**Dener Lizot Rech**

# DESEMPENHO DE EQUAÇÕES BASEADAS NA CREATININA PLASMÁTICA PARA ESTIMAR A TAXA DE FILTRAÇÃO GLOMERULAR EM IDOSOS

Dissertação apresentada à Universidade de Caxias do Sul, para obtenção do Título de Mestre em Ciências da Saúde.

Caxias do Sul

2018

**Dener Lizot Rech**

# DESEMPENHO DE EQUAÇÕES BASEADAS NA CREATININA PLASMÁTICA PARA ESTIMAR A TAXA DE FILTRAÇÃO GLOMERULAR EM IDOSOS

Dissertação apresentada à Universidade de Caxias do Sul, para obtenção do Título de Mestre em Ciências da Saúde.

Orientador: Prof. Dr. Luciano da Silva Selistre

Co-Orientador: Profª. Drª. Laurence Dubourg

Caxias do Sul

2018

**UNIVERSIDADE DE CAXIAS DO SUL**

**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE**

**COORDENADOR DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE**

PROF. DR. ASDRUBAL FALAVIGNA

**Dener Lizot Rech**

Dados Internacionais de Catalogação na Publicação (CIP)
Universidade de Caxias do Sul
UCS - BICE - Processamento Técnico

R296d Rech, Dener Lizot, 1982-
Desempenho de equações baseadas na creatinina plasmática para estimar a taxa de filtração glomerular em idosos / Dener Lizot Rech. – 2018.
viii, 46 f. ; 30 cm

Apresenta bibliografia.
Dissertação (Mestrado) – Universidade de Caxias do Sul, Programa de Pós-Graduação em Ciências da Saúde, 2018.
Orientação: Prof. Dr. Luciano da Silva Selistre.
Coorientação: Profa. Dra. Laurence Dubourg.

1. Insuficiência renal crônica. 2. Doenças em idosos. 3. Taxa de filtração glomerular. I. Selistre, Luciano da Silva, orient. II. Dubourg, Laurence, coorient. III. Título.

CDU 2. ed.: 616.61

Índice para o catálogo sistemático:

| | |
|---|---|
| 1. Insuficiência renal crônica | 616.61 |
| 2. Doenças em idosos | 616-053.9 |
| 3. Taxa de filtração glomerular | 616.611 |

Catalogação na fonte elaborada pela bibliotecária
Carolina Machado Quadros – CRB 10/2236.

# QUAL A FÓRUMA MAIS ADEQUADA PARA AVALIAR A FUNÇAO RENAL EM IDODOS?

*Dener Lizot Rech*

Dissertação de Mestrado submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graducação em Ciências da Saúde da Universiade de Caxias do Sul, como parte dos requisitos nescessários para a obtenção do título de Mestre em Ciências da Saúde, Linha de Pesquisa: Investigação Clínica e Epidemiológica.

Aprovado em 23 de março de 2018.

Banca Examinadora:

Dr. Carlos Eduardo Poli de Figueiredo
Pontifícia Universidade Católica do Rio Grande do Sul

Dr.Dagoberto Vanoni de Godoy
Universidade de Caxias do Sul

Dr. Leandro Tasso
Universidade de Caxias do Sul

Dr. Luciano da Silva Selistre
Universidade de Caxias do Sul
Orientador

## Dedicatória

Dedico esta dissertação a minha esposa, meus pais e irmãos, que foram fonte de incentivo e apoio constante. Também, aos meus pacientes, aos idosos e doentes renais crônicos.

## Agradecimentos

Aos colegas do programa de pós-graduação, pelo agradável convívio e discussões construtivas.

Aos professores, pela maneira com que passaram e instigaram buscar e construir conhecimento e, sobretudo, pelo exemplo de profissionais.

Ao meu orientador, Prof. Dr. Luciano Selistre, pela motivação a enveredar nesses caminhos da pós-graduação, pela paciência e disponibilidade; pelo carinho, também com seus alunos e pacientes; e pela amizade que vai além dos corredores acadêmicos e nosocomiais.

Aos meus pais, Neiva e Domingos, por, desde muito cedo, instigar e incentivar a curiosidade, a ousar e a questionar, o que me direcionou aos caminhos da medicina, da clínica médica, da geriatria e das ciências da saúde.

Aos meus irmãos, Darwin e Mellina, os quais tiveram a satisfação de compartilhar o exemplo, valores e o amor dos mesmos pais, pelo companheirismo e apoio sem limites.

E, por fim, à Fabrícia, sinônimo de amor, compaixão, paciência e companheirismo. O meu porto seguro.

## Sumário

Esta dissertação de Mestrado Acadêmico Stricto Sensu é apresentada no formato exigido pelo Programa de Pós-Graduação em Ciências da Saúde da Universidade de Caxias do Sul. A mesma é constituída da secção de “Introdução com referências bibliográficas”, a inclusão do artigo original submetido/publicado em periódico Qualis A na classificação da Coordenação de Aperfeiçoamento de Pessoal em Nível Superior (CAPES), e as “Considerações Finais e Perspectivas”.

# 1 INTRODUÇÃO

A Doença Renal Crônica (DRC) é um importante problema de saúde pública mundial, com elevados custos de tratamento, bem como risco de complicações e morte [1,2]. Embora tenha uma prevalência de 13% da população adulta norte-americana, atinge 47% daqueles com mais de 70 anos [3]. Sua definição e classificação foi introduzida pelo *National Kidney Foundation Kidney Disease Outcomes Quality Initiative* (NKF-KDOQI) em 2002, e posteriormente confirmada pela diretriz internacional *Kidney Disease Improving Global Outcomes* (KDIGO) em 2004. Este consenso prevê a presença de dano renal ou o valor da Taxa de Filtração Glomerular (TFG) menor que 60mL/min/1,73m$^2$ por 3 ou mais meses, este o melhor parâmetro de função renal e determinante do estágio de DRC [1]. Assim, simplificou-se o conceito, trazendo uma melhor comunicação entre todos os profissionais da saúde, mesmo não especialistas, demandando estratégias de prevenção, detecção precoce e manejo nos diferentes estágios.

Os principais fatores de risco para DRC são: hipertensão arterial sistêmica, diabetes mellitus, doença cardiovascular, história familiar da doença e idade maior que 60 anos. A detecção precoce da DRC, em indivíduos sob risco, mostra-se importante para definir a desordem subjacente e prevenir, especialmente em idosos**,** complicações como: anemia, alterações hidro-eletrolíticas, morte prematura cardiovascular, deterioração física e cognitiva, infecções, agudização da doença e evolução da DRC para estágio final [1].

A mensuração direta da TFG por depuração de substância exógena exclusivamente excretada por filtração glomerular (inulina, ioexol, etc), embora seja o padrão-ouro, não é realizada de maneira rotineira devido ao custo e complexidade, salvo em situações muito particulares como: protocolos de transplante renal ou necessidade de ajuste de dose de drogas com índice terapêutico muito estreito [4]. Por essa problemática, existe um constante esforço em desenvolver equações capazes de estimar, confiavelmente, a TFG através de marcadores endógenos como a Creatinina Plasmática (CrP) [5-8]. Esta, um produto da degradação da fosfocreatina muscular, é produzida de maneira relativamente constante, o que a torna um adequado marcador para estimar a

TFG. Entretanto, sendo o envelhecimento associado tanto a alterações estruturais e fisiológicas renais quanto à perda de massa muscular, o cálculo da estimativa da TFG com este marcador fica prejudicado, podendo potencialmente afetar os cuidados clínicos [9-11].

Para a população adulta, a principal equação baseada na CrP, e sugerida pelo KDIGO, é a "*Chronic Kidney Disease Epidemiology Collaboration Creatinine Equation"* (CKD-EPI), embora tenha sido elaborada com baixa representatividade de idosos [5]. Estudos tem questionado seu desempenho entre os idosos [6-8,12-18]. A equação "*Berlin Initiative Study creatinine equation"* (BIS1) foi a única equação desenvolvida exclusivamente entre maiores de 70 anos, tendo tido adequado desempenho [7]. As equações *"Lund-Malmö"* revisada (LMR) e "*Full Age Spectrum Equation"* (FAS), vem tendo bom desempenho em estudos com idosos, embora, ainda com pouca validade externa [6,8].

Portanto, os objetivos deste estudo são: (1) avaliar a confiabilidade de 4 equações baseadas na CrP: CKD-EPI, LMR, BIS1 e FAS para estimar a TFG quando comparadas com a TFG mensurada por um método padrão referência (depuração urinária da inulinal), entre diferentes idosos de diversas apresentações clínicas; (2) avaliar o desempenho destas equações entre 2 grupos de TFG (<45 e ≥45mL/min/1,73m$^2$) [9].

## 2 REFERÊNCIAS


1. Group KDIGOKCW. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. 2013;3(1):1-150.

2. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: global dimension and perspectives. *Lancet.* 2013;382(9888):260-272.

3. Eckardt KU, Coresh J, Devuyst O, et al. Evolving importance of kidney disease: from subspecialty to global health burden. *Lancet.* 2013;382(9887):158-169.

4. Soveri I, Berg UB, Bjork J, et al. Measuring GFR: a systematic review. *Am J Kidney Dis.* 2014;64(3):411-424.

5. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-612.

6. Björk J, Grubb A, Sterner G, Nyman U. Revised equations for estimating glomerular filtration rate based on the Lund-Malmö Study cohort. *Scand J Clin Lab Invest.* 2011;71(3):232-239.

7. Schaeffner ES, Ebert N, Delanaye P, et al. Two novel equations to estimate kidney function in persons aged 70 years or older. *Ann Intern Med.* 2012;157(7):471-481.

8. Pottel H, Hoste L, Dubourg L, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol Dial Transplant.* 2016;31(5):798-806.

9. Glassock R, Delanaye P, El Nahas M. An Age-Calibrated Classification of Chronic Kidney Disease. *JAMA.* 2015;314(6):559-560.

10. United States Renal Data System. Chapter 1: CKD in the General Population. 2015 USRDS annual data report: Epidemiology of Kidney Disease in the United States. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2015. In:2015.

11. Dowling TC, Wang ES, Ferrucci L, Sorkin JD. Glomerular filtration rate equations overestimate creatinine clearance in older individuals enrolled in the Baltimore Longitudinal Study on Aging: impact on renal drug dosing. *Pharmacotherapy.* 2013;33(9):912-921.

12. Lopes MB, Araújo LQ, Passos MT, et al. Estimation of glomerular filtration rate from serum creatinine and cystatin C in octogenarians and nonagenarians. *BMC Nephrol.* 2013;14:265.

13. Koppe L, Klich A, Dubourg L, Ecochard R, Hadj-Aissa A. Performance of creatinine-based equations compared in older patients. *J Nephrol.* 2013;26(4):716-723.

14. Alshaer IM, Kilbride HS, Stevens PE, et al. External validation of the Berlin equations for estimation of GFR in the elderly. *Am J Kidney Dis.* 2014;63(5):862-865.

15. Vidal-Petiot E, Haymann JP, Letavernier E, et al. External validation of the BIS (Berlin Initiative Study)-1 GFR estimating equation in the elderly. *Am J Kidney Dis.* 2014;63(5):865-867.

16. Fan L, Levey AS, Gudnason V, et al. Comparing GFR Estimating Equations Using Cystatin C and Creatinine in Elderly Individuals. *J Am Soc Nephrol.* 2015;26(8):1982-1989.

17. Bjork J, Grubb A, Gudnason V, et al. Comparison of glomerular filtration rate estimating equations derived from creatinine and cystatin C: validation in the Age, Gene/Environment Susceptibility-Reykjavik elderly cohort. *Nephrol Dial Transplant.* 2017.

18. Bjork J, Back SE, Ebert N, et al. GFR estimation based on standardized creatinine and cystatin C: a European multicenter analysis in older adults. *Clin Chem Lab Med.* 2017.

# 3 ARTIGOS

# GLOMERULAR FILTRATION IN OLDER PEOPLE - Performance of creatinine-based GFR estimating equations

Luciano Selistre[1,2,3,4,5], Dener L. Rech[2], Vandréa de Souza[1,2,4,5], Sandrine Lemoine[1], Laurence Dubourg[1,2,4,5]

[1] Department of Nephrology and Renal Functional Explorations, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

[2] Universidade de Caxias do Sul, Programa de Pós-graduação em Ciências da Saúde, Caxias do Sul, Brazil.

[3] CAPES Foundation, Ministry of Education of Brazil

[4]Université de Lyon, Lyon, France.

[5] Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique, UMR5305 CNRS/Université Lyon 1, Lyon, France

**ABSTRACT**

**Importance:** Estimating kidney glomerular filtration rate (GFR) is of utmost importance in many clinical conditions. However, very few studies have evaluated the performance of GFR estimating equations over elderly person and degrees of kidney impairment.

**Objective:** To determine the performance of the four plasma creatinine-based equations (CKD-EPI, LMR, BIS 1 and FAS) in a large cohort of elderly person with a wide range of age and a broad spectrum of GFRs

**Designs:** GFR was measured by urinary inulin clearance in 2,247 patients and jointly estimated with four equations: CKD-EPI, LMR, BIS 1 and FAS.

**Setting:** The measure performance was assessed using bias (median of difference measured and estimated GFR), precision (interquartile range of the ratio), accuracy $P_{30}$ (percentage of estimates 30% higher or lower than the measured GFR) and probability of diagnostics (ROC operative).

**Participants:** Patients were referred for GFR measurement for suspected renal dysfunction, renal risk or before kidney donation. The patients' ages ranged from 65 to 90 years and the measured GFRs from 5 to 147 mL/min/1.73 $m^2$

**Main Outcome(s) and Measure(s):** GFR was measured by renal inuline clearance and blood creatinine assays were based on international standards *and calibrators*

**Results:** In whole population, CKD-EPI showed comparable performance with LMR, BIS and FAS (median bias: -2.0 [3.0; -1.0] vs. 2.0 [1.5; 3.5], -2.0 [3.5; -1.5] and 0.0 [-0.5;.05] 0.24 [-0.20; 0.68]; IQR: 14 [13.0; 15.5] vs. 13 [12.0; 13.0], 14 [13.0; 15.0]] and 14 [13.0; 15.0]; and $P_{30}$: 78.0 [76.0; 80.0] vs. 81.5 [80.0; 83.0], 77.5 [75.5; 79.0] and 79.0 [76.0; 80.0], *P= NS*). In the oldest patients

(aged > 75 years), contrary to patients with GFR > 45, LMR performed slightly better than others equations in patients with GFR <45 mL/min/1.73 $m^2$ (median bias: -1.0 [-2.0; 0.0]; IQR: IQR: 11 [9.0; 12.5], and $P_{30}$: 72.0 [69.0; 76.0], <.001). However; the receiver operating characteristic (ROC) curve analysis (cut-off for GFR 45 mL/min/1.73 $m^2$) showed no significant differences in diagnostic accuracy between all the aforementioned equations. Overall, it has to be noted, that in patients over 65 years with GFR < 45 mL/min/1.73$m^2$, accuracy ($P_{30}$) of all tested GFR estimating equations is clearly lower than the 80% limit of KDIGO recommendations.

**Conclusion:** the current study comparing the LMR, FAS and BIS1 with the CKD-EPI equations suggests that none of the equations are clearly superior in the elderly population and all equations demonstrated a weak performance when GFR < 45 ml/min/1.73$m^2$. We recommended maintaining the use of CKD-EPI in elderly to estimated GFR with a cautious interpretation of results. Specific equations should be developed in this specific population.

**Keywords:** glomerular filtration rate, creatinine-IDMS, chronic kidney disease, older people.

## INTRODUCTION

Accurate estimation of glomerular filtration rate (GFR) is essential in the elderly population for correct classification and management of chronic kidney disease (CKD) and for adjusting drug dosage[1-3]. Ideally, GFR should be measured by renal clearance of an exogenous marker that is exclusively removed by glomerular filtration (e.g: inulin, iohexol, EDTA); however, for practical reasons, such GFR measurements (mGFR) can not be performed in routine clinical practice[3,4]. For this reason, there has been a constant strive to develop equations which can estimate GFR (eGFR) reliably from blood biochemical markers such as Plasma Creatinine (PCr)[5-8].

Aging is associated with structural and physiological change in the kidney as well as loss of muscle mass, both of which may afect eGFR calculation[9-11]. Therefore, the eGFR may be less reliable in older patients and can therefore potentially adversely affect their clinical care. The prevalence of CKD as currently defined by an eGFR<60 mL/min/1.73 $m^2$ or albuminuria (urine albumin-to-creatinine ratio >30 mg/g) persisting for 3 months or more is reportedly 13% of the United States adult population. However, the prevalence by these criteria markedly increases with age from 4% for adults ages 20–39 years old to 47% for adults ages 70 years old and older [3,12]. PCr based equations underestimated eGFR at values close to the diagnostic threshold of 60 mL/min/1.73 $m^2$ with overdiagnosis in healthy older populations[13]. .Nevertheless, it has been demonstrated that the current definition of CKD (<60 mL/min/1.73$m^2$) does not reduce life expectancy or is a reliable prediction of an excess mortality or end stage renal disease (ESRD)[9,11,13]. Therefore, Glassock

et al suggested to modify the definition of CKD for a lower GFR (<45 mL/min/1.73m$^2$) in older person[9].

The Kidney Disease Improving Global Outcomes guidelines (KDIGO) recommend using PCr standardized assays to calculate eGFR with the *Chronic Kidney Disease Epidemiology Collaboration equation* (CKD-EPI) to adults, including older individuals[1,5,14]. However, some studies have cast doubt that CKD-EPI may be useful in older people [6-8,15-21].

Therefore, the objectives of our study were the following: (1) to assess the reliability of three PCr-based equations IDMS-calibrated compared to CKD-EPI equation (Full Age Spectrum (FAS), Berlin Initiative Study (BIS1) and Lund-Malmö Revised (LMR) equations to estimate GFR versus measured GFR (mGFR) by urinary inulin clearance across different older adults of various CKD levels and (2) to assess the ability to detect CKD in elderly (GFR <45 mL/min/1.73 m$^2$)

## MATERIALS AND METHODS

### Study population

The study considered a cross-sectional retrospective cohort of 2,247 participants. It included all eligible patients 65 to 90 years old referred between July 2003 and July 2017 to a single university hospital (*Renal and Metabolic Function Exploration Unit of Edouard Herriot Hospital, Lyon, France*) to undergo GFR measurement for suspected or established renal dysfunction, renal risk, or before kidney donation. All the procedures were carried out in accordance with the ethical standards of the institutional and/or national research committees

and of the 2013 Helsinki Declaration and its later amendments or comparable ethical standards. Precisely, an appropriate informed consent was obtained from all the patients or their legal representatives. The consent form contained information on the procedure itself as well as on the possibility of later use of the data for research purposes. According to the current French law, an observational study that does not change routine management of patients does not need to be declared or submitted to the opinion of a research ethics board (*Loi Huriet-Sérusclat 88-1138, 20 December 1988 and its subsequent amendments, text available at http://www.chu-toulouse.fr/IMG/pdf/loihuriet.pdf*).

**Data collection**

Performance assessment and comparisons between the four eGFR equations were carried out on different GFR categories. The modest reduction of GFR (45 to 59 ml/min/1.73m$^2$) seen in the elderly does not seem to have much effect on risk for shortened life expectancy or other adverse events so long as it is not accompanied by overt proteinuria, we considered only two groups of GFR: <45 and ≥45 mL/min/1.73 m$^2$ [9,22].

**Laboratory assessments**

**Reference method for mGFR**

The classic inulin clearance is still considered as the gold-standard method for GFR measurement. The renal clearance of inulin used a polyfructosan-based method (Inutest®, Fresenius Kabi, Graz, Austria). A standard technique was used by a trained staff with a continuous infusion after a 30 mg/kg priming dose of polyfructosan. Water diuresis was induced by a first

oral administration of 5 mL/kg of water followed by 3 mL/kg every 30 minutes combined with an intravenous infusion of 0.9 % sodium chloride. Three to four urine samples were collected and a blood sample was drawn mid-way through each collection period. The clearance value, calculated by the usual UV/P equation, was the mean value of three to four clearance periods. The measurements of plasma and urine polyfructosan were performed using the same enzymatic method that demonstrated very good specificity and reproducibility (within-run precision <1% and between-run precision <3.5%) [23].

The results were expressed per 1.73 m² according to the Dubois equation: BSA = height0.725 x weight0.425 x 0.007184.

All PCr measurements were performed with methods traceable to the National Institute of Standards and Technology creatinine standard reference (IDMS-calibrated). From October 2003 to June 2010, PCr was obtained by a kinetic colorimetric compensated *Jaffé* technique (Roche Modular) whose results were standardized by linear regression adjustment versus the concentrations obtained by liquid chromatography mass spectrometry (LCMS). The calibration equation was: standardized PCr = 0.9395 x *Jaffé* compensated serum creatinine in µmol/L + 4.6964. The coefficient of correlation was 0.97. From June 2010, all PCr values were obtained by an enzymatic method traceable to the National Institute of Standards and Technology. According to the KDIGO, the two techniques are considered similar [2]. PCr is expressed in µmol/L.

**GFR estimating equations.**

The following equations were evaluated for elderly population:

**CKD-EPI** Female; PCr ≤61.88, **eGFR** = 144 x $[PCr/61.88]^{-0.329}$ x $[0.993]^{Age}$ x [1.159 if black]

Female; PCr >61.88, **eGFR** = 144 x $[PCr/61.88]^{-1.209}$ x $[0.993]^{Age}$ x [1.159 if black]

Male; PCr ≤79.56, **eGFR** = 141 x $[PCr/79.56]^{-0.411}$ x $[0.993]^{Age}$ x [1.159 if black]

Male; PCr >79.56, **eGFR** = 141 x $[PCr/79.56]^{-1.209}$ x $[0.993]^{Age}$ x [1.159 if black]

**Lund-Malmö Revised** **eGFR = $e^{X-0.0158\times Age+0.438\times \ln(Age)}$**

Female; PCr <150, $X=2.50+0.0121\times(150-PCr)$

Female; PCr ≥150, $X=2.50-0.926\times \ln(PCr/150)$

Male; PCr <150, $X=2.56+0.00968\times(180-PCr)$

Male; PCr ≥180, $X=2.56-0.926\times \ln(PCr/180)$

**FAS** **eGFR**= $\frac{107.3}{PCr/Q} \times 0.988^{(Age-40)}$ for age≥40

Male, Q=80 µmol/L Female, Q=62 µmol/L

**BIS 1** **eGFR** = 3,736 x $PCr^{-0.87}$ x $age^{-0.95}$ x 0.82 (if female)

**Statistical analyses**

The bias was defined as the median of between mGFR and eGFR. Precision was assessed as the interquartile range (IQR) of the differences between mGFR and eGFR. The 30% accuracies ($P_{30}$) according to the KDIGO guidelines were calculated[1]. These are defined as the proportions of the estimates falling respectively within the interval the interval mGFR±30%. The root mean squared error (RMSE) was calculated as the square root of the squared differences between mGFR and eGFR. The 95% confidence intervals (CIs) around the median difference, IQR of the difference, $P_{30}$ and RMSE were calculated using the bootstrap method (2,000 bootstraps).

In order to assess and compare the two equations according to the age and the level of GFR, the analysis was carried out on different age groups and different categories of mGFR. The results are presented for two categories of age (65 to 75 and ≥76 years) and for two categories of renal function defined here by the (<45 and ≥45 mL/min/1.73 $m^2$)[9].

We used area under the ROC curves (AUC) to determine the ability of the eGFR to discriminate between patients with and without CKD in elderly (defined by a mGFR <45 ml/min/1.73 $m^2$). The Delong Clarke-Pearson method is used to compare AUCs[24].

The comparisons of the bias, IQR and the $P_{30}$ between used, respectively, *Kruskal-Wallis*, the *Westenberg-Mood* and *Cochran* Q tests with *Dunn* and pairwise *McNemar* permutation tests as a post-hoc[25]. Method of *Holm-Bonferroni* was used to correct for multiple comparisons. The nominal *P*-value used to conclude to a statistical significance was <.005 [26].

The analyses were performed with R for Windows, version 3.4.4 (R-Cran project, http://cran.r-project.org/).

## RESULTS

### Participants' characteristics

The clinical characteristics of the 2,247 participants are shown in Table 1. At inclusion in the cohort, the mean (±SD) of the participants was 71.3 ± 5.2 years. Female patients and kidney transplant recipients represented 47% and 14% of the population, respectively. The mean (±SD) of GFRs was 44.5 ± 21 mL/min/1.73 $m^2$. Within the GFR range of 5 to 147 mL/min/1.73 $m^2$, 43.5% of the measurements had values <45 mL/min/1.73 $m^2$. We compared the measurement GFR of the population of renal transplants with the general population and did not find statistical difference.

### Performance according to equation type, CKD class and age

In the whole population, no equation demonstrated a significant higher performance compared to CKD-EPI according to bias, precision (IQR), accuracy ($P_{30}$ and RMSE) (table 2).In the whole population, all the equations had similar performed and without difference statistical (median bias: -2.0, 95% CI [-3.0; -1.0]; 2.0, 95% CI [1.5; 3.5]; 0.0, 95% CI [-0.5; 0.5] and -2.0, 95% CI [-3.5; -1.5] *P*=.75, for CKD-EPI, LMR, FAS and BIS1, respectively). In the subjects between 65 and 74 years, we had similar results (median bias: 2.0, 95% CI [0.8; 2.5]; -2.0, 95% CI [-3.0; -1.6]; 0.0, 95% CI [-0.5; 0.5], and 2.0, 95% CI [-0.5; 1.5]) *P*=.42, for CKD-EPI, LMR, FAS and BIS1 (Table 2) (Figure 1). Same resultants showed for the individuals ≥75 years (mean bias: 3.0, 95% CI

[2.5; 5.0]; -2.0, 95% CI [-3.5; -1.5], 0.0, 95% CI [-1.0, 0.5] and 2.0, 95% CI [1.5; 3.5] *P*=.72, for CKD-EPI, LMR, FAS and BIS1, respectively) (Table 3).

The medians differences by the four equations have slightly changes along with the GFR (Table 4). Moreover, in GFR <45 mL/min/1.73 $m^2$, LMR equation were significantly less biased than the other equations (bias -1, 95% CI: [-2.0; 0.0], P<.001, ANOVA). In GFR ≥45 mL/min/1.73 $m^2$, there were no differences between equations (Table 3).

**Equations precision and accuracy**

All the equations performed slightly similar regarding accuracy $P_{30}$ (78.0, 95% CI [76.0; 80.0], 81.5, 95% CI [80.0; 83.0], 79.0, 95% CI [78.0; 82.0], 77.5, 95% CI [75.5; 79.0] *P*=.78, for CKD-EPI, LMR, FAS and BIS1, respectively) (Table 2).

There were some differences regarding the accuracy $P_{30}$ in the individuals with mGFR<45 mL/min/1.73 $m^2$. LMR was superior and BIS1 was inferior to CKD-EPI in those between 65 and 75 years: accuracy $P_{30}$ (72.0, 95% CI [69.0; 76.0] and 59.0, 95% CI [55.0; 63.0] for LMR and BIS1, respectively, vs. 67.0 95% CI [64.0; 70.5] and 69.0, 95% CI [65.5; 72.5] for CKD-EPI and FAS, respectively, P<.001). For the oldest individuals (>75 years), both LMR and BIS1 showed the best accuracy ($P_{30}$: 74.5, 95% CI [70.0; 79.5] and 73.0, 95% CI [68.0; 78.0] for LMR and BIS1, vs. 69.0 95% CI [64.5; 74.0] and 69.0, 95% CI [65.5; 72.0] P<.001, for CKD-EPI and FAS, respectively).

Nevertheless, analysis of ability to correctly predict patient's GFR above 45 mL/min per 1.73 $m^2$ showed similar ability for all equations (P = .0–0.89). All

equations are equally accurate for estimating GFR in elderly Caucasian CKD patients. All GFR equations demonstrated a similar ability to detect mGFR<45 mL/min/1.73 $m^2$ as assessed by the area under the ROC >0.90 (table 3 and figure 3).

**Bland and Altman plots**

The difference between calculated and measured values of GFR was illustrated using a graphical technique according to Bland and Altman and regression (Figure 1). These figures display the span between 11.96 and –1.96 SD of the mean difference (limits of agreement), which represents the 95% CI. The limits of agreements were similar between equations equation: CKD-EPI (47 mL/min/1.73 $m^2$), LMR (44 mL/min/1.73 $m^2$), BIS 1 (45 mL/min/1.73 $m^2$) and FAS (47 mL/min/1.73 $m^2$).

**DISCUSSION**

The GFR is regarded as the best indicator for kidney function[1]. Late referrals for the management of CKD may result in suboptimal outcomes related to increased mortality, higher rates of hospitalization, decreased rates of kidney transplantation, and higher rates of catheter use for dialysis in aging. However, very few equations have been specifically determined in elderly (except the BIS equation) and in clinical practice the question of which equation should be used in this population is debated. Indeed, early detection of significant CKD in elderly notably in GFR <45 mL/min/1.73 $m^2$ may allow prevent/slow down evolution to end-stage renal disease and specific care (drug dosing, avoidance of intravenous iodinated contrasts, etc.) can be implemented[1,2,32,33]

The CKD-EPI equation is recommended for eGFR reporting in all age groups of adults in North America, Europe, and Australia. This equation were developed in a North American and European study population with a wide age range and took into account in addition to age and gender, ethnicity. However, the proportion of patients >65 years within the CKD-EPI development and internal validation data sets was only 12.0%[5]. We have shown that the CKD-EPI equation has an adequate performance for the diagnosis of CKD in geriatric practice.

The LMR equation was based on a cohort with 28% of the patients aged 70 years or older [6]. This equation performed better than CKD-EPI in an older population and in a large European population, predominantly (67%) from a single country (Sweden) without external validation [20,21,29,30]. In our study, the performance of the LMR equation was slightly more accurate ($P_{30}$) in the mGFR <45 mL/min/1.73 $m^2$ with the corresponding CKD-EPI equation.

Rare studies done specifically in geriatric population has reasonably performs, usually with poor accuracy in GFR greater than 60 mL/min/1.73 $m^2$ [6-8,15,16,19-21,27,28]. An equation projected specifically to eGFR in older people was by the Berlin Iniative Study (BIS)[7]. The development study population had aged >70 years (mean, 78.5 years). The results showed surprising performance, with better bias, precision and accuracy ($P_{30}$: 95.1%), appearing promising for eGFR in the elderly [7]. Other studies that attempted to validate this equation, however, showed conflicting results, some with acceptable performance[8,15,17-19]. However, our study the BIS1 equation had a poor performance in GFR <45 mL/min/1.73 $m^2$. These findings are corroborated by other studies [16,21,31].

The FAS equation was designated for eGFR across the children to older adults[8]. It was developed in a predominantly European multicentre study, with a total of 1,764 older people. In this study, the equation FAS performed better than both BIS1 and CKD-EPI in older people ($P_{30}$: 86.1%)[8]. However, we did not find an evident superior accuracy in our study between FAS and others equations.

Late referrals for the management of CKD may result in suboptimal outcomes related to increased mortality, higher rates of hospitalization, decreased rates of kidney transplantation, and higher rates of catheter use for dialysis in aging. Nevertheless, it has been demonstrated that the current definition of CKD in elderly (<60 mL/min/1,73m$^2$) does not reduce life expectancy or reliable predict an excess mortality or end stage renal disease (ESRD)[9,11,34]. Therefore, *Glassock* et al suggested to modify the definition of CKD for a lower GFR (<45 ml/min/1,73m$^2$) in older person[9]. We agree that the current cut-off for the diagnosis of CKD has created an epidemic in elderly individuals, because the most equations used the age as an exponential factor for the eGFR.

The prior probability of disease before the test is applied the ROC curve analysis (cut-off for GFR <45 mL/min/1.73 m$^2$) has no statistically significant difference between all the equations in our study. The *Bland-Altman* analysis for the same cut-off value showed that all equations are similar in their limits of agreements.

The strengths of the present study are: a pooled data set unique in size and large proportion of subjects with various ages and degrees of GFR; use of

PCr assays calibrated on standardized values; use of exogenous clearance with Gold Standard, urinary clearance of inulin; and rigor statistical techniques.

Despite its large scale, the present study has some limitations. First, this single centre study, which included few non-white participants, could not assess the effect of ethnicity, the same problem for other Europeans studies (LMR, BIS1 and FAS), not for CKD-EPI. Recent studies, however, have reported that GFR is independent of ethnicity and that the use of population-specific corrections for PCr provides robust adjustments for standard eGFR equations. Second, the performance of eGFR equations in participants with GFR <30 mL/min/1.73 m² could not be independently examined because of the small number of participants with severe CKD. Thirdly, the use of PCr alone as an endogenous marker has some limitations being widely known, especially among the elderly with sarcopenia [35,36]..

In conclusion, although falling short of the $P_{30}$ (>80%) aspiration of the KDIGO guideline[1], eGFR with PCr are acceptable to older populations. Moreover, we found no evidence that the news equations have the better performance than the CKD-EPI equations, particularly at values of GFR <45 mL/min/1.73 $m^2$. We recommended maintaining the use of CKD-EPI in elderly to estimated GFR with a cautious interpretation of results. Specific equations should be developed in this specific population in multicentre (Africa, America, Asia and Europe).

**AKNOWLEDGEMENTS**

**Conflicts of Interest:** The authors have no conflicts.

**Financial Disclosure:** None.

**Author Contributions**: Study concept and design: LSS, LD, DR; Acquisition of data: LSS, LD, VS, SL; Data analysis and interpretation: LSS, LD, VS; DR; Drafting and revising of the manuscript: LSS, DR, LD, VS, SL; Statistical analysis: LSS, VS. All authors had access to the data.

**Table 2 - Characteristics of the patients**

| Characteristic | Whole cohort | <45 mL/min/1.73 $m^2$ | ≥45 mL/min/1.73 $m^2$ | *P* value |
|---|---|---|---|---|
| **N of participants – (%)** | 2,247 (100.0) | 991 (43.5) | 1,256 (56.5) | |
| **Mean age in years** | 71.5 ± 5.0 | 72.0± 5.5 | 70.5 ± 4.5 | <.001 |
| **Age Group – n. (%)** | | | | |
| **65 – 75 yrs.** | 1.710 (76.0) | 670 (67.5) | 1040 (83.0) | <.001 |
| **>75 yrs.** | 537 (24.0) | 321 (32.5) | 216 (17.0) | <.001 |
| **Female sex, n°. (%)** | 1,055 (47.0) | 450 (45.5) | 605 (48.0) | 0.2 |
| **Transplant Kidney, n°. (%)** | 311 (14.0) | 156 (16.0) | 155 (12.5) | .03 |
| **Mean Weight, Kg** | 71.5 ± 18.0 | 73.0 ± 17.5 | 68.5 ± 16.5 | <.001 |
| **Mean Height, cm** | 163.5 ± 9.5 | 163.5 ± 9.5 | 163.0 ± 9.0 | .3 |
| **Mean BSA, $m^2$** | 1.75 ± 0.20 | 1.78 ± 0.20 | 1.73 ± 0.20 | <.001 |
| **Mean BMI (Kg/$m^2$)** | 26.5 ± 5.5 | 27.3 ± 6.0 | 25.7 ± 5.0 | <.001 |
| **BMI ≥30.0, n°. (%)** | 466 (21.0) | 257 (26.0) | 209 (16.5) | <.001 |
| **Median PCr, µmol /L [IQR]** | 113 [86; 145] | 150 [123; 187] | 92 [74; 111] | <.001 |
| **GFR, mL/min/1.73 $m^2$** | 44.5 ± 21.0 | 31.0 ± 9.0 | 64.0 ± 15.5 | <.001 |
| **Transplant Kidney, GFR** | 47.0 ± 17.0 | 34.0 ± 7.5 | 60.0 ± 14.0 | <.001 |
| **Median albuminuria, mg/g [IQR]** | 4.5 [1.5;22.5] | 9.5 [3.0;50.0] | 2.5 [1.0;10.0] | <.001 |
| **Albuminuria Categories, n(%)** | | | | |
| **<30 mg/g** | 940 (42.0) | 255 (26.0) | 685 (54.5) | <.001 |
| **30-300 mg/g** | 827 (37.0) | 427 (43.0) | 400 (32.0) | <.001 |
| **>300 mg/g** | 480 (21.0) | 309 (31.0) | 171 (13.5) | <.001 |

GFR: Glomerular filtration rate. BMI: Body-mass index. BSA: Body-surface area. IQR: interquartile range. PCr: Plasma Creatinine.

**Table 3 – Bias, precision (RMSE), and accuracy of the four estimating GFR equations**

| | CKD-EPI | LMR | FAS | BIS 1 |
|---|---|---|---|---|
| **A. All measurements (N=2,247; mGFR = 44.5 ± 21.0 mL/min/1.73m²)** | | | | |
| **Bias (95% CI)** | -2.0 (-3.0; -1.0) | 2.0 (1.5; 3.5) | 0.0 (-0.5; 0.5) | -2.0 (-3.5; -1.5) |
| **IQR (95% CI)** | 14.0 (13.0; 15.5) | 13.0 (12.0; 13.0) | 14.0 (13.0; 15.0) | 14.0 (13.0; 15.0) |
| **$P_{30}$ (95% CI)** | 78.0 (76.0; 80.0) | 81.5 (80.0; 83.0)¥ | 79.0 (78.0; 82.0) | 77.5 (75.5; 79.0) |
| **RMSE (95% CI)** | 0.195 (0.188; 0.203) | 0.185 (0.178; 0.190) | 0.188 (0.182; 0.196) | 0.189 (0.183; 0.196) |
| **B. Group 1 - 65-74 years (N=1,710; mGFR = 52.0 ± 21.0 mL/min/1.73m²)** | | | | |
| **Bias (95% CI)** | 2.0 (0.8; 2.5) | -2.0 (-3.0; -1.5) | 0.0 (-0.5; 0.5) | 2.0 (-0.5; 1.5) |
| **IQR (95% CI)** | 15.0 (14.5; 17.0) | 14.0 (13.0; 15.5) | 14.0 (12.5; 15.0) | 15.0 (14.0; 16.5) |
| **$P_{30}$ (95% CI)** | 79.5 (76.0; 83.0) | 82.0 (80.5; 84.0) | 80.5 (78.5; 82.0) | 78.0 (76.0; 80.0) |
| **RMSE (95% CI)** | 0.202 (0.194; 0.211) | 0.190 (0.182; 0.198) | 0.196 (0.187; 0.204) | 0.195 (0.188; 0.204) |
| **C. Group 2 mGFR ≥ 75 years (N=537; mGFR = 41.0 ± 19.0 mL/min/1.73m²)** | | | | |
| **Bias (95% CI)** | 3.0 (2.5; 5.0) | -2.0 (-3.5; -1.5) | 0.0 (-1.0; 0.5) | 2.0 (1.5; 3.5) |
| **IQR (95% CI)** | 13.0 (12.0; 15.0) | 12.0 (11.0; 14.0) | 13.0 (12.0; 15.0) | 13.0 (11.0; 15.0) |
| **$P_{30}$ (95% CI)** | 77.0 (73.0; 80.0) | 80.0 (76.5; 83.5)¥ | 78.5 (75.0; 82.0) | 76.0 (72.5; 79.5) |
| **RMSE (95% CI)** | 0.173 (0.160; 0.185) | 0.164 (0.152; 0.176) | 0.166 (0.154; 0.179) | 0.171 (0.159; 0.183) |

¥P <.005 for difference between CKD-EPI equation and other equations. GFR and eGFR are expressed in mean ± standard deviation. mGFR= Measured glomerular filtration rate by inulin or iohexol clearance, eGFR= estimated glomerular filtration rate. RMSE: Root mean square error

**Table 4 – Bias, precision, accuracy and AUC of the four estimating GFR equations**



| | Age range 65-75 years | | | | | Age range >75 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **mGFR <45 mL/min/1.73 m² (N=991; mGFR = 30.5 ± 9.0 mL/min/1.73m²)** | | | | | | | | | | |
| **Equations** | **Bias (95% CI)** | **IQR (95% CI)** | **AUC (95% CI)** | **RMSE (95% CI)** | **$P_{30}$ (95% CI)** | **Bias (95% CI)** | **IQR (95% CI)** | **AUC (95% CI)** | **RMSE (95% CI)** | **$P_{30}$ (95% CI)** |
| **CKD-EPI** | -4.0 (-5.0;-3.0) | 11.0 (9.0;12.0) | 0.92 (0.90;0.93) | 0.168 (0.156;0.180) | 67.0 (64.0;70.5) | -4.0 (-5.0;-3.0) | 11.0 (10.0;13.0) | 0.92 (0.90;0.94) | 0.150 (0.136;0.164) | 69.0 (64.5;74.0) |
| **LMR** | -1.0 (-2.0;0.0) | 11.0 (9.0;12.5) | 0.92 (0.90;0.93) | 0.150 (0.140;0.160) | 72.0 (69.0;76.0)¥ | -1.0 (-2.5;-0.5) | 10.0 (8.5;12.0) | 0.92 (0.90;0.94) | 0.127 (0.116;0.138) | 74.5 (70.0;79.5)¥ |
| **BIS 1** | -7.0 (-8.0;-6.0) | 10.0 (9.0;11.0) | 0.92 (0.90;0.93) | 0.178 (0.169;0.189) | 59.0 (55.0;63.0)¥ | -5.0 (-6.0;-4.0) | 8.0 (6.0;9.5) | 0.92 (0.90;0.94) | 0.140 (0.129;0.151) | 73.0 (68.0;78.0)¥ |
| **FAS** | -4.0 (-4.6;-2.5) | 10.0 (8.5;11.0) | 0.91 (0.90;0.93) | 0.153 (0.144;0.163) | 69.0 (65.5;72.5) | -3.0 (-4.0;-2.0) | 9.0 (7.5;10.5) | 0.92 (0.90;0.94) | 0.123 (0.112;0.134) | 69.0 (65.5;72.0) |
| **mGFR ≥45 mL/min/1.73 m² (N=1,256; mGFR = 64.5 ± 15.5 mL/min/1.73m²)** | | | | | | | | | | |
| **CKD-EPI** | -1.0 (-2.5; 0.0) | 16.0 (15.0;17.5) | 0.92 (0.90;0.93) | 0.224 (0.213;0.236) | 85.0 (83.0;87.0) | 1.0 (-1.0;2.5) | 15.0 (12.5;17.5) | 0.92 (0.90;0.94) | 0.207 (0.184;0.230) | 88.0 (84.0;92.0) |
| **LMR** | 4.0 (3.0;4.5) | 14.0 (12.0;15.0) | 0.92 (0.90;0.93) | 0.216 (0.205;0.227) | 88.5 (86.5;90.5) | 7.0¥ (6.0;8.5) | 14.0 (12.0;16.5) | 0.92 (0.90;0.94) | 0.219 (0.196;0.241) | 88.0 (84.0;92.0) |
| **BIS 1** | 4.0 (3.5;5.5) | 14.0 (13.0;15.5) | 0.92 (0.90;0.93) | 0.206 (0.196;0.218) | 90.0 (88.0;92.0)¥ | 7.0¥ (5.5;8.5) | 13.0 (11.0;16.0) | 0.92 (0.90;0.94) | 0.217 (0.194;0.241) | 89.5 (86.5;93.5) |
| **FAS** | 4.0 (3.0;5.0) | 15.0 (14.0;17.0) | 0.91 (0.90;0.93) | 0.222 (0.211;0.234) | 88.0 (86.0;90.0) | 7.0¥ (5.0;8.0) | 13.0 (10.0;15.0) | 0.92 (0.90;0.94) | 0.229 (0.205;0.255) | 86.5 (82.5; 92.0) |

¥P <.005 for difference between CKD-EPI equation and other equations. GFR and eGFR are expressed in mean ± standard deviation. mGFR= Measured glomerular filtration rate by inulin clearance, eGFR= estimated glomerular filtration rate. RMSE: Root mean square error. AUC: the area under a receiver curve operating characteristic

## REFERENCES


1. Group KDIGOKCW. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. 2013;3(1):1-150.
2. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: global dimension and perspectives. *Lancet.* 2013;382(9888):260-272.
3. Eckardt KU, Coresh J, Devuyst O, et al. Evolving importance of kidney disease: from subspecialty to global health burden. *Lancet.* 2013;382(9887):158-169.
4. Soveri I, Berg UB, Bjork J, et al. Measuring GFR: a systematic review. *Am J Kidney Dis.* 2014;64(3):411-424.
5. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-612.
6. Björk J, Grubb A, Sterner G, Nyman U. Revised equations for estimating glomerular filtration rate based on the Lund-Malmö Study cohort. *Scand J Clin Lab Invest.* 2011;71(3):232-239.
7. Schaeffner ES, Ebert N, Delanaye P, et al. Two novel equations to estimate kidney function in persons aged 70 years or older. *Ann Intern Med.* 2012;157(7):471-481.
8. Pottel H, Hoste L, Dubourg L, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol Dial Transplant.* 2016;31(5):798-806.
9. Glassock R, Delanaye P, El Nahas M. An Age-Calibrated Classification of Chronic Kidney Disease. *JAMA.* 2015;314(6):559-560.
10. Bevc S, Hojs N, Hojs R, Ekart R, Gorenjak M, Puklavec L. Estimation of Glomerular Filtration Rate in Elderly Chronic Kidney Disease Patients:

Comparison of Three Novel Sophisticated Equations and Simple Cystatin C Equation. *Ther Apher Dial.* 2017;21(2):126-132.

11. Garasto S, Fusco S, Corica F, et al. Estimating glomerular filtration rate in older people. *Biomed Res Int.* 2014;2014:916542.
12. United States Renal Data System. Chapter 1: CKD in the General Population. 2015 USRDS annual data report: Epidemiology of Kidney Disease in the United States. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2015. In:2015.
13. Dowling TC, Wang ES, Ferrucci L, Sorkin JD. Glomerular filtration rate equations overestimate creatinine clearance in older individuals enrolled in the Baltimore Longitudinal Study on Aging: impact on renal drug dosing. *Pharmacotherapy.* 2013;33(9):912-921.
14. Raman M, Middleton RJ, Kalra PA, Green D. Estimating renal function in old people: an in-depth review. *Int Urol Nephrol.* 2017;49(11):1979-1988.
15. Lopes MB, Araújo LQ, Passos MT, et al. Estimation of glomerular filtration rate from serum creatinine and cystatin C in octogenarians and nonagenarians. *BMC Nephrol.* 2013;14:265.
16. Koppe L, Klich A, Dubourg L, Ecochard R, Hadj-Aissa A. Performance of creatinine-based equations compared in older patients. *J Nephrol.* 2013;26(4):716-723.
17. Alshaer IM, Kilbride HS, Stevens PE, et al. External validation of the Berlin equations for estimation of GFR in the elderly. *Am J Kidney Dis.* 2014;63(5):862-865.
18. Vidal-Petiot E, Haymann JP, Letavernier E, et al. External validation of the BIS (Berlin Initiative Study)-1 GFR estimating equation in the elderly. *Am J Kidney Dis.* 2014;63(5):865-867.

19. Fan L, Levey AS, Gudnason V, et al. Comparing GFR Estimating Equations Using Cystatin C and Creatinine in Elderly Individuals. *J Am Soc Nephrol.* 2015;26(8):1982-1989.

20. Bjork J, Grubb A, Gudnason V, et al. Comparison of glomerular filtration rate estimating equations derived from creatinine and cystatin C: validation in the Age, Gene/Environment Susceptibility-Reykjavik elderly cohort. *Nephrol Dial Transplant.* 2017.

21. Bjork J, Back SE, Ebert N, et al. GFR estimation based on standardized creatinine and cystatin C: a European multicenter analysis in older adults. *Clin Chem Lab Med.* 2017.

22. Glassock RJ, Rule AD. The implications of anatomical and functional changes of the aging kidney: with an emphasis on the glomeruli. *Kidney Int.* 2012;82(3):270-277.

23. Selistre L, De Souza V, Cochat P, et al. GFR estimation in adolescents and young adults. *J Am Soc Nephrol.* 2012;23(6):989-996.

24. Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics.* 2011;12:77.

25. Seeger P, Gabrielsson A. Applicability of the Cochran Q test and the F test for statistical analysis of dichotomous data for dependent samples. *Psychol Bull.* 1968;69(4):269-277.

26. Ioannidis JPA. The Proposal to Lower P Value Thresholds to .005. *JAMA.* 2018;319(14):1429-1430.

27. Stevens LA, Schmid CH, Greene T, et al. Comparative performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 m2. *Am J Kidney Dis.* 2010;56(3):486-495.

28. Kilbride HS, Stevens PE, Eaglestone G, et al. Accuracy of the MDRD (Modification of Diet in Renal Disease) study and CKD-EPI (CKD Epidemiology Collaboration) equations for estimation of GFR in the elderly. *Am J Kidney Dis.* 2013;61(1):57-66.

29. Björk J, Jones I, Nyman U, Sjöström P. Validation of the Lund-Malmö, Chronic Kidney Disease Epidemiology (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) equations to estimate glomerular filtration rate in a large Swedish clinical population. *Scand J Urol Nephrol.* 2012;46(3):212-222.

30. Nyman U, Grubb A, Larsson A, et al. The revised Lund-Malmo GFR estimating equation outperforms MDRD and CKD-EPI across GFR, age and BMI intervals in a large Swedish population. *Clin Chem Lab Med.* 2014;52(6):815-824.

31. Liu X, Cheng MH, Shi CG, et al. Variability of glomerular filtration rate estimation equations in elderly Chinese patients with chronic kidney disease. *Clin Interv Aging.* 2012;7:409-415.

32. Etgen T, Chonchol M, Förstl H, Sander D. Chronic kidney disease and cognitive impairment: a systematic review and meta-analysis. *Am J Nephrol.* 2012;35(5):474-482.

33. Musso CG, Jauregui JR, Macías Núñez JF. Frailty phenotype and chronic kidney disease: a review of the literature. *Int Urol Nephrol.* 2015;47(11):1801-1807.

34. Hallan SI, Matsushita K, Sang Y, et al. Age and association of kidney measures with mortality and end-stage renal disease. *JAMA.* 2012;308(22):2349-2360.

35. Epstein M. Aging and the kidney. *J Am Soc Nephrol.* 1996;7(8):1106-1122.

36. Baxmann AC, Ahmed MS, Marques NC, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol.* 2008;3(2):348-354.

Braz. J. Nephrol. (J. Bras. Nefrol.) 2018;40(1):xxx-xxx

**Short Communication** | Comunicações Breves

# *Association between high-performance liquid chromatography with iohexol (glomerular filtration rate) and plasma oxalate*

**Título abreviado: Glomerular filtration rate and plasma oxalate**

**Luciano da Silva Selistre[1], Pierre Cochat[2], Dener Iizot Rech[1], François Parant[3], Vandréa Carla de Souza[1], Laurence Dubourg[4]**

[1]Universidade de Caxias do Sul, Caxias do Sul, Brasil

[2]Université Claude-Bernard Lyon, Centre de Référence des Maladies Rénales Rares Nephrogones, Service de Néphrologie et Rhumatologie Pédiatriques, Lyon, France

[3]Hospices Civils de Lyon, GHS - Centre de Biologie Sud, UM Pharmacologie - Toxicologie, F-69495, Pierre Bénite, France

[4]Université Claude-Bernard, Groupement Hospitalier Edouard Herriot, Hospices Civils de Lyon,UMR 5305, Rhone-Alpes, Lyon, France

## ABSTRACT

Introduction. Secondary hyperoxalemia is a multifactorial disease affecting several organs and tissues, native and transplanted kidneys. Plasma oxalate may increase during renal failure because oxalate is cleared from the body by the kidneys. However, there is rare evidence evaluating the association between glomerular filtration rate and plasma oxalate, especially in the early stages of chronic renal disease. Methods: A case series focuses on the description of variations in clinical presentation. A pilot study was conducted using cross-sectional analyses with 72 subjects. The glomerular filtration rate (GFR) and plasma oxalate levels were measured for all patients.Results: Median (IQR) GFR was 70.50 [39.0; 91.0] mL/min/1.73 m2. Plasma oxalate was <5.0µmol/L in all patients with a GFR > 30 mL/min/1.73m2. Among the 14 patients with severe CKD (GFR < 30 mL/min/1.73 m2) only 4 patients showed a plasma oxalate slightly increased (between 6 and 12 µmol/L).Conclusion: In non-primary hyperoxaluria, plasma oxalate concentration increase when GFR < 30mL/min/1.73 m2 and, in our opinion, values greater than 5 µmol/L with a GFR> 30 mL/min/1.73 m2 are suggestive of primary hyperoxaluria. Further studies are necessary to confirm plasma oxalate kinetic increase in lowest level of GFR (<30mL/min/1.73 m2).

**Keywords:** Glomerular Filtration Rate; Hyperoxaluria; Renal Insufficiency, Chronic.

**Correspondence to:**
Luciano da Silva Selistre.
E-mail: lsselist@ucs.br

DOI: 10.1590/1678-4685-JBN-3743

## INTRODUCTION

Oxalate is the ionic form of plasma oxalate (POx) and is a highly insoluble end product of metabolism in humans and derived from various animal and plant sources. Increase of POx and oxalosis, i.e., calcium oxalate deposition in the tissues, could occur and is classified as primary or secondary hyperoxaluria (SH) (1, 2). Primary hyperoxalurias (PH) are a group of rare autosomal recessive metabolic disorder resulting in overproduction of oxalate (3). There are three forms of primary hyperoxaluria in which the underlying defects have been identified; they are designated as primary hyperoxaluria types 1, 2, and 3. Each is caused by an enzyme deficiency, and each affects a different intracellular organelle (1,2).

SH may occur either as a result of excessive ingestion of oxalate or oxalate precursors such as ethylene glycol or through decreased excretion of oxalate by the kidney (1). Excessive intake or increased absorption (intestinal disorders) of oxalate are widespread causes of urinary oxalate excretion increase and urolithiasis, but is rarely associated to an increase of POx with normal glomerular filtration rate (GFR) (3-5). Thus, it cannot be further processed, being totally eliminated through GFR by the kidneys. In patients with CKD, POx accumulates 10–30 times above normal levels as a result of its reduced excretion. Neither hemodialysis nor peritoneal dialysis is able to normalize POx levels in CKD patients. Only a 60% reduction of POx pool is expected to happen after a usual hemodialysis procedure, but POx was

found to return to pre-dialysis levels within 48 h (7). In contrast to PH, clinical manifestations of uremic oxalosis, such as nephrolithiasis, fractures and bone pain, are not common (1,7).

Nevertheless, a decline of GFR could increased POx, and linked to cardiovascular complications (1, 2, 6, 7). Salye et al reported that SH, i.e. renal and myocardial calcium oxalate deposition, in association with renal insufficiency is frequent and often extensive (8). In addition, they demonstrated that the incidence and severity of the oxalate deposition are related to the duration of renal insufficiency (8, 9). Rechet et al described an association between high levels of POx and endothelial injury leading to atherogenic effects by elevating intracellular calcium in endothelial cells (7). In transplanted renal, the POx may overload the graft with potential tubular damage his function (3, 6, 11).

Therefore, we initiated a pilot study with measured POx, GFR measurement (mGFR, iohexol clearance) and estimated GFR (eGFR, plasma creatinine) in a series of patients with various stages of CKD.

## MATERIALS AND METHODS

### PATIENTS

This pilot study includes 72 CKD patients without PH between October 2014 and November 2014 to undergo GFR measurement (iohexol clearance). Ten patients with a severe CKD (Stage IV-V) have POX without a mGFR. The population was divided into groups of GFR according to the KDIGO classification (12).

## OXALATE

POx was measured on a Pentra 400 analyser (HORIBA) by a modified sensitive oxalate oxidase colorimetric assay as reported by Petrarulo et al. Briefly, the oxalate is converted to hydrogen peroxide, which reacts in presence of peroxidase (POD) with MBTH (3-methyl-2-benzothiazolinone hydrazone) and DMAB (3-dimethylamino benzoic acid) forming a blue quinone compound. The intensity of colour is proportional to the concentration of POx in the sample and it is read at 600 nm with 700 nm as reference wavelength. The optimization of the assay included plasma deproteinization by the sulfosalicylic acid (SSA) method and treatment with choarcal. The reference values are < 5 µmol/L (13).

## GFR MEASUREMENT BY IOHEXOL CLEARANCE

Iohexol clearance was performed a standard technique with single-bolus injection. Briefly, an IV injection of 6 mL (Omnipaque, 300 mg/mL) was administered and 3 blood samples were drawn from the contra lateral arm after 120, 180, and 240 minutes. The mGFR was calculated from the slope of plasma concentrations using a one-compartment model corrected with the Bröchner-Mortensen formula (13). Plasma iohexol concentration was determined with an HPLC method adapted from Cavalier et al (15).The results were expressed in mL/min/1.73 $m^2$.

## ESTIMATION OF GFR

Plasma creatinine (PCr) was enzymatic IDMS standardized measurement and eGFR was calculated with the CKD-EPI equation (12).

## STATISTICAL ANALYSES

We evaluated the distribution of continuous variables by assessing mean ± standard deviation and categorical variables by number (percentage) in the whole data set, as well as in subgroups according to study population characteristics and candidate for living kidney donation. We defined error as mGFR minus eGFR (mGFR – eGFR) for each individual and percent error as this difference relative to mGFR, ie, (mGFR - eGFR)/mGFR. We computed bias as the average error with mean used as appropriate for the distribution.

Bias, an expression of systemic error in estimated GFR, is defined as the median or mean of the differences between estimated and measured GFR. The analysis was performed using R for windows, version 3.1.1 (*R-Cran project*, http://cran.r-project.org/).

## ETHICAL APPROVAL

All the procedures were carried out in accordance with the ethical standards of the institutional and/or national research committees and of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Precisely, an appropriate informed consent writer was obtained from all the participants or their legal representatives. The consent form contained information on the procedure itself as well as on the possibility of later use of the data for research purposes. According to the current French laws, an observational study that does not change routine management of patients does not need to be declared or submitted to the opinion of a research ethics board (Loi Huriet-Sérusclat 88-1138, 20 December 1988 and its subsequent amendments, text available at http://www.chu-toulouse.fr/IMG/pdf/loihuriet.pdf).

## RESULTS

The patient clinical characteristics are listed in Table. The mean age and BMI were 50.0 [IQR, 40.0-63.0] years and 25.3 [IQR, 22.3-32.0] kg/$m^2$, respectively (**Table)**.

Table and figure shows the performance of the CKD-EPI equation versus iohexol. CKD-EPI equation had a mean error of -3.0 (95% CI, -7.0 to -0.5) mL/min/1.73 $m^2$ without statistical difference to GFR (**Table and figure)**. Therefore, the use of CKDEPI in patients without mGFR could be adequate for evaluation of POx levels.

For all but one patient with mGFR the POx concentration was <5µmol/L. Only a patient had abnormal values of POx (7µmol/L) with a mGFR of 30 mL/min/1.73 $m^{2.}$ Among the 14 patients with severe CKD (GFR < 30 mL/min/1.73 $m^2$), four patients showed a POx slightly increased (between 6 and 12 µmol/L) (**Table)**.

## DISCUSSION

POx could increase in CKD due to the reduction GFR and secretion of the proximal renal tubules (1, 2, 15-18). However, few authors have specifically studied the correlation between POx and GFR stage (7,15-20). In addition, some authors established a threshold POx level that differentiate PH from other causes of POx increase (1, 2).

Constable et al. reported that POx was raised by a factor of 10 in PH subjects who still had good renal function (18). By contrast, Morgan et al. demonstrated in patients with non-PH-CKD that oxalate retention is increased when GFR is below 20 mL/min/1.73$m^2$ (20). In the same way, Constable et al. reported that the oxalate

metabolic pool began to expand rapidly when the GFR is under 25 mL/min/1.73 $m^2$(19) which is in accord with our results. Barratt et al. found that POx is also increased in end stage renal disease (ESRD) (19). Bhasin et al. reported that POx was higher than 80 µmol/L in PH patients with ESRD(1, 2). Elgstoen et al. found that median POx before transplantation was 35.0 µmol/L (95% CI: 10.4 to 93.9) and 98% of the values were above normal limits (11).

In the present study, we found a POx slightly increased when GFR < 30 mL/min per 1.73 $m^2$ which is well above the level at which renal replacement is needed. However, we were not able to demonstrate a correlation between GFR and POx.

The strengths of the present study are i) the reference method (iohexol) for direct measurement of GFR for most of the patients; and ii) the wide ranges for GFR levels (7 to 139 mL/min/1.73 $m^2$)

Its limitations are: i) the study population included few patients with GFR <30 mL/min/1.73 $m^2$, and could not allow establishing a correlation between POx and GFR.

## CONCLUSION

This study suggests that POx increases significantly only in the advanced stages of CKD. In our opinion, values greater than 5 µmol/L with an eGFR> 30 mL/min/1.73 $m^2$ are suggestive of PH. However, news studies should determine the kinetic of POx in advance CKD and dialysis patients.

## CONFLICT OF INTEREST

The authors state that they have no conflicts of interest to disclose.

**Table** Characteristics of the Patients

| | |
|---|---|
| Number of participants | 72 |
| Age (years) | 50 [40; 63] |
| Male | 40 (56.3) |
| Diagnostic | |
| CKD | 60 (83) |
| Candidate for living kidney *donation* | 12 (17) |
| Weight (kg) | 72.0 [60.5; 90.0] |
| Height (cm) | 168.0 [160.0; 174.5] |
| Body-surface area ($m^{2)}$ | 1.83 [1.63; 2.01] |
| Body-mass index ($kg/m^2$) | 25.3 [22.3; 32.0] |
| mGFR (mL/min/1.73 $m^2$) (n=62) | 74.5 [53.0; 91.0] |
| eGFR (mL/min/1.73 $m^2$) | 70.0 [39.0; 96.0] |
| mean mGFR-eGFR (95%CI), mL/min/1.73 $m^2$(N=62) | -3.8 (-7.0; -0.5) |
| Plasma oxalate | |
| Plasma oxalate < 5 µmol/L | 67 (93.0) |
| Plasma oxalate ≥ 5 µmol/L | 5 (7.0) |
| CKD stages | |
| Stage I | 16 (22.2) |
| Stage II | 28 (38.9) |
| Stage IIIa | 4 (5.5) |
| Stage IIIb | 10 (13.9) |
| Stage IV | 11 (15.3) |
| Stage V | 3 (4.0) |

Values are median (IQR) or n (%) unless otherwise specified CKD: chronic kidney disease - GFR: glomerular filtration rate·- IQR: interquartile range, Mean, *P<0.05- CKD stages were determined according to mGFR or eGFR if not available)

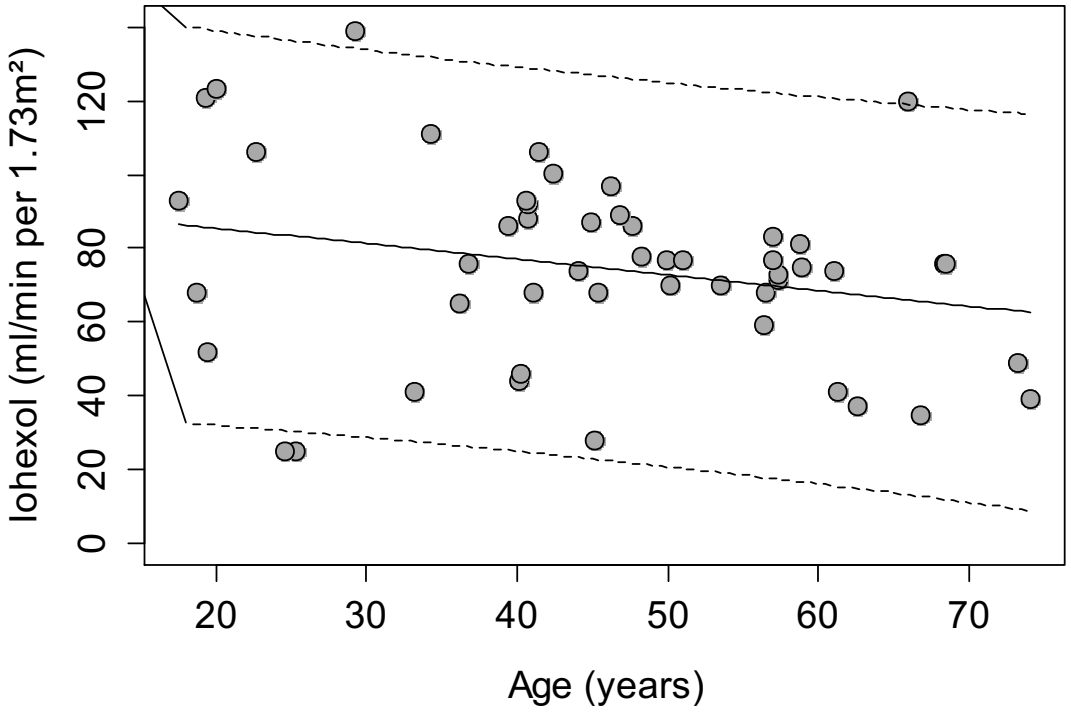


**Figure - Gromerular Filtration by Iohexol and Age (years).**

## REFERENCES

1. Bhasin B, Urekli HM, Atta MG. Primary and secondary hyperoxaluria: Understanding the enigma. World J Nephrol. 2015;4(2):235-44.

2. Cochat P, Rumsby G. Primary hyperoxaluria. N Engl J Med. 2013;369(7):649-58.

3. Bagnasco SM, Mohammed BS, Mani H, Gandolfo MT, Haas M, Racusen LC, et al. Oxalate deposits in biopsies from native and transplanted kidneys, and impact on graft function. Nephrol Dial Transplant. 2009;24(4):1319-25.

4. Rankin AC, Walsh SB, Summers SA, Owen MP, Mansell MA. Acute oxalate nephropathy causing late renal transplant dysfunction due to enteric hyperoxaluria. Am J Transplant. 2008;8(8):1755-8.

5. Gregoriou S, Kalapothakou K, Kontochristopoulos G, Belyayeva H, Chatziolou E, Rigopoulos D. Subungual oxalate deposits in a patient with secondary hyperoxaluria. Acta Derm Venereol. 2011;91(2):195-6.

6. Truong LD, Yakupoglu U, Feig D, Hicks J, Cartwight J, Sheikh-Hamad D, et al. Calcium oxalate deposition in renal allografts: morphologic spectrum and clinical implications. Am J Transplant. 2004;4(8):1338-44.

7. Pinheiro HS, Câmara NO, Osaki KS, De Moura LA, Pacheco-Silva A. Early presence of calcium oxalate deposition in kidney graft biopsies is associated with poor long-term graft survival. Am J Transplant. 2005 Feb;5(2):323-9.

8. Recht PA, Tepedino GJ, Siecke NW, Buckley MT, Mandeville JT, Maxfield FR, et al. Oxalic acid alters intracellular calcium in endothelial cells. Atherosclerosis. 2004;173(2):321-8.

9. Salyer WR, Salyer DC. Oxaluric renal failure. N Engl J Med. 1973;288(17):913.

10. Salyer WR, Keren D. Oxalosis as a complication of chronic renal failure. Kidney Int. 1973;4(1):61-6.

11. Elgstoen KB, Johnsen LF, Woldseth B, Morkrid L, Hartmann A. Plasma oxalate following kidney transplantation in patients without primary hyperoxaluria. Nephrol Dial Transplant. 2010;25(7):2341-5.

12. Group KDIGOKCW. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. 2013;3(1):1-150.

13. Petrarulo M, Cerelli E, Marangella M, Cosseddu D, Vitale C, Linari F. Assay of plasma oxalate with soluble oxalate oxidase. Clin Chem. 1994;40(11 Pt 1):2030-4.

14. Brochner-Mortensen J. A simple method for the determination of glomerular filtration rate. Scand J Clin Lab Invest. 1972;30(3):271-4.

15. Cavalier E, Rozet E, Dubois N, Charlier C, Hubert P, Chapelle JP, et al. Performance of iohexol determination in serum and urine by HPLC: validation, risk and uncertainty assessment. Clinica chimica acta; international journal of clinical chemistry. 2008;396(1-2):80-5.

16. Sikora P, Beck B, Zajaczkowska M, Hoppe B. Plasma oxalate level in pediatric calcium stone formers with or without secondary hyperoxaluria. Urol Res. 2009;37(2):101-5.

17. Kadlec AO, Greco KA, Fridirici ZC, Gerber D, Turk TM. Effect of renal function on urinary mineral excretion and stone composition. Urology. 2011;78(4):744-7.

18. Kasidas GP, Nemat S, Rose GA. Plasma oxalate and creatinine and oxalate/creatinine clearance ratios in normal subjects and in primary hyperoxaluria. Evidence for renal hyperoxaluria. Clin Chim Acta. 1990;191(1-2):67-77.

19. Constable AR, Joekes AM, Kasidas GP, O'Regan P, Rose GA. Plasma level and renal clearance of oxalate in normal subjects and in patients with primary hyperoxaluria or chronic renal failure or both. Clin Sci (Lond). 1979;56(4):299-304.

20. Barratt TM, van't Hoff WG. Are there guidelines for a strategy according to glomerular filtration rate, plasma oxalate determination and the risk of oxalate accumulation? Nephrol Dial Transplant. 1995;10 Suppl 8:22-3.

21. Morgan SH, Maher ER, Purkiss P, Watts RW, Curtis JR. Oxalate metabolism in end-stage renal disease: the effect of ascorbic acid and pyridoxine. Nephrol Dial Transplant. 1988;3(1):28

# 4 CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Os resultados do nosso estudo mostram que, considerando toda a população estudada, houve uma pequena melhora de desempenho da equação LMR em relação as equações BIS1, FAS e CKD-EPI, sendo mais nítido no subgrupo com TFG estimada <45mL/min/1,73m$^2$. Isso reflete que, o estudo de coorte de Lund-Malmö (elaboração da equação LMR), com uma menor TFG em geral (mediana 55mL/min/1,73m$^2$), comparado com o estudo de desenvolvimento CKD-EPI (média 68mL/min/1,73m$^2$), é mais representativo em idosos. Entretanto, pode não ser o ideal para estimar a TFG no subgrupo da população com maior TFG.

Apesar da vantagem da equação LMR em nosso estudo, os autores concluem que a ampla e globalmente utilizada equação CKD-EPI não teve um desempenho tão inferior que justifique ser substituída como preferência para reportar a TFG estimada em idosos. Manter o uso de CKD-EPI para adultos de qualquer idade facilita, e uniformiza, o informe da TFG estimada nos laboratórios de análises clínicas e a comunicação entre profissionais, pesquisadores e pacientes.

Deve-se atentar, entretanto, que este estudo, assim como a totalidade dos estudos que elaboraram ou tentaram validar as equações avaliadas em nosso estudo, foram realizados em população européia e predominantemente branca. Também, neste estudo, não se estudou equações que utilizem outro marcador endógeno bastante conhecido, embora menos utilizado devido ao custo, a Cistatina C, associada ou não a CrP, o que poderia trazer vantagem em idosos. Há, portanto, necessidade de replicar tal projeto baseado em outras populações, como a brasileira, melhorando assim a validade externa das equações citadas, assim como testar equações que contemplem a Cistatina C.

Por fim, chama a atenção que, desde o desenvolvimento das primeiras equações, há mais de 60 anos, todas as equações desenvolvidas para estimar a TFG baseadas em marcadores endógenos, tem tido um desempenho bastante modesto quando comparadas com modelo referência de mensuração da TFG, geralmente com uma acurácia P30 <80%, assim como em nosso estudo. Tal fato mostra o paradoxo desta questão em tempos de medicina de precisão. Urge que se

evolua na melhora do desempenho de equações que estimem a TFG, ou até que se facilite e simplifique  a mensuração direta da TFG por método referência.