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Fibrinogen A Alpha-Chain Amyloidosis: the landscape of dialysis and kidney transplantation in patients with the *FGA* p.Glu545Val variant and Portuguese ancestry

José Pedro Escaleira

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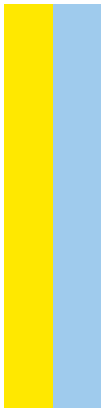

Fibrinogen A Alpha-Chain Amyloidosis: the landscape of dialysis and kidney transplantation in patients with the *FGA* p.Glu545Val variant and Portuguese ancestry

Dissertação de candidatura ao grau de Mestre em Medicina submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

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Dedication

Faço minhas as palavras escritas pela minha irmã, na dedicatória da sua tese de mestrado:

“À Família, por me continuar a ensinar “o amor pelas coisas belas”.
Aos Avós, o melhor do mundo e permanente fonte de inspiração.
Aos meus Amigos, que transformaram o ICBAS na minha nova casa e me fizeram viajar tantas vezes, sem sair do bairro.
À saudade do Porto e daqueles dias felizes, dos quais teimo em não me querer separar.”

Acknowledgements

Não podia deixar de agradecer à Professora Luísa Lobato o seu entusiasmo permanente. Senti que os desafios colocados despertaram em mim uma vocação que julguei acomodada. Foi graças ao seu espírito crítico e conhecimento profundo que uma verdadeira sinergia se criou, o que permitiu alargar os limites do trabalho à medida que novas "pistas" iam sendo encontradas.

Quero agradecer igualmente à Dra. Isabel Tavares, pela ajuda na colheita dos dados, mostrando sempre uma disponibilidade total. Os seus passos na caracterização da Amiloidose por Fibrinogénio em Portugal foram inovadores e uma fonte de inspiração.

Agradeço também à Dra. Josefina Santos e à Dra. Sofia Pedroso o tempo prestado em esclarecer dados importantes relativos a alguns doentes. O Dr. Pedro Gonçalves merece, adicionalmente, uma palavra de agradecimento.

Por fim, agradeço a toda a equipa do DEFI a simpatia e hospitalidade mostradas nos muitos dias lá passados.

Funding Sources

This work did not receive funds from research agencies or scientific societies. Collection of epidemiological and genetic data was possible by preceding support of the Portuguese Society of Nephrology and from UMIB-Unit for Multidisciplinary Research in Biomedicine, Instituto de Ciências Biomédicas Abel Salazar, University of Porto, granting research works referred along this dissertation.

RESUMO

Introdução: A amiloidose da cadeia Aα do fibrinogénio (Amiloidose AFib) compreende formas raras de amiloidose sistémica devido a mutações no gene codificador da cadeia Aα do fibrinogénio (*FGA*). O tipo de mutações no gene FGA pode influenciar a história natural da doença. A progressão para doença renal terminal é inevitável, sendo acompanhada por um atingimento sistémico extra-renal com uma relevância clínica desconhecida. No norte de Portugal, foi identificado, nas últimas duas décadas, um *focus* da amiloidose AFib causada pela mutação p.Glu545Val do gene *FGA.* Este estudo pretende, assim, descrever e clarificar o fenótipo da Amiloidose AFib p.Glu545Val nos doentes com ancestralidade portuguesa, abrangendo a relação entre o genótipo e os principais eventos clínicos ocorridos durante a terapia de substituição renal, quer seja a diálise ou o transplante renal.

Métodos: Realizámos um estudo retrospetivo dos doentes com amiloidose AFib seguidos no Centro Hospitalar Universitário do Porto desde o primeiro registo (1990) até maio de 2022. O primeiro doente registado foi diagnosticado definitivamente 11 anos após o follow-up. As informações recolhidas incluíram características demográficas e clínicas, bem como exames analíticos e imagiológicos disponíveis desde a apresentação até ao fim do follow-up. Adicionalmente, avaliámos as complicações a curto e longo prazo após o início da terapia de substituição renal, incluindo a transplantação renal.

Resultados: Foram incluídos 27 doentes (14 mulheres), pertencentes a 19 famílias diferentes, tratados com hemodiálise, diálise peritoneal, ou transplante renal. A mediana do follow-up foi de 15 anos. A maioria dos doentes era proveniente das regiões do Norte (Braga) e do Ave. Um novo local de origem, contudo, foi identificado em Trás-os-Montes. A existência de um diagnóstico inicial incorreto com Amiloidose AL/AH foi comum. A apresentação clínica englobou essencialmente hipertensão, proteinúria nefrótica e insuficiência renal. As principais alterações nos ecocardiogramas foram a hipertrofia ventricular esquerda, a dilatação da aurícula esquerda e a hipertrofia do septo intraventricular. A neuropatia, para além, da doença do trato digestivo superior, foi uma manifestação importante de atingimento sistémico extra-renal. A idade média de início da diálise foi aos 62.84 ± 8.94 anos. A rutura espontânea do baço correspondeu a uma complicação severa do período dialítico. O tempo mediano de follow-up após a transplantação renal foi de 4.9 anos, com sete enxertos funcionais até essa data (sobrevivência cumulativa de 68.26%). A taxa de nefrectomia dos enxertos renais foi de 30% após a transplantação, essencialmente devido a graves complicações hemorrágicas nos primeiros seis meses. A morbilidade cardiovascular progrediu durante o follow-up, evidenciada principalmente através da distensão da aurícula esquerda, fibrilhação auricular e acidentes vasculares cerebrais isquémicos.

Conclusões: A amiloidose AFib p.Glu545Val manifesta-se através de um envolvimento sistémico lento, mas sustentado, a par do atingimento renal durante a terapia de substituição renal. A diálise constitui uma boa opção de tratamento para os doentes com doença renal terminal. A recorrência de amiloide não foi uma causa de perda dos enxertos renais. Contrariamente, foram as hemorragias severas que levaram à realização de nefrectomias precoces dos enxertos. Este estudo ampliou as regiões de origem da doença, encorajando uma maior pesquisa da amiloidose AFib em Portugal.

Palavras-Chave: AFib E526V, coagulação, diálise, doença renal terminal, amiloidose da cadeia Aα do fibrinogénio, FGA p.Glu545Val, coração, hereditariedade, transplante renal, sobrevida, sistémico

ABSTRACT

Background: Fibrinogen A alpha-chain amyloidosis (AFib amyloidosis) comprises rare forms of systemic amyloidosis due to mutations in the fibrinogen A alpha-chain gene (*FGA*). The type of *FGA* gene mutation appears to influence the natural history of the disease. Progression to end-stage kidney disease (ESKD) is almost universal, accompanied by an extra-renal involvement of uncertain clinical relevance. In northern Portugal, a focus of AFib amyloidosis due to p.Glu545Val *FGA* mutation was identified in the last two decades. This study aims to describe and clarify the phenotype of AFib p.Glu545Val amyloidosis in patients of Portuguese ancestry, encompassing a relationship between the genotype and the main clinical events under renal replacement therapy (RRT), either dialysis or kidney transplantation.

Methods: We conducted a retrospective study of AFib amyloidosis patients observed at Centro Hospitalar Universitário do Porto from the first registry (1990) to May 2022. The first registered patient had the definitive diagnosis established 11 years after follow-up. Data included demographic and clinical features, along with available analytical and imagiologic exams at the presentation during the follow-up. In addition, short and long-term complications after RRT, including kidney transplantation, were assessed.

Results: The registry included 27 patients (14 females), belonging to 19 unrelated families, treated by hemodialysis, peritoneal dialysis, or kidney transplantation, with a median follow-up of 15 years. Braga/North region and Ave sub-region was the origin of the majority of patients. A new origin, in Trás-os-Montes, was also identified. Misdiagnosis with AL/AH amyloidosis was common. Clinical presentation consisted of hypertension, nephrotic proteinuria, and kidney failure. Echocardiographic alterations at the presentation were left ventricular hypertrophy, left atrial distension, and intraventricular septal hypertrophy. Neuropathy, besides upper gastrointestinal tract disease, was recognised important extra-renal involvement. The average age at dialysis onset was 62.84 ± 8.94 years. Spontaneous spleen rupture was a severe complication during the dialytic period. Median patient follow-up time after renal transplantation was 4.9 years, with seven still functional allografts at censor (cumulative survival rate of 68.26%). There was a graft nephrectomy rate of 30%, essentially due to uncontrolled allograft bleeding in the first six months after transplantation. Cardiovascular morbidity progressed during the follow-up, mainly with left atrial enlargement, atrial fibrillation, and ischemic strokes.

Conclusions: AFib p.Glu545Val amyloidosis evolves with a slow but steady systemic involvement along with kidney attainment and during RRT. Dialysis represents a good treatment choice for

patients with ESKD. Amyloid recurrence was not the cause of kidney graft lost, instead, uncontrolled hemorragic events lead to early graft nephrectomy. The study amplified the regions of origin of disease, encouraging a broader survey of AFib in Portugal.

Keywords: AFib E526V, clotting, dialysis, end-stage kidney disease, fibrinogen A alpha-chain amyloidosis, FGA p.Glu545Val, heart, hereditary, kidney transplantation, survival, systemic

List of Abbreviations

AA: Serum amyloid A protein
AAS: Acetylsalicylic acid (aspirin)
AF: Atrial fibrillation
AFib: Amyloid fibril fibrinogen Aα chain
AL: Amyloid fibril immunoglobulin light chain
ATTR: Amyloid fibril transthyretin
AVF: Arteriovenous fistula
AVP: Arteriovenous prosthesis
CHUPorto: Centro Hospitalar Universitário do Porto
CKD: Chronic kidney disease
CT: Computerized tomography
CVC: Central vein catheter
CVD: Cardiovascular disease
ECG: Electrocardiogram
EDLV: End diastolic left ventricular volume
EMG: Electromyography
ESKD: End-stage kidney disease
FGA: Fibrinogen Aα chain gene
FGA: Fibrinogen Aα chain protein
HCDD: Heavy chain deposition disease
HD: Hemodialysis
HF: Heart failure
IHC: Immunohistochemistry
ISA: International Society of Amyloidosis
IVS: Intraventricular hypertrophy
KT: kidney transplantation
LA: Left auricle
LKT: Liver and kidney transplant
LVEF: Left ventricle ejection fraction
LVH: Left ventricle hypertrophy
LVPW: Left ventricle posterior wall
MTHFR: Methylenetetrahydrofolate reductase gene
PD: Peritoneal dialysis
PER: Protein excretion rate
RRT: Renal replacement therapy
TIA: Transient ischemic attack
TTR: Transthyretin gene
UPER: Urine and serum protein electrophoresis

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INTRODUCTION

Amyloidosis is a group of diseases that result from the systemic or localized deposition of insoluble fibrils, derived from precursor proteins, in the extracellular spaces of tissues causing organ dysfunction.[1] So far, 36 different amyloid precursor proteins have been identified, nearly half associated with inherited genetic mutations.[2] These comprise just 10% of all cases, being rare and underdiagnosed forms of systemic amyloidosis.[3]

Kidney amyloid deposits may be derived from several precursor proteins, inherited or not, including immunoglobulin light chains (AL amyloidosis), amyloid A, fibrinogen Aα-chain, lysozyme, gelsolin, apolipoprotein A-I, apolipoprotein A-II, apolipoprotein A-IV, apolipoprotein C2, apolipoprotein C3, transthyretin (TTR) and leukocyte chemotactic factor 2.

However, fibrinogen A α-chain (AFib) amyloidosis[4,5] is emerging as the most common hereditary amyloid disease in Europe, apart from ATTR amyloidosis. Caused by autosomal dominant mutations in the gene encoding fibrinogen alpha subunit (*FGA*)[6], it targets a key component of the coagulation factor fibrinogen, structured by two sets of disulfide-bridged Aα-, Bβ-, γ- chains.[7]

Currently, 18 amyloidogenic *FGA* variants have been reported[8], the most common being the E526V (p.Glu545Val).[9] The origin of this amyloidogenic variant in diverse regions (United States, Europe, and Asia more rarely)[10] and its migration in the different populations are still unclear, albeit common *FGA* haplotypes amid Portuguese and Brazilian families, beyond others, were detected.[11]

Additionally, the first genotype-phenotype correlations have been made, indicating that the clinical expression in patients with AFib amyloidosis may be determined by the mutation type.[12] Renal amyloidosis is the predominant presentation[13], with patients typically developing late-onset chronic kidney disease (CKD) and end-stage kidney disease (ESKD) a few years after, regarding p.Glu545Val mutation.[14] Extra-renal amyloid involvement seems more vast than previously thought (heart, liver, spleen, nervous system, and large vessels)[15-19], despite the lack of consensus about its clinical relevance.[20-22]

Once fibrinogen plays an essential role in hemostasis, congenital fibrinogen disorders, either quantitative (afibrinogenemia, hypofibrinogenemia) or functional (dysfibrinogenemia), may cause bleeding or thrombosis.[23] This seems not to be the case of AFib amyloidosis, as functional and antigen fibrinogen levels are normal. However, as a mutant variant is circulating AFib amyloidosis some authors considered it a dysfibrinognemia.[24]

Renal replacement therapy and kidney transplantation (KT) are the mainstays of AFib amyloidosis treatment.[25] Even though KT outcome in patients with p.Glu545Val mutation seems

favourable[12], the risk of graft loss from recurrent amyloid remains.[26] Moreover, multiple organ short-term complications have been reported[27], but the correlation between long-term dialysis and KT outcome is poorly defined.[28] On the other hand, combined liver (where amyloidogenic AFib is produced) and kidney transplantation (KLT) carries a high burden of mortality and perioperative comorbidities.[29]

Therefore, this study aims to clarify the comorbid conditions and the main clinical events in Portuguese and Portuguese ancestry patients with AFib p.Glu545Val amyloidosis under renal replacement therapy, either dialysis or KT. The study expects to represent a benefit to guide the treatment choice, enclosing the relationship between phenotype and patient ancestry, especially from the most extensive known endemic *focus* worldwide (district of Braga, Portugal).[30]

METHODS

Study design and patients

We performed a retrospective study of AFib amyloidosis patients observed at Centro Hospitalar Universitário do Porto (CHUPorto), Portugal, from the first registry (1990) to May 2022. The present analysis included the patients who progressed to ESKD.

A skilled nephrologist team of CHUPorto established clinical connection with expertise nephrologists from Centro Hospitalar de São João and Hospital de Braga, both Hospitals in northern Portugal, to obtain supplementary information concerning patient's follow-up. Whenever possible, to complete records, clinical contacts with nephrologists from other regions of Portugal or abroad were established. Registries included also information from Brasil, France, United States of America and Switzerland.

The patients were over 18 years of age and had a genetically confirmed FGA amyloidogenic mutation. When genotyping was not possible to obtain, the patients were included in the presence of AFib amyloidosis documented in tissues (kidney or other specimen) and a first degree relative with the FGA amiloidogenic mutation and CKD.

The FGA mutation documented in all patients/families was the pathogenic variant FGA E526V (p.Glu545Val). All patients and families had Portuguese ancestry confirmed by the patient or/and their relatives.

The onset of follow-up was established as "clinical presentation", defined by the date of the first referral to a nephrologist. Demographic data and family history were collected by electronic health records and supplemented by directly review from the patients' registers by L.L. and I.T. The first record had the definitive diagnosis established 11 years after follow-up.

Clinical picture was evaluated based on kidney, cardiovascular, gastrointestinal, and neurologic morbidity; laboratory values; the first and last available electrocardiograms, upper endoscopy and colonoscopy, or radiologic exams reports. The registry included echocardiograms, kidney and abdominal computerized tomography (CT) or ultrasounds, and brain CT. Furthermore, we assessed the short and long-term complications after RRT, including kidney transplantation. Concerning kidney transplantation, we evaluated the survival and causes of graft losses and causes and events associated to death.

Supplementary definitions

ESKD corresponded to the late stage of chronic kidney failure, causing the patient's death if RRT was not initiated[31]. Nephrotic range proteinuria was defined as protein excretion rate (PER)

> 3500 mg/24 hours or PCR (protein to-creatinine ratio) > 3500 mg/g[32]. CKD was present if there was proteinuria with protein excretion ≥ 0.15g/24h or estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m2 for three or more months, as calculated by the CKD-EPI (CKD Epidemiology Collaboration) creatinine equation6. Fibrinogen levels were considered high if > 500 mg/mL and low if < 200 mg/mL[33].

Dimensions of cardiac chambers followed the measurements established by international recommendations[34], with left ventricle hypertrophy defined if septal or posterior wall thickness > 12 mm)[35]. Left atrial (LA) enlargement was present when LA with ≥ 38 mm, LA area ≥ 20 cm2, or LA volume ≥ 55 mL[36]. Regarding splenomegaly and hepatomegaly, criteria for its presence included: clinically palpable spleen or liver by abdominal examination on two occasions or by two physicians on the same occasion; longest spleen diameter ≥13 cm by US or >10 cm by CT[37]; and liver size > 15 cm by US or CT[38]. A regular kidney length was defined as between 10–12 cm[39].

Ethical statement

The study was performed in accordance with the ethical standards of the Declaration of Helsinki and it was approved by the Centro Hospitalar Universitário do Porto-ICBAS Ethics Committee [study reference 2021-299(245-DEFI/253-CE]. The use of data in this project was permitted by the Responsible for Personal Health Information of CHUPorto through the form *Reuse of Clinical Records for Research and Development*.

Statistical Analyses

Categorical variables are expressed as numbers and percentages; continuous variables are given as average ± one standard deviation if normally distributed and median [interquartile range] when non-normally distributed. The relationship between left atria (LA) size, intraventricular septum thickness (IVS), left ventricle posterior wall (LVPW), end diastolic left ventricular volume (EDLVV), left ventricle ejection fraction (LVEF), and liver size since first available echocardiogram/CT or echography were analyzed by Pearson correlation coefficient. Patient survival after the presentation was censored at the time of loss to follow-up, end of the study, or death. Patient survival and time from clinical presentation to ESKD were estimated by the Kaplan-Meier method. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, N.Y., USA), and a two-sided P-value < 0.05 was considered statistically significant.

RESULTS

Studied population

Demographic and genealogical features

There were 27 patients on RRT treated by hemodialysis, peritoneal dialysis, or kidney transplantation (14 females), as displayed in Table I. These belonged to 19 unrelated families, all Portuguese, except one Brazilian family though with Portuguese ancestry (subject 10). Patients 1, 2, and 18 were siblings (family 1), like patients 8 and 22 (family 2); 16 and 23 (family 3); 11 and 27 (family 4). Patient 24 was cousin of the latest mentioned subjects. Figure 1 represents a selected family tree.

In addition, during our research, one new patient was referred to L.L., despite not having CKD. We decided to include her exceptionally due to first reporting demographic and clinical characteristics. Hence, she will be mentioned in parallel whenever appropriate by the letter A.

Most subjects were born in Cantelães (Vieira do Minho), in the District of Braga (Figure 2). In this study, a new city, Murça, was added to patients origin in Portugal; the proband (patient A) had no previously known links to AFib amyloidosis in our country. Her sister, with ESKD and the same mutation, was identified in Switzerland, a country unreported to have Portuguese diagnosed patients. This way, Figure 3 summarizes the global widespread of the p.Glu545Val mutation, connecting all patients with Portuguese ancestry included in our study and other cases reported so far.

Mode of diagnosis

Kidney biopsy was performed in two-thirds of the cases (18 patients). Kidney histopathology showed a glomerular enlargement by extensive amyloid deposition, sparing tubular interstitium, and vessels. These characteristics are typically seen in AFib amyloidosis. Alternatively, four patients did salivary gland biopsy, but only one reported amyloid presence. Finally, two patients used other tissue samples for amyloid detection (ileum or abdominal fat).

Of note, kidney biopsy was not carried out in four patients. However, three of them had the p.Glu545Val mutation reported, and one had a first-degree family history of AFib p.Glu545Val amyloidosis. Mass spectrometry was only performed on one patient for amyloid type characterization. Instead, direct immunohistochemical staining of the amyloid deposits with an antibody against the fibrinogen A-chain was the primary mode for its characterisation. The mean age at genetic diagnosis was 64.05 ± 10.33 years (n=21), which highlights the late definitive genotype definition. Only patient 3 was homozygous for p.Glu545Val mutation.

Misdiagnosed and missed diagnosis cases

Nearly one-quarter of all AFib amyloidosis diagnoses were incorrect at the beginning of the follow-up by nephrologists, internal medicine or hematologists. Two patients (1 and 2) were initially suspected of having Heavy Chain Deposition Disease (HCDD). Patient 1 serum protein immunofixation showed monoclonal gammopathy (IgG *kappa*/*lambda*); serum and urine protein electrophoresis (UPEP) were normal. Patient 2 had increased urine *kappa* chain levels. Bone medullar biopsies were normal in both patients. Patient 1 kidney biopsy was interpreted as heavy chain deposition disease. Later, that biopsy was revisited, with AFib amyloidosis being identified. The other subject`s kidney biopsy showed amyloid deposition (AFib), ruling out the initial diagnosis.

AL amyloidosis was the first diagnosis in three cases (3, 4, and 6). No monoclonal immunoglobulin or plasma cell disorder was identified in patient 3. However, kidney biopsy showed abundant glomerular amyloid deposition, initially ascribed to AL. Patient 4 had a slight plasmacytosis in the medullar bone biopsy, and first interpretation of kidney biopsy was AL *kappa* amyloidosis. Patient 10 diagnostic exams were regular, apart from amyloid detection on kidney biopsy, also attributed to AL/AH amyloidosis. Moreover, the misdiagnosis motived that patients 3 and 10 started chemotherapy with melphalan and bortezomib, respectively, associated with corticoids.

Misdiagnosis did not resume to heavy or light chain nephropathies. Patient 9 first diagnostic suspicion was interstitial nephritis, presumably because of chronic non-steroid anti-inflammatory drugs (NSAIDs) therapy. Regarding subject 25, a nephrotic syndrome caused by lithium uptake for schizoaffective disorder was the primary explanation. Curiously, kidney biopsy was firstly understood as AA amyloidosis. Case A. was initially suspected of having a mitochondrial cytopathy.

Clinical landscape at presentation

Kidney findings and fibrinogen levels

Table I summarizes the main clinical features at the presentation. Hypertension (100%, n=26) and nephrotic proteinuria (85.2%) were the hallmark features, as well as chronic kidney failure (85.2%). Hypertension was diagnosed at a mean age of 53.7 ± 9.4 years (n=24), preceding in 5 years the presentation age (59.0 ± 8.9 years), on average. Mean proteinuria and serum creatinine consisted of 5.84 ± 3.17 g/day (n=23) and 2.6 ± 2.0 mg/dL (n=22), respectively. More than two-thirds of patients (70.4%, n=26) had a familial history of CKD. Features of CKD on ultrasound (US)

were detected in 41.1% of cases (n=17), divided between kidney atrophy (23.5 %) and increased cortical echogenicity (23.5%). No kidney enlargement was detected.

Among 11 patients, 10 had average fibrinogen values (mean of 406.6 ± 65.4 mg/dL, n=9), contrasting to the homozygous low levels. No deviations in coagulation assays were detected.

Heart morbidity

Ischemic heart disease was diagnosed in 25% of male patients (n=12) by heart scintigraphy or angiography in two cases. One patient suffered a myocardial infarction at 56 years (n=24). One year after, he suffered an episode of unstable angina while still on double anticoagulation. Rhythm disturbances had a low prevalence in our series, especially atrial fibrillation (5.6%, n=18). We did not detect any low voltage pattern on the patient's ECG.

On the other hand, morphological heart alterations ranked first in heart morbidity. Out of 18 cases, left ventricle hypertrophy affected two-thirds of the patients, followed by IVS hypertrophy (42.1%) and LA enlargement (31.6%). Of note, only one patient with IVS hypertrophy had increased LV volume.

Mild valvar disease was also common, mainly mitral valve regurgitation (31.6%). However, two patients underwent valve replacement surgery due to mitral stenosis or aortic stenosis. Notability, there were few cases of systolic (2) or diastolic dysfunction (3). Restrictive cardiomyopathy was not detected. No endomyocardial biopsies were performed. Further cardiovascular and other systems parameters are detailed in Table II.

Vascular disease

This aspect was possible to be studied in 24 patients. Among 24 patients, three (12.5%) had an ischemic brain stroke, one at 62 years in the right median cerebral artery; and the others at 61 and 63 years (patient 21 was anticoagulated with warfarin at the time). Six subjects had a registry of brain CT previous to presentation, one-third of which evidenced past lacunar strokes. One was detected at 54 years during a brain CT for suspected TIA in a patient medicated with warfarin. By comparing brain CTs, we defined that other patient must have had a lacunar stroke from 59 to 64 years, despite anticoagulation therapy with warfarin (patient 21).

Regarding systemic vascular disease, lower limb intermittent claudication was described in just one case (n=24). In addition, nine subjects had performed a cervical echo-Doppler, showing no signs of carotidal stenosis. One case of pulmonary embolism was reported at 49 years (no history of anticoagulation at the time). He (patient 7) had had a deep venous thrombosis in the past and was found to be heterozygous for *MTHFR* gene mutation.

Gastrointestinal and abdominal features

Upper digestive tract disease was the most relevant finding, combining more than 40% of cases (8) among 19 with this involvement. There was a registry of three subjects with known gastric biopsies (patients 5, 11, and 27), with H. pylori infection detected in one. Other, with no infection, the specimen was stained with Congo red for amyloid detection, albeit no deposits were found.

Hepatomegaly and splenomegaly with massive calcification deposits (Figure 4) were present in two (9.1%) and one subject (4.5%) among 22 patients, respectively. Nearly one-third (7 out of 22) showed a heterogenic liver aspect or increased echogenicity in US. Cholestatic hepatitis was the established diagnose in patient 3.

Neurologic involvement

About 6 (25%) among 24 subjects suffered from lower limb sensory peripheral neuropathy, manifested by paresthesia. Two patients performed electromyography (EMG), one of which was abnormal. In addition, carpal tunnel syndrome affected one patient. Regarding possible autonomic nervous involvement, one subject had a functional gastric motility disorder, 27.3% of male subjects had erectile dysfunction (n=11) associated with urinary incontinence, dizziness, or diarrhea in two of them.

Follow-up after renal replacement therapy

Dialysis course

Table III summarizes the ESKD patient's main features and complications. Two cases had no registry of the main clinical features on dialysis starting (n=25). Patients progressed to ESKD two years after presentation, in median (9 to 46 months). The average age at dialysis onset was 62.84 ± 8.94 years. None underwent preemptive kidney transplantation. Hemodialysis was the first modality choice in 22 patients. Three cases received peritoneal dialysis initially, but all eventually changed to hemodialysis due to recurrent peritoneal catheter infections or a peritoneal-pleural fistula development.

On the other hand, two patients (7 and 23) started peritoneal dialysis after hemodialysis mainly because of recurrent AVF or CVC thrombosis, both developing SVC syndrome and medicated with warfarin thereafter. Lupic anticoagulant and antithrombin III deficits were consequently detected in patient 8. However, there is no registry of a prothrombotic study in the other subject.

Regarding the dialytic period, four patients had no data (n=21). Overall, the most common complications were infections (28.6%), dialysis access thrombosis (23.8%), either in AVF/AVP (four

patients) or CVC (two patients), and AVF stenosis (23.8%). Patient 7, who had had recurrent thrombosis, was the heterozygous for the *MTHFR* gene mutation after. Indeed, no patient was anticoagulated when thrombosis occurred. Aneurysmal dilatation of vascular access was also common (23.8%), mainly related to AVF (four cases). AVF and CVC failures occurred in two and one patient, respectively. Post or intra-dialytic hypotension was identified in 19.0% of cases, followed by intra-dialytic hypertension (14.3%). Syncope or acute pulmonary edema occurred in four patients. AV hematomas were present in 14.3% of the subjects, two-thirds medicated with sole or combined aspirin with warfarin. However, two patients medicated with aspirin or clopidogrel and aspirin had no hemorrhagic complications. Curiously, only one patient had both thrombotic and hemorrhagic events during dialysis.

Despite rare, the most severe complications were splenic rupture immediately after hemodialysis start (patient 14). He developed hemorrhagic shock and underwent emergent splenectomy. Spleen's biopsy later showed large amyloid deposits. Of note, he was probably anticoagulated because of a previous iliac vein thrombosis three months. Patient 8 suffered from a right ventricle rupture during CVC related procedure. She underwent emergent cardiac surgery and developed Dressler syndrome after.

KT outcome

Nine patients underwent KT after a median of 4.1 years [2.9, 5.7] on dialysis. In total, 10 KT were performed at an average age of 60.8 ± 4.4 years. Median patient follow-up time after renal transplantation was 4.9 years, with seven still functional allografts at censor (cumulative survival rate of 68.26%). According to Kaplan-Meier analysis, the median allograft survival was 6.1 [0.5-16.3] years. There was a graft nephrectomy rate of 30%, essentially due to uncontrolled allograft bleeding in the first six months (patients 1, 2, and 5). Hemorrhagic events tended to occur soon after surgery while on hospital stay, but recurrent bleeding was also seen in one case (patient 1). Presentation with hemorrhagic shock or hematuria was universal. Allograft hematomas also contributed to allograft dysfunction, ending up infected or causing obstruction. Recurrent ureteral-hydronephrosis on patient 1 also decreased graft function.

Notably, all unsuccessful KT patients shared a history of anti-aggregating/anticoagulation therapy before or during the hospital stay, dictated by thrombotic/ischemic events: patient 1 suffered a myocardial infarction 48h after surgery and started clopidogrel and acetylsalicylic consequently; patient 2 was administered intraoperative heparin due to allograft artery lack of flux, besides acetylsalicylic acid; and patient 5 was already under combined warfarin with acetylsalicylic acid medication during the hospital stay because of past pulmonary embolism. In

addition, two biopsies were performed regarding the nephrectomised allografts. Patient 5 samples showed evidence of chronic pyelonephritis, renal vein thrombosis, and amyloid absence, and patient 2 histology showed 20% of infarcted area.

Other significant short-term complications reported were mainly renal allograft vein thrombosis (patient 2) and, surprisingly, native kidney spontaneous bleeding (patient 8), conditioning emergent nephrectomy. There was no rejection of kidney grafts. Table IV describes additional complications, including later ones (mainly infections). Two patients died during follow-up; in the case of homozygous patient (patient 3) the kidney allograft showed no dysfunction during all survival time (196 months), whereas patient 6 died 6.1 years after KT. He was submitted to a kidney biopsy before deceased, which showed no amyloid recurrence.

Patient comorbidity during ESKD: enclosing the big picture

Heart disease progression

Table II shows the main clinical features at the end of the follow-up. LA enlargement rose during that period, reaching 56.5% (13 out of 23 subjects), with a mean size of 47.1 ± 6.0 mm (n=11). Indeed, there seems to be a significant positive correlation between its size and the duration of the disease (figure 5). Four *de novo* cases of atrial fibrillation were detected, comprising 26.3% of patients (5 among 19). There was an 87.0% of LVH at the end of the study, followed by a 69.6% prevalence of IVS septal hypertrophy, with a mean overall IVS thickness of 13.0 ± 2.1 mm (n=16). Moreover, LV dilatation was uncommon, as only two patients were shown to have it. Figures 6 to 9 analyse the evolution of other echocardiographic parameters during follow-up. The mitral valve was again the most affected regarding the valvar disease, although closely followed by the aortic valve—one *de novo* mitral insufficiency complicated with acute pulmonary edema, leading to a programmed valve replacement.

Ischemic and bleeding events

At the end of the follow-up, 11 ischemic and one hemorrhagic stroke were detected in 11 patients aged 68.7 ± 6.7 years. (n=24), Three patients were medicated with warfarin or clopidogrel and aspirin at the time of the events. Another patient had had a previous deep vein thrombosis seven months before, but no registry of posterior anticoagulation was found. A similar case was seen in another patient diagnosed with a central retinal artery occlusion a few years after. Considering other shared features, 63,6% had left atrial enlargement, with 3 cases of AF also. *De novo* TIA was detected in just one patient (patient 1). Patient 1 suffered from a recurrent myocardial

infarction, at the age of 66. Central retinal artery occlusion occurred in two patients (at 49 and 67 years).

Concerning main bleeding events, lower digestive bleeding was diagnosed in two patients (angiodysplasia and diverticular hemorrhage), all under antiaggregating or anticoagulation. Patient 5 developed a retroperitoneal hematoma while on hospital stay after orthopedical surgery for a fracture in the upper limb. He also suffered from recurrent arm hematomas at the surgery local area. He was already medicated with warfarin and aspirin at the time. There were two spleen hemorrhages during a colectomy procedure, and the other was caused by spontaneous rupture.

Other systems review

Lower limb sensory neuropathy affected 8 out of 21 subjects at the end of the follow-up. There was a 28.6% prevalence of splenomegaly (n=21), three of which *were de novo*. The homozygous patient`s spleen atrophied during follow-up, curiously. Figure 10 shows the spleen evolution in two patients. Hepatomegaly was identified in four among 21 patients. There is a significant positive correlation between time and liver size. This feature is explored in figure 11.

Overall follow-up

Median follow-up time and survival were 15 and 11.4 [2.0-23.7] years, respectively, by the Kaplan-Meier analysis (figure 12); 15 patients died at an average age of 71 ± 9.2 years, one-third due to ischemic stroke. Other causes identified were two cases of sepsis, one case of pseudomembranous colitis associated with urosepsis; one plasmablastic lymphoma; one endocarditis; and one intestinal ischemia. Four patients had no cause elucidated.

DISCUSSION

Portuguese ancestry: new boundaries

AFib amyloidosis Portuguese patients were mainly clustered in the district of Braga, making it the most extensive focus of p.Glu545Val mutation in the world, as previously reported by I.T. and L.L.[6,11]. According to the same authors, a possible explanation for this may be the mountainous geography, leading to the population isolation in this northern area of Portugal.[11] Indeed, the discovery of Murça as a new patient's origin stretches AFib amyloidosis boundaries deep into the Trás-os-Montes region, another very mountainous area. This region could, therefore, have more unknown cases.

Interestingly, Trás-os-Montes and the districts of Viseu (patient 6 was from S. João da Pesqueira) and Braga make up the Portuguese part of the former Suevian Kingdom of Gallaecia.[40,41] The Iberian Suevians (Quadi tribe) migrated from the north of the middle Danube (what is now lower Austria and western Slovakia) to the western roman empire during the Great Migration period.[42] They were among the many Germanic tribes that fled the Huns, like the angles (originally from Poland) and Saxons. The latter two moved to England, one of the countries with the most reported cases of AFib p.Glu545Val Amyloidosis.[14,43]

The relationship between the Suevians and Saxons tribes is poorly understood, although it is known that at least a group of Suevians coming from West Scleswinbg also invaded Britain in the fifth century[42]. *These* Germanic tribes could, therefore, be a possible explanation for the European widespread of the disease, *as* a common founder for AFib p.Glu545Val amyloidosis has been recently suggested by the same haplotype found in Polish, Portuguese, English, Irish, and German families.[11]

Moreover, the following globalization with the expansion of the Portuguese and British Empires may have helped spread the p.Glu545Val mutation, namely to Brazil (patient 10) and other countries such as China, the USA, Canada, Australia, and New Zealand (figure 2). Besides that, Portuguese people living in the district of Braga have a continuous tradition of emigration, mainly to other European countries. This way, this first report of an AFib pGlu545Val Amyloidosis Portuguese patient living in Switzerland could unravel a new disease *focus*.

AFib amyloidosis diagnostic pitfalls

Our study highlights the insidious way a physician has to walk to achieve the correct diagnosis. AL amyloidosis misdiagnosis is common in AFib amyloidosis,[5,14,44,45] as monoclonal

gammopathy is not infrequent in the general population.[46] However, only one patient had such a feature and was not even suspected of having AL amyloidosis.

The main misleading feature in our series was the kidney biopsy immunofluorescence/immunohistochemistry staining. Nearly one-third of systemic amyloidosis is ambiguously diagnosed by IHC mainly due to high background staining, lack of epitope specificity, and/or epitope masking.[47] This could explain, for example, the cross-reaction of AFib amyloid with anti-*kappa* and anti-*lambda* antibodies. Besides that, the salivary gland seems not to be a good specimen for amyloid detection, as three out of four cases were negative; on contrast to ATTR amyloidosis, the salivary gland may be common local of deposition. Similar findings have been reported in another AFib amyloidosis patients.[26]

To our knowledge, we are the first to report two unusual presumed diagnoses- interstitial nephritis and lithium toxicity, later changed to AA amyloidosis due to kidney biopsy. These findings highlight the broad spectrum of differential diagnoses considered at the presentation and the importance of clinical and family history. These, together with the typical glomerular enlargement caused by selective amyloid deposition seen in kidney biopsy histology and the detection of the p.Glu545Val mutation detection, remain the best reliable way of diagnosing AFib amyloidosis.[47]

Clinical presentation: beyond nephropathy

Our study reaffirms the clinical profile commonly seen in the most extensive AFib p.Glu545Val amyloidosis studies to date:[5,6,12,14] patients with long-standing hypertension (5 years), presenting with nephrotic proteinuria and CKD at the end of the fifth decade of life, with average fibrinogen levels. We add one more important feature: the normality of the kidney ultrassonography in more than half of the cases. Besides that, a family history of CKD, when accurately collected is usually present, as I.T. and L.L. have previously reported.[6]

In our series, symptomatic extra-renal involvement at presentation was not unusual. It included mainly upper digestive tract disease, lower limb sensory neuropathy (25% of cases), erectile dysfunction in males (possibly related to dysautonomia), and ischemic brain strokes (3 patients). We, therefore, report a larger number of patients with sensory neuropathy than Meyer et al. [12] and tavareset al.[14], who detected no cases. However, our study did not find the enteric nervous system so extensively involved, as mentioned by Stangou et al. [5], that reported a wider spectrum symptoms.

The atherosclerotic disease seemed to be clinically less relevant, as only one patient reported a MI, besides two others diagnosed with IHD. In Stangou et al. series[5], only two MI cases before presentation were reported among 21 AFib p.Glu545Val amyloidosis patients. IHD

prevalence was higher, although overlooked as the presence of one or more risk factors for ischemic heart disease prompted a coronary angiography.

On the other hand, there were evident alterations in our patients' echocardiograms at presentation, like LVH (73.7%), LA enlargement (31.6%), and mitral valve regurgitation (31.6%). These findings were also described by Stangou et al. [5] during patient assessment for LKT, at a median age of 57 years, and by Gillmore et al. [14]. We, as in other studies, confirmed the absence of the classical electrocardiographic features seen in amyloidosis, reinforcing the deviations that this type of amyloidosis may have in relation to what is expected for these diseases. [5,6,12,14]

Renal replacement therapy complications spectrum

Dialysis is a good treatment choice

The median time from presentation to ESKD was two years, evidencing patients' fast lost of kidney function after the presentation. Other studies that estimated median times from AFib p.Glu545Val amyloidosis reported a range from 8[5] months, 1.4[47] years, 2[12] years, to 4.6[14] years. However, the latter is shortened to two years when was considered after the diagnosis.

The main complications were infections and those related to the vascular access (either thrombosis or stenosis) in hemodialysis or the technique failure in PD, which is in line with the general population receiving dialysis.[48-50] Recurrent thrombotic events were associated with a pro-thrombotic state in two patients. These included a heterozygous mutation in the *MTHFR* gene, lupic anticoagulant, and antithrombin III deficit. Hemorrhagic complications could be explained by concurrent anticoagulation/anti-aggregating medication (as seen in the two-thirds of patients who had AV hematomas).

The only complication linked to amyloid deposition detected during the dialytic period was spontaneous spleen rupture. Curiously, such findings have already been reported in one case during hemodialysis.[5] Therefore, physicians should pay attention to this rare but serious potential complication.

Kidney allograft survival

In our series, the median allograft survival was 6.1 years. A similar result was found by Gillmore et al. [14] (5.6 years) and Stangou et al. [5] (5.9 years). Meyer et al.[12] reported a significantly larger survival of 8.5 years, however.

We have shown that the short-term complications after KT play a significant role in the graft failure rate, as previously detected by I.T. and L.L.[27]. Indeed, three out of ten ended up in nephrectomy due to severe hemorrhagic events in the first six months.

One possible explanation could be the use of anti-aggregating/anticoagulation drugs in the perioperative period, a shared feature seen in all our unsuccessful KT patients. There are conflicting conclusions regarding its influence on bleeding events: one extensive study states that postoperative heparin infusion and vitamin K antagonists are associated with increased bleeding risk, contrary to intraoperative heparin and antiplatelet therapy.[51] Another study finds only postoperative heparin infusion as a risk factor[52], whereas two other studies concluded that chronic perioperative anticoagulation led to an increased risk of bleeding.[53,54]

An alternative explanation, could instead be supported by a particular susceptibility to anticoagulation/anti-aggregating therapy. It is known that AFib amyloidosis does not present with a clear hemorrhagic or thrombotic tendency. However, the previously described events may resemble the hemorrhagic and/or thrombotic phenotype seen in some congenital dysfibrinogenemia.[55] In fact, the occurrence of thrombotic events shortly after KT (MI and renal vein thrombosis) increases, even more, our doubts regarding the clotting behaviour in this form of amyloidosis following major procedures like surgeries. Short-term complications included three spontaneous perioperative splenic rupture cases and two deep vein thrombosis in the other series.[5] Meyer et al.[12] also reported a sudden cardiac arrest after KT but do not specify when it exactly happened. In the Gillmore et al. study[14] , three grafts failed for technical reasons, again without explicit characterization.

We conclude, therefore, that amyloid recurrence was not a cause of graft loss in our series. However, our median follow-up after K.T. was 4.9 years, and recurrence of AFib amyloidosis and subsequent graft loss occurs on average 7.3 years after transplantation.[29] There is even a case of sequentially graft failure due to recurrence.[14] In our series, the homozygous patient, lived almost 16 years with a functional kidney allograft, and patient 6 had no amyloid deposition in kidney graft biopsy at the time of death (6.1 years after K.T.) The reason why some patients suffer from amyloid recurrence and others apparently do not remains unknown, although in case of frame-shift mutations, on contrary to single amino acid substitutions as p.Glu545Val, a rapid reoccurrence of amyloid in kidney transplants seems more likely.

AFib amyloidosis puzzle: a new way to put together

The clinical picture taken at the end of the follow-up, after a median of 15 years, differs from the one seen at the presentation (figure 13). Heart morbidity was higher, with de novo cases being detected in multiple features: left atrial enlargement was present in more than half of the patients, intraventricular septal hypertrophy was quite prevalent, as well left ventricular

hypertrophy. Ischemic stroke followed the same rising tendency, with seven de novo cases. Three new cases of heart failure were detected.

There are currently two ways of interpreting the high burden of cardiovascular disease seen in AFib amyloidosis. On the one hand, we cannot underestimate the dramatic impact that ESKD has on accelerating atherosclerosis.[56] In fact, the mortality due to CVD is 20 times higher among patients in hemodialysis than in the general population.[57] Gillmore et al.[14] , hence, compare the high prevalence of atherosclerotic cardiovascular disease among AFib patients to patients with chronic kidney disease.[14] Additionally, they do not link the echocardiographic changes seen by Stangou et al.[5] (impaired relaxation pattern, increased wall thickness, and/or reduced ejection fraction) to cardiac amyloidosis.

On the other hand, the later authors carried out a more systematic clinical assessment of 20 among 21 patients included in the series of Gillmore et al.[14] They concluded that the atheromatous disease seen could be provoked by AFib amyloidosis itself, once they discovered the presence of AFib amyloid in the vascular walls and atheromatous plaques. Cardiac amyloidosis was, in this way, an important extra-renal feature. I.T. and L.L[19], who have previously studied many of the patients included in our series over a median follow-up period of eight years, have also proposed a similar explanation.

From our perspective, there can be a middle term depending on what time perspective is chosen. Indeed, the cardiac morbidity detected at presentation did not translate into a clear clinical relevance. The median follow-up in Gillmore et al.[14] was just four years, which may have precipitated their conclusions. In contrast, our median follow-up time was 15 years, which enabled us to get a wider clinical landscape of the disease progression. This is the furthermost extensive follow-up time reported in an AFib amyloidosis patient series.

This way, the widespread atherosclerotic deposition may not be so important as first thought because the leading cause of cardiovascular morbidity was probably embolic. Nearly 63.6% of patients with ischemic stroke had left atrial enlargement, with three cases of AF diagnosed de novo. Besides that, the intestinal ischemia and central retinal eye occlusion could be manifestations of atrial fibrillation. Atrial enlargement, together with a probably even higher rate of atrial fibrillation (underdiagnosed at presentation), was a hallmark of the AFib amyloidosis in ESKD patients from our series. Mitral valve disease was also common, mainly mitral regurgitation. This, together with an apparently main endocardiac and perivascular AFib deposition pattern (evidenced by low intraventricular conduction disturbances, absence of low voltage QRS pattern, and a few systolic dysfunctions), could be explained by atrial cardiac amyloidosis.[58] Moreover, the most known pro-thrombotic factors in systemic amyloidosis are heart failure, atrial fibrillation, and atrial

myopathy.[59] Overall, cardiac involvement in AFib amyloidosis may have a slow but steady progression similar to the one seen in the kidneys.

The choice to anticoagulate these patients when needed may be challenging, as, in our series, there were bleeding events associated with anti-aggregating/anticoagulation medication. Besides that, in many cases, such therapy could not prevent major thrombotic events. Whether this is linked to a fibrinogen dysfunction itself remains unknown. Furthermore, another essential extra-renal involvement found during the follow-up was splenomegaly, which can complicate spleen rupture, as previously explained. Considering the risk of systemic AFib amyloid deposition, as seen, and its recurrence in the kidney graft, LKT has been proposed as a choice. However, the price to pay for a definitive cure[5] is a high burden of perioperative mortality.[29] Furthermore, if the LKT is performed in ESKD, the extent of amyloid widespread cannot be reverted, together with the cardiovascular morbidity. A possible solution may be a preemptive LKT at the first signs of CKD, as proposed by Delabre et al, but the procedure in a asymptomatic gene carrier or in a patient with very mild disease it is not an easy option.[26]

In conclusion, our series reinforces that AFib amyloidosis is not solely nephropathy but a systemic disease with a broad spectrum of extra-renal involvement. Dialysis is a good option for ESKD treatment, even though it does not prevent AFib amyloid deposition. In addition, there is a hemorrhagic/thrombotic phenotype trait that must be more deeply studied to improve KT outcomes. Finally, we further characterized and augmented the Portuguese regions of disease, promoting new ways to broaden our focus and encouraging the exploration of specific areas of the Iberian Peninsula.

APPENDIX

Table I. Patient demographics and clinical characteristics at presentation. Abbreviations: PT, patient; Y; years; bx, biopsy; HTN, hypertension; pCr, plasmatic creatinine; MNG, multinodular goiter; In echo, increased kidney echogenicity; KA, kidney atrophy; AP, acute pancreatitis; EE-erosive esophagitis; CG, Chronic gastritis; EG, erosive gastritis; Hm, Hepatomegaly; Sm, splenomegaly; DH, digestive hemorrhage; LLP, lower limb paresthesia; ICla, Intermittent claudication; HF, heart failure; HT, Hashimoto thyroiditis; AVB, atrioventricular block; AoS, aortic stenosis; LAFB, left anterior fascicular block; AoI, Aortic insufficiency; FGD, function gastric disorder; ED, erectile disfunction; UI, urinary incontinence; Inc, incomplete; RBB, right bundle block; D; diabetes *mellitus;* TIA, transient ischemic attack; PE, pulmonary emboly; DVT, deep vein thrombosis; SG, salivary gland biopsy; LBhB, left bundle hemiblock; DD, diastolic disfunction; AF, atrial fibrillation; MvF, Mitral valve insuffiency; IS, ischemic stroke; End, endocarditis; AbF, abdominal fat; Mech, mechanical; MvP; mitral mechanical prostheis; Echo, echocardiogram; ECG, electrocardiogram; IVS, intraventricular septum; LVPW, left ventricle posterior wall; AG, atrophic gastritis; MS, mitral stenosis; n.a., non-available; LA, left auricle. Notes: y, had nephrotic proteinuria; z, had CKD; x, had HTN; d, no other data; *, no amyloid deposits found. Patients 10, 14, 15, 16, 17, 18, 20, 24, 27 had no registry of an ECG made until or at presentation. Concerning the first available echocardiogram, patients 12, 14, 15, 16, 17, 18, 24, 25. No data about LLP for patients 15, 17, 18, 21, and 24.

TABLE I. PATIENT DEMOGRAPHICS AND CLINICAL CHARACTERISTICS AT PRESENTATION.

PT NO.	SEX	AGE AT HTN DIAGNOSIS, Y	AGE, Y	PROTEINURIA, G/24H	PCR, MG/DL	COMORBIDITIES	RENAL ECHO	CARDIAC ECHO/ECG FINDINGS (IVS, LVPW IN MM)	MODE OF DIAGNOSIS	RENAL FAMILY HISTORY
1	M	57	60	7.80	7.5	AP, MNG, ICla, MI, EG, EE, Hm	Normal	LVH, EF 50%, LA 48, IVS 12, LAFB, MvI, MvS, AoI	Renal bx	Positive
2	F	50	52	4.00	0.7	MNG, asthma, leukopenia, EG, EE	Normal	Normal	Renal bx	Positive
3	F	44	44	7.50	4	Hm, Sm, superior DH, LLP	KA	LVH	Renal bx	Negative
4	F	56	60	5.10	1.6	MNG, HT, asthma	In echo	DD, MvI	Renal bx	Negative
5	M	51	51	8.92	1.38	EG, EE, FGD, LLP	Normal	LVH, AVB, , IVS 13, AoS, AoI	Renal bx	Negative
6	M	47	50	5.80	5.9	ED, Diahrrea, UI, LLP	Normal	LVH,LA 42,inc RBB, MvI, MvS, AoS	Renal, SG bx	Negative
7	M	47	57	8.20	2.78	CG, Pre-D, TIA, PE, DVT, ED, UI	Normal	LVH, LA 54, IVS 13, MvI	SG Bx*	Positive
8	F	52	57	4.30	1.8	LLP, carpal tunnel syndrome	Normal	LVH, Pulmonary HTN	Renal bx	Positive
9	F	n.a.[x]	63	n.a.[y]	7.8	Femoral head necrosis	KA	LVH, LBhB, LA 46, IVS 13, MvI, AoI	SG Bx*	Negative
10	F	n.a.[x]	56	4.00	2.4	LLP	n.a.	Normal	Renal bx	Positive
11	M	48	62	2.80	1.79	CG	n.a.	LVH, IVS 12	Ileum bx	Positive
12	M	71	71	3.50	n.a[z].	pancitopenia, gastric ulcer	n.a.	n.a.	Renal bx	Negative
13	F	47	52	4.72	1.3	D	n.a.	LVH, IVS 12	Renal bx	Positive
14	M	56	77	5.70	1.2	Pacemaker, deafness, LLP	n.a.	AVB	Renal bx	Negative
15	M	n.a.	47	5.00	Normal	Ischemic heart disease.[d]	n.a.	n.a.	Renal bx	n.a.
16	M	30	62	5.70	n.a.	IS	In echo	n.a.	No bx	Positive
17	F	50	55	2.00	n.a.	Normal	n.a.	n.a.	Renal bx	Positive
18	M	n.a.[x]	48	n.a.[y]	2.6	n.a.	n.a.	n.a.	Renal bx	Positive
19	M	64	68	8.87	n.a[z].	HF, ischemic heart disease, IS	Normal	Normal	SG Bx*	Negative
20	M	58	58	11.00	3.1	EG	KA	LVH, EF 36%, DD, LA 44, MvI	Renal bx	Positive
21	F	55	65	7.06	1.61	MvP, D, IS, End, AF	Normal	LVH, Dilated LA,MS	No bx	Positive
22	F	58	69	1.63	1.86	Normal	n.a.	LVH, IVS ≥ 12	No bx	Positive
23	F	63	63	3.20	n.a.[z]	MNG	In echo	Normal	AbF bx	Positive
24	F	71	71	2.50	0.8	n.a.	Normal	n.a.	Renal bx	Positive
25	F	46	58	n.a.[y]	1.24	HF	Normal	n.a.	Renal bx	Positive
26	M	45	45	15.05	2	D	n.a.	LVH, IVS 15	Renal bx	Positive
27	F	67	73	n.a[y]	2.3	CG, gastric ulcer	KA/In echo	LVH, pericardic effusion	No bx	Positive

Table II. Clinical features of the studied patients at presentation and/or at the end of the follow-up. Abbreviations: ECG, electrocardiogram. Notes: values for categorical variables give as mean; for continuous variables, as count (%). a-one patient performed the first ECG after presentation only. On the other hand, three subjects have an ECG report just at presentation; b-From all selected echocardiograms, four subjects performed the first after presentation; c-Three upper and lower endoscopies were performed only at the end of follow-up; d- One patient did not have the registry of the first abdominal echography/computerized tomography.

Table II. Clinical features of the studied patients at presentation and/or at the end of the follow-up

Characteristic	At the presentation	Until the follow-up end	*De novo*[1]
Cardiovascular morbidity	**N=24**	**N=24**	
Acute coronary syndrome/ Heart failure	1/1 (4.2%)	2/4 (8.3/16.3%)	1/3
Ischemic heart disease **(N=25)**	3 (12%)	3 (12%)	0
Ischemic stroke/Hemorrhagic stroke	3/0 (12.5%)	11/1 (45.8/4.2%)	7/1
	N=6	**N=12**	
Lacunar stroke	2 (33.3%)	4 (33.3%)	-
Conduction disturbances	**N=18**	**N=19**	**a**
Atrial fibrillation	1 (5.6%)	5 (26.3%)	4
Atrioventricular block	1 (5.6%)	2 (10.5%)	1
Left bundle block or hemiblock	2 (11.1%)	3 (15.8%)	0
Right bundle block	1 (5.6%)	1 (5.3%)	0
Pacemaker **(N=24)**	1 (4.2%)	1 (4.2%)	0
Morphological alterations	**N=19**	**N=23**	**b**
Left ventricular hiperthrophy	14 (73.7%)	20 (87,0%)	1
Intra ventricular septal hipertrophy	8 (42.1%)	16 (69.6%)	5
Mean intra ventricular septal thickness (mm)	11.6 ± 2.0 (N=11)	13.0 ± 2.1 (n=16)	-
Left atrial enlargement	6 (31.6%)	13 (56.5%)	5
Mean left atrial size (mm)	42.7 ± 8.1 (N=7)	47.1 ± 6.0 (n=11)	-
Increased posterior wall thickness	0	5 (21.7%)	4
Mean posterior wall thickness (mm)	9.8 ± 1.2 (N=6)	11.4 ± 2.1 (n=10)	-
Systolic dysfunction	2 (10.5%)	5 (21.7%)	1
Diastolic dysfunction	3 (15.8%)	5 (21.7%)	1
Valvar disease			
Mitral insufficiency	6 (31.6%)	10 (43.5%)	1
Mitral stenosis	3 (15.8%)	5 (21.7%)	2
Aortic insufficiency	3 (15.8%)	8 (34.8%)	4
Aortic stenosis	2 (10.5%)	8 (34.8%)	4
Systemic vasculature	**N=24**	**N=24**	
Intermittent claudication	1 (4.2%)	2 (8.3%)	1
Deep/superficial vein thrombosis	1 (4.2%)	2/1 (8.3/4.2%)	1/1
Pulmonary emboly	1 (4.2%)	1 (4.2%)	0
Central retinal artery occlusion	0	2 (8.3%)	2
Gastrointestinal disorders	**N=19**	**N=21**	**c**
Erosive gastritis	3 (15.8%)	4 (19.0%)	3
Peptic ulcer	2 (10.5%)	3 (14.3%)	0
Erosive esophagitis	3 (15.8%)	3 (14.3%)	1
Chronic gastritis	3 (15.8%)	7 (33.3%)	2
	N=24	**N=24**	
Ischemic Colitis/ Mesenteric Ischemia	0	1/1 (4.2%)	1/1
Upper or lower digestive bleeding	0	3 (12.5%)	3
Organomegalies	**N=22**	**N=21**	**d**
Hepatomegaly	2 (9.1%)	4 (19%)	1
Splenomegaly	1 (4.5%)	6 (28.6%)	3
Spleen hemorrhage **(N=24)**	1 (4.2%)	2 (8.3%)	1
Neurological morbidity	**N=24**	**N=24**	
Peripheric nervous system			
Lower limb sensory neuropathy	6 (25.0%)	8 (33.3%)	2
Carpal tunnel syndrome	1 (4.2%)	1 (4.2%)	0
Autonomic nervous system			
Erectile dysfunction **(N=11)**	3 (27.3%)	3 (27.3%)	0
Gastric motility disorder/ Diarrhea	1/1 (4.2%)	2/1 (8.3/4.2%)	1/0
Urinary incontinence	2 (8.3%)	2 (8.3%)	0
Other systems review			
Multinodular goiter	3 (12.5%)	4 (16.7%)	1
Diabetes *mellitus*	3 (12.5%)	3 (12.5%)	0

Table III. Patient characteristics and complications during dialysis. Abbreviations: Pt, patient; y; years; mo; months; CV, cephalic vein; PPF, peritoneal-pleural fistula; SVC, superior vena cava; LTRI, lower tract respiratory infection; AVF, arteriovenous fistula; AVP, arteriovenous prosthesis; PD, peritoneal dialysis; HD, hemodialysis; n.a., non-available; CVC, central venous catheter. Notes: Patients 18 and 15 had no data concerning dialysis. For the cases that returned to dialysis after an unsuccessful KT, the dialysis period is divided between the time before and after KT.

Table III. Patient characteristics and complications during dialysis

PT NO.	AGE AT DIALYSIS ONSET, Y	TIME FROM PRESENTATION TO ESKD, MO	DIALYSIS MODALITY	MAIN COMPLICATIONS	DIALYSIS DURATION, YEARS	KT
1	60	1	HD	Acute pulmonary edema, recurrent LRTI	4.5+5.3	Yes
2	56	49	PD, HD	Recurrent CVC and PD access infections	2.8 + 1.9	Yes
3	44	20	HD	None	5.6	Yes
4	64	48	HD	Intra and post-dialytic hypotension, intradialytic hypertension, syncope, AVF hematoma and CV aneurism	1.8	No
5	57	72	HD	Syncope, recurrent post-dialytic hypotension, AVF hematoma, brachial artery pseudoaneurysm	3.8 + 5.1	Yes
6	51	1	HD	AVF thrombosis, intra and post dialytic hypotension, intradialytic hypertension	6.3	Yes
7	59	21	PD, HD	Recurrent AVP thrombosis, AVP stenosis, acute pulmonary edema, abdominal hernia, PPF	3.2	Yes
8	59	22	HD, DP	Recurrent AVF thrombosis, CVC thrombosis, AVF stenosis, recurrent AVF aneurisms, CVC infection, SVC syndrome LRTI, ventricular rupture	5.9	Yes
9	63	0	HD	LRTI	3.0	Yes
10	57	25	HD	None	5.4	Yes
11	70	88	HD	None	4.0	No
12	73	30	HD	AVF thrombosis, AVF stenosis, AVF hematoma, AVF pseudoaneurysm	8.3	No
13	53	10	HD	None	8.1	No
14	79	32	HD	Splenic rupture immediately after HD	0.4	No
16	66	44	HD	AVF failure*	7.7	No
17	69	165	HD	n.a.	10.3	No
19	69	7	HD	AVF stenosis, AVF aneurism	1.8	No
20	60	17	HD	AVF stenosis	8.0	No
21	68	28	HD	None	8.1	No
22	72	36	HD	None	0.7	No
23	70	89	HD, PD	CVC thrombosis, recurrent CVC failure, AVF failure, PD access infection, SVC syndrome	4.2	No
24	72	17	HD	n.a.	27.3	No
25	60	24	HD	Recurrent intradialytic hypo and hypertension, pneumonia, CVC infection	8.8	No
26	46	8	PD, HD	n.a.	6.6	No
27	74	7	HD	n.a.	0.6	No

Table IV. KT outcome and anticoagulation/anti-aggregating therapy interface. Abbreviations: Pt, patient; y; years; mo, months, KT, kidney transplantation; AAS, aspirin; UFH, unfractionated heparin; MI, myocardial infarction; UA, unstable angina; UrH, ureterohydronephrosis; PN, pyelonephritis; UTI, urinary tract infection; CMV, cytomegalovirus Notes: Patient 7 had bladder rupture during surgery due to the previous retention in ureteroneocystostomy.

Table IV. KT outcome and anticoagulation/anti-aggregating therapy interface

Pt no.	Age at KT, y	Anticoagulation/ Anti-aggregating before KT	Intraoperative heparin	Anticoagulation/ Anti-aggregating after KT	Main complications during the hospital stay	Anticoagulation/ Anti-aggregating after charge	Later complications	Graft survival time/mo
1	64	AAS	No	AAS + UFH	MI 48h after KT, UrH, and hematuria, with hemorrhagic shock after renal allograft nephrostomy, renal allograft hematoma and abscess, LTRI	AAS + clopidogrel (started after MI)	Recurrent hematuria and UrH, renal allograft bleeding leading to emergent nephrectomy	6
2	59	AAS	Yes	AAS	Allograft bleeding 24h after KT, renal allograft hematoma and abscess, renal allograft vein thrombosis and infarction	AAS	Absence of renal allograft function recovery leading to nephrectomy	<2
2	61	None	No	None	Renal allograft lymphocele and infarction	None	CMV infection, recurrent renal allograft PN and UTI	-
3	51	None	n.a.	None	None	None	None	195
5	60	AAS + Warfarin	No	AAS + UFH	Renal allograft bleeding eight days after KT leads to emergent nephrectomy, native kidney spontaneous bleeding leading emergent nephrectomy, abscesses at surgery *loca*	AAS + Warfarin	-	<1
6	57	None	No	None	LTRI, moderate renal allograft dysfunction (no need for dialysis)	None	Renal allograft lymphocele, BK nephropathy	73
7	62	Aspirin	No	Aspirin	None	Aspirin	None	-
8	65	Warfarin	No	UFH	None	Warfarin	Recurrent UTI	-
9	66	None	No	None	Slow renal allograft function recovery (need for dialysis); surgical wound infection		Recurrent UTI, urinary obstruction	-
10	63	None	n.a.	None	None	None	CMV infection	-

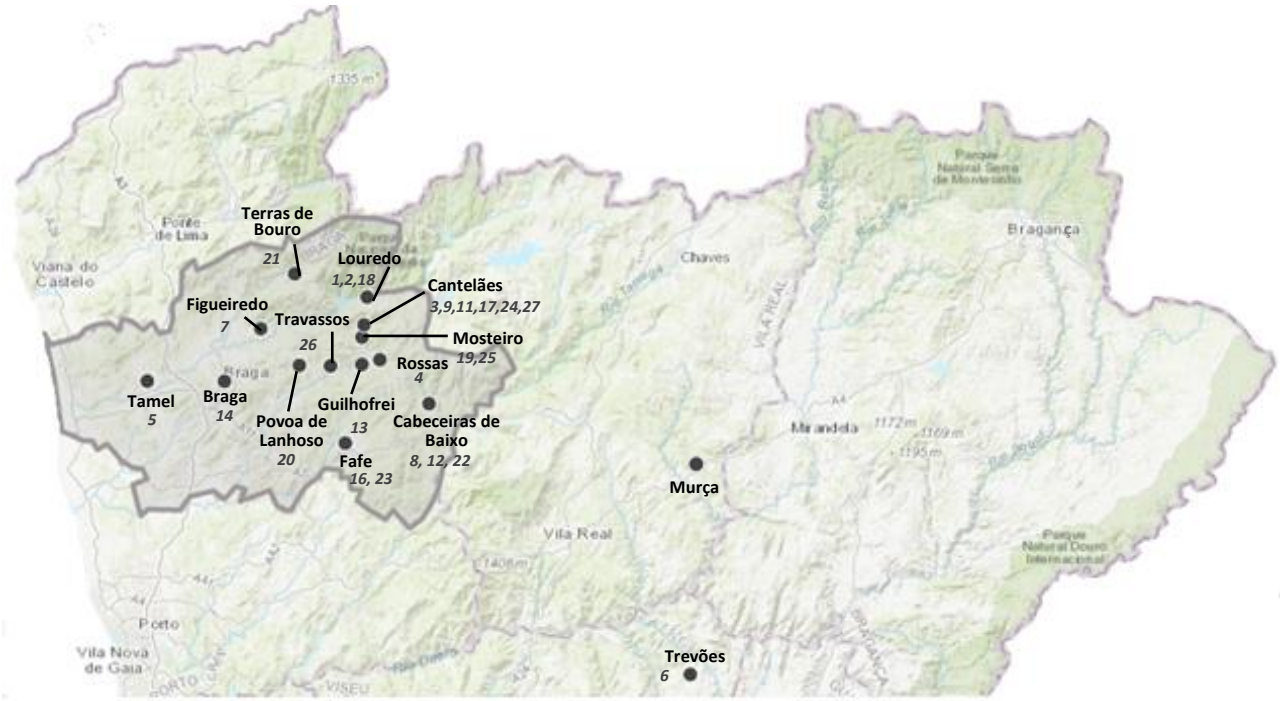

Figure 1. AFib amyloidosis Portuguese resident patient's birthplaces, with respective patient numbers underneath. The newly found patient was born in Murça, where no known cases were previously reported, stretching AFib amyloidosis boundaries deep into the Trás-os-Montes region. The District of Braga comprises the demarked area.

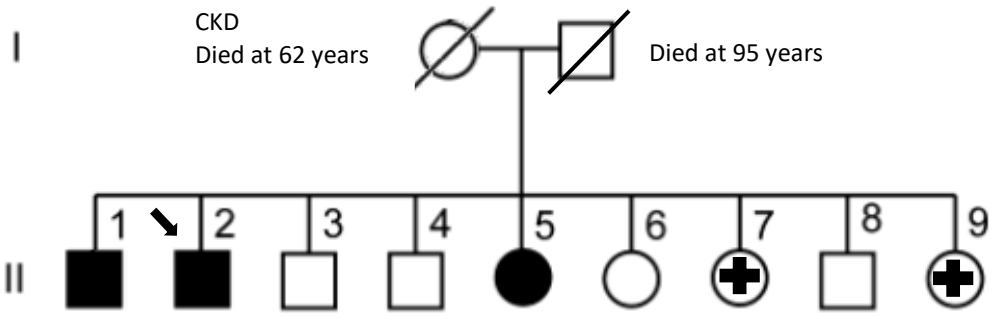

Figure 2. Family 1 tree. Patients 1, 2, and 3 correspond to patients 18, 1 (dark arrow), and 2 of our study. The dark cross identifies two of the other three tested sisters with the p. Glu545Val mutation. They lived in Luxembourg, with no known CKD until 2017.

Figure 3. Global distribution of the p.Glu545Val mutation in the *FGA* gene. Notes: countries coloured in yellow have AFib p-Glu545Val amyloidosis cases reported. Dotted lines connect widespread AFib amyloidosis patients with that mutation and Portuguese ancestrally

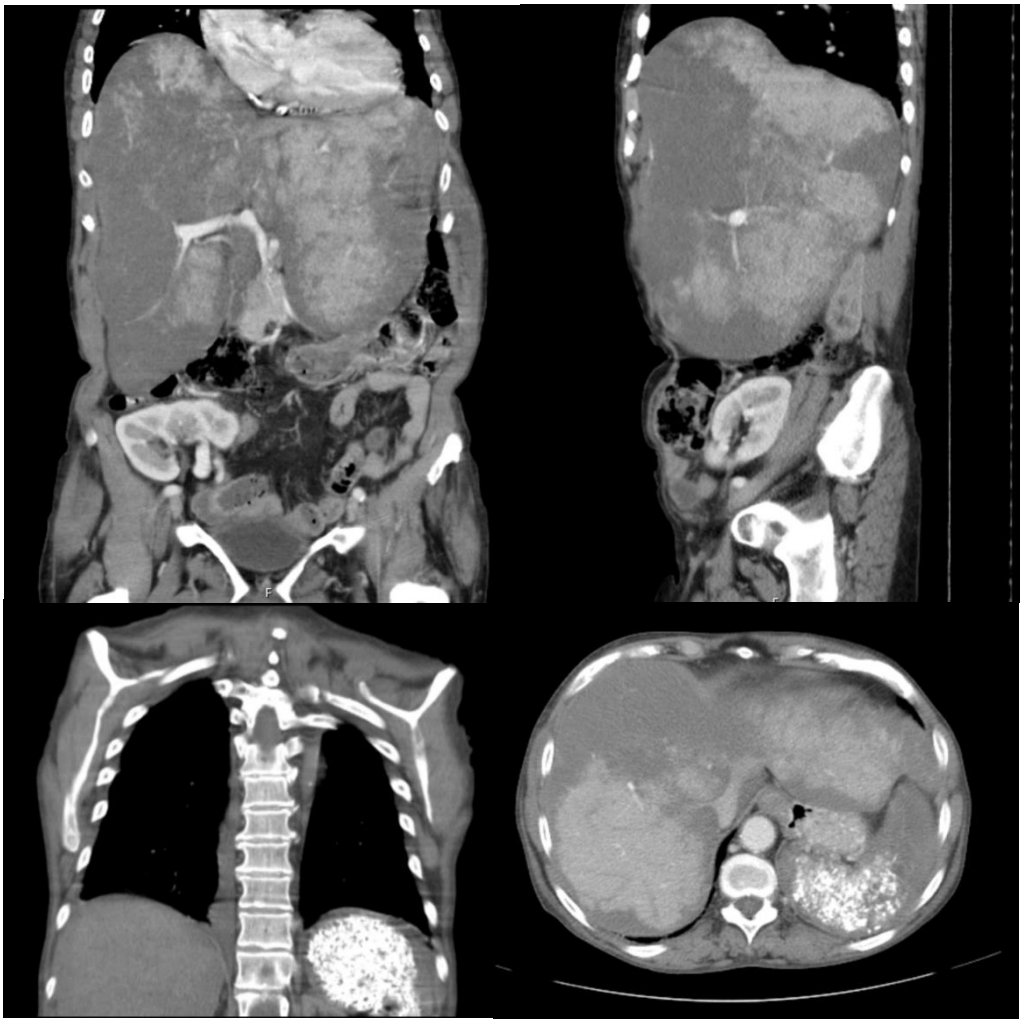

Figure 4. Coronal, sagittal, and axial CT slices from patient 3. There is massive hepatomegaly and calcified spleen, besides signs of chronic liver disease. These slices were taken after kidney transplantation, as evidenced by the allograft presence in the right iliac fossa.

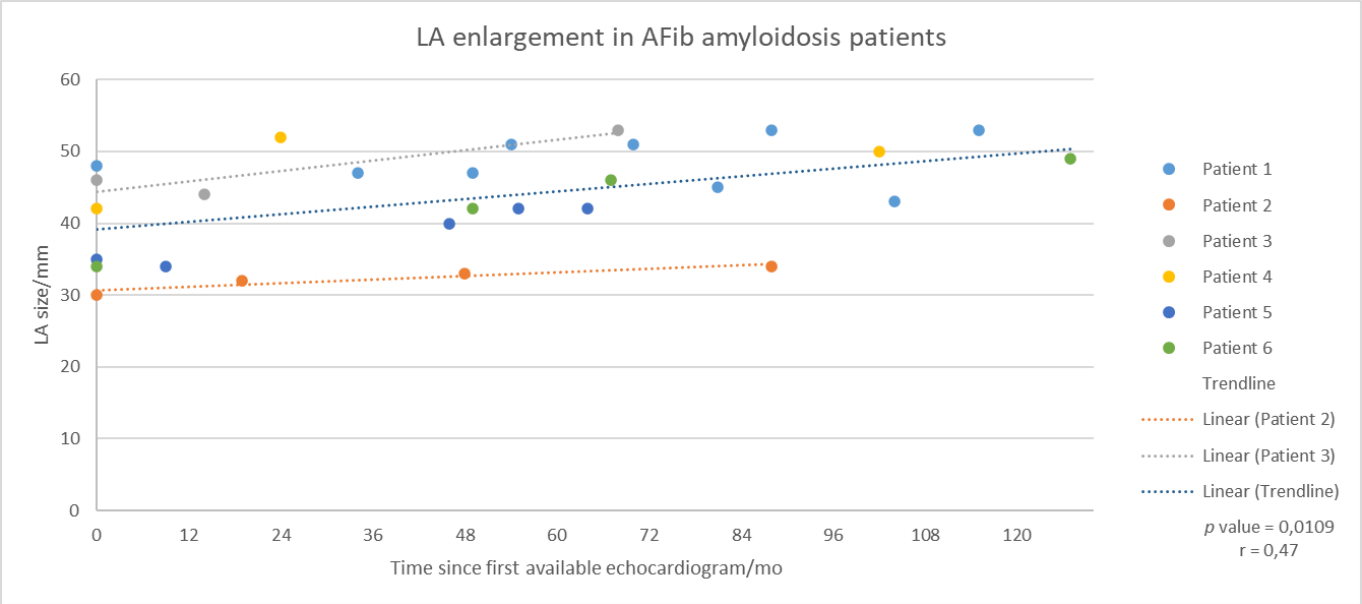

Figure 5. Correlation between time since first available echocardiogram and LA size (r=0,47, p=0,01). Abbreviations: LA, left atrium; mo, month; mm, millimetres. There is a significant positive correlation.

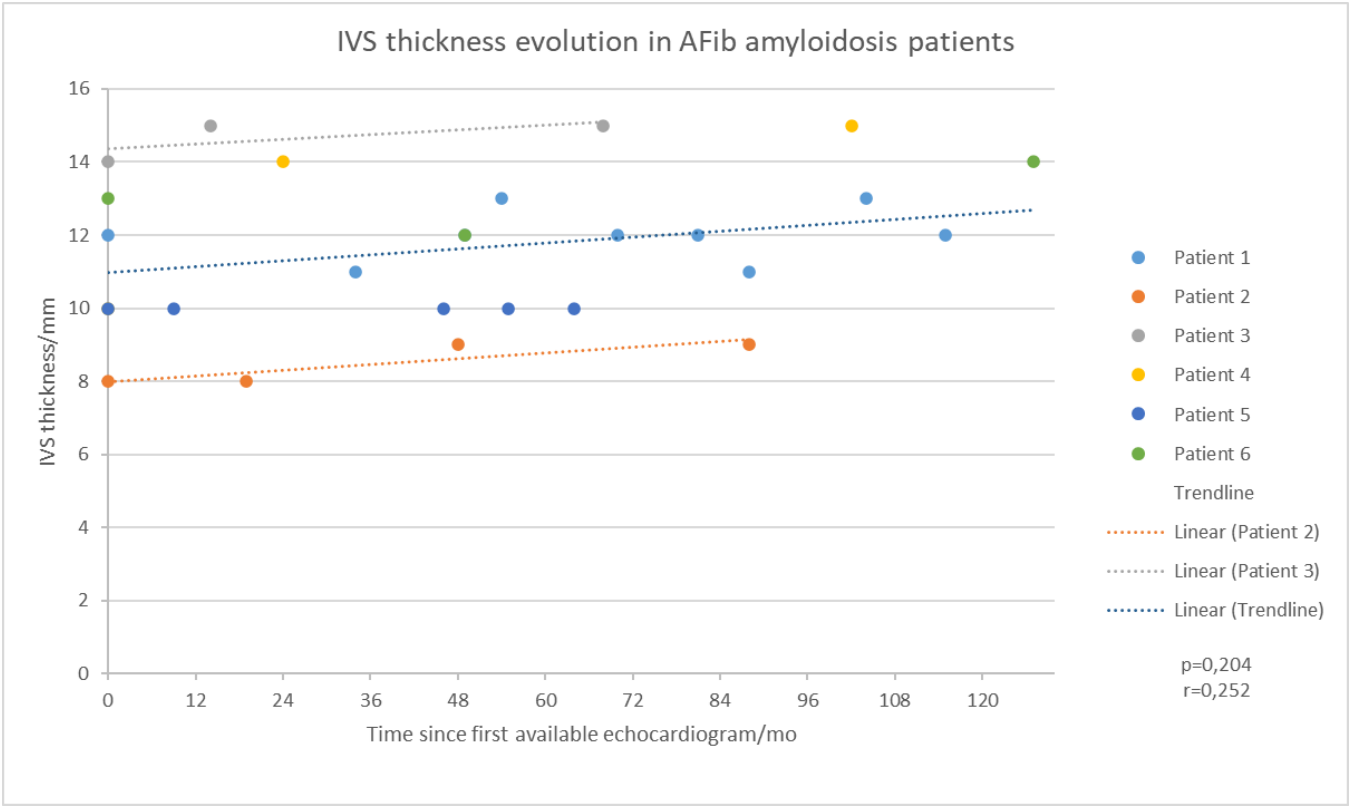

Figure 6. Correlation between time since first available echocardiogram and IVS thickness (r=0,25, p=0,2). Abbreviations: IVS, intraventricular septum; mo, month; mm, millimetres.

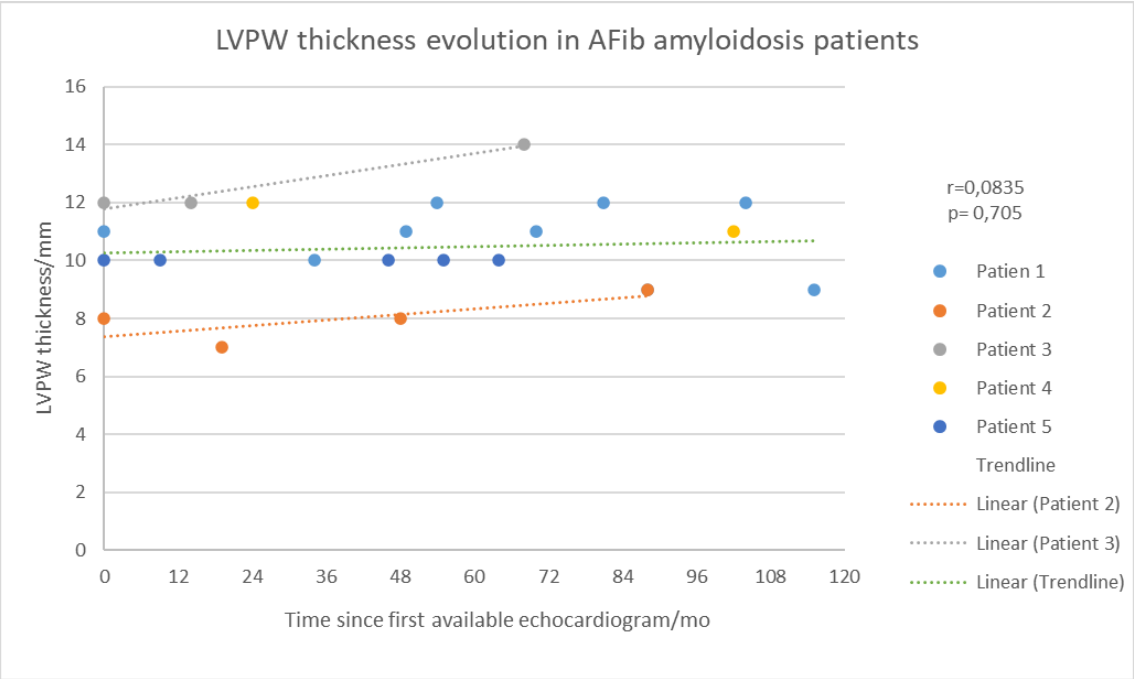

Figure 7. Correlation between time since first available echocardiogram and LVPW thickness. Abbreviations: LVPW, left ventricle posterior wall; mo, month; mm, millimetres.

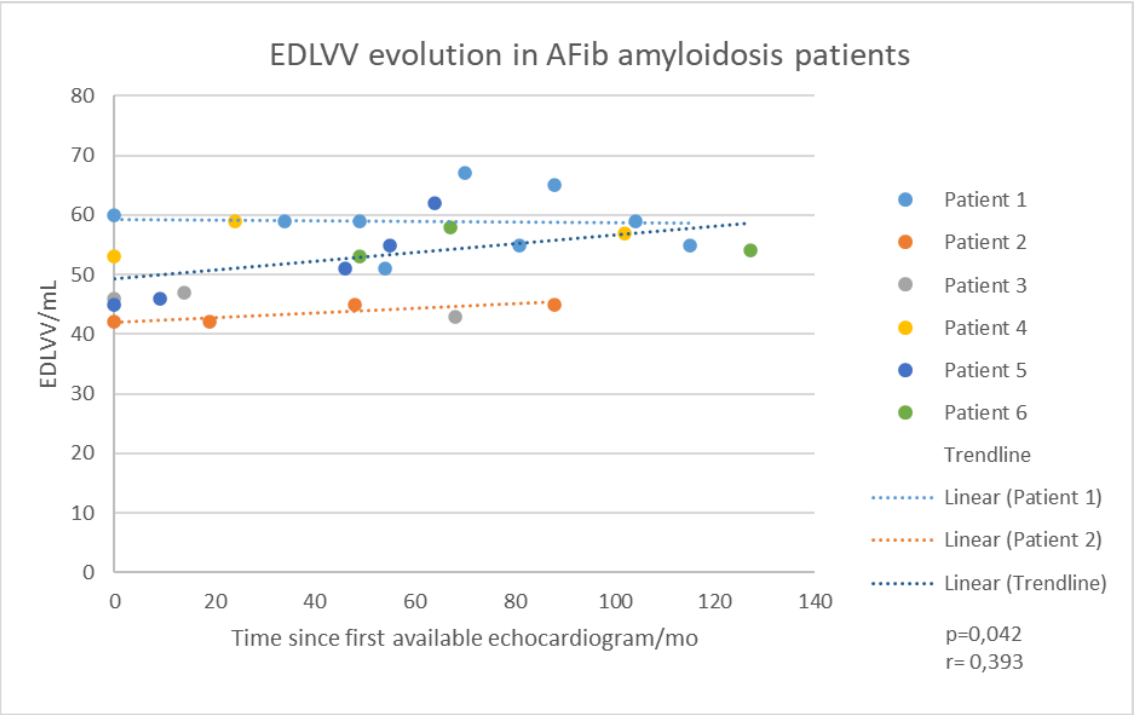

Figure 8. Correlation between time since first available echocardiogram and LEDVV (r=0,39, p=0,04). Abbreviations: LEDVV, left end diastolic ventricle volume; mo, month; mm, milliliters

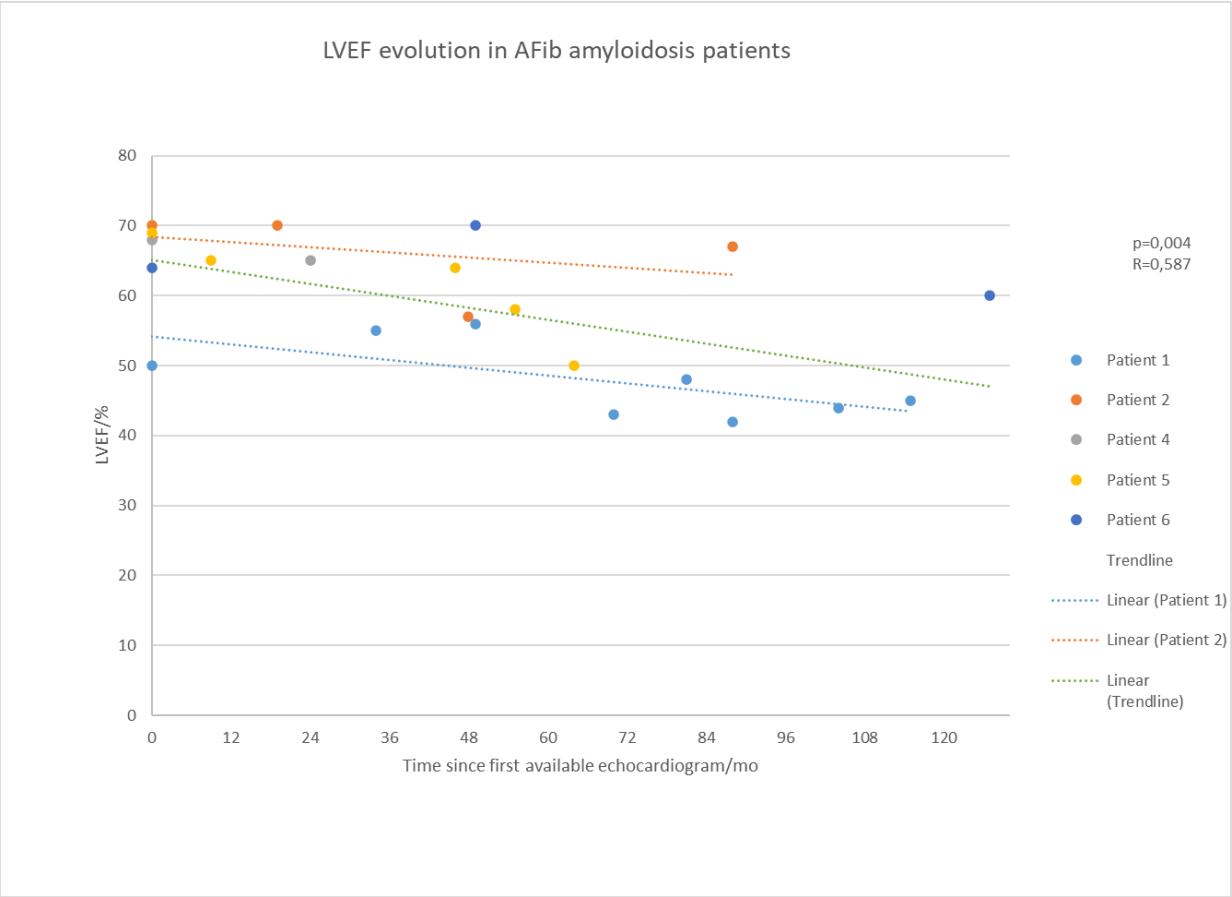

Figure 9. Correlation between time since first available echocardiogram and LVEF. Abbreviations: LVEF, left ventricle ejection fraction; mo, month; %, percentage. There is a significant negative correlation.

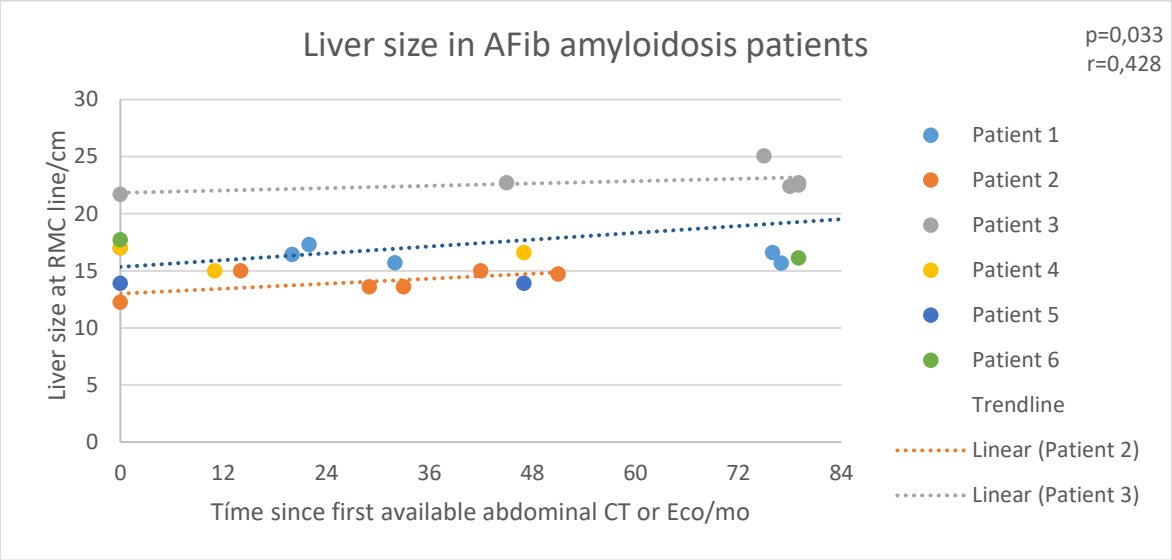

Figure 10. Correlation between time since first available abdominal CT/echography and liver size at RMC line. Abbreviations: Echo, echography, CT, computerized tomography; mo, month; cm, centimetres; RMC, right middle clavicular. There is a significant positive correlation.

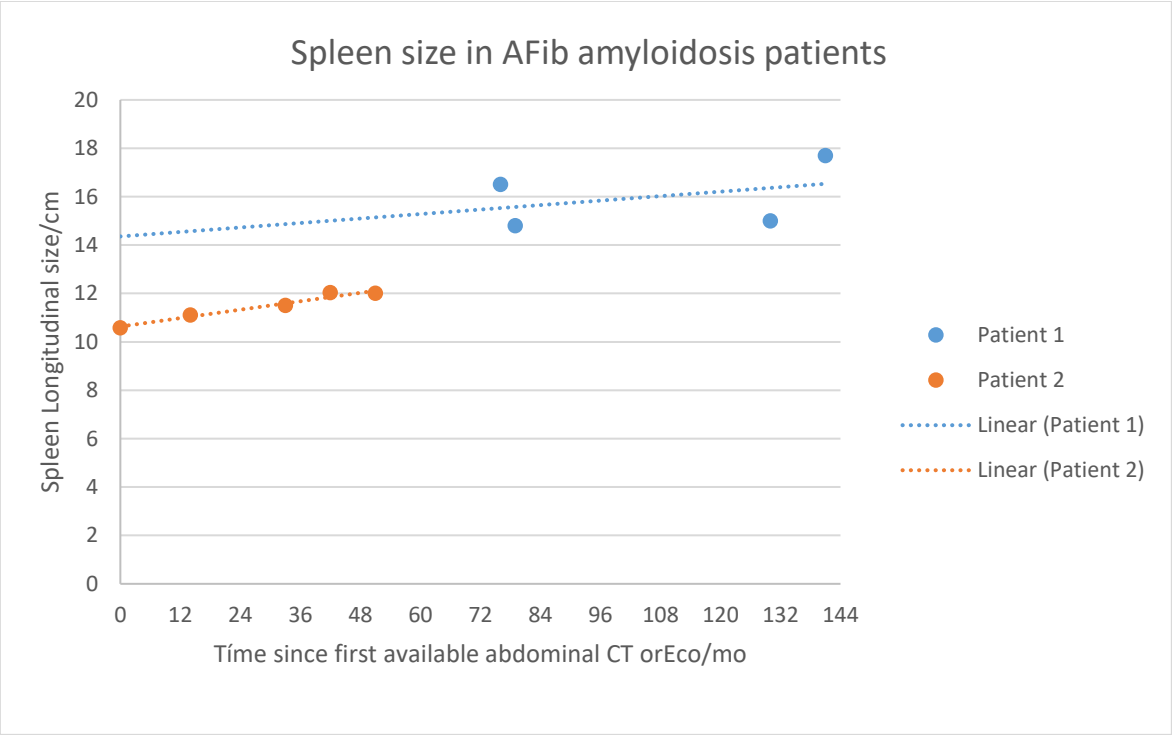

Figure 11. Correlation between time since first available abdominal CT/echography and spleen longitudinal size. Abbreviations: Echo, echography; CT, computerized tomography; mo, month; cm, centimetres.

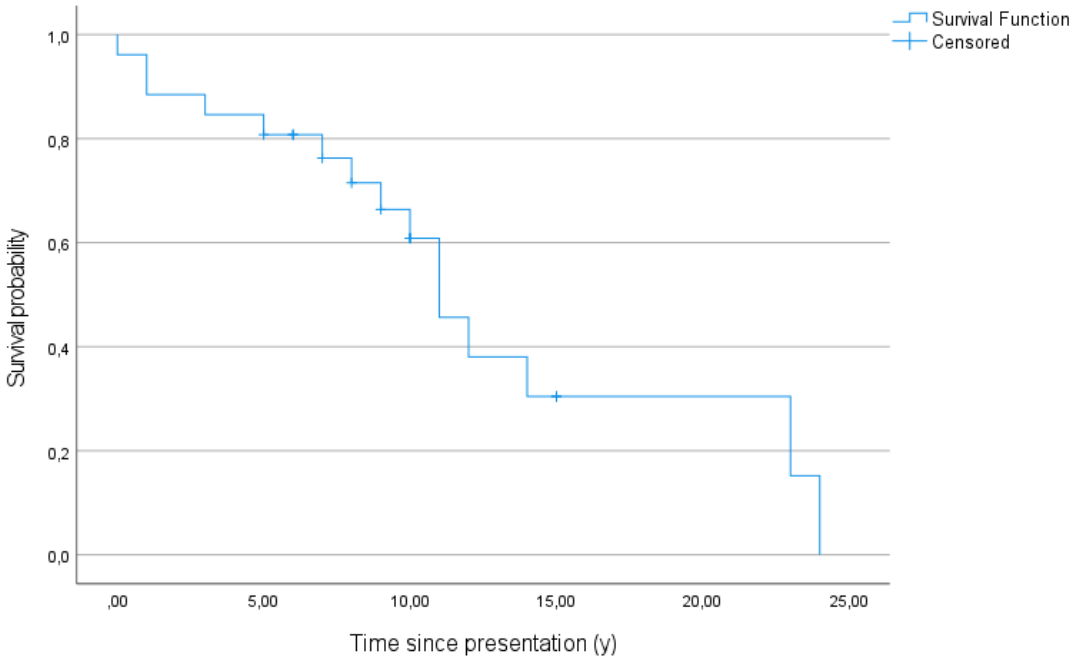

Figure 12. AFib amyloidosis patients' survival probability censored at death or lost to follow-up. Abbreviations: y, years.

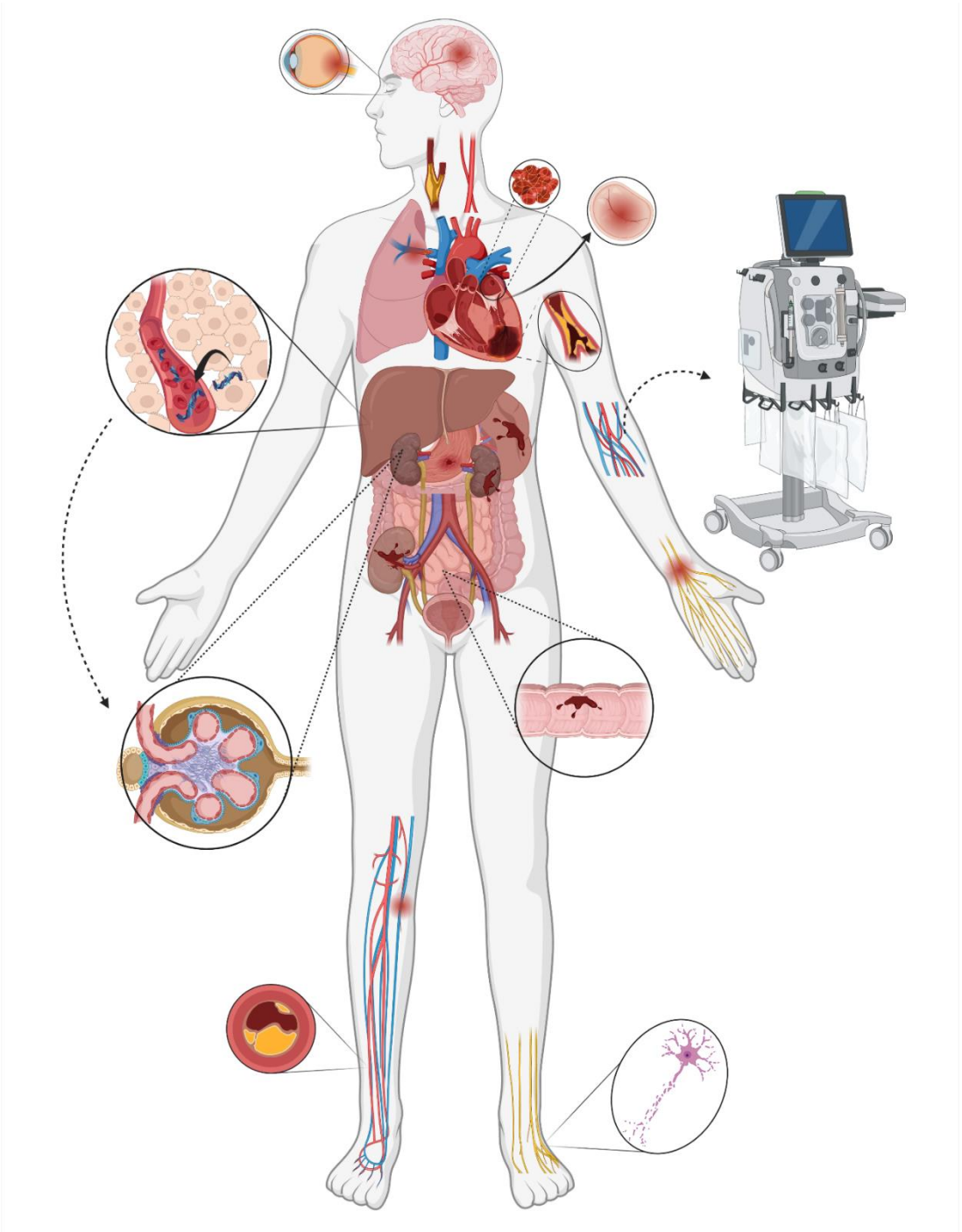

Figure 13. AFib p.Glu545Val amyloidosis clinical landscape. AFib is produced in the liver and distributed across the body via blood vessels. The primary amyloid deposition occurs in the kidneys. However, our study also suggests a slow but steady systemic involvement, mainly in the heart. Intraventricular septal hypertrophy was common, besides left auricle enlargement and mitral valve disease. Indeed, there was a significant presence of atrial fibrillation, which increased the risk of thromboembolic events: ischemic strokes mainly, but other rare presentations were also reported, like central retinal artery occlusion and intestinal ischemia. RRT accelerates atherosclerosis, which increases cardiovascular risk. Bleeding complications, either related to KT (graft and native kidney hemorrhage) or dialysis (splenic rupture) can develop. Thrombotic events like myocardial infarction were also described. Lower limb sensory neuropathy was a common extra-renal feature, besides others as carpal tunnel syndrome.

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