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The estimation of glomerular filtration rate in chronic kidney disease stage III-V pediatric patients: a retrospective study

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RESUMO

Introdução e objetivos: A incidência da doença renal crónica (DRC) tem aumentado em crianças e adolescentes, tendo um impacto significativo no seu crescimento e desenvolvimento. A taxa de filtração glomerular (TFG) é o indicador mais preciso para avaliar e monitorizar a função renal. O uso da *clearance* de creatinina (ClCr) em urina 24 horas é uma alternativa fiável para estimar a TFG, no entanto em pediatria pode ser difícil a sua obtenção. Diversas fórmulas foram propostas usando marcadores endógenos conhecidos como creatinina (Cr), cistatina C (CisC) ou uma combinação, para estimar a TFG. O objetivo deste estudo passa pela avaliação da precisão das várias fórmulas selecionadas para cálculo da taxa de filtração glomerular estimada (TFGe), em doentes com DRC estádio III-V, em idade pediátrica, em termos de estimativa de TFG e estadiamento, comparando com a *clearance* de creatinina 24-horas (ClCr-24h).

Métodos: Foi realizado um estudo retrospetivo em doentes pediátricos, entre os 2-18 anos, com diagnóstico de DRC estádio III-V. A TFG foi calculada com recurso a ClCr em urina 24-horas, sendo usada como referência. A TFGe foi calculada com base em diferentes fórmulas baseadas na Cr, CisC ou numa combinação. Os resultados de cada fórmula foram comparados, como um todo e pelos diferentes estádios de DRC, com a ClCr-24h.

Resultados: Foram incluídos um total de 31 crianças/adolescentes (61,3% masculinos), com uma idade média (percentil 25º-75º) de 14,0 (12,0-16,0), com estádio de DRC III-V. As fórmulas Larsson-CisC, Zappitelli-CisC e Zappitelli-combinada produziram TFGe significativamente diferentes em toda a amostra, quando comparadas com a referência. Ao analisar cada estádio separadamente, no estádio III todas as fórmulas, exceto Schwartz-Cr, apresentaram diferenças significativas; enquanto no estádio IV, as fórmulas baseadas em CisC e Schwartz-combinada, e no estádio V Schwartz-Cr, Larsson-CisC e Zappitelli-combinada não demonstraram diferenças significativas. A CKiD-CisC foi a única fórmula que categorizou os pacientes de forma semelhante à referência, com todas as outras fórmulas a sobrestimar o estádio de DRC. Os resultados demonstraram que Schwartz-Cr tem o desempenho mais próximo da ClCr-24h, apresentando maior precisão e correlação; seguido pelas fórmulas combinadas.

Conclusões: O estudo reforçou que as fórmulas baseadas na Cr, especialmente a Schwartz-Cr, fornecem estimativas bastante precisas da TFG, especialmente em estádios mais avançadas de DRC. Em suma, em estádios iniciais de DRC, as fórmulas baseadas na CisC demonstraram ser mais precisas e deveriam ser utilizadas, sempre que disponíveis.

Palavras-chave: Taxa de filtração glomerular, taxa de filtração glomerular estimada, doença renal crónica, creatinina, cistatina C.

ABSTRACT

Background and objective: Chronic kidney disease (CKD) incidence has been rising in children and adolescents, having a significant impact on their growth and development. Glomerular filtration rate (GFR) is the most accurate indicator for evaluating and monitoring kidney function. The use of 24-hour urine creatinine clearance (CrCl) is a valuable option to estimate GFR, yet in pediatric patients it can be challenging and somehow unpractical. Many formulas have been proposed using known endogenous markers as creatinine (Cr), cystatin C (CysC) or a combination, to estimate GFR. In the present study we aimed to evaluate the accuracy of selected equations of estimated glomerular filtration rate (eGFR) in pediatric CKD patients, stage III-V, in terms of GFR estimation and CKD staging, by comparing it to 24-hour urinary CrCl.

Methods: A retrospective study was conducted with pediatric patients, aged 2 to 18 years, diagnosed with stages III to V CKD. GFR was calculated using 24-hour urine CrCl, to be used as a reference. eGFR was calculated using different formulas based on Cr, CysC or a combination. The results of each formula were compared, as a whole and by the different stages of CKD, with the 24-hour creatinine clearance (24h-CrCl).

Results: A total of 31 children and adolescents (61.3% male), with a median (25^{th}-75^{th} percentile) age of 14.0 (12.0-16.0) years, with CKD stages III to V, were included. Larsson-CysC, Zappitelli-CysC and Zappitelli-Combined formulas yielded significantly different GFR estimations in the whole sample when compared to the reference. When analyzing each stage separately, in stage III all formulas, except Schwartz-Cr, displayed significant differences; while in stage IV, all CysC-based formulas and Schwartz-Combined did not display significant differences and in stage V, only Schwartz-Cr, Larsson-CysC and Zappitelli-combined did not display significant differences. Regarding CKD staging, the CKiD-CysC was the only formula that categorized patients similarly to the reference, with all other formulas overestimating the CKD staging. Overall, our results demonstrated that Schwartz-Cr has the closest performances to 24h-CrCl, showing higher accuracy and stronger correlation; followed by the combined formulas.

Conclusion: Our study reinforced that Cr-based formulas, especially Schwartz-Cr, remains a good estimate, providing fairly accurate estimations of GFR, especially in more advanced stages of CKD. Additionally, we also showed that in earlier CKD stages (stage III), CysC-based formulas were more accurate and should be used, whenever available.

Keywords: Glomerular filtration rate, Estimated glomerular filtration rate, Chronic Kidney Diseases, creatinine, cystatin C.

ABBREVIATIONS

24h-CrCl, 24-hour creatinine clearance
BMI, body mass index
BSA, body surface area
BUN, blood urea nitrogen
CKD, chronic kidney disease
CKiD, refers to the designation of the multicentre prospective cohort study Chronic Kidney Disease in Children
CMIN, *Centro Materno Infantil Norte*
Cr, creatinine
CrCl, creatinine clearance
CysC, cystatin C
eGFR, estimated glomerular filtration rate
GFR, glomerular filtration rate
KDIGO, Kidney Disease: Improving Global Outcomes
SD, standard deviation

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INTRODUCTION

During the last decade, chronic kidney disease (CKD) incidence has been rising in children and adolescents, which is calling attention to the importance of early recognition of at-risk individuals, in order to prevent further progression.[1] An European-wide study, comprising data provided by different pediatric nephrology societies in several countries, concluded that the incidence of stage III-V CKD in pediatric age is around 11-12 cases per million of the age-related population, with a prevalence of 55-60 cases per million of the age-related population, with a predominance in males, given the greater predisposition to renal and urinary tract congenital anomalies, one of the main causes of CKD in this age group.[1,2]

Although CKD in pediatric age share the same pathophysiology as in adults, it acquires different dimensions in this age group, as distinct etiologies and clinical impact, mainly on growth and development.[2,3]

The glomerular filtration rate (GFR) is the most accurate indicator for evaluating and monitoring kidney function, being essential in the diagnosis and staging, but also on evaluating disease progression, of acute and chronic kidney disease, in both children/adolescents and adults.[4,5]

As in any other laboratory test, GFR measurement should be performed using a precise, safe, simple and inexpensive method. A direct measurement is not practicable as the filtration process occurs in multiple glomeruli simultaneously.[5] Given that it is not possible to measure GFR by a direct method, the urinary clearance of a filtration marker turns out to be the most precise method to define GFR, due to the fact that it outlines the rate at which a substance is removed from a certain volume of serum per unit of time, in a 24-hour urine sample.[5] The marker used should be a substance that is freely filtered by the glomeruli, free of reabsorption or tubular secretion, should have a constant rate of production, be inert and exclusively renal excreted.[5,6]

Considering the characteristics of an ideal marker, GFR can be measured using exogenous substances such as inulin, iohexol, among others.[6,7,8] These exogenous methods have been used as gold-standard for GFR determination, however, since they are invasive, expensive and complex methods to apply, currently these methods are more often used for research purposes.[5,7] In clinical practice, endogenous markers are preferred given the ease of their measurement. Creatinine (Cr) continues to be the most frequently used marker, but it presents several limitations.[9,10] Cr is known to be influenced by several factors besides glomerular filtration, such as muscle mass, diet, age, race, gender and CKD stage, and to overcome some of these limitations, recent studies have proposed the use of cystatin C (CysC).[7,9,10] CysC is also an endogenous marker but it is reported not to be significantly affected by age, gender or muscle mass, currently starting to gain importance in

clinical practice.[7] However, there is no data that withstand CysC better efficacy compared to Cr, except in children under two years of age or with low muscle mass.[5,7,10,11,12]

The GFR estimation based in the clearance of this endogenous markers requires a 24-hour urine sample, which is often difficult in children, especially in those without sphincter continence.[10] As a result, several formulas have been proposed to estimate GFR, representing an important asset in clinical practice.[7,10]

In 1976, Schwartz proposed the first formula for estimated glomerular filtration rate (eGFR) in children, based on height, serum Cr and a constant value, and it has been used in clinical practice for several decades.[6,13] More recently, several other formulas have been proposed, including some with the use of other biomarkers, such as CysC, or a combination of markers.[12]

Several studies have tried to evaluate the accuracy of these formulas and since exogenous GFR determination are usually unavailable, many of these studies consider 24-hour creatinine clearance (24h-CrCl) as the gold standard. The performance of these formulas depends on the type of setting in which they are used, with some formulas yielding better estimates in healthy children, others in CKD patients and others in transplant patients. Since the CKD staging depends on the GFR estimation, it is not infrequent that the use of different formulas lead to the categorization of the same patients in different CKD stages, which represents either over or underestimation of the actual GFR.[12,13]

Despite all previous studies, there is not yet any evidence-based recommendation on the best formula to be used in pediatric patients, mostly, due to the variety of results obtained by the different studies. Some authors defend the preference for formulas using a combination of Cr, CysC and blood urea nitrogen (BUN), as the use of combined markers seems to strengthen the accuracy of the estimations.[10,13] Nonetheless, especially in populations with low muscle mass, some other authors believe that CysC based formulas would provide a more accurate estimation.[14] Despite Schwartz-Cr and Schwartz-combined being the most frequently used bedside formulas, still, routinely many laboratories prefer to calculate eGFR with formulas developed for adult patients, which puts in evidence the need for more pediatric studies further exploring the performance of different formulas in each specific setting.[6,10,12,13]

Considering all of the above, in the present study we aimed to evaluate the accuracy of selected equations of eGFR in pediatric CKD patients, stage III-V, in terms of GFR estimation and CKD staging, when compared to 24h-CrCl.

METHODS

We conducted a retrospective study of all children and adolescents, aged 2 to 18 years, with the diagnosis of CKD, stages III to V, and sphincter control, followed at a Pediatric Nephrology unit, in a tertiary center, *Centro Materno-Infantil Norte* (CMIN).

All CKD patients with a previous 24h-CrCl determination and serum CysC, less than 30 days apart, were eligible for inclusion in the present analysis. Only the most recent 24h-CrCl and serum CysC measurements of each patient were considered for analysis. Regarding CKD patients that had undergone kidney transplant, the last results prior to kidney transplantation, thus representing a CKD patient stage V, were considered for analysis.

Clinical data was collected, by consulting the clinical records of each patient, and the following variables were gathered: gender, age, height, weight, CKD etiology, treatment data (renal transplantation, hemodialysis peritoneal dialysis, conservative treatment), serum urea, Cr and CysC values, and urine volume and Cr in a 24-hour urine sample.

Serum Cr assay was analyzed using a calibrator for automated system (Roche Diagnostics).[15] Urinary Cr was determined with the same clinical chemistry analyzer. Serum CysC was assayed using a particle-enhanced nephelometric assay (DADE - Behring, Siemens Company, European Format).[15]

To estimate GFR, in mL/min/1.73m^2, several formulas were used: revised Schwartz formula (Schwartz-Cr)[13], k x (height(cm)/serum Cr(mg/dL)), using a k constant of 0.413; Filler formula (Filler-CysC)[16], Log(GFR) = 1.962 + (1.123 x log(1/CysC(mg/L))); Larsson formula (Larsson-CysC)[17], 77.24 × CysC(mg/L)$^{-1.2623}$; Zappitelli formula (Zappitelli-CysC)[14], 75.94 × CysC(mg/L)$^{-1.17}$; CKiD formula (CKiD-CysC)[18], 70.69 x CysC(mg/L)$^{-0.931}$; combined Schwartz formula (Schwartz-Combined)[19], 39.8 x (height(cm)/serum Cr(mg/dL))$^{0.456}$ x (1.8/CysC(mg/L))$^{0.418}$ x (30/BUN)$^{0.079}$ x (height(cm)/1.4)$^{0.179}$ (x1.076 if female); and combined Zappitelli formula (Zappitelli-Combined)[14], (507.76 x e$^{0.003}$ x height(cm))/(CysC(mg/L)$^{0.635}$ x serum Cr(mg/dL)$^{0.547}$).

All 24-hour urine collections performed in the consultation followed a written information, given to children's parents, on the correct method of collection. All samples considered for the present analysis, had a urinary Cr within the range of 11.3‑28.0 mg/kg/d, according to age and sex-specific reference values.[20]

CKD staging was performed according to KDIGO (Kidney Disease: Improving Global Outcomes) criteria [18], considering GFR estimated by 24h-CrCl, assumed as gold-standard.

The 24h-CrCl was calculated using the standard formula and was normalized to 1.73m^2 of body surface area (BSA), determined by the Haycock formula.[21]

This project was approved by the *Departamento de Ensino, Formação e Investigação* and Ethics Committee of *Centro Hospitalar Universitário do Porto*.

Standard statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (Chicago, IL). Differences between groups in continuous variables were evaluated with Mann–Whitney tests. Bivariate associations were assessed by Spearman correlation tests. To evaluate the agreement between methods of eGFR, calculation of differences (means ± standard deviation (SD)), accuracies (within 10, 30, and 50%), and absolute bias (i.e., average difference between eGFR and creatinine clearance (CrCl)) were performed and Bland Altman charts were plotted. Two-sided $p<0.05$ was considered significant.

RESULTS

The present analysis included 31 children and adolescents (61.3% male), with a median (25^{th}-75^{th} percentile) age of 14.0 (12.0-16.0) years, with CKD stages III to V. General characteristics and renal function parameters are presented, as a whole and according to stages of CKD, in table I. No significant differences were observed between the three stages of CKD in terms of age or anthropometric variables (weight, height or body mass index (BMI)). The GFR estimated by 24h-CrCl was considered the reference for CKD staging. The median values of 24h-CrCl and the eGFR by all the formulas considered are presented in Table I. The eGFR formulas of Larsson-CysC, Zappitelli-CysC and Zappitelli-Combined yielded significantly different estimations in the whole sample when compared to 24h-CrCl. When analyzing each CKD stage separately, in stage III all formulas, except Schwartz-Cr, displayed significant differences; in stage IV, all CysC-based formulas and Schwartz-Combined did not display significant differences and in stage V, only Schwartz-Cr, Larsson-CysC and Zappitelli-combined did not display significant differences.

The table II shows the distribution of the studied sample by three CKD stages, according to 24h-CrCl (reference) and all the eGFR formulas considered. All formulas, except CKiD-CysC, distribute the patients differently by CKD stages; all formulas classify a lower number of patients in the stage III (and a higher number of patients in stage IV), when compared to the reference classification, thus overestimating the CKD staging. In stage V, the distribution of patients using eGFR by Schwartz-Cr, Larsson-CysC and Zappitelli-Combined formulas appear to be similar to the reference categorization with 24h-CrCl (29.0%).

The Spearman correlation between 24h-CrCl and eGFR formulas are shown in table III. The strongest correlations, in the whole sample, were found with Schwartz-Cr eGFR (p=0,892) and with the combined formulas (Schwartz-Combined and Zappitelli-Combined, both with p=0,865). When considering the CKD stages separately, in stage III, the correlation with 24h-CrCl are stronger with CysC-based formulas (p=0,732), while in stage V no significant correlations were found.

In table IV, the summary of the agreement between different formulas to estimate GFR and 24h-CrCl, in the whole sample, is shown. The formulas that seemed to have the closest performances to 24h-CrCl (both higher correlation coefficients and higher accuracy values) were the Schwartz-Cr and the combined formulas, especially Schwartz-Combined. The Bland-Altman plots for each formula, using 24h-CrCl as the reference GFR value are presented in Figure 1.

DISCUSSION

In the present study we were able to compare the performance of several formulas for GFR estimation and CKD staging, when compared to 24h-CrCl, in CKD pediatric patients. The eGFR formulas of Larsson-CysC, Zappitelli-CysC and Zappitelli-Combined yielded significantly different estimations in the whole sample when compared to 24h-CrCl. When analyzing each CKD stage separately, compared to 24h-CrCl, in stage III all formulas, except Schwartz-Cr, display significant differences; while in stage IV Schwartz-Cr and Zappitelli-combined, and in stage V, Filler-CysC, Zappitelli-CysC, CKiD-CysC and Schwartz-Combined show significant differences. Regarding CKD staging, the CKiD-CysC was the only formula that categorized patients similarly to 24h-CrCl, with all other formulas classifying a lower number of patients in the stage III (and a higher number of patients in stage IV), when compared to the reference classification, thus overestimating the CKD staging. Overall, our results demonstrated that the formula that seemed to have closest performances to 24h-CrCl, showing a higher accuracy and stronger correlation, was the Schwartz-Cr, followed by the combined formulas, especially Schwartz-combined.

We reported that Schwartz-Cr GFR estimation yielded the strongest correlation with 24h-CrCl, although it tended to underestimate eGFR, even in earlier phases of kidney dysfunction. A study conducted on 2014, comparing eGFR using different formulas with iohexol clearance, concluded that Schwartz formula shows a good accuracy when GFR is inferior to 60 mL/min/1.73m^2, which is in line with our findings.[12] However, some authors have reported that Cr-based formulas, especially at higher GFR levels, underestimate GFR, supporting that in early stages of CKD these formulas might be less accurate on GFR estimation.[12]

Acknowledging the well-known limitations of Cr, previous studies have explored the performance of combined formulas, using both Cr and CysC, defending that these formulas tend to provide more accurate GFR estimations.[10,22] Since the relationship between height and serum Cr has a strong correlation with GFR, by introducing CysC and BUN, into the formula, they defend it strengthens the calculations and accuracy of eGFR.[10,13] In fact, in our study, we found that combined formulas showed strong concordance with 24h-CrCl, especially when considering the Schwartz-combined.

A study conducted in 2015, with a cohort of obese pediatric patients, concluded that eGFR calculated using Zappitelli-Combined formula presented the strongest correlation with 24h-CrCl, while Schwartz-Cr had poor performance in this group of patients. The authors emphasize that BMI might be an important factor to consider, due to its influence on GFR.[23] In this study, patients from all CKD stages were included, but in comparison to our study, we should underline that our sample present a median BMI lower than 25 kg/m^2 throughout all categories, which could in part explain

our different results. We believe that BMI should be taking into account in future studies evaluating the performance of eGFR formulas, since the presence of overweigh/obesity might introduce important bias to consider and account for.

Regarding CysC-based formulas, in general, we found these to have a weaker correlation with 24h-CrCl but, in line with what we just stated, it should be noted that none of the equations include anthropometric data on the calculations. It has been proposed that Cr-based formulas should be replaced by CysC-based formulas, due to the variation of Cr with body mass and the inconstant increase of Cr secretion with GFR decrease, with previous studies showing stronger correlation of CysC formulas (especially Filler-CysC) with serum iothalamate clearance, reporting precisions around 30% higher, when compared to the Schwartz-Cr.[14] The authors of the same study also reported that CysC-based formulas generally presented to be more sensitive, but less specific, in identifying those with GFR inferior than 90 mL/min/1.73m^2, when compared to Schwartz-Cr.[14]

In what concerns to CKD staging, CKiD-CysC was the only formula that categorized patients similarly to 24h-CrCl, with all other formulas classifying a lower number of patients in the stage III (and a higher number of patients in stage IV). This overestimation of CKD stage in our sample is concordant to the fact that CysC might be more sensitive in early phases of kidney degradation.[23]

The role of formulas including CysC is believed particularly important in populations with low muscle mass, such as in hematopoietic stem cell transplant recipients, oncology patients, and spinal cord injury patients but technical limitations, namely in the access to the measurement of this marker, still limits its use in many centers worldwide, where Cr remains the only kidney function marker available in the clinical practice.[10]

The conclusions of our study are limited by its retrospective nature and by the small number of patients included. Nonetheless, we believe to have contributed to reinforce that Cr-based formulas, especially Schwartz-Cr, a classical and very studied formula, validated in different setting of patients, remains a good estimate, providing fairly accurate estimations of GFR, especially in more advanced CKD stages. Additionally, we also showed that in earlier CKD stages (stage III), CysC-based formulas were more accurate and should be used, whenever available.

An accurate determination of GFR is essential to diagnose CKD as early as possible, and to follow-up the disease in all stages, in order to allow the delivery of adequate treatment and prevent deterioration and complications. Therefore, especially in pediatric age, due to its challenges and adversities, and due to the arise in incidence of CKD in this age group, it is crucial to identify the best formulas to accurately estimate GFR. Multicentric prospective studies, if possible comparing GFR formulas with methods based on exogenous markers, are of utmost importance to increase the knowledge on this subject and to allow for better staging and follow-up of CKD patients.

APPENDIX

TABLES

Table I. General characteristics, renal function markers and estimated glomerular filtration rates, according to the chronic kidney disease stage (III-V).

BMI, body mass index; GFR, glomerular filtration rate.
The values presented are median (25^{th} - 75^{th} percentile) or n (%).
* The p values presented in this section of the table refer to the comparison between 24h-CrCl and estimated glomerular filtration rates, by each formula, in the whole sample and separately, in each chronic kidney disease stage.

	All	Stage III	Stage IV	Stage V	*p* value
Demographic and anthropometric variables					
Age (years)	14.0(12.0-16.0)	14.0(12.0-16.0)	15.0(7.0-17.0)	13.0(10.5-16.0)	0.961
Male sex	19(61.3%)	9(60%)	6(85.7%)	4(44.4%)	0.495
Height (cm)	154.7(142.0-166.5)	159.0(145.0-167.5)	155.0(124.0-170.0)	148.5(133.3-160.5)	0.515
Weight (kg)	47.8(36.7-56-8)	49.5(36.7-60.0)	47.8(25.6-56.0)	45.8(32.1-64.2)	0.961
BMI (kg/m^2)	18.7(17.4-21.4)	18.2(17.4-21.4)	18.6(16.6-22.1)	20.8(16.4-22.7)	0.867
Renal Function Markers					
Creatinine (mg/dL)	2.9 (1.7-5.3)	1.7 (1.4-2.5)	2.9 (2.8-4.3)	7.3 (4.7-7.8)	-
Urea (mg/dL)	116.0 (88.0-134.0)	91.0 (60.0-105.0)	127.0 (99.0-132.0)	134.0 (121.0-182.0)	-
Cystatin C (mg/L)	2.8 (2.2-3.7)	2.6 (2.0-2.8)	3.1 (2.6-3.8)	3.7 (3.0-5.5)	-
Estimated GFR ($mL/min/1.73m^2$)*					
24h Creatinine Clearance (Reference)	29.6 (12.5-42.2)	42.2 (32.0-50.0)	21.8 (18.4-27.7)	7.4 (4.2-12.0)	-
Schwartz-Cr	21.7 (12.6-37.7) (*p*=0.096)	37.7 (27.8-45.7) (*p*=0.125)	21.6 (15.9-22.9) (*p*=0.028)	8.8 (7.9-11.3) (*p*=0.139)	-
Filler-CysC	29.4 (21.0-37.2) (*p*=0.624)	31.6 (29.3-41.6) (*p*=0.003)	25.5 (20.6-31.9) (*p*=0.128)	21.0 (13.5-26.5) (*p*= 0.008)	-
Larsson-CysC	21.5 (14.7-28.1) (*p*= 0.006)	23.3 (21.4-31.8) (*p*=0.001)	18.4 (14.4-23.6) (*p*=0.063)	14.7 (8.9-19.2) (*p*= 0.066)	-
Zappitelli-CysC	23.3 (16.3-29.7) (*p*= 0.038)	25.1 (23.2-33.4) (*p*=0.001)	20.1 (16.0-25.3) (*p*=0.237)	16.3 (10.3-20.9) (*p*= 0.021)	-
CKiD-CysC	28.2 (21.4-34.1) (*p*= 0.845)	29.8 (28.1-37.3) (*p*=0.001)	25.1 (21.1-30.1) (*p*=0.128)	21.4 (14.9-25.9) (*p*= 0.008)	-
Schwartz-Combined	24.4 (15.8-31.3) (*p*= 0.055)	31.3 (26.6-38.6) (*p*=0.001)	21.4 (16.3-24.4) (*p*=0.128)	12.1 (11.2-15.2) (*p*= 0.015)	-
Zappitelli-Combined	22.7 (11.9-28.7) (*p*= 0.001)	28.7 (23.2-34.9) (*p*=0.001)	18.2 (14.4-22.7) (*p*=0.028)	9.4 (7.8-13.3) (*p*= 0.173)	-

Table II. Chronic kidney disease staging of the studied sample, in stages III to V, according to 24-hour creatinine clearance (reference for study inclusion) and to the different formulas of estimated glomerular filtration rate.

The values presented are n (%).

	Stage III	**Stage IV**	**Stage V**	***p value***
24h Creatinine clearance	15 (48.4%)	7 (22.6%)	9 (29.0%)	-
Schwartz-Cr	8 (25.8%)	14 (45.2%)	9 (29.0%)	0.000
Filler-CysC	13 (41.9%)	15 (48.4%)	3 (9.7%)	0.009
Larsson-CysC	6 (19.4%)	16 (51.6%)	9 (29.0%)	0.015
Zappitelli-CysC	7 (22.6%)	20 (64.5%)	4 (12.9%)	0.003
CKiD-CysC	10 (32.3%)	19 (61.3%)	2 (6.5%)	0.114
Schwartz-Combined	9 (29.0%)	15 (48.4%)	7 (22.6%)	0.000
Zappitelli-Combined	6 (19.4%)	16 (51.6%)	9 (29.0%)	0.000

Table III. Spearman correlations between 24-hour creatinine clearance and estimated glomerular filtration rates, by the different formulas, according to the chronic kidney disease stage (III-V).

**p<0.05; *p<0.01

	24h Creatinine Clearance			
	All	**Stage III**	**Stage IV**	**Stage V**
Schwartz-Cr	0.892*	0.436	0.821**	0.533
Filler-CysC	0.661*	0.732*	0.500	-0.233
Larsson-CysC	0.661*	0.732*	0.500	-0.233
Zappitelli-CysC	0.661*	0.732*	0.500	-0.233
CKiD-CysC	0.661*	0.732*	0.500	-0.233
Schwartz-Combined	0.865*	0.639**	0.786**	-0.117
Zappitelli-Combined	0.865*	0.539**	0.679	-0.117

Table IV. Accuracy and bias of the different formulas for glomerular filtration rate estimation compared to 24-hour creatinine clearance (mean 27.31 mL/min/1.73m^2).

*p<0.01.

	Cystatin C-based formulas			
	Filler-CysC	**Larsson-CysC**	**Zappitelli-CysC**	**CKiD-CysC**
eGFR (mL/min/1.73m2), mean ±SD	29.38 ±10.44	21.70 ±8.6	23.28 ±8.60	27.86 ±8.12
CrCl-eGFR(mL/min/1.73m2), mean ±SD	-2.07 ±11.86	5.61 ±12.12	4.02 ±12.10	-0.55 ±12.14
Accuracy 10% (%)	25.8	6.5	12.9	9.7
Accuracy 30% (%)	58.1	45.2	58.1	64.5
Accuracy 50% (%)	77.4	77.4	80.6	77.4
Correlation coefficient (CrCl-eGFR)	0.661*	0.661*	0.661*	0.661*

	Creatinine-based formula	*Combined formulas*	
	Schwartz-Cr	**Schwartz-Combined**	**Zappitelli-Combined**
eGFR (mL/min/1.73m2), mean ±SD	24.94 ±14.60	24.35 ±10.12	21.84 ±10.54
CrCl-eGFR(mL/min/1.73m2), mean ±SD	2.37 ±8.04	2.96 ±8.70	5.46 ±8.77
Accuracy 10% (%)	29.0	12.9	22.6
Accuracy 30% (%)	67.7	51.6	67.7
Accuracy 50% (%)	90.3	90.3	83.9
Correlation coefficient (CrCl-eGFR)	0.892*	0.865*	0.865*

FIGURES

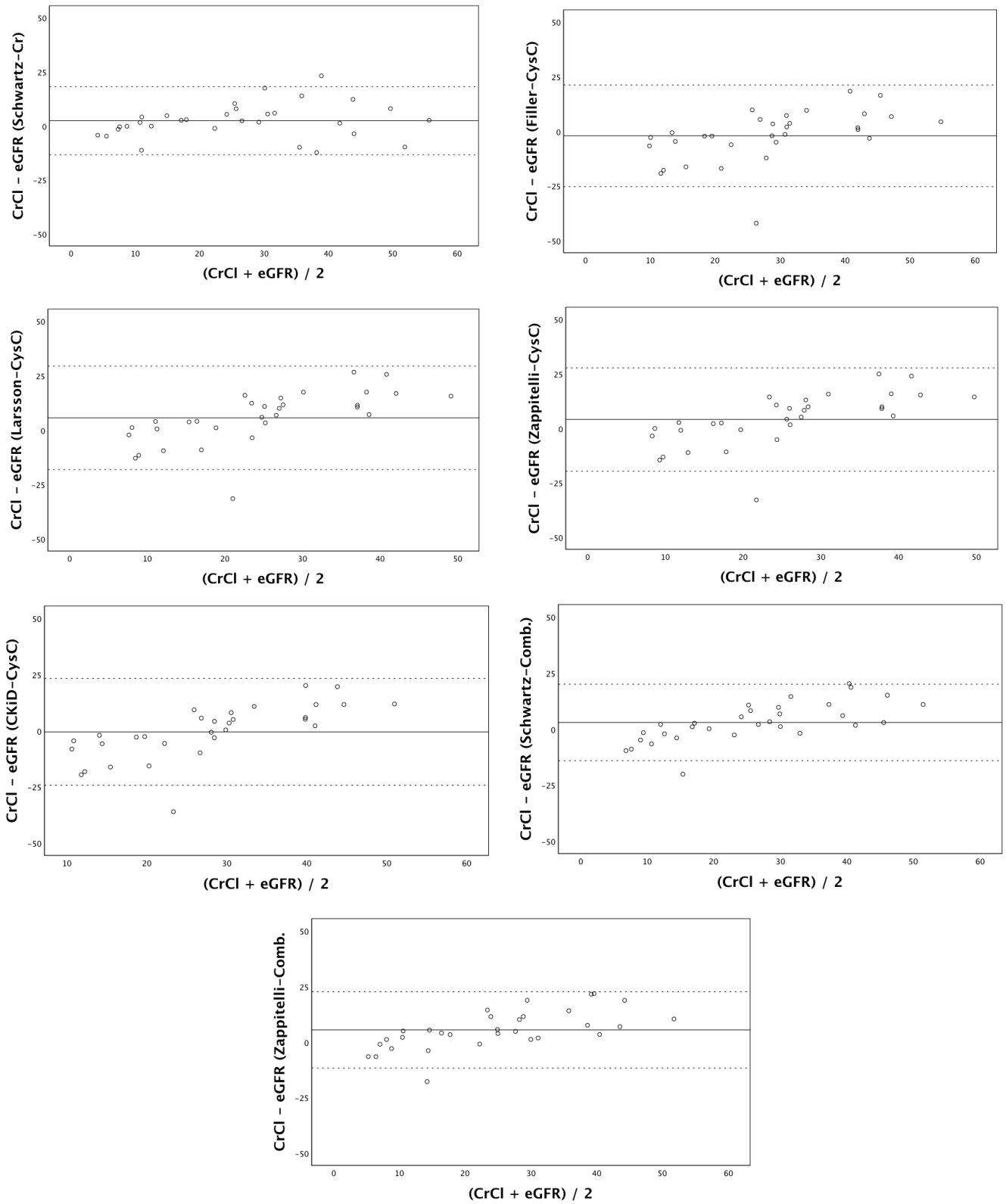

Figure 1. Bland-Altman plots of the different formulas for glomerular filtration rate estimation in comparison with 24-hour urine creatinine clearance.

The horizontal full line represents the mean difference between 24h creatinine clearance and glomerular filtration rates, by each formula, and the horizontal dashed lines the limits of agreement**.**

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