
Lipid Profile			
Total Cholesterol (mg/dL)	183.4 ± 34.76	189.0 ± 34.91	0.64
Triglycerides (mg/dL)	91.61 ± 33.00	100.3 ± 37.36	0.46
HDL (mg/dL)	57.83 ± 13.48	56.40 ± 12.24	0.75
LDL (mg/dL)	108.3 ± 26.80	114.3 ± 29.65	0.55
Proteins			
Cystatin C (mg/L)	0.83 ± 0.13	1.14 ± 0.19	<0.0001
Albumin (g/dL)	4.38 ± 0.42	4.42 ± 0.31	0.95
Total Proteins (g/dL)	6.65 ± 0.44	6.85 ± 0.48	0.24
Hormones			
PTH (pg/mL)	54.56 ± 17.71	72.61 ± 44.91	0.21
TSH (μUI/mL)	1.77 ± 0.86	2.11 ± 1.24	0.53
T3 (ng/ml)	2.30 ± 1.00	2.30 ± 1.00	>0.99
T4(μg/mL)	3.54 ± 3.54	6.04 ± 3.67	0.04
Urine			
Albumin (mg/mg creat)	8.91 ± 12.35	9.06 ± 12.91	0.25
Creatinine (g/L)	113.2 ± 59.63	109.2 ± 68.97	0.68
Proteins (g/L)	0.13 ± 0.18	0.08 ± 0.07	0.34
Proteins (g/g creat)	0.09 ± 0.07	0.07 ± 0.03	0.06
Volumetry			
KV (Total) (cm ³)	346.9 ± 58.45	175.4 ± 32.19	<0.0001
KV/W (cm ³ /Kg)	5.42 ± 1.06	2.69 ± 0.58	<0.0001

Abbreviations: BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; T4, total thyroxine; T3- Total triiodothyronine; KV, kidney volume; KV/W, kidney volume indexed to body weight.

Supplementary Table 1**Table S2.** Resonance assignment in the ¹H-NMR spectra of urine sample with the listening of all the metabolites identified.

Metabolite	δ ¹ H ppm (multiplicity)
Acetate	1.90 (s)
Alanine	1.48 (d); 3.77 (q)
Benzoate	7.86 (d), 7.54 (t), 7.47 (dd)
Betaine	3.26 (s), 3.90 (s)
Citrate	2.51 (d); 2.67 (d)
Creatine	3.01 (s); 3.91 (s)
Dimethylamine	2.50 (s)
Formate	8.46 (s)
Glycine	3.54 (s)
Glycolate	3.93 (s)
Hydroxyacetone	2.27 (s); 4.40 (s)
Hypoxanthine	8.19 (s), 8.21 (s)
Isobutyrate	1.05 (td); 1.11 (dt); 1.11 (dd); 1.13 (dd); 2.37 (m); 3.70 (s)
Lactate	1.31 (d); 4.10 (q)
Pyruvate	2.46 (s)
Succinate	2.40 (s)
Taurine	3.26 (t), 3.43 (t)
Tartrate	4.30 (s)
Valine	0.97 (d); 1.02 (d); 2.25 (m); 3.59 (d)

s- *singlet*; d-*doublet*; q-*quartet*; t-*triplet*; dd-*double of doublets*, tt- *triple triplet*, m- *multiplot*, td- *triple duplet*; dt- *double triplet*

Figure 1**Figure 1.** Illustration of the experimental design of the study

First-morning urine and blood samples were collected from healthy individuals recruited as living kidney donors before (T0) and six months after donation (T6). The study included 18 donors (4 men and 14 women). Urine samples were analyzed using untargeted ¹H-NMR spectroscopy, and metabolite data were correlated with clinical and biochemical parameters obtained from paired blood and urine analyses.

Figure 2

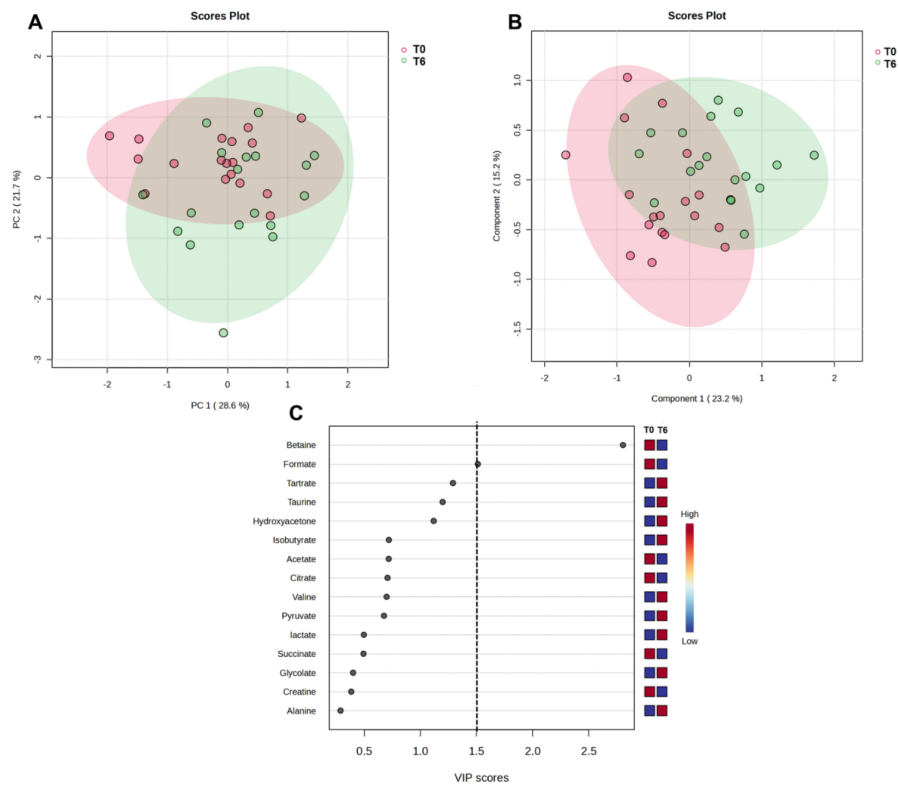


Figure 2. Multivariate analysis of the integrated urinary metabolome of living kidney donors before (T0) and after kidney donation (T6). Score scatterplots resulting from (A) Principal Component Analysis (PCA) and (B) supervised Partial Least Square Discriminant Analysis (B). Obtained Variable Importance in Projection (VIP) scores of the 15 most relevant metabolites represented in C. A VIP> 1.5 (dotted line) was considered as relevant for group separation.

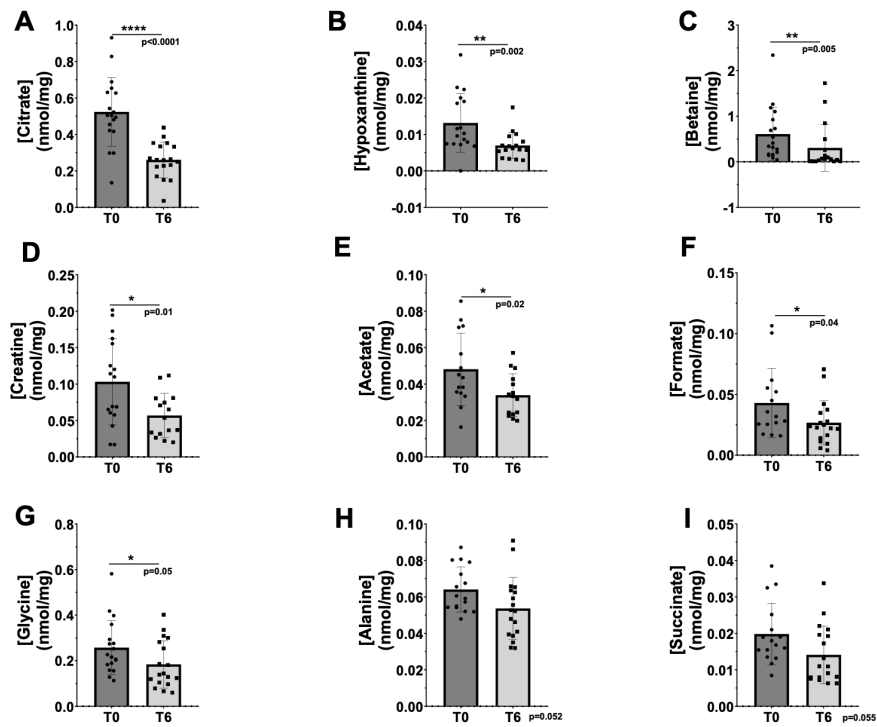
Figure 3.

Figure 3. Metabolites altered in urinary metabolome of healthy individuals recruited for living kidney donation before (T0) and after surgery (T6). Results are expressed as mean $\pm$ SD. Data were tested using paired t-test or Wilcoxon signed-rank test, depending on normality (Shapiro–Wilk test). Significance was considered at $P < 0.05$. * $P < 0.05$, ** $P < 0.005$ and *** $P < 0.0005$.

Figure 4

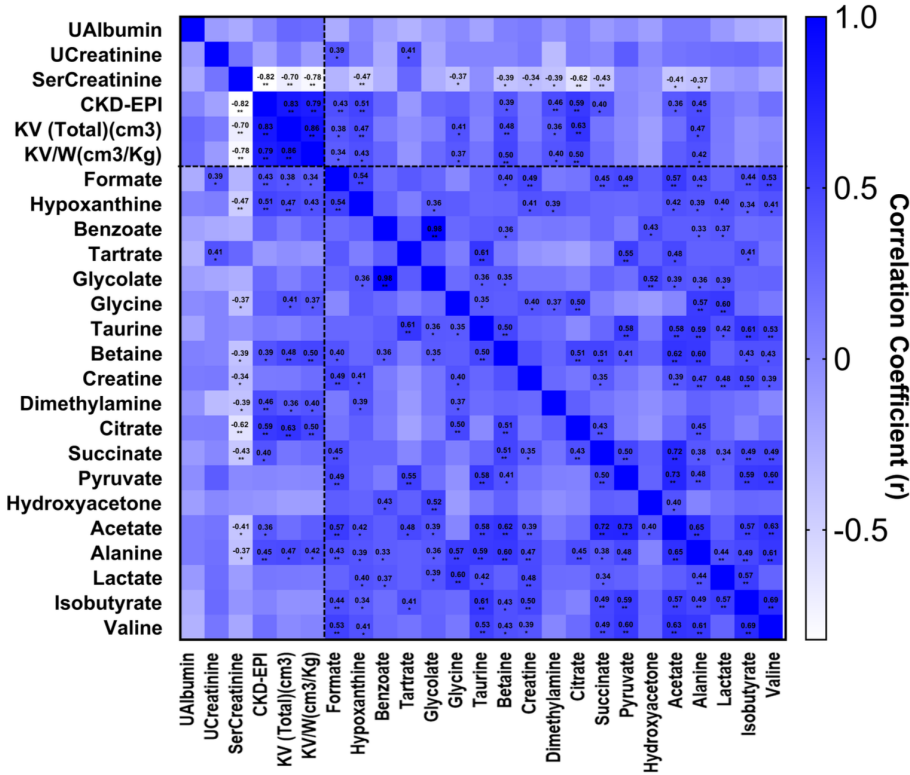


Figure 4. Correlation analysis heatmap for the combined data of metabolite and clinical biomarkers of kidney function from living kidney donors before (T0, n=18) and after (T6, N=18) surgery.

Corresponding Pearson or Spearman correlation coefficient (r) were represented when $p < 0.05$ (*) and $p < 0.01$ (**). CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; KV, kidney volume; KV/W, kidney volume indexed to body weight; UAlb, urinary albumin; UCreat, urinary creatinine.

Figure 5

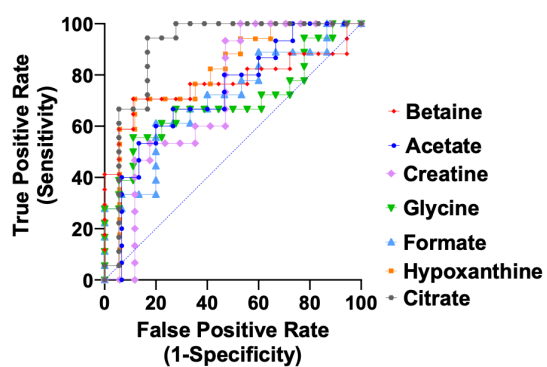
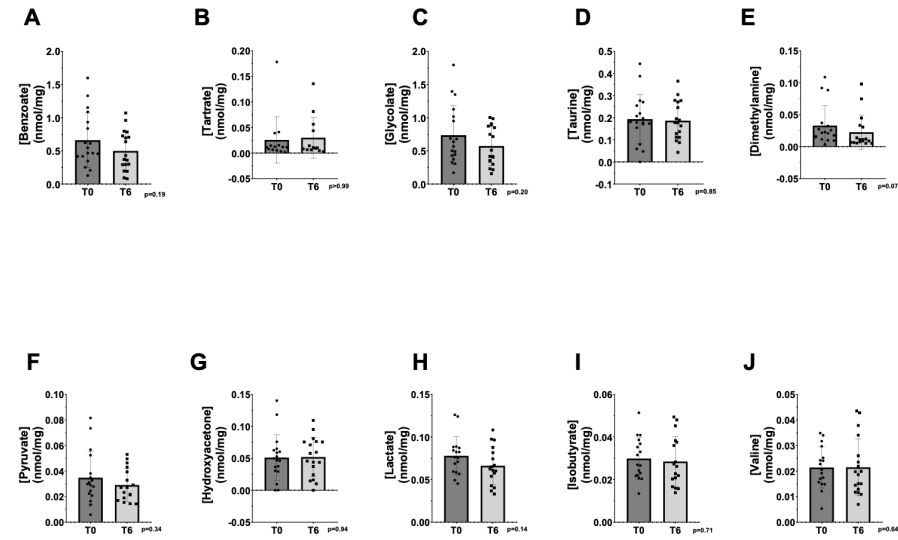


Figure 5. Receiver operation Characteristic (ROC) curve analysis for assessing the potential of using metabolic fingerprint as biomarkers of alteration after kidney donation. The obtained AUC curves for living kidney donors. Betaine (•), Acetate (•), Creatine (•), Glycine (•), Formate (•), Hypoxanthine (•) and Citrate (•). **ROC curves of the main metabolites identified with p value ≤ 0.05 .**

Supplementary Figure 1



Supplementary Figure 1. Metabolites altered in urinary metabolome of healthy individuals recruited for living kidney donation before (T0) and after surgery (T6). Results are expressed as mean $\pm$ SD. Data were tested using paired t-test or Wilcoxon signed-rank test, depending on normality (Shapiro–Wilk test). Significance was considered at $P < 0.05$. * $P < 0.05$, ** $P < 0.005$ and *** $P < 0.0005$.

APPENDIX 7 – Psychological Outcomes in a Cohort of Portuguese Living Kidney Donors

Almeida, M., Frade, IC., Sousa, A., Martins, LS., Ramos, MS., Malheiro, J., Fonseca, I. and Lopes, A.

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Psychological Outcomes in a Cohort of Portuguese Living Kidney Donors

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ABSTRACT

Background. The psychosocial aspects of living kidney donation remain a challenge nowadays. We sought to evaluate the psychological outcomes of donation in a cohort of living kidney donors (LKD).

Material and methods. LKDs who underwent nephrectomy from January 2018 to December 2019 completed a predonation psychosocial assessment followed by a postdonation evaluation using 36-Item Short Form (SF-36) and Hospital Anxiety and Depression Scale forms in May 2022. Mean scores were compared before and after the donation using the paired *t*-test. Pearson's correlation tested the relationship between anxiety and depression scores and relevant variables.

Results. Forty-four LKDs were included. Most were female (68.2%), aged 47.3 ± 10.3 years, 52.3% were genetically related, and 63.6% were compatible and donated directly to their recipients. Overall, we observed a statistically significant reduction postdonation in various components of SF-36: physical functioning ($P = .037$), bodily pain ($P = .003$), vitality ($P = .023$), social functioning ($P = .023$), and mental health ($P = .006$). Only LKDs who experienced stressful life events ($n = 19$), showed significant reductions in some SF-36 domains: physical functioning ($P = .012$), physical role ($P = .028$), bodily pain ($P = .006$), and mental health ($P = .009$). Depression scores increased significantly postdonation ($P = .029$) but lacked clinical significance. Additionally, there was a positive and significant correlation between predonation depression scores and postdonation anxiety levels postdonation.

Conclusions. Living donation is usually a safe procedure regarding psychosocial aspects. Nonetheless, some LKDs, particularly those with medical complications or stressful live events, may face adverse emotional impacts. Therefore, transplant professionals must screen, assess, and provide psychosocial support before and after donation.

A kidney transplant from a living donor is a distinctive medical procedure that involves removing an organ from a healthy person to benefit someone else. Although much of the literature on living donors emphasizes the physical effects of nephrectomy [1–4], the unique psychosocial aspects of this procedure merit equal attention. While living kidney donors (LKDs) receive no personal medical benefit from the donation, some may experience

psychosocial benefits from helping to improve the health and well-being of another person [5].

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Living donation is commonly regarded as a secure psychosocial option. However, some LKDs might encounter negative effects [6,7]. Evaluating published studies presents challenges. Many have methodological concerns and often lack sufficient information; most studies are retrospective with limited sample sizes. Additionally, although quantitative research tends to examine straightforward concepts, qualitative studies uncover that the process of living donation is far more complex and nuanced, involving renegotiations of identity, roles, and relationships [8]. Consequently, transplant professionals are responsible for screening, assessing, and offering aftercare to living organ donors, particularly regarding psychosocial matters.

While not mandatory, most LKDs guidelines suggest a psychosocial evaluation [9–11]. Unfortunately, there are limited clear directions for performing this evaluation [7,12], and many institutions often lack consistent follow-up after donation. In Portugal, under ordinance 003/INF-IPST, IP/18, it is recommended that all LKDs undergo both pre- and postdonation evaluations, regardless of their relationship to the recipients [13]. However, implementing these recommendations can be challenging due to the limited availability of dedicated psychological and psychiatric teams in transplantation units.

We sought to evaluate the psychological outcomes of donation in a cohort of individuals submitted to nephrectomy for living donor kidney transplantation in our unit, assessing the aspects of health-related quality of life (HRQoL), anxiety, and depression through the evaluation of psychometrically validated scales for the Portuguese population. We hypothesized that donors with medical or other outcomes postdonation would have the highest reduction in HRQoL scores and increased anxiety and depression scores postdonation; predonation anxiety and depression scores would relate to postdonation psychosocial outcomes.

SUBJECTS AND METHODS

We retrospectively reviewed the clinical data of all adult LKDs who underwent nephrectomy at our center between January 2018 and December 2019 ($n = 60$). Inclusion criteria included the complete predonation psychosocial evaluation protocol of the donor-recipient pairs for comparison, which was available for 46 LKD pairs (3 pairs were evaluated at another transplant center, and 11 pairs had not fully completed the protocol). Forty-four LDs completed the follow-up protocol conducted in May 2022, defining our study cohort.

The Institutional Review Board at Unidade Local de Saúde de Santo António (ULSddSA) approved this cross-sectional observational study, conducted according to the Helsinki Declaration. All donors provided written informed consent to participate.

The study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Living Donors

Following international guidelines, all donors were subjected to a standard evaluation protocol [10,11]. Baseline demographic, anthropomorphic, analytical, and clinical data were collected

from the LKDs. A dedicated team of Psychologists and Psychiatrists performed the psychosocial evaluation. The final approval for kidney donation was reviewed in a multidisciplinary meeting, and ethical approval was mandatory.

In May 2022, all donors submitted to KT during 2018 and 2019 who met the inclusion criteria were scheduled for psychology and living donor appointments and routine analysis on the same day to avoid extra hospital dislocations. According to current legislation, they were reimbursed for the dislocation expenses [14]. The psychologist (ICF) explained that the questionnaire they had to answer was self-administered. The same investigator (MA) performed the medical consultation. Some LDs who live abroad received the questionnaire by email or postmail with a prepaid envelope to send it back.

Psychosocial Evaluation Protocol

A sociodemographic questionnaire included age, gender, school level, professional activity, current employment situation, marital status, and relatedness to the recipient. The psychometrics tools included the 36-Item Short Form (SF-36), administered as an HRQoL evaluation [15], and the Hospital Anxiety and Depression Scale (HADS), designed to assess anxiety and depression status.

The SF-36 is a well-validated generic questionnaire to assess quality of life. It includes 36 items divided into eight domains: physical functioning, physical role, bodily pain, general health, vitality, social functioning, emotional role, and mental health. Scores can be obtained for each scale or aggregated into two summary scores: a Mental Component Summary and a Physical Component Summary. Likert scales and yes/no options assess function and well-being on this 36-item questionnaire. To score the SF-36 v2, scales are standardized with a scoring algorithm. Higher scores indicate better health status. Ferreira et al [16], translated and validated it for the Portuguese population.

Snaith and Zigmond described the HADS [17] to aid the clinician in recognizing the emotional components of physical illness. It is a recognized, reliable, and valid psychological screening tool. HADS consists of two subscales: one measuring anxiety, with seven items, and one measuring depression, with seven items scored separately, each item on a 4-point (0-3) response category. The possible scores range from 0 to 21 for anxiety and from 0 to 21 for depression. The HADS manual indicates that a score between 0 and 7 is “normal,” between 8 and 10, “mild,” between 11 and 14, “moderate,” and between 15 and 21, “severe.” Pais-Ribeiro et al [18] validated its psychometric properties in the Portuguese population.

Statistical Analysis

A baseline sample description was obtained using frequencies, means, and standard deviations or medians and interquartile ranges (IQR). The normality of variable distribution was determined using the Kolmogorov–Smirnov test. Mean HDAS anxiety, depression, and SF-36 subdomains scores were compared before and after transplantation using the paired *t*-test. The

relationship between anxiety and depression scores and other relevant variables was tested by Pearson's correlation.

A P value $< .05$ was considered statistically significant. Statistical calculations were performed using SPSS Statistics, version 28 (SPSS Inc.).

RESULTS

Donor Demographic and Social Data

The baseline characteristics for our study cohort are summarized in Table 1. The mean age of the population was 47.3 ± 10.32 years, and most were female (68.2%). All donors were genetically or emotionally related to their recipients. Most donors ($n = 24$, 54.5%) were genetically related, and most unrelated to their recipients were spouses ($n = 19$, 40.9%). Most LDs attended only primary school (52.4%, $n = 23$), and 27.3% ($n = 12$) completed a university degree.

In predonation, most of the donors were married or in a non-marital partnership ($n = 36$, 81.8%). Most were employed ($n = 32$, 72.1%), 14% were retired, and 11.6% ($n = 5$) were unemployed.

Twenty-eight donors were compatible with their recipients, 5 were ABO incompatible, and 11 (25%) were transplanted in the Kidney-Paired Exchange Program.

Living donors were evaluated 5.800 (IQR 3.217, 13.508) months before donation. A postdonation evaluation was performed 3.350 (IQR 2.724-3.725) years after the donation.

Complications Following Living Donor Transplantation

No donor had major postsurgical events. Fifteen donors (34.1%) reported medical complications after donation, mostly pain complaints related to the surgical procedure ($n = 6$). Nineteen patients (43.2%) experienced stressful life events after donation unrelated to the procedure; 25% of the donors had medical causes, three had psychosocial causes, and five had both medical and psychosocial causes.

One recipient had a graft loss due to thrombosis in the early postoperative period. Two recipients experienced acute kidney rejections, which were treated successfully, and one patient was readmitted to the hospital because of a urinary fistula.

HRQoL Scores Before and After Kidney Donation (SF-36)

All SF-36 component scores lowered after nephrectomy, as depicted in Table 2. We find a statistically significant reduction after donation in the components of physical functioning (from

Table 1. Sociodemographic Characteristics of the Study Cohort

			Number	%
Age (y)		47.3 (± 10.3)		
Sex		Male	14	31.8
		Female	30	68.2
Relationship with the recipient		Partners	18	40.9
		Brothers	13	29.5
		Parents	7	15.8
		Nephews	2	4.6
		Sons	1	2.3
		Brothers-in-law	1	2.3
		Cousins	1	2.3
		Friends	1	2.3
Educational level		Primary school	23	52.4
		Secondary school	9	20.3
		Licentiate degree	12	27.3
Marital status	Predonation	Married/nonmarital partnership	36	81.8
		Single	5	11.4
		Divorced	3	6.8
	Postdonation	Married/nonmarital partnership	31	70.5
		Single	7	15.9
		Divorced	6	13.6
Professional situation	Predonation	Active	31	70.5
		Unemployed	5	11.4
		Medical leave	1	2.3
		Retired	6	13.6
	Postdonation	Active	30	68.2
		Unemployed	5	11.4
		Medical leave	1	2.3
		Retired	8	18.2
Type of donation		Compatible	28	63.6
		KPE program	11	25
		ABOi program	5	11.4

ABOi, ABO incompatible; KPE, kidney paired exchange.

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Table 2. Donors' HRQoL Assessment Pre- and After-Donation, Overall Population

	Predonation	After-Donation	P
Physical functioning	93.64 ± 8.784	86.45 ± 20.956	.037*
Physical role	90.75 ± 25.138	79.95 ± 34.986	.055
Bodily pain	86.70 ± 17.922	74.52 ± 27.032	.003*
General health	76.84 ± 18.767	70.23 ± 24.256	.146
Vitality	70.45 ± 12.381	61.61 ± 23.755	.023*
Social functioning	91.48 ± 12.865	80.81 ± 26.773	.018*
Emotional role	88.29 ± 27.597	79.40 ± 36.144	.159
Mental health	80.00 ± 14.280	67.64 ± 26.129	.006*

HRQoL, health-related quality of life.

* Statistically significant.

93.64 ± 8.784 to 86.45 ± 20.956, $P = .037$), bodily pain (from 86.70 ± 17.922 to 74.52 ± 27.032, $P = .003$), vitality (from 70.45 ± 12.381 to 61.61 ± 23.755, $P = .023$), social functioning (from 91.48 ± 12.865 to 80.81 ± 26.773, $P = .023$), and mental health (from 80.00 ± 14.280 to 67.64 ± 26.129, $P = .006$).

Compatible donors who donated directly to their recipients did not score significantly different from those who were incompatible or donated in a kidney-paired program, except for mental health. Compatible donors scored significantly lower postdonation: 61.82 ± 26.07 vs 77.81 ± 23.671 predonation, $P = .005$.

We then stratified the analysis considering the occurrence of medical complications during donation or a stressful life event after donation.

In those LDs with medical complications after nephrectomy, the scores for bodily pain and mental health diminished significantly after donation (91.60 ± 13.271 to 77.73 ± 18.359, $P = .010$; and 80.80 ± 13.539 to 65.93 ± 26.111, $P = .034$). In those LDs without medical complications, only the bodily pain scores diminished significantly after the nephrectomy (from 84.17 ± 19.640 to 72.86 ± 30.745, $P = .047$). All other scores diminished in both populations after donation, but not significantly.

Considering significant life events after donation, we find different patterns in those with and without events. While the latter did not have significant reductions in the SF-36 scores in any of its domains those with stressful life events presented statistically significant reductions in the scores of physical functioning (93.42 ± 6.678 to 79.47 ± 21.724, $P = .012$), physical role (93.89 ± 21.341 to 71.05 ± 41.048, $P = .028$), bodily pain (86.26 ± 18.720 to 67.89 ± 25.591, $P = .006$), and mental health (74.74 ± 15.552 to 55.89 ± 24.642, $P = .009$).

Anxiety and Depression Scores-HDAS

Most donors ($n = 40$, 90.9%) did not present scores suggesting an anxiety diagnosis in the predonation evaluation. Of those who scored positive on the anxiety scale, one was classified as suffering from severe anxious symptoms, three (6.8%) with moderate symptoms, and five (11.4%) with mild symptoms (Table 3). Post-donation, most donors ($n = 36$, 81.6%) remained without scores suggesting an anxiety diagnosis. Still, more

Table 3. Donors' HADS Assessment Pre- and After-Donation, Overall Population

	Predonation	After-Donation	P
Global scores			
Anxiety	5.16 ± 3.585	5.79 ± 4.454	.308
Depression	2.05 ± 2.104	3.37 ± 3.761	.029*
Dicotomic analysis			
Anxiety yes (11-21)	4	7	
Anxiety no (0-10)	40	36	
Depression yes (11-21)	0	1	
Depression no (0-10)	44	41	
Anxiety scores			
No symptoms	35	26	
Mild symptoms	5	10	
Moderate symptoms	3	5	
Severe symptoms	1	2	
Depression scores			
No symptoms	43	34	
Mild symptoms	1	8	
Moderate symptoms	0	1	
Severe symptoms	0	0	

HADS, Hospital Anxiety and Depression Scale.

* Statistically significant.

donors scored on the anxiety scale: two with severe anxiety-suggestive symptoms, five (11.4%) with moderate, and 10 (22.7%) with mild symptoms. Postdonation anxiety scores increased compared to predonation ones from 5.16 ± 3.585 to 5.79 ± 4.454, although it was not statistically significant ($P = .308$) (Table 3).

No donor presented a score of suggestive depression diagnosis in the predonation evaluation, although one had mild symptoms on the HDAS scale. Postdonation, most donors ($n = 41$, 93.2%) did not have a score suggestive of a depression diagnosis, but one (2.3%) had it. For the donors who scored on the depression scale, most ($n = 8$) had mild symptoms, and one had moderate symptoms. No donor had severe symptoms. Postdonation, the depression scores increased significantly from 2.05 ± 2.104 predonation to 3.37 ± 3.761, $P = .029$ (Table 3).

The occurrence of medical complications from the nephrectomy reported in 15 donors was associated with increased scores of anxiety and depression, although not statistically significant. Those without medical complications after surgery did not present any remarkable changes in the scores for anxiety and depression pre- and postdonation.

The occurrence of stressful life events after donation in 19 donors was associated with a significant increase in depression scores from 2.79 ± 2.463 to 5.79 ± 4.036, $P = .015$, and a non-significant increase in anxiety scores from 6.89 ± 3.740 to 8.84 ± 4.311, $P = .071$.

Predonation anxiety scores were positively and significantly correlated with depression scores predonation ($r = 0.602$, $P < .001$) and anxiety scores postdonation ($r = 0.526$, $P < .001$). Postdonation anxiety was positively correlated with depression postdonation ($r = 0.788$, $P < .001$). Predonation depression was positively and significantly correlated with anxiety postdonation ($r = 0.309$, $P = .049$).

Additionally, we find a significant negative correlation between anxiety scores (before and postdonation) and the time passed between evaluation and donation: the longer the time, the lower the anxiety ($r = -0.367$, $P = .015$).

DISCUSSION

In our group of LKDs, the psychosocial results from living donations were encouraging, particularly in terms of psychosocial complications. However, we cannot definitively comment on the psychological advantages of living donation. There is a lack of clarity regarding what constitutes a negative psychosocial outcome, including whether it merely reflects a poorer postdonation score compared to baseline. Nevertheless, they underscored the importance of ongoing psychosocial support, especially for donors facing certain risk factors such as medical complications, stressful life events, anxiety, or depressive symptoms. Postdonation, the analysis of HRQoL indicates a statistically significant decrease in physical functioning, body pain, vitality, social functioning, and mental health scores. However, no changes were observed in any SF-36 dimensions among individuals not impacted by stressful life events. Remarkably, postdonation, the scores were still elevated in comparison to the normative values for the Portuguese population [16].

Postdonation anxiety and depression scores rose compared to predonation assessments; however, only the latter showed a significant increase, which lacked clinical relevance. Correlations were found between predonation depression scores and postdonation anxiety scores, indicating a positive and significant relationship with anxiety following donation.

The literature generally shows that most LKDs had positive experiences with donation, with few postoperative psychosocial complications [6]. However, methodological issues in these studies made the findings unreliable. There were inconsistencies between different studies and a disconnect between qualitative and quantitative literature [6,8]. Detailed quantitative studies on donor benefit and comprehensive psychosocial factor measurements are lacking [7,19].

Most research on the psychosocial effects of donation on living donors focuses on assessing HRQoL scores [6]. The SF-36 questionnaire is frequently used in studies, either alone or in combination with other tools. Its popularity in living donor research increased after being employed in studies that compared different surgical methods for living donor nephrectomy. Over time, these assessment tools have been incorporated into the literature on psychosocial outcomes. They are regarded as reliable, standardized measures that are user-friendly, available in various languages, and widely referenced in living donor studies. However, although these generic measures allow for comparisons among diverse populations, they might lack the sensitivity needed to address the unique complexities of the living donor group. A systematic review and meta-analysis assessing the psychosocial well-being of LKDs following donation found that most donors had high long-term HRQoL scores [20]. Those donors who exhibited low psychological functioning at baseline are at the highest risk for experiencing diminished long-term quality of life [20].

Studies comparing HRQoL scores 1 year after and predonation found no significant difference [19,21,22] or increased quality of life scores after donation [23,24]. Early evaluations after nephrectomy (days to weeks) are associated with significantly decreased HRQoL scores, particularly of the physical component [19]. Risk factors associated with decreased donor quality of life include donor fatigue, anxiety, depression, lack of social support, the nature of the donor-recipient bond, and postoperative complications or recipient graft loss [21,22,25,26]. In a multicenter, prospective study of 228 living donors, perioperative complications were unsurprisingly associated with clinically and statistically significant reductions in HRQoL measure [27]. Our results align with these findings. We found a statistically significant decrease in some SF-36 components after donations. Still, this only holds in donors with some significant intercurrent and is not necessarily clinically relevant. We could not link it to transplant failure, as we encountered only a single instance of graft loss. Additionally, the few other complications experienced by recipients were effectively managed during the early postoperative period, well ahead of our psychosocial reevaluation. In previous research by our team, Frade et al [28] found predonation SF-36 scores similar to those seen 1 year after donation, except for social functioning, which experienced a significant increase. The study consisted of a younger demographic (41.2 years old on average vs 47.3), had a smaller proportion of female participants, and only up to third-degree blood relative donations were allowed [28].

A psychosocial re-evaluation was performed at a median of 3.35 (IQR 2.724-3.725) years postdonation. A full physical recovery is expected at 1-year postdonation [11]. Although our evaluation was scheduled later, SF-36 physical scores were lower than at the predonation evaluation. Only donors with stressful life events, including medical complications, scored significantly worse. The same was true for depression scores. Anxiety scores were inversely correlated with the time that passed since donation, pointing to the necessity of scheduling follow-up appointments covering different times postdonation, covering from the early periods postdonation to long-term follow-up (more than 5-10 years after donation).

Kroencke et al [22] compared HRQoL in donors to healthy individuals and the general population. They found that preoperatively, donor scores were similar to healthy controls and significantly higher than those of the general population. Postoperative physical donor scores decreased and were significantly worse than healthy controls, yet they were not statistically different from the general population. A year following the donation, physical donor scores regained their predonation levels. Relying on the general population as a control group instead of healthy individuals may lead to misleading interpretations regarding the quality of life among living donors. If postoperative donors are compared to the general population, it might falsely suggest that there has been no change in quality of life despite an actual decline. This method may fail to adequately reflect the intricate psychosocial dimensions of living donation. One potential solution to these issues involves using donors as a control and comparing their preoperative scores with one or more postoperative scores from various psychosocial questionnaires, as used in our cohort.

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Numerous studies have examined the states of depression and anxiety following donation, but making comparisons among these studies proves to be a nearly impossible challenge. Overall, the depression rates after donation are low, and most donors experienced no symptoms of depression after donation [6,19,22,29]. In the studies that evaluated the depression scores prospectively, it means, before and after donation, different findings were described: no difference between pre- and postdonation scores [19,22] and worsening of mood postdonation [29]. The rates of anxiety postdonation are also usually low. Still, the true incidence is difficult to ascertain, as explained. In the prospective evaluations, contradictory results were found when comparing pre and postdonation scores: improvement in rates of anxiety [30], no difference in rates of anxiety [31], and an increase in anxiety scores [32].

Factors associated with an increase in symptoms of depression and anxiety include donors with postoperative complications or recipients with graft loss [19,33,34]. Our results were unsurprising and are consistent with previous findings [19,29,32–34], identifying medical and other donor complications as risk factors for donors and correlating anxiety and depression scores with HRQoL scores in the way it was hypothesized.

Moreover, during our evaluation, the COVID-19 pandemic ensued. It has greatly affected our lives. We cannot entirely dismiss the extra anxiety this situation has caused. Indeed, the COVID-19 pandemic has been a prolonged, stressful event, inflicting a detrimental impact on individuals. Moderate-to-severe symptoms of depression, anxiety, and stress have been described in different stages of the COVID-19 pandemic worldwide [35,36]. Pais-Ribeiro et al [37], reported that up to 36% of unselected Portuguese adults showed signs of at least mild psychological discomfort (ie, depression, anxiety, and stress) during the first mandatory lockdown in 2020 [37]. Vieira et al [38], in an unselected adult Portuguese population during the first lockdown, found much lower emotional function and mental health dimension scores compared to the general population normative data scores.

We could not identify different scores among living donors who were compatible and donated directly to their recipients vs those who were part of an ABO-incompatible desensitization program or the National Kidney Paired Exchange Program. Our sample size was limited, hindering further analysis. At the very least, these various transplant options did not visibly harm the psychosocial health of the living donors. Maple et al [39], in a cross-sectional study in the UK, demonstrated that undirected kidney donors have comparable physical and psychosocial outcomes to directed LKD. These favorable outcomes may be because of the rigorous evaluation process, which includes a mental health assessment. The authors concluded that the program could continue to expand safely across the United Kingdom.

Our study has several limitations. Because of the cross-sectional study, donors were evaluated at different time points after donation. Still, all were evaluated at least 16 months after donation, at a time when complete physical recovery from donation was expected and no more than 60 months after surgery,

making recall bias not probable. As strengths, it is the first comprehensive evaluation of postdonation systematic evaluations of living donors after the legislation changed in Portugal. The tools used with recognized psychometric properties are not specific to living donor evaluation, but they should maintain the impact of the conclusions. A larger sample would be more informative. A control group would be desirable, but as has already been discussed in the literature, LKDs have unique characteristics, making matched group design very difficult [19]. A big limitation that must be recognized is that we could not evaluate the impact of nondonation if those potential donors were disclosed for medical or psychosocial reasons [40].

Transplant procedures, particularly donation, can be a distressing experience that leads to psychosocial challenges. Alongside the importance of psychosocial evaluation prior to donation, establishing a protocol for continued assessment after donation is essential.

CONCLUSIONS

These results underscore the importance of serial psychosocial evaluation. Although living donors generally have a good quality of life, some experience psychosocial difficulties. Our study emphasizes the role of life events in triggering worse values in HRQoL, anxiety, and depression items, making clearer the importance of psychosocial follow-up of donors. Systematic evaluation can enhance informed consent, LDKT care quality, and the development of interventions to mitigate psychosocial risks.

AUTHOR CONTRIBUTIONS

MA, ICF, AS, IF, and AL Research design, data acquisition, data analysis, and paper writing. LSM, MR, and JM were involved in the research design and data analysis. All the authors approved the submitted version.

ETHICAL STATEMENT

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The institutional review board at our institution approved this study (Ref.: 147-21 (119-DEFI/122-CE)).

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DATA AVAILABILITY

Data will be made available on request.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

REFERENCES

- [1] Muzaale AD, Massie AB, Wang MC, et al. Risk of end-stage renal disease following live kidney donation. *JAMA* 2014;311(6):579–86.
- [2] Mjoen G, Hallan S, Hartmann A, et al. Long-term risks for kidney donors. *Kidney Int* 2014;86(1):162–7.
- [3] Wainright JL, Robinson AM, Wilk AR, et al. Risk of ESRD in prior living kidney donors. *Am J Transplant* 2018;18(5):1129–39.
- [4] Janki S, Steyerberg EW, Hofman A, Jzermans JN. Live kidney donation: are concerns about long-term safety justified? A methodological review. *Eur J Epidemiol* 2017;32(2):103–11.
- [5] Reese PP, Boudville N, Garg AX. Living kidney donation: outcomes, ethics, and uncertainty. *Lancet* 2015;385(9981):2003–13.
- [6] Cazauevillh V, Moal V, Prudhomme T, et al. Psychological Impact of Living Kidney Donation: A Systematic Review by the EAU-YAU Kidney Transplant Working Group. *Transpl Int* 2023;36:11827.
- [7] Menjivar A, Torres X, Manyalich M, et al. Psychosocial risk factors for impaired health-related quality of life in living kidney donors: results from the ELIPSY prospective study. *Sci Rep* 2020;10(1):21343.
- [8] Tong A, Chapman JR, Wong J, et al. The motivations and experiences of living kidney donors: a thematic synthesis. *Am J Kidney Dis* 2012;60(1):15–26.
- [9] Frutos MA, Crespo M, de la Oliva Valentin M, et al. Living-donor kidney transplant: guidelines with updated evidence. *Nefrologia (Engl Ed)* 2022;42(Suppl 2):1–4.
- [10] Lentine KL, Kasiske BL, Levey AS, et al. KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* 2017;101(8S Suppl 1):S1–S109.
- [11] Andrews PA, Burnapp L. British Transplantation Society /Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: Summary of Updated Guidance. *Transplantation* 2018;102(7):e307.
- [12] Massey EK, Timmerman L, Ismail SY, Duerinckx N, et al. The ELPAT living organ donor Psychosocial Assessment Tool (EPAT): from 'what' to 'how' of psychosocial screening - a pilot study. *Transpl Int* 2018;31(1):56–70.
- [13] Transplantação IPdSe. *Protocolo de Avaliação Psicológica Pré e Pós Doação em Vida*, in 003/INF-IPSR, IP/18, IPST, Editor http://www.ipst.pt/files/TRANSPLANTACAO/transplantaacao_Circular_Normativa_03_de_2018.pdf; 2018.
- [14] Saúde MdS-GdSdEAdMd. In: Saúde MdS-GdSdEAdMd, editor. Editor, 2015, editor. *Despacho n.º 2055/2015*, in *Despacho n.º 2055/2015*, de 26 de fevereiro. *Diário da República*; 2015. Editor <https://diariodarepublica.pt/dr/detalhe/despacho/2055-2015-6660200>.
- [15] McHorney CA, Ware Jr. JE, Raczek AE. The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. *Med Care* 1993;31(3):247–63.
- [16] Ferreira PL, Ferreira LN, Pereira LN. Medidas sumário física e mental de estado de saúde para a população portuguesa. *Revista Portuguesa de Saúde Pública* 2012;30(2):163–71.
- [17] Zigmund AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;67(6):361–70.
- [18] Pais-Ribeiro J, Silva I, Ferreira T, et al. Validation study of a Portuguese version of the Hospital Anxiety and Depression Scale. *Psychol Health Med* 2007;12(2):225–35 quiz 235-7.
- [19] Maple H, Chilcot J, Weinman J, Mamode N. Psychosocial wellbeing after living kidney donation - a longitudinal, prospective study. *Transpl Int* 2017;30(10):987–1001.
- [20] Wirken L, van Middendorp H, Hooghof CW, et al. The Course and Predictors of Health-Related Quality of Life in Living Kidney Donors: A Systematic Review and Meta-Analysis. *Am J Transplant* 2015;15(12):3041–54.
- [21] Lopes A, Frade IC, Teixeira L, et al. Quality of life assessment in a living donor kidney transplantation program: evaluation of recipients and donors. *Transplant Proc* 2013;45(3):1106–9.
- [22] Kroencke S, Fisher L, Nashan B, et al. A prospective study on living related kidney donors' quality of life in the first year: choosing appropriate reference data. *Clin Transplant* 2012;26(4):E418–27.
- [23] Garcia MF, Andrade LG, Carvalho MF. Living kidney donors —a prospective study of quality of life before and after kidney donation. *Clin Transplant* 2013;27(1):9–14.
- [24] Sommerer C, Estelmann S, Metzendorf NG, et al. Gender disparity in health-related quality of life and fatigue after living renal donation. *BMC Nephrol* 2018;19(1):377.
- [25] Chen P, Luo Q, Peng L. Anxiety and decreased social support underline poorer quality of life of parent living kidney donors. *Asia Pac Psychiatry* 2015;7(2):197–205.
- [26] Meyer K, Wahl AK, Bjork IT, et al. Long-term, self-reported health outcomes in kidney donors. *BMC Nephrol* 2016;17:8.
- [27] Hosseini K, Omorou AY, Hubert J, et al. Nephrectomy Complication Is a Risk Factor of Clinically Meaningful Decrease in Health Utility among Living Kidney Donors. *Value Health* 2017;20(10):1376–82.
- [28] Frade IC, Fonseca I, Dias L, et al. Impact assessment in living kidney donation: psychosocial aspects in the donor. *Transplant Proc* 2008;40(3):677–81.
- [29] Lopes A, Frade IC, Teixeira L, et al. Depression and anxiety in living kidney donation: evaluation of donors and recipients. *Transplant Proc* 2011;43(1):131–6.
- [30] Minz M, Ud giri N, Sharma A, et al. Prospective psychosocial evaluation of related kidney donors: Indian perspective. *Transplant Proc* 2005;37(5):2001–3.
- [31] Virzi A, Signorelli MS, Veroux M, et al. Depression and quality of life in living related renal transplantation. *Transplant Proc* 2007;39(6):1791–3.
- [32] Yoo HJ, Han DJ, Kim SY, et al. Attitudes and psychological characteristics of kidney donors toward organ donation. *Transplant Proc* 1996;28(3):1932–3.
- [33] Holscher CM, Leanza J, Thomas AJ, et al. Anxiety, depression, and regret of donation in living kidney donors. *BMC Nephrol* 2018;19(1):218.
- [34] Lentine KL, Shnitzler MA, Xiao H, et al. Depression diagnoses after living kidney donation: linking U.S. Registry data and administrative claims. *Transplantation* 2012;94(1):77–83.
- [35] Wang C, Pan R, Wan X, et al. A longitudinal study on the mental health of general population during the COVID-19 epidemic in China. *Brain Behav Immun* 2020;87:40–8.
- [36] Hyland P, Shevlin M, Murphy J, et al. A longitudinal assessment of depression and anxiety in the Republic of Ireland before and during the COVID-19 pandemic. *Psychiatry Res* 2021;300:113905.
- [37] Pais-Ribeiro J, Ferreira-Valente A, Jarego M, et al. COVID-19 Pandemic in Portugal: Psychosocial and Health-Related Factors Associated with Psychological Discomfort. *Int J Environ Res Public Health* 2022;19(6).
- [38] Vieira DA, Meirinhos V. COVID-19 Lockdown in Portugal: Challenges, Strategies and Effects on Mental Health 2021;29(2):354–74.
- [39] Maple H, Chilcot J, Burnapp L, et al. Motivations, outcomes, and characteristics of unspecified (nondirected altruistic) kidney donors in the United Kingdom. *Transplantation* 2014;98(11):1182–9.
- [40] Allen MB, Abt PL, Reese PP. What are the harms of refusing to allow living kidney donation? An expanded view of risks and benefits. *Am J Transplant* 2014;14(3):531–7.

APPENDIX 8 – Living Kidney Donor Perceptions About Organ Donation: Who are They, and How Do They Feel About the Process?

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Living Kidney Donor Perceptions About Organ Donation: Who are They, and How Do They Feel About the Process?

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ABSTRACT

Background. The panorama of living kidney donors (LKD) in Portugal has changed over the past decade, including kidney-paired exchange programs, undirected donations, and more complex kidney transplants, which could affect the predisposition to donation. We sought to describe the sociodemographic characteristics of a cohort of LKDs at our center and assess their perceptions of the donation process.

Material and Methods. LKDs who donated between January 2018 and December 2019 underwent a predonation psychosocial evaluation and a follow-up evaluation in May 2022, which included questionnaires about perceptions regarding donation. We analyzed the results descriptively.

Results. Forty-four LKDs were included, aged 47.3 ± 10.3 years. Most were female (68.2%). A total of 52.4% only attended primary school; 52.3% were genetically related to the recipients, and 40.9% were spouses. In 86.4% of the LKDs, the decision to donate was easy; in 13.6%, it was a little difficult, with some doubts. All donors said that the information provided was adequate. Most LDs (79.5%) stated that the relationship with the recipient did not change, and most (88.6%) believed that the recipient's health state improved significantly. Only one LD would not donate again, and 77.3% would strongly encourage others to do it.

Conclusions. The perceptions about donation in our cohort were very positive, and most donors would encourage others to donate. All felt well-informed about the process, and the regret rate was very low. Although these results must be studied in larger cohorts, sharing previous donors' experiences can add value to the dissemination of LKD programs.

A living donor kidney transplant is the best treatment for selected end-stage renal disease patients (ESRD) [1–3]. Although recipients receive significant benefits, living kidney donors (LKDs) are submitted to major surgery without self-benefits. The perceived physical risks for the LKDs are considered overall low and ethically acceptable [3–5]. Moreover, a psychosocial benefit from the act of donation could justify the act [3], mainly if there is a relationship between the donor and the recipient and the donor testifies to an improvement in the recipient's health. Nevertheless, some donors experience poor

psychosocial outcomes, and risk factors are yet to be fully clarified [6,7].

Research in living donation predominantly focuses on the physical aspects of living kidney donation and is the basis of

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current guidelines for LKD evaluation and selection [8–10]. Although the guidelines recommend psychosocial studies and follow-up, a dearth of research exists on the psychosocial dimensions of living kidney donation [11]. Two primary categories of studies are identified: qualitative studies, predominantly anchored in interviews and investigator-oriented approaches, and quantitative studies, relying on standardized questionnaires. Furthermore, the methodologies employed are highly variable, encompassing only predonation evaluations, various postdonation assessment time points, and a heterogeneity of utilized questionnaires. This variability presents significant challenges for making comparisons and drawing definitive conclusions. A recent systematic review [6] concluded that quantitative studies reported that donor quality of life remained unchanged or improved after donation. On the other hand, qualitative studies reported more complex and multifaceted donation experiences [6]. Tong et al. [12], in their systematic review, elucidated that living donors need to renegotiate their identities, roles, and relationships following surgery and develop strategies to protect them from adverse adjustments.

Furthermore, the landscape of living donor kidney transplantation has undergone significant evolution in Portugal and globally. Only the recipient's biological relatives were historically recognized as eligible living donors. However, advancements in immunosuppressive therapies, evidence supporting comparable outcomes with unrelated donors, and the increasing scarcity of available organs have contributed to the broader acceptance of nonrelated donors [13]; in 2007, Portugal enacted laws permitting transplantation between genetically unrelated individuals. The National Kidney Paired Exchange Program was established in 2010 [14], and in 2022, legislation was passed for nondirected kidney donation [15]. Conversely, more intricate, and high-risk kidney transplants, including those involving ABO incompatibility as well as medically complex donors and recipients, are increasingly prevalent, thereby impacting the propensity towards living donation.

Psychosocial evaluations are significant in assessing living donors. Since 2003, every potential LKD from our Unit has undergone a systematic psychosocial evaluation prior to and following donation. This evaluation has been enhanced through the European Living Donor Psychosocial Follow-Up project [16], which examined the psychosocial outcomes and implications of the donation process for LKDs, thereby establishing high standards within the Living Kidney Transplant programs. Furthermore, since 2018, a National Directive has advocated for standardized pre- and postdonation evaluations for all living donors in Portugal [17].

We aim to study LKDs social and demographic characteristics and assess their feelings and perspectives regarding the donation process. A better understanding of the perspectives of LKDs can help shape health services and policies to improve outcomes for potential and actual LKDs.

MATERIAL AND METHODS

We retrospectively reviewed the clinical data of all adult LKDs who underwent nephrectomy at our center between January

2018 and December 2019 ($n = 60$). Inclusion criteria included a complete predonation psychosocial evaluation (available for 46 LKD: 3 pairs were evaluated in another transplant center, and 11 pairs had not fully completed the questionnaire) and a follow-up questionnaire conducted in May 2022. Forty-four LKDs completed both questionnaires and defined our cohort.

The Institutional Review Board at Unidade Local de Saúde de Santo António (ULSddSA) approved this cross-sectional observational study, which was conducted according to the Helsinki Declaration. All donors provided written informed consent to participate.

The study was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Living Donors

Following international guidelines, all donors were subjected to a standard evaluation protocol [8,10]. Baseline demographic and clinical data were collected from LKDs. A dedicated team of Psychologists and Psychiatrists performed the psychosocial evaluation. The final approval for kidney donation was reviewed in a multidisciplinary meeting, and independent ethical approval was mandatory.

In May 2022, all donors submitted to nephrectomy during 2018 and 2019 who met the inclusion criteria were scheduled for psychological and living donor appointments and routine analysis on the same day to avoid extra hospital dislocations. According to current legislation, they were reimbursed for the relocation expenses [18]. The psychologist (ICF) explained that the questionnaire they had to answer was self-administered. The same investigator (MA) also performed the medical consultation. Some LDs who live abroad received the questionnaire by email or postmail with a prepaid envelope to send it back.

Questionnaire

A sociodemographic survey was conducted to gather information regarding participants' age, gender, level of education, employment status, marital status, and their relationship to the recipient. Participants completed a donation perception questionnaire comprising ten multiple-choice questions, in addition to a satisfaction questionnaire that contained 14 multiple-choice questions along with one open-ended question pertaining to their emotions and feelings associated with the act of donation. Furthermore, participants provided responses to a questionnaire designed to assess their relationship with the recipient, which included five multiple-choice questions and one open-ended question inviting further elaboration. Data regarding the postoperative course and complications for both donors and recipients were acquired from the participants' medical records.

Statistical Analysis

A baseline sample description was obtained using frequencies, means, and SD or medians and IQR. The normality of the variable distribution was determined using the Kolmogorov–Smirnov test.

A *P*-value <.05 was considered to be statistically significant. Statistical calculations were performed using SPSS Statistics, version 28 (SPSS Inc.).

RESULTS

Donor Sociodemographic Characteristics- Predonation

The baseline characteristics for our study cohort are summarized in Table 1. The mean age of the population was 47.3 ± 10.32 years, and most were female (68.2%). All the donors were genetically or emotionally related to their recipients. Most donors (n = 24, 54.5%) were genetically related, and most unrelated to their recipients were spouses (n = 19, 40.9%). Most LDs attended only primary school (52.4%, n = 23), and 27.3% (n = 12) completed a university degree.

In predonation, most of the donors were married or in a non-marital partnership (n = 36, 81.8%). Most were employed (n = 32, 72.1%), 14% were retired, and 11.6% (n = 5) were unemployed.

Twenty-eight donors were compatible with their recipients, 5 were ABO incompatible, and 11 (25%) were transplanted in the Kidney-Paired Exchange Program.

Living donors were evaluated at 5.800 (IQR: 3.217 to 13.508) months predonation.

Psychiatric Evaluation

After the psychological interview, 14 donors (31.8%) were referred for psychiatric evaluation, primarily for mental health history or current significant anxiety, depression, or other relevant symptoms. Six donors (42.9%) maintained regular psychiatric follow-up per indication.

Donor Sociodemographic Characteristics- Postdonation

A postdonation evaluation was performed at 3.350 (IQR: 2.724–3.725) years postdonation.

In the postdonation evaluation, most donors were married or in nonmarital partnerships (n = 31, 70.5%), but this was less than previously.

Most were employed (n = 30; 68.2%), 18.2% were retired, and 11.4% (n = 5) were unemployed. None described professional problems related to the donation. One member was on medical leave due to an acute medical condition unrelated to the previous nephrectomy.

Complications Following Living Donor Kidney Transplantation

No major complications have been described among the donor group. Fifteen donors (34.1%) reported medical issues

Table 1. Sociodemographic Characteristics of the Study Cohort

		Number	%
Age (years)		47.3 (±10.3)	
Sex			
	Male	14	31.8
	Female	30	68.2
Relationship with the recipient			
	Partners	18	40.9
	Brothers	13	29.5
	Parents	7	15.8
	Nephews	2	4.6
	Sons	1	2.3
	Brothers-in-law	1	2.3
	Cousins	1	2.3
	Friends	1	2.3
Educational level			
	Primary school	23	52.4
	Secondary school	9	20.3
	Licenciate degree	12	27.3
Marital status	Predonation		
	Married/nonmarital partnership	36	81.8
	Single	5	11.4
	Divorced	3	6.8
	Postdonation		
	Married/nonmarital partnership	31	70.5
	Single	7	15.9
	Divorced	6	13.6
Professional situation	Predonation		
	Active	31	70.5
	Unemployed	5	11.4
	Medical leave	1	2.3
	Retired	6	13.6
	Postdonation		
	Active	30	68.2
	Unemployed	5	11.4
	Medical leave	1	2.3
	Retired	8	18.2
Type of donation			
	Compatible	28	63.6
	KPE Program	11	25
	ABOi Program	5	11.4

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postdonation, primarily pain associated with the surgical procedure ($n = 6$, 13.6%). Additionally, nineteen donors (43.1%) faced stressful life events unrelated to the donation.

One recipient experienced graft loss due to thrombosis in the early postoperative period. Two recipients had acute kidney rejections, which were treated successfully, and one patient was readmitted to the hospital because of a urinary tract infection fistula.

Donors' Perceptions of Donation

Table 2 presents examples of questions and answers from the Perceptions questionnaire. For 90.9% of donors ($n = 40$), the decision to donate was their own will, but one donor felt some pressure to donate. Most donors ($n = 38$, 86.4%) considered the decision-making process easy. No donor felt that the decision was difficult or had many doubts. In any event, over one-third of the donors ($n = 15$) expressed at least some fears that the surgery donation could diminish their life expectancy. 27 donors (61.3%) were afraid that complications could occur during the surgery, and 22 donors (50%) had concerns that the donation could prejudice their health. During the process, 33 out of 44 donors (75%) expressed concerns that their donations might be in vain. Only one donor (2.27%) completely agreed that he did not trust the recipient to take care of his kidney after the transplant, but four more donors (9.09%) moderately or strongly agreed with that (Table 2).

Before the multidisciplinary living donor evaluation, 15 donors (34.1%) indicated they lacked sufficient information about the medical procedures, which hindered their ability to make an informed choice. Notably, every donor expressed contentment with the information they received regarding the donation process, surgery, and associated risks (Table 2).

Thirty-five donors (79.5%) stated that their donation had no financial repercussions, but two donors (4.5%) categorized it as high.

Most donors experienced acknowledgment and support from their families ($n = 32$, 72.7%), whereas 11 donors (26%) expressed concerns about lacking such support. A significant number of donors ($n = 30$, 68.2%) did not report feeling closer to their families after the donation. Despite this, most donors ($n = 36$, 81.8%) indicated that their family and friends are proud of them for the donation.

A total of 79.5% of donors ($n = 35$) claimed that their relationship with the recipient remained unchanged after the donation. No donors reported a deterioration in their relationship. Most donors ($n = 29$, 64.9%) strongly believe the recipient is healthy following transplantation (Table 2).

Notably, most donors are not the primary caregivers for their recipients ($n = 31$, 70.5%). While many do not experience feelings of debt or obligation from the recipient ($n = 27$, 61.4%), 19 donors (43.2%) mentioned that sometimes their recipients express excessive gratitude, unlike one donor (2.3%) who feels the recipient lacks appreciation for the gift.

A total of 61.4% of donors ($n = 27$) viewed the donation process as a challenging physical and emotional experience, while 15 (34%) disagreed. Among the donors, 38.4% ($n = 17$)

reported the surgery was as painful as they anticipated, 36.4% ($n = 16$) found it less painful, and the remaining 25% ($n = 11$) considered it more painful. Most donors felt that donation positively influenced their lifestyle and daily habits ($n = 30$, 68.2%). More than half of the participants ($n = 23$, 52.27%) harbored some concerns about the possibility of unsuccessful donations; however, 37 donors (84.2%) affirmed that the donation process was worthwhile, and 77.3% ($n = 34$) would strongly recommend it to others. Others emphasized the importance of individual decision-making for potential donors, with none discouraging donation. A total of 42 donors (95.5%) indicated they would donate again; only one expressed doubts, while another chose not to donate again (Table 2).

The predominant postdonation emotion was joy, experienced by 23 donors (52.3%). This was followed by pride, reported by 13 donors (29.5%), and satisfaction, noted by the remainder. Notably, none of the donors reported any negative emotions.

DISCUSSION

In our cohort of LKDs, the perception of donation was mainly positive, either regarding the physical health of the donor and the recipient or the social and emotional aspects. The LKDs considered the information about the donation process adequate, and most would strongly encourage others to donate. Most did not have adverse financial consequences or overall health impacts. Nevertheless, some negative experiences deserve appropriate attention and follow-up. These results reinforce the importance of routine psychosocial evaluation beyond physical evaluation predonation and the continuity of support after donation. These results mostly agree with our previous assessment of donors' and recipients' perceptions of living donor transplants, although the national panorama has changed [19,20].

One significant finding of our study is that our donors felt the information concerning the donation process was adequate. A recent study involving our center, conducted by Menjivar et al. [7], identified a subgroup of donors who require more information about surgical risks as a risk factor for poorer psychosocial outcomes. The need for more details can unmask more doubts or ambivalence about the donation process, but it can, in fact, be low-perceived information. The absence of adequate information predonation has been reported as high as 31% of LKDs [21,22]. Moreover, uniform informed consent is far from reality [23]. The importance of teamwork, where psychosocial evaluation accompanies side-by-side physical and medical assessment by dedicated teams, as practiced in our center, is an example of improving LKD evaluation and expected results. Applying additional tools to evaluate knowledge and comprehension gaps could improve these achievements [24].

The quality of the donor-recipient relationship remains a significant concern during evaluations of living kidney donations. If the prospective donor harbors doubt or regrets regarding their decision to donate, the medical team typically ascribes a medical reason for the nondonation to maintain the integrity of the relationship, as many of these pairs are familiar with one another or have emotional ties. This situation underscores the

Table 2. Examples of Questions and Answers Provided in the Perception Questionnaire

Questions	Not Answer	I Don't Know	No	Yes
Did you feel any kind of pressure to donate?	0 (0)	3 (6.8)	40 (90.9)	1 (2.3)
The decision-making process of donation was				
Easy, without thinking a lot	38 (86.4)	6 (13.6)	0 (0)	
Difficult with a lot of doubts				
Not answer	1 (2.3)	28 (63.6)	12 (27.3)	
Moderately agree				2 (4.5)
Agree a lot				1 (2.3)
Completely agree				0 (0)
I felt that I had little information about the medical and surgical procedures to make a decision	1 (2.3)	21 (47.7)	19 (43.2)	2 (4.5)
I thought that donate a kidney could prejudice my health	1 (2.3)	21 (47.7)	19 (43.2)	2 (4.5)
Donate a kidney could reduce my life expectancy	2 (4.5)	15 (34.1)	15 (34.1)	9 (20.5)
I was afraid the surgery could go wrong	1 (2.3)	20 (45.5)	12 (27.3)	6 (13.6)
I was afraid that the donation might be in vain.	1 (2.3)	2 (4.5)	2 (4.5)	11 (25)
I felt that I did not have confidence that the recipient could take good care of the donated kidney				27 (61.4)
What do you think about the information provided?	Not answer 0 (0)	Insufficient 0 (0)	Partly insufficient 0 (0)	Suitable 44 (100)
What implications did the donation had on your financial situation?	Not answer 0 (0)	None 35 (79.6)	Some 5 (11.9)	A lot 2 (4.5)
Do you think that the relationship with the recipient after the donation is	Not answer 0 (0)	Much worse 0 (0)	Worse 0 (0)	The same 35 (79.5)
I feel that there are feelings of gratitude/debt in the relationship with the recipient	4 (9.1)	Yes 9 (20.5)	No 27 (61)	A little better 3 (6.8)
Sometimes I feel that the recipient feels too much gratitude towards me	4 (9.1)	19 (43.2)	21 (47.7)	Much better 6 (13.6)
Sometimes I feel like the recipient doesn't show enough appreciation for my donation	4 (9.1)	1 (2.3)	39 (88.6)	
The entire donation process was a physical and emotional challenge for me	2 (4.5)	5 (11.4)	10 (22.7)	Completely agree 8 (18.8)
Did you think about stopping the donation process at any time?	1 (2.3)	36 (81.8)	4 (9.1)	2 (4.3)
The recipient is currently healthy	0 (0)	0 (0)	1 (2.3)	14 (31.8)
The donation was worth it	1 (2.3)	0 (0)	0 (0)	29 (65.9)
If you could, would you donate again	Not answer 0 (0)	Yes, definitely 42 (95.5)	No 1 (2.3)	37 (84.1)
Would you recommend living donor donation to other people?	0 (0)	Discouraged 0 (0)	Every potential donor should make an individualized decision 10 (13.6)	Yes, strongly 34 (77.4)

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necessity of a dedicated evaluation team working closely with both parties. As reported in most studies, the relationships between the pairs remained unchanged after the donation [6,7,25] or even improved. Surprisingly, none reported that it deteriorated, although it has rarely been reported [6]. Most of our donors were not primary caregivers, which could have reduced the stress impact on the relations. Furthermore, a qualitative analysis of the changes in their relationships would be more enlightening. Qualitative studies describe a “renegotiation of roles” [26,27] and “reshaping” [12,28] of the relations after donations.

Another significant issue to consider is the financial burden associated with donations. In Portugal, organ transplantation is comprehensively supported by the national health system, which encompasses the process of living donation. Additionally, according to directive 2055/2015 [18], compensation for justifiable expenditures associated with the donation is mandatory, including travel for tests, appointments, hospital stays, supplementary medical costs, and the financial consequences of diminished work capacity. Most of our LKDs did not report any economic impact on their donations; however, two individuals indicated a substantial effect. None reported experiencing unemployment because of their donations. Although health insurance and housing credit were not explicitly mentioned, it is reasonable to expect potential challenges, especially when one donor is part of a couple, and the recipient has received a kidney transplant.

Only one of our donors would not donate again, and another doubted it. The studies agree that, in most cases, donors are satisfied with their donation process [6], with low regret rates. Regret was understandably associated with graft failure [29], recipient death, deterioration of the relationship, donor anxiety, and depression, but not always [6]. Indeed, the very definition of regret presents considerable challenges. An individual may experience regret regarding an event yet still advocate for its value or opt to undertake it. As demonstrated in the results section, the donation process posed physical and emotional challenges for most donors, significantly affecting their lifestyles and daily routines. Unsurprisingly, these reactions are not dichotomous; instead, they are intricate and multifaceted.

Most donors within our cohort would actively encourage others to participate in donations. It is reasonable to assert that these individuals should be involved in informational sessions aimed at patients with chronic kidney disease, enabling them to share their experiences, including their apprehensions, concerns, and doubts. Noteworthy, although we have passed in the questionnaire validated psychometric tools, namely SF-36 and HADS, beyond the specific donation questionnaire, their analysis is beyond the scope of this work. The overall positive perceptions about donation and low levels of regret described here did not translate into improved psychometric scores after donation. Maple et al. [25] also found this in a prospective longitudinal study of 93 LDs in England. Overall, there was no significant change in well-being, life satisfaction, self-esteem, social comparison, distress, depression, stress, anxiety, or social support scores at 3 or 12 months. Despite this, questions specific to LKD indicated that donors felt optimistic about the

donation and had low levels of regret. The authors hypothesized that the validated psychometric tests could be insufficiently sensitive to capture the complex dynamics of living donation, the possibility of social desirability bias when answering the questionnaires, and the necessity of repeating the evaluation in different timeframes [25].

Our study has several limitations. It is a descriptive study of perceptions in a cohort of previous LKDs. Although the donors filled out the questionnaires alone, one cannot exclude a social desirability bias [6]; this is when the donors give the answers they think are expected from them. Although this would be higher in predonation questionnaires as they could be afraid not to be accepted, the same attitude could be present after donation. Because of the cross-sectional study, donors were evaluated at different time points after donation. We should also acknowledge that the COVID-19 pandemic has dramatically affected our lives and relationships during the period between the two evaluations. A larger sample would be more informative.

CONCLUSIONS

Considering the physical, psychosocial, and financial aspects, most donors felt the donation process was a positive experience. They had all the relevant information about the process and the surgery and would encourage others to donate, although some negative experiences have been recorded. Psychosocial evaluations and follow-up must accompany regular medical evaluations of LKDs for optimal results and a safe expansion of living kidney donor programs.

DATA AVAILABILITY

Data will be made available on request.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTIONS

MA, ICF, AS, IF, and AL Research design, data acquisition, data analysis, and paper writing. LSM, MR, and JM were involved in the research design and data analysis. All the authors approved the submitted version.

ETHICAL COMPLIANCE

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

The institutional review board at our institution approved this study (Ref.: 147-21 [119-DEFI/122-CE]).

REFERENCES

- [1] Gruessner RWG, Gruessner AC. Solid-organ transplants from living donors: cumulative United States Experience on 140,156 living donor transplants over 28 years. *Transplant Proc* 2018;50(10):3025–35.
- [2] Park YH, Min SK, Lee JN, Lee HH, Jung WK, et al. Comparison of survival probabilities for living-unrelated versus cadaveric renal transplant recipients. *Transplant Proc* 2004;36(7):2020–2.
- [3] Reese PP, Boudville N, Garg AX. Living kidney donation: outcomes, ethics, and uncertainty. *Lancet*. 2015;385(9981):2003–13.
- [4] Mjoen G, Hallan S, Hartmann A, Foss A, Midvedt K, et al. Long-term risks for kidney donors. *Kidney Int* 2014;86(1):162–7.
- [5] Muzaale AD, Massie AB, Wang MC, Montgomery RA, MacBride MA, et al. Risk of end-stage renal disease following live kidney donation. *JAMA* 2014;311(6):579–86.
- [6] Cazaueville V, Moal V, Prudhomme T, Pecoraro A, Piana A, et al. Psychological impact of living kidney donation: a systematic review by the EAU-YAU Kidney Transplant Working Group. *Transpl Int* 2023;36:11827.
- [7] Menjivar A, Torres X, Manyalich M, Fehrman-Eckholm I, Papachristou C, et al. Psychosocial risk factors for impaired health-related quality of life in living kidney donors: results from the ELIPSY prospective study. *Sci Rep* 2020;10(1):21343.
- [8] Andrews PA, Burnapp L. British Transplantation Society /Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: summary of updated guidance. *Transplantation* 2018;102(7):e307.
- [9] Frutos MA, Crespo M, de la Oliva Valentin M, Hernandez D, de Sequera P, et al. Living-donor kidney transplant: guidelines with updated evidence. *Nefrologia (Engl Ed)* 2022;42(Suppl 2):1–4.
- [10] Lentine KL, Kasike BL, Levey AS, Adams PL, Aiberu J, et al. KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* 2017;101(8S Suppl 1):S1–S109.
- [11] Massey EK, Timmerman L, Ismail SY, Duerinckx N, Lopes A, et al. The ELPAT living organ donor Psychosocial Assessment Tool (EPAT): from 'what' to 'how' of psychosocial screening - a pilot study. *Transpl Int* 2018;31(1):56–70.
- [12] Tong A, Chapman JR, Wong G, Kanellis J, McCarthy J, Craig JC. The motivations and experiences of living kidney donors: a thematic synthesis. *Am J Kidney Dis* 2012;60(1):15–26.
- [13] Simforoosh N, Shemshaki H, Nadjafi-Semnani M, Sotoudeh M. Living related and living unrelated kidney transplantations: a systematic review and meta-analysis. *World J Transplant* 2017;7(2):152–60.
- [14] Saúde, M.d., Portaria n.º 802/2010 de 23 de Agosto, in Portaria n.º 802/2010 de 23 de Agosto. 2010: Diário da República, available at <https://diariodarepublica.pt/dr/detalhe/portaria/802-2010-343588>
- [15] Transplantação, I.P.d.S.e., Programa Nacional de Doação Renal Não Dirigida ou Anónima. 2022(002/CN/2022).
- [16] Manyalich M, Menjivar A, Yucetin L, Peri JM, Torres X. Living donor psychosocial assessment/follow-up practices in the partners' countries of the ELIPSY project. *Transplant Proc* 2012;44(7):2246–9.
- [17] Transplantação, I.P.d.S.e., Protocolo de Avaliação Psicológica Pré e Pós Doação em Vida, in 003/INF-IPSR, IP/18, IPST, Editor. 2018., available at http://www.ipst.pt/files/TRANSPLANTACAO/transplantaao_Circular_Normativa_03_de_2018.pdf
- [18] Saúde, M.d.S.-G.d.S.d.E.A.d.M.d., Despacho n.º 2055/2015, in Despacho n.º 2055/2015, de 26 de fevereiro, M.d.S.-G.d.S.d.E.A.d.M.d. Saúde, Editor. 2015: Diário da República, available at <https://diariodarepublica.pt/dr/detalhe/despacho/2055-2015-66602020>
- [19] Frade IC, Lopes A, Teixeira L, Rodrigues J, Almeida M, et al. Perceptions in living kidney donation: what protagonists think and feel. *Transplant Proc* 2011;43(1):39–42.
- [20] Frade IC, Fonseca I, Dias L, Henriques AC, Martins LS, et al. Impact assessment in living kidney donation: psychosocial aspects in the donor. *Transplant Proc* 2008;40(3):677–81.
- [21] Clemens K, Boudville N, Dew MA, Geddes C, Gill JS. The long-term quality of life of living kidney donors: a multicenter cohort study. *Am J Transplant* 2011;11(3):463–9.
- [22] Schover LR, Strem SB, Boparai N, Duriak K, Novick AC. The psychosocial impact of donating a kidney: long-term follow-up from a urology based center. *J Urol* 1997;157(5):1596–601.
- [23] Kortram K, Lafranca JL, I. Jzermans JN, Dor FJ. The need for a standardized informed consent procedure in live donor nephrectomy: a systematic review. *Transplantation* 2014;98(11):1134–43.
- [24] Grossi AA, Sever MS, Hellemans R, Mariat C, Crespo M. The 3-Step Model of informed consent for living kidney donation: a proposal on behalf of the DESCARTES Working Group of the European Renal Association. *Nephrol Dial Transplant* 2023;38(7):1613–22.
- [25] Maple H, Chilcot J, Weinman J, Mamode N. Psychosocial wellbeing after living kidney donation - a longitudinal, prospective study. *Transpl Int* 2017;30(10):987–1001.
- [26] Hildebrand L, Melchert TP, Anderson RC. Impression management during evaluation and psychological reactions post-donation of living kidney donors. *Clin Transplant* 2014;28(8):855–61.
- [27] Andersen MH, Bruserud F, Mathisen L, Wahl AK, Hanestad BR, Fosse E. Follow-up interviews of 12 living kidney donors one yr after open donor nephrectomy. *Clin Transplant* 2007;21(6):702–9.
- [28] Andersen MH, Mathisen L, Oyen O, Wahl AK, Hanestad BR, Fosse E. Living donors' experiences 1 wk after donating a kidney. *Clin Transplant* 2005;19(1):90–6.
- [29] Meyer KB, Bjork IT, Wahl AK, Lennerling A, Andersen MH. Long-term experiences of Norwegian live kidney donors: qualitative in-depth interviews. *BMJ Open* 2017;7(2):e014072.

APPENDIX 9 – Clinical performance of the iPREDICTLIVING tool for the prediction of the post-transplant recipient and living donor outcomes in a European cohort

Almeida, M.; Ribeiro, C.; Silvano, J.; Pedroso, S.; Tafulo, S.; Martins, LS., Ramos, MS.; and Malheiro, J.

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Clinical performance of the iPREDICTLIVING tool for the prediction of the post-transplant recipient and living donor outcomes in a European cohort

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Abstract

A living donor kidney transplant (LDKT) is the best treatment for ESRD. A prediction tool based on clinical and demographic data available pre-KT was developed in a Norwegian cohort with three different models to predict graft loss, recipient death, and donor candidate's risk of death, the iPREDICTLIVING tool. No external validations are yet available. We sought to evaluate its predictive performance in our cohort of 352 pairs LKDT submitted to KT from 1998 to 2019. The model for censored graft failure (CGF) showed the worse discriminative performance with Harrell's C of .665 and a time-dependent AUC of .566, with a calibration slope of .998. For recipient death, at 10 years, the model had a Harrell's C of .776, a time-dependent AUC of .773, and a calibration slope of 1.003. The models for donor death were reasonably discriminative, although with a poor calibration, particularly for 20 years of death, with a Harrell's C of .712 and AUC of .694 with a calibration slope of .955. These models have moderate discriminative and calibration performance in our population. The tool was validated in this Northern Portuguese cohort, Caucasian, with a low incidence of diabetes and other comorbidities. It can improve the informed decision-making process at the living donor consultation joining clinical and other relevant information.

KEYWORDS

graft survival, kidney transplant, living donor, outcomes, patient survival, prediction tools

1 | INTRODUCTION

A living donor (LD) kidney transplant (KT) is the preferred treatment for selected patients with end-stage renal disease (ESRD).¹⁻³ It is associated with increased graft and patient survival.¹⁻³ Also, it increases organ availability and preemptive transplant and decreases waiting list time.^{1,2,4} Although most LD organs are better than most deceased donors' (DD) organs, it is well established that there is a spectrum of quality among LD organs. The importance of risk stratification for

DDKT based on donors' characteristics has long been acknowledged. Applying statistical models that could classify LD organ quality and predict graft and patient survival, following LDKT is attractive.⁵⁻⁷ It could guide the informed consent process, as recommended by current LD evaluation guidelines.⁸

Massie et al. developed the living kidney donor profile index (LKDPI), a risk index for LDKT,⁵ based on data from the US Scientific Registry of Transplant Recipients (SRTR). It incorporates weighted donor factors such as donor age, cigarette use, African American race,

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BMI, and eGFR, as well as donor and recipient interaction factors, such as if they are biologically related, ABO compatibility, HLA B and DR match, or the donor/recipient weight ratio. It was created on the same scale as the DD KDPI, and it was proposed as a valuable tool for comparing LD kidneys to each other and to DD kidneys⁵. It was externally validated in several populations^{9–11} and is used in literature to compare LD quality.^{12–15} However, its predictive performance (discrimination) measured by the C-statistic was relatively low at .59 (95% CI .55–.62).

Ashby et al.⁶ also developed risk prediction models for graft loss based on US SRTR data. Models were adjusted for the year of transplant and donor and recipient characteristics, with particular attention to mismatches in age, sex, human leukocyte antigens (HLA), body size, and weight. An online calculator was created for estimation of overall and censored graft failure 5 and 10 years after transplant and the 5-year recipient risk of death (<https://kecc.shinyapps.io/SurvivalCalculator/>). The model's discriminative ability was also relatively low, with a C-statistic of .64. External validations have yet to be available, limiting their applicability.

The iPREDICTLIVING tool⁷ was developed by Haller et al. in a European Norwegian population, using data from 1854 LD and from 837 recipients that performed LDKT at Oslo University. Different models were developed for predicting donor mortality for up to 20 years, recipient death, and graft loss for up to 10 years. For donor mortality, the model considered age and year of donation, smoking habits, cholesterol values, and serum creatinine. Donor outcomes were not predicted in previous tools. Further, linear predictors of donor mortality served as a summary of donor prognosis in recipient models. The model performed well in the internal validation set for donor mortality, with a C-statistic of .81. The recipient mortality model comprised age, sex, year of KT, dialysis vintage, primary renal disease, cerebrovascular and peripheral vascular disease, and class II HLA mismatch. The recipient mortality model C-statistic was .77. The graft loss model relied on age, dialysis vintage, linear prediction of donor mortality, HLA mismatch, peripheral vascular, and heart disease. The C-statistic was .66. Calibrations were reliable in all models. No external validations are available.

We sought to appraise the predictive performance of the iPREDICTLIVING tool in our cohort of LDKT, evaluating the three different models separately: (a) donor candidate's risk of death within twenty years after donation, (b) recipient death within ten years after KT, and (c) censored graft loss up to ten years after KT. Additionally, we evaluated the performance of the most used prediction model, LKDPI,⁵ and compared it with that of the more similar model of the iPREDICTLIVING tool in our cohort.

2 | MATERIAL AND METHODS

We retrospectively reviewed the clinical data of all LDKT pairs that were performed at our Center between January 1998 and January 2020 ($n = 365$), followed until December 31, 2021, death, or loss to follow-up. Eleven cases of early graft failure (GF) (<48 h after KT) and

two of early recipient death were excluded from the analysis, with the remaining 352 pairs defining the studied cohort. The Institutional Review Board at Centro Hospitalar Universitário de Santo António (CHUdSA) approved this retrospective cohort validation study, conducted according to the Helsinki Declaration.

2.1 | Donor variables

Following international guidelines, all donors were subjected to a standard evaluation protocol. Baseline demographic, anthropomorphic, analytical, and clinical data were collected from the LD. Serum creatinine-based CKD-EPI equation¹⁶ was used to predict eGFR. Smoking habits were recorded. Potential donors with a history of malignancy, class II or higher obesity, diabetes mellitus, uncontrolled hypertension, or evidence of end-organ damage were excluded. The final approval for kidney donation was reviewed in a multidisciplinary meeting, and ethical approval was mandatory.

Left-side procurement was preferred for anatomical reasons except for complex vessel anatomy or when a significant renal asymmetry was found. A transperitoneal laparoscopic approach was performed in most donors. Lifetime annual follow-up appointments are available for all donors.

2.2 | Recipient variables

Demographic, analytical, and clinical data were collected from all the recipients, including dialysis modality and dialysis vintage. Comorbidities such as cerebrovascular, peripheral vascular, and heart disease were recorded. Chronic kidney disease (CKD) etiologies were grouped into four categories: diabetic nephropathy (DN), chronic glomerulonephritis (CGN), vascular, and other causes. The recipients were followed until death, graft failure (GF), or December 31, 2021.

2.3 | Transplant variables

The transplant year, peak percentage calculated panel reactive antibody (cPRA), and the number of human leukocyte antigen (HLA) A, B, and DR mismatches were collected. Induction therapy was used in most patients with an anti-IL-2 receptor monoclonal antibody or a polyclonal anti-thymocyte globulin. ATG was primarily used in HLA-incompatible KT, sensitized patients, and retransplants. All enrolled recipients had similar triple maintenance immunosuppression, consisting of a calcineurin inhibitor (most of them tacrolimus or, less often, cyclosporine), mycophenolate mofetil, and prednisolone.¹⁷

Incompatible KT includes cases of HLA-incompatible KT, in which a transplant occurred with preformed donor-specific antibodies (DSA), and cases of ABO-incompatible KT, in which there was a blood group incompatibility. Both situations were submitted to desensitization protocols that included treatment with rituximab, plasmaphereses, and

intravenous immunoglobulin. Further details of our immunosuppressive regimen have already been published.¹⁷

2.4 | Statistical analysis

Continuous variables were expressed by the mean and standard deviation (SD) and by the median and interquartile range (IQR) in the case of non-parametric distributed variables. Categorical variables were presented by frequencies and percentages.

Most predictor variables were available for the full cohort, except for donor fasting serum cholesterol (missing, $n = 13$), which were replaced by multiple imputations with chained equations.

We evaluated the predictive performance of the three models of the iPREDICTLIVING tool in our cohort of LKDT, as by Haller et al.⁷ and are available in the online tool iPREDICTLIVING at <http://www.meduniwien.ac.at/nephrogene/index.php/data/iPREDICTLIVING>. According to each model studied, the outcomes of interest were donor death, recipient death, and censored graft failure (GF).

The predictive performance of the LKDPI, as described by Massie et al.⁵ ($LKDPI = -11.30 + 1.85 * [(age - 50) \text{ if } age > 50] - 0.381 * eGFR + 1.17 * BMI + 22.34 \text{ if African-American} + 14.33 \text{ if history of cigarette use} + 0.44 * SBP (-21.68 \text{ if donor and recipient both male}) + 27.30 \text{ if ABO incompatible} (-10.61 \text{ if unrelated}) + 8.57 * (\# \text{ HLA-B mismatches}) + 8.26 * (\# \text{ HLA-DR mismatches}) - 50.87 * [\min(D/RWR, 0.9)]$) was also evaluated in our population to assess the risk of overall and censored GF.

Models' performances were evaluated by discrimination and calibration performance. Discrimination measures the ability of prediction models to separate individuals with different outcomes. To obtain a more comprehensive understanding of this metric, we analyzed discrimination using Harrell's C-statistic (time-independent approach) and area under receiver operating characteristic curve (AUC) or time-dependent AUC analysis, which accounts for the censoring of the time-to-event outcome.¹⁹ The C index (or AUC) is a proportion that can vary from 0 to 1. Values near 1 indicate high performance and a value near .5 indicates that the model's performance is a random concordance (the same as a coin flip). Values below .5 would exclude the predictive ability of the model. Generally, a C statistic of .7 indicates acceptable discrimination, .8 indicates good discrimination, and .9 indicates excellent discrimination.¹⁸ At time-dependent AUC analysis, the area under the receiver operating characteristic (ROC) curve, optimal cutoff (Youden), and sensitivity and specificity values were recorded using the STATA command *stroccurve*.

Calibration refers to the agreement between observed and predicted probabilities by the model. We categorized the predicted probabilities by their quintiles and computed the mean predicted probabilities and the Kaplan-Meier estimates of observed cumulative incidence in each quintile group. A model that calibrates well should have similar predicted and observed probabilities. The analysis of the calibration slope evaluated calibration performance. A good calibration

model has a slope of 1 (and an intercept close to 0); slope >1 means overestimating the predictive values, and slope <1 means underestimating the predictive values. A good discriminative performance does not always ensure good calibration performance and vice versa.¹⁸

A 2-sided p -value <.05 was considered as statistically significant. Statistical calculations were performed using STATA/MP, version 15.1 (Stata Corp, College Station, TX).

3 | RESULTS

3.1 | Baseline characteristics of the cohort

A total of 352 pairs were included in the study. Clinical characteristics of the cohort are shown in Table 1, including donor and recipient characteristics and transplant data. The mean donor age was 46.8 ± 10.7 years (19.4–76.2), and 69% were female. The median donor body mass index (BMI) was 25.0 (IQR 22.7–27.7) kg/m^2 , the median systolic blood pressure was 120 (IQR 113–130) mmHg, and the median diastolic blood pressure was 74 (IQR 70–80) mmHg. The median pre-donation total cholesterol was 194 (IQR 170–218) mg/dL. The median pre-donation serum creatinine was $.72$ (IQR .64–.88) mg/dL with a mean pre-donation eGFR of 100.5 ± 14.8 mL/min/1.73 m^2 . Sixty-five percent of living kidney donors were biologically related to their recipient (most first-degree relatives), and most donors (96%) were ABO compatible with their recipients. The median donor follow-up was 7.1 (IQR 4.2–11.8) years.

The mean recipient age was 40.4 ± 13.6 years, and most were male (67%). The median recipient BMI was 23.0 (IQR 21.0–26.1) kg/m^2 . Glomerulonephritis was the most common cause of CKD (47%). Most recipients were submitted to a first KT (82%), and the median waiting time was 1.2 (IQR .3–2.6) years, whether 20% received a preemptive KT. Hemodialysis was the most prevalent renal replacement therapy in 58% of recipients. Diabetes, cardiovascular, cerebrovascular, and peripheral vascular disease affected less than 8% of the recipients. Most of the recipients had a cPRA of zero. Albeit that, most recipients received induction therapy, mostly with basiliximab. The median follow-up of the graft was 7.5 (IQR 4.9–11.4) years and 8.1 (IQR 5.1–12.7) years for the recipient.

3.2 | Observed and predicted outcomes in the cohort

Six (1.7%) donors and 17 (5%) recipients died, and 44 (13%) recipients experienced censored GF during the observation period until December 2021.

Observed donor death at 10 and 20 years was 1.4% ($n = 3$) and 1.7% ($n = 4$). Predicted donor death was .2 (IQR .1–.5) % at 10 years and 1.4 (IQR .6–3.1) % at 20 years (Table 2). We observed recipient death at 10 years of 2.5% ($n = 9$). Predicted recipient death was 6.8% (IQR 3.5–13.9) % (Table 2). Observed censored graft failure (CGF) at 10 years was

TABLE 1 Donor, recipient, and the transplant data from the cohort studied.

	Total, N = 352
<i>Donor</i>	
Age, mean $\pm$ SD	46.8 $\pm$ 10.7
Age >50, n (%)	143 (41)
Female R, n (%)	244 (69)
BMI, median (IQR)	25.0 (22.7–27.7)
Weight, median (IQR)	68 (60–76)
Height, median (IQR)	163 (158–170)
SBP, median (IQR)	120 (113–130)
DBP, median (IQR)	74 (70–80)
Hypertension before donation, n (%)	53 (15)
Total cholesterol, median (IQR)	194 (170–218)
Smoking habits, n (%)	56 (16)
Serum creatinine, median (IQR)	.72 (.64–.88)
eGFR, mean $\pm$ SD	100.5 $\pm$ 14.8
Proteinuria, median (IQR)	.11 (.07–.16)
Missing = 25	
Donor ESRD, n (%)	1 (.3)
Donor death, n (%)	6 (2)
Years of follow-up (donor), median (IQR) [Min–Max]	7.1 (4.2–11.8) [.3–30.9]
<i>Recipient</i>	
Age, mean $\pm$ SD	40.4 $\pm$ 13.6
Age <18, n (%)	14 (4)
Female R, n (%)	117 (33)
BMI, median (IQR)	23.0 (21.0–26.1)
Weight, median (IQR)	67 (59–75)
Height, median (IQR)	170 (161–175)
Prior KT, n (%)	64 (18)
Years on dialysis, median (IQR)	1.2 (.3–2.6)
<i>Vintage, n (%)</i>	
Preemptive	69 (20)
HD	203 (58)
PD	80 (23)
Raw EPTS, mean $\pm$ SD	1.14 $\pm$.76
Diabetes, n (%)	20 (6)
Cardiovascular disease, n (%)	26 (7)
Cerebrovascular disease, n (%)	5 (1)
Peripheral vascular Disease, n (%)	3 (1)
HCV+, n (%)	7 (2)
<i>CKD etiology, n (%)</i>	
Diabetes	10 (3)
GN	166 (47)
Hereditary	16 (5)
Polycystic	36 (10)

(Continues)

TABLE 1 (Continued)

	Total, N = 352
<i>Urologic</i>	
Urologic	57 (16)
Unknown	27 (16)
Other	10 (3)
<i>Transplant</i>	
Transplant year	2013 (2009–2016)
Living-related 1st degree, n (%)	214 (61)
Living-related 2nd degree, n (%)	15 (4)
Living-unrelated, n (%)	123 (35)
MM A, mean $\pm$ SD	.90 $\pm$.66
MM B, mean $\pm$ SD	1.13 $\pm$.69
MM DR, mean $\pm$ SD	.97 $\pm$.68
<i>PRA-CDC, n (%)</i>	
0%	307 (87)
0%–50%	35 (10)
$\geq$ 50%	10 (3)
<i>PRA-virtual, n (%)</i>	
0%	222 (63)
0%–50%	80 (23)
$\geq$ 50%	25 (7)
Missing	25 (7)
ABOi, n (%)	15 (4)
HLAi, n (%)	26 (7)
<i>Paired donation, n (%)</i>	
Paired donation, n (%)	19 (5)
<i>Induction, n (%)</i>	
No	16 (5)
Basiliximab	273 (78)
ATG	63 (18)
Years of follow-up (graft), median (IQR) [Min–Max]	7.5 (4.9–11.4) [2.1–22.3]
Years of follow-up (recipient), median (IQR) [Min–Max]	8.1 (5.1–12.7) [2.1–22.3]
<i>Post-transplant outcomes</i>	
Overall graft failure, n (%)	55 (16)
Censored graft failure, n (%)	44 (13)
Recipient death, n (%)	17 (5)

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated Glomerular Filtration Rate; EPTS, estimated post-transplant survival; ESRD, end-stage renal disease; GN, glomerulonephritis; HD, hemodialysis; IQR, inter quartile range; KT, kidney transplant; PD, peritoneal dialysis; PRA-CDC, Panel Reactive Antibody by cytotoxicity; R, recipient; SBP, systolic blood pressure; SD, standard deviation.

7.9%, while the predicted CGF at that time was 12 (IQR 8.7–18.1) % (Table 2).

We observed a significantly reduced number of events concerning recipients' deaths in the first 5 years ($n = 1$) and donor deaths in the first 5 years ($n = 1$), and the global number of events is

TABLE 2 Scores of the iPREDICTLIVING tool and Living Kidney Donor Profile Index (LKDPI) in our study cohort.

	Median (IQR)
Predicted failure donor 10-years (%)—iPREDICTLIVING	.2 (.1–.5)
Predicted failure donor 20-years (%)—iPREDICTLIVING	1.4 (.6–3.1)
Predicted failure recipient 10-years (%)—iPREDICTLIVING	6.8 (3.5–13.9)
Predicted censored failure graft 10-years (%)—iPREDICTLIVING	12.3 (8.7–18.1)
LKDPI	7.9 (–5.2–23.1)

Abbreviation: LKDPI, Living Kidney Donor Profile Index.

otherwise low in the first 5 years after LDKT. It was not unexpected as a predicted unfavorable outcome to the recipient is a relative contraindication to LDKT and donors' candidates are selected as healthy candidates.

3.3 | Performance assessment of the iPREDICTLING tool in an external cohort of LDKT

3.3.1 | Model A—Donor mortality model-donor death following LDKT

Better discrimination was observed in 20-year prediction in a time-dependent (AUC of .694 vs. .636) and -independent approach (Harrell's C of .712 vs. .699), albeit with a slightly worse calibration (calibration slope of .955 vs. .992), as shown in Table 3 and Figure 1.

3.3.2 | Model B – Recipient mortality model – Recipient death following LDKT

Considering the predictive performance of recipient death at 10 years, this tool had very acceptable discrimination, both in a time-independent (Harrell's C .776) and time-dependent manner (AUC of .773) (Table 3) although without excellent calibration (calibration slope of 1.003) as shown in Figure 1.

3.3.3 | Model C – Graft failure model – Graft failure following LDKT

This model was elaborated for predicting censored GF. Overall, the predictive performance of this model was worse than previous models, which has already been verified in the internal validation set. At 10 years, the tool had a low discrimination value, particularly in a time-dependent manner (AUC of .566), with a sensitivity of 41% and specificity of 79%. The discriminative performance was better in the time-independent method (Harrell's C .665) (Table 3). However, in living donor selection, censored GF in a time-dependent manner should

be prioritized. The calibration slope was .998, and the calibration curve is depicted in Figure 1.

3.4 | Performance assessment of the LKDPI in the cohort and comparison with iPREDICTLIVING tool for GF

LKDPI was developed to predict overall GF, for which it has a low discriminative performance at ten years, especially at a time-dependent approach (AUC of .579) (Table 3). Interestingly it performs better as a predictor of censored GF at both approaches (Harrell's C .633 and AUC .617) with excellent calibration (calibration slope 1.000). These results are comparable to those of the iPREDICTLIVING tool model C.

4 | DISCUSSION

In our cohort of 352 LDKT pairs, the iPREDICTLIVING tool performed differently according to the model tested, being worse for the prediction of censored GF, but the tool was successfully validated. It was its first external validation joining the armamentarium of the available tools in LD consultation with other well-known tools like the LKDPI.⁵ Both discrimination and calibration performance were reported for the three tool models. The performance was globally worse than that at the internal validation, but it was unexpected as it was developed and validated in the same dataset.⁷ The performance of LKDPI for the prediction of censored GF at 10 years and the performance of the model of the iPREDICTLIVING tool for this endpoint were comparable.

A Kidney Disease: Improving Global Outcomes (KDIGO) work group published in 2017 an extensive clinical practice guideline for evaluating LD candidates. A comprehensive approach to risk assessment was recommended to replace decisions based on the assessment of isolated single risk factors. Transplant programs were recommended to provide each donor candidate with individualized quantitative estimates of short-term and long-term risks from a donation, anticipated recipient outcomes, such as graft and patient survival, and treatment alternatives available to the intended recipient, including opportunities for kidney-paired donation. Furthermore, each center was recommended to define its threshold for acceptable post-donation risk.⁸ Nevertheless, reliable, externally validated risk prediction models with low risk of bias,¹⁹ to guide clinical decisions in LDKT are yet to be available, making accomplishing these guidelines difficult. Although the performance of the iPREDICTLIVING tool at the external validation was not outstanding, it was acceptable. Implementing this model in LD consultation would represent an added value in decision-making to clinical judgment alone, with an expected improved informed consent process. Further application to Kidney paired exchange (KPE) programs could be explored.

Individualized prediction of donor outcomes is available for end-stage renal disease or impaired renal function after kidney donation.^{3,20–22} Several analyses compared the risk of donor mortality to the general population.^{3,20,21} The Norwegian study described an

TABLE 3 iPREDICTLIVING tool models and LKDPI – Time-independent (Harrell's C) and time dependent Receiver Operating Characteristic (ROC) analysis.

	Time-independent analysis		Time-dependent ROC		
	Discrimination AUC Harrell's C	Calibration slope	AUC	Optimal cutoff (youden)	Sensitivity (%) Specificity (%)
Model A- Donor death following LDKT					
Predicted donor death 10-years (%)	.699	.992	.636	.1	10030
Predicted donor death 20-years (%)	.712	.955	.694	3.1	6477
Model B- Recipient death following LDKT					
Predicted recipient death 10-years (%)	.776	1.003	.773	8.3	9462
Model C- Graft failure following LDKT					
Predicted censored failure graft 10-years (%)	.665	.998	.566	18.6	4179
LKDPI					
LKDPI- overall graft failure at 10-years	.591	1.002	.579	9.3	6557
LKDPI- censored graft failure at 10-years	.633	1.000	.617	10	7560

Note: Model A- Donor mortality model-donor death following LDKT: predicted donor death at 10 and 20 years. Model B- Recipient mortality model- Recipient death following LDKT: predicted recipient death at 10 years. Model C- Graft failure model- Graft failure following LDKT: predicted censored graft failure at 10 years. LKDPI- Living Kidney Donor Profile Index: overall and censored graft failure at 10 years.

increased risk of global and cardiovascular death in donors compared to matched healthy non-donors.²¹ Albeit we had a small number of events, this tool performed well in predicting donor death in our cohort, particularly in the long term (20 years), considering discrimination issues (Harrell's C of .712 and AUC of .699), with less reliable calibration (calibration slope of .955). Further, the linear predictor of donor mortality contributes to the other models, particularly for graft loss.

LDKT is the preferred treatment for ESRD. While donor safety must be assured, favorable outcomes for the recipient are pivotal at the same time to justify nephrectomy in a healthy individual. Concerning recipient mortality, the iPREDICTLIVING tool was acceptable in predicting recipient death at ten years in our cohort. Although we cannot directly compare the different measures of discriminative performance used in our external validation and in the original study (10 years' time-dependent AUC of .773 vs. optimism corrected C-statistic of .77), they represent similar performance,¹⁸ with reliable calibration. Undoubtedly, it would place in clinical practice and generate information that could improve the informed consent process at pre-transplant evaluation.

The censored GF model had the worst discriminative performance at the external validation (time-dependent AUC of .665) and the worst performance at the internal validation model (optimism corrected C statistic of .66). It was unexpected. Many immune and nonimmune processes may lead to graft loss, most of which develop after transplantation and, as such, past the time point of decision-making that cannot be predicted before the transplant. This tool is designed to be used at the pre-transplant consultation. It would be better than the clinical judgment alone.

Previous risk tools of LDKT have been published, but most have yet to be validated and, except for LKDPI, are not used in current

practice.¹⁹ In the European Cohort, Rehse et al.⁹ provided external validation of the LKDPI to predict overall GS and confirmed the comparability of the LKDPI with the KDPI to discriminate post-transplant outcome. However, they found a C-statistic for the LKDPI of .52 and discussed that the moderate discriminative ability indicates limited clinical use for the guidance of the individual patient. A higher predictive value was found for censored GS and donors with the worse LKDPI, making this tool more valuable for "marginal donors" We evaluated its performance in our cohort and, like Rehse et al., we found a better performance of LKDPI to predict censored than overall GF in our cohort (Table 3).

Considering the prediction of censored GF, at 10 years iPREDICTLIVING tool had a better discriminative performance in a time-independent manner, with a Harrell's C of 0.663 versus .633 of LKDPI, but not at time-dependent analysis (AUC of 0.566 vs. .617). The calibration was worse for the iPREDICTLIVING tool (calibration slope 1.004 vs. 1.000). We conclude that the two tools have similar predictive performance in our population, albeit being different. They were developed in different populations and incorporated different predictors. The LKDPI⁵ was developed in the American population, and most of the predictors consist of demographical and anthropometric data of the donor as well as histocompatibility and ABO compatibility data. In view of the population characteristics, the LDKT recipients had a median age of 50 (IQR 39–60), BMI 27 (IQR 24–31) kg/m² median time on RRT .7 (.0–1.7) years, 37.6% were female and 27.7% were not Caucasians. The iPREDICTLIVING tool⁷ was developed in a Norwegian Caucasian population. The mean age of donors was 48.1 ± 12.5 years with a BMI of 24.9 ± 3.3 kg/m². The recipients had a mean age of 47.2 ± 14.5 years, 1.2% had diabetes mellitus, and 9.6% had cardiovascular disease. The model for GF had significant input from the model of donor mortality. Although the HLA mismatch accounts

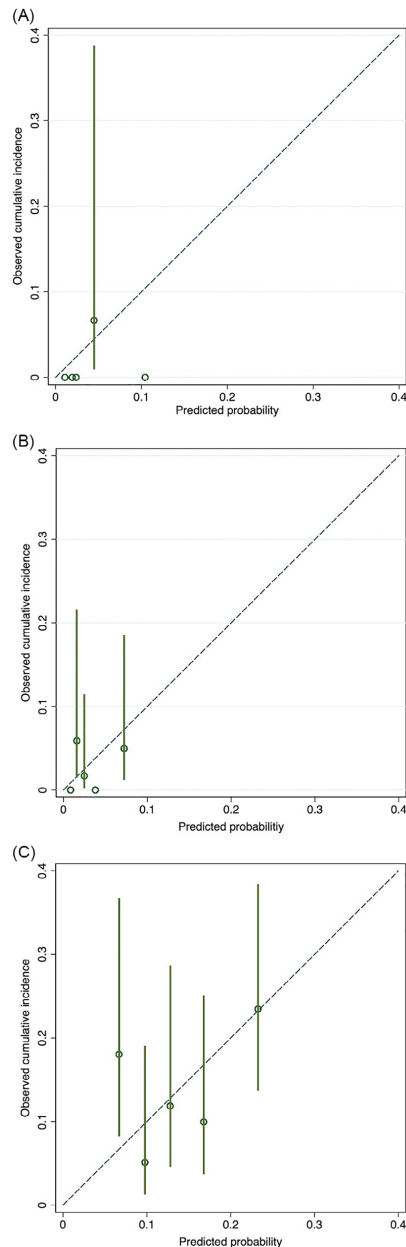


FIGURE 1 Calibration plots for the prediction models of the iPREDICTLIVING tool. (A) Calibration plot for prediction of donor death at 20 years. (B) Calibration plot for prediction of recipient death at 10 years. (C) Calibration plot for prediction of censored graft failure at 10 years.

for, the other factors rely on dialysis vintage and peripheral vascular and heart disease. They can represent different lenses looking at the same phenomenon. Surprisingly their performance was very similar, as shown by our data. Our cohort was more similar to the Norwegian, also consisting of a Caucasian population, albeit our recipients are younger (mean age 40.4 ± 13.6 years) and had less diabetes (6%). We assume that no predictor of graft outcomes applicable pre-donation will have an excellent discriminative performance since most outcomes after LDKT will be determined to be recipient-specific characteristics and post-transplant intercurrents. Although we must be cautious about applying the results to individual decision-making based on the limited predictive value of the models, their utility in combining clinical and other information in the decision-making process must be recognized. It does not warrant utilizing this metric as an isolated decision metric but incorporates many other important considerations associated with living donor transplantation. It pushes the community to investigate further into factors influencing the variability in recipient outcomes.

Overall, we can consider two competing events in the analysis of graft and recipient death with functioning graft. In the iPREDICTLIVING tool, Haller et al.⁷ deliberately do not account for the competing risk of death when analyzing graft failure, considering that a patient is interested in graft survival whether he is alive. For most young recipients, graft loss is more probable than death with a functioning graft, but the opposite can be expected when we evaluate older recipients.

The increasing scarcity of organs and the number of hypersensitized patients renewed the interest in increasing KPE programs by introducing compatible pairs. Its efficacy has been described precociously by Gentry et al.²³ when they showed that compatible pairs could double the match rate within KPE networks. Although increased altruism may guide this compatible pair's decision, we must look for some time of compatibility beyond classic ABO and HLA match.¹² The LKDPI is already used in some programs to compare the donor's quality and justify some gain or, at least, equipoise, albeit its limitations.¹⁵ Nevertheless, in a recent work, Irish et al., using data from Australia and New Zealand Dialysis and Transplant Registry and from the SRTTR, concluded that the LKDPI does not discriminate death-censored graft survival and should not be used to promote compatible pairs participation in KPE programs. The iPREDICTLIVING tool was purposed to be used to inform candidates with compatible donors on the advisability of joining a KPE program.⁷ Its utilizations need to be further explored by the transplant community.

Limitations of this study must be recognized. It was a single-center study with a retrospective observational design, involving a Caucasian population. It is representative of the population of the North of Portugal but may compromise the generalizability of the results as other races and ethnic origins are not represented. We cannot predict its performance in other populations with more ethnic variety and comorbidities in the donor and recipient populations. Such external validation in different populations and, if necessary, recalibration and further development of the model to adapt to changing demographics and more contemporary eras should precede any general application in

clinical practice. Few events were verified in the validation population, particularly considering donor and recipient deaths. It was unexpected in a selected LDKT population, with younger recipients and a low rate of comorbidities (6% diabetes). Furthermore, the tools considering these events had good discrimination. Excluding from the analysis very early graft failures and recipient deaths because of technical complications was a deliberate choice but could cause some limitations considering the validation sample.

Additionally, it must be acknowledged that a prediction model is not developed to predict whether an individual will have the event or not, but it represents a mathematical approach to quantify the chance of the event for a group of individuals with similar baseline characteristics.

As strengths, both discrimination and calibration are reported for the three models (calibration is poorly reported in most validations).

5 | CONCLUSIONS

These tools have moderate discriminative and calibration performance in our population, especially for the censored graft loss model, which also had worse discrimination in the original model. It may represent the unneglectable effect of recipient factors on graft outcomes. The tool was validated in in this Northern Portuguese cohort, Caucasian, with a low incidence of diabetes and other comorbidities to assess living donor survival, recipient survival, and censored graft failure. It can assist the informed decision-making process at the living donor and pre-transplant consultation, joining clinical, and other relevant information.

AUTHOR CONTRIBUTIONS

Manuela Almeida, Catarina Ribeiro, and Jorge Malheiro: Research design, data acquisition, data analysis, and paper writing. José Silvano, Sofia Pedroso, and Sandra Tafulo were engaged in the data acquisition and analysis. Miguel Ramos and La Salette Martins were involved in the research design and data analysis. All the authors approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Gruessner RWG, Gruessner AC. Solid-organ transplants from living donors: cumulative United States experience on 140,156 living donor transplants over 28 years. *Transplant Proc.* 2018;50(10):3025-3035.
2. Park YH, Min SK, Lee JN, et al. Comparison of survival probabilities for living-unrelated versus cadaveric renal transplant recipients. *Transplant Proc.* 2004;36(7):2020-2022.
3. Reese PP, Boudville N, Garg AX. Living kidney donation: outcomes, ethics, and uncertainty. *Lancet.* 2015;385(9981):2003-2013.
4. Meier-Kriesche H-U, Kaplan B. Waiting time on dialysis as the strongest modifiable risk factor for renal transplant outcomes: a paired donor kidney analysis. *Transplantation.* 2002;74(10):1377-1381.
5. Massie AB, Leanza J, Fahmy LM, et al. A risk index for living donor kidney transplantation. *Am J Transplant.* 2016;16(7):2077-2084.
6. Ashby VB, Leichtman AB, Rees MA, et al. A kidney graft survival calculator that accounts for mismatches in age, sex, HLA, and body size. *Clin J Am Soc Nephrol.* 2017;12(7):1148-1160.
7. Haller MC, Wallisch C, Mjøs G, et al. Predicting donor, recipient and graft survival in living donor kidney transplantation to inform pretransplant counselling: the donor and recipient linked iPREDICTLIVING tool—a retrospective study. *Transpl Int.* 2020;33(7):729-739.
8. Lentine KL, Kasiske BL, Levey AS, et al. Summary of kidney disease: improving global outcomes (KDIGO) clinical practice guideline on the evaluation and care of living kidney donors. *Transplantation.* 2017;101(8):1783-1792.
9. Rehse G, Halleck F, Khadzhyrov D, et al. Validation of the Living Kidney Donor Profile Index in a European cohort and comparison of long-term outcomes with US results. *Nephrol Dial Transplant.* 2019;34(6):1063-1070.
10. Okumura K, Yamanaga S, Tanaka K, et al. Is the living donor kidney profile index valuable in Japanese patients? *Transplant Proc.* 2020;52(6):1650-1654.
11. Shantier M, Li Y, Ashwin M, Famure O, Singh SK. Use of the Living Kidney Donor Profile Index in the Canadian kidney transplant recipient population: a validation study. *Can J Kidney Health Dis.* 2020;7:2054358120906976.
12. Jackson KR, Segev DL. Rethinking incompatibility in kidney transplantation. *Am J Transplant.* 2022;22(4):1031-1036.
13. Chipman V, Cooper M, Thomas AG, et al. Motivations and outcomes of compatible living donor-recipient pairs in paired exchange. *Am J Transplant.* 2022;22(1):266-273.
14. Furian L, Nicolò A, Di Bella C, Cardillo M, Cozzi E, Rigotti P. Kidney exchange strategies: new aspects and applications with a focus on deceased donor-initiated chains. *Transpl Int.* 2020;33(10):1177-1184.
15. Basu A, Prieto M, Kosberg C, et al. Ten years of kidney paired donation at mayo clinic: the benefits of incorporating ABO/HLA compatible pairs. *Transplantation.* 2020;104(6):1229-1238.
16. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-612.
17. Silva F, Malheiro J, Pestana N, et al. Lower donated kidney volume is associated with increased risk of lower graft function and acute rejection at 1 year after living donor kidney—a retrospective study. *Transpl Int.* 2020;33(12):1711-1722.
18. Park SY, Park JE, Kim H, Park SH. Review of statistical methods for evaluating the performance of survival or other time-to-event prediction models (from conventional to deep learning approaches). *Korean J Radiol.* 2021;22(10):1697-1707.
19. Haller MC, Aschauer C, Wallisch C, et al. Prediction models for living organ transplantation are poorly developed, reported, and validated: a systematic review. *J Clin Epidemiol.* 2022;145:126-135.
20. Muzaale AD, Massie AB, Wang M-C, et al. Risk of end-stage renal disease following live kidney donation. *JAMA.* 2014;311(6):579-586.

21. Mjølén G, Hallan S, Hartmann A, et al. Long-term risks for kidney donors. *Kidney Int.* 2014;86(1):162-167.
22. Andrews PA, Burnapp L. British Transplantation Society/Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: summary of updated guidance. *Transplantation.* 2018;102(7):e307.
23. Gentry SE, Segev DL, Simmerling M, Montgomery RA. Expanding kidney paired donation through participation by compatible pairs. *Am J Transplant.* 2007;7(10):2361-2370.

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APPENDIX 10 – Living Donors' Age Modifies the Impact of Pre-Donation Estimated Glomerular Filtration Rate on Graft Survival.

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Article

Living Donors' Age Modifies the Impact of Pre-Donation Estimated Glomerular Filtration Rate on Graft Survival

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Abstract: Background: The global scarcity of organs for kidney transplants (KTs) has led to the increased acceptance of living donors (LDs) with minor abnormalities to increase the donor pool. We sought to evaluate the effects of some of these LDs' clinical characteristics (older age, borderline renal function, hypertension, dyslipidemia, smoking, and obesity) on graft outcomes. Methods: We studied 352 recipients of LDKTs (1998–2020). Firstly, considering the recipients and KT variables, we identified relevant predictors of overall and censored graft failure (GF). Then, adjusting for these predictors, we explored LD variables as predictors of overall and censored GF in a multivariable Cox model. Results: The recipients from LD with higher eGFR (≥ 90 mL/min/1.73 m²) had significantly better overall and censored graft survival (GS) at 15 y after KT (respectively, 67 and 75% vs. 46 and 46%, $p < 0.001$). Importantly, none of the remaining LD factors which were evaluated (hypertension, dyslipidemia, smoking, proteinuria, and obesity) were independent predictors of GF. In recipients from LDs < 50 y, having an eGFR < 90 was an independent predictor of overall GF [adjusted HR (95%CI) of 2.578 (1.120–5.795)] and censored GF [adjusted HR (95%CI) of 3.216 (1.300–7.959)], compared to recipients from LDs with eGFR ≥ 90 . Contrarily, when donors were older, no difference in the risk of GF was observed between eGFR categories. Conclusion: In our cohort, lower pre-donation eGFR had an impact on GS only in younger LDs. An age-adjusted eGFR cutoff may be pursued for improved donor admissibility.

Keywords: age; graft survival; living donor; glomerular filtration rate; kidney transplantation; medically complex living donors

1. Introduction

Chronic kidney disease (CKD) is a global health problem affecting more than 10% of the general population worldwide, amounting to >800 million individuals [1]. CKD progresses to end-stage renal disease (ESRD) in approximately 2% of patients overall [1,2]. Kidney transplantation (KT) is the best treatment for ESRD. It is associated with improved survival and quality of life [2,3]. After KT, an increase in overall lifespan of 10 years is expected, from 3 years in older recipients to 17 years in the younger ones who would benefit the most from KT [3]. Unfortunately, severe organ shortage for kidney transplants is a worldwide problem [1,4]. Concerted efforts have been taken to increase the donor pool. However, the supply of donors (both living and deceased) is far lower than the

need, resulting in an enormous number of qualified patients remaining on the wait-list and thousands being removed from the list every year because of death or becoming too sick for transplantation [2,4]. Portugal has a median wait-list time of around 5 years, and the mortality rate in wait-listed individuals is more than 5% each year [5]. Although this country has one of the highest deceased donor rates of kidney transplant worldwide, at 38.5 pmp in 2021, the rate of living donor is not impressive, at 3.5 pmp the same year [6].

A living donor (LD) kidney transplant (KT) is the preferred treatment for end-stage renal disease (ESRD) [7]. Living donor (LD) KTs increase organ availability, decrease time on the waiting list, allow for pre-emptive transplantation, and improve graft and patient survival [7,8]. Not all LD organs have the same quality, and donor specificities may affect recipient outcomes [9].

Increasingly, the shortage of organs leads to organ acceptance from LDs with borderline medical or surgical abnormalities, potentially associated with worse graft and recipient outcomes [7,9]. These donors are usually referred to as medically complex living donors (MCLDs), such as the elderly or those with obesity, vascular complexity, or a family history of ESRD [10].

The practice patterns of acceptance of MCLDs are very variable between transplant centers [11,12], but it is estimated that they comprise at least 25% of LDKT programs [11]. Considering the donor perspective, two landmark studies from the last decade have shown an increased risk of ESRD in kidney donors compared with matched healthy non-donors [13,14]. These risks could be higher in MCLDs. The absence of high-quality studies with long-term follow-ups for recipient and donor outcomes makes this issue highly relevant to the transplant community [9,15].

A Kidney Disease: Improving Global Outcomes (KDIGO) work group published an extensive clinical practice guideline for evaluating LD candidates in 2017 [16]. A comprehensive approach to risk assessment is recommended in order to replace decisions based on the evaluation of isolated single risk factors, but precise orientations concerning MCLDs are lacking [16]. Data on organ admissibility for the intended recipients are even scarcer. Most of the available studies are retrospective, based on observational registry data or small cohort samples, or have considerable heterogeneity in design and results.

We sought to evaluate the effects on the graft outcomes (overall and censored graft failure (GF) and graft function) of clinical characteristics of LDs of medical complexity in our cohort, namely, donor age, reduced pre-donation eGFR, hypertension, dyslipidemia, smoking, proteinuria, and obesity. We hypothesize that these characteristics could be associated with worse outcomes.

2. Materials and Methods

We retrospectively reviewed the clinical data of all LDKT pairs that were performed at our center between January 1998 and January 2020 ($n = 365$). Inclusion criteria included at least one month of follow-up after KT. Thirteen patients were excluded from the analysis, eleven because of primary non-function due to perioperative problems, and two due to deaths within 30 days of the transplant (in all situations, donor-related characteristics causing the events were excluded). The remaining 352 pairs defined our study cohort.

The Institutional Review Board at our center, Centro Hospitalar Universitário de Santo António (CHUdSA), approved this retrospective observational study (Ref.: 147-21(119-DEFI/122-CE)), which was conducted according to the Helsinki declaration.

2.1. Donor Variables

Following international guidelines, all donors were subjected to a standard evaluation protocol [16,17]. Baseline demographic, anthropomorphic, analytical, and clinical data were collected from the LD. Serum creatinine-based CKD-EPI equation [18] was used to predict eGFR. Renal function was further evaluated by creatinine clearance in 24 h urine samples and serum C-cystatin in more recent donors. The final approval for kidney donation was reviewed in a multidisciplinary meeting, and ethical approval was mandatory.

In this study, we decided to use 90 mL/min/1.73 m² as the eGFR cutoff, as the KDIGO guidelines propose [16]. In terms of donor age, the optimal cut-off point for the prediction of the outcomes of interest was set at 50 years through a univariate, time-dependent ROC analysis performed in this cohort (stroccurve STATA command).

Hypertension was defined by blood pressure (BP) in the consultation > 140/90 mmHg, 24 h ambulatory BP > 135/85 mmHg, and past hypertension diagnosis or antihypertensive medication [16]. Dyslipidemia was defined by the laboratory criteria (had total cholesterol > 200 mg/dL, LDL > 130 mg/dL, triglycerides > 150 mg/dL, or HDL < 40 mg/dL) and/or the use of hypolipidemic agents. Upon urinary analysis, proteinuria was defined by random urine protein 0.15 to 0.5 g/g [16], and was confirmed by determination using a 24 h sample. Donors with confirmed proteinuria over 300 mg/day were discarded.

Left-side procurement was preferred for anatomical reasons, unless complex vessel anatomy or significant renal asymmetry were present. A transperitoneal laparoscopic approach was utilized in most donors. Lifetime annual follow-up appointments were available for all donors.

2.2. Recipient Variables

Demographic, analytical, and clinical data were collected from all the recipients. Comorbidities were registered. Chronic kidney disease (CKD) etiologies were grouped into six categories: diabetic nephropathy (DN), chronic glomerulonephritis (CGN), cystic diseases, urologic pathologies, unknown etiologies, and other causes. A serum creatinine based on the CKD-EPI equation [18] was used to predict the eGFR. Graft biopsies were performed for cause. Acute rejection (AR) was defined as per biopsy criteria. The recipients were followed until death, GF, or reaching 15 years of follow-up. Graft survival (GS) comprised the time from transplantation to GF, defined in the case of overall GS as a return to dialysis, retransplantation, or death with a functioning graft. Alternatively, in death-censored GS, GS was censored at the time of death with a functioning graft, but not imputed as a failure.

2.3. Transplant Variables

Two different transplant eras were considered in our cohort: 1998–2009 and 2010–2019. The peak-percentage-calculated panel-reactive antibody (cPRA) and the number of human leukocyte antigen (HLA) A, B, and DR mismatches were collected. Induction therapy was used in most patients with an anti-IL-2 receptor monoclonal antibody or a polyclonal anti-thymocyte globulin (ATG). ATG was primarily used in HLA-incompatible KT, sensitized patients, and retransplants. All enrolled recipients underwent similar triple-maintenance immunosuppression therapy consisting of a calcineurin inhibitor, mycophenolate mofetil, and prednisolone.

The incompatible group KT included cases of HLA-incompatible and ABO-incompatible KT. Both situations were submitted to desensitization protocols that included treatment with rituximab, plasmaphereses, and intravenous immunoglobulin. Further details of our immunosuppression protocols have been described previously [19].

2.4. Statistical Analysis

Continuous data were described using the mean and standard deviation (SD) or the median (interquartile range [IQR]), and categorical data were expressed as numbers (and percentages) as appropriate. Categorical data, including demographic, clinical, and immunological features, were compared using Pearson χ^2 or Fisher's exact tests. Continuous variables were compared using the Student *t* test or Mann–Whitney *U*, as appropriate. A comparison of annual graft function according to donor eGFR and age was performed by means of one-way ANOVA.

Overall and censored GS curves were visualized using the Kaplan–Meier method, with comparisons between the patients' groups being carried out by log-rank tests. As our main purpose was to identify the clinical characteristics of LD associated with worse graft

outcomes, all of these potential predictors were explored by multivariable Cox proportional hazards models adjusted to recipient and transplant variables and selected by a separate multivariable Cox model, in which a p value less than 0.157 was necessary for retention using a backward elimination method, as previously proposed [20]. Additionally, a double interaction term was included in the univariate and multivariable Cox models to examine the potential for effect modification between donor age and eGFR categories, given that they were identified as the main independent predictors of the outcomes of interest. Hazard proportionality was checked by plotting a log minus log of the distribution hazard and using Schoenfeld residuals of distribution hazards.

Statistical calculations were performed using STATA/MP, version 15.1 (Stata Corp, College Station, TX, USA). A 2-sided p -value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline Characteristics

The baseline characteristics of our study cohort are summarized in Table 1. It was divided into two populations according to LD eGFR pre-donation: <90 vs. ≥ 90 mL/min/1.73 m². Most donors (77%) had eGFR ≥ 90 mL/min/1.73 m². Most recipients were male (67%), with a mean age of 40.4 ± 13.6 years old. The recipients from LD with higher eGFR (≥ 90 mL/min/1.73 m²) were significantly younger (39.6 ± 13.1 vs. 43.1 ± 14.6 , $p = 0.04$) and less frequently had histories of coronary artery disease (6 vs. 13%, $p = 0.017$). Their donors were significantly younger (44.9 ± 10.4 vs. 52.8 ± 9.5 , $p < 0.001$) and had fewer diagnoses of hypertension (13 vs. 23%, $p = 0.019$) and dyslipidemia (9 vs. 26%, $p < 0.001$).

Table 1. Baseline characteristics of the cohort.

	Total <i>n</i> = 352	eGFR < 90 mL/min/1.73 m ² <i>n</i> = 82 (23%)	eGFR ≥ 90 mL/min/1.73 m ² <i>n</i> = 270 (77%)	<i>p</i>
Recipient				
Age, mean $\pm$ SD	40.4 $\pm$ 13.6	43.1 $\pm$ 14.6	39.6 $\pm$ 13.1	0.040
F sex, <i>n</i> (%)	117 (33)	24 (29)	93 (34)	0.384
BMI, mean $\pm$ SD	23.6 $\pm$ 3.7	23.4 $\pm$ 3.6	23.6 $\pm$ 3.8	0.581
DM, <i>n</i> (%)	20 (6)	7 (9)	13 (5)	0.202
Smoking habits, <i>n</i> (%)	80 (23)	17 (21)	63 (23)	0.622
Coronary heart disease, <i>n</i> (%)	26 (7)	11 (13)	15 (6)	0.017
Cerebrovascular disease, <i>n</i> (%)	5 (1)	2 (2)	3 (1)	0.331
CKD etiology, <i>n</i> (%)				
DN	10 (3)	3 (4)	7 (3)	
CGN	166 (47)	40 (49)	126 (47)	
Hereditary	16 (5)	4 (5)	12 (4)	
Cystic disease	36 (10)	8 (10)	28 (10)	0.614
Urologic	57 (16)	13 (16)	44 (16)	
Unknown	57 (16)	10 (12)	47 (17)	
Others	10 (3)	4 (5)	6 (2)	
Vintage (years), median (IQR)	1.17 (0.28–2.59)	1.19 (0.10–2.52)	1.17 (0.29–2.70)	0.656
Previous RRT, <i>n</i> (%)				
HD	200 (57)	44 (54)	156 (58)	
PD	79 (22)	18 (22)	61 (23)	0.642
Preemptive	73 (21)	20 (24)	53 (20)	
Donor				
Age, mean $\pm$ SD	46.8 $\pm$ 10.7	52.8 $\pm$ 9.5	44.9 $\pm$ 10.4	<0.001
Age ≥ 50 , <i>n</i> (%)	143 (41)	53 (65)	90 (33)	<0.001
F sex, <i>n</i> (%)	244 (69)	55 (67)	189 (70)	0.615
BMI, mean $\pm$ SD	25.2 $\pm$ 3.3	25.8 $\pm$ 3.1	25.0 $\pm$ 3.4	0.067
BMI ≥ 30 , <i>n</i> (%)	33 (9)	10 (12)	23 (9)	0.317
Pre-donation SCr, median (IQR)	0.72 (0.64–0.88)	0.90 (0.80–1.02)	0.69 (0.61–0.78)	<0.001

Table 1. Cont.

	Total <i>n</i> = 352	eGFR < 90 mL/min/1.73 m ² <i>n</i> = 82 (23%)	eGFR ≥ 90 mL/min/1.73 m ² <i>n</i> = 270 (77%)	<i>p</i>
Pre-donation eGFR, mean ± SD	100.5 ± 14.7	79.7 ± 8.4	106.8 ± 9.5	<0.001
Hypertension, <i>n</i> (%)	53 (15)	19 (23)	34 (13)	0.019
Dyslipidemia, <i>n</i> (%)	46 (13)	21 (26)	25 (9)	<0.001
Smoking habits <i>n</i> (%)	56 (16)	9 (11)	47 (17)	0.163
ProtU 0.15–0.5 g/g, <i>n</i> (%)	98 (28)	23 (28)	75 (28)	0.962
Transplant				
Transplant era, <i>n</i> (%)				
1998–2009	102 (29)	22 (27)	80 (30)	0.624
2010–2019	250 (71)	60 (73)	190 (70)	
LUR, <i>n</i> (%)	123 (35)	31 (38)	92 (34)	0.535
Previous transplant, <i>n</i> (%)	64 (18)	18 (22)	46 (17)	0.312
PRA-CDC, <i>n</i> (%)				
0–4	314 (89)	74 (90)	240 (89)	0.695
5–49	28 (8)	5 (6)	23 (9)	
50–100	10 (3)	3 (4)	7 (3)	
HLA-incompatible, <i>n</i> (%)	26 (7)	7 (9)	19 (7)	0.649
ABO-incompatible, <i>n</i> (%)	15 (4)	2 (2)	13 (5)	0.535
HLA AB MM, mean ± SD	2.03 ± 1.18	1.98 ± 1.18	2.04 ± 1.18	0.648
HLA DR MM, mean ± SD	0.97 ± 0.68	0.99 ± 0.69	0.96 ± 0.68	0.776
Induction, <i>n</i> (%)				
No	16 (5)	3 (4)	13 (5)	0.699
Basiliximab	273 (78)	62 (76)	211 (78)	
ATG	63 (18)	17 (21)	46 (17)	
Acute rejection, <i>n</i> (%)	47 (13)	12 (15)	35 (13)	0.697
Censored graft failure <i>n</i> (%)	42 (12)	17 (21)	25 (9)	0.005
Overall graft failure <i>n</i> (%)	52 (15)	17 (21)	35 (13)	0.082
Years of follow-up, median (IQR)	7.4 (4.9–11.5)	6.6 (4.5–9.5)	7.8 (5.1–12.0)	0.041

F—female; BMI—body mass index; DM—diabetes mellitus; CKD—chronic kidney disease; DN—diabetic nephropathy; CGN—chronic glomerulonephritis; SD—standard deviation; IQR—interquartile range; ProtU—proteinuria; LUR—living unrelated donor; PRA—panel-reactive antibody; CDC—complement-dependent cytotoxicity; ATG—anti-thymocyte thymoglobulin; eGFR—estimated glomerular filtration rate; statistically significant values were represented on bold.

The population was further stratified by donor age (< vs. ≥50 years) and eGFR pre-donation (<90 vs. ≥90 mL/min/1.73 m²), as depicted in Table 2. The population of recipients of younger donors (<50 years) with lower eGFRs presented significantly more frequent history of coronary artery disease vs. those with higher eGFRs (17 vs. 4%, *p* = 0.014). Remarkably, these donors were older (42.8 ± 5.4 vs. 39.1 ± 7.3, *p* = 0.011) and had significantly more frequent pre-donation diagnoses of hypertension (21 vs. 6%, *p* = 0.004).

Table 2. Baseline characteristics according to eGFR and stratified by donor age.

	Donor Age < 50 <i>n</i> = 209		<i>p</i>	Donor Age ≥ 50 <i>n</i> = 143		<i>p</i>
	eGFR < 90 mL/min/1.73 m ² <i>n</i> = 29 (14%)	eGFR ≥ 90 mL/min/1.73 m ² <i>n</i> = 180 (86%)		eGFR < 90 mL/min/1.73 m ² <i>n</i> = 53 (37%)	eGFR ≥ 90 mL/min/1.73 m ² <i>n</i> = 90 (63%)	
Recipient						
Age, mean ± SD	38.0 ± 13.0	38.0 ± 12.2	0.985	46.0 ± 14.8	42.9 ± 14.3	0.234
F sex, <i>n</i> (%)	8 (28)	64 (36)	0.402	16 (30)	29 (32)	0.800
BMI, mean ± SD	23.2 ± 2.8	23.6 ± 3.8	0.525	23.6 ± 3.9	23.7 ± 3.8	0.914
DM, <i>n</i> (%)	1 (4)	9 (5)	1	6 (11)	4 (4)	0.173
Smoking habits, <i>n</i> (%)	2 (7)	41 (23)	0.051	15 (28)	22 (24)	0.611
Coronary heart disease, <i>n</i> (%)	5 (17)	7 (4)	0.014	6 (11)	8 (9)	0.636

Table 2. Cont.

	Donor Age < 50 n = 209			Donor Age ≥ 50 n = 143		
	eGFR < 90 mL/min/1.73 m ² n = 29 (14%)	eGFR ≥ 90 mL/min/1.73 m ² n = 180 (86%)	p	eGFR < 90 mL/min/1.73 m ² n = 53 (37%)	eGFR ≥ 90 mL/min/1.73 m ² n = 90 (63%)	p
Cerebrovascular disease, n (%)	1 (3)	3 (2)	0.452	1 (2)	0 (0)	0.371
CKD etiology, n (%)						
DM	1 (3)	4 (2)		2 (4)	3 (3)	
CGN	15 (52)	90 (50)		25 (47)	36 (40)	
Hereditary	3 (10)	10 (6)	0.865	1 (2)	2 (2)	0.475
Cystic disease	1 (3)	11 (6)		7 (13)	17 (19)	
Urologic	5 (17)	27 (15)		8 (15)	17 (19)	
Unknown	4 (14)	33 (18)		6 (11)	14 (16)	
Others	0 (0)	5 (3)		4 (8)	1 (1)	
Vintage (years), median (IQR)	1.36 (0.78–2.52)	1.11 (0.29–2.35)	0.213	1.14 (0–1.83)	1.40 (0.31–4.11)	0.082
Previous RRT, n (%)						
HD	17 (59)	106 (59)		27 (51)	50 (56)	
PD	7 (24)	41 (23)	0.642	11 (21)	20 (22)	0.737
Preemptive	5 (17)	33 (18)		15 (28)	20 (22)	
Donor						
Age, mean ± SD	42.8 ± 5.4	39.1 ± 7.3	0.011	58.4 ± 6.0	56.5 ± 4.2	0.029
F sex n (%)	18 (62)	119 (66)	0.671	37 (70)	70 (78)	0.289
BMI, mean ± SD	25.3 ± 2.9	24.8 ± 3.4	0.483	26.1 ± 3.1	25.5 ± 3.4	0.313
BMI ≥ 30, n (%)	3 (10)	15 (8)	0.721	7 (13)	8 (9)	0.416
Pre-donation SCr, median (IQR)	1.00 (0.88–1.06)	0.70 (0.63–0.80)	<0.001	0.88 (0.69–0.97)	0.66 (0.60–0.74)	<0.001
Pre-donation eGFR, mean ± SD	80.9 ± 7.2	110.3 ± 9.2	<0.001	79.1 ± 9.0	99.9 ± 5.6	<0.001
Hypertension, n (%)	6 (21)	10 (6)	0.004	13 (25)	24 (27)	0.778
Dyslipidemia, n (%)	3 (10)	10 (6)	0.397	18 (34)	15 (17)	0.018
Smoking habits, n (%)	3 (10)	38 (21)	0.215	6 (11)	9 (10)	0.803
ProtU 0.15–0.5 g/g, n (%)	9 (31)	53 (29)	0.862	14 (26)	22 (24)	0.793
Transplant						
Transplant era, n (%)						
1998–2009	14 (48)	59 (33)	0.104	8 (15)	21 (23)	0.237
2010–2019	15 (52)	121 (67)		45 (85)	69 (77)	
LUR, n (%)	8 (28)	57 (32)	0.660	23 (43)	35 (39)	0.596
Previous transplant, n (%)	6 (21)	25 (14)	0.339	12 (23)	21 (23)	0.924
PRA-CDC, n (%)						
0–4	25 (86)	161 (89)		49 (92)	79 (88)	
5–49	2 (7)	14 (8)	0.439	3 (6)	9 (10)	0.793
50–100	2 (7)	5 (3)		1 (2)	2 (2)	
HLA-incompatible, n (%)	2 (7)	12 (7)	1	5 (9)	7 (8)	0.761
ABO-incompatible, n (%)	2 (7)	10 (6)	0.675	0 (0)	3 (3)	0.295
HLA AB MM, mean ± SD	2.17 ± 0.97	2.06 ± 1.20	0.668	1.87 ± 1.27	2.02 ± 1.15	0.464
HLA DR MM, mean ± SD	1.03 ± 0.63	0.97 ± 0.69	0.641	0.96 ± 0.73	0.94 ± 0.68	0.898
Induction, n (%)						
No	3 (10)	11 (6)		0 (0)	2 (2)	
Basiliximab	16 (55)	133 (74)	0.102	46 (87)	78 (87)	0.648
ATG	10 (34)	36 (20)		7 (13)	10 (11)	
Acute rejection, n (%)	6 (21)	24 (13)	0.294	6 (11)	11 (12)	0.872
Censored graft failure n (%)	10 (34)	13 (7)	<0.001	7 (13)	12 (13)	0.983
Overall graft failure n (%)	10 (34)	19 (11)	0.001	7 (13)	16 (18)	0.472
Years of follow-up, median (IQR)	7.8 (5.3–12.1)	8.1 (4.9–12.5)	0.687	5.6 (4.0–8.5)	7.8 (5.4–10.8)	0.008

F—female; BMI—body mass index; DM—diabetes mellitus; CKD—chronic kidney disease; CGN—chronic glomerulonephritis; SD—standard deviation; IQR—interquartile range; ProtU—proteinuria; LUR—living unrelated donor; PRA—panel-reactive antibody; CDC—complement-dependent cytotoxicity; ATG—anti-thymocyte thymoglobulin; eGFR—estimated glomerular filtration rate, statistically significant values were represented on bold.

3.1.1. Graft Survival by Donor eGFR Pre-Donation

The overall GS rates in the group of recipients of transplants from LDs with higher vs. lower eGFRs ($\geq$ vs. <90 mL/min/1.73 m²) at 5, 10, and 15 years were, respectively, 98/97%, 86/72%, and 67/46% ($p = 0.013$). The censored GS rates in the group of recipients of transplants from LDs with higher vs. lower eGFR ($\geq$ vs. <90 mL/min/1.73 m²) at 5, 10, and 15 years were, respectively, 98/97%, 90/72%, and 75/46% ($p = 0.0004$). For both outcomes, the in-between groups' differences were observed at a later stage (>5 years) of the follow-up (Figure 1).

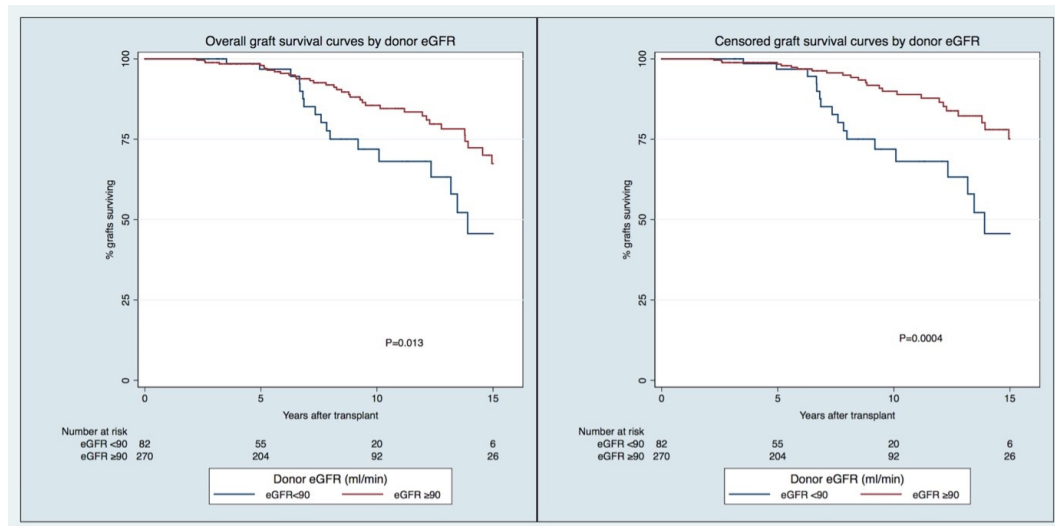


Figure 1. Overall (right) and censored (left) graft survival curves by donor eGFR pre-donation.

3.1.2. Independent Predictors of Graft Failure

Independent predictors of overall and censored GF, according to the recipient and KT variables, are shown in Tables 3 and 4, respectively. Acute rejection and retransplant were significant predictors of both overall and censored GF. In contrast, recipient age, mismatch in DR, and CGN as the cause of CKD were only significant for censored GF.

Table 3. Predictors of overall graft failure included if $p < 0.157$ in a stepwise backward selection (variables from the recipient/kidney transplant).

	HR	95% CI	<i>p</i>
Acute rejection	3.115	1.834–5.991	<0.001
Female recipient	1.584	0.898–2.793	0.112
Retransplant	2.672	1.483–4.816	0.001

HR—hazard rate; CI—confidence interval.

Table 4. Predictors of censored graft failure included if $p < 0.157$ in a stepwise backward selection (variables from the recipient/kidney transplant).

	HR	95% CI	<i>p</i>
Acute rejection	2.095	1.017–4.314	0.045
Retransplant	3.385	1.718–6.670	<0.001
Recipient age	0.943	0.911–0.976	0.001
MM DR	1.631	0.913–2.912	0.098
CGN etiology	2.041	1.004–4.147	0.049

HR—hazard rate; CI—confidence interval; MM—mismatch; CGN—chronic glomerulonephritis.

Only donor age ≥ 50 years and pre-donation eGFR < 90 mL/min/ 1.73 m^2 were identified as donor-derived independent predictors of overall or censored GF in the multivariable Cox models (Tables 5 and 6). Remarkably, none of the remaining donor factors which were evaluated (hypertension, dyslipidemia, smoking, proteinuria, and obesity) were independent predictors for the outcomes of interest.

Table 5. Predictors of overall graft failure; multivariable Cox model.

	HR	95% CI	p
Pre-donation eGFR < 90 mL/min/1.73 m ²	1.893	1.030–3.478	0.040
Donor age ≥ 50 years	1.730	0.947–3.159	0.075
Hypertension	0.523	0.200–1.370	0.187
Dyslipidemia	0.653	0.233–1.825	0.416
Smoking habits	1.159	0.452–2.971	0.759
Donor BMI ≥ 30 kg/m ²	2.087	0.774–5.626	0.146
Proteinuria 0.15–0.5 g/24 h	1.551	0.846–2.843	0.156
Female donor	1.227	0.594–2.538	0.581
Acute rejection	3.316	1.826–6.020	<0.001
Female recipient	1.662	0.935–2.956	0.084
Retransplant	2.643	1.453–4.810	0.001

HR—hazard rate; CI—confidence interval; eGFR—estimated glomerular filtration rate; BMI—body mass index.

Table 6. Predictors of censored graft failure; multivariable Cox model.

	HR	95% CI	p
Pre-donation eGFR < 90 mL/min 1.73 m ²	2.378	1.224–4.622	0.011
Donor age ≥ 50 years	2.108	1.043–4.260	0.038
Hypertension	1.081	0.385–3.034	0.883
Dyslipidemia	0.658	0.218–1.985	0.457
Smoking habits	2.093	0.682–6.424	0.197
Donor BMI ≥ 30 kg/m ²	2.103	0.750–5.894	0.158
Proteinuria 0.15–0.5 g/24 h	1.250	0.595–2.626	0.556
Female donor	1.400	0.597–3.288	0.439
Acute rejection	2.137	1.109–4.479	0.044
Retransplant	3.458	1.715–6.975	0.001
Recipient age	0.945	0.911–0.980	0.002
MM DR	1.538	0.847–2.795	0.158
CGN etiology	2.529	1.202–5.323	0.015

HR—hazard rate; CI—confidence interval; eGFR—estimated glomerular filtration rate; BMI: body mass index; MM DR: mismatch in DR; CGN—chronic glomerulonephritis.

3.1.3. Interaction of Pre-Donation eGFR × Donor Age

The interaction of LDs' pre-donation eGFRs and ages was studied for both models (Tables 7 and 8).

Table 7. Interaction between donor age and eGFR categories for the prediction of overall graft failure: multivariate Cox model (adjusted to the same variables as Table 5).

Multivariate Cox Model P Interaction = 0.317	HR	95% CI	p
eGFR < 90 vs. ≥90 and donor age < 50 years	2.578	1.120–5.795	0.026
GFR < 90 vs. ≥90 and donor age ≥ 50 years	1.328	0.548–3.405	0.555

HR—hazard rate; CI—confidence interval eGFR—estimated glomerular filtration rate. Units: mL/min/1.73 m².**Table 8.** Interaction between donor age and eGFR categories for the prediction of censored graft failure: multivariate Cox model (adjusted to the same variables as Table 6).

Multivariate Cox Model P Interaction = 0.349	HR	95% CI	p
eGFR < 90 vs. ≥90 and donor age < 50 years	3.216	1.300–7.959	0.012
GFR < 90 vs. ≥90 and donor age ≥ 50 years	1.667	0.607–4.582	0.322

eGFR—estimated glomerular filtration Rate. Units: mL/min/1.73 m².

For the overall GF model, recipients of transplants from LDs < 50 years of age and those with eGFRs < 90 mL/min/1.73 m² had the highest risk of overall GF, with an adjusted HR [95% CI] of 2.578 [1.120–5.795], compared to recipients of transplants from LDs < 50 years old and those with eGFRs ≥ 90 mL/min/1.73 m². For the recipients of transplants from older donors (age ≥ 50), no difference in the risk of GF was observed between the eGFR categories.

Similarly, in the death-censored GF model, in younger donors (age < 50), recipients of transplants from LDs with eGFRs < 90 mL/min/1.73 m² experienced the greatest risk of censored GF, with an adjusted HR [95%CI] of 3.216 [1.300–7.959], compared to recipients of transplants from LDs with eGFRs ≥ 90 mL/min/1.73 m². In contrast, when the donors were older (age ≥ 50), no difference in the risk of GF was observed between the eGFR categories.

The interaction held when, in the univariate Cox model, the patients with acute rejection were excluded (Supplemental Materials). The multivariate models were adjusted for acute rejection (Tables 7 and 8).

3.1.4. The Longitudinal Pattern of Graft Function by LD eGFR and Age

The longitudinal pattern of graft function by LD eGFR pre-donation and age (Figure 2 and Supplemental Table S4) has displayed, since the first years after KT in recipients from younger LDs (age < 50), that significant differences exist between those with lower and higher eGFRs (eGFR < 90 vs. ≥ 90 mL/min/1.73 m²) pre-donation. In comparison, these differences do not hold in recipients from older LDs. The differences in prognoses in the recipients of transplants from LDs < 50 years of age with different eGFR categories are foreseeable early post-KT, and not just after five years, when there were different GS rates between groups (Figure 3).

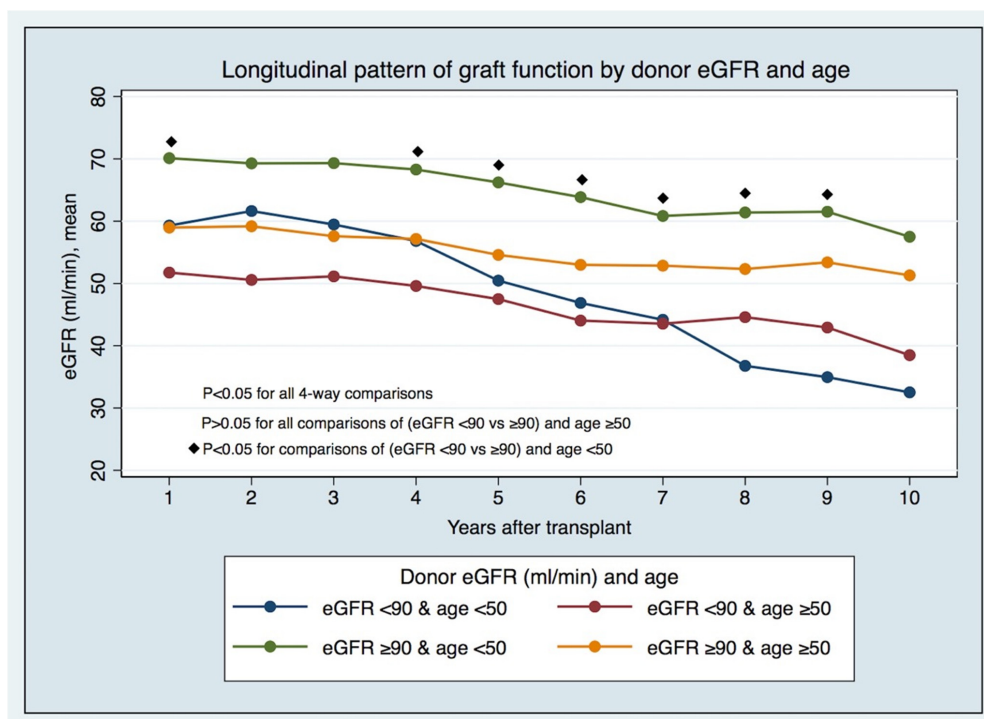


Figure 2. Longitudinal pattern of graft function by donor eGFR and age.

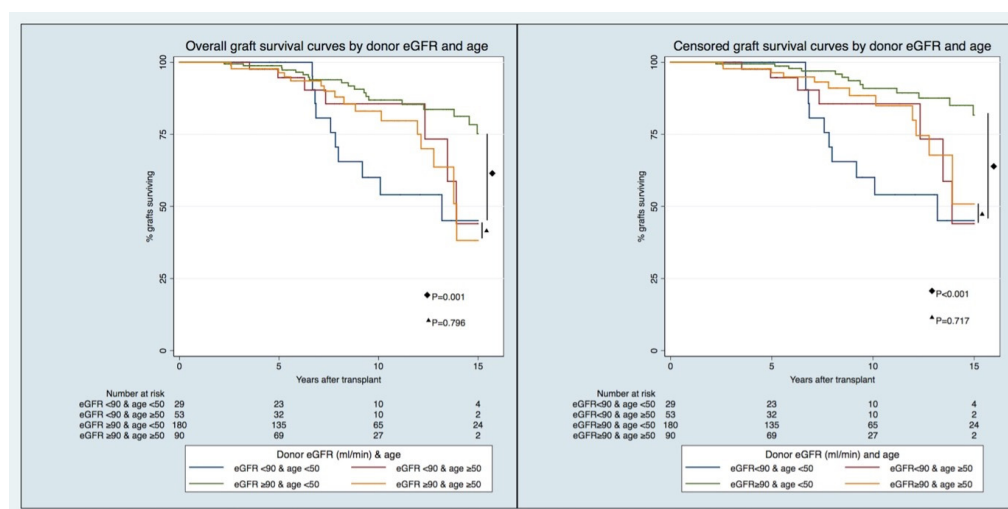


Figure 3. Overall (right) and censored (left) graft survival curves by donor eGFR and age.

4. Discussion

In this cohort of LDKT recipients, recipients from LDs with higher pre-donation eGFRs (≥ 90 mL/min/1.73 m²) had higher overall and censored GS rates compared to the recipients of those LDs with lower eGFRs (< 90 mL/min/1.73 m²). Nevertheless, when the interaction between LD age and pre-donation eGFR was studied, recipients of transplants from younger LDs (< 50) with lower eGFRs pre-donation experienced the highest risk of overall (adjusted HR [95% CI] 2.578 [1.120–5.795]) and censored GF (adjusted HR [95% CI] 3.216 [1.300–7.959]) compared to those with higher eGFRs pre-donation. Otherwise, when donors were older (≥ 50), no difference in the risk of GF was observed between eGFR categories. By further evaluating the longitudinal pattern of eGFR in the recipients, these differences between groups became evident from the first year after KT. No other analyzed factors (hypertension, obesity, proteinuria, or smoking habits) significantly influenced recipient outcomes in this cohort in either the univariate or multivariate analyses.

Lim et al. [21], in an analysis of 11,095 recipients of deceased donors and LDs, described an increased risk of overall GF in recipients with eGFRs < 30 mL/min/1.73 m² vs. > 60 mL/min/1.73 m² at 12 months after KT, and the magnitude of the increased risk was higher among recipients from younger donors. In contrast, the donor type did not affect the risk, suggesting a possible donor-intrinsic pathology [21]. Our study showed a different relationship between LDs' pre-donation eGFRs and graft outcomes modulated by LD age. The effect of pre-donation eGFR ($<$ vs. ≥ 90 mL/min/1.73 m²) was different according to the LD subgroup age (< 50 vs. ≥ 50), and this association has not been described yet. Our results suggest that the range of eGFR thresholds pursued for LD should be adjusted to their age.

The age cutoff for “old” LDs remains unsolved. If the Japanese Transplantation Committee were to define standard LDs as those aged up to 70 years [22], other guidelines would use thresholds such as 45 [23], 50 [24], 55 [25], and 60 years [26]. We used 50 years as an exploratory cutoff based on the statistical approach mentioned above. The reported outcomes also differ. Some studies have described inferior graft and patient survival [27], death-censored graft survival [28], and higher AR [27], or no significant difference [29,30], among the recipients of kidneys from “older” LDs. In their metaanalysis, Bellini et al. refer to high heterogeneity between studies [9].

Our results are different from previous publications. Although, as expected, our donors' ages impacted our recipients' outcomes, when an interaction with pre-donation eGFR was studied, a dissimilarity in outcomes driven by pre-donation renal function between younger and older LDs became evident. This interaction held true when the impact of AR was further analyzed in a univariate Cox model excluding patients who experienced AR. AR was a variable that was accounted for in the multivariate Cox model. This may result from different pathophysiologic statuses being measured as pre-donation eGFR.

The evaluation of renal function is critical in LD evaluation. It is used to screen for kidney disease and aid in predicting graft function and long-term kidney failure risk after donation. The measurement of GFR using an "ideal" filtration marker is unfeasible in clinical practice; determining eGFR from serum levels of endogenous filtration markers, such as creatinine [16], is the most widely used approach, and it is recommended by the current guidelines [16,17]. The KDIGO guidelines consider an eGFR of ≥ 90 mL/min/1.73 m² acceptable for donation, and an eGFR $60 < \text{mL/min/1.73 m}^2$ is considered a contraindication for donation [16]. Our results remind us to focus on younger LDs, in whom we should be alert to lower, yet almost normal, pre-donation eGFRs, as these could translate into unrecognized pathologies.

Based on the KDIGO guidelines [31], the current definition of CKD includes the diminution of GFR below 60 mL/min/1.73 m². This threshold does not consider the physiologic decline of GRF with healthy aging. The available evidence suggests that the GFR threshold below which the risk of mortality is increased is different across ages, from 75 mL/min/1.73 m² in younger persons to values as low as 45 mL/min/1.73 m² in elderly people [32]. Structural differences are identified between aging kidneys and CKD. In aging kidneys, substantial nephron loss is expected, albeit with no compensation by the remaining nephrons. In CKD, beyond disease-specific pathology, it is expected that associated glomerular enlargement, segmental glomerulosclerosis, and higher single-nephron GFR in intact nonsclerotic glomeruli will occur as adaptive changes [32]. The fixed GFR threshold may result in the overdiagnosis of CKD in older adults, but it may also lead to missed diagnoses of CKD in younger individuals without obvious signs of kidney damage and who have a GFR well above that threshold. This group may include young people with low nephron reserves, those born pre-term, or those with other unrecognized risk factors who may have a risk of developing CKD and associated comorbidities [32]. Some of our younger LDs with lower eGFR could be part of this group.

Some transplant centers perform routine implantation biopsies. Structural findings in the kidney could provide insight into the pathology of early CKD and help us to understand our findings. Still, this practice is not universal; it has some risks, and the biopsies are performed after donor selection. A study by Elsherbiny et al. [33] evaluated 1395 LDKT implantation biopsies of donated kidneys. It concluded that kidney function and CKD risk factors had similar associations with different morphologic measures of nephron hypertrophy, but different associations with glomerulosclerosis [33]. The authors suggested that different biologic pathways may contribute to the risk of CKD [33]. Exploring biomarkers and metabolic pathways beyond serum creatinine and eGFR that could help us to understand and predict the strength of these correlations before donation, thus helping in terms of accepting or discarding some donors, are currently major challenges of LDKT programs.

The available evidence regarding the outcomes of grafts with lower baseline function is scarce. Norden et al. [34] reported that a donor with low GFR was associated with a higher graft loss risk, establishing a lower acceptance limit of 80 mL/min in many transplant centers. Young et al. [35], in an analysis of 2057 LDKT pairs, reported no differences in the adjusted hazard ratios for GF amongst recipients of LD kidneys with eGFRs ≥ 110 mL/min/1.73 m² vs. < 80 mL/min/1.73 m². In our cohort, GS was significantly higher in the group of recipients of LD with higher pre-donation eGFRs (eGFR ≥ 90 mL/min/1.73 m²) after the first five years. Nevertheless, when the interaction between LD age and pre-donation eGFR was studied, this held only for recipients of transplants from younger LDs (< 50 years).

We did not find a significant effect on graft outcomes for the other factors studied (hypertension, dyslipidemia, smoking, proteinuria, and obesity), or for the association of factors, although their impact has been described in other cohorts [9,23,36]. Remarkably, in the “problem” group (age < 50 and eGFR < 90 mL/min/1.73 m²), LDs had significantly more diagnoses of hypertension (21 vs. 6%, $p = 0.004$) vs. those in the same age group = with higher eGFRs, which could also indicate unrecognized kidney disease. Their recipients had significantly more frequent coronary artery disease, but they had more GF, not more deaths. Hypertension is the strongest cardiovascular risk factor worldwide, and is also significantly associated with CKD [37]. Conversely, there are limited data on the association between LD hypertension and recipient outcomes. Both a significant impact [23] and no impact [38] in the recipients of hypertensive LD kidneys after KT have been described. Hypertensive donors with well-controlled hypertension, without evidence of end organ damage or metabolic syndrome, were accepted after a comprehensive evaluation and informed consent in our cohort, according to published guidelines [16,17]. We did not find significant effects of LD hypertension on graft outcomes as other studies did [38]. We can hypothesize that LDs are otherwise healthy people without evidence of end-organ damage, and only selected hypertensive subjects were effective donors.

Obesity is a worldwide pandemic. It is associated with structural, hemodynamic, and metabolic alterations in the kidneys. Despite this, several studies, including ours, have failed to find significance in the immediate and short-term post-transplant recipient outcomes between normal-weight and overweight and obese donors [36]. We must note that we only accepted those obese donors with BMIs near 30 who showed the ability to lose weight and maintain a lower weight before donating. Other cardiovascular risk factors, such as concomitant dyslipidemia, smoking habits, and impaired glucose balance, can influence the decision to accept an LD.

Often, potential donors exhibit several medical complexities. Strong outcome data are not available. In clinical practice, we perform a global risk assessment considering the global risk for the donor, including the expected lifetime exposure to the risk and the adequacy of the graft for the recipient. Theoretically, the more medically complex a donor is, the more likely the donor or the corresponding recipient is to have an inferior outcome. We were unable to demonstrate this in our cohort analysis.

Finally, we should emphasize the implications of our study results. Recipients from LDs with lower eGFRs (<90 mL/min/1.73 m²) had worse outcomes, but this effect was modulated by the LDs’ ages, and was only significant in recipients from younger donors (<50 y). This effect was evident from the first year after KT onward in the evolution of kidney function. These results agree with the current guidelines for LD evaluation [16,17], which recommend a comprehensive approach to risk assessment in order to replace decisions based on assessments of single risk factors in isolation. Although these recommendations are centered on donor safety, the same strategy should be followed in the recipient pair selection process to optimize the results of the procedure.

Our study has several limitations. First, donors were evaluated retrospectively, and unobserved confounders may have introduced bias. Second, our cohort consisted only of Caucasian patients, limiting the generality of our results. In addition, using eGFR to assess kidney function by means of estimation equations has limitations, but it is the common practice in most transplant centers and agrees with the International Guidelines [16,17]. The defined cohorts were not wholly balanced considering the preoperative variables, as depicted in Tables 1 and 2. A multivariable analysis was performed, considering transplant, recipient, and donor factors, to overcome this limitation. In addition, added strengths of our study cohort are its larger size and reasonable length of follow-up. Considering two time periods can partially offset some heterogeneity about immunosuppressive protocols during those periods. Nevertheless, longer follow-up studies must be required; prospective studies are necessary to allow for a cause–effect analysis of the studied parameters. We recognize that the most important issue in LDKT is assuring LDs’ safety, but this goes beyond the scope of this article and deserves a separate analysis. Additionally, we did not seek to

compare the effects of declining a “complex” living donor and causing a patient to remain on the waiting list, but rather sought to better characterize that phenotype.

5. Conclusions

In our cohort, pre-donation eGFR was associated with post-transplant outcomes, but the donor’s age significantly interacted with this effect. In younger donors only, an eGFR < 90 vs. ≥ 90 mL/min/1.73 m² had a detrimental impact on graft survival, drawing attention to the need for an age-adjusted eGFR cutoff for donor admissibility. The dissimilarity in outcomes driven by pre-donation renal function between younger and older donors may result from different pathophysiologic statuses being measured as eGFR pre-donation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jcm12216777/s1>, Table S1. Overall and censored graft and survival by pre-donation LD eGFR and Age, Table S2. Interaction between donor age and eGFR categories for the prediction of overall graft failure, Table S3. Interaction between donor age and eGFR categories for the prediction of censored graft failure, Table S4. Longitudinal pattern of graft function by graft eGFR and age.

Author Contributions: M.A., C.R. and J.M.: research design, data acquisition, data analysis, and paper writing. J.S., S.P. and S.T. were engaged in the data acquisition and analysis. M.R. and L.S.M. were involved in the research design and data analysis. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, as well as with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The institutional review board at our institution approved this study (Ref.: 147-21 (119-DEFI/122-CE)).

Data Availability Statement: The data underlying this article will be shared upon reasonable request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kovesdy, C.P. Epidemiology of chronic kidney disease: An update 2022. *Kidney Int. Suppl.* **2022**, *12*, 7–11. [\[CrossRef\]](#)
2. Gupta, R.; Woo, K.; Yi, J.A. Epidemiology of end-stage kidney disease. *Semin. Vasc. Surg.* **2021**, *34*, 71–78. [\[CrossRef\]](#)
3. Wolfe, R.A.; Ashby, V.B.; Milford, E.L.; Ojo, A.O.; Ettenger, R.E.; Agodoa, L.Y.C.; Held, P.J.; Port, F.K. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N. Engl. J. Med.* **1999**, *341*, 1725–1730. [\[CrossRef\]](#)
4. McCormick, F.; Held, P.J.; Chertow, G.M. The Terrible Toll of the Kidney Shortage. *J. Am. Soc. Nephrol.* **2018**, *29*, 2775–2776. [\[CrossRef\]](#) [\[PubMed\]](#)
5. IPST. Atividade Nacional Anual. 2021. Available online: https://www.ipst.pt/files/TRANSPLANTACAO/DOACAOETRANSPLANTACAO/Dados_Anuais_Atividade_Doacao_Transplantacao2021_versao_integral_para_publicacao.pdf (accessed on 10 October 2023).
6. GODAT. International Report on Organ Donation and Transplantation. 2021. Available online: <https://www.transplant-observatory.org/2021-global-report-5/> (accessed on 10 October 2023).
7. Reese, P.P.; Boudville, N.; Garg, A.X. Living kidney donation: Outcomes, ethics, and uncertainty. *Lancet* **2015**, *385*, 2003–2013. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Meier-Kriesche, H.U.; Kaplan, B. Waiting time on dialysis as the strongest modifiable risk factor for renal transplant outcomes: A paired donor kidney analysis. *Transplantation* **2002**, *74*, 1377–1381. [\[CrossRef\]](#) [\[PubMed\]](#)

9. Bellini, M.I.; Nozdrin, M.; Pengel, L.; Knight, S.; Papalois, V. How good is a living donor? Systematic review and meta-analysis of the effect of donor demographics on post kidney transplant outcomes. *J. Nephrol.* **2022**, *35*, 807–820. [\[CrossRef\]](#)
10. Reese, P.P.; Caplan, A.L.; Kesselheim, A.S.; Bloom, R.D. Creating a medical, ethical, and legal framework for complex living kidney donors. *Clin. J. Am. Soc. Nephrol.* **2006**, *1*, 1148–1153. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Reese, P.P.; Feldman, H.I.; McBride, M.A.; Anderson, K.; Asch, D.A.; Bloom, R.D. Substantial variation in the acceptance of medically complex live kidney donors across US renal transplant centers. *Am. J. Transplant.* **2008**, *8*, 2062–2070. [\[CrossRef\]](#)
12. Arabi, Z.; Hamad, A.; Bukhari, M.; Altheaby, A.; Kaysi, S. Practice Patterns for the Acceptance of Medically Complex Living Kidney Donors with Hematuria, Sick Cell Trait, Smoking, Illegal Drug Use, or Urological Issues: A Multinational Survey. *Avicenna J. Med.* **2021**, *11*, 185–195. [\[CrossRef\]](#)
13. Muzaale, A.D.; Massie, A.B.; Wang, M.-C.; Montgomery, R.A.; McBride, M.A.; Wainright, J.L.; Segev, D.L. Risk of end-stage renal disease following live kidney donation. *JAMA* **2014**, *311*, 579–586. [\[CrossRef\]](#)
14. Mjoen, G.; Hallan, S.; Hartmann, A.; Foss, A.; Midtvedt, K.; Øyen, O.; Reisaeter, A.; Pfeffer, P.; Jenssen, T.; Leivestad, T.; et al. Long-term risks for kidney donors. *Kidney Int.* **2014**, *86*, 162–167. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Hiramitsu, T.; Tomosugi, T.; Futamura, K.; Okada, M.; Tsujita, M.; Goto, N.; Ichimori, T.; Narumi, S.; Takeda, A.; Watarai, Y. Preoperative Comorbidities and Outcomes of Medically Complex Living Kidney Donors. *Kidney Int. Rep.* **2020**, *5*, 13–27. [\[CrossRef\]](#)
16. Lentine, K.L.; Kasiske, B.L.; Levey, A.S.; Adams, P.L.; Alberú, J.; Bakr, M.A.; Gallon, L.; Garvey, C.A.; Guleria, S.; Li, P.K.T.; et al. Summary of Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* **2017**, *101*, 1783–1792. [\[CrossRef\]](#)
17. Andrews, P.A.; Burnapp, L. British Transplantation Society/Renal Association UK Guidelines for Living Donor Kidney Transplantation 2018: Summary of Updated Guidance. *Transplantation* **2018**, *102*, e307. [\[CrossRef\]](#)
18. Levey, A.S.; Stevens, L.A.; Schmid, C.H.; Zhang, Y.L.; Castro, A.F., III; Feldman, H.I.; Kusek, J.W.; Eggers, P.; Van Lente, F.; Greene, T.; et al. A new equation to estimate glomerular filtration rate. *Ann. Intern. Med.* **2009**, *150*, 604–612. [\[CrossRef\]](#)
19. Silva, F.; Malheiro, J.; Pestana, N.; Ribeiro, C.; Nunes-Carneiro, D.; Mandanelo, M.; Tavares, J.; Pedrosa, S.; Almeida, M.; Silva-Ramos, M.; et al. Lower donated kidney volume is associated with increased risk of lower graft function and acute rejection at 1 year after living donor kidney—A retrospective study. *Transpl. Int.* **2020**, *33*, 1711–1722. [\[CrossRef\]](#)
20. Heinze, G.; Dunkler, D. Five myths about variable selection. *Transpl. Int.* **2017**, *30*, 6–10. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Lim, W.H.; Ooi, E.; Pilmore, H.L.; Johnson, D.W.; McDonald, S.P.; Clayton, P.; Hawley, C.; Mulley, W.R.; Francis, R.; Collins, M.G.; et al. Interactions Between Donor Age and 12-Month Estimated Glomerular Filtration Rate on Allograft and Patient Outcomes after Kidney Transplantation. *Transpl. Int.* **2022**, *35*, 10199. [\[CrossRef\]](#)
22. Kinoshita, Y.; Yagisawa, T.; Sugihara, T.; Hara, K.; Takeshima, S.; Kubo, T.; Shinzato, T.; Shimizu, T.; Suzuki, M.; Maeshima, A.; et al. Clinical outcomes in donors and recipients of kidney transplantations involving medically complex living donors—A retrospective study. *Transpl. Int.* **2020**, *33*, 1417–1423. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Issa, N.; Stephany, B.; Fatica, R.; Nurko, S.; Krishnamurthi, V.; Goldfarb, D.A.; Braun, W.E.; Dennis, V.W.; Heeger, P.S.; Poggio, E.D. Donor factors influencing graft outcomes in live donor kidney transplantation. *Transplantation* **2007**, *83*, 593–599. [\[CrossRef\]](#)
24. Busson, M.; Benoit, G. Is matching for sex and age beneficial to kidney graft survival? Societe Francaise de Transplantation and Association France Transplant. *Clin. Transpl.* **1997**, *11*, 15–18.
25. Gill, J.; Bunnapradist, S.; Danovitch, G.M.; Gjertson, D.; Gill, J.S.; Cecka, M. Outcomes of kidney transplantation from older living donors to older recipients. *Am. J. Kidney Dis.* **2008**, *52*, 541–552. [\[CrossRef\]](#)
26. Fuggle, S.V.; Allen, J.E.; Johnson, R.J.; Collett, D.; Mason, P.D.; Dudley, C.; Rudge, C.J.; Bradley, J.A.; Watson, C.J.; Kidney Advisory Group of NHS Blood and Transplant. Factors affecting graft and patient survival after live donor kidney transplantation in the UK. *Transplantation* **2010**, *89*, 694–701. [\[PubMed\]](#)
27. Rizzari, M.D.; Suszynski, T.M.; Gillingham, K.J.; Matas, A.J. Consideration of donor age and human leukocyte antigen matching in the setting of multiple potential living kidney donors. *Transplantation* **2011**, *92*, 70–75. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Noppakun, K.; Cosio, F.G.; Dean, P.G.; Taler, S.J.; Wauters, R.; Grande, J.P. Living donor age and kidney transplant outcomes. *Am. J. Transpl.* **2011**, *11*, 1279–1286. [\[CrossRef\]](#)
29. Balachandran, V.P.; Aull, M.J.; Charlton, M.; Afaneh, C.; Serur, D.; Leeser, D.B.; Del Pizzo, J.; Kapur, S. Kidneys from older living donors provide excellent intermediate-term outcomes after transplantation. *Transplantation* **2012**, *94*, 499–505. [\[CrossRef\]](#)
30. Ferrari, P.; Lim, W.; Dent, H.; McDonald, S.P. Effect of donor-recipient age difference on graft function and survival in live-donor kidney transplantation. *Nephrol. Dial. Transpl.* **2011**, *26*, 702–708. [\[CrossRef\]](#)
31. Levin, A.; Stevens, P.E. Summary of KDIGO 2012 CKD Guideline: Behind the scenes, need for guidance, and a framework for moving forward. *Kidney Int.* **2014**, *85*, 49–61. [\[CrossRef\]](#)
32. Delanay, P.; Jager, K.J.; Bökenkamp, A.; Christensson, A.; Dubourg, L.; Eriksen, B.O.; Gaillard, F.; Gambaro, G.; van der Giet, M.; Glasscock, R.J.; et al. CKD: A Call for an Age-Adapted Definition. *J. Am. Soc. Nephrol.* **2019**, *30*, 1785–1805. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Elsherbiny, H.E.; Alexander, M.P.; Kremers, W.K.; Park, W.D.; Poggio, E.D.; Prieto, M.; Lieske, J.C.; Rule, A.D. Nephron hypertrophy and glomerulosclerosis and their association with kidney function and risk factors among living kidney donors. *Clin. J. Am. Soc. Nephrol.* **2014**, *9*, 1892–1902. [\[CrossRef\]](#)
34. Norden, G.; Lennerling, A.; Nyberg, G. Low absolute glomerular filtration rate in the living kidney donor: A risk factor for graft loss. *Transplantation* **2000**, *70*, 1360–1362. [\[CrossRef\]](#) [\[PubMed\]](#)

35. Young, A.; Kim, S.J.; Garg, A.X.; Huang, A.; Knoll, G.; Treleaven, D.; Lok, C.E.; Arnold, J.; Boudville, N.; Bugeya, A.; et al. Living kidney donor estimated glomerular filtration rate and recipient graft survival. *Nephrol. Dial. Transpl.* **2014**, *29*, 188–195. [[CrossRef](#)] [[PubMed](#)]
36. Mohamed, M.M.; Daoud, A.; Quadri, S.; Casey, M.J.; Salas, M.A.P.; Rao, V.; Fülöp, T.; Soliman, K.M. Hypertension and obesity in living kidney donors. *World J. Transpl.* **2021**, *11*, 180–186. [[CrossRef](#)] [[PubMed](#)]
37. Kjeldsen, S.E. Hypertension and cardiovascular risk: General aspects. *Pharmacol. Res.* **2018**, *129*, 95–99. [[CrossRef](#)]
38. Iordanous, Y.; Seymour, N.; Young, A.; Johnson, J.; Iansavichus, A.V.; Cuerden, M.S.; Gill, J.S.; Poggio, E.; Garg, A.X.; Iordanous, Y.; et al. Recipient outcomes for expanded criteria living kidney donors: The disconnect between current evidence and practice. *Am. J. Transpl.* **2009**, *9*, 1558–1573. [[CrossRef](#)]

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APPENDIX 11 – Biologically Unrelated Living Donor for Kidney Transplantation Associated with Higher Acute Rejection Rate but With Similar Graft Survival of a Related Living Donor.

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Biologically Unrelated Living Donor for Kidney Transplantation Associated With Higher Acute Rejection Rate but With Similar Graft Survival of a Related Living Donor

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Abstract

Introduction: Kidney transplantation (KT) from living donors has been shown to have multiple benefits compared to those from deceased donors. We sought to compare significant graft outcomes, namely acute rejection (AR), graft function, and survival between transplant recipients who received a kidney from living related donor (LRD) and living unrelated donor (LURD).

Methods: Our cohort comprised 198 donor and recipient pairs undergoing living-donor KT at our center over 10 years. The LRD recipients were compared with LURD recipients according to demographic and clinical characteristics, transplant variables (including immunosuppression), graft function, survival, and AR rate.

Results: The estimated glomerular filtration rate (eGFR) was similar in both groups over the follow-up time i.e., 60-65 mL/min ($p > 0.05$ over 10 years). Censored graft survival was similar between LRD and LURD recipients (96.9% vs. 98.0% at five years and 87.8% vs. 79.4% at 10 years, respectively; $p = 0.837$). The LURD recipients had a higher incidence of AR, although LURD recipient status was not an independent risk factor for AR. Multivariate analysis showed that human leukocyte antigen (HLA)-DR mismatch (MM) was an independent predictor of AR (hazard ratio (HR) 2.256, $p < 0.05$). Both HLA-A and HLA-B MM did not affect the AR HR between the groups.

Conclusion: Graft function and censored graft survival rates were similar between LURD and LRD KT recipients in our study. The AR was higher in LURD recipients, although the LURD recipient status was not an independent risk factor for AR. The HLA-DR MM was an independent predictor of AR, while HLA-A and HLA-B MM did not affect AR HR between groups of patients.

Categories: Allergy/Immunology, Nephrology, Transplantation

Keywords: living-unrelated donor, immunosuppression, acute rejection, graft survival, kidney transplant, living-related donor

Introduction

Kidney transplantation (KT) has been shown to improve survival and long-term outcomes in patients with end-stage kidney failure [1]. Over the last decades, the number of patients on the KT waitlist has been steadily increasing [2]. In the setting of organ scarcity, living KT allows for an increase in the donor pool and reduces waiting time for KT [3].

Living donors can be classified as living related donors (LRD) or as living unrelated donors (LURD). An LRD is defined as being genetically related to the transplant recipient, such as parents, siblings, or children. A LURD, on the other hand, is not genetically related to the transplant recipient. However, they could be someone with whom the recipient has an emotional connection, such as a spouse or a friend, or an unacquainted person, such as an altruistic donor or a donor from a kidney paired exchange (KPE) program. With policies and legislative issues varying between countries, some countries do not allow LURD KT or KPE programs. In Portugal, legislation allowing genetically unrelated transplantation was passed in 2007, and is based on evidence showing that transplants from unrelated living donors too, have better outcomes compared with transplants from deceased donors [4].

Several studies have focused on comparing the outcomes between LRD and LURD transplant

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recipients. Most studies have shown similar graft survival between recipients of these two types of living donation [5-11], while some studies have shown a better survival of LRD recipients compared to LURD recipients [12,13]. A recent study with 14,370 patients reported similar patient and overall graft survival in LRD and LURD recipients, while a higher death-censored graft failure in LURD recipients was noticeable [14]. Moreover, some studies have reported higher rates of vascular rejection in LURD recipients [10,15], while others observed similar rates of acute rejection (AR) between both types of living KT [8]. Incidence of chronic allograft nephropathy has also been shown not to be different between LRD and LURD recipients, as well as rates of other post-transplant complications [15].

In this study we aimed to compare graft function and survival, as well as rates of AR in transplant recipients from LRD and LURD, evaluating the first 10 years of our center's experience after the introduction of LURD KT in Portugal.

Materials And Methods

Study population

We retrospectively reviewed the clinical data of adult donor and recipient pairs undergoing living donor KT (LDKT) at our institution between January 2008 and December 2017 (n=210). After the exclusion of seven recipients who had been submitted to ABO-incompatible KT, and of five (2.5%) patients who had a primary loss of KT, the remaining 198 recipients defined our study cohort. Of these primary non-function cases, four (1.9%) were LRD recipients and one (0.5%) was a LURD recipient (p=0.650).

Baseline data and graft outcomes

Baseline demographic, anthropomorphic, analytical, and clinical data were collected from both recipients and donors. Transplant data were also analyzed. Human leukocyte antigen (HLA)-incompatible KT refers to cases in which transplants are performed in the presence of preformed donor-specific antibodies (DSA). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to predict the estimated glomerular filtration rate (eGFR). Delayed graft function (DGF) was defined as the need for dialysis in the first week after transplantation. Graft biopsies were performed for indication. Each recipient was followed up until the end of June 2019, the date of death, graft loss, or loss during follow-up. The study protocol was reviewed and approved by the institutional ethical review and hospital administration boards (approval no. 147/21 [119-DEFI/122-Ce]) as per the recommendations of the Declaration of Helsinki and European Data Protection Regulations.

Immunosuppression (IS) and desensitization protocols

Induction therapy was used in most patients, with an anti-interleukin (IL)-2 receptor monoclonal antibody (basiliximab (Novartis), 20 mg twice on days 0 and 4) or polyclonal anti-thymocyte globulin (ATG) (Fresenius, 3 mg/kg for five to seven days). The ATG was primarily used in HLA-incompatible KT and retransplants. All enrolled recipients had similar triple maintenance immunosuppression consisting of oral tacrolimus, mycophenolate mofetil (MMF), and methylprednisolone (MP)/prednisolone. Further details of our regimen have already been published [16].

The HLA-incompatible KT received desensitization with intravenous immunoglobulin (2 g/kg) at transplant (0.5 g/kg immediately before transplant, and on days 1, 2, and 3) and one month after transplantation (1 g/kg in two consecutive days), and a dose of rituximab (375 mg/ m²) on day 3 post-transplant. Given the strength of preformed anti-HLA DSA and flow cytometry crossmatch results, six patients also underwent plasmapheresis every other day (the first session three days before the transplant, for a total of six to nine sessions) [16].

Statistical analysis

Continuous data are described as mean $\pm$ standard deviation (SD) or median (interquartile range (IQR)), and categorical data are expressed as numbers and percentages. Categorical data were compared using Pearson's chi-square test or Fisher's exact test, and continuous variables were compared using Student's t-test or Mann-Whitney U-test, as appropriate.

The AR and graft survival curves were visualized using the Kaplan-Meier method. Comparisons between patient groups were performed using the log-rank test. In cases of death with a functioning graft, the time was censored at the time of death. Potential predictors of AR and graft failure were explored using univariate and multivariate Cox proportional hazard models. In all multivariable models, independent predictors were identified using a backward elimination method, with a P-value < 0.05 necessary for retention in the model.

A two-sided P-value of < 0.05 was considered statistically significant. Statistical calculations were performed using Stata that can perform symmetric multiprocessing (STATA/MP), version 15.1 (Stata Corp, College Station, TX, USA).

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Results

Characteristics of patients

Our study cohort comprised 198 recipients; 59% (n=116) had LRD KT, and 41% (n=82) had LURD KT. The main group characteristics of living donor pairs and transplants based on LRD and LURD are shown in Table 1.

	Total N=198	LRD N=116 (59%)	LURD N=82 (41%)	P-value
Recipient				
Age of R, mean $\pm$ SD	41.1 $\pm$ 13.2	35.9 $\pm$ 12.2	48.5 $\pm$ 10.9	<0.001
Sex of R (F), n (%)	56 (28)	36 (31)	20 (24)	0.307
BMI of R, mean $\pm$ SD	23.9 $\pm$ 3.9	23.2 $\pm$ 3.9	24.9 $\pm$ 3.9	0.004
Time on dialysis before KT (months), median (IQR)	13.9 (0-30.3)	12.6 (0-27.2)	16.3 (3.9-32.3)	0.125
RRT pre-KT, n (%)				0.061
Preemptive	50 (25)	33 (28)	17 (21)	
HD	106 (54)	54 (47)	52 (63)	
PD	42 (21)	29 (25)	13 (16)	
Donor				
Age of D, mean $\pm$ SD	48.1 $\pm$ 10.5	47.4 $\pm$ 11.4	49.1 $\pm$ 9.2	0.265
Sex of D (F), n (%)	143 (72)	80 (69)	63 (77)	0.224
BMI of D, mean $\pm$ SD	25.3 $\pm$ 3.5	25.2 $\pm$ 3.5	25.4 $\pm$ 3.5	0.798
Predonation eGFR, mean $\pm$ SD	100.2 $\pm$ 14.3	101.4 $\pm$ 14.0	98.4 $\pm$ 14.6	0.144
Left kidney donated, n (%) / Missing: 9	156 (83)	89 (81)	67 (85)	0.486
Transplant				
Year of KT, n (%)				0.903
2008-2012	71 (36)	42 (36)	29 (35)	
2013-2017	127 (64)	74 (64)	53 (65)	
Retransplant, n (%)	27 (14)	18 (16)	9 (11)	0.359
Calculated PRA >0%, n (%)	60 (30)	37 (32)	23 (28)	0.562
HLA-incompatible KT, n (%)	22 (11)	14 (12)	8 (10)	0.610
HLA-A MM, mean $\pm$ SD	0.94 $\pm$ 0.68	0.63 $\pm$ 0.52	1.39 $\pm$ 0.62	<0.001
HLA-A MM, n (%)				<0.001
0	51 (26)	45 (39)	6 (7)	
1	107 (54)	69 (59)	38 (46)	
2	40 (20)	2 (2)	38 (46)	
HLA-B MM, mean $\pm$ SD	1.16 $\pm$ 0.72	0.80 $\pm$ 0.64	1.66 $\pm$ 0.50	<0.001
HLA-B MM, n (%)				<0.001
0	38 (19)	37 (32)	1 (1)	
1	91 (46)	65 (56)	26 (32)	
2	69 (35)	14 (12)	55 (67)	
HLA-DR MM, mean $\pm$ SD	0.97 $\pm$ 0.69	0.67 $\pm$ 0.59	1.40 $\pm$ 0.61	<0.001

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HLA-DR MM, n (%)				<0.001
0	50 (25)	45 (39)	5 (6)	
1	103 (52)	64 (55)	39 (48)	
2	45 (23)	7 (6)	38 (46)	
IS Induction, n (%)				0.614
Without	5 (3)	4 (3)	1 (1)	
Basiliximab	172 (87)	100 (86)	72 (88)	
ATG	21 (11)	12 (10)	9 (11)	
Maintenance IS, n (%)				1
TAC + MMF+pred	192 (97)	112 (97)	80 (98)	
Others	6 (3)	4 (3)	2 (2)	
DGF, n (%)	8 (4)	5 (4)	3 (4)	1
Follow-up (years), median (IQR)	5.1 (3.3-7.2)	5.2 (3.4-8.3)	4.9 (3.0-6.9)	0.327

TABLE 1: Baseline characteristics of living donor pairs and transplants, based on LRD and LURD.

LRD: Living related donor; LURD: Living unrelated donor; R: Recipient; SD: Standard deviation; F: Female; BMI: Body mass index; KT: Kidney transplant; IQR: Interquartile range; RRT: Renal replacement therapy; HD: Hemodialysis; PD: Peritoneal dialysis; D: Donor; eGFR: Estimated glomerular filtration rate; PRA: Panel-reactive antibodies; HLA: Human leukocyte antigen; MM: Mismatch; ATG: Antithymocyte globulin; TAC: Tacrolimus; MMF: Mofetil mycophenolate; pred: Prednisone; DGF: Delayed graft function

The mean recipient age at the time of KT was lower in LRD compared to LURD recipients (35.9±12.2 vs. 48.5±10.9 years old, $p < 0.001$). The percentage of preemptive KT was 28% (n=33) in the LRD group and 21% (n=17) in the LURD group ($p=0.061$). The HLA-A mismatches (MM) were significantly higher in LURD recipients at 1.40±0.61 compared to 0.67±0.59 in LRD recipients. The HLA-B MM was also higher in LURD recipients at 1.66±0.50 compared to 0.80±0.64 in LRD recipients, as well as HLA-DR MM, which was 0.67±0.59 for LURD recipients and 0.67±0.59 for LRD recipients ($P < 0.001$ for all HLA MM comparisons).

The immunosuppression induction regimen included basiliximab in 86% (n=100) of LRD recipients and 88% (n=72) of LURD recipients, ATG in 10% (n=12) of LRD recipients and 11% (n=9) of LURD recipients; 3% (n=4) of LRD recipients, and 1% (n=1) of LURD recipients had no induction immunosuppression ($p=0.614$). The maintenance immunosuppression regimen included triple immunosuppression with tacrolimus, mofetil mycophenolate, and prednisone in 97% (n=113) of the LRD recipients and 98% (n=79) of the LURD recipients. Median follow-up was of 5.0 years (IQR: 3.3-7.2).

Acute rejection

The AR was observed in 23 recipients (12%), of which 13 had acute cellular rejection (ACR) and 10 had antibody-mediated rejection (ABMR) (Table 2). Nine cases (8%) of AR were identified in LRD recipients, while LURD recipients had 14 cases (17%) of AR ($p=0.044$). The cumulative incidence of AR during the follow-up period is shown in Figure 7. The days until AR and the incidence of ABMR and T cell-mediated (cellular) rejection (TCMR) were similar in both groups.

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	Total N=198	LRD N=116 (59%)	LURD N=82 (41%)	P-value
Acute rejection (AR) n (%)	23 (12)	9 (8)	14 (17)	0.044
Days to AR, median (IQR)	21 (9-154)	34 (12-100)	18 (9-154)	0.900
ACR (%)	13 (7)	5 (4)	8 (10)	0.128
Days to ACR, median (IQR)	16 (12-91)	34 (16-91)	14 (7-92)	0.305
Antibody-mediated rejection (ABMR), n (%)	10 (5)	4 (3)	6 (7)	0.324
Days to ABMR, median (IQR)	61 (9-1480)	53 (6-790)	613 (9-1502)	0.284

TABLE 2: Impact of LURD vs. LRD transplants in acute rejection

LRD: Living related donor; LURD: Living unrelated donor; AR: Acute rejection; ACR: Acute cellular rejection; ABMR: Antibody-mediated rejection; IQR: Interquartile range

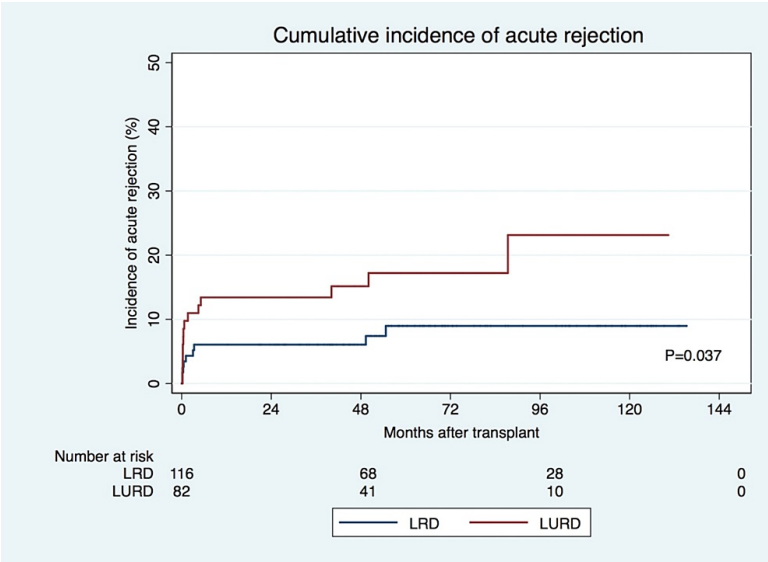


FIGURE 1: Cumulative incidence of acute rejection according to LRD or LURD KT

LRD: Living related donor; LURD: Living unrelated donor; KT: Kidney transplant

In the univariate analysis, LURD recipients were at an increased risk of AR (HR=2.348; p=0.046). However, multivariate analysis showed that LURD was not an independent risk factor for AR (after adjustment for the recipient and donor age, sex, immunosuppression induction regimen, previous time on kidney replacement therapy (KRT), type of previous KRT, re-transplantation rate, donor eGFR, the prevalence of HLA-A MM, HLA-B-MM, and receptor BMI) (Table 3). In contrast, HLA-DR MM increased the HR of AR in both groups (HR=2.256, p=0.011). Both HLA-A and HLA-B MM did not affect the AR HR between the groups of patients. Additionally, KT occurring in the 2008 to 2012 time frame was associated with a significantly higher risk of AR (HR=2.480, p=0.039).

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	HR (CI 95%)	P-value
Univariate analysis		
LURD	2.348 (1.016-5.427)	0.046
Multivariate analysis 1*		
HLA-DR MM	2.256 (1.205-4.223)	0.011
Year of KT, 2008-2012	2.480 (1.047-5.874)	0.039

TABLE 3: Predictors of acute rejection

*Adjusted to recipient age, donor age, sex, induction of IS therapy, IS maintenance, retransplant rate, months on RRT, type of dialysis/preemptive, donor eGFR, HLA A MM, HLA B MM, KT HLAI, and recipient BMI.

HR: Hazard ratio; LURD: Living unrelated donor; HLA: Human leukocyte antigen; HLAI: Human leukocyte antigen incompatibility; MM: Mismatch; KT: Kidney transplant; IS: Immunosuppression; BMI: Body mass index; RRT: Renal replacement therapy; eGFR: Estimated glomerular filtration rate; CI: Confidence interval

When ACR and ABMR were analyzed separately, higher BMI was associated with a higher risk of ACR (HR=1.179, p=0.013) (Table 4), and HLA-DR MM had an independent impact on ABMR (HR=2.892, p=0.045) and HLA incompatibility (HR=5.070, p=0.012) (Table 5). The LURD KT was not significantly associated with any of the rejection types in either univariate or multivariate analysis.

	HR (CI 95%)	P-value
Univariate analysis		
LURD	2.345 (0.767-7.169)	0.135
Multivariate analysis*		
BMI R	1.179 (1.035-1.344)	0.013

TABLE 4: Predictors of acute cellular rejection (ACR)

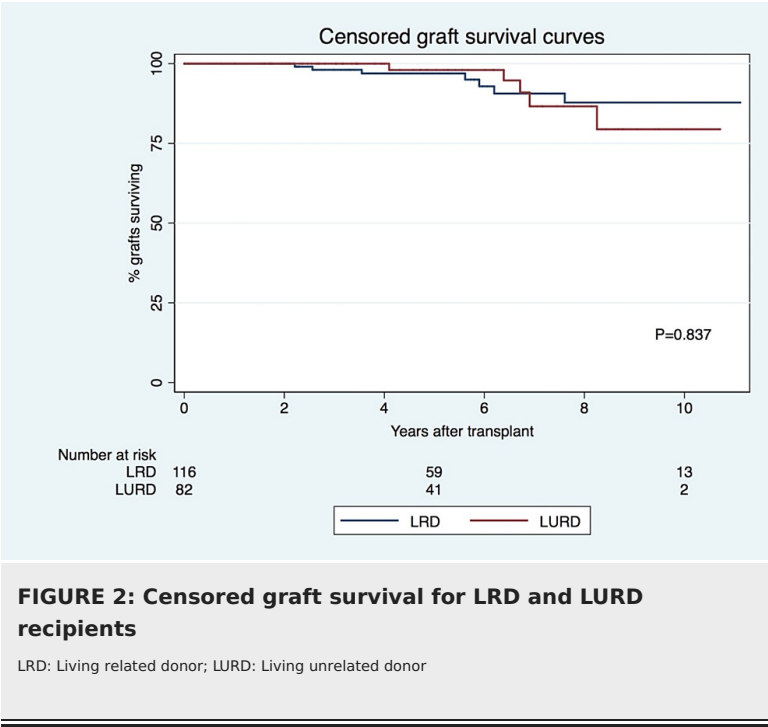
* Adjusted to recipient age, donor age, recipient sex, donor sex, induction of IS therapy, IS maintenance, retransplant rate, months on RRT, type of dialysis/preemptive, donor eGFR, HLA-A MM, HLA-B MM, KT HLAI, and KT time period.

HR: Hazard ratio; LURD: Living unrelated donor; HLA: Human leukocyte antigen; HLAI: Human leukocyte antigen incompatibility; MM: Mismatch; KT: Kidney transplant; IS: Immunosuppression; BMI: Body mass index; RRT: Renal replacement therapy; eGFR: Estimated glomerular filtration rate; CI: Confidence interval

Graft and patient survival

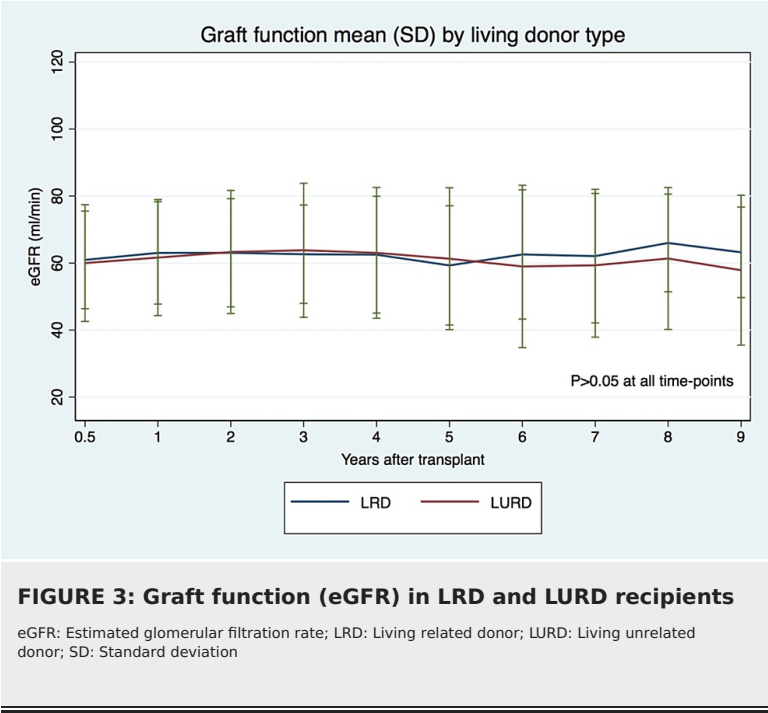
Censored graft survival was similar for LRD and LURD recipients (96.9% vs. 98.0% at five years and 87.8% vs. 79.4% at 10 years, p=0.837, respectively) (Figure 2), which remained true after adjustment for several factors. Recipient age (HR=0.938, p<0.05), the occurrence of AR (HR 16.576, p< 0.001), and the presence of preformed DSA (HR=3.387, p<0.05) were identified as predictors of censored graft failure (Table 6). Graft survival rates at five and 10 years were 99%/94% for LRD recipients with no AR, 78%/47% for LURD recipients with AR, 100%/100% for LURD recipients with no AR, and 91%/28% for LURD recipients and AR (overall p<0.001). Patient survival was similar in both groups (one (1%) death in the LRD group and one (1%) death in the LURD group, p=0.422).

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Graft function

Graft function was similar in both groups over the follow-up period: around 60-65 mL/min ($p>0.05$, in all evaluated time-points) (Figure 3).



Discussion

Living KT can mitigate organ scarcity and help reduce KT waitlists[3]. Evaluating the impact of living donor sources on long-term outcomes may potentially allow the optimization of donor matching and immunosuppression to improve results [17]. In this study, we aimed to compare graft function and survival between LURD and LRD transplant recipients. Additionally, we studied AR occurrence in both groups.

In our study, graft function was similar in both groups during the medium and long-term follow-up periods. Other studies have found that the donor source does not significantly influence graft function in living donation [10,14]. Censored graft survival was also comparable between LURD and LRD patients, which is in line with most previous studies suggesting graft survival to be similar for both groups of patients [7]. In a recent large-scale study, however, LURD had higher death-censored graft failure than LRD recipients [14], which contrasts with our results; although the authors stated that they could not explain this finding, they speculated that it could be due to old age and a high proportion of patients with type II diabetes mellitus and hypertension as primary kidney disease among LURD transplant recipients. Long-term follow-up and large-scale studies are necessary to confirm these results.

Patient survival was also similar between both groups, with one (1%) death in the LRD group and one (1%) death in the LURD group. A United States study from 1998 [12] showed that 10-year patient survival among recipients of LURD transplants was worse than that of LRD transplants (86% vs. 63%, respectively), although these findings have not been replicated in other more recent studies, which have consistently shown similar survival rates between LRD and LURD KT recipients [10,14].

In our study, the univariate analysis showed that LURD recipients had a higher incidence of AR. However, in the multivariate analysis, LURD recipient status was not found to be an independent risk factor for AR, consistent with findings from previous studies [8]. However, the presence of HLA-DR MM predicted an increased risk of AR, regardless of the donor origin. The LURD recipients had higher HLA-DR MM, which explains why, despite having a higher rate of AR in multivariate analysis, LURD recipient status did not predict AR, while HLA-DR MM did (Tables 1, 3). In contrast, HLA-A and HLA-B MM did not affect the AR between the groups. The HLA-MM is recognized as a strong risk factor for the development of AR [18,19] and HLA-DR MM, in particular, has been shown to strongly influence KT outcomes [20], namely AR [21,22]. More recently, HLA-DR epitope mismatch is an independent predictor of ABMR [23]. A recent study using random forest analysis in the United Network for Organ Sharing (UNOS) database identified HLA-DR as an important variable for acute rejection among Black kidney transplant recipients in the United States [24]. In pancreatic transplantation, HLA-DR MM has been shown to independently predict acute rejection [25], an effect that might be reproducible in KT. The number of days until AR after KT was similar between LURD and LRD recipients. Other adverse outcomes are associated with HLA-MM. In the case of deceased donors, HLA matching has been shown to correlate with renal allograft and patient survival, even in the absence of preformed DSA [26,27], but few studies have evaluated this relationship in living donor transplants. In one study with first adult transplants from deceased donors in the United States between 1987 and 2013, a significant linear relationship between HLA MM and graft survival were identified, with one MM conferring a 13% higher risk and six MM conferring a 64% higher risk of allograft failure [26]. In another study, 83 0-HLA MM patients were matched to 407 controls with more than 0-HLA MM, with the authors reporting no differences in death-censored graft survival or patient survival for both groups [28]. Our data reinforce the importance of HLA-DR matching and its association with graft survival and incidence of rejection [25].

The hurdle associated with high HLA MM in LDKT may potentially be managed by the introduction of compatible pairs in a KPE program, which has been possible in Portugal since legislation concerning the National KPE program was amended in 2021. Careful immunological risk profiling, including improved HLA and epitope analysis, could also improve these results. In addition, a clear definition of the inclusion criteria for compatible pairs in KPE is crucial. A recent report that reviewed the first nine years of KPE transplants from the National Kidney Registry in the United States showed a 27% lower five-year graft failure rate compared to traditional direct living donor transplants [29], and improved transplant outcomes have been attributed to improved antibody avoidance. In the setting of an increasingly hypersensitized population of KT candidates worldwide, surely the optimal choice type of KT is a low HLA mismatch transplant. We should also refer to our finding that KT occurring in the 2008 to 2012 time frame was associated with a significantly higher risk of AR (HR=2.480, p=0.039). We hypothesize this is most likely due to changes in our center's immunosuppression protocol, which was mainly based on cyclosporine for the majority of patients before 2012, and increasingly became tacrolimus-based in the most recent years, justifying a higher incidence of AR in the aforementioned period.

In the multivariate analysis with separate ACR and ABMR, BMI was associated with a higher risk of ACR (HR=1.179, p=0.013). Other studies have reported recipient obesity as a risk factor for AR [30]. This has been attributed to more difficult dosage-adjustment immunosuppressant medications in this population and to the low-grade inflammatory state that characterizes obesity, which has also been hypothesized as a factor that may impact the efficacy of immunosuppressants. Adapted immunosuppressant targets/regimens to the obese population might improve results in this specific group of patients, in the future.

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In our population, the preemptive KT rate was similar in both groups, 28% (n=33) for LRD recipients and 21% (n=12) for LURD recipients. As waiting time on dialysis is considered the strongest modifiable risk factor for KT outcomes, increasing this rate would certainly improve our results.

The major limitation of this study is the sample size. However, it should be emphasized that our data refers to a single-center population, with similar background demographic and clinical features, submitted to KT by the same multidisciplinary team, which delivered the same standards of patient care. This allowed the retrieval of robust data for statistical analysis; thus, valid conclusions can still be ascertained. In addition, the fact that this was a retrospective study rather than a prospective study designed to assess KT outcomes with formal event adjudication implies that the level of evidence is not as high as would be derived from a clinical trial. On the other hand, our results are significant, as our center is currently responsible for more than half of all LDKT performed in Portugal.

Conclusions

In conclusion, our study of LURD and LRD KT recipients showed similar graft function over time and similar censored-death survival rates. The AR was higher in LURD recipients compared to LRD recipients. However, multivariate analysis showed that LURD recipient status was not an independent risk factor for AR. The HLA-DR MM was an independent predictor of AR, while HLA-A and HLA-B MM did not affect AR HR between groups of patients, reinforcing the importance of HLA-DR matching and its association with graft survival and incidence of rejection.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Ethical Committee of Centro Hospitalar Universitario do Porto issued approval NA. The study protocol was reviewed and approved by the institutional ethical review and hospital administration boards in accordance with the recommendations of the Declaration of Helsinki and European Data Protection Regulations. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

References

- Wolfe RA, Ashby VB, Milford EL, et al.: Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med*. 1999, 341:1725-1730. [10.1056/NEJM199912023412303](https://doi.org/10.1056/NEJM199912023412303)
- Clayton LM, Rizzolo D, Nair V: Kidney transplant wait list: review and current trends. *JAAPA*. 2018, 31:1-5. [10.1097/01.JAA.0000545074.86472.d4](https://doi.org/10.1097/01.JAA.0000545074.86472.d4)
- Hou S: Expanding the kidney donor pool: ethical and medical considerations. *Kidney Int*. 2000, 58:1820-1836. [10.1046/j.1523-1755.2000.00345.x](https://doi.org/10.1046/j.1523-1755.2000.00345.x)
- Organ Harvest and Transplantation - AESOP. (2007). <https://www.aesop-enfermeiros.org/colheitaetransplante/>.
- Taylor GS, Prather JC, Norman DJ, de Mattos AM, Mogilishetty G, Conlin MJ, Barry JM: Living unrelated donor renal transplantation: a single center experience. *J Urol*. 2005, 174:223-225. [10.1097/01.ju.0000162058.64983.b9](https://doi.org/10.1097/01.ju.0000162058.64983.b9)
- Simforoosh N, Basiri A, Fattahi MR, et al.: Living unrelated versus living related kidney transplantation: 20 years' experience with 2155 cases. *Transplant Proc*. 2006, 38:422-425. [10.1016/j.transproceed.2006.01.012](https://doi.org/10.1016/j.transproceed.2006.01.012)
- Simforoosh N, Basiri A, Tabibi A, et al.: Living unrelated versus related kidney transplantation: a 25-year experience with 3716 cases. *Urol J*. 2016, 13:2546-2551. [10.22037/uj.v13i1.3196](https://doi.org/10.22037/uj.v13i1.3196)
- Ahmad N, Ahmed K, Khan MS, Calder F, Mamode N, Taylor J, Koffman G: Living-unrelated donor renal transplantation: an alternative to living-related donor transplantation?. *Ann R Coll Surg Engl*. 2008, 90:247-250. [10.1308/003588408X261636](https://doi.org/10.1308/003588408X261636)
- Kizilisik AT, Ray JM, Nylander WA, Langone AJ, Helderma JH, Shaffer D: Living donor kidney transplantation in a veterans administration medical center. *Am J Surg*. 2004, 188:611-613. [10.1016/j.amjsurg.2004.07.022](https://doi.org/10.1016/j.amjsurg.2004.07.022)
- Matter YE, Nagib AM, Lotfy OE, et al.: Impact of donor source on the outcome of live donor kidney transplantation: a single center experience. *Nephrourol Mon*. 2016, 8:e34770. [10.5812/numonthly.34770](https://doi.org/10.5812/numonthly.34770)
- Santori G, Barocci S, Fontana I, et al.: Kidney transplantation from living donors genetically related or unrelated to the recipients: a single-center analysis. *Transplant Proc*. 2012, 44:1892-1896. [10.1016/j.transproceed.2012.05.061](https://doi.org/10.1016/j.transproceed.2012.05.061)
- D'Alessandro AM, Pirsch JD, Knechtle SJ, et al.: Living unrelated renal donation: the University of Wisconsin experience. *Surgery*. 1998, 124:604-610; [10.1067/msy.1998.91482](https://doi.org/10.1067/msy.1998.91482)
- Bellini MI, Nozdrin M, Pengel L, Knight S, Papalouis V: How good is a living donor? Systematic review and meta-analysis of the effect of donor demographics on post kidney transplant outcomes. *J Nephrol*. 2022, 35:807-820. [10.1007/s40620-021-01231-7](https://doi.org/10.1007/s40620-021-01231-7)

14. Abd ElHafeez S, Noordzij M, Kramer A, et al.: The association of living donor source with patient and graft survival among kidney transplant recipients in the ERA-EDTA registry - a retrospective study. *Transpl Int*. 2021, 34:76-86. [10.1111/tri.13759](#)
15. Gheith O, Sabry A, El-Baset SA, Hassan N, Sheashaa H, Bahgat S, El-Shahawy el-M: Study of the effect of donor source on graft and patient survival in pediatric renal transplant recipients. *Pediatr Nephrol*. 2008, 23:2075-2079. [10.1007/s00467-008-0760-y](#)
16. Silva F, Malheiro J, Pestana N, et al.: Lower donated kidney volume is associated with increased risk of lower graft function and acute rejection at 1 year after living donor kidney-a retrospective study. *Transpl Int*. 2020, 33:1711-1722. [10.1111/tri.13740](#)
17. Montgomery RA, Tatapudi VS, Leffell MS, Zachary AA: HLA in transplantation. *Nat Rev Nephrol*. 2018, 14:558-570. [10.1038/s41581-018-0039-x](#)
18. Mota A, Figueiredo A, Cunha MF, Bastos M, Pratas J, Furtado L: Risk factors for acute rejection in 806 cyclosporine-treated renal transplants: a multivariate analysis. *Transplant Proc*. 2003, 35:1061-1063. [10.1016/s0041-1345\(03\)00306-3](#)
19. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group: KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant*. 2009, 9 Suppl 3:S1-155. [10.1111/j.1600-6143.2009.02834.x](#)
20. Wiebe C, Pochinco D, Blydt-Hansen TD, et al.: Class II HLA epitope matching-A strategy to minimize de novo donor-specific antibody development and improve outcomes. *Am J Transplant*. 2013, 13:3114-3122. [10.1111/ajt.12478](#)
21. McKenna RM, Lee KR, Gough JC, Jeffery JR, Grimm PC, Rush DN, Nickerson P: Matching for private or public HLA epitopes reduces acute rejection episodes and improves two-year renal allograft function. *Transplantation*. 1998, 66:38-43. [10.1097/00007890-199807150-00006](#)
22. Wiebe C, Nevins TE, Robiner WN, Thomas W, Matas AJ, Nickerson PW: The synergistic effect of class II HLA epitope-mismatch and nonadherence on acute rejection and graft survival. *Am J Transplant*. 2015, 15:2197-2202. [10.1111/ajt.13341](#)
23. Tafulo S, Malheiro J, Santos S, et al.: Degree of HLA class II eplet mismatch load improves prediction of antibody-mediated rejection in living donor kidney transplantation. *Hum Immunol*. 2019, 80:966-975. [10.1016/j.humimm.2019.09.010](#)
24. Thongprayoon C, Jadlowiec CC, Leeaphorn N, et al.: Feature importance of acute rejection among Black kidney transplant recipients by utilizing random forest analysis: an analysis of the UNOS database. *Medicines (Basel)*. 2021, 8:10.3390/medicines8110066
25. Rudolph EN, Dunn TB, Mauer D, Noreen H, Sutherland DE, Kandaswamy R, Finger EB: HLA-A, -B, -C, -DR, and -DQ matching in pancreas transplantation: effect on graft rejection and survival. *Am J Transplant*. 2016, 16:2401-2412. [10.1111/ajt.13734](#)
26. Williams RC, Opelz G, McGarvey CJ, Weil EJ, Chakkera HA: The risk of transplant failure with HLA mismatch in first adult kidney allografts from deceased donors. *Transplantation*. 2016, 100:1094-1102. [10.1097/TP.0000000000001115](#)
27. Zachary AA, Leffell MS: HLA mismatching strategies for solid organ transplantation - a balancing act. *Front Immunol*. 2016, 7:575. [10.3389/fimmu.2016.00575](#)
28. Casey MJ, Wen X, Rehman S, Santos AH, Andreoni KA: Rethinking the advantage of zero-HLA mismatches in unrelated living donor kidney transplantation: implications on kidney paired donation. *Transpl Int*. 2015, 28:401-409. [10.1111/tri.12495](#)
29. Flechner SM, Thomas AG, Ronin M, et al.: The first 9 years of kidney paired donation through the National Kidney Registry: characteristics of donors and recipients compared with National Live Donor Transplant Registries. *Am J Transplant*. 2018, 18:2730-2738. [10.1111/ajt.14744](#)
30. Scheuermann U, Babel J, Pietsch UC, et al.: Recipient obesity as a risk factor in kidney transplantation. *BMC Nephrol*. 2022, 23:37. [10.1186/s12882-022-02668-z](#)

APPENDIX 12 – Exploring the Complex Ethical Challenges Associated with Living Donor Kidney Transplantation

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Exploring the Complex Ethical Challenges Associated with Living Donor Kidney Transplantation

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Abstract

Living donor kidney transplantation is regarded as the best treatment for selected patients with end-stage renal disease, associated with improved survival and quality of life. However, it should be recognized as a distinct medical procedure during which an organ is procured from a healthy individual for the benefit of another patient. Seven decades after the first successful procedure, ethical constraints persist, even though the procedure has become routine worldwide. The perceived risks for donors are considered low and ethically acceptable; however, recent studies have identified increased long-term risks of end-stage renal disease for living donors compared to healthy non-donors, raised hypertensive disorders during pregnancy, and a possible increase in mortality. Additionally, in most centers, there is a growing acceptance of donors with minor abnormalities, which were previously declined, yet long-term follow-up data on these donors remains scarce. Furthermore, various risks and benefits must be evaluated, including the potential risks from refusing donations and possible lifesaving opportunities arising from conditions identified during the evaluation process. A thorough assessment of the candidate donor's risks is essential. While current literature helps us mitigate significant risks for the candidate donor, it is important to elaborate on these risks with greater precision. Maintaining ethical practices and ensuring informed consent requires an updated registry of all living donors and candidates, along with long-term follow-up that includes ongoing data reviews.

Keywords: Informed Consent; Kidney Transplantation/ethics; Living Donors; Personal Autonomy

INTRODUCTION

Living kidney donation constitutes a distinct medical procedure in which an organ is taken from a healthy individual to benefit another patient and, more broadly, the wider society. The tension between bodily integrity and human solidarity is fundamental to the ethics of transplantation. Joseph Murray elucidated this in his Nobel Lecture on December 8, 1990: "The only remaining problem was the ethical decision concerning the removal of a healthy organ from a normal person for the benefit of someone else. For the first time in medical history, a normal healthy person was to be subjected to a major surgical operation, not for his benefit".¹ He referred to the first successful kidney transplant from a living donor between identical

twins, Herrick, which took place on December 23, 1954. This pioneering procedure was carried out by Murray and his team at Peter Bent Brigham Hospital in Boston, Massachusetts. This marked a milestone in the history of transplantation.

Seven decades after this procedure became routine, ethical constraints still persist, albeit each year, more than 35 000 people worldwide become living kidney donors.² Overall, perioperative mortality has declined significantly in the past decade compared to earlier periods, now down to fewer than one event per 10 000 donations.³ Long-term increased risks of end-stage renal disease have been emphasized in two pivotal studies,^{4,5} along with potential higher mortality rates.⁴ Additionally, there have

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been reports of increased complications related to pregnancy, which adds to the overall uncertainty.⁶ It is essential to assess the risks associated with donor nephrectomy to protect this population.

The global shortage of organs has led to an increase in kidney transplants from living donors. In recent decades, Portugal has emerged as a global leader in organ donation and transplantation. Portugal has one of the highest rates of incident and prevalent treated end-stage renal patients worldwide, with 240.9 per million population (pmp) and 2055 pmp as of December 31, 2023, respectively. It also has a significantly lower rate of living donor kidney transplants at 7 pmp, compared to deceased donor transplants, which stands at 53.6 pmp.⁷⁻⁹ When comparing internationally, data from the Global Observatory on Donation and Transplantation indicates that in 2023, we ranked sixth in transplants per million population, following the United States, Spain, Finland, the Netherlands, and France. While we placed fourth in deceased donor kidney transplants, our ranking was significantly lower for living donor transplants, where we held the 35th position out of 89.⁸ While optimizing and enhancing deceased organ donation must consistently remain a priority, boosting our living donor program is also a national priority. Addressing the ethical issues related to living donor kidney transplantation is therefore a significant concern.

ETHICAL CHALLENGES IN LIVING KIDNEY DONATION

Living kidney donation directly contradicts the primary principle of medical ethics, “*primum non nocere*,” and poses a considerable ethical challenge. Most of the ethical issues related to transplantation have been discussed in the context of principlism theory by Beauchamp and Childress.¹⁰ It comprises four principles: 1) autonomy (respecting personal choices), 2) beneficence (producing benefits), 3) nonmaleficence (avoiding harm), and 4) justice (distributing benefits and burdens equitably). Although imperfect and not without critics, principlism remains the dominant approach to medical ethics.² It provides a set of tools for guiding the living donor evaluation and is, therefore, the ethical theory outlined here.

THE PRINCIPLE OF AUTONOMY

The principle of autonomy states that an individual, in this situation, a living kidney donor candidate, has the right to make their own choices. But this concept is inextricably linked to that of informed consent. Informed consent requires that a person be informed of an intervention's nature, risks, and probable outcomes before giving their consent. The donor must be competent to understand the relevant information.

Potential living donors should be provided with all relevant information concerning the donation procedure, including the evaluation process, risks of the procedure

and of the evaluation itself (such as the discovery of an unknown health problem), other available treatments for the recipient, such as the deceased organ transplant or the dialysis programs, and the expected outcome for both the recipient and the donor.¹¹⁻¹³ Research revealed significant variation in the written and oral information provided among different countries, transplant centers, and even providers at the same centers, particularly regarding long-term risks.¹⁴ Although achieving complete standardization of information may not be possible, research has highlighted the necessity of a standardized process to ensure fair educational and decision-making opportunities, thereby preventing disparities across transplant centers.^{14,15} More recent findings of a slightly absolute (i) increased risk of end-stage renal disease compared with healthy non-donors, (ii) increased complications of donor's pregnancy, and (iii) possible decreased survival for a long time, must be transmitted and discussed with the potential donor.^{4,5,11-13,16} The uncertainties about outcomes in certain medical conditions must also be considered.¹⁶ Some uncertainties prevail and probably will increase in future years as accepting complex medical donors is an increasing practice.¹⁷ Developing communication strategies to clarify these uncertainties is a major challenge, as well as keeping living donor registries up to date, following up long-term, including all donors, and facilitating research and investigations, namely in developing countries.^{5,14,17,18} Furthermore, the panorama of living kidney donation is rapidly changing. Last decade's new strategies to increase living donation include ABO incompatible transplants and Kidney paired exchange programs with several variations, including non-directed or “altruistic” donation, each with specific ethical dilemmas. Communicating all the possibilities and implications is a major challenge in a living donor consultation.¹⁸

In Portugal, the informed consent process is not considered complete without an evaluation with an independent donor advocate or a representative of the local ethics committee to further ensure the donor's safety and obtain proper ethics authorization. This practice aligns with international guidelines recommending an independent living donor advocate to verify that donor candidates have the necessary information to make a voluntary and informed decision about donating, confirm consent for donation, and function independently from the recipient candidate's team.¹² Other countries might employ different procedures, such as conducting an external review of planned donations, to ensure that independence, advocacy for donor rights, and voluntarism are respected.¹² The donors should be informed that they can withdraw consent at any time before the procedure without any recrimination. In that situation, the evaluation team must ensure that medical and individual reasons for not proceeding with a donation remain confidential.^{12,13} The decision to donate should be voluntary and not coerced.

Exhaustive psychosocial evaluation is mandatory.^{11,12} Another important issue is how the donor's autonomy should be restricted in case of excessive risk. The increasing shortage of organs led to the evaluation of a broader range of potential living donors that would not have been evaluated before: the complex medical donors.¹⁷ Donors with an increased risk of complications above the predefined threshold risk established by each Unit should not donate, regardless of the relationship with the receptor.¹² However, defining acceptable risk in many situations is often challenging, and precise metrics may not be applicable. A multidisciplinary team discussion, including the psychosocial team, is necessary.

End-stage renal patients are excluded as transplant candidates when no expected benefits are foreseen due to serious comorbidities.

In less obvious situations, the living donation should additionally be questioned when we expect low results for the receptor because of comorbidities or a high risk of recurrence of the primary renal disease with graft loss. A transplantation failure causes significant emotional stress for the donor-recipient pair, family, and the team involved. In both scenarios, there are instances of paternalism. Often, this involves a person in authority (such as a medical doctor) conditioning decisions for a subordinate person (like a living donor) to act in the subordinate person's best interest. From another perspective, in some instances, the principles of beneficence/nonmaleficence, based on the best interest argument, prevail over the principle of autonomy.

In some challenging cases, such as those involving complex vascular donor anatomy or rare disorders in the recipient, referring patients to specialized centers for "high-risk" cases can help to balance the tensions between paternalism and patient autonomy. This should only be done with the patient's full understanding and consent and with appropriate safeguards in place to address the increased risks. Transparent audits, support for medical personnel, and a focus on improvement rather than punitive measures in the case of below-average outcomes should be included.¹⁹

THE PRINCIPLES OF BENEFICENCE AND NONMALEFICENCE

The principles of beneficence and nonmaleficence are often discussed together in medical practice, focusing on the benefits and potential harms of a particular treatment. When considering the pair donor-recipient, the living donor kidney transplant is the best treatment for the recipient,^{6,20,21} but it can harm another individual. Although the risks of nephrectomy are small, they are real, including short-term complications such as infections, pain, and even death,²² and long-term risks do exist.^{4,5,18} Many people who donate a kidney to a relative experience important psychological benefits from donating, including lasting increases in self-esteem and happiness after

donating.²³⁻²⁶ However, a small number of donors had an adverse outcome.²⁷ Concern for donor health, defined broadly from a medical and psychological perspective, is a central ethical consideration when physicians subject healthy individuals to a procedure with medical-physical, and psychological - risks. Minimizing possible harmful aspects depends on careful selection, informed consent, and follow-up of donation candidates.

An expanded view of the risks and benefits of living donation must include a discussion of the harms of refusing to allow it.²⁸ In their elegant study, Reese *et al* reported that potential donors who were discarded for donation were more likely to report that their lives were worse and that they experienced more stress, anxiety, and emotional changes. This highlights the importance of looking at the benefits of donation.²⁹ This additional point in the equation will redefine the beneficence/nonmaleficence discussion.³⁰ Many potential donors do not donate.³¹ In addition to tackling the reasons for non-donation and enhancing those strategies, future efforts should include more in-depth psychosocial assessment, characterization, and support for this group. This group is expected to be quite diverse, but we anticipate that the findings will help improve the care provided to living donors.

In a qualitative study of 56 living donors, Rasmussen *et al*³² pointed out some tangible benefits identified by donors that are currently overlooked in living donor evaluation, including reduced caregiving burden, increased wage earnings, and improved donor independence. They argue that these qualitative findings should inform quantitative research on the tangible benefits of donation, as these additional benefits may alter present living donor risk-benefit calculations. Further research in this area will be welcomed and reinforce the practice of living kidney donation.

Another often overlooked benefit is the potential for lives saved while assessing living donor candidates. This evaluation can lead to the early diagnosis of various abnormalities, including previously undetected life-threatening illnesses, which should be considered one of the key advantages of living donation programs.³³

THE PRINCIPLE OF JUSTICE

Finally, the principle of justice reflects a concern for the fair distribution of the limited resources in the healthcare system. It is particularly important in transplantation, where demand far exceeds supply. The presence of international guidelines, particularly the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors,¹² helps guide clinicians in the selection, evaluation, and follow-up of living donors. It ensures that all donors undergo almost identical tests and appointments. In practice, each Unit should set its acceptable risk threshold that can be consistently reproduced,¹² although this is not always true.³⁴

The principle of justice can also be applied to the entire society. Improving living donor programs will reduce the competition for deceased donor organs. Lately, the waiting list for a deceased-procured organ has been reduced, and so has the economic burden of dialysis.³⁵ Portugal has one of the highest rates of incident and prevalent treated end-stage renal disease,^{8,9} as well as deceased donor kidney transplants worldwide.³⁶ However, there is potential for a significant increase in living donor kidney donations. It is crucial to emphasize that establishing living kidney transplant programs should not undermine the deceased donor program, as that would be inequitable for patients without living donors. Instead, by developing both programs simultaneously, patients can benefit more from this scarce resource.

Research indicates that women are less likely than men to receive kidney transplants, yet they constitute most of the kidney donors.^{37,38} Gender inequality in transplantation is unfair and needs to be tackled. Transplant centers must investigate the underlying causes of this disparity, and educational programs should be actively promoted. It is essential to consider both biological and sociocultural factors. The biological factors often cited include the sex distributions of various potential biological risk factors for diseases, such as smoking, along with a higher incidence of hypertension and ischemic heart disease, which may result in the more frequent exclusion of male candidates. Conversely, while women show a higher prevalence of chronic kidney disease compared to men, the incidence of end-stage renal disease is higher in males than in females.³⁷ Notably, even women can often be more incompatible with their spouse or child due to the immunological consequences of pregnancy; they may become living donors, albeit that.

Socio-cultural aspects should be considered, as they hold significant importance in many cultures. It is expected that women's heightened altruism will arise from their traditional role as caregivers within the family.³⁸⁻⁴⁰ Family expectations often place the burden of being a living donor on her, while men are more commonly expected to keep working and provide for the family. Furthermore, access to living donor transplants can differ among various populations due to professional motivations, availability of information, and financial concerns. Identifying and addressing these disparities is a crucial task for living donor programs.

There is a consensus in European countries that international and national laws should strictly maintain the general prohibition on organ commercialism. In Portugal, law 36/2013,⁴¹ for the transposition of the EU directive 2010/53/EU⁴² on living organ donation practice, has determined that living organ donation must be voluntary and unpaid. No case of suspected commercialism in the transplantation field has ever been identified in Portugal. However, donors should not incur financial losses and should receive compensation for additional expenses such as travel costs or lost income.⁴³

LONG-TERM FOLLOW-UP OF DONORS AND NATIONAL REGISTRIES

Long-term follow-up of all donors and national and international registries are essential. Collecting follow-up information on donors' health status is vital for understanding the risks and consequences of donation. This information is crucial for individual donors who may need timely intervention if health issues arise. It also plays a significant role in educating potential donors and assisting them in making informed decisions about whether to donate. The literature concurs that we do not fully understand the long-term medical outcomes following kidney donation. This underscores the urgent need for continued data collection efforts, as it is only through such efforts that we can bridge this knowledge gap and ensure the safety of living donors.^{6,17,18,44,45} The best way to address concerns about long-term risks in previous donors, especially those with medical complexities, is through longitudinal prospective cohort studies. These studies would compare clinical outcomes in organ donors with non-donor controls, evaluating the donation's effect on these outcomes. Defining appropriate controls presents a significant challenge. Additionally, low event rates in living kidney donors hinder the detection of meaningful differences in clinical outcomes. Ongoing prospective studies are encountering difficulties in recruiting and retaining enough control subjects. The Living Donor Collective Project in the US aims to ensure long-term follow-up of living donors and potential candidates who did not proceed with donation.⁴⁶ If successful, the information gathered will be invaluable.

Retrospective cohort studies are a more efficient and cost-effective method for examining donor risk and are the main source of our current knowledge of LKD outcomes. However, most retrospective cohort studies suffer from low inclusion rates, short observation period rates, and limited racial diversity, making them susceptible to selection bias and limited conclusions.

A national donor follow-up registry offers a more comprehensive assessment of risks to living kidney donors than other studies since it focuses on the same community. It should provide outcome data on a much larger and more diverse population of LKDs than retrospective or prospective cohort studies. This approach offers sufficient power to determine if baseline characteristics predict worsened long-term health outcomes in subgroups of LKDs. In contrast to data linkage studies, a registry would not be limited to a small number of hard outcomes but could examine the broader scope of important health outcomes.⁴⁷ Additionally, it ensures quality assurance at all transplant centers, maintaining public trust in the system of living organ donation.⁴⁷

Some countries have successful models for tracking the long-term outcomes of living organ donors, especially those with universal healthcare systems. For example, all living donors in Switzerland are documented in the Swiss

Organ Living Donor Health Registry.⁴⁸ This registry collects information from general practice doctors one year after donation and then every two years. In Norway, donors receive free, lifelong medical follow-up, and details about each donor are stored in the Norwegian Living Kidney Donor Registry.⁴⁹ Australia and New Zealand also have similar universal healthcare systems and registries.⁵⁰ In Portugal, all LKD receive free lifelong medical follow-up, but a similar comprehensive registry does not yet exist.

The European Commission's EULID project was designed to establish consensus on living donor practices. It stated that "any registry proposal must be realistic and, therefore, simple; its implementation must be feasible".⁵¹ However, the European Union nations have national healthcare systems that ensure healthcare for all living donors and the protection of donors on an individual level. The same is not true for US donors, 18% of whom are uninsured, with higher rates among groups of donors who face more significant long-term health risks, including Blacks and Hispanics.⁵²

The Directive 2010/53/EU on standards of quality and safety of human organs intended for transplantation addresses these concerns. It advocates the establishment of National Living Donor Registries to facilitate monitoring and follow-up for Living Organ Donation Practices.⁴² It introduced a significant organizational change by establishing central registries in each Member State, overseen by the national competent authority. These registries will need two types of data from transplant centers. The first type concerns the number of living donors and the organs procured, transplanted, or disposed of. The national competent authority will use this data to create an annual report. The second type of data is related to post-transplantation information, focusing on the quality and safety of transplanted organs, including long-term follow-up of living donors. Transplant centers must collect and report this data based on guidance from the national competent authority.⁴² The directive was implemented into law through law 36/2013, dated 12th June in Portugal.⁴¹ The competent authority responsible for ensuring compliance with the law is the Direção Geral de Saúde, in collaboration with the Instituto Português de Sangue e Transplantação (IPST). The national registries for living kidney donors, including post-donation follow-up, would be carried out by local transplant Units in the Portuguese Registry of Transplantation (RPT). Unfortunately, this tool has yet to be available. Local registries of long-term follow-up of living donors are maintained at the

Transplant Centers without a Central Registry. This hinders a better understanding, monitoring, and analysis of living donor long-term outcomes beyond organ failure and death. The Registry of Centro Hospitalar Universitário de Santo António represents about 50% of the living donors in Portugal.^{53,54} It predominantly features individuals from the North of Portugal and only Caucasians, representing center-specific practices and limiting their generality. The authors argue that incorporating comprehensive and detailed national data on all living donors can improve the informed consent process.

Collecting data from living donors over a longer period could significantly enhance living donations. Monitoring the health of living donors throughout their lifetime helps us better understand the risks and benefits of living organ donation for the donor. This aligns with the recent World Health Organization Resolution (WHA 77.4, 2024) on increasing availability, ensuring ethical access, and overseeing the transplantation of human cells, tissues, and organs.⁵⁵ Additionally, it can promote the long-term well-being and safety of living donors by identifying risk factors and long-term outcomes, which can then inform living donor policy. This could lead to a more evidence-based approach to expanding opportunities for living donation while safeguarding donors.

CONCLUSION

Living kidney transplantation is the most effective treatment for patients with end-stage renal disease without severe comorbidities.

An assessment, as in-depth as possible, of the candidate donor's risks is mandatory.

Although current literature data allows us to avoid high risks for the candidate donor, efforts should be made to detail these risks more precisely. This can be achieved through retrospective and prospective registries of kidney donors, with comprehensive and long-term data.

Respect for the autonomy of the candidate donor requires that he or she be informed of both the prognosis of the person to whom he or she proposes to give a kidney and of his or her own risks. Only with this clear and complete knowledge can a person provide true informed consent.

Living kidney transplantation, to be correct, requires both great technical differentiation and high ethical demands. Only when these two requirements are present is it possible to overcome the Hippocratic principle *primum non nocere* and subject "a normal healthy person (...)" to a major surgical operation, not for his own benefit".¹

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29. Reese PP, Allen MB, Carney C, Leidy D, Levsky S, Pendse R, et al. Outcomes for individuals turned down for living kidney donation. *Clin Transplant*. 2018;32:e13408. doi: 10.1111/ctr.13408.
30. Van Pilsom Rasmussen SE, Henderson ML, Kahn J, Segev DL. Considering Tangible Benefit for Interdependent Donors: Extending a Risk-Benefit Framework in Donor Selection. *Am J Transplant*. 2017;17:2567-71. doi: 10.1111/ajt.14319.
31. Veríssimo R, Almeida M, Oliveira JP, Pereira PR, Ribeiro BO, Pedroso S, et al. Why Donors Do Not Donate: A Living Donation Program Experience. *Transplant Proc*. 2022;54:1197-201. doi: 10.1016/j.transproceed.2022.02.066.
32. Van Pilsom Rasmussen SE, Robin M, Saha A, Eno A, Lifshitz R, Waldram MM, et al. The Tangible Benefits of Living Donation: Results of a Qualitative Study of Living Kidney Donors. *Transplant Direct*. 2020;6:e626. doi: 10.1097/TXD.0000000000001068.
33. Park SC, Shapiro R, Good D, Lopez R, Shah N, Sood P, et al. The living donor evaluation as a life-saving event. *Clin Transplant*. 2023;37:e14885. doi: 10.1111/ctr.14885.
34. Lafranca JA, Spoon EQ, van de Wetering J, Ilzermans JN, Dor FJ. Attitudes among transplant professionals regarding shifting paradigms in eligibility criteria for live kidney donation. *PLoS One*. 2017;12:e0181846. doi: 10.1371/journal.pone.0181846.
35. Katz-Greenberg G, Shah S. Sex and Gender Differences in Kidney Transplantation. *Semin Nephrol*. 2022;42:219-29. doi: 10.1016/j.semnephrol.2022.04.011.
36. Global Observatory on Donation and Transplantation. International Report on Organ Donation and Transplantation 2021. [accessed Dez 2024] Available from: https://www.transplant-observatory.org/wp-content/uploads/2023/11/2022-data-global-report_VF_2.pdf
37. Katz-Greenberg G, Shah S. Sex and Gender Differences in Kidney Transplantation. *Semin Nephrol*. 2022;42:219-29. doi: 10.1016/j.semnephrol.2022.04.011.
38. Carrero JJ, Hecking M, Chesnaye NC, Jager KJ. Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease. *Nat Rev Nephrol*. 2018;14:151-64. doi: 10.1038/nrneph.2017.181.
39. Brar A, Markell M. Impact of gender and gender disparities in patients with kidney disease. *Curr Opin Nephrol Hypertens*. 2019;28:178-82. doi: 10.1097/MNH.0000000000000482.
40. Kurnikowski A, Krenn S, Lewandowski MJ, Schwaiger E, Tong A, Jager KJ, et al. Country-specific sex disparities in living kidney donation. *Nephrol Dial Transplant*. 2022;37:595-8. doi: 10.1093/ndt/gfab305.
41. Portugal. Lei n.º 2/2015 – Diário da República n.º 5/2015, Série I de 2015-01-08
42. European Union. Directive 2010/45/EU of the European Parliament and of the Council of 7 July 2010 on standards of quality and safety of human organs intended for transplantation. [accessed Jan 2025] Available at: <https://eur-lex.europa.eu/eli/dir/2010/53/oj/eng>
43. Ministério da Saúde, Gabinete do Secretário de Estado Adjunto do Ministro da Saúde. Diário da República, 2.ª série — N.º 40 — 26 de fevereiro de 2015 Despacho n.º 2055/2015, 26 de fevereiro.
44. Lentine KL, Schnitzler MA, Xiao H, Saab G, Salvalaggio PR, Axelrod D, et al. Racial variation in medical outcomes among living kidney donors. *N Engl J Med*. 2010;363:724-32. doi: 10.1056/NEJMoa1000950.
45. Cazauviel V, Moal V, Prudhomme T, Pecoraro A, Piana A, Campi R, et al. Psychological Impact of Living Kidney Donation: A Systematic Review by the EAU-YAU Kidney Transplant Working Group. *Transpl Int*. 2023;36:11827. doi: 10.3389/ti.2023.11827.
46. Kasiske BL, Ahn YS, Conboy M, Dew MA, Folken C, Levan M, et al. Outcomes of Living Kidney Donor Candidate Evaluations in the Living Donor Collective Pilot Registry. *Transplant Direct*. 2021;7:e689. doi: 10.1097/TXD.0000000000001143.
47. Lentine KL, Lam NN, Segev DL. Risks of Living Kidney Donation: Current State of Knowledge on Outcomes Important to Donors. *Clin J Am Soc Nephrol*. 2019;14:597-608. doi: 10.2215/CJN.11220918.
48. Thiel GT, Nolte C, Tsinalis D. Prospective Swiss cohort study of living-kidney donors: study protocol. *BMJ Open*. 2011;1:e000202. doi: 10.1136/bmjopen-2011-000202.
49. Haugen AJ, Hallan S, Langberg NE, Dahle DO, Pihlstrøm H, Birkeland KI, et al. Increased risk of ischaemic heart disease after kidney donation. *Nephrol Dial Transplant*. 2022;37:928-36. doi: 10.1093/ndt/gfab054.
50. Clayton PA, Saunders JR, McDonald SP, Allen RD, Pilmore H, Saunderson A, Boudville N, Chadban SJ. Risk-Factor Profile of Living Kidney Donors: The Australia and New Zealand Dialysis and Transplant Living Kidney Donor Registry 2004-2012. *Transplantation*. 2016;100:1278-83. doi: 10.1097/TP.0000000000000877..
51. Manyalich M, Ricart A, Martínez I, Balleste C, Paredes D, Vilardell J, et al. EULID project: European living donation and public health. *Transplant Proc*. 2009 Jul-Aug;41(6):2021-4. doi: 10.1016/j.transproceed.2009.05.021.
52. Lentine KL, Schnitzler MA, Garg AX, Xiao H, Axelrod D, Tuttle-Newhall JE, et al. Race, Relationship and Renal Diagnoses After Living Kidney Donation. *Transplantation*. 2015;99:1723-9. doi: 10.1097/TP.0000000000000733. .
53. Instituto Português do Sangue e da Transplantação [accessed Dez 2024] Available from: https://www.ipst.pt/files/PressReleaseIPST_AtividadeDoacaoTransplantacao2023_21Maio2024.pdf.
54. Sociedade Portuguesa de Transplantação. Annual Registry 2020. [accessed Dez 2024] Available from: <https://spt.pt/wp-content/uploads/2022/03/RSPT2020-RIM.pdf>.
55. World Health Organization. Increasing availability, ethical access and oversight of transplantation of human cells, tissues and organs. Geneva: WHO;2024.

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