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Editor-in-chief : Prof. Gábor L. Kovács, MD, PhD, DSc
Department of Laboratory Medicine, Faculty of Medicine, University of Pecs, Hungary
e-mail: ejifcc@ifcc.org

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

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Farewell message from the editor-in-chief

Gábor L. Kovács, M.D., Ph.D., DSc

Dear Colleagues,

This is the last issue of the eJIFCC that I edit in my capacity as the editor-in-chief of the journal. The two terms, i.e. a six-year period of service I held in the Communication and Publication Division of the IFCC, have elapsed rapidly. Looking back, a lot of things have happened to us including a large variety of changes we were involved in, or we initiated. 14.000 laboratorians from 93 countries receive the journal mass-mailed to their computers quarterly. Most importantly, eJIFCC is now indexed by PubMed Central, a development that has largely increased our visibility. A year ago, we submitted our application to Web of Science as well as to Scopus indexing; these processes, however, take years. We have also become member of the Committee of Publication Ethics (COPE).

The Editorial Board has been extended considerably during this period. In addition to the "old" members, twenty new members accepted to volunteer for the journal's editorial activities, all of them renowned laboratory scientists from different continents. Thanks to them, I almost never received a reviewer invitation rejected. The editorial board members were also most helpful in reviewing the presentations of IFCC's e-Academy, a new and highly successful product of the Communication and Publication Division. I specially thank the help of my two associate editors, Dr. Harjit Pal Bhattoa (Hungary), responsible for the linguistic editing, and Dr. Reinhard B. Raggam (Austria), the case-report editor of the journal.

The journal, the editorial board and the editor has always had the full support of Dr. Ellis Jacobs and Dr. Khosrow Adeli, the two chairs of the Communication and Publication Division, who followed each other in my editorial period.

The professional help of Insoft Digital in the e-publication of the journal needs to be acknowledged as well. They have greatly improved the professional appearance of the journal.

Last, but not least, I have to thank Mrs. Silvia Colli-Lanzi at the IFCC Office in Milan. Without her enthusiastic and highly professional assistance, neither the IFCC, nor the e-journal would be the same.

I wish the new editor and especially the laboratorians out there on all the continents further great success with the eJIFCC!

Pécs, November 13th, 2017
Gabor L. Kovacs

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Foreword of the editor

Editor-in-Chief: Gábor L. Kovács, MD, PhD, DSc

This special issue has been dedicated to the laboratory diagnosis of chronic kidney diseases. The IFCC Task Force on Chronic Kidney Disease sheds light on several aspects of the field, from basic research to daily clinical practices, uniting many IFCC member countries working in different aspects of the laboratory in chronic kidney care. Dr. Flavio Ferraz de Paes e Alcantara (Brazil), the chair of the task force and Dr. Vanja Radišić Biljak (Croatia), a member of the task force, were asked to guest-edit the issue.

Dr. Flavio Ferraz de Paes e Alcantara graduated in medicine in Santos, Brazil. He earned the specialization degree in clinical pathology after finishing medical residency at the clinical hospital of the University of Sao Paulo. Worked as a post-doctoral fellow at The Scripps Research Institute (USA), from 1996 to 2001. In 2001, he came back to Brazil as associated director of a medium size private clinical laboratory (IACS), working there part time, and as of 2012 became the leading director of IACS. Dr. Alcantara also works part time at the University of São Paulo. Initially as a research fellow, since 2006 he holds a tenure position as assistant physician at the Central Laboratory Division - Molecular Biology Section, the largest public hospital in South America. Dr. Alcantara is an active member of the Brazilian Society for Clinical Pathology and Laboratory Medicine, where he is frequently invited for lectures and meetings. In 2010, Dr. Alcantara started a term at the IFCC-WASPaLM Task Force on Chronic Kidney Diseases, and became the chair person for the 2016-2018 term.

Dr. Vanja Radišić Biljak, PhD, from Zagreb, Croatia, studied medical biochemistry at the Faculty of Pharmacy and Biochemistry, University of Zagreb (1999-2004). In 2005, she started her postgraduate doctoral study in medical biochemistry and defended her doctoral thesis „Chronic obstructive pulmonary disease and glutathione cycle" in 2010, gaining her PhD degree. Since 2010 she has been employed in Merkur University Hospital where she started her residency in Medical Biochemistry and Laboratory Medicine and graduated in 2014. Her major interest shifted towards nephrology, diabetes, and medical informatics. In 2010, she got a scholarship for EASD Young Scientists Training Course "Reactive metabolites in late diabetic complications" (University Hospital Heidelberg, Dept. of Medicine I and Clinical Chemistry) in Heidelberg, Germany. In 2013, she received a travel grant for the EFLM Postgraduate Course in Clinical Chemistry and Laboratory Medicine New Trends in Diagnosis and Monitoring using POC Instruments. In 2016, she was awarded as the best young scientist for 2015. The award was presented by the Croatian Society for Medical Biochemistry and Laboratory Medicine.

TF-CKD special issue: The laboratory diagnostics of chronic kidney diseases

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IFCC Task Force on Chronic Kidney Disease (Integrated Project) – (TF-CKD) special issue

Guest editors: Flavio F. Alcantara[1], Vanja Radišić Biljak[2]

[1] *Central Laboratory Division, Clinical Hospital, University of São Paulo, Medical School, Brazil*

[2] *Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Croatia*

ARTICLE INFO

Corresponding author:
Flavio F. Alcantara
Hospital das Clínicas
Av Eneas Carvalho Aguiar
155. Bloco 4, 2o andar
Sao Paulo 05403-000 SP
Brazil
Phone: +55 11 2661 7275
E-mail: flavio.alcantara@hc.fm.usp.br

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EDITORIAL

This series of articles is a milestone not only for the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the World Association of Societies of Pathology and Laboratory Medicine (WASPaLM) Task Force on Chronic Kidney Disease (TF-CKD) integrated project, but also an important outline on the role of the laboratory on assessment of the renal function in different scenarios. It sheds light on several aspects of the field, from basic research to daily clinical practices, uniting many IFCC member countries working with different aspects of the laboratory in CKD care.

In the initial article “We have come a long way” (1), a brief history of the Task Force is narrated. The IFCC leadership with WASPaLM partnership formulated a TF which amalgated the two major International scientific societies in Laboratory Medicine. The initial idea of “forging a global consensus” has shifted onto “helping to create and implement national guidelines in each country through corresponding members”, and later using the KDIGO 2012 guidelines (2) as a frame document.

IFCC has succeeded in gaining acceptance as a global partner for CKD care (3).

KDIGO 2012 guidelines classifies CKD based on cause, Glomerular Filtration Rate (GFR) category, and albuminuria category (CGA) (2), thus emphasizing the role of laboratory medicine in management of CKD. One of the major laboratory tests involved in CKD management is certainly creatinine and consequently estimation of GFR via estimating equations (2). The group of authors on behalf of the Société Française de Biologie Clinique in the article "Did creatinine standardization give benefits to the evaluation of glomerular filtration rate" (4) evaluate some limitations of creatinine and emphasize the importance on using IDMS traceable enzymatic assays and the reporting of eGFR.

Regarding albuminuria measurement, the article "Moving Toward Standardization of Urine Albumin Measurements" (5) reports on the continuing effort undertaken by the NKDEP Laboratory Working Group following their success on the standardization of creatinine measurement. It mentions their work on pre-analytical issues, the current state of measurements evaluating their precision and accuracy, the strategy undertaken defining a candidate reference method and for producing certified reference materials, including an evaluation of several albumin methods as previously published (6).

KDIGO 2012 guidelines also recognize the value of estimating GFR using Cystatin C measurements as a biomarker alternative to creatinine (2). In the article "Cystatin C is indispensable for evaluation of kidney disease" (7) a good case is made for using Cystatin C instead of other biomarkers for GFR. In fact, given their expertise, the Swedish have realize the importance of using both $eGFR_{crea}$ and $eGFR_{cysC}$, providing not only the best estimate GFR, but more importantly, yielding the mean $eGFR_{crea}$ and $eGFR_{cysC}$ value. This $eGFR_{mean}$, when in close agreement with each of the single eGFR results, is the best evaluation of GFR; in situations where the disagreement is above ~1/3 ($eGFR_{cysC} \leq 60\%\ eGFR_{crea}$), the decreased cystatin C filtration signals the presence of the recently described "Shrunken Pore Syndrome", predicting increase in mortality and morbidity while elucidating key pathophysiologic aspects on kidney diseases.

Nevertheless, neither creatinine nor Cystatine C present the ideal marker for estimating GFR. The "Novel Filtration Markers for GFR estimation" (8) article includes an update on the past and present research on two Glomerular Filtration Rate markers: the 11.8 kDa Beta Trace Protein (BTP) and the 23-29 kDa Beta 2 Microglobulin (B2M), some equations designed for their use in GFR estimation and the experience in specific patient cohorts using these markers, comments on approaches using panels of markers such as $eGFR_{cre}$, $eGFR_{cys}$, $eGFR_{BTP}$, and $eGFR_{B2M}$. Additionally, there is a glimpse on the use of metabolomic on studies searching for markers associated with $eGFR_{cre}$.

"A pathway to national guidelines for laboratory diagnostics of chronic kidney disease – examples from diverse European countries" take us on the path travelled by several countries toward improvement of CKD care (9). Various scenarios on developing and implementing national CKD guidelines are described, ranging from as early as 2002 when the Sociéte Francaise de Biologie Clinique (SFBC) formed the "Creatinine Working Group", later joined by Sociéte de Nephrologie, until 2017 and releasing the newest recommendations from Croatian Working Group for Laboratory Diagnostic of CKD.

The last article of the series "A summary of worldwide activities in chronic kidney disease (CKD) testing" (10) includes examples of countries with different settings. National CKD activities from almost every continent are presented, which makes the very first step in achieving the national CKD guidelines as a final goal.

A world of CKD has been depicted and different activities have been summarized. Unfortunately,

a lot has not been told and several fundamental authors and settings were not included, due to time and space constraints. Still, different levels of maturity on CKD care can be grasped upon. In laboratory, the saying goes that "quality is not an end point but a journey", we hope the road ahead may now have some additional marks for the travelers.

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TF-CKD special issue: The laboratory diagnostics of chronic kidney diseases

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Task Force on CKD – we have come a long way

Graham R. D. Jones

Department of Chemical Pathology, SydPath, St Vincent's Hospital, Sydney, Australia

ARTICLE INFO

Corresponding author:
Graham R. D. Jones
Department of Chemical Pathology,
SydPath, St Vincent's Hospital
Victoria St, Darlinghurst
NSW 2010, Sydney
Australia
E-mail: Graham.jones@svha.org.au

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ABSTRACT

Chronic Kidney Disease (CKD) is an important medical condition where diagnosis, staging and monitoring is largely based on routine laboratory tests. During the last 15 years there have been many important changes in the clinical management of CKD described in key international guidelines. In order to successfully implement these guidelines, laboratories must collaborate with clinicians to provide a co-ordinated service, including accurate measurements and of creatinine and urine albumin and reporting of an estimated glomerular filtration rate (eGFR). The IFCC/WASPaLM Task Force on Chronic Kidney Disease (TF-CKD) was established in 2008 and since that time has worked to improve laboratory testing in CKD. Key aspects of the work of the TF-CKD include supporting national laboratory medicine organisations to develop CKD testing guidelines, recognition of the vital role of collaboration between laboratory and clinical organisations, the importance of accurate measurements, and endorsement of the KDIGO 2012 CKD guidelines. A key function of the TF-CKD has been to facilitate sharing and learning between countries to provide the best outcomes.

INTRODUCTION

One of the great ways that progress is made is through the power of people working together. The current practices for laboratory testing for Chronic Kidney Disease (CKD) in many countries is the product of many different collaborations. The players involved in these collaborations include laboratory scientists, chemical pathologists, nephrologists, general practitioners, researchers, diagnostic manufacturers and many others. Often the mechanism for these collaborations is through professional societies and other organised structures.

I believe the IFCC Task Force on Chronic Kidney Disease (TF-CKD) has played an important role in promoting good laboratory practice in this field through collaboration on a range of levels. In this paper I outline the activities of the TF-CKD and its role in laboratory testing for CKD.

For this purpose I will consider three separate aspects: the formation and early years; the recommended approach to organising CKD testing; and the effects of sharing.

A BRIEF HISTORY OF THE TF-CKD

The TF-CKD was formed in 2008 on the initiative of then IFCC President Mathias Müller who recommended the formation of a Working Group on Screening for Chronic Kidney Disease (WG-CKD). The members of the WG included laboratory scientists, nephrologists and a chemical pathologist. In 2009 the terminology was changed to "Task Force" (TF-CKD) and an invitation was extended to the World Association of Societies of Pathology and Laboratory Medicine (WASPaLM) to be a joint sponsor of the TF, recognising the importance of pathologists as well as laboratory scientists and with the aim of getting the widest professional and organisational coverage. The invitation was accepted and the initial full membership is shown in Table 1.

These members had experience with developing and implementing CKD testing guidelines, in research in the field of CKD testing, of the measurement of serum creatinine and in the clinical application of the laboratory tests. Importantly the membership also had key roles in clinical, research and guideline organisations outside the

Table 1 WG-CKD initial membership

IFCC Nominees	
Graham JONES (AUS)	Joe CORESH (USA)
Edmund LAMB (UK)	Andy NARVA (USA)
David SECCOMBE (CAN)	Mauro PANTEGHINI (IT)
Joris DELANGHE (BEL)	
WASPaLM Nominees	
John ECKFELDT (USA)	Adagmar ANDRIOLO (BRA) *(replaced by Flavio ALCANTARA (BRA) during 2010)*

laboratory medicine community allowing communication and collaboration. The membership remained very similar for the first six years and some original members remain active today.

The following **Terms of Reference** were adopted at the first meeting:

- To achieve global consensus on the laboratory strategy (including reporting) for the identification, diagnosis, and monitoring of chronic kidney disease.
- To collaborate in the preparation of international diagnostic guidelines with relevant clinical organisations by providing guidance on laboratory aspects of chronic kidney disease testing.
- To facilitate the guideline implementation within IFCC member organizations and reach improvement over the current situation.

EARLY ACTIVITIES OF THE TF-CKD

While the initial plans for the TF-CKD were aimed at preparing a global guidance document, the first major activity was a survey of current practice in laboratory testing related to CKD. At that time the latest international guidelines were the United States National Kidney Foundation Kidney Disease Outcome Quality Initiative (K/DOQI) 2002 guidelines which provided, amongst many items, a clear definition of CKD and also recommended routine reporting of an eGFR with serum creatinine (1). The survey was conducted in 2010 and distributed amongst IFCC and WASPaLM member organisations with 25 responses with the aim to assess uptake of the K-DOQI recommendations. It is likely that the results were skewed to countries with an interest in the topic. Of the respondents 42% had national guidelines on eGFR reporting, with the guidelines being produced either by, or in collaboration with a renal medicine organisation. Fewer than half the responding countries estimated that over 80% of laboratories routinely reported an eGFR and many were using creatinine assays which were not aligned to the reference method (isotope dilution mass spectrometry, IDMS). A key feature was a strong positive reaction to questions about willingness to share experience and to receive assistance in this area.

Members of the Task Force were also independently active in developing guidelines in their own countries, speaking at national and international meetings, and with involvement in research on laboratory and clinical issues. For example, in 2011 Task Force members presented at meetings in Berlin, China, Malaysia and Mexico.

ORGANISING CKD TESTING

As stated above, the original goals of the TF-CKD included “to achieve global consensus on the laboratory strategy (including reporting) for the identification, diagnosis, and monitoring of chronic kidney disease.” However over time it became apparent to the membership that CKD testing programs are best organised at the national rather than global level. The examples of structured CKD testing that were in place were organised at the national rather than the international level. For example by 2010 the survey showed that at least nine countries were known to have national CKD testing programs. Other organisational categories may be regional (a number of countries acting together) or at a state or provincial level within a country. There are many reasons for thinking that way. Firstly the available resources, including laboratory facilities, doctors, medicine, are often very different in different parts of the world. Importantly an organisational structure is required to formulate then implement change. The relevant structures include laboratory and clinical professional organisations, medical education (pre- and post-graduate), medical and laboratory funding and governments.

Thus the recommendation of the TF-CKD became to assist countries (or regions or states) to develop and implement CKD testing programs, as opposed to recommending the same approach for everyone. The issues that need to be considered can be addressed by, and owned by, local organisations and people. The role of the TF-CKD then is to support these national activities.

The other major international event in the field of CKD was the publication of the Kidney Disease Improving Global Outcomes (KDIGO) 2012 Guideline on the diagnosis and management of CKD (2). It is hard to overestimate the quality and importance of this document in the field of CKD testing and management. Building on the 2002 KDOQI guideline, it contains a balanced and evidence based-approach to achieving the aims in the document title. Importantly it also describes key aspects of laboratory testing including creatinine standardisation, eGFR reporting and interpretation and urine albumin measurement. The document also provides a way of approaching the data, for example recommending the use of the CKD-EPI formula for estimation of GFR, unless there is evidence that an alternative formula can improve the accuracy of the result. The use of a common guideline to support both clinical and laboratory activities ensures that laboratory testing is supportive of the clinical goals in caring for patients with kidney disease.

In response to the publication of this document, the TF-CKD formally recommended that any CKD testing programs should be based on this document. To put these last two items together, the TF-CKD, in 2013, recommended that CKD testing programs should be organised nationally, using the 2012 KDIGO guidelines as a basis with changes as required for local adoption.

SUPPORT FOR NATIONAL ACTIVITIES

The original membership of the TF-CKD was limited to individuals with known expertise in the area. In 2012 the concept was raise of inviting “corresponding members” from as many member organisations as wished to join. A corresponding member needed to have an interest in the field and the support of the relevant national biochemistry or pathology organisation. The effect of this was to markedly expand the membership and include people with an active interest, but possibly limited specific knowledge in the field.

One assessment of this expansion is that it has produced the greatest effects of the TF-CKD. By becoming a member and participating at meetings and in e-mail discussions, there was an opportunity to learn and then facilitate activities in the home country. A key example of this approach in action was the TF open meeting held at the Paris IFCC congress in 2015. The format of the meeting was presentations from members about the state of progress in CKD testing in their home country. There were presentations from thirteen countries from six continents enabling a period of sharing experiences and creating new contacts. The countries presented were at many stages of the process of developing or implementing CKD guidelines. Following on from this meeting members have played key roles in the development of CKD guidelines in Croatia (3) and Turkey (4).

In an offshoot from the TF-CKD, a similar process has started under the auspices of the Asia Pacific Federation of Clinical Biochemistry (APFCB). At their regional meeting in Taiwan in 2016, a meeting of national representatives of seven Asian countries again shared experiences and challenges in the area.

THE FUTURE OF THE TF-CKD

With the rise of the numbers of corresponding members, as well as corporate representatives, in 2017 the TF includes 26 members representing 23 countries (5).

There remains much work to be done. A recent international survey by the International Society of Nephrology has indicated that measurement of serum creatinine with eGFR reporting was either not available or only rarely available in 63% of countries worldwide, and creatinine alone either not available or only rarely available in 35% of 119 countries assessed (6). A follow-up paper has identified the IFCC as a partner organisation for improvement in laboratory testing in CKD (7).

There also persists a need for activity to promote improvements in assay quality. Specifically creatinine is the basis of GFR assessment in most of the world and clinicians rely on laboratories for quality results. While the quality of assays used in the developed world has improved markedly, in the developing world it is often difficult for a laboratory scientist to even identify whether a creatinine assay is traceable to international standards (8).

I believe that the TF-CKD has been, and will continue to be an active force for change in improving the use of laboratory testing to identify and manage patients with CKD. The mechanisms are to promote the development of appropriate national programs through collaboration of laboratory medicine and other organisations. This assistance may be through providing a list of issues to address, partnering with individual countries, advising on the processes or technical issues, providing guest speakers or other ways.

It has been my pleasure to be involved in CKD testing for over 12 years during which it is fair to say the world has changed (9). The improvement of laboratory medicine is an adventure we all should play a part in.

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TF-CKD special issue: The laboratory diagnostics of chronic kidney diseases

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Did creatinine standardization give benefits to the evaluation of glomerular filtration rate?

Laurence Piéroni[1,4], Anne-Sophie Bargnoux[1,4], Jean-Paul Cristol[1,4], Etienne Cavalier[2,4], Pierre Delanaye[3]

[1] *Department of Biochemistry and Hormonology, CHU de Montpellier, PhyMedExp, University of Montpellier, France*

[2] *Department of Clinical Chemistry, Sart Tilman Hospital and University of Liège, Belgium*

[3] *Department of Nephrology Dialysis Transplantation, Sart Tilman Hospital and University of Liège, Belgium*

[4] *On behalf of the Société Française de Biologie Clinique*

ARTICLE INFO

Corresponding author:
Jean-Paul Cristol
Department of Biochemistry
and Hormonology
CHU de Montpellier
371, Avenue du Doyen Gaston Giraud
34295 Montpellier Cedex 5
France
Email: jp-cristol@chu-montpellier.fr

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ABSTRACT

During the last decade, a lot of efforts has been made to improve the evaluation of renal functions. Measured Glomerular Filtration Rate (GFR) remains the only valuable test to confirm or confute the status of chronic kidney disease (CKD) and is recommended by Kidney Disease Global Outcomes guidelines when estimation of GFR is not reliable. However, in routine clinical practice, serum creatinine remains the one of the most prescribed biological parameters and is an undeniable factor, alone or in association with other parameters, of the estimation of GFR. Since many years, a great improvement in the creatinine measurements was realized because of the standardization of the methods and fabrication of an international standard with concentration near to physiological ones (SRM967). Standardization according to Isotopic Dilution Mass Spectrometry dramatically improves the analytical performances of creatinine assays resulting in a more accurate estimation of GFR using creatinine based equations. Indeed, the standardization of creatinine improves the analytical performance by reducing the bias and removing the influence of the interfering substances.

However, biological variability of creatinine is not affected by analytical standardization and remains a limitation to the use of creatinine in some selected populations, having extreme ages or weights like children, elderly subjects, obese or malnourished populations. Standardization of creatinine assays result in a clear improvement of estimated GFR in general population but alternative methods should be used when creatinine production or metabolism is impaired.

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INTRODUCTION

Today, serum creatinine (SCr) is still one of the most prescribed analyses in medical laboratories to estimate the glomerular filtration rate (GFR) [1] and it is now recommended to integrate its value in a predictive equations. But creatinine is still used in some parts of the world to evaluate kidney function. Since methods for measuring SCr is potentially prone to several interferences, e.g. with bilirubin or pseudochromogens [2-4], the imprecision of the SCr measurement has been improved from the initial manual Jaffe method with important innovations. Earlier in the 1970s, the automatization of the methods began [5-7], followed by the development of kinetic measurements and by the emergence of enzymatic methods, almost free from interference by pseudochromogens like proteins [2-4, 8, 9]. Finally, the development of GC-IDMS or LC-IDMS as reference methods allowed the emergence of IDMS traceable assays [10].

However, limitations of creatinine as a potential GFR biomarker is not restricted to analytical considerations. First, creatinine levels are dependent of muscle mass since creatinine is a product of muscle catabolism of creatine phosphate [11, 12, 13]. Extremely low or extremely high muscular mass could result in a misinterpretation [14, 15]. Secondly, a tubular secretion of creatinine exists and this secretion could be responsible for an overestimation of GFR especially during the course of chronic kidney disease [11, 16-18]. Third, Serum creatinine can also be influenced by diet. Meals rich in proteins such as cooked red meat can increase the serum creatinine. The GFR itself also increases with such food intakes [11, 13, 19-21]. Fourth, some authors have described extrarenal clearance of serum creatinine, possibly by intestinal bacteria, which could be relevant in advanced chronic kidney disease (CKD) [22]. Finally, the production of creatinine, from muscular creatine, could be influenced negatively in severe hepatic disease and positively in rhabdomyolysis [11, 23].

These sources of imprecision are “physiologic limitations” of serum creatinine and one can only be conscious of them. But the standardization of methods is actually required for reducing analytical errors like bias in the creatinine measurement. We present here the actions made during the last decade resulting in standardization of creatinine measurements and their possible consequences on GFR estimation.

HOW CAN WE STANDARDIZE CREATININE MEASUREMENT METHODS?

The concept of the standardization of creatinine measurement was simple. The Creatinine Standardization Program was created by NKDEP’s Laboratory Working Group in collaboration with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the European Communities Confederation of Clinical Chemistry (now called the European Federation of Clinical Chemistry and Laboratory Medicine) to reduce interlaboratory variation in creatinine assay calibration. The National Institute for Standards and Technology (NIST) has released a standard reference material (SRM 967 Creatinine in Frozen Human Serum) for use in establishing calibrations

for routine creatinine measurement procedures, with demonstrated commutability with native clinical specimens in routine methods. These materials were value-assigned with the gas chromatography (GC) -isotope dilution mass spectrometry (IDMS) and liquid chromatography (LC)-IDMS reference measurement procedures [24]. A concentration of 88,4 µmol/L (1mg/dL) was chosen since this value is comprised in the critical range 1.0-1.5 mg/dL that allows clinical laboratories to verify that method performances follow recommendations (Total error in creatinine measurement should not increase the variability in eGFR more than 10% in eGFR at a serum creatinine concentration of 1.0 mg/dL) [3]. A new SRM 967a was prepared with two sub-pools, with one having normal levels of creatinine (Level 1, 0.8 mg/dL±0.1 mg/dL), and the other spiked with crystalline creatinine to achieve an elevated level of creatinine (Level 2, 4.0 mg/dL±0.2 mg/dL) to explore a wide range of creatinine values.

Since the Creatinine Standardization Program has requested the manufacturers to standardize their creatinine assays to an IDMS reference measurement procedure, we can theoretically expect that the same sample will give the same result in any laboratory in the world, whatever the method (Jaffe or enzymatic) and manufacturer, since the calibrators will all be "traceable" to the higher-order method [25, 26].

But several independent studies have shown that results obtained with so-called IDMS traceable methods (notably Jaffe assays and some dry enzymatic methods) still provide results that were quite far away from the "true value," as determined with a reference method [27, 28]. Importantly, this occurs most of the times when dealing with lower creatinine values, whereas, once again, this is the range of values with the largest impact on eGFR variability. Finally, we can assert that most enzymatic assays on the market in 2017 are IDMS-calibrated [29]. Enzymatic assays have reached the goal to decrease the inter-assay variability and thus to decrease systematic differences (i.e., bias) between assays [30]. However, the systematic error due to the bias inherent to calibration is only one part of the potential error linked to the serum creatinine measurement.

WHY CREATININE STANDARDIZATION LED TO REDUCING INTERFERENCES IN CREATININE METHODS?

The first goal to reach when you try to standardize a method is to find a process which allows you to get a specific method. Two types of methods are used to determine creatinine concentrations: enzymatic and Jaffe's methods. Both are colorimetric methods but since the first ones are using enzymatic reactions, they are more specific than the Jaffe's ones [27].

In 1886, Jaffe [31] described complex formation between picric acid and creatinine in an alkaline environment. Since then, several colorimetric methods based on Jaffe's observation were commercialized [32]. The total error budget of colorimetric methods was rather due to bias than to imprecision, in particular for low creatinine concentrations. This bias is due to the analytical interference by pseudo-chromogens for the Jaffe group [33] or to the calibration used in the dry chemistry method [34]. The earlier processes to reduce the interference of pseudo-chromogen effect of proteins [35] on the reactions based on alkaline picrate were deproteinization or dialysis. Today, however, analyzers use untreated serum or plasma, making creatinine assays using alkaline picrate reaction prone to the so-called ''protein error'' [33]. On average, this effect produces a positive difference of 27 µmol/L creatinine compared with enzymatic methods [33]. Moreover, before standardization, each assay was calibrated with specific material provided by the manufacturers

and particular processes. For example, different Jaffe assays would lead to different serum creatinine results [3, 25, 34, 36, 37]. Compared to non-calibrated assays, using IDMS traceable creatinine (and creatinine-based equations specifically developed for such standardized assays) leads to a modest but significantly better performance for eGFR [38].

However, harmonization of creatinine measurement between laboratories is especially important in population studies and on the longitudinal monitoring of renal function in individuals, with great influence on the establishment of reference intervals. Ceriotti et al., when trying to identify universally applicable reference intervals for creatinine via a systematic review of the literature, concluded that only data obtained with enzymatic assays had to be considered because of the higher specificity of this analytical approach [39]. They explained their choice because the subtraction of 18–25 µmol/L to eliminate protein-related unspecific interference on alkaline picrate assays significantly improves the correlation of these assays with enzymatic ones. In this situation, the obtained reference intervals are very similar to those of the enzymatic methods. However, on individual samples, especially at the low creatinine concentrations found in children, large differences can be seen.

Indeed, since the relationship between sCr and eGFR is actually exponential, it implies that small sCr differences will greatly impact the GFR values at low SCr values (corresponding to high GFR values) but the same difference will have minimal impact at high SCr values (corresponding to low GFR values). Therefore, if we consider an analytical error of 17.6 µmol/L in creatinine measurement for a 60 year-old man presenting a creatinine value of 98.6 µmol/L, this value is not different from 116.2 µmol/L. The corresponding GFR values with the CKD EPI study equation will be 71 or 58 ml/min/1.73 m2, respectively. The same example with a serum creatinine of 264 µmol/L and 281.6 µmol/L with the other assay will give CKD-EPI results of 22 and 20 mL/min/1.73 m2, respectively [3, 40-43]. A relative low analytical error of 17.6 µmol/L creatinine can therefore be responsible for a misclassification in the staging of CKD.

Is standardization responsible for the improvement of the imprecision of creatinine assays?

Comparing the analytical imprecision of both methods, the coefficient of variation (CV) is systematically better for the enzymatic assays [2, 44]. For low creatinine concentrations presented by children [2], the serum creatinine concentrations measured with the Jaffe reaction will be higher than with the enzymatic assay. Therefore, one may prefer enzymatic assays in specific populations like in children or in patients with hyperfiltration but also in specific situations where some Jaffe's methods are subject to interferences like bilirubin, ketoacidosis etc.

The gain in imprecision (due to a smaller random error) with the enzymatic assays as compared to Jaffe assays is an intrinsic characteristic of the assay and is totally independent of the standardization procedure, which only improves the systematic error.

DID STANDARDIZATION GIVE BENEFIT TO EGFR?

Another source of variability of creatinine is biological variation expressed in an intra-individual CV. This variation is physiological, independent of the analytical CV and cannot be reduced by standardization [44].

Indeed, when combining the intra individual CV (5.95%) and analytical CV for Jaffe (5.5%) and enzymatic (2%) methods, in a 60-year old man, this means that for a given GFR, the serum creatinine concentration may vary for a creatinine

concentration of 88.4µmol/L between 80.1 and 117 µmol/L if the Jaffe assay is used or between 85.4 and 111.8 µmol/L if the enzymatic assay is used. Using the CKD-EPI equations, this range of non-different sCr values leads to eGFR values that may vary between 58 and 92 mL/min/1.73 m2 for Jaffe serum creatinine and between 61 and 84 mL/min/1.73 m2 for the enzymatic assay results. The intrinsic variability of creatinine is thus not so negligible when it is used in the eGFR equation. The relevance of this variation will be, once again, important in adults with normal or close to normal serum creatinine values and especially in children.

CONCLUSION

Standardization of creatinine assays is effective in 2017. This improvement in creatinine measurements has decreased the analytical component of creatinine variability and for assessing the transferability of creatinine results, a relatively simple recommendation is to use enzymatic assays (to decrease the random error) and IDMS traceable assays (to decrease the systematic error). Today enzymatic methods have shown to be effectively calibrated to IDMS [29, 44]. However, with an analytical imprecision of 2% (for usual assays), the error due to intra-individual biological variation still remains. Thus, to overcome this limitation in selected populations (extreme age or body size, muscle diseases including severe denutrition, vegetarian diet…) recommendation is to measure GFR [1].

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Moving toward standardization of urine albumin measurements

Jesse C. Seegmiller[1], W. Greg Miller[2], Lorin M. Bachmann[2]

[1] *University of Minnesota, Department of Laboratory Medicine and Pathology, Minneapolis, Minnesota, USA*
[2] *Virginia Commonwealth University, Department of Pathology, Richmond, Virginia, USA*

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Corresponding author:
Jesse C. Seegmiller
University of Minnesota
Department of Laboratory Medicine
and Pathology
420 Delaware Street S.E.
Minneapolis, MN 55455
United States of America
Phone: 1-612-625-6198
Fax: 1-612-625-4831
E-mail: jseegmil@umn.edu

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ABSTRACT

Measurement of urine albumin is important for detecting and monitoring kidney disease. At the present time, measurement of urine albumin is not standardized due to the lack of a reference system, which includes both a reference measurement procedure and certified reference materials. Developing a reference system will provide a means for clinical laboratory measurement procedures to become standardized and will enable successful use of uniform clinical decision points. Currently, urine albumin results vary in excess of 40% depending on which commercially available measurement procedure is utilized for measurement. Clinicians may struggle with classification of kidney disease in part due to differences in measurements from lack of agreement among laboratory methodologies employed when assessing urine albumin concentrations. This report focuses on current findings in urine albumin testing, highlights important measurement and reporting considerations, and presents strategies for developing a reference measurement procedure to enable standardization of urine albumin measurements.

INTRODUCTION

Urine albumin is a diagnostic and prognostic marker for chronic kidney disease (CKD), diabetes and cardiovascular disease.[1,2] When interpreting measurements of urine albumin, providers must consider the type of urine collection and the methodology used for analytical measurement. The historical standard for measuring the amount of albumin excreted into the urine, known as the urine albumin excretion rate, has been to measure the albumin concentration obtained from a 24-hour urine collection.[3] In clinical practice, 24-hour urine collections present problems in terms of specimen storage and timing accuracy. Thus, assessment of urine albumin from shorter collection times is a common clinical practice and presents a more convenient collection option. In untimed situations, the urine albumin result should be indexed to urine creatinine concentration and reported as the albumin to creatinine ratio (ACR). The ACR accounts for hydration and produces a ratio that has similar diagnostic performance as a 24-hour urine albumin excretion rate.[4-6] A caveat to these different timing approaches is differences in classification of albuminuria depending on timing of collection. Therefore, the collection method should remain consistent throughout studies.[7] Recommendations are to report the ACR along with the albumin concentration, preferably collect the first morning void specimen, and follow-up findings from random urine collections with first morning void collections.[4,8-10]

A variety of testing methodologies have been employed to monitor urine albumin including turbidimetry[11,12], dipstick[13], radioimmunoassay[14,15], immunoturbidimetry[16], immunonephelometry[17,18], high performance liquid chromatography[19], liquid chromatography mass spectrometry[20,21], and liquid chromatography tandem mass spectrometry (LC-MS/MS)[22].

Some of these methods are known to have issues with analytical specificity when measuring urine albumin. One essential attribute for a reference measurement procedure is that it must be specific for the measurand it is intended to quantify and not be influenced by matrix effects or interfering substances that can be present in patient urine.

This report highlights standardization recommendations for urine albumin measurements and focuses on methodologies likely suitable for use as a reference measurement procedure for standardizing such measurement results.

PREANALYTICAL AND STORAGE CONSIDERATIONS FOR URINE ALBUMIN

Several precollection factors have been shown to increase urine albumin excretion such as exercise[23], posture[24] and fever[25]. These factors should be considered when assessing albuminuria for comprehensive renal workups. Interventions may not be indicated in patients with the above conditions unless the albuminuria persists when the confounding physiological conditions are no longer present. Nonspecific binding of albumin to urine collection containers does not contribute to measurement error, as binding to the container has been estimated to be <1% depending on the container hydrophobicity, which is considered inconsequential.[26]

A fresh midstream collection for urine albumin measurement is preferred.[8,27,28] Albumin can remain stable in urine for up to 8 weeks when stored under refrigerated conditions at 4 °C.[29] For long term frozen storage of urine albumin samples, a temperature -70 °C or lower is required. Degradation of albumin in urine causing measurement issues has been reported when stored at -20 °C over periods of 2 weeks to 3 years.[29,30] Therefore, careful attention must be paid to the storage conditions for urine specimens particularly for investigations using stored samples.

CURRENT STATE OF URINE ALBUMIN MEASUREMENTS

While the utility of this biomarker is clear, applying disease specific cutoffs for albuminuria becomes compromised near the decision values due to non-standardized measurement procedures used in clinical laboratories. In a study that evaluated the state of agreement among 16 quantitative clinical laboratory immunoassay measurement procedures from in-vitro diagnostics manufacturers, who distribute globally, results from 332 freshly collected non-frozen urine albumin samples had total coefficients of variation (CVs) of 5.2-8.1% and the effects of sample-specific influences were < 10% for most measurement procedures.[31]

However, bias was found to cause a significant lack of agreement among measurement procedures. The median difference range for routine measurement procedures vs. a comparator LC-MS/MS procedure was approximately 40%. Mean biases ranged from -35% to +34% for concentrations near 15 mg/L and -15% to +18% for concentrations near 30 mg/L. The results of this study demonstrate that fixed decision thresholds cannot be effectively utilized due to lack of agreement among routine measurement procedures and therefore standardization is needed.

The College of American Pathologists offers an Accuracy Based Urine Survey that uses unaltered pooled frozen human urine as the samples.

Table 1 Results from the College of American Pathologists - Accuracy Based Urine Survey first mailing in 2017[a]

Sample	Methods	N. Labs	Median, mg/L	Median bias vs. LC-MS/MS, %	Low value, mg/L	High value, mg/L
A	Siemens Dimension Vista (IN)	7	16	-1.8	16	18
	Abbott Architect c Systems (IT)	10	13	-20.2	11	13
	Beckman AU Series (IT)	8	13	-20.2	11	14
	Roche cobas c500 Series	9	12	-26.4	10	13
	Vitros 5.1 FS/4600/5600	5	15	-8.0	8	16
	All methods	58	13	-20.2	8	18
	LC-MS/MS	-	16.3	-	-	-

B	Siemens Dimension Vista (IN)	7	38	4.1	37	38
	Abbott Architect c Systems (IT)	10	32	-12.3	30	33
	Beckman AU Series (IT)	7	31	-15.1	30	32
	Roche cobas c500 Series	11	32	-12.3	30	34
	Vitros 5.1 FS/4600/5600	5	37	1.4	25	38
	All methods	59	32	-12.3	25	39
	LC-MS/MS	-	36.5	-	-	-

C	Siemens Dimension Vista (IN)	7	192	4.1	178	195
	Abbott Architect c Systems (IT)	9	164	-11.1	161	167
	Beckman AU Series (IT)	7	167	-9.4	149	169
	Roche cobas c500 Series	11	155	-15.9	130	173
	Vitros 5.1 FS/4600/5600	5	166	-10.0	133	180
	All methods	58	164	-11.1	130	195
	LC-MS/MS	-	184.4	-	-	-

[a] Data used with permission from the College of American Pathologists
(IN) - immunonephelometric, (IT) - immunoturbidimetric

Table 1 shows participant results compared to an LC-MS/MS candidate reference measurement procedure. Although there were a small number of participants, the information is representative and consistent with the previously mentioned larger study based on individual patient urine samples.[31] The median bias vs. the comparative method was larger at lower concentrations of urine albumin with the all methods bias -20% at 16 mg/L, -12% at 36 mg/L, and -11% at 184 mg/L. The joint committee of the Laboratory Working Group of the National Kidney Disease Education Program and the International Federation of Clinical Chemistry and Laboratory Medicine Working Group for Standardization of Albumin in Urine recommended desirable and optimal bias goals of ≤13% and ≤7%, respectively, vs. a reference measurement procedure.[32]

These survey results suggest that some measurement procedures can meet these bias goals, but many do not. The survey also reported ACR values. Reference measurement procedure results were not available for urine creatinine but comparison of mean results among different methods in the survey had differences of 17%, 8.8% and 14% at mean concentrations of 55 mg/dL, 69 mg/dL and 89 mg/dL (4.8 mmol/L, 6.1 mmol/L and 7.9 mmol/L), respectively. When both creatinine and albumin were used to calculate the ACR, the differences between the lowest and highest ACR values for all methods combined were 76% at 15 mg/g, 49% at 60 mg/g, and 65% at 237 mg/g. These differences will cause misclassification of risk of kidney disease at the commonly used albuminuria decision values of 30 and 300 mg/g creatinine (3.4 and 34 mg/mmol creatinine).

A reference system is in place for urine creatinine and perhaps needs to be more stringently implemented. However, a reference system does not yet exist for urine albumin and is the focus of this report.

METHODS FOR MEASURING URINE ALBUMIN

To improve the analytical selectivity in the measurement of urine albumin, liquid chromatography mass spectrometry (LC-MS) methods were utilized.[20,21] A comparison study of one LC-MS method to an immunoturbidimetric method found the comparability between the methodologies greatly improved when both methods employed the same calibrators with the same calibrator value assignments. Mean bias improved from -37.8% to 2.2% using the same calibrators on both platforms.[33] A potential shortcoming of the LC-MS urine albumin method was the lower limit of quantitation of 10-20 mg/L, which is above the level expected in specimens with normal albumin concentrations.[20,33] Other possibilities that could introduce error with this methodology are the presence of urine albumin fragments containing the N-terminal fragment used in the analysis, which could falsely elevate albumin levels or modification to the N-terminal portion used in analysis that would change the mass, which could falsely lower albumin levels.

In an effort to improve the lower limit of quantitation for urine albumin, a LC-MS/MS method was developed.[22] This method employed proteolysis of urinary proteins to produce peptides of albumin as well as peptides from other proteins present in urine. Large variations in pH (4.5-8) and specific gravity are expected in the urine of patients with or without a kidney abnormality.[34] pH variations could adversely affect the trypsin proteolysis process, which is a critical preanalytic step that occurs prior to LC-MS/MS measurement. Therefore, buffering conditions and dilutions were employed that provide an optimal environment for trypsin proteolysis. Peptides known to be unique to albumin were analyzed and quantitated to represent the quantity of intact albumin. One

of the key components of this method was the incorporation of an internal standard that consisted of a recombinant form of human serum albumin isotopically labeled with ^{15}N.

The internal standard served dual purpose:

1. to normalize for any differences in the proteolytic processing among specimens;
2. to provide normalization for LC-MS/MS analysis.

Several peptides unique to human serum albumin were quantitated and referenced to a calibration curve. The lower limit of quantitation for the LC-MS/MS measurement procedure was found to be 3.13 mg/L.[22] Method comparison studies have been performed examining commercially available immunoassay platforms to the LC-MS/MS method.[31,35] The LC-MS/MS measurement procedure was used to perform the comparison study of 16 commercially available measurement procedures previously described.

Potential challenges for a LC-MS/MS method include the possibility of albumin fragments in the urine, post-translational modifications of the unique peptides monitored, or factors that inhibit albumin proteolysis. Further investigation of this technique compared urine albumin concentrations before and after ultrafiltration using a 10 kDa molecular weight cutoff filter where differences in the results were small and suggested minimal signal contribution from fragments of albumin.[36] With the above cautions appropriately addressed in the measurement procedure details, the LC-MS/MS method is a good candidate reference measurement procedure for urine albumin. This method provides the necessary sensitivity to assess urine albumin concentrations <5 mg/L. The ability to quantitate the albumin molecule with a high degree of analytical specificity by using proteotypic peptides of albumin that are not known to be subject to modification and do not appear in other human proteins, provides support for use of the LC-MS/MS method as a reference measurement procedure. To ensure high quality results, the LC-MS/MS measurement procedure requires an isotopically enriched form of albumin as an internal standard. Procedures for making labeled albumin have been described.[21]

A HIGHER ORDER REFERENCE SYSTEM FOR CALIBRATION TRACEABILITY

A higher order reference system is needed to enable all measurement procedures to implement common calibration traceability to achieve equivalent results for urine albumin irrespective of the measurement procedure used. A reference system for urine albumin that follows the International Organization for Standardization standard 17511 for calibration traceability hierarchy[37] includes three main components:

1. A pure human albumin primary reference material.
2. A reference measurement procedure.
3. A human urine matrix based secondary reference material.

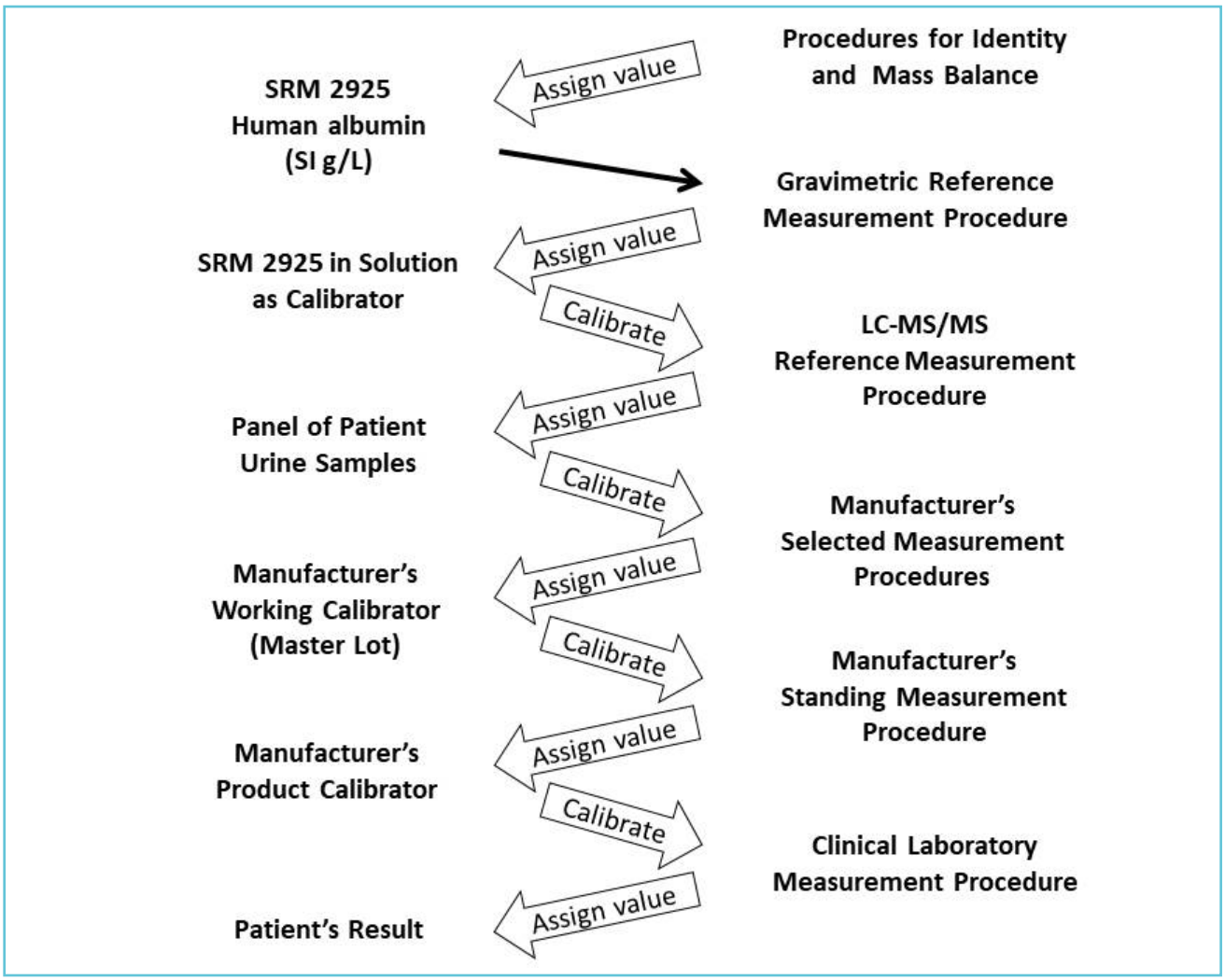
The National Institute for Standards and Technology (NIST) in the USA is qualifying a recombinant human albumin certified primary reference material expected to be released in 2018 as SRM 2925. SRM 2925 will be a highly purified solid substance intended to be used to prepare calibrators for a mass spectrometry based reference measurement procedure. SRM 2925 is not intended to be used to prepare calibrators for immunoassays. NIST is also preparing an albumin in frozen human urine certified reference material designated SRM 3666 that will include four concentrations intended to be used to establish the metrological traceability of calibration for clinical laboratory measurement procedures, including immunoassays.

SRM 3666 will be value assigned using a NIST reference measurement procedure that is currently in development. The commutability of NIST SRM 3666 will be validated to ensure it is suitable for use as a calibrator for manufacturer's selected measurement procedures as well as for clinical laboratory measurement procedures. It is not known at this time when either the reference measurement procedure or the SRM 3666 will be available from NIST.

Since SRM 2925 pure albumin will be available soon, development of suitable reference measurement procedures will provide the essential components of a reference system to allow standardized calibration traceability for commercially available clinical laboratory urine albumin immunoassay procedures.

A reference measurement procedure intended for use in a calibration traceability hierarchy for clinical laboratory measurement procedures must have performance characteristics to ensure acceptable uncertainty in values assigned to patient samples used as calibrators in the traceability hierarchy, as described below. In addition, a reference measurement procedure must be operational in at least two sites to

Figure 1 Metrologic traceability hierarchy for calibration of urine albumin measurement procedures



validate equivalent performance to qualify for listing by the Joint Committee for Traceability in Laboratory Medicine.

Metrologic traceability of calibration is described in the International Organization for Standardization 17511 standard.[37] Figure 1 shows how the reference system components being developed for urine albumin fit into the traceability hierarchy. NIST SRM 2925 is a pure substance primary reference material that is used with a gravimetric reference measurement procedure to prepare calibrators for an LC-MS/MS reference measurement procedure. The LC-MS/MS reference measurement procedure is used to assign values to a panel of patient's urine samples that are used as calibrators for a manufacturer's selected measurement procedure that is used to assign values to the manufacturer's working, or master lot, calibrator.

In the case of urine albumin, there will be several concentrations of working calibrator used to calibrate the manufacturer's standing immunoassay measurement procedure. The working calibrators can be prepared as dilutions of a single master lot of working calibrator or as a set of concentrations of working calibrators, with each value assigned by the selected measurement procedure. The manufacturer's standing measurement procedure is then used to value assign sequential lots or batches of the manufacturer's product calibrator that is used to calibrate the clinical laboratory measurement procedures. In many cases, the manufacturer's selected and standing measurement procedures will be the same as the clinical laboratory measurement procedure but operated with a more stringent protocol for items such as maintenance, calibration, replicate measurements, multiple reagent lots and/or instruments to reduce the uncertainty of the value assignment steps. Thus, metrologic traceability is established from patient results to the pure substance primary reference material.

When NIST SRM 3666, albumin in frozen human urine, becomes available it can replace the panel of patient urine samples to simplify the traceability process. In addition, SRM 3666 can be used by clinical laboratories to verify calibration of their immunoassay measurement procedures for urine albumin.

CONCLUSIONS

The need for standardization of urine albumin measurements is clear. Standardization of this measurand will assist in applying uniform clinical decision points for various diseases and conditions based on urine albumin to creatinine ratio values. Standardization of urine albumin measurements requires development of both a certified primary reference material and a reference measurement procedure. LC-MS/MS measurement of albumin-specific peptides after proteolytic digestion under carefully controlled conditions provides a suitable methodology for a reference measurement procedure. When available, these reference system components can be used by immunoassay measurement procedure manufacturers to achieve metrologic traceability of calibration to a common reference system. Availability of a commutable frozen human urine reference material will also be useful as a common calibrator for immunoassays. In addition to standardized metrologic traceability, urine collection and storage conditions influence the suitability of urine albumin measurements and therefore preanalytical processing procedures should be standardized.

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Cystatin C is indispensable for evaluation of kidney disease

Anders Grubb

Department of Clinical Chemistry and Pharmacology, Laboratory Medicine, Lund University, Skåne University Hospital, Lund, Sweden

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Corresponding author:
Anders Grubb
Department of Clinical Chemistry
and Pharmacology
Laboratory Medicine
Lund University
Skåne University Hospital
SE-22185 Lund
Sweden
Phone: +46 702393773
E-mail: anders.grubb@med.lu.se

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The author declares that there is no conflict of interest regarding the publication of this article.

ABSTRACT

The present minireview of the place of cystatin C in clinical medicine emphasizes, and discuss the evidence, that cystatin C-based GFR-estimating equations do not require the use of vague terms like race and sex, that cystatin C-based GFR-estimating equations are useful for both children and adults, including the elderly, that the best GFR-estimation requires simultaneous use of both cystatin C- and creatinine-based equations, that cystatin C-based GFR-estimating equations are superior to creatinine-based equations in predicting end-stage renal disease, cardiovascular manifestations, hospitalisation and death, and, finally that cystatin C is required to diagnose the new syndrome "Shrunken Pore Syndrome" with its high mortality and morbidity, even in the absence of reduced GFR. When automated laboratory equipment is available, the cost of cystatin C is comparable to that of enzymatically determined creatinine.

The conclusion is that cystatin C should be used at least as often as creatinine in clinical medicine.

INTRODUCTION

The introduction of creatinine as a marker of GFR started in 1926 with the publication of an article by Poul Brandt Rehberg: "Studies on kidney function. The rate of filtration and reabsorption in the human kidney" (1). Since then the use of creatinine has been a vital element of clinical medicine. Cystatin C was suggested to be a marker of GFR in 1979 (2) and a few articles published before 1994 supported its use as a GFR-marker (3-5). In 1994 an article with the title "Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate" was published (6), which initiated widespread studies of cystatin C as a marker of GFR. Today, October 2017, the search string in PubMed "Cystatin C AND (renal OR kidney)" produces more than 3500 titles. The information obtained in these 3500 investigations allows the conclusion that the low-cost analysis of cystatin C should be an integral part of the analysis spectrum for optimal evaluation of the kidney status of a patient.

This is because:

A. Cystatin C-based GFR-estimating equations do not require the use of vague terms like race and sex
B. Cystatin C-based GFR-estimating equations are useful for both children and adults, including the elderly
C. The best GFR-estimation requires simultaneous use of both cystatin C- and creatinine-based equations
D. Cystatin C-based GFR-estimating equations are superior to creatinine-based equations in predicting end-stage renal disease, cardiovascular manifestations, hospitalisation and death
E. Cystatin C is required to diagnose the new syndrome "Shrunken Pore Syndrome" with its high mortality and morbidity, even in the absence of reduced GFR.

CYSTATIN C-BASED GFR-ESTIMATING EQUATIONS DO NOT REQUIRE THE USE OF VAGUE TERMS LIKE RACE AND SEX

One of the main advantages of cystatin C compared to creatinine as a GFR-marker is that it is less dependent upon the body composition of a patient. For example, while muscle mass strongly influences creatinine, it does not, or only marginally, affect cystatin C (7-11).

Creatinine-based GFR-estimating equations therefore contain terms aiming at evaluating the muscle mass of a specific patient. These terms refer to the race and sex of the patient. Specific race-factors have been suggested for Afro-Americans (12), Japanese (13,14), Chinese (15), Koreans (16), and native Americans and Hispanics (17). But "race" is a very vague term, difficult to define and does not consider the problem that a major part of the world population represents persons with mixed ethnicity. In contrast, the cystatin C concentration varies only marginally with ethnicity and no vague race terms are therefore required in cystatin C-based GFR-estimating equations (18-20).

The mean muscle mass of females is lower than that of males and creatinine-based GFR-estimating equations therefore requires significant sex-related factors for females (21). However, the world is less and less sex-dichotomized and the existence of more than two sexes is now acknowledged in several countries (22). This ambiguity in applying creatinine-based GFR-estimating equations does not apply for some cystatin C-based GFR-estimating equations, since muscle mass only marginally, or not at all, influences the cystatin C-level and thus cystatin C-based GFR-estimating equations do not require factors for sex (20).

CYSTATIN C-BASED GFR-ESTIMATING EQUATIONS ARE USEFUL FOR BOTH CHILDREN AND ADULTS, INCLUDING THE ELDERLY

The strong correlation between muscle mass and creatinine poses a special problem concerning the use of creatinine-based GFR-estimating equations in childhood, since the muscle mass strongly increases with age. As a consequence, different equations generally have to be used for adults and children (23-25). In contrast, since the muscle mass does not influence cystatin C significantly many cystatin C-based equations work for both children and adults (20, 23-25). One of them is the CAPA-equation which has been shown to work from 1 - 50 years of age (20, 25 and unpublished observations by Grubb A, et al.). Another problem related to the use of creatinine-based equations is that the muscle mass in the elderly is often considerably reduced, so that it negatively affects the ability of these equations to demonstrate a reduced GFR in the elderly. In contrast, cystatin C-based equations are not significantly influenced by muscle mass and therefore useful in identifying reduced GFR also in the elderly with low muscle mass (26).

THE BEST GFR-ESTIMATION REQUIRES SIMULTANEOUS USE OF BOTH CYSTATIN C- AND CREATININE-BASED EQUATIONS

Although creatinine-based GFR-estimating equations are inferior in diagnostic performance compared to cystatin C-based equations for several populations, it has generally been shown that the best GFR-estimation requires use of both cystatin C and creatinine in the equation (27-31). The best estimates of GFR, produced by cystatin C-based equations, $eGFR_{cystatin\ C}$, produce values of which 80-85% are within ±30% of GFR measured by invasive gold-standard methods and similar figures are valid for the corresponding estimates, $eGFR_{creatinine}$, obtained by creatinine-based equations (27-31). Equations using both cystatin C and creatinine might produce values of which 90-91% are within ±30% of GFR measured by invasive gold-standard methods (30,32). Still better results are obtained if the mean, $eGFR_{mean} = (eGFR_{cystatin\ C} + eGFR_{creatinine})/2$ of the estimates obtained by a cystatin C-and a creatinine-based equation are used, rather than complex equations containing both cystain C and creatinine (32-34). This is due to that combined equations do not perform optimally in a number of clinical situations, for example, if the patient has an abnormally low muscle mass or is treated with a high dose of glucocorticoids. A strategy for GFR estimation based on the automatic use of a combined cystatin C and creatinine-based equation will, in these cases, have a worse diagnostic performance than a strategy that only uses the cystatin C- or creatinine-based GFR-estimating equation *not* influenced by the specific patient characteristics (33,34). Such a strategy thus requires that GFR is estimated by both a cystatin C- and a creatinine-based equation, producing $eGFR_{cystatin\ C}$ or $eGFR_{creatinine}$, and that the results are compared. If the two equations produce similar estimates, their average is a very reliable estimate of GFR. If the estimates do not agree and a specific factor known to disturb either the cystatin C- or creatinine-based estimate is present, only the estimate produced by the equation *not* disturbed by this factor, is used (33,34). As a matter of fact, since 1994, when cystatin C-based estimations of GFR were introduced in Lund in parallel with creatinine-based estimations, we have had 20-30 cases for which $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ agreed, but disagreed with GFR measured by our invasive gold-standard procedure (plasma clearance of iohexol). In all cases, in which relevant information was available, the error was caused by technical problems in the execution of the gold-standard procedure. We therefore consider that, in practice, $eGFR_{mean}$ based upon agreeing

$eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ is at least as reliable as GFR measured by invasive gold-standard procedures (33,34). This strategy is described at the multilingual site www.egfr.se (35), which can also be implemented to calculate absolute GFR from relative GFR, which might be required in, *e.g.,* dosing of medicines cleared by the kidneys.

CYSTATIN C – BASED GFR-ESTIMATING EQUATIONS ARE SUPERIOR TO CREATININE-BASED EQUATIONS IN PREDICTING END-STAGE RENAL DISEASE, CARDIOVASCULAR MANIFESTATIONS, HOSPITALISATION AND DEATH

One important reason to estimate GFR in a patient is to decide whether the patient suffers from chronic kidney disease or not, and to classify the degree of the chronic kidney disease, if present. Both $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ work well for this purpose. However, another important aspect of the estimation is how well it predicts the consequences of kidney disease, *e.g.,* end-stage renal disease, cardiovascular manifestations, hospitalisation and death, since this knowledge influences decisions concerning the intensity of the treatment modalities. In this respect, $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ differ, because the published scientific studies virtually unanimously show that $eGFR_{cystatin\ C}$ is significantly superior to $eGFR_{creatinine}$ (36-39).

The cause for the superiority of $eGFR_{cystatin\ C}$ as a risk marker is unknown, but observational studies have shown that inflammation, old age, male gender, greater weight, and cigarette smoking correlate with higher cystatin C levels (40). But statistical correlations in observational studies do not prove causal connections. A study of elective surgery of patients demonstrated a postoperative sharp rise in inflammation of the patients, with large increases in the levels of CRP of all patients, but with no increase in the cystatin C levels, thus rejecting the hypothesis that inflammation causes a raise in the production of cystatin C (41). The correlations between inflammation, old age, male gender, greater weight, and cigarette smoking and cystatin C might be due to that all these factors promote the development of atherosclerosis, also in the renal arteries, thus producing a decrease in GFR and an increase in cystatin C (41). These correlations therefore speak in favour of cystatin C as a GFR-marker and not against it.

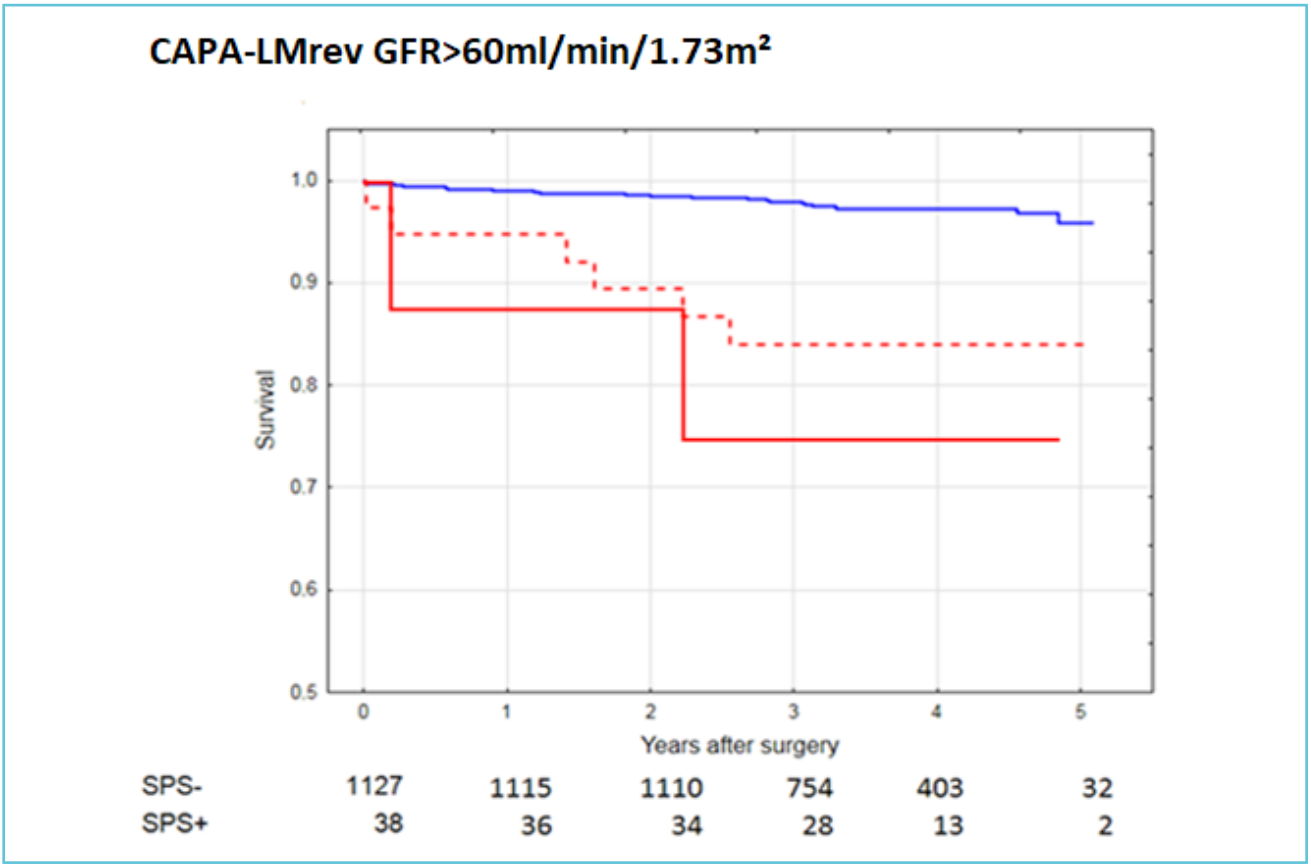
CYSTATIN C IS REQUIRED TO DIAGNOSE THE NEW SYNDROME "SHRUNKEN PORE SYNDROME" WITH ITS HIGH MORTALITY AND MORBIDITY, EVEN IN THE ABSENCE OF REDUCED GFR

The use of $eGFR_{mean}$ and the simultaneous comparison of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, as the best way to estimate GFR in clinical practice (32-34,42) identifies a number of patients with significant differences between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$. Part of these differences can be explained by factors, such as muscle wasting or treatment with large doses of glucocorticoids, known to invalidate the GFR estimations based on creatinine or cystatin C (33). But the majority of the patients with such differences between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, do not display any known such factor and their $eGFR_{mean}$ is, despite the differences between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, still the best way to estimate GFR (41).

Most of the patients displaying these differences has a pattern of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ in which $eGFR_{cystatin\ C}$ is lower than $eGFR_{creatinine}$ (42,43). When the levels of low-molecular mass proteins other than cystatin C, *e.g.,* β_2-microglobulin, β-trace protein, and retinol-binding protein, were determined in patients with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$, it was observed that the concentration ratios of these proteins to creatinine were, like the cystatin C-creatinine ratio, higher, than in patients in whom $eGFR_{cystatin\ C} \approx eGFR_{creatinine}$ (43).

The genes for these proteins are located at different chromosomes and have different regulation elements and the synthesis of these proteins is not generally known to be influenced by factors affecting the production of cystatin C (43). This strongly indicates that the production of these proteins and cystatin C is not co-regulated and therefore cannot explain the concordant increases of their plasma levels. But the concurrent increase can be explained if the proteins have a common clearance mechanism by glomerular filtration and that this is reduced by shrinking of the glomerular pores (43). Therefore, the observation that $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ in a patient indicates the presence of a new syndrome, tentatively called "Shrunken Pore Syndrome" (43). The explanation that creatinine and other small molecules do not simultaneously increase in concentration would then be, that their sieving coefficients are still close to unity (*i.e.,* one) despite the shrunken pores resulting in reduced sieving coefficients for proteins similar in size to cystatin C (43-45).

Figure 1A **Survival after coronary artery bypass surgery for patients with GFR > 60 mL/min per 1.73 m² with and without Shrunken Pore Syndrome (SPS)**



$eGFR_{cystatin\ C}$ was estimated using the CAPA equation and $eGFR_{creatinine}$ using the LMrev equation.

The cut-off level for SPS was $eGFR_{cystatin\ C} \leq 70\%$ of $eGFR_{creatinine}$ (red broken line) or $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ (red unbroken line).

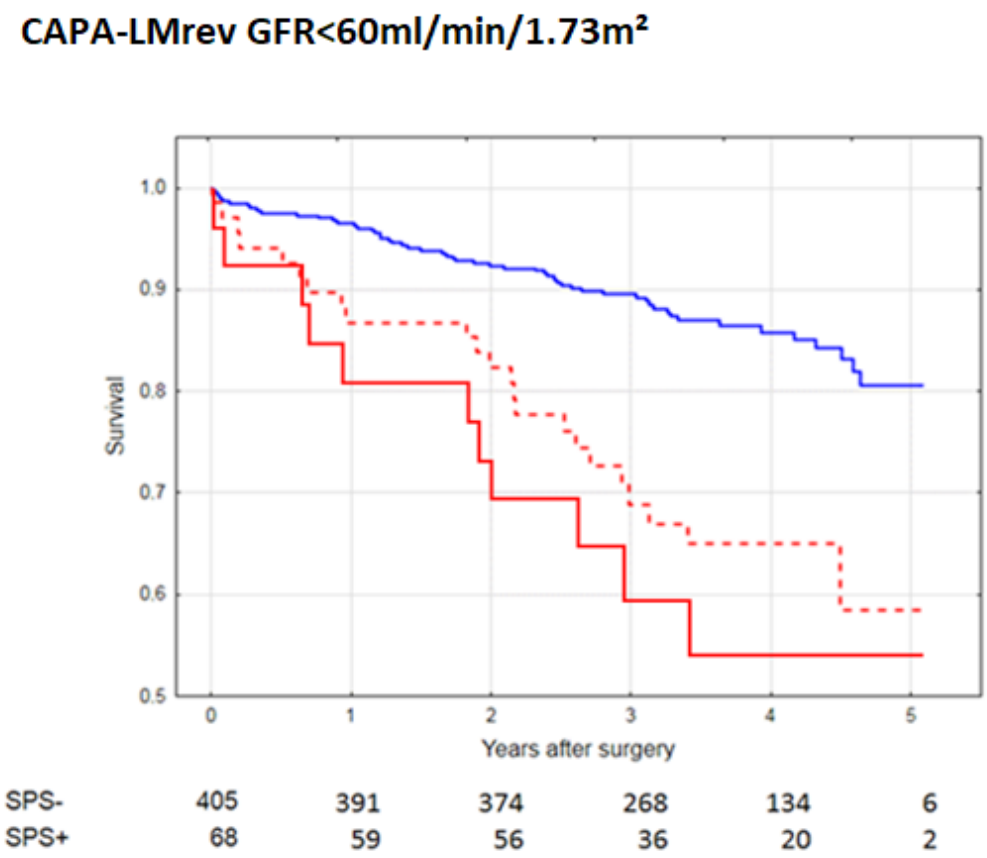
The unbroken blue line indicates the mortality of patients without SPS ($0.90 < eGFR_{cystatin\ c}/eGFR_{creatinine} < 1.10$).

The numbers below indicate patients with and without SPS, when the cut-off level was 70%.

It is noteworthy, that a similar mechanism previously has been suggested for the increase in plasma levels of low-molecular mass proteins in the third trimester of pregnancy (46-48) and for the development of still higher concentrations of low-molecular mass proteins in preeclampsia (49,50). This suggests that the (patho-)physiologic changes in late pregnancy and preeclampsia are similar to those occurring in patients with "Shrunken Pore Syndrome."

As "Shrunken Pore Syndrome" was identified recently (43), only a few studies of its clinical consequences have been performed. The first investigation showed, that the long-term mortality in patients undergoing elective coronary artery bypass grafting was much higher in patients suffering from "Shrunken Pore Syndrome" than in patients without the syndrome (51). This was true both when the preoperative GFR was normal or reduced (Figure 1A and B). In this study, the

Figure 1B **Survival after coronary artery bypass surgery for patients with GFR < 60 mL/min per 1.73 m² with and without Shrunken Pore Syndrome (SPS)**



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The unbroken blue line indicates the mortality of patients without SPS ($0.90 < eGFR_{cystatin\ c}/eGFR_{creatinine} < 1.10$).

The numbers below indicate patients with and without SPS, when the cut-off level was 70%.

cystatin C-based CAPA-equation was used to produce $eGFR_{cystatin\ C}$ and the creatinine-based LMrev-equation to produce $eGFR_{creatinine}$, as both these equations work not only for adults, but also for children (20,52,53). Interestingly, an increase in mortality was not only observed when $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$, but also when $eGFR_{cystatin\ C} \leq 70\%$ of $eGFR_{creatinine}$ (Figure 1 A and B). Ongoing studies demonstrate that the long-term mortality in "Shrunken Pore Syndrome" increases inversely with the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio, starting at 0.90. Recently published and ongoing studies in several different types of populations corroborate, that "Shrunken Pore Syndrome" is associated with significantly increased mortality and morbidity (54,55) and indicate that the syndrome also predicts higher risks for development of end-stage renal disease, cardiovascular manifestations and for hospitalisation.

CONCLUSION

The use of cystatin C (or $eGFR_{cystatin\ C}$) in addition to creatinine improves the estimation of GFR, makes it independent of vague terms like race and sex, and facilitates its use for children and the elderly. It also allows the identification of a new syndrome (Shrunken Pore Syndrome) associated with a high morbidity and mortality. When automated laboratory equipment is available, the cost of cystatin C is comparable to that of enzymatically determined creatinine. Cystatin C should therefore be used at least as often as creatinine in the clinical routine.

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Novel filtration markers for GFR estimation

Amy B. Karger[1], Lesley A. Inker[2], Josef Coresh[3], Andrew S. Levey[2], John H. Eckfeldt[1]

[1] *Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA*
[2] *William B. Schwartz Division of Nephrology, Tufts Medical Center, Department of Medicine, Tufts University School of Medicine, Boston, MA, USA*
[3] *Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Medical Institutions, Baltimore, MD, USA*

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Corresponding author:
Amy B. Karger
Department of Laboratory Medicine and Pathology
University of Minnesota
420 Delaware St SE MMC 609,
Minneapolis, MN 55455
United States of America
E-mail: karge026@umn.edu

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ABSTRACT

Creatinine-based glomerular filtration rate estimation ($eGFR_{cr}$) has been improved and refined since the 1970s through both the Modification of Diet in Renal Disease (MDRD) Study equation in 1999 and the CKD Epidemiology Collaboration (CKD-EPI) equation in 2009, with current clinical practice dependent primarily on $eGFR_{cr}$ for accurate assessment of GFR. However, researchers and clinicians have recognized limitations of relying on creatinine as the only filtration marker, which can lead to inaccurate GFR estimates in certain populations due to the influence of non-GFR determinants of serum or plasma creatinine. Therefore, recent literature has proposed incorporation of multiple serum or plasma filtration markers into GFR estimation to improve precision and accuracy and decrease the impact of non-GFR determinants for any individual biomarker. To this end, the CKD-EPI combined creatinine-cystatin C equation ($eGFR_{cr\text{-}cys}$) was developed in 2012 and demonstrated superior accuracy to equations relying on creatinine or cystatin C alone ($eGFR_{cr}$ or $eGFR_{cys}$). Now, the focus has broadened to include additional novel filtration markers to further refine and improve GFR estimation. Beta-2-microglobulin (B2M) and beta-trace-protein (BTP)

are two filtration markers with established assays that have been proposed as candidates for improving both GFR estimation and risk prediction. GFR estimating equations based on B2M and BTP have been developed and validated, with the CKD-EPI combined BTP-B2M equation ($eGFR_{BTP-B2M}$) demonstrating similar performance to $eGFR_{cr}$ and $eGFR_{cys}$. Additionally, several studies have demonstrated that both B2M and BTP are associated with outcomes in CKD patients, including cardiovascular events, ESRD and mortality. This review will primarily focus on these two biomarkers, and will highlight efforts to identify additional candidate biomarkers through metabolomics-based approaches.

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INTRODUCTION

It is currently estimated that 15% of US adults, or about 30 million people, have chronic kidney disease (CKD)[1]. CKD is defined as the presence of kidney damage, or estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2, for a duration of at least 3 months[2]. Once diagnosed, CKD is staged based on cause of disease, level of GFR and albuminuria, to provide guidance for disease management and risk stratification[2]. GFR is accepted as the best overall measure of kidney function in health and disease and reflects the product of the number of nephrons and the average single nephron GFR[3]. Measured glomerular filtration rate (mGFR) via quantification of urinary or plasma clearance of an exogenous filtration marker remains the gold standard for assessing GFR in patients with CKD. However, GFR measurement is burdensome for patients as well as clinical laboratories. Therefore, clinicians instead routinely use GFR estimates to diagnose and manage patients with CKD. The 2012 Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend GFR estimation based on serum or plasma creatinine ($eGFR_{Cr}$) as the first line test, with eGFR based on cystatin C ($eGFR_{cys}$) or the combination of the two ($eGFR_{cr-cys}$) as a confirmatory test, particularly when there is concern for inaccurate $eGFR_{cr}$ results in individuals impacted by known non-GFR determinants of creatinine, such as extremes of muscle mass, a high meat-containing diet, or some dietary supplements such as creatine[4].

GFR estimating equations were developed as early as the 1970s, but it was the 4-variable Modification of Diet in Renal Disease (MDRD) Study equation developed in 2000 and re-expressed for use with standardized creatinine[5] that was the first estimating equation to become widely integrated into routine clinical laboratory reports for assessment of kidney function, due to its reliance on creatinine and readily available demographic metrics (age, gender and race)[6,7]. While this MDRD Study equation was useful for estimating GFR < 60 ml/min/1.73 m^2, it was found to systematically underestimate GFR at levels > 60 ml/min/1.73 m^2.

Therefore, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) was formed in 2003 and set forth to improve the accuracy of GFR estimating equations by development and validation of equations based on creatinine or cystatin C in a diverse population that included participants across the range of GFR and age[3], with and without CKD, diabetes and transplants. Cystatin C was selected as a complimentary candidate filtration marker to creatinine because it is less affected by non-GFR determinants that impact creatinine[8], and several studies have demonstrated that it is a better prognostic marker for predicting development of cardiovascular disease and mortality than creatinine[9-11].

The CKD-EPI group developed and validated a new CKD-EPI creatinine-based eGFR equation, which was found to have lesser bias compared

to measured GFR at GFR > 60 than the MDRD Study equation, and therefore its use was recommended as an improvement over the MDRD Study equation[12]. This work was followed by publication of two papers which demonstrated that estimating equations which relied on both creatinine and cystatin C were superior in precision to equations that relied on only one biomarker alone[13,14]. These studies laid the groundwork for the main hypothesis driving current efforts to improve GFR estimation – that incorporation of additional biomarkers into estimation of GFR diminishes the impact of non-GFR determinants for any given biomarker and improves overall equation performance. Based on this hypothesis, research in the area of GFR estimation has moved from fine-tuning current creatinine and cystatin C-based equations to identifying new endogenous filtration markers that can be incorporated into GFR estimation to improve precision.

Beta-2-microglobulin (B2M) and beta-trace protein (BTP) have been identified as two endogenous low molecular weight protein filtration markers with established assays that have the potential to improve the accuracy of GFR estimations. Additionally, due to technologic advances in the field of metabolomics, work is currently in progress to identify and validate the utility of additional, novel filtration markers, with subsequent development of validated assays.

BETA-2-MICROGLOBULIN (B2M)

B2M is a 11.8 kD protein which associates with both classical and non-classical MHC Class I molecules on the surface of all cells and is critical for antigen presentation[15]. It is freely filtered by the glomerulus, with more than 99.9% reabsorbed and metabolized in the proximal tubule[15]. Serum/plasma B2M concentrations are impacted by the amount generated and shed by nucleated cells, body distribution kinetics, and the amount eliminated through glomerular filtration and tubular metabolism. Due to its ubiquitous presence on the surface of all cells, B2M elevation is seen with diseases associated with high cell turnover, such as many malignancies. Therefore, B2M is most commonly measured along with serum albumin to risk stratify multiple myeloma patients using the International Staging System (ISS)[16], with higher levels of B2M associated with higher tumor burden and more aggressive subtypes, due to increased shedding of B2M[15].

B2M was first suggested as a biomarker for glomerular filtration in the 1980s[17,18], however, as an acute phase reactant that increases in a variety of inflammatory and infectious disorders, its potential as a candidate for a single-marker equation was limited[19,20]. Despite this shortcoming a handful of research groups derived GFR estimating equations based on B2M alone, but data supporting the performance and validity of these equations is lacking[21-24].

Elevation of B2M in patients with CKD, especially end stage renal disease (ESRD), has been traditionally attributed to impaired removal secondary to decreased glomerular filtration. However recent literature has put forth the hypothesis that an additional source of B2M elevation in patients with CKD may be the interference of uremic solutes with the non-covalent binding of B2M to MHC molecules, leading to an increase in shedding of B2M into the circulation[15].

Due to its established use as a prognostic marker for multiple myeloma, B2M is routinely measured in many clinical laboratories by a variety of methods – nephelometry, turbidimetry, or immunoassay[25]. However, studies have demonstrated that B2M assays are not harmonized or standardized leading to discordance between methods[25,26]. While the WHO 1st International Standard for B2M was developed in 1985[27],

and a B2M certified reference value in the serum protein standard ERM-DA470k/IFCC was assigned in 2015 by the Institute for Reference Materials and Measurements (IRMM)[28], manufacturers have not universally adopted use of ERM-DA470k/IFCC for calibration of their measurement procedures[25].

BETA-TRACE PROTEIN (BTP)

BTP, also known as lipocalin prostaglandin D_2 synthase (L-PGDS), is a 23-29 kDa protein. The variation in size depends on the degree of post-translational glycosylation[29], with the larger isoforms of BTP in serum and urine, and smaller isoforms with truncated side chains in cerebrospinal fluid (CSF)[29]. BTP was first noted to be elevated in patients with CKD in 1987, as an incidental finding in a study focused on BTP as a marker for CSF leak[30]. Its specific potential as a filtration marker was not suggested until 1997, in a study that observed very high levels of BTP in patients on hemodialysis[31].

The first GFR estimating equations based on BTP were derived in 2007 by White and colleagues, in a cohort of 163 adult kidney transplant patients with measured GFR. These equations, known as the White equations, performed comparably to the MDRD Study equation, with evidence of improved performance at higher GFRs[32]. The following year, researchers led by Dr. Uwe Pöge developed 3 additional BTP-based GFR estimating equations from a cohort of 85 kidney transplant patients validated in a separate cohort of 102 kidney transplant patients[33]. The three Pöge equations were compared to the re-expressed MDRD Study equation and White equation 1 (based on BTP and urea). The Pöge BTP-formula 3 had better accuracy and precision than White equation 1, and demonstrated a slightly smaller bias and higher 10% accuracy when compared to the re-expressed MDRD Study equation[33]. The generalizability of these equations to clinical populations other than kidney transplant recipients has not been

Table 1 GFR estimating equations based on BTP developed by White[32] and Pöge[33]

Description	Development population	Equation
White Equation 1 (BTP & urea)	N = 163, kidney transplant patients	$eGFR = 112.1 \times BTP^{-0.662} \times Urea^{-0.280} \times (0.880 \text{ if female})$
White Equation 2 (BTP & Cr)	N = 163, kidney transplant patients	$eGFR = 167.8 \times BTP^{-0.758} \times Cr^{-0.204} \times (0.871 \text{ if female})$
Pöge BTP-formula 1 (BTP alone)	N = 85, kidney transplant patients	$eGFR = 47.17 \times BTP^{-0.7933}$
Pöge BTP-formula 2 (BTP & Cr)	N = 85, kidney transplant patients	$eGFR = 974.31 \times BTP^{-0.2594} \times Cr^{-0.647}$
Pöge BTP-formula 3 (BTP & urea)	N = 85, kidney transplant patients	$eGFR = 89.85 \times BTP^{-0.5541} \times Urea^{-0.3018}$

White and Pöge formulas utilize units of mg/L for BTP, mmol/L for creatinine, and mmol/L for urea.

established, and these minor differences were not deemed sufficient enough to recommend replacement of the MDRD Study equation for routine clinical practice[33]. (Table 1)

While GFR estimating equations based on BTP appear promising, a major hurdle involves the lack of standardization amongst currently available BTP assays[29,34]. Unlike creatinine, cystatin C and B2M, there are currently no certified reference materials available for BTP. Additionally, given the known variation in post-translational modification which creates a variety of glycoprotein epitopes, immunoassays utilizing different antibodies would be expected to give disparate BTP results.

USING BTP AND B2M TO IMPROVE GFR ESTIMATION

Given the various shortcomings of relying on BTP or B2M alone for GFR estimation, the CKD-EPI investigators evaluated the utility of combining the markers[35]. Data was pooled from 3 separate research studies involving a total of 3,551 subjects with CKD due to a variety of causes, each with GFR measured based on urinary clearance of iothalamate[35]. Equations were developed using either BTP or B2M concentrations alone and in combination (Table 2).

The performance of the three equations was compared to the CKD-EPI creatinine- and cystatin C-based equations based on precision (Table 3). Their analysis demonstrated that the combined BTP-B2M equation had similar performance to both the creatinine and cystatin C equations but did not represent an improvement over either equation[35]. Additionally, the combined BTP-B2M equation was not as accurate as the combined creatinine-cystatin C equation[35]. Lastly, averaging the BTP-B2M equation with the creatinine-cystatin C equation did not lead to improvement in equation performance[35]. Limitations of this work included the absence of participants without CKD and an external validation population. (Table 3)

While the non-GFR determinants of creatinine were already well-established, it was important to more fully characterize the non-GFR determinants of cystatin C, BTP and B2M. Preliminary studies demonstrated evidence for non-GFR determinants of cystatin C, including inflammation, immunosuppressive therapies, thyroid disease and obesity[36-39], but there were few studies that had evaluated the non-GFR determinants of BTP and B2M. Therefore, in 2016 the CKD-EPI investigators published a cross-sectional analysis of these same CKD cohorts which characterized the non-GFR determinants for these three biomarkers[40]. Their analysis showed that

Table 2 CKD-EPI BTP and B2M equations[35]

Description	Development population	Equation
BTP	N = 2,380, chronic kidney disease patients	$GFR = 55 \times BTP^{-0.695} \times 0.998^{age} \times 0.899$ if female
B2M	N = 2,380, chronic kidney disease patients	$GFR = 133 \times B2M^{-0.852}$
BTP-B2M	N = 2,380, chronic kidney disease patients	$GFR = 96 \times BTP^{-0.278} \times B2M^{-0.588}$

Table 3 Performance of CKD-EPI GFR Estimating Equations (Adapted from Inker et al.[35])

Description	Inter-quartile range (95% CI)	1-P_{30} (%)(95% CI)	1-P_{20} (%) (95% CI)
BTP	15.0 (14.1, 15.9)	23.6 (21.3, 26.1)*	43.6 (40.8, 46.5)
B2M	12.9 (12.2, 13.8)	18.4 (16.2, 20.8)*	37.2 (34.6, 40.1)
BTP-B2M	12.1 (11.4, 13.0)	15.5 (13,3, 17.7)*	35.4 (32.5, 38.1)
Creatinine	11.6 (10.9, 12.4)	16.4 (14.2, 18.6)*	34.5 (31.7, 37.3)
Cystatin C	11.4 (10.6, 12.4)	16.9 (14.9, 18.6)*	34.8 (32.1, 37.6)
Creatinine-Cystatin C	9.3 (8.7, 10.1)	11.3 (9.5, 13.2)	25.5 (23.1, 28.0)
Average of Creatinine-Cystatin C + BTP-B2M	10.2 (9.5, 11.0)	9.6 (8.0, 11.4)	25.0 (22.6, 27.6)

P_{30} and P_{20} are the percentage of GFR estimates > 30% and > 20% from measured GFR
**P < 0.001 when compared to the creatinine-cystatin C equation*

Table 4 Summary of major non-GFR determinants for filtration markers[40,41]

GFR biomarker	Non-GFR determinant profile
Creatinine	Male sex, black race, elevated urine creatinine, age
Cystatin C	Male sex[1], smoking, body mass index (BMI) and C-reactive protein (CRP)
BTP	Male sex[1], urine protein excretion, non-black race, body mass index (BMI)
B2M	Urine protein excretion, smoking and C-reactive protein (CRP)

[1]The association between male sex and creatinine was stronger than the associations between male sex and BTP or cystatin C

creatinine was more strongly associated with male sex, black race and elevated urine creatinine than BTP, B2M or cystatin C. In addition, each filtration marker exhibited unique profiles of non-GFR determinants (Table 4).

In 2017, non-GFR determinants of these filtration markers were further characterized in 2 community-based, predominantly elderly cohorts (Table 4)[41]. Again, creatinine was found to more strongly associate with age and sex than

cystatin C, BTP or B2M. Additionally, both cystatin C and B2M had significant associations with CRP, confirming prior studies demonstrating a relationship between inflammation and inflammatory diseases and these biomarkers[15,42]. Not all associations were duplicated between the two studies, and therefore more research is needed. Both studies did provide evidence that each filtration marker has unique non-GFR determinant profiles, providing a foundation of support for the hypothesis that combining multiple markers with differing non-GFR determinants for GFR estimation has the potential to minimize bias and imprecision, thereby improving accuracy. Additionally, $eGFR_{cys}$, $eGFR_{BTP}$ and $eGFR_{B2M}$ were less influenced by race than $eGFR_{cr}$, thus introducing the possibility of developing a multiple marker estimating equation without creatinine which would eliminate the need for race specification.

PROGNOSTIC VALUE OF BTP AND B2M

Like cystatin C, BTP and B2M are promising biomarkers in CKD not only due to their potential role in improving GFR estimation, but also due to their role as prognostic indicators. Patients with CKD have a significantly increased risk for cardiovascular disease, hospitalization and mortality compared to the general population, and therefore there is interest in predicting these outcomes[43-45].

In 2005, cystatin C was found to be a stronger predictor of mortality and cardiovascular outcomes than creatinine and $eGFR_{cr}$[10]. Additionally there is a marked discordance in mortality prediction between $eGFR_{cr}$ and $eGFR_{cys}$ at higher eGFRs, with higher $eGFR_{cr}$ associated with increased mortality while higher $eGFR_{cys}$ is associated with decreased mortality[46]. This discordance is thought to be due to non-GFR determinants of creatinine such as muscle wasting that would confound its association with outcomes in individuals in poor health, but could also be due to confounding by non-GFR determinants of cystatin C [46]. These results raised the question of whether B2M and BTP have prognostic value beyond creatinine or $eGFR_{cr}$ alone[10]. The first study proposing B2M as a prognostic marker was published in 2008, and demonstrated that B2M was an independent predictor of overall mortality in a community-based elderly population[47]. BTP was first proposed as a prognostic marker in a 2010 study which found that it was a strong predictor for future CKD progression[48].

Based on the promise of these initial studies, in 2012 researchers took a more comprehensive look at BTP and B2M as prognostic markers, by examining their association with risks for mortality, cardiovascular disease and kidney failure in a large group of subjects from the Atherosclerosis Risk in Communities (ARIC) study (n = 9,988), a middle-aged general population cohort[49].

The study found that, similar to cystatin C, B2M is a stronger predictive marker than $eGFR_{cr}$ for outcomes such as cardiovascular disease, kidney failure and mortality[49]. BTP levels also predicted these outcomes more strongly than $eGFR_{cr}$, although not to the degree of cystatin C and B2M levels[49]. This study was followed up by a similar analysis performed on 6,445 subjects from the Third National Health and Nutrition Examination Survey (NHANES III), a general population cohort spanning the range of adulthood, ages 20 and older[50]. This study also demonstrated that BTP and B2M were stronger prognostic markers than $eGFR_{cr}$, for all-cause mortality, cardiovascular disease, and coronary heart disease mortality[50]. Additionally, incorporating 4 markers – creatinine, cystatin C, B2M and BTP – into a risk prediction model led to moderate improvement in 10-year risk prediction compared to $eGFR_{cr}$, when adjusted for mortality and cardiovascular risk factors[50].

While these studies supported the utility of B2M and BTP as prognostic markers in the general population, they did not examine their utility in clinically relevant sub-populations, such as diabetics or patients with chronic kidney disease, or in racial groups other than Whites or African-Americans. Therefore, their role as risk predictors in a type 2 diabetic Pima Indian cohort was examined in 2015[51].

This study found that both BTP and B2M were associated with ESRD, with BTP having the stronger association[51]. Interestingly in this study only B2M, and not BTP, was associated with mortality, after adjustment for other mortality risk factors and kidney function markers[51]. Therefore, B2M may be a more useful prognostic marker than BTP in this subpopulation of Pima Indian diabetics. To further address the potential role of BTP and B2M in clinically significant subpopulations, a cohort of CKD patients was examined to specifically look at B2M and BTP's role in predicting cardiovascular events, ESRD and mortality[52]. This study demonstrated that both B2M and BTP were independently associated with ESRD and all-cause mortality, and B2M was associated with risk for cardiovascular events in these patients with mild or moderate CKD[52]. Additionally, a 4-marker composite score generated from $eGFR_{cr}$, $eGFR_{cys}$, B2M and BTP levels was independently associated with all three outcomes – ESRD, all-cause mortality and cardiovascular events[52]. Of note, this analysis showed that BTP and B2M are associated with ESRD, and B2M and the 4-marker composite score were significantly associated with all-cause mortality and cardiovascular events even after adjustment for mGFR, indicating that non-GFR determinants contribute to risk prediction[52]. These findings support prior studies that have shown that B2M or BTP have prognostic value beyond measured GFR[51,53].

Lastly, a recent individual patient meta-analysis from the CKD Biomarkers Consortium study also examined the association between eGFR based on the four filtration markers (creatinine, cystatin C, BTP and B2M) alone and in combination with each other, through analysis of the three cohorts described above (ARIC, NHANES III, Pima) combined with three CKD study populations–Chronic Renal Insufficiency Cohort (CRIC), Modification of Diet in Renal Disease (MDRD) study and African American Study of Kidney Disease (AASK)[54].

Consistent with the data supporting association of B2M and BTP with risk outcomes, this study found that $eGFR_{B2M}$ and $eGFR_{BTP}$ modestly improved prediction of ESRD and mortality over $eGFR_{Cr}$[54]. Additionally, this meta-analysis demonstrated that higher $eGFR_{B2M}$ and $eGFR_{BTP}$ are associated with lower mortality, similar to $eGFR_{cys}$[54], consistent with the hypothesis that increased mortality associated with higher $eGFR_{cr}$ reflects confounding by non-GFR determinants of creatinine such as muscle wasting in patients in poor health. Additionally, eGFR based on the average of the estimated GFRs from all 4 biomarkers provided the best overall performance for risk prediction, albeit only a modest improvement over $eGFR_{Cr}$[54].

This study and others together demonstrate that combining multiple filtration markers provides the best overall performance for predicting risk outcomes.

LOOKING TO THE FUTURE —KIDNEY METABOLOMICS

Advances over the last decade in mass spectrometry and associated chromatography methods have led to an explosion of metabolomics studies aimed at discovering novel biomarkers for various diseases[55].

In 2010, the first targeted metabolomics studies in CKD patients identified novel uremic toxins, but the studies were too limited in size and power to draw firm conclusions about the identified metabolites[56-58].

In 2012, the first large-scale targeted metabolomics study in subjects spanning the range of GFR was performed using 3,011 samples from the KORA F4 study for metabolite discovery, and 984 samples from the TwinsUK study for metabolite validation[59]. A total of 22 metabolites and 516 metabolite ratios were identified as having a significant association with $eGFR_{cr}$, with acylcarnitines having the strongest association[59].

This cross-sectional analysis was soon followed by a targeted longitudinal metabolomics study in 2013, aimed at determining whether the same or different metabolites and metabolite ratios were associated with development of $eGFR_{cr}$ decline over time independent of baseline eGFR[60]. The study examined associations between 140 metabolites and 19,460 ratios with the incidence of decreased $eGFR_{cr}$ and $eGFR_{cr}$ decline over a 7 year period in 1,104 subjects from the KORA study[60]. This longitudinal analysis demonstrated that the acylcarnitines overall did not significantly associate with $eGFR_{cr}$ decline over time. Rather, the study identified one metabolite and two ratios that had a significant association with change in $eGFR_{cr}$ over time – spermidine, the kynurenine-to-tryptophan ratio, and the phosphatidylcholine diacyl C42:5-to-phosphatidylcholine acylalkyl C36:0 ratio – all of which were supported by smaller, prior studies[61-64].

In 2016, the first large-scale non-targeted metabolomics study was published, with metabolite discovery performed on samples from 1735 Kora study subjects, and validated in 1164 samples from the TwinsUK study[65]. A non-targeted approach has the advantage of identifying previously unrecognized CKD-associated metabolites. Of the 493 small molecules quantified in the study, 54 metabolites had a validated significant association with $eGFR_{cr}$, with 6 metabolites demonstrating a significant pairwise correlation: C-mannosyltryptophan, pseudouridine, N-acetylalanine, erythronate, myo-inositol and N-acetylcarnosine[65]. Additionally, three metabolites (C-mannosyltryptophan, pseudouridine, and O-sulfo-L-tyrosine) were significantly associated with development of low $eGFR_{cr}$[65]. Studies comparing metabolites to measured GFR have been reported and could yield more accurate estimates of GFR whose generalizability and robustness will need to be tested.

CONCLUSION

While there have been marked improvements in the accuracy of GFR estimation using serum- or plasma-based biomarkers over the last 20 years with refinement of equations based on creatinine and cystatin C, inaccuracy of estimated GFR remains a challenge due to the impact of non-GFR determinants of these biomarkers. B2M and BTP hold promise as candidate endogenous filtration markers that have the potential to improve the accuracy of both GFR estimation and risk prediction.

Additionally, cystatin C, B2M and BTP are less affected by race than creatinine, and therefore provide the potential opportunity to estimate GFR without the need for race specification. Kidney metabolomics research is in the early phases of metabolite discovery and validation, with work on the horizon to assess the clinical feasibility of using additional, new biomarkers for improved GFR estimation and risk prediction. Thus, the focus is shifting to the concept of estimating GFR with a panel of several serum or plasma biomarkers, to minimize the impact of each individual biomarker's non-GFR determinants.

Additionally, multiple studies on BTP and B2M as prognostic markers support the idea that risk prediction also improves when multiple markers are combined. Therefore, novel biomarkers identified via metabolomics profiling in chronic kidney disease patients will likely be combined with biomarkers such as creatinine, cystatin C, B2M and BTP, for future incorporation into multi-biomarker estimating equations for GFR and multi-biomarker risk prediction models.

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TF-CKD special issue: The laboratory diagnostics of chronic kidney diseases

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A pathway to national guidelines for laboratory diagnostics of chronic kidney disease – examples from diverse European countries

Vanja Radišić Biljak[1], Kristin Moberg Aakre[2], Dogan Yucel[3], Anne-Sophie Bargnoux[4], Jean-Paul Cristol[4], Laurence Piéroni[4]

[1] *Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Croatia, on behalf of the Croatian Society of Medical Biochemistry and Laboratory Medicine (CSMBLM)*

[2] *Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway*

[3] *Department of Medical Biochemistry, Ankara Training and Research Hospital, University of Health Sciences, Altindag, Ankara and Turkish Biochemical Society*

[4] *Laboratoire de Biochimie, CHRU de Montpellier, PhyMedExp, University of Montpellier, France, on behalf of the Société Française de Biologie Clinique (SFBC)*

ARTICLE INFO

Corresponding author:
Vanja Radišić Biljak
Department of Medical Biochemistry and Laboratory Medicine
Merkur University Hospital
Zajčeva 19
10000-Zagreb
Croatia
Phone: +385917967867
E-mail: vanja.radisic@gmail.com

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ABSTRACT

The principal benefit of guidelines is to improve the quality of care received by patients. In the 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease (KDIGO) was released and it is designed to provide information and assist decision making. This review gives a brief overview of a various national CKD guidelines that rely on the newly released KDIGO guidelines. All of the included countries (France, Turkey, Norway and Croatia) are non-English speaking countries and they differ in population and socio economic aspects. Examples shown in this review may provide valuable experience for countries that are in process of creating their national CKD guidelines.

INTRODUCTION

For patients (and almost everyone else in health care), the greatest benefit that could be achieved by guidelines is to improve health outcomes (1). In the 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease (2) was released by the The Kidney Disease: Improving Global Outcomes (KDIGO). This guideline serves to update the 2002 KDOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification following a decade of focused research and clinical practice in CKD. Although it is designed to provide information and assist decision making, it is not intended to define a standard of care, and should not be construed as one, nor should it be interpreted as prescribing an exclusive course of management (2).

As a comparison to worldwide recognized British (3), Australian (4, 5) and American CKD guidelines (2), this article gives a brief overview of various national CKD guidelines from diverse European countries: France, Turkey, Norway and Croatia. All of the included countries are non-English speaking countries and they differ in population and socio economic aspects. Through these examples variations in practice will be shown when the needs of individual patients, available resources, and limitations unique to a specific country, were taken into account.

FRENCH GUIDELINES FOR ESTIMATION OF GLOMERULAR FILTRATION RATE (EGFR) – PAST, PRESENT AND THE FUTURE

I) Analytical evaluation and improvement of creatinine measurement: involvement of the scientific societies

As early as 2002, the "Société Française de Biologie Clinique" (SFBC) recognized the glomerular filtration rate (GFR) estimation as a major health problem (6) and created the "Creatinine" Working Group. This laboratory working group initiated a multicentric study to evaluate inter-assay variation and accuracy of 17 creatinine assays of which 14 were commercially available automated assays (4 enzyme assays, 1 compensated Jaffe assay, and 9 non-compensated Jaffe assays) (7). Using 30 frozen human samples, they demonstrated that a very high inter-assay variation persisted since the median inter-assay coefficient of variation (CV) was 14.2% for 20 low samples (45–150 µM) and 7.7% for 10 high samples (250–350 mM). In addition, the inaccuracy, assessed with three certified reference materials, appears to be relatively high, especially for the lowest concentration with biases ranging from –2.9% to +57.5% for the low level (68.7 µM) (8).

In 2008 the newly formed working Group «Biologie des fonctions rénales et de l'insuffisance rénale» involving Nephrologists and medical Biologists was supported by both the SFBC and the "Sociéte de Néphrologie". This group decided to perform a new study after the publication of the "Laboratory Working Group of the National Kidney Disease Education Program" recommendations (NKDEP) for in-vitro diagnostic (IVD) manufacturers (9), highlighting the need for developing methods for creatinine measurement that are reproducible and traceable to isotope dilution mass spectrometry (IDMS). Our evaluation involved 25 clinical laboratories, 12 enzymatic and 4 compensated Jaffe creatinine automated assays. Creatinine was measured in serum pools ranging from 35.9±0.9 µmol/L to 174.5±3.1 µmol/L (IDMS determination). This study demonstrates substantial improvements in the calibration, traceability and precision of the enzymatic methods, reaching the total analytical error of 8% for the majority of enzymatic methods (10). Moreover, most of these assays allowed accurate creatinine measurements for creatinine levels lower than 40 µmol/L. By contrast, this requirement was never obtained for the compensated Jaffe methods at the critical level of 74.4±1.4 µmol/L (11).

II) Time to recommendations: a step by step improvement

Based on the international recommendations and our own French studies, we were able to publish some French recommendations. The first recommendation (12), published in 2002 by the «Agence National d'Accréditation et d'Evaluation en Santé» (ANAES, former name of Haute Autorité de Santé, HAS) recommended that laboratory analysts should provide an estimation of GFR value using the Cockcroft-Gault formula for every request for serum creatinine, but no analytical guidelines for creatinine measurement were suggested.

However, as soon as the Kidney Disease Outcome Quality Initiative (K/DOQI) classification was proposed (6) following a position statement from Kidney Disease: Improving Global Outcomes (KDIGO) (13), an update of the French position statement about estimation of GFR and proteinuria has been developed by the «Société de Néphrologie» in 2009.

For renal function measurement, it is recommended to estimate GFR from serum creatinine using IDMS traceable simplified modification of diet in renal disease (MDRD) equation.

These recommendations were published in the French journal of the Société Française de Néphrologie: Néphrologie Therapeutique (14).

This guidelines was further supported by the report of «Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS)» in 2010 recommend an IDMS traceable creatinine assay. They advised the use of enzymatic assays in specific populations like in pediatric patients or in specific situations where Jaffe assays are known to be subject of interferences (15).

Through their collaborative works, the SFBC working group published its own recommendations in the French journal of the SFBC: "Les Annales de Biologie Clinique", highlighting the use of IDMS-traceable creatinine assay and the use of CKD-EPI equation (16). Finally, the «Haute Autorité de Santé», driven by the French Ministry of Health, meet an expert panel involving clinical biologists, nephrologists, and geriatricians.

These guidelines, available on the Web site of the HAS in 2011-2012, recommend an IDMS traceable enzymatic creatinine assay in all clinical situations because of better analytical specificity, sensitivity and performances of enzymatic assays compared to Jaffe assays (17).

Further to the publication of KDIGO 2012 clinical practice (2), the HAS recommend that French clinical laboratories report eGFR in adults using the 2009 CKD-EPI creatinine equation which gives the best performance in terms of accuracy. Pending the full adoption of the CKD-EPI equation by health professionals, the 175 MDRD formula may be used in the meantime but Cockroft and Gault formula should be omitted. It should be noted that the ethnic adjustment factor into the equation does not apply in France due to the non-validation of this correction in non-American black people and to the French law for preventing any ethnic discrimination.

Since it has been demonstrated that mild to moderate CKD is associated with adverse clinical outcomes, the KDIGO working group decided not to combine stage 1–2 CKD.

A precise eGFR above 60 mL/min/1.73 m² is thus valuable. Results of the SFBC study support the use of CKD-EPI equation rather than MDRD and found that accurate enzymatic assays allow estimation of eGFR until 90 mL/min/1.73 m² with MDRD and 120 mL/min/1.73 m² with CKD-EPI equation.

In all cases, compensated Jaffe creatinine assays lead to important errors in eGFR and should be avoided (18).

III) Results and limitations after more than 10 years of scientific communication

Data from the French biochemistry external quality assessment scheme «ProBioQual» (Centre lyonnais d'études pour laPROmotion de la BIOlogie et du contrôle de QUALité, LYON - FRANCE) underline the emergence of standardized Jaffe assays in 2008, and a gradual implementation of enzymatic assays in French laboratories since the creation of these guidelines (Figures 1 and 2).

In 2017, about 65% of French laboratories use enzymatic creatinine assays. In addition, all laboratories give the creatinine result with an estimation of GFR. Some of them add the staging of CKD according to the KDIGO from stage I to V. As a limitation, these recommendations are clearly designed for adult population, no French recommendations are edited for children.

Reliable estimates of high eGFR are important for drug dosing, nevertheless, the HAS draws attention to the difficulties of drug dosage adjustments. For example, there are currently conflicting recommendations between the current CKD guidelines (19) and the «Groupe Français d'Etude sur l'Hémostase et la Thrombose (GEHT)» regarding the use of direct oral anticoagulants for the prevention and treatment of thromboembolic disease in patients with reduced renal function.

Clinicians are reluctant to use the MDRD or CKD-EPI formula reported by the lab since the estimation of renal function with the Cockcroft and Gault formula is used for the pharmacokinetics studies and the development of drug dosing guidelines. In addition, the values obtained with the Cockcroft formula in patients >75 years are systematically lower than the values obtained with the MDRD formula. This should allow a safe use of prescription of direct oral anticoagulants.

Figure 1 Creatinine assays before French recommendations

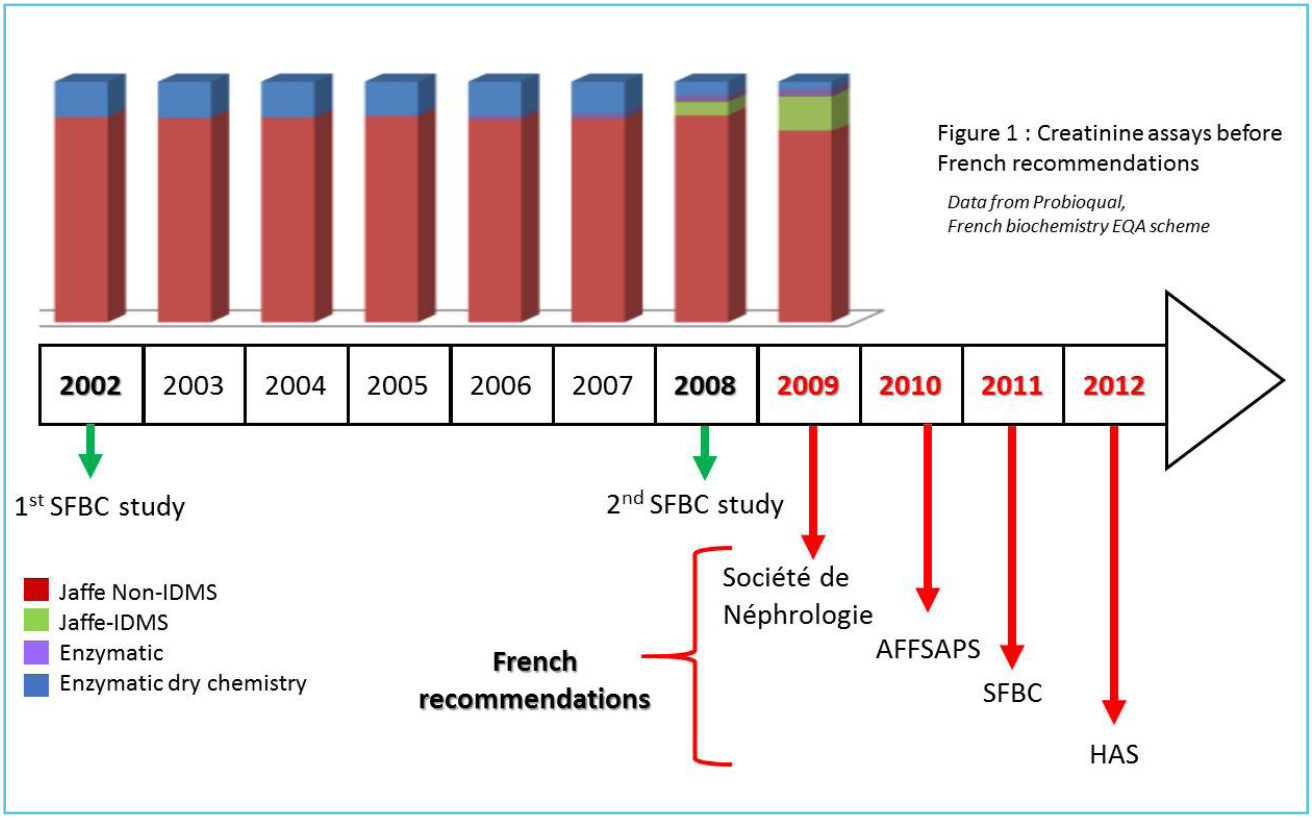
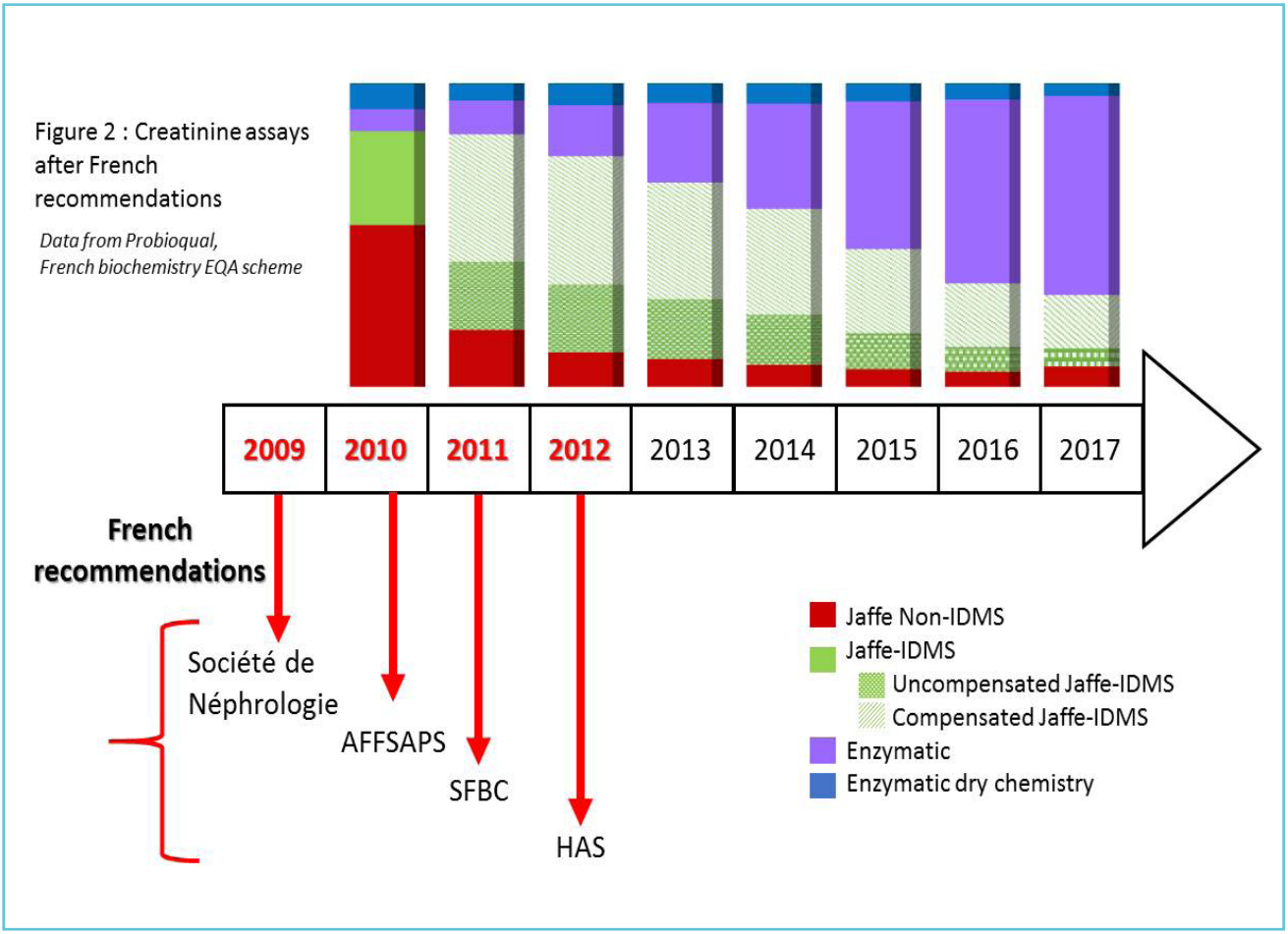


Figure 2 Creatinine assays after French recommendations



IV) Lessons and perspectives

The strong involvement of the French scientific societies, mainly the "Société de néphrologie" and the SFBC leading to the constitution of joint working group associating clinicians and medical biologists, allows the creation of the French recommendations for GFR estimation. In addition, these collaborative groups allow the initiation and realization of multicentric studies. Similarly to the creatinine study, the SFBC group conducted a multicentric evaluation of automated cystatin C assays before (2008) and after (2015) standardization using the certified reference material ERM-DA 471. In the latter study, we showed that bias remains the major component of the combined uncertainty because of possible problems associated with the implementation of traceability (20). Although some manufacturers have clearly improved their calibration protocols relative to ERM-DA471, most of them failed to meet the criteria for acceptable cystatin C measurements. As a result, no recommendations are currently available in France for cystatin C.

To date, the SFBC and Société de Néphrologie have initiated two working groups, one about the biomarkers of Chronic Kidney Disease – Bone and Mineral disorders and one about urinary markers of renal dysfunctions. These groups produced reviews for the journal of the SFBC ("Les Annales de Biologie Clinique") and organized multicentric evaluations of albuminuria and urinary calcium and phosphate determinations.

INCREASING AWARENESS ABOUT CHRONIC KIDNEY DISEASE AND IMPLEMENTATION OF A NEW PRACTICE GUIDELINE IN TURKEY

CKD is an important and growing public health problem in Turkey like in all over the World. For the year 2015, general incidence and general prevalence of end stage renal disease in Turkey were 147.3 and 935.4 per million population. But the prevalence of CKD is very high, 15.7% in Turkey. Currently, there are about 100 000 patients undergoing hemodialysis or peritoneal dialysis in our country. Hence, the awareness level should be increased. Currently, awareness level is <10% in the World and not more than 2% in Turkey.

Although the laboratory examinations are sine quo non for screening, diagnosis, evaluation, staging and monitoring of CKD, these examinations are mostly analysed by using different analytical methods and techniques and therefore different results can be obtained and reported with different units. For this reason, a uniform and standard approach is required for laboratory practice. From this point of view, the Turkish Biochemical Society (TBS) planned a strategy through implementation of a series of steps. These steps are presented as follows.

The working group

TBS organized a working group on CKD (WG-CKD) in 2014. The WG-CKD was essentially consisted of laboratory specialists from different level hospital laboratories and the representatives of main diagnostic companies.

The survey

The WG-CKD at first coordinated a questionnaire for Turkish laboratories. The survey included questions addressing the assessment of awareness about the CKD and especially on creatinine and urinary albumin measurements such as instrument use, creatinine and urine albumin methods and their traceability, calibration and control procedures, external quality assessment scheme, reporting of eGFR, reporting of creatinine and albumin results. There were similar questions also relating to serum cystatin C and urine total protein in the survey.

About 100 specialists from different hospital laboratories, a total of 94 labs, participated in the survey. The major analytical systems and reagents for creatinine were of Roche Diagnostics (29.4%), Abbott Diagnostics (28.24%), Beckman-Coulter Inc. (27.06%), Siemens Healthcare (5.88%), and Mindray (1.18%). More than 90% of the laboratories were using the Jaffe method and only 8% were using the enzymatic creatinine method. The methods were traceable to SRM 967 (50.79%), SRM 914 (33.33%) and SRM 909 (11.11%), essentially. Creatinine results were mostly reported with conventional units (mg/dL, 95.18%).

Reference ranges recommended by diagnostic companies were used (about 80%) and age - and/or sex-related reference ranges were reported (89.73%) by a majority of the labs. Only 49.30% and 18.31% of the laboratories were reporting eGFR for adult and pediatric population, respectively. Mostly CKD-EPI formula was used (44.74%) for eGFR, and cystatin C use was only 10.53%). Cystatin C was measured by nephelometric and turbidemetric methods and only 5.2% of the labs were participate in an EQAS. Urine albumin was measured by turbidimetric (86.00%) and nephelometric (12%) methods and all specimen types, 24-h urine, random, first morning and second morning, were accepted. The majority of the laboratories (88.37%) did not use decision limits for urine albumin.

The guideline

The WG-CKD decided to prepare a short guideline based on KDIGO 2012 clinical practice guideline for the evaluation and management of CKD for laboratory specialists. The guideline was completed and published in 2015 and included the following key recommendations (21).

1. Creatinine assays should be traceable to a reference material which creatinine concentration assigned by GC-IDMS technique.
2. When reporting the creatinine result, eGFR should also be reported in adult (>18 years) population. A warning expression should be included in the report form if eGFR result is <60 mL/min/1.73 m^2.
3. eGFR values should be expressed quantitatively up to 90 mL/min/1.73 m^2 by CKD-EPI equation. Above 90 mL/min/1.73 m^2, eGFR values can be expressed quantitatively or >90 mL/min/1.73 m^2.
4. eGFR equations of the adult population should not be used for pediatric population. Different equations utilizing also patient height should be used. The enzymatic creatinine assay should be preferred. eGFR based on cystatin C can be used for confirmation in the pediatric population.
5. Cystatin C measurements, at least when eGFR based on creatinine is not reliable and for confirmation should be encouraged.
6. Proteinuria or albuminuria values should be reported in proportion to creatinine.

Implementation of the guideline

The guideline was was accepted by the Ministry of Health and it was circulated by Department of Elderly Health and Disables, Public Health Institution of Turkey, under the Turkey's Prevention and Control Program of Kidney Diseases (2014 – 2017) in December 2015. The guideline was also announced from the website of the Department of Laboratory Services, Ministry of Health (http://dosyamerkez.saglik.gov.tr/Eklenti/2621,kbh1pdf.pdf?).

Currently, the guideline is implemented by all public and private medical laboratories at all levels, primary, secondary, and tertiary health institutions across Turkey. In this connection, eGFR is reported with CKD-EPI formula through serum creatinine in adult population and with Schwartz formula in the pediatric population; proteinuria and albuminuria are also interpreted and reported according to the guideline.

We hope, the implementation of the guideline by all medical laboratories, will have important consequences on standardisation and harmonisation of laboratory tests relating to CKD and of course on patient safety.

NORWEGIAN RECOMMENDATIONS FOR DIAGNOSING CKD

In Norway, two recommendations regarding diagnosing CKD have been published within the last years. One is the recommendation from the Norwegian Society of Medical Biochemistry (NSMB) regarding estimation of GFR based on creatinine measurements (22), and the other is the Diabetes Guideline (23) from the Norwegian Directorate of Health that includes a chapter on diagnosis and follow-up on diabetes nephropathy. Amongst other things this guideline describes how urinary albumin testing should be undertaken.

NSMBs recommendations for estimating GFR

The recommendation was worked through by a working group consisting of five specialists in laboratory medicine and one laboratory technician, and was published in 2016. The group got feedback from local nephrologists during their work.

The main messages in the recommendations

- Creatinine should be measured using an enzymatic assay
- eGFR should be calculated using the CKD-EPI formula
- eGFR results should be multiplied by 1.15 if the patient is Afro-American
- Renal disease should be classified according to the guideline from KDIGO (2)

Implementation of the recommendation

In 2017 most Norwegian laboratories use an enzymatic assay. The last numbers from the Norwegian EQA scheme (NOKLUS) shows that 57 laboratories use enzymatic assays and only two uses the Jaffe method. This is an improvement from before the recommendations were produced, when 8 laboratories used the Jaffe method. NSMB has not yet evaluated if Norwegian laboratories have changed formula for eGFR calculations from the MDRD formula to the CKD-EPI formula, but oral communications with the main laboratories in Norway indicate that this change has been undertaken.

The guideline for diagnosing and follow up of Diabetes Nephropathy

This guideline is part of the official Norwegian Diabetes Guideline. It was produced by a working group established by the Norwegian Directorate of Health and gives recommendations regarding the diagnosis and follow-up of renal disease in diabetes patients. The group members were endocrinologists, nephrologists, general practitioners and a specialist in laboratory medicine. Recommendations related to diagnosing renal disease focused on eGFR and measurement of urinary albumin. The group recommended that these tests were conducted on a yearly basis and more often if positive results or progressive disease were detected.

The laboratory specialist was also a member of the eGFR working group described above, so recommendations related to eGFR were harmonized between the two groups and are identically to those described above.

Some information was available regarding followed up and diagnosis of albuminuria in diabetes patients in Norway when the work started. This task is primarily done in primary care, and > 95% of general practitioners screen diabetes patients for albuminuria (24). General practitioners commonly use high quality quantitative point of care instruments that measure albumin/creatinine ratio in morning or spot samples (25).

The main recommendations related to urine albumin measurements

- Urine albumin should be measured as albumin/creatinine ratio.
- A morning sample or a random spot sample should be used.
- Two positive samples are necessary to diagnose albuminuria. The second sample should be taken within 3 months from the first sample.
- Albuminuria should be classified as recommended by KDIGO (2).
- Physical activity, acute inflammatory response and urinary tract infection may lead to false positive results and should therefore be avoided during testing.
- Biological variation is high and reference change values of 100-200% may be expected.

Implementation of the guideline

The implementation of this guideline has not yet been evaluated by the Norwegian health care authorities.

ADVANTAGES AND OBSTACLES IN CREATING NATIONAL CHRONIC KIDNEY DISEASE LABORATORY RECOMMENDATIONS IN CROATIA

In 2013, the Joint Croatian Working Group (JCWG) for laboratory diagnostic of CKD on the behalf of Croatian society of medical biochemistry and laboratory medicine (CSMBLM) and Croatian chamber of medical biochemists (CCMB) conducted a survey across Croatian medical-biochemistry laboratories to assess the current practice in this area of laboratory medicine. The results from the survey were published in the presented article in the first issue of national Biochemia Medica Journal in 2015 (26).

The results of the survey showed that there is a large heterogeneity among Croatian laboratories regarding measuring methods, reporting units and reference intervals (cut-off values), both for creatinine and urine albumin or protein. The two key prerequisites for CKD screening, automatic reporting of eGFR and albuminuria or proteinuria assessment, are not implemented nationwide. There is a need for harmonization in laboratory diagnostics of CKD in Croatia (26). There is still a substantial number of laboratories that use the non-standardized uncompensated Jaffe method, almost one quarter of all Croatian medical-biochemistry labs. Only about 11% of laboratories use enzymatic method. The rest of laboratories measure creatinine with compensated Jaffe method traceable to IDMS method and Standard Reference Material 967.

The majority of laboratories that participated in the survey generally do not report results for eGFR (75%). Among laboratories that report eGFR, there is a statistically signifcant difference in distribution by type of institution ($P < 0.001$), with the lowest number of laboratories from primary health care institutions.

The most prevalent equation for calculating eGFR, at the time point when the survey was conducted, was MDRD equation for standardized creatinine, which was in accordance with the recommendations of Croatian Chamber at that time. However, there were some answers indicating using the MDRD equation for standardized creatinine with the results of serum creatinine measured with non-standardized uncompensated Jaffé method, and reporting of results for eGFR calculated with MDRD equation as an exact number regardless of eGFR value.

Majority of laboratories that participated in the survey do not measure urine albumin or protein (75%), predominantly in primary health care laboratories. There is a large heterogeneity among type of sample recommended for measuring urine albumin or protein and reporting units, consequently. The results indicate that assessment of albuminuria and proteinuria in a large number of laboratories is still performed in 24-hour urine samples.

The most important issue that occured is the fact that laboratories still use non-standardized methods for creatinine results and do not report eGFR values. Also, the majority of laboratories do not measure urine albumin, especially in primary care health setting. These facts set the background for the process of standardization and harmonization in this area of laboratory medicine which resulted in issuing first national recommendations for laboratory diagnostics of chronic kidney disease in Croatia (27). These national recommendations, based on the relevant 2012 KDIGO Guideline, represent the first step in accomplishing the goal of standardization and harmonization in this area of laboratory medicine. The recommendations were published on English language, however Croatian translation was printed in a form of a booklet and distributed to every medical-biochemistry laboratory in Croatia (Figure 3).

Figure 3 The main page of a Croatian national recommendations for laboratory diagnostics of chronic kidney disease (booklet)

The national recommendations are mainly based on the KDIGO 2012 guidelines, however, novel literature findings are also incorporated. Considering the results obtained via conducted survey, our main goal was to provide recommendations that can be easily applied in every medical biochemistry laboratory in Croatia. We, as a WG and authors of recommendations, decided to start at the basic laboratory tests used in laboratory diagnostics of CKD: creatinine, eGFR, urine albumin-to-creatinine ratio (ACR) and urine protein-to-creatinine ratio (PCR). The text of the national recommendations is organized to identify critical points in four major laboratory tests used in basic laboratory diagnostics of CKD. The draft of the recommendations was sent to numerous national and international experts for their comments. The manuscript was also made available for public consultation. All comments were carefully considered and incorporated into the final version of the recommendations.

It is rather difficult to give unique and uniform recommendations, regarding a large heterogeneity amongst methods and populations. Our intention was to point out to some weak points in pre-, post- and analytical phase, as well as some basic pediatric considerations, but every laboratory must set their own specifications for method performance and handling the specimens, according to their possibilities and conditions.

The main messages in the recommendations are as follows:

- Creatinine should be measured using an enzymatic assay
- eGFR should be calculated using the CKD-EPI formula
- Urine albumin should be measured as albumin/creatinine ratio.
- A morning sample or a random spot sample should be used.

1. Creatinine assays should be traceable to a reference material which creatinine concentration assigned by GC-IDMS technique.
2. When reporting the creatinine result, eGFR should also be reported in adult (>18 years) population. A warning expression should be included in the report form if eGFR result is <60 mL/min/1.73 m^2.
3. eGFR values should be expressed quantitatively up to 90 mL/min/1.73 m^2 by CKD-EPI equation. Above 90 mL/min/1.73 m^2, eGFR values can be expressed quantitatively or >90 mL/min/1.73 m^2.
4. eGFR equations of the adult population should not be used for pediatric population. Different equations utilizing also patient height should be used. The enzymatic creatinine assay should be preferred. eGFR based on cystatin C can be used for confirmation in the pediatric population.

So, our final goal for 2017, as a Joint Working Group, will be a complete implementation of national guidelines.

Every member of the WG participates in the implementation process. We inted to provide relevant information to every medical biochemist in our geografically diverse country. To facilitate implementation of national guidelines the members of a national WG gave a series of lectures entitled: „The role of laboratory testing in detection and classificaton of chronic kidney disease: national recommendations".

To assess the national recommendations implementation process, our subsequent actions include repeating a slightly modified survey by the end of 2017. The biggest challenge remains introduction of albuminuria measurement in primary health care laboratories. This is a regulatory issue that requires the involment of the State and our health care system to finance the introduction of the new tests in primary health care labs that represent about 70% of medical-biochemistry labs in Croatia. This problem is already presented twice to responsible regulatory bodies, however no agreement was made so far.

Future plans also include cooperation with Croatian Society for Nephrology, Dialysis and Transplantation of Croatian Medical Association – initial contact has already been established and there is good will to continue with this project in the future.

CONCLUSION

Guidelines call attention to increasing awareness to CKD and implementation of a new guideline for medical laboratories (Turkey), clinical services, and preventive interventions (France, Norway).

As seen in Croatian example, services that were not previously offered to patients may be available as a response to newly released guidelines.

Explicit guidelines improve clinical practice; however clinical guidelines will achieve the full potential only if appropriate strategies are selected at each stage of the implementation (28). Examples shown in this review may provide valuable experience for countries that are in process of creating their national CKD guidelines.

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TF-CKD special issue: The laboratory diagnostics of chronic kidney diseases

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

A summary of worldwide national activities in Chronic Kidney Disease (CKD) testing

Roberto Ruiz-Arenas[1], Rosa Sierra-Amor[1], David Seccombe[2], Stella Raymondo[3], Maria Stella Graziani[4], Mauro Panteghini[4], Tewogbade A. Adedeji[5], Shanthi Naidu Kamatham[6], Vanja Radišić Biljak[7]

[1] *Alianza Mexicana para Prevenir las Enfermedades Crónicas, A.C. (AMPEC)*
[2] *CEQAL Inc., Vancouver, British Columbia, Canada*
[3] *Asociación Bioquímica Uruguaya (ABU)*
[4] *Italian Society of Clinical Chemistry and Clinical Molecular Biology (SIBioC)*
[5] *Association of Clinical Chemists of Nigeria (ACCN)*
[6] *Association of Medical Biochemists of India (AMBI)*
[7] *Croatian Society of Medical Biochemistry and Laboratory Medicine (CSMBLM)*

ARTICLE INFO

Corresponding author:
Vanja Radišić Biljak
Department of Medical Biochemistry
and Laboratory Medicine
Merkur University Hospital
Zajčeva 19
10000-Zagreb
Croatia
Phone: +385917967867
E-mail: vanja.radisic@gmail.com

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KDIGO

ABSTRACT

Chronic kidney disease (CKD) is a major public health issue worldwide and is associated with adverse health outcomes, especially in low- and middle-income countries. In a cash limited healthcare system, guidelines that improve the efficiency of health care free up resources needed for other healthcare services. This short review presents some examples from national acitivities in CKD testing, including countries throughout the globe: Mexico in North America, Uruguay in South America, Italy in Europe, Nigeria in Africa and India in Asia. Considering the fact that treatment of CKD is cost-effective and improves outcomes, this observation argue in favor of including CKD in national guidelines and noncommunicable chronic disease (NCD) programs. This diverse example of national activities fullfil the very first step in achieving this goal.

INTRODUCTION

Chronic kidney disease (CKD) is a major public health issue worldwide and is associated with adverse health outcomes, especially in low- and middle-income countries (1-4). Considering the fact that CKD is associated with high health-care costs, CKD is readily identifiable, treatment of CKD is cost-effective and improves outcomes (5), many countries are developing or refining national strategies for CKD (6). Howevever, despite two decades of widely accepted CKD clinical practice guidelines, such as the Kidney Disease Outcomes Quality Initiative (KDOQI) and recently The Kidney Disease: Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease (7) and continuing medical education for physicians, recent reports from many developed countries indicate that CKD care remains suboptimal (8).

In a cash limited healthcare system, guidelines that improve the efficiency of health care free up recources needed for other healthcare services (9). Perhaps the intial step to create a guideline is to explore the current status of CKD testing in a national enviroment. Therefore, the aim of this article was to present a summary of worldwide national activities in CKD testing in various countries without already developed national CKD guidelines.

NORTH AMERICA

Creatinine standardization Mexican Pilot Study to determine accuracy and trueness

In Mexico, a national end-stage renal disease registry has not been developed. However, data from single state registries (10) and from the US Renal Data System (11) indicate that some Mexican states have an unusually high incidence and prevalence of CKD. Early recognition of CKD in the Mexican population will provide opportunities for slowing and in some cases preventing the natural progression of this disease to end-stage and the need for dialysis.

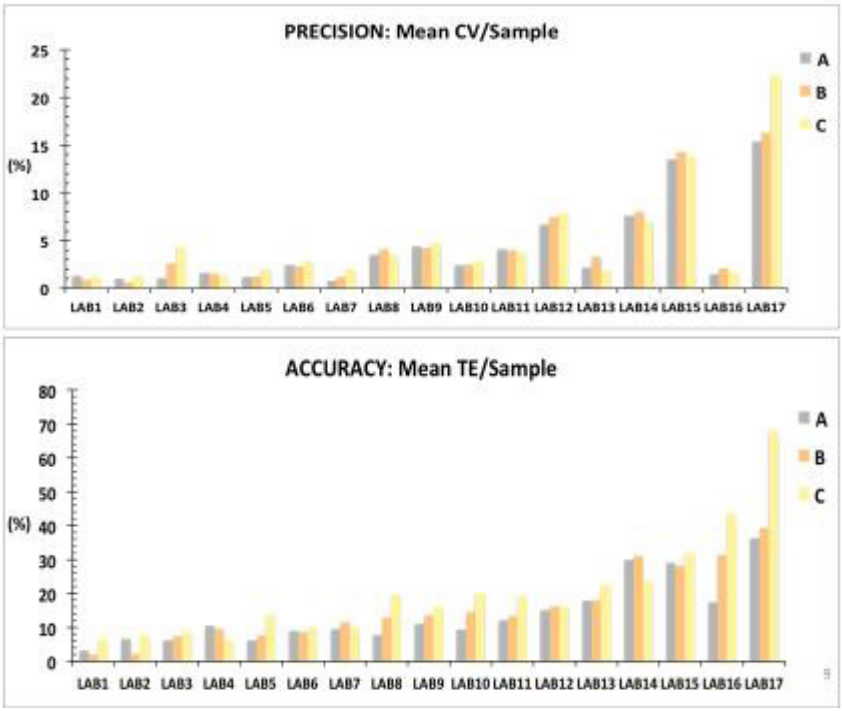
The early recognition of CKD may be achieved through an estimation of glomerular filtration rate (eGFR) – a well-recognized index of renal function. The calculation of eGFR for a given patient is based upon their age, gender and serum Creatinine (Cr) test result. The accurate measurement of creatinine is therefore an essential requirement for the accurate assessment of renal function. Significant differences exist between clinical laboratories (CL) for the measurement of creatinine. Standardizing the measurement and reporting of this analyte by CLs is an essential pre-requisite for the accurate diagnosis and management of CKD. In this short report, we examined the accuracy of creatinine measurements from some CLs in Mexico.

CLs nationwide were invited to participate, and a questionnaire was distributed. The CLs that voluntarily accepted to participate received 3 sets of human serum samples (3 samples/set) with differing concentrations of creatinine provided by CEQAL. The creatinine reference values in these samples had been assigned by the ID/MS reference method for the measurement of creatinine (12).

Each CLs recorded the measurement of Cr in each set of samples (one sample set analyzed on each of three separate days), the methodology, and the manufacturer´s information were also provided. Intra and inter-run coefficient of variation (CV) as well as total error percentage (TE %) were calculated and used for comparison.

A total of 17 CLs, 5 from public and 12 from private sector participated voluntarily. The mean CV% was 4.56 (1 to 18.04 %) and mean TE% was 16.6 (3.9 to 47.9%). When grouped, public CLs had a mean CV% of 3.93 and a mean TE% of 19.0, and private CLs of 4.82% and 15.7%, respectively.

Figure 1 Precision and accuracy of 17 Mexican clinical laboratories measuring creatinine using different methods and instruments

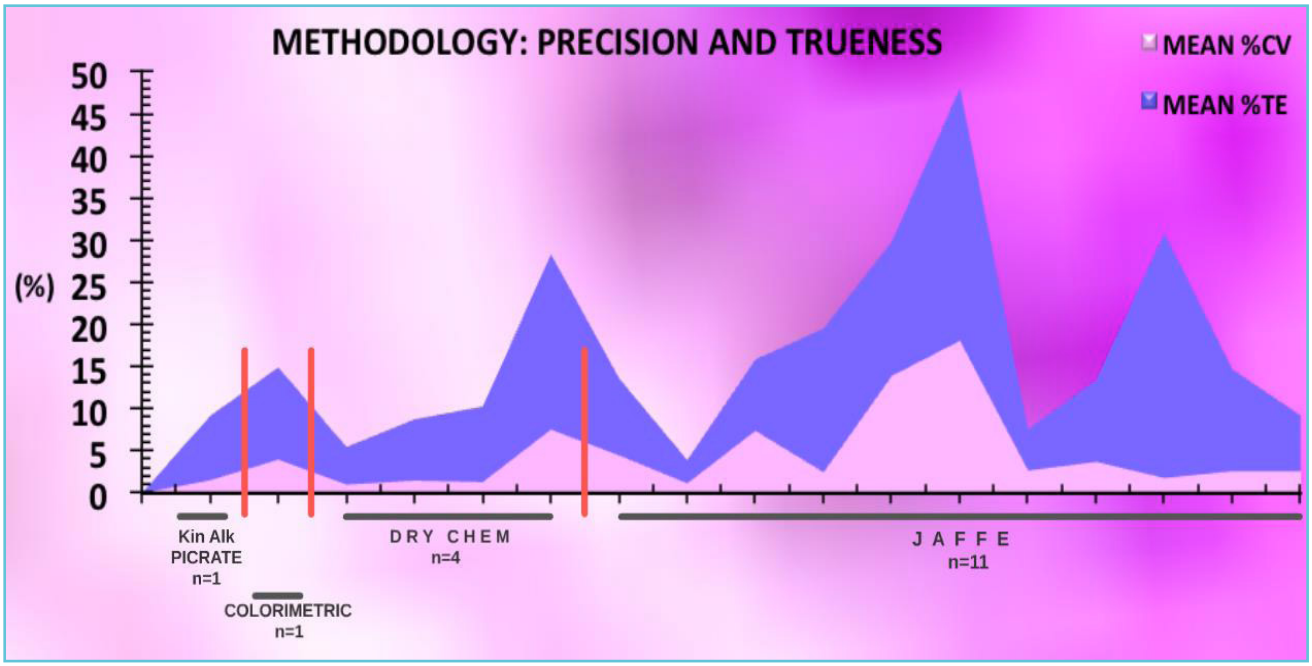


When compared individually to international standards, 4 CLs had a "minimum acceptable performance" (≤11.4 TE %), 3 a "desirable performance" (≤7.6 TE %), and 10 an "undesirable performance" (between 13.41 and 47.93 TE %). None had an "optimum performance" (≤3.8 TE %) (see Figure 1).

Most of the participating laboratories were operating Jaffe methods (see Figure 2). The bimodal nature of the Jaffe results may reflect differences between Jaffe and Jaffe compensated methods. This level of detail was not captured in this study. Some of the observed between method differences may have been due in part to the non-specificity of the Jaffe method. The between method precision data show that better precision can be achieved with a closed system (dry chemistry) as opposed to an open system (Jaffe). These data serve to highlight the methodological differences (in addition to calibration issues) that can exist between laboratories and the significant impact that they can have on reported test results.

The performance data from this accuracy based assessment pilot study serve to highlight the inaccuracy and variability of creatinine test results from a small sampling of Mexican laboratories. A desirable and/or minimum level of performance was achieved by 41% of the laboratories whereas an unacceptable level of performance was recorded for 59% of the participating laboratories. The methods used were alkaline picrate (n=1), colorimetric (n=1), dry chemistry (n=4) and Jaffe (n=11). These data demonstrate that there is an urgent need for a nationwide creatinine standardization program in Mexico that is directed towards improving the accuracy and reporting of creatinine test

Figure 2 Precision and trueness data sorted according to creatinine method used



results throughout the country. Standardized measurements of creatinine in Mexico will identify opportunities for preventing CKD while at the same time making sure that "most at risk" Mexicans are accurately identified, diagnosed and managed appropriately.

Recommendations for improving serum Cr measurement (13) as well as IFCC/WASPaLM TF-CKD survey results guided us to initiate this pilot study.

SOUTH AMERICA

Standardization of creatinine in Uruguay

CKD in Uruguay has a prevalence of 7%.

Interaction between laboratories and clinicians has taken place in a unique way through the External Evaluation Program conducted by the Committee for Standardization and Quality Control (CECC).

The Integrated Health System (SIS) in Uruguay has a National Fund of Resources (FNR), whose mission is to provide financing for highly specialized medical services, allowing them to be available with equity for the entire population.

Our country has a Renal Health Program (PSR), dependent on the FNR and the Ministry of Public Health (MSP), executed by the Honorary Committee on Renal Health (CHSR). The main purpose of PSR is to prevent CKD and improve the quality of life of the patients who carry it, seeking their integration into the SIS.

In view of the enormous economic losses the country was suffering, the CHSR contacted the CECC, looking for our help , with the hypothesis that the diagnosis of CKD could be improved as the quality of the creatinine result improved. Creatinine is the main tool for diagnosis of CKD throughout the glomerular filtration rate calculation. It was through the CECC that they could integrate all the laboratories in the country to standardize creatinine.

This Project was declared of National Interest by the Government, and CECC obtained a grant from the National Innovation Research Agency (ANII).

Given the need to have a Reference Laboratory to obtain target values of creatinine with traceability to reference methods and advice for the design of standardization procedures, the CECC incorporated into the national project the Reference Laboratory of the Argentine Biochemical Foundation (LARESBIC), which was formalized by an agreement that was signed in 2010.

The standardization was done using a reference method calibrated with certified materials by NIST and DGKL, with traceable values to the primary method of Isotopic Dilution and Mass Spectrometry.

The preparation, packaging and distribution in dry ice of the material used (native serum), was in charge of the CECC. An aliquot of vials was sent to LARESBIC Argentina under the same conditions, for the assignment of reference values (creatininase/sarcosine oxidase).

Four surveys were made

Level I: 98 Laboratories. 3 creatinine levels/3 samples/3days Date: March 2008

Level II: 73 Laboratories. 3 creatinine levels/3 samples/3days Date: November 2008

Level III: 101 Laboratories. 5 creatinine levels/2 samples/3days Date: September 2009

Level IV: Laboratories. 5 creatinine levels/2 samples/3days Date: March 2011

Imprecision, Systematic Error, Total Error and Regression Parameters were obtained.

Eleven years after the start of these activities, and with four surveys conducted and continuous monitoring through our EQAs, the evolution of the analyte is considered to have been favorable in statistically significant terms.

The analytical quality parameters have improved since the first distribution, but in the last two the situation has been maintained. This positive impact will deepen in the measure that the laboratories can incorporate the corrections that arise from the standardization, for which it will be necessary to lower their intra-laboratorial CVs through the Internal and External Control and using homogenous systems with traceability to the primary reference method.

EUROPE

STATUS OF LABORATORY TESTS FOR CHRONIC KIDNEY DISEASE IN ITALY

1. Educational activities by the Italian Society of Clinical Biochemistry and Laboratory Medicine (SIBioC)

After the issuing of the 2012 Kidney Disease Improving Global Outcomes Initiative (KDIGO) guideline (7), which included specific recommendations for the clinical laboratory, SIBioC has worked to diffuse the guideline content among the professional community in Italy, with the aim to help laboratories to implement the recommendations in their daily practice.

This was obtained through the publication of a number of papers in *Biochimica Clinica*, the SIBioC official journal, and the efforts of SIBioC Working Groups dealing with renal disease, that organized training courses using both traditional meeting and e-learning approaches.

2. Cooperation between SIBioC and clinical organisations

In 2009, a joint SIBioC-Italian Society on Nephrology recommendation was released (14) and, more recently, the Italian Minister of Health has issued a national guideline on identification and prevention of CKD in adults, with the proactive contribution of laboratory experts (15).

3. Laboratory tests

a. Serum creatinine

Two national evaluations have been recently published on the status of creatinine measurement

in Italy, both using commutable control materials with target values assigned by a higher-order reference procedure (16,17). Data seem to indicate that the standardization efforts are still having effects lower than expected. In 2014, only 41% of laboratories showed optimal performance [i.e., a total error (TE)<4.5%], while 16.6% were unable to reach even the minimum quality goal (i.e., TE<13.3%). It should be noted that enzymatic methods, although strongly promoted by SIBioC (18,19), are used in a minority of laboratories (~25%).

Table 1 **Main results of the 2015 national survey on the urine albumin measurement, compared with the results obtained in a similar survey performed in 2007 (Italy)**

	2007	2015
Type of sample		
24-h collection	43%	16%
First morning void	9%	59%
Second morning void	-	6%
Random	30%	19%
Partecipation to an EQAS		
Yes	28%	44%
No	72%	56%
Measurement unit		
mg/mmol creatinine or mg/g creatinine	15%	52%
µg/min	9%	5%
mg/24 h	33%	9%
mg/L	29%	26%

b. eGFR

Data on the use of equations to derive the GFR are still sparse and heterogeneous (17).

In 2013, employed equations were MDRD (69%), CKD-EPI (15%), Cockcroft-Gault (7%) and Schwartz (1%).

More importantly, ~25% of laboratories did not offer any eGFR option.

c. Urine albumin

In 2007 and 2015, SIBioC did two surveys to check the use of this parameter. As reported in the Table 1, results were encouraging in showing the improvement of adherence to the KDIGO recommendations.

EQAS show some variability among different commercial measuring systems, even if the whole performance is not so bad.

In 2014, 3.6% of laboratories were unable to fulfil the minimum quality level, while 88.6% showed good performance (defined as a TE<11%) (17).

4. Final remarks

SIBioC is dedicating many efforts to the standardisation of laboratory procedures for CKD diagnostics, through the action of its Working Groups and the publication of recommendations.

The situation is, however, far from optimal with large room for improvement, as indicated by EQAS and surveys results. In particular, some goals, indicated by Graziani et al. (17) with the corresponding indicators, have been identified for being pursued in a relatively short time.

It is also worthy to note that many of these objectives (availability of eGFR and use of CKD-EPI equation, report of albuminuria in the recommended terminology and unit, use of the recommended sample for urine albumin) can be achieved at no additional costs under the direct control of the clinical laboratory.

AFRICA

NATIONAL ACTIVITIES IN CKD TESTING: THE NIGERIAN CURRENT SITUATION

Prevalence

Various studies with different results possibly because mild-moderate cases excluded; meta-analysis for Saharan Africa gives prevalence as 13.9% (20). Prevalence of CKD in a Nigerian family practice population: 250 consecutive, newly registered patients during 2005-2006; 45% had increased urine albumin on first testing, persistent in 12.4%; 20% had low e-GFR on first testing, persistent in 10% (21).

Causes of CKD in Nigeria

Hypertension 30%; chronic glomerulonephritis 28%; diabetes 3%; obstructive uropathy 5%; others 3.9%; unknown 30% (Arogundade et al 2005); 37% CKD in a population of diabetic subjects (22); 38% CKD in HIV positive population in outpatient clinic (23); 24% CKD (e-GFR MDRD) in a HIV/AIDS population in tertiary referral unit (24,25).

The enormity of CKD in Nigeria

The Situation in a Teaching Hospital in South-East Nigeria showed CKD accounts for 8-10% of hospital admissions; death from end-stage renal disease constituted 22% of medical deaths (26).

Current/existing practices

Since information on activities in CKD testing at national level on how nephrologists/laboratories investigate patients can mainly be provided by national surveys, most of which is questionnaire-based with voluntary participation.

A questionnaire was sent to the membership of Association of Clinical Chemists of Nigeria (ACCN) in different hospitals across Nigeria (representing a complex mixture of private and public clinical laboratories) to seek information

on the current practices. The information obtained was then collated, analysed, and summarized for this submission.

Not surprisingly, there is evidence of differences in practice across laboratories in different parts of Nigeria. These differences are seen in all aspects of CKD testing including methods being used, commercial assay kits, commercial standards, commercial controls (SRMs or CRMs), and even some slight variations exist in creatinine reference interval values across laboratories in different states.

Chronic Kidney Disease testing in Nigeria (Summarized in Table 2)

1. ***Serum creatinine:*** in routine use, mostly commercial enzymatic and kinetic assay kits
2. ***eGFR (Cockcroft-Gault):*** still being used by some (though very few), based on some validation studies done in some population groups
3. ***eGFR (creatinine-based, MDRD):*** widely in routine use
4. ***eGFR (creatinine-based CKD-EPI):*** widely in routine use
5. ***Albumin/creatinine ratio:*** in routine use
6. ***Urine protein*****:** in routine use
7. ***Urinalysis* (*dipstick):*** mainly in routine screening/medical tests
8. ***Conventional 24-hour urine profile*****:** still being used in some institutions
9. ***eGFR (Cystatin C based CKD-EPI):*** currently for research use only, not yet routine
10. ***eGFR (Creatinine with cystatin C-based CKD-EPI):*** currently for research use only, not yet routine
11. ***Kidney injury molecule 1 (KIM 1):*** currently for research use only, not yet routine

Creatinine assay

Most institutions use commercial enzymatic (kinetic) assay kits.

Use of automation in creatinine assay

This is also widely distributed across the country, though less widely than the Roche Reflotron Point-of-Care machine. Automation systems in current use in different parts of the country include: ARCHITECT C4000, ABBOTT LABORATORIES, USA, Miura 200, ISE, Italy and TC-Matrix, Teco Diagnostics, USA etc.

Use of Point-of-Care Testing (POCT) in creatinine assay

Roche Reflotron machine, a point of care clinical chemistry equipment that measures creatinine with the result being made available within 5 minutes is widely distributed and used across the country (available in 196 health institutions across the country). Also, point of care testing equipment for measuring albumin to creatinine ratio is being used in some centers.

External quality assurance of creatinine (and other analytes) assays

A few public/governmental institutions partake in EQA (e.g UKNEQAS, with satisfactory results); several of the modern private institutions/laboratories are partakers with satisfactory results.

Routine reporting of eGFR/ standardisation

In Nigeria, some clinical chemists and clinicians have worked on use of eGFR to determine/evaluate renal function, both in the normal or apparently healthy population and in various diseases - “the studies cited under the introduction above”. A cut-off value of ≥60ml/min/1.73m^2 for the apparently healthy (non-CKD subjects) population using the 4-variable MDRD equation has been established in some regions/

Table 2 Summary of CKD testing in Nigeria

CKD testing approach	Current assay methods/practice	Currently in routine use	Currently for research only	Remarks
Serum creatinine	Automation, Enzymatic, Kinetic Spectrophotometry, POCT (Roche Reflotron) **Jaffe End point still done in some health institutions*	YES	NO	IDMS Traceable; Commercial enzymatic assay kits; Commercial standards and controls (SRMs and CRMs), e.g. Randox; EQA results satisfactory in institutions that engage
eGFR (creatinine-based, Cockcroft-Gault)	Automation, Enzymatic, Kinetic Spectrophotometry, POCT (Roche Reflotron)	YES	NO	Still being used by some (very few) based on validation studies done in some population groups
eGFR (creatinine-based, MDRD)	Automation, Enzymatic, Kinetic Spectrophotometry, POCT (Roche Reflotron)	YES	NO	Mostly 4-variable MDRD; IDMS Traceable
eGFR (creatinine-based CKD-EPI)	Automation, Enzymatic, Kinetic Spectrophotometry, POCT (Roche Reflotron)	YES	NO	Mostly IDMS Traceable
eGFR (Cystatin C based CKD-EPI	ELISA assays	NO	YES	Mostly commercial ELISA kits with controls and calibrators
eGFR (Creatinine with cystatin C-based CKD-EPI)	Creatinine (Automation, Enzymatic, Kinetic spectrophotometry); Cystatin C: ELISA	NO	YES	Mostly commercial ELISA kits with controls and calibrators
Kidney injury molecule 1 (KIM 1)	ELISA	NO	YES	Mostly commercial ELISA kits with controls and calibrators

Urine Albumin/ creatinine ratio	Albumin: Automation, ELISA, POCT equipment	YES	NO	Mostly commercial ELISA kits with controls and calibrators
24 hour Urine protein	Spectrophotometric	YES	NO	In cases that require 24 hour quantification (e. g Nephrotic syndrome)
Urinalysis (dipstick with/without microalbuminuria template)	Strips	YES	NO	Mostly at Point-of-Care and in routine screening/medical tests

zones of the country. Available data from different studies done in Nigerian population groups is consistent with the cut-off value of <60mL/min/1.73m^2 for CKD, using the 4-varaible MDRD equation. Sanusi et al. in Ile-Ife, South- western Nigeria (27, 28), Adebisi in Ilorin, North-central Nigeria (29), and Agaba et al. in Jos, North-eastern Nigeria (30) have proved that the MDRD equation is reliable alternative to measurement of endogenous creatinine clearance (Crcl) in the estimation of GFR in Nigerians. Currently, only a few clinical chemistry laboratories report eGFR. However, in many/most institutions, the nephrologists commonly use the creatinine result from the laboratories for eGFR (mostly MDRD and CKD-EPIcr equations).

CKD task force in Nigeria

ACCN will constitute a task force.

Collaboration with nephrologists

The Nigerian Association of Nephrology (NAN) developed ***Guidelines for the Detection and Management of Chronic Kidney Disease (CKD), 2nd May, 2011,*** though without involving the clinical chemists.

However, there is a level of collaboration being practised by nephrologists and clinical chemists in some centres/institutions in reporting eGFR, especially in the aspect of creatinine assay method, standardisation and traceability to IDMS, even though there is no Laboratory/ Nephrology Working Group put in place yet.

Conclusion and way forward

Need for ACCN to have a CKD Task Force who will work towards standardization of assays of creatinine and other markers of CKD across the country, and also collaborate with the nephrologists (Nigerian Association of Nephrology) in the following areas: agreement to recommend the routine reporting of e-GFR; agreement on clinical practice guidelines; joint initiative to promote the benefits of testing for CKD; preparation of educational support materials for laboratory personnel, family doctors and patients, renal physicians (31).

ASIA

SUMMARY OF NATIONAL ACTIVITIES IN CKD TESTING IN INDIA

In the large ethnicity of India, uniformity in testing is not available for CKD, and many a time not affordable. There is no one statutory control for the laboratory diagnosis in correlation with clinical diagnosis for various reasons at present.

The testing can be categorized into the following types of laboratories generally available in line with scope of services, Basic composite/ Medium/Advanced laboratories.

Awareness and protocol of testing is available, but actual performance is seen in some teaching, corporate and centres of excellence hospitals which are not in the reach of the population specially in outreach places and lower socio economic strata. Hence most of the understanding and publications are from these laboratories which may not reflect the accurate incidence. International guidelines are followed in the best possible way available. Most of the publications are from the advanced laboratories.

The IDMS traceability creatinine is used in majority of the Medium and Advanced Laboratories (32). Though MDRD equation is used quite commonly, the appropriateness of the same in line with creatinine traceability is many a time questionable. There is variation in use of the formula of MDRD among the laboratories

In the higher centres, creatinine is reported with eGFR and vice versa. Protein creatinine ratio is done in all 3 categories of laboratories and albumin creatinine ratio in the medium and advanced laboratories. Standardization of the eGFR in institutes have been done initially by comparison with isotope renogram and found to be superior to CG formula (33). The Schwartz formula for pediatric population is well accepted clinically, CKD-EPI children has not yet taken off (34).

Serum Cystatin usage is minimal as a regular tool of evaluation mainly due to cost and eGFR using the same, is sporadic in publication.

Both medium and large laboratories have good results of proficiency testing. Laboratories are under a national accreditation programme (though not yet mandatory). With different ethnicities to arrive at national reference interval, would take time. Though study evaluations are done in different areas of the country, these may be considered cross-sectional due to the diversity of individuals living in different areas.

The distinct features to be addressed in the country under statutory guidance

1. ***Uniformity of use in creatinine assay***
2. ***Biological reference interval*** of the serum creatinine, protein creatinine ratio and albumin creatinine ratio, sex and gender wise
3. ***Selection of MDRD and CKD EPI equation uniformity*** (35)
4. ***Awareness of general medical practitioners in use of the equation*** only with standardized IDMS creatinine assay
5. ***To always correlate with regional higher centres on knowhow of*** assays, reference intervals with national and international guidelines
6. ***A regional state wise registry*** to know the CKD burden in the country (36)
7. ***To work with instrument and reagent vendors*** to help in this achievement on a Government statutory.

CONCLUSION

This short review presents some examples from worldwide national acitivities in CKD testing. Considering the fact that already mentioned treatment of CKD is cost-effective and improves outcomes, this observation argue in favor of including CKD in national guidelines and noncommunicable chronic disease (NCD) programs (5). The described activities fullfil the very first step in achieving this goal.

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Mohammed[7], Chinelo Onyenekwu[8], Ify D. Nnakenyi[9], Alaje A.[10], Ayinbuomwan E[11]

[1] *Hospital de Especialidades, CMNO, IMSS, México*
[2] *Hospital Civil de Gudalajara, SSA, México*
[3] *Alianza Mexicana para Prevenir las Enfermedades Crónicas, A.C. (AMPEC), México*
[4] *Department of Chemical Pathology, Benue State University, Makurdi, Nigeria*
[5] *Department of Chemical Pathology, Lagos University Teaching Hospital, Lagos, Nigeria*
[6] *Department of Chemical Pathology, Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria*
[7] *Department of Chemical pathology, Aminu Kano Teaching Tospital, Kano State, Nigeria*
[8] *Department of Chemical Pathology, Babcock University Teaching Hospital, Lishan-Remo, Ogun State, Nigeria*
[9] *Department of Chemical Pathology University of Nigeria Teaching Hospital, Enugun, Enugun State Nigeria*
[10] *Department of Chemical Pathology, Obafemi Awolowo University/Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun State, Nigeria*
[11] *Department of Chemical Pathology University of Benin Teaching Hospital, Benin, Edo State Nigeria.*

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Evaluation of the correlation coefficient of polyethylene glycol treated and direct prolactin results and comparability with different assay system results

Shyamali Pal

R B Diagnostic Private Limited

INFO

Corresponding author:
Shyamali Pal
Technical Director & Consultant Biochemist
R B Diagnostic Private Limited
P- 857, Block-A, Lake Town
Kolkata-700089
India
Phone: +91-9874415344
Email: shy23_pal@yahoo.co.in

Key words:
macro prolactin, bioactive prolactin, correlation coefficient, regression coefficient

ABSTRACT

The presence of Macro prolactin is a significant cause of elevated prolactin resulting in misdiagnosis in all automated systems. Poly ethylene glycol (PEG) pretreatment is the preventive process but such process includes the probability of loss of a fraction of bioactive prolactin.

Surprisingly, PEG treated EQAS & IQAS samples in Cobas e 411 are found out to be correlating with direct results of at least 3 immunoassay systems and treated and untreated Cobas e 411 results are comparable by a correlation coefficient. Comparison of EQAS, IQAS and patient samples were done to find out the trueness of such correlation factor. Study with patient's results have established the correlation coefficient is valid for very small concentration of prolactin also.

Materials and methods

EQAS, IQAS and 150 patient samples were treated with PEG and prolactin results of treated and untreated samples obtained from Roche Cobas e 411. 25 patient's results (treated) were compared with direct results in Advia Centaur, Architect I & Access2 systems.

Statistical calculations

Correlation coefficient was obtained from trend line of the treated and untreated results. Two tailed p-value obtained from regression coefficient(r) and sample size.

Results and discussion

The correlation coefficient is in the range (0.761-0.771). Reverse correlation range is (1.289-1.301). r value of two sets of calculated results were 0.995. Two tailed p- value is zero approving dismissal of null hypothesis.

Conclusion

- The z-score of EQAS does not always assure authenticity of results
- PEG precipitation is correlated by the factor 0.761 even in very small concentrations

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Abbreviations

***GFC**: gel filtration chromatography*
***PEG:** polyethylene glycol*
***EQAS:** external quality assurance system*
***M-PRL:** macro prolactin*
***PRL:** prolactin*
***ECLIA:** electro-chemiluminescence immunoassay*
***CLIA:** clinical laboratory improvement amendments*
***IQAS:** internal quality assurance system*
***r:** regression coefficient*

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INTRODUCTION

The presence of Macro prolactin (M-PRL) is a known cause of misdiagnosis, unnecessary investigation and inappropriate treatment. M-PRL in human blood consists of monomeric bioactive prolactin (PRL) of molecular mass 23kDa and a non reactive immunoglobulin G molecule with a molecular mass of approximately 150-170kDa causing a prolonged clearance rate. Though M-PRL is non reactive but it interferes with prolactin immunoassay and causes false elevation of prolactin (1, 2, 3).

The probable reasons for elevation may be:

- The assay antibodies are probably having affinity to different epitopes on PRL with which they react. The elevation of result is dependent on the availability of such epitopes on the M-PRL complex (4)
- The coupling of same pair of antibodies to different solid phase and signal generating system (5, 6)
- Incubation time has also been shown to be directly related to the reactivity with M-PRL (6). It was observed that Roche Elecsys System showed maximum elevated results (5, 7)

A study was done to examine the frequency of Macroprolactinemia in clinical practice and the ability of immunoassay systems to distinguish between M-PRL and PRL using 300 hyperprolactinemic serum samples. Overall, 71 results dropped to within the normal range following treatment of serum samples with PEG, indicating that 24% of hyperprolactinemia are approximately misdiagnosed due to interference by M-PRL. Ten out of these samples where elevation of results was due to interference of M-PRL were tested at 18 clinical laboratories. Two sets of PRL measurements of these serum samples were obtained from each of the nine most commonly used immunoassay systems. Across the nine assay systems, differences in the PRL estimates ranged from 2.3- to 7.8-fold. Elecsys users reported the highest PRL levels. Somewhat lower values were reported for DELFIA systems followed by Immuno-1, AxSYM, and Architect assay system.

The Immulite 2000 assay generated PRL levels equivalent to approximately 50% of those reported by the high-reading methods. The lowest PRL levels were reported by Access, ACS: 180, and Centaur systems (4, 8).

Two system of separation of M-PRL from bioactive PRL became popular, PEG precipitation, and Gel Filtration Chromatography (GFC)(9,10). A HPLC method has been developed using Agilent Zorbax GF-250 Column, tris buffer with saline at pH 7.2 which was found to have equal efficiency of GFC but still not very popular(11). GFC is time consuming and expensive, so not suitable for regular clinical laboratory performances. Therefore, PEG precipitation became common method of precipitation of M-PRL. Karolina et al (12) assessed elevation effect of M-PRL in 27 patients among which 19 with functional hyperprolactinemia and 8 with prolactinoma between PEG precipitation and ultracentrifugation. A high diagnostic agreement (95.9%) and positive correlation coefficient ($r=0.506$, $p<0.001$) was found out between two precipitation method. Both precipitation methods showed equal efficacy in functional hyperprolactinemia, and PEG precipitation was better method in prolactinoma.

Kit inserts of different systems (13, 14, 15, 16) mentioned that the PRL results may get affected due to the presence of M-PRL and PEG precipitation has been suggested where elevated result is obtained. It was also stated that a fraction (approximately 14%) of active prolactin may get co precipitated during PEG pre- treatment. The dilution effect also to be taken into consideration. No instruction on cutoff value above which PEG pretreatment to be done was mentioned in the inserts. Hence, a correlation study was felt to be necessary to get a guideline regarding such cutoff value.

In the current study, the author treated all samples twice, direct assay and after PEG precipitation. A reverse correlation of both results was done and regression coefficient (r) was calculated. The reverse correlation was necessary to substantiate the authenticity of correlation coefficient. The reason of finding out such correlation was to find out:

- Should PEG precipitation be done for all samples irrespective of normal/or elevated results? (i.e., whether there should be a lower cutoff?)
- Does the fraction of PRL being co precipitated with M-PRL affect patients' clinical status?
- Though M-PRL is still being mentioned as interfering molecule in all inserts but PEG pretreated results of Cobas e 411 are in agreement with untreated results of Access-2 system. Hence, the underlying problem of elevated prolactin results is still a matter of concern in Cobas e 411.
- Whether the correlation coefficients are within an acceptable uncertainty both in direct and reverse direction?

MATERIALS AND METHODS

Materials

A total of 150 patient samples were collected at random with direct prolactin results from 0.25-300 ng/ml. As the basic aim of the study was to check the dilution effect during PEG treatment and transferability of the expected values so clinical case history has not been considered to make the study a blind trial. Prolactin has been measured twice, direct measurement & after PEG precipitation in EQAS samples (BIORAD, Cycle 13), BIORAD immunoassay control levels 1, 2 & 3, lot 40330 and above mentioned patient samples.

Total 25 number samples were selected and tested in 4 different systems Roche Cobas e 411, Abbott Architect I, Advia Centaur and Access 2.

The laboratory performed tests in Cobas e 411 & Access 2 and outsourced in accredited laboratories having Advia Centaur & Abbott Architect I systems. It was informed not to treat the samples with PEG. In Access 2 and Cobas e 411 these 25 samples were measured twice ie, direct estimation and after PEG precipitation.

Methods

Cobas e 411

Electro Chemiluminescence Immunoassay (ECLIA) (13, 17).

Access 2

Chemiluminescence Immunoassay (CLIA) (14). The Access Prolactin assay is a simultaneous one-step immunoenzymatic ("sandwich") assay using Lumiphos* 530 as Chemiluminescent substrate (14).

Abbott, Architect i

Prolactin assay is a two-step immunoassay using Chemiluminescent Micro particle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex (15).

Advia Centaur

Chemiluminescence Immuno assay (CLIA) (16).

Precipitation using PEG

25% solution of poly ethylene glycol 6000(PEG) is prepared using deionized water. It is stable for 7 days. Sample and PEG solution was mixed in equal volume. Mixed for 10 seconds and centrifuged for 5-30 minutes between 1500-10000g (13).

Table 1 Comparison of EQAS values, BIORAD, Cycle-13

Sample no.	N#	Lab results* (ng/mL)	z-score	Peer mean of compared systems(ng/mL)				PEG treated results (Cobas-e 411) (ng/mL)	PEG X 1.289 (ng/mL)	Direct result X0.771 (ng/mL)
				Cobas e 411	Advia Centaur**	Access 2**	Architect**			
1	165	13.3	-0.67	13.9	9.01	9.9	10.6	10.6	10.57	10.25
2	217	19	-0.71	19.9	12.3	13.3	14.7	15.1	15.14	14.65
3	232	33.75	-0.56	35	22.2	23.3	25.7	26.6	26.63	26.02
4	236	47.16	-1.48	52.7	33.2	35.4	39.8	40.2	40.1	36.36
5	235	19.9	-0.25	20.2	11.9	13.4	14.3	15.4	15.37	15.34
6	243	54.63	0.40	53.3	33.6	35	39.3	40.34	40.56	42.12
7	238	14.96	0.75	14.2	8.84	9.91	10.5	11	10.8	11.53

8	232	19.84	-0.41	20.4	11.8	13.2	14.7	16.2	15.52	15.3
9	241	36.24	0.16	35.9	22.1	23.2	26.5	26.9	27.32	28
10	232	54.11	-0.03	54.2	34	34.6	40.6	41.85	41.24	41.71
11	246	14.56	0.01	14.6	8.91	9.67	10.5	11.55	11.11	11.45
12	225	37.03	0.43	36.1	22.1	23	26.6	28.2	27.47	28.55
r***	-	-	-	-	0.998	0.997	0.999	-	-	0.9995

p-value: <0.0001

**The lab has enrolled for Roche Cobas e 411 only.*

***Peer mean obtained from BIORAD, monthly EQAS assessment sheet.*
 # Participant laboratories of Roche Cobas e 411.

*r***- When compared with PEG pretreated results*

Figure 1 Correlation of EQAS results of Cycle 13

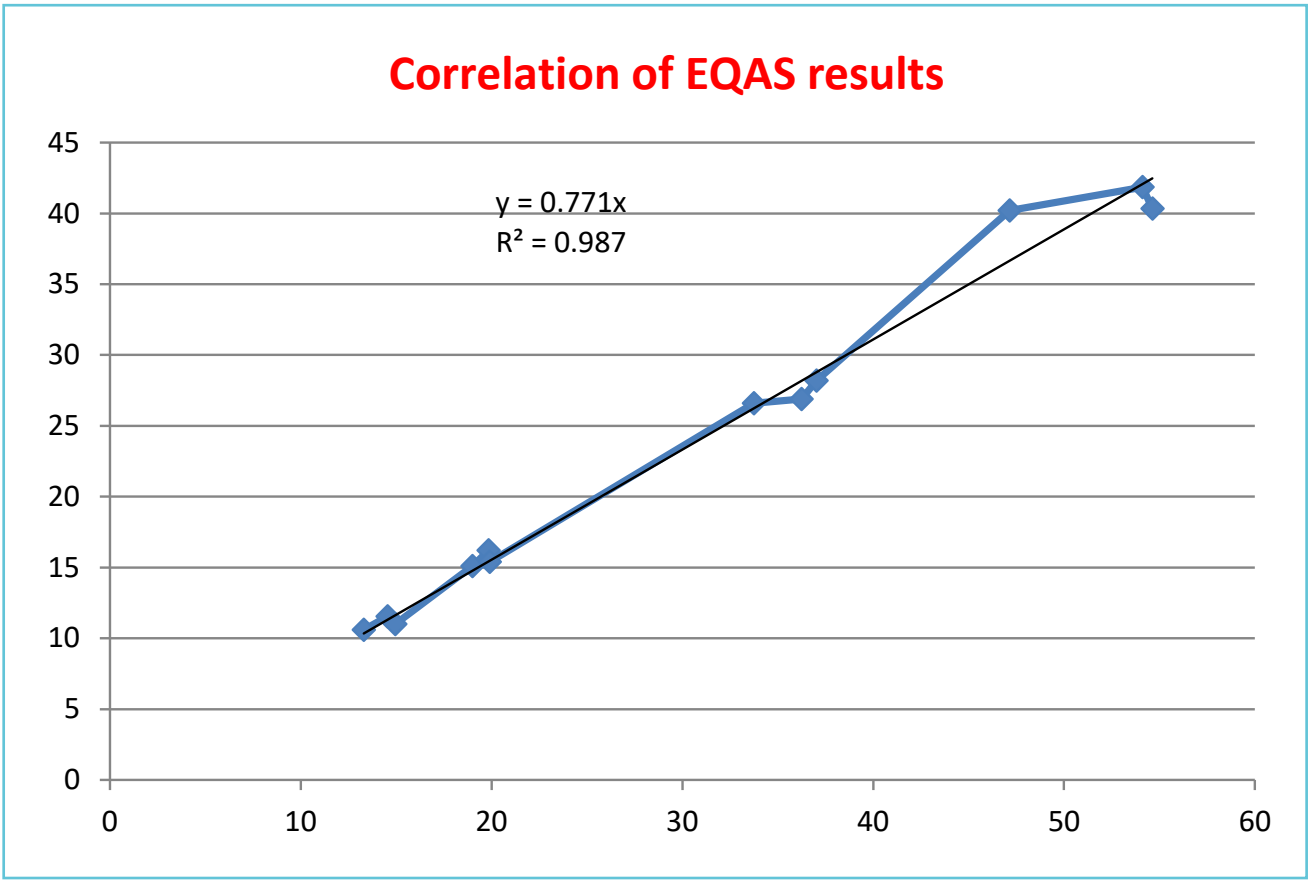
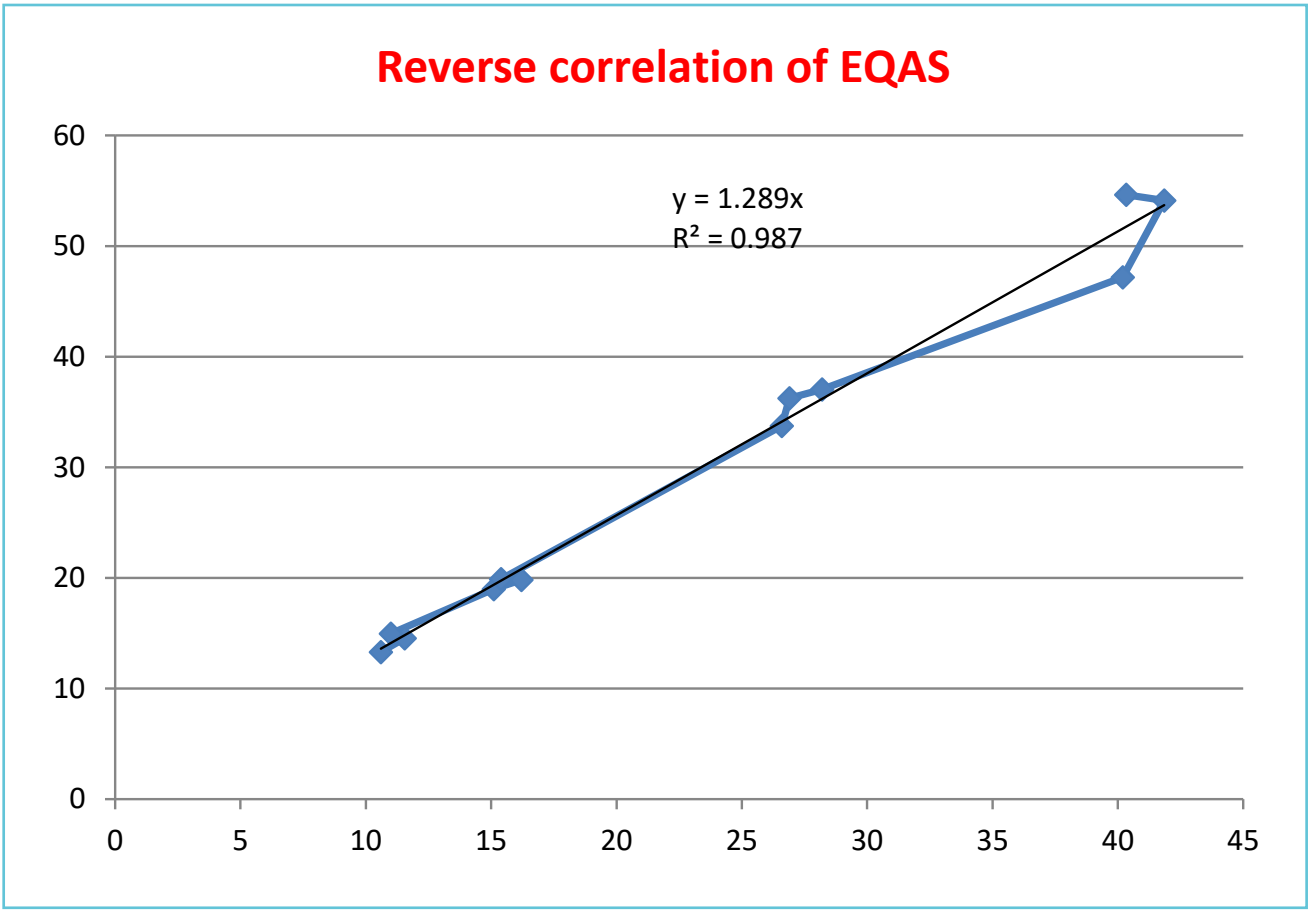


Figure 2 Reverse correlation of EQAS results Cycle 13



Calculations

PEG treated results were multiplied by 2(1+1ratio). EQAS results of direct and PEG treated values were compared twice, putting PEG results on Y-axis and direct result on X-axis and reversing the axis.

Correlation factors obtained from trend lines (Table 1, Figures 1 & 2). The factors were 0.761 & 1.306.

In a similar manner correlation factors obtained for Trilevel Immunoassay controls. Correlation factors were 0.762 & 1.307 (Table 2, Figures 3 & 4).

Such correlation was checked from 150 patient's data ranging from (0.298-355) ng/ml. Factors were 0.761 & 1.306 (Table 4, Figures 5 & 6)

Results and discussion

The z-scores of EQAS results (Immunoassay, BIORAD, Cycle 13) showed no outlying score in the complete cycle but remarkable discrepancy was observed with the peer mean of other immunoassay systems.

The laboratory observed recurrent complaints of elevated prolactin results from the patients though EQAS and IQAS results were appropriate to the peer mean values.

The patients obtained results mainly from Abbott Architect, Advia Centaur systems and laboratory started comparing results with Access 2 system.

The difference in results from Cobas e 411 and comparability of results of other systems were

Table 2 Correlation of IQC value (Lot 40330, BIORAD, immunoassay trilevel)

Control	Without PEG (ng/mL)	After pretreatment with PEG (ng/mL)	Access-2 (ng/mL)	Advia Centaur (ng/mL)	Architect-i (ng/mL)
L1	9.05	6.86	6.54	5.75	7.37
L2	27.3	21.1	17.9	15.6	21.5
L3	55.8	42.4	39.2	37.7	49.4

p-value: <0.01

Figure 3 Correlation of BIORAD trilevel immunoassay control (Lot 40330)

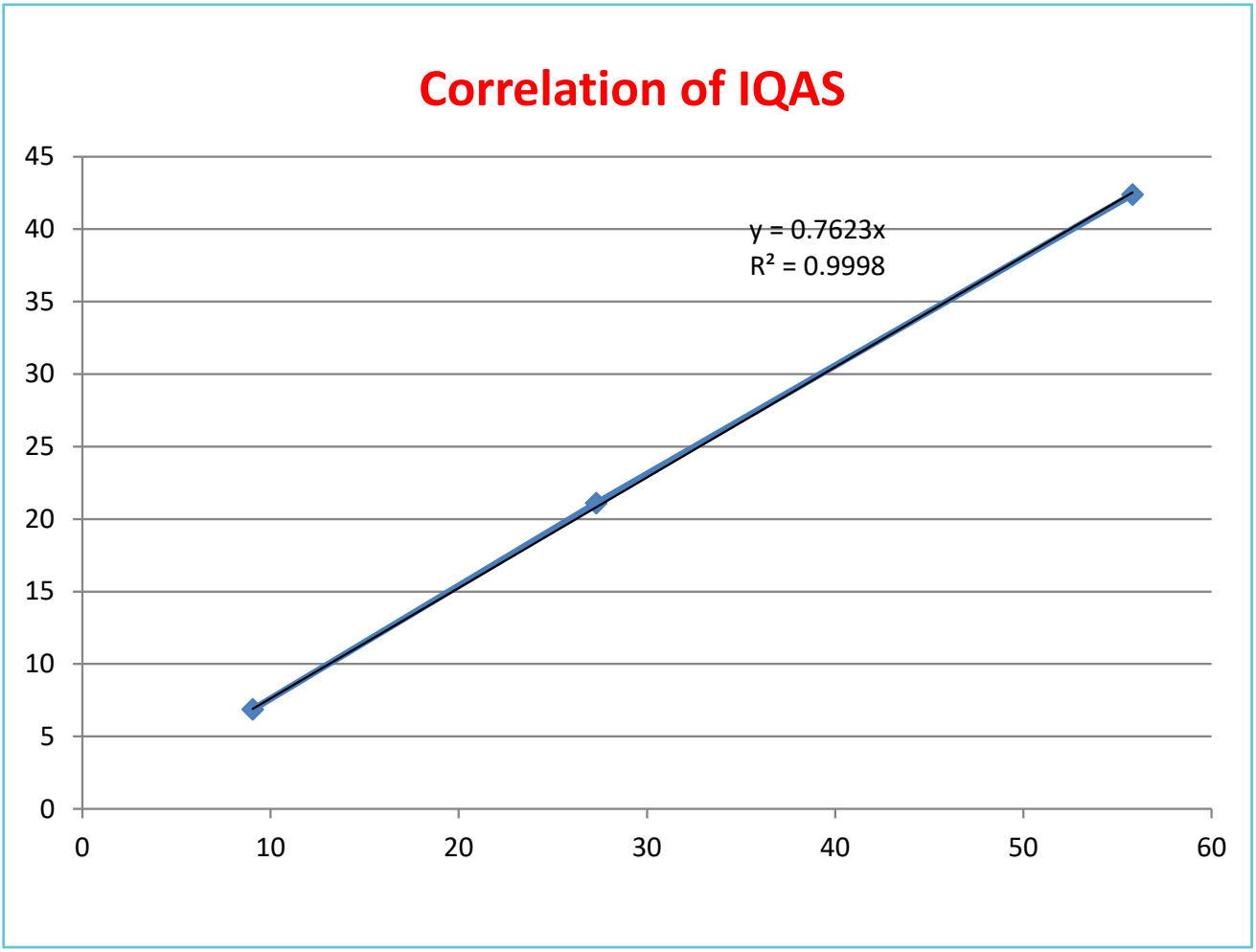


Figure 4 Reverse correlation of BIORAD trilevel immunoassay control (Lot 40330)

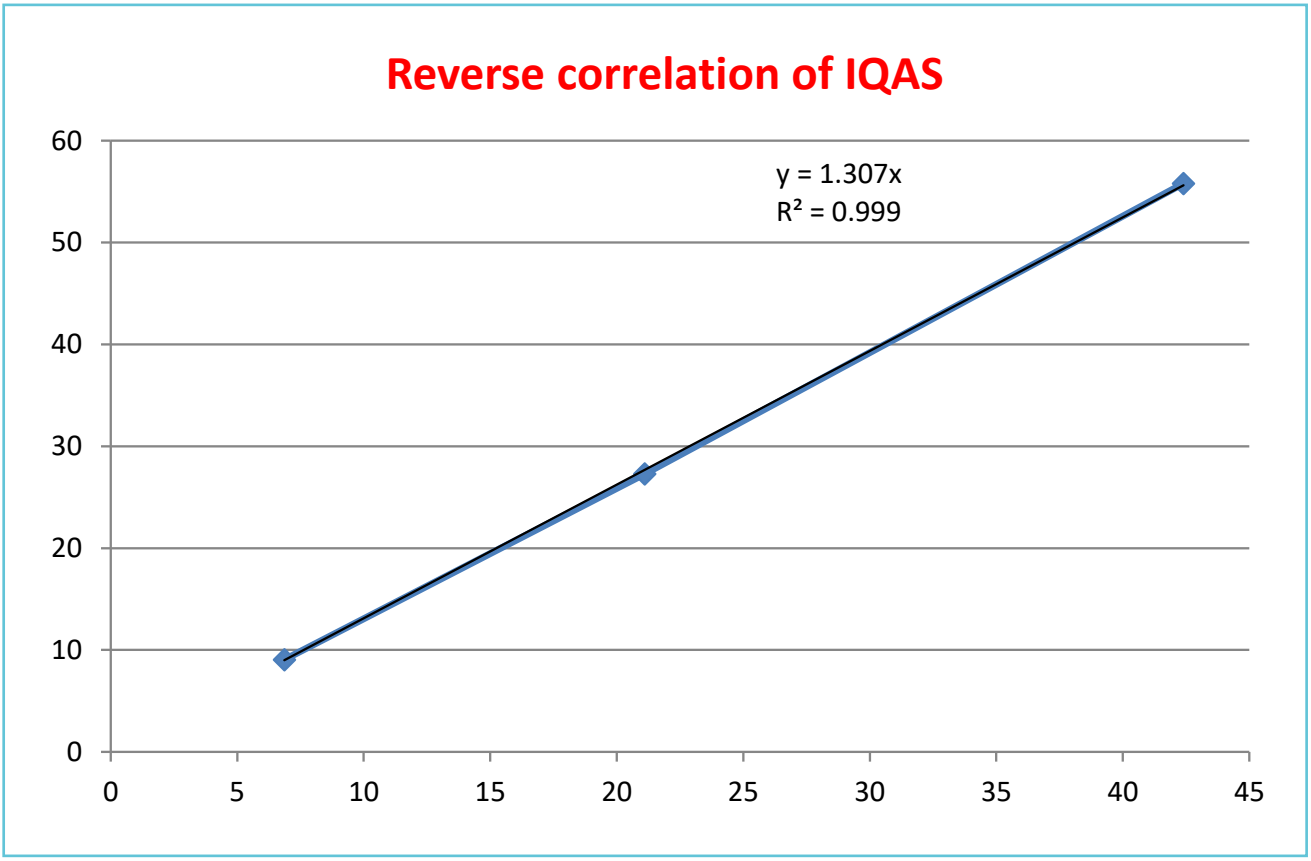


Table 3 Comparison of PEG treated PRL values of Roche Cobas e 411 with direct results of other Immunoassay systems
Sample: Patient sample chosen at random of concentration range 6ng/mL - 365ng/mL

Sl.no.	Roche Cobas e 411 (ng/mL)		Abbott Architect (direct) (ng/mL)	Access 2 Beckman (ng/mL)		Advia Centaur (direct) (ng/mL)
	Direct result	PEG treated result		Direct result	PEG treated result	
1	8.5	6.3	6.8	7.1	6.9	6.0
2	10.2	7.6	7.9	7.5	7.1	8.5
3	9.8	8	7.2	7.3	7.7	7.8
4	10.1	8	7.9	8.3	8.8	9.1

5	18.8	14.8	15.6	14.3	13.8	15.8
6	20.1	16.6	14.8	15.9	14.8	17.1
7	28	20.4	21.1	19.2	20.5	18.5
8	29	21.1	22.5	18.5	19	22.5
9	30.5	24	26.8	23.8	24	23.6
10	41	30	31.1	28.8	27.5	32.7
11	38.8	32	29.4	31.5	30.8	29
12	52	39.4	41.6	40.8	41.5	42.5
13	58.9	46.2	43.5	48.3	49	45.1
14	66.2	50.5	55	49.1	50.2	47
15	66.8	50.6	53.8	48.5	47.9	53
16	73	56	55	55.5	54.7	51
17	75	57	60.1	61.2	58.9	62.8
18	87	65	66	69	71.2	59
19	108	84.8	80	75	73.2	78
20	110	86	78	92	90	90
21	168	124	118	132	124	125
22	139	103	112	119	115	114
23	302	235	228	215	209	230
24	103	78	82	75	70	81

25	471	355	341	362	355	358
r*	-	-	0.9988	0.9967	0.997	0.9985

p-value: <0.001

r -- Each series of instrument results compared with Cobas e 411 after PEG treatment results*

Table 4 Correlation of prolactin direct & PEG treated results

Total no. of patients	Range of analysis (ng/mL)	Correlation factor of PEG to direct results	Correlation factor of direct to PEG results	Regr. of direct result & PEGX1.306	Regr. of Di rectx0.761 & PEG results
150	0.298-355	0.761	1.306	0.995	0.995

Figure 5 Comparison of data with PEG on Y-axis

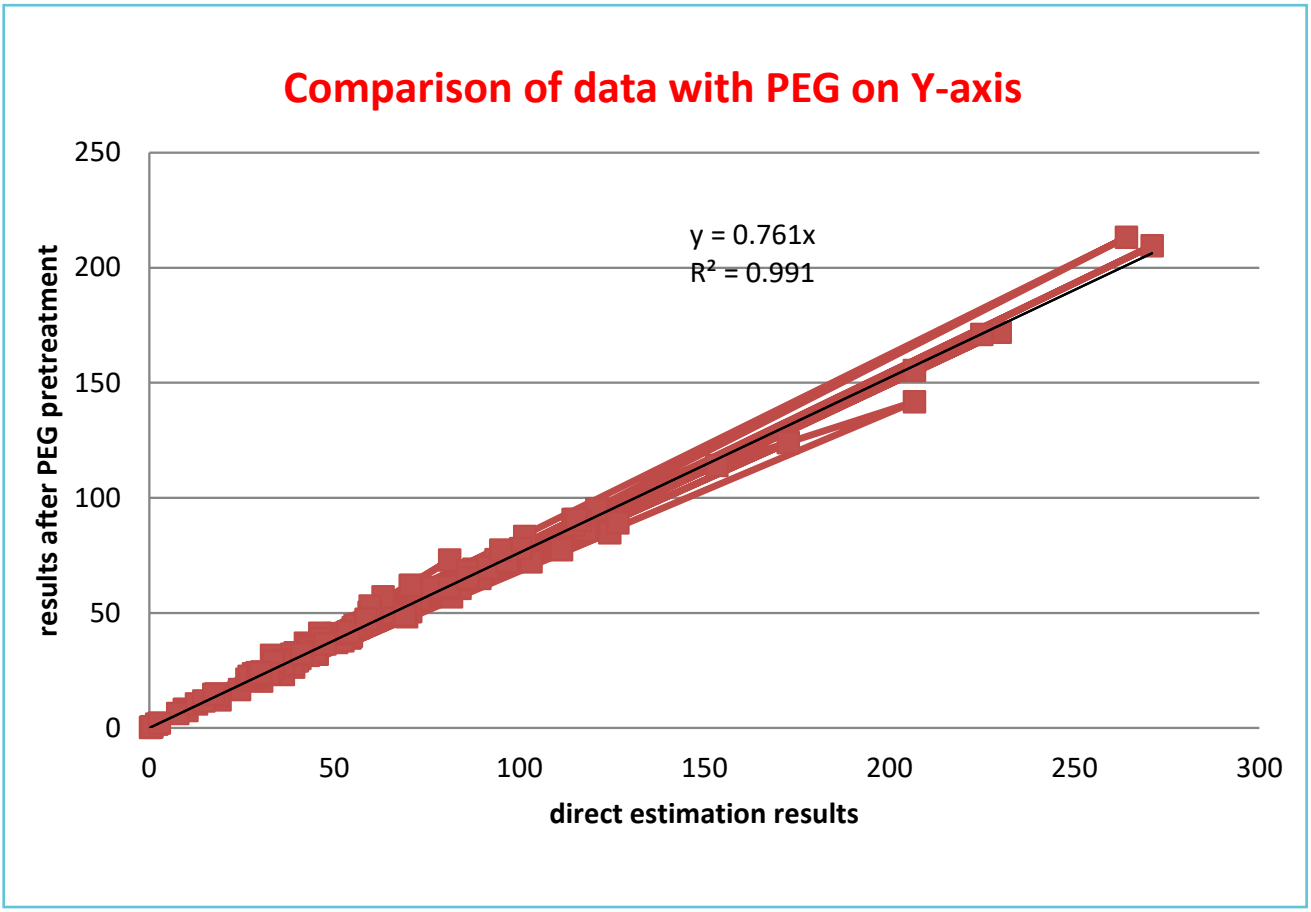
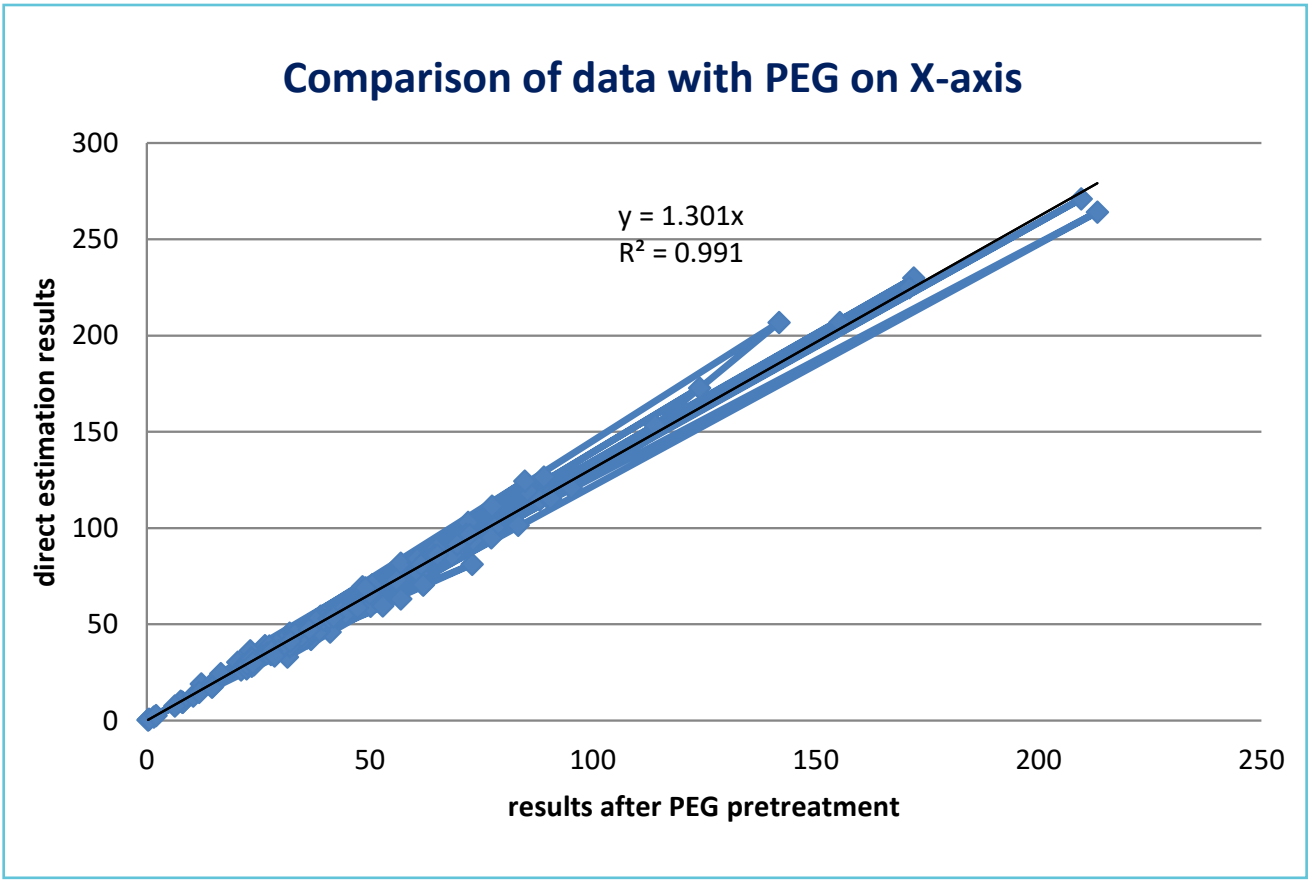


Figure 6 Comparison of data with PEG on X-axis (reverse comparison)



observed. Hence, the laboratory started comparing peer mean of EQAS and IQAS results in all chemiluminescent immunoassay systems. EQAS samples pretreated with PEG and compared the results with direct results. The correlation coefficients obtained from the slope twice.

i. ***Plotting PEG results on Y axis and direct results on X-axis:***
The correlation coefficient was 0.761 for EQAS results of Cycle 16 (Table 1, Fig. 1)

ii. ***Plotting PEG results on X axis and direct results on Y-axis:***
The correlation coefficient was 1.289 for EQAS results of Cycle 16 (Table 1, Fig. 2) i.e. reverse comparison.

The two step crosschecking was done and correlation coefficients were evaluated to confirm validity of the same within a limit of acceptable uncertainty.

It was also observed that PEG treated Cobas e 411 results are in accordance with Immunoassay systems Abbott Architect, Access 2 & Advia Centaur, r being 0.998, 0.997 & 0.999. The reason may be the EQAS samples either pretreated with PEG or the methods in other systems were modified so that involvement of M-PRL is not affecting the patient's results like Cobas e 411.

The insert of Lot 40330, BIORAD reflected similar consistency in results of above mentioned Systems and elevated prolactin results in Cobas e 411. So Trilevel immunoassay results were obtained and evaluated following the same procedure.

The correlation coefficients were found out to be 0.762 and 1.307 with R^2 0.999 (Table 2, Fig. 3, Fig. 4). The correlation coefficients of EQAS and IQAS both in direct and reverse direction are within the limit of acceptable uncertainty.

Now, to exclude PEG precipitation effect 25 samples were treated in the laboratory in Cobas e 411 and Access 2. In both systems direct and PEG treated sample results were recorded. The

Advia Centaur & Abbott Architect results obtained from accredited laboratories and it was instructed to send direct results only (Table 3). No remarkable deviation in results were noted between direct and after PEG treatment results in Access 2. The r value of Abbott Architect compared to PEG treated results of Cobas e 411 was 0.9988. The same for Access direct was 0.9967, for Access-PEG 0.997 and Advia Centaur was 0.9985. Though more than 25 samples could not be compared considering financial viability the range of prolactin results sent for comparison was extended from 6.0-365.0 ng/mL (Table 3).

The previous and current PRL insert of Roche Diagnostics were compared. No amendment in the procedure was observed (17). Though the inserts mentioned PEG treatment but no cutoff was instructed. To assess the validity of the laboratory defined correlation constant 150 samples were tested directly and after PEG precipitation. Correlation constants obtained are 0.761 & 1.301 with R^2 0.991 (Table 4, Figures 5, 6). Hence, the correlation constant is almost same for IQAS & human sample (0.761 & 0.762). Slightly different for EQAS samples (0.771) but such deviation is negligible and well within acceptable range. The EQAS cycle is a current one and difference in peers values confirm that Cobas e 411 values are still elevated and such elevation is measurable by a correlation coefficient.

CONCLUSION

- Correlation coefficient 0.761 is validated.
- When results are correlated in a wide range it can be concluded that PEG treatment to be done irrespective of concentration of prolactin within normal reference interval or above the biological reference interval. Within normal reference range result does not exclude the presence of M-PRL.
- As the PEG treated results are in correlation with other systems hence chance of inappropriate diagnosis is less. At least it may be concluded the precipitation of PRL is so small that it will not affect the results and interpretation.
- Possibility of systematic bias for the elevated results in Cobas e 411 cannot be excluded as it is not expected that presence M-PRL will be measurable by a correlation coefficient.
- Current peer mean results and range of IQAS and comparison data of EQAS, BIORAD is affirmative of the fact that the results of PRL in Cobas e 411 is yet elevated than other systems and needs modification/amendment of method. As peer mean of EQAS and IQAS are worldwide data hence the problem is a universal one and needs immediate resolution.

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Lab test findings in the elderly

Barna Vásárhelyi[1], Lóránd A. Debreczeni[2]

[1] *Department of Laboratory Medicine, Faculty of Medicine of Semmelweis University, Budapest, Hungary*
[2] *Unit of Laboratory Medicine, "Szent Imre" Teaching Hospital, Budapest, Hungary (retired)*

INFO

Corresponding authors:
Barna Vásárhelyi
E-mail: vasarhelyi.barna@med.semmelweis-univ.hu

Lóránd A. Debreczeni
E-mail: loranddebreczeni@gmail.com

Key words:
reference intervals of analytes in elderly, risk of chronic morbidities

LETTER TO THE EDITOR

A specific issue of the eJIFCC, with several papers [i.e. 1-6], focuses on the harmonization of laboratory information provided by different services with different clinical settings. Hopefully, these publications will also consider the fact that the reference interval of several analytes is different in elderly patients compared to the younger age groups.

A common accompanying phenomenon in the elderly (above 75 years of age) is the increased risk of chronic morbidities. This is associated with an increased requirement for health care activities that include the patients' assessment and monitoring. Blood sampling and laboratory testing are an inherent part of this process.

When a clinician considers the results of laboratory tests (as 'good' or 'bad'), the reference range used by the laboratory is the cornerstone and the basis of further evaluations. In the majority of laboratories healthy reference range is declared according to test descriptions and literature; just a minority perform a systematic analysis among individuals of a population considered as healthy. However, this is where the problem lies: in general, blood donors or other adults aged 20-40 years are enrolled to assess

healthy reference range. And very rarely elder (and completely healthy) subjects are asked to participate. This is not an issue until the analyte to be tested is not affected by the age itself. This factor, however, has a direct or indirect effect on a number of laboratory tests, as it is highlighted in a recent review [7]. In addition to age and comorbidities (e.g. diabetes mellitus, rheumatism, osteoporosis, etc.) elder patients also have various disadvantages such as obesity, low socioeconomic status, disabilities and poor (unhealthy) diet. These all may have an impact on laboratory results. (see Table 1)

Therefore, it is a common scenario that the laboratory reports of elderly patients may present a number of flags indicating a deviation from 'normal reference range' [8, 9]. That is often alarming for the patient (and his or her physician). A resultant consequence (provided that the doctor is not fully aware, whether a slight abnormality is acceptable for the patient) is the prescription of novel laboratory tests that generate novel questions.

The direction of anticipated abnormalities (compared to the reference range obtained in younger subjects) is summarized in Table 2.

SOME ANALYTES WITH PARTICULAR IMPORTANCE

Decreased hemoglobin/hematocrit

In the elderly, the impairment of the intestinal absorbance of iron and vitamin B12 may lead to a decrease in hemoglobin and erythrocyte synthesis. Occult blood loss is also common. There is an increased tendency for hemolysis. Therefore, it is recommended to decrease the lower level of reference range of hemoglobin (e.g. 115 g/l and 110 g/l for males and females, respectively). However, it is challenging to differentiate real anemia from the effect of aging. In the majority of patients the cause of anemia

Table 1 Factors having an effect on the result of lab tests in the elderly

Physiological changes gonadal hormones' levels are decreasing, bone loss is increasing; renal function is impaired; blood fat levels are increased.
Life style modification inactivity and associated alteration of body compartments; muscular mass is decreased; less supply of vitamin D due to less exposure to sun.
Dietary factors problems with digestion and absorption, dental problems lead to insufficient intake of nutrient rich food – vitamin and mineral trace deficiency. Further risks are alcoholosim and obesity. In US patients (according to CDC) aged 50 – 74 years up to 40 per cent of cancers are linked to obesity. Increased body weight is a risk factor for at least 13 cancers (i.e. esophageal, thyroid, breast, gall-bladder, gastric, liver, pancreas, renal, ovarian, uterine and colorectal cancers). [10]
Medicinal therapy (due to co-morbidities) may also cause abnormality in some lab test results.
Advertisement e.g. dietary supplements.

Table 2 Some analytes with altered results in the elderly

Increasing
alkaline phosphatase, antinuclear antibody, fibrinogen, FSH, LH, SHBG, gamma glutamil transferase, gastrin, uric acid, interleukin-6, insulin, cholesterol, parathormone (PTH), prostate specific antigen (PSA), rheuma factor, copper, triglycerol, ESR
Decreasing
aldosterone, vitamin B12, dihydroepiandrosterone (DHEA), vitamin D, ferritin, phosphate, HDL-cholesterol, IGF-1, interleukin-1, calcium (total), creatinine clearence, creatine kinase, magnesium, growth hormone, estradiol, free testosterone, T3, iron

is a chronic disease e.g. occult blood loss or renal failure. Anemia is of particular importance as elderly patients with anemia are at higher risk for circulatory and oxygenation problems hallmarked by fatigue, dyspnea, paresthesia (that are often attributed to elder age and, therefore, is not treated.)

The area of gas exchanging alveolar surface is also decreased, leading to a decrease in arterial oxygen tension by 4 mmHg per decade; this process results in latent hypoxia. Hypoxia is often associated with cognitive problems (that are further aggravated by the side effects of medicines used commonly).

Increase in blood glucose levels

Serum glucose levels increase proportionally to age, while glucose tolerance is decreasing. The reference range of fasting glucose is wider in the elderly (3.9 – 6.7 mmol/l). However, blood glucose levels are often low due to decreased body weight and dietary problems. Simultaneously, serum insulin levels also increase indicating insulin resistance; this is responsible for impaired glucose tolerance observed in up to 25% of patients above 75 years. Therefore, postprandial blood glucose levels are often higher when performing an oral glucose tolerance test (upper limit = 5.5 mmol/l + [age in years /18].

Increased erythrocyte sedimentation rate (ESR)

The ESR is increasing proportionally with age (in general by 0.22 mm/h per year above 20 years of age), but its exact cause is not known. Therefore, the upper limit of reference range in the eldery is 40 mm/h and 45 mm/h in males and females, respectively. (The contribution of the common occurrence of systemic inflammation in the elderly to high ESR is not fully clear. One should remember not to use ESR as a basis of diagnosis of inflammation in the elderly.)

Decrease of iron levels and stores

Serum iron levels decrease in the elderly, probably due to impaired production of gastric juice. Simultaneously, iron stores decrease also. The other common cause of low iron levels and systemic iron deficiency is chronic blood loss; therefore, malignancy should be searched for.

Increase of total cholesterol and triglyceride levels

Total cholesterol levels increase by up to 1 mmol/L in 60 years of age. No further elevation is anticipated thereafter; rather, in very old subjects the level of this analyte decreases. Triglyceride levels increase by 30 and 50 per cent in males and females, respectively, between 30 and 80 years of age. HDL-levels increase somewhat in aged men, while decrease in aged women.

Decreased renal function

In general, aged people take several medicines simultaneously. The metabolites are partly excreted via the kidney. Therefore, it would be of utmost importance to assess renal function to establish optimal dosage. In the elderly the number of functioning nephrons decreases by 30 – 45%; this is accompanied by the decrease of glomerular filtration rate. However, creatinine levels change rarely, as the lean body mass decreases. Therefore, net BUN and creatinine levels are not appropriate to estimate renal function; instead, eGFR calculation is required that incorporates patient's age.

Low albumin levels

Simultaneously with aging the level of some specific proteins, particularly that of albumin decrease (leading to a decrease of total protein levels). This is partly due to impairment of liver functions and an inappropriate diet. As albumin is the major carrier protein in blood, you should not be surprised, if a patient with low albumin levels presents with low calcium or hormone levels.

Thyroid function impairment is common

Hypothyreosis is not an inevitable consequence of ageing; however, it is a quite common phenomenon in aged patients (of note, its signs and symptoms include weakness, slowness and tiredness that are falsely attributed to old age). Therefore, it is recommended to screen patients' TSH levels. Roughly, TSH reference range is comparable to that in younger age. One should remember that medicines used commonly in old patients may influence thyroid hormone levels (eg. glucocorticoid hormones suppress TSH, while lithium inhibits thyroxin secretion).

WHAT SHOULD BE DONE?

Analytes changing with age are challenging as the doctor should decide whether a laboratory test result deviates from the 'healthy' (younger) reference range due to physiology or due to a disease. Unfortunately, there is no clear-cut answer, just our common advice: one should never establish a diagnosis exclusively on laboratory test results. Instead, laboratory test results can be used as an aid and prior results along with clinical history should be always considered.

The determination of old age-specific reference range would be an enormous support for the evaluation. This is, however, not an easy task. Subjects encounter the doctor (mostly) if they have complaints; healthy subjects normally avoid the doctor. The major question with the subject presenting at the clinic is not that (s)he has any problem (the answer: yes, (s)he has, otherwise (s)he would not have come); instead: what is the cause of the problem. Indeed, the goal of laboratory investigation is the exclusion or reinforcement of a diagnosis in an otherwise diseased person.

Therefore, it is not a healthy reference range that is required for this population; instead, a 'non-affected' reference range that is characteristic for said population (that is representative for the given subject). (e.g. the 'non-affected' reference range of troponin is different from the healthy reference range in an old patient with moderate renal failure.) The routine establishment of such reference ranges even for routine

laboratory tests, however, is not performed in domestic and foreign laboratories. Furthermore, the age-adjusted reference range for any analyte is also affected by the analytical environment; therefore, one cannot provide general exact numbers.

Therefore, it is recommended for physicians caring for older patients to request as few laboratory tests as they can. The major risk with large number of laboratory tests: if more laboratory tests are performed, the risk of false-positivity (and associated diagnostic doubts) is increased. The premise of fewer laboratory test is that the doctor should be clear with the anticipated information hoped from the test result, i.e. how the laboratory test result will improve his/her clinical decision making.

If you still decide to ask a laboratory test, it is recommended to strictly adhere to standardized sampling conditions.

Unfortunately, the laboratory and age specific reference ranges (that would improve the patient's assessment) are not commonly available. The use of individual reference tables available in literature is also controversial as reference ranges depend on local laboratory settings including instrument and reagents.

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Erratum

(1) Pediatric obesity and cardiometabolic disorders: risk factors and biomarkers

E. Levy, A.K. Saenger, M.W. Steffes, E. Delvin

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An error occurred in the numbering of the references presented in Table 2 of the review "Pediatric Obesity and Cardiometabolic Disorders: Risk factors and Biomarkers" Levy E, Saenger AK, Steffes MW, **Delvin E**. eJIFCC. 2017 Mar 8;28(1):6-24. eCollection 2017 Mar. PMID: 28439216.

The table should read as follows:

Table 2 Cut-off points for defining insulin resistance (IR)

Insulin measurement	Population Studied	Age (years)	Gender	HOMA-IR 95th percentile	Ref
Immunoassay (Access, Beckman Coulter)	French Canadian	9 13 16	M/F	1.88/2.07 3.28/3.86 3.31/3.10	(80)
Fluoroimmunoassay (AutoDelfia, Pharmacia)	Brazilian	10-19	M/F	>2.93	(81)
Chemiluminescence Immunoassay (Immulite, Siemens)	American	11-14	M/F	≥2.7	(82)
Chemiluminescence Immunoassay (Cobas, Roche Diagnostics)	Spanish	8-18	M/F	≥3.6	(83)

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