Review Article



# New biomarkers for estimating glomerular filtration rate

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**Abstract:** Glomerular filtration rate (GFR) is still considered the best indicator for kidney function assessment. In clinical routine, creatinine-based GFR estimation is the most widely used method for the evaluation of kidney function in healthy and kidney-diseased individuals. In recent years, researchers and clinicians have become aware of the limitations when using solely serum creatinine for kidney function assessment as it can lead to inaccurate estimation of GFR, particularly in populations with abnormal muscle mass distribution or generation. Several novel biomarkers have been proposed to improve accuracy and precision of GFR estimation, of which cystatin C, a low molecular weight protein, has proven to contribute considerable benefit to kidney function assessment when combined with creatinine. Importantly, the use of cystatin C as filtration marker has gained even more clinical relevance since the introduction of international reference standards available for assay manufacturers led to a harmonization of cystatin C analysis by minimizing inter- and intra-laboratory variability. Beta-trace protein (BTP) and beta-2 microglobulin (B2M) are two renal biomarkers with established but non-standardized assays that have been proposed as promising novel candidates for improving GFR estimation. In summary, a variety of new filtration markers and methods are available to assist clinicians in the evaluation of kidney function. The ongoing investigations of novel markers and metabolomics will help to identify their utility and may clarify whether they have the potential to improve the care of patients with and without kidney disease. This review will discuss the physiology, measurement and clinical potential of cystatin C, BTP, and B2M, will give a brief overview of current achievements in the field of kidney metabolomics and a short introduction to the concept of "rescaling" renal biomarkers.

**Keywords:** Filtration markers; new biomarkers; cystatin C; beta-trace protein (BTP); beta-2 microglobulin (B2M); glomerular filtration rate estimation (GFR estimation)

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## Introduction

The kidney filtration capacity or glomerular filtration rate (GFR) (1) is still considered the most preferable indicator of kidney function (2). GFR is best understood through the concept of renal clearance of a plasma solute. Ideally, this plasma solute should be produced at a constant rate, should not be bound to protein in plasma or metabolized outside the nephron-system, should be freely filtered through the glomerular barrier and neither secreted nor reabsorbed when passaging the tubulus-system (3). A substance with the above mentioned characteristics is called an "ideal filtration marker" and generally, renal biomarkers meet most of these characteristics but—as we will see—unfortunately, never all of them.

The assessment of GFR plays an important role in clinical routine as it forms part of the criteria (together

with albuminuria) for diagnosing chronic kidney disease (CKD). Additionally, the level of GFR defines the severity of CKD due to the KDIGO staging system (4). GFR is also important for making drug dosing decisions for a variety of different therapeutic agents that are either excreted by the kidney or have a direct nephrotoxic effect (5).

There are different ways to assess GFR: either estimated (eGFR) using GFR equations with endogenous filtration markers like creatinine that can be measured in the blood, serum as well as plasma, or it can be directly measured (mGFR) using invasive methods based on the injection of exogenous markers such as e.g., inulin, iothalamate, iohexol, EDTA or DTPA. Measured GFR is also considered the reference method for GFR assessment.

Using creatinine, a 113 Dalton molecule produced in the muscle from the precursor creatine, as a single endogenous filtration marker to assess GFR can be problematic as its production is tightly linked to muscle metabolism which in turn is dependent on muscle mass of patients and can vary between individuals. As a result, serum creatinine concentration of 1.1 mg/dL may represent perfectly normal kidney function in a healthy young man whereas in the presence of severe muscle wasting in an elderly lady it may mean the presence of clinically relevant kidney disease. This is one example of the possible influence of so-called non-GFR determinants when assessing GFR with endogenous filtration markers.

Several novel biomarkers have emerged as alternatives to creatinine and cystatin C with the goal to improve estimation of mGFR (6,7), of which beta-trace protein (BTP) and beta-2-microglobulin (B2M) are the two most intensively discussed new biomarkers in terms of their potential to optimize further kidney function assessment (8). Most recently, metabolomics studies have been performed to discover novel metabolites and metabolite ratios with a significant association with creatinine-based eGFR or mGFR, aiming for a more accurate estimate of GFR (9). The goal of these large-scale metabolomics studies is to provide a comprehensive list of kidney function-associated metabolites, a predefined "GFR biomarker-panel", for a quick and more accurate estimate of mGFR (10). Up to now this has been rather a vision for the future (11).

## New biomarkers for assessing GFR

### *Cystatin C*

Cystatin C, a 13 KDalton low molecular weight protein, is produced in all nucleated body cells at a constant rate and fulfills the above mentioned criteria for a filtration marker. In 1985, Grubb and colleagues first described the value of cystatin C as a biomarker for GFR (1,12). Although more than 30 years have passed, cystatin C can still be called rather "new" since the biomarker is far from being established as a routine marker in clinical practice. Since then, cystatin C has gained increasingly importance in clinical nephrology as large-scale cohort studies have demonstrated the added value to estimate GFR using cystatin C-based equations or combined equations (including serum creatinine and cystatin C) over creatinine-based equations (13-15). With regard to its non-GFR determinants cystatin C exhibits a lower dependency on muscular mass, is less influenced by gender and shows superior predictability of mortality and ESRD risk as compared to creatinine (16-18). On the other hand, the use of cystatin C as renal marker may be discouraged when patients are treated with high dose steroid therapy (19), in obese individuals, tobacco smokers or patients with hyperthyroidism or inflammation (20-23). There seem to be conflicting results in oncology: whereas in 134 oncology patients a malignancy and treatment-mediated effect on cystatin C measures could be found as potential confounder for cystatin C-based eGFR (24) this effect could not be observed in a smaller sample of patients with myeloma (25).

When included into GFR estimating equations cystatin C has led to higher accuracy of GFR estimates as compared to measured GFR, especially in children and older adults (14,15,26). Therefore, in certain situations, where creatinine-based eGFR (*Table 1*) alone might not be appropriate [e.g., in children, at very old age (33-35), in individuals with very low or very high body mass (36), patients with muscle dystrophy (37)], it has been shown that cystatin C might be the preferred endogenous biomarker. Also, the current "Kidney Disease Improving Global Outcome" (KDIGO) guidelines (4) recommend to estimate GFR with a cystatin C-based equation as a confirmative test when creatinine-based eGFR is between 45 and 59 mL/min/1.73 $m^2$ and urine albumin is <30 mg/g creatinine, which corresponds to the KDIGO CKD-stage IIIa.

Importantly, cystatin C analysis should be performed with assays that were calibrated against an international reference material (38). To our knowledge, this recommendation is not yet implemented area-wide into common practice (39).

There are a number of estimating GFR equations that include either cystatin C alone or cystatin C (*Table 2*) in

 

**Table 1** Selection of creatinine-based GFR estimating equations currently in use to calculate eGFR (mL/min/1.73 $m^2$)

| Study | Equations |
|---|---|
| BIS1 (15)* | $3{,}736 \times \text{creatinine}^{-0.87} \times \text{age}^{-0.95} \times 0.82$ (if female) |
| CKD-EPI$_{(crea)}$ (27) | Female with creatinine ≤62 μmol/L (≤0.7 mg/dL): $144 \times (\text{creatinine}/0.7)^{-0.329} \times 0.993^{age}$ (×1.159 if black) |
| | Female >62 μmol/L (>0.7 mg/dL): $144 \times (\text{creatinine}/0.7)^{-1.209} \times 0.993^{age}$ (×1.159 if black) |
| | Male ≤80 μmol/L (≤0.9 mg/dL): $141 \times (\text{creatinine}/0.9)^{-0.411} \times 0.993^{age}$ (×1.159 if black) |
| | Male >80 μmol/L (>0.9 mg/dL): $141 \times (\text{creatinine}/0.9)^{-1.209} \times 0.993^{age}$ (×1.159 if black) |
| Cockcroft-Gault (28)* | (140 – age) × weight/(72 × creatinine) |
| FAS$_{(crea)}$ (29)* | $107.3/(\text{creatinine}/Qcr) \times [0.988^{(age-40)}$ when age <40 years]: Qcr = 0.70 mg/dL for females and 0.90 for males |
| LM-REV (30)** | $e^{X-0.0158\times age+0.438\times \ln(age)}$ |
| | Female with creatinine <150 μmol/L (<1.7 mg/dL): X =2.50 + 0.0121 × (150 – creatinine) |
| | Female with creatinine ≥150 μmol/L (≥1.7 mg/dL): X =2.50 – 0.926 × ln(creatinine/150) |
| | Male with creatinine <180 (≥2.0 mg/dL): X =2.56 + 0.00968 × (180 – creatinine) |
| | Male with creatinine ≥180 (≥2.0 mg/dL): X =2.56 – 0.926 × ln(creatinine/180) |
| MDRD (31)* | $175 \times \text{creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female) |
| Schwartz bedside (32)* | 0.413 × (height/creatinine) if height is expressed in centimeters or 41.3 × (height/creatinine) if height is expressed in meters |

*creatinine in mg/dL; **creatinine in μmol/L. GFR, glomerular filtration rate; BIS, Berlin Initiative Study; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; FAS, full-age spectrum; LM-REV, Revised Lund Malmo; MDRD, Modifikation of Diet in Renal Disease; Qcr, Q-value for creatinine.

**Table 2** Selection of cystatin C-based GFR estimating equations currently in use to calculate eGFR (mL/min/1.73 $m^2$)

| Study | Equations |
|---|---|
| CKD-EPI$_{cys}$ (14) | Female or male with cystatin C ≤0.8 mg/L: $133 \times (\text{cystatin C}/0.8)^{-0.499} \times 0.996^{age}$ (×0.932 if female) |
| | Female or male with cystatin C >0.8 mg/L: $133 \times (\text{cystatin C}/0.8)^{-1.328} \times 0.996^{age}$ (×0.932 if female) |
| CAPA$_{cys}$ (40) | $130 \times \text{cystatin C}^{-1.069} \times \text{age}^{-0.117} - 7$ |
| FAS$_{cys}$ (41) | $107.3/(\text{cystatin C}/Qcys) \times [0.988^{(age-40)}$ when age >40]: Qcys =0.82 mg/L for ages <70; for age ≥70 Qcys =0.95 |
| Schwartz$_{cys}$ (42) | $70.69 \times (\text{cystatin C})^{-0.931}$ |

GFR, glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CAPA, Caucasian, Asian, pediatric, and adult; FAS, full-age spectrum.

combination with serum creatinine (*Table 3*). These GFR equations are available at a variety of websites as "GFR calculators" so that GFR can be calculated with serum values of creatinine and/or cystatin C, age and gender: (I) creatinine- and cystatin C-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (14); (II) full-age spectrum (FAS) equation (41); (III) Caucasian, Asian, pediatric, and adult (CAPA) equation (40); (IV) creatinine-cystatin C-based CKID equation [2012] by Schwartz for pediatric patients (42); and (V) Berlin Initiative Study (BIS2) equation (15).

### *BTP*

BTP, another low molecular weight glycoprotein with 168 amino acids, also known as prostaglandin D2 synthase (L-PGDS), is produced at a constant rate by glial cells in the central nervous system (43). It is a heterogeneous monomeric 23–29 KDa glycoprotein. The different sizes are a result of post-translational N-glycosylation. The larger isoforms are found in serum and urine whereas the smaller "brain" isoforms are present in the central nervous system. Interestingly, the functional significance

 

**Table 3** Selection of creatinine- and cystatin C-based GFR estimating equations currently in use to calculate eGFR (mL/min/1.73 $m^2$)

| Study | Equations |
|---|---|
| BIS2 (15) | $767 \times \text{cystatin C}^{-0.61} \times \text{creatinine}^{-0.40} \times \text{age}^{-0.57} \times 0.87$ (if female) |
| CKD-EPI$_{(crea/cys)}$ (14) | $135 \times \min(\text{creatinine}/\kappa, 1)^{\alpha} \times \max(\text{creatinine}/\kappa, 1)^{-0.601} \times \min(\text{cystatin C}/0.8, 1)^{-0.375} \times \max(\text{cystatin C}/0.8, 1)^{-0.711} \times 0.995^{age}$ (×0.969 if female) (×1.08 if black). α is −0.248 for females and −0.207 for males, min indicates the minimum of creatinine/κ or 1, and max indicates the maximum of creatinine/κ or 1 |
| FAS$_{(crea/cysC)}$ (41) | $107.3/\alpha \times \text{cr}/\text{Qcr} + (1-\alpha) \times \text{cystatin C}/\text{Qcys} \times [0.988^{(age-40)}$ when age >40]: Qcr =0.70 mg/dL for females and 0.90 for males; Qcys =0.82 mg/L for ages <70; for age ≥70 Qcys =0.95 mg/L; α can take a value between 0–1, if creatinine and cystatin C should be weighed equally then α =0.5 |
| Schwartz$_{(crea/cysC)}$ (42) | $39.8 \times (\text{height}/\text{creatinine})^{0.456} \times (1.8/\text{cystatin C})^{0.418} \times (30/\text{BUN})^{0.079} \times$ (1.076 if male) or (1.00 if female) $\times (\text{height}/1.4)^{0.179}$ |

GFR, glomerular filtration rate; BIS, Berlin Initiative Study; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; FAS, full-age spectrum; BUN, (blood urea nitrogen) in mg/dL; creatinine in mg/dL; cystatin C in mg/L; height in meters.

**Table 4** BTP-and B2M-based GFR estimating equations

| Study | Equations |
|---|---|
| Inker$_{(BTP)}$ (8) | $55 \times \text{BTP}^{-0.695} \times 0.998^{age}$ (×0.899 if female) |
| Inker$_{(B2M)}$ (8) | $133 \times \text{B2M}^{-0.852}$ |
| Inker$_{(B2M/BTP)}$ (6) | $96 \times \text{BTM}^{-0.278} \times \text{B2M}^{-0.588}$ |
| Pöge$_{(BTP)}$ (51) | $47.17 \times \text{BTP}^{-0.7933}$ |
| Pöge$_{(BTP/crea)}$ (51) | $\text{eGFR} = 974.31 \times \text{BTP}^{-0.2594} \times \text{creatinine}^{-0.6}$ |
| White$_{(BTP/crea)}$(50) | $\text{eGFR} = 167.8 \times \text{BTP}^{-0.758} \times \text{creatinine}^{-0.204}$ (×0.871 if female) |

BTP, beta-trace protein; B2M, beta-2 microglobulin; GFR, glomerular filtration rate.

of the different isoforms of BTP has not been understood yet but the existence of these post-translational different isoforms are a challenge for the measurement of BTP with immunoassays (44). It has been shown that BTP is freely filtered by the glomerulus with little if any tubular reabsorption or non-renal elimination (45). In clinical practice, BTP had been found to be a marker for cerebrospinal fluid fistula (46) and was established as a marker for the diagnosis of liquor leakage syndrome in the late eighties. About ten years later, in 1997, a study by Hoffmann *et al.* discovered elevated serum concentration of BTP in hemodialysis patients and suggested it as a potential diagnostic marker for renal disease (47).

In contrast to creatinine and cystatin C, only little is known about potential non-GFR determinants and the impact of race on BTP serum levels. It has been described in a population of adult kidney transplant recipients (48) and older adults (49) that women have a lower mean BTP concentration than men. Also, age seems to have an impact on the BTP level as was seen in datasets of children (50) and older adults (49) where both groups were found to exhibit higher mean BTP concentrations compared to middle aged adults.

For the estimation of GFR it is important to know whether a renal marker performs equally well in different patient populations, such as e.g., children, older adults, kidney transplant recipients or individuals with specific conditions such as liver cirrhosis or muscle wasting. With regard to using BTP in special populations, two research groups, White *et al.* and Poege *et al.*, have shown potentially superior properties for assessing kidney function in renal transplant recipients (50,51). Currently, there are six GFR estimating equations published for adults, including either serum BTP alone or in combination with serum creatinine or urea: the Inker$_{(BTP)}$ equation (8), the Poege$_{(BTP)}$, Poege$_{(BTP/Crea)}$, Poege$_{(BTP/Urea)}$ equations (51), the White$_{(BTP/Crea)}$ and White$_{(BTP/Urea)}$ equations (50) (*Table 4*). It is important to point out that both, Poege and White, have developed their equations in relatively small patient samples all of whom were kidney transplant recipients. In an external validation study performed in a population-based cohort of older adults, the Inker$_{(BTP)}$ equation showed the best performance

 

compared to the other BTP-based equations (49). In children and older adults, BTP alone or the addition of BTP did not outperform current biomarkers such as creatinine and cystatin C for GFR estimation (49,52). Especially the use of cystatin C seemed to render the addition of BTP unnecessary.

One additional aspect of BTP has been published recently by Shafi and colleagues; they could show that in patients on hemodialysis, serum BTP appeared to be in steady state during the interdialytic interval which lead to the conclusion that BTP equations, developed for the calculation of residual kidney function of patients with end-stage kidney disease, may not be influenced by diet and dialysis schedules compared with equations using other filtration markers such as creatinine or urea (53). However, further research and a standardized assay are necessary to reconfirm that BTP is a reliable filtration marker for the estimation of residual kidney function in patients on dialysis.

### *B2M*

B2M is a 100-amino acid protein component that is the light chain of the class I major histocompatibility (MHC) molecules expressed on the cell surface of all nucleated cells (54). Just like BTP and cystatin C it was also already discovered about 30 years ago and is the third classical low molecular weight protein, that has been found to be highly correlated with measured GFR (mGFR) (55) and, like BTP, is less affected by age, sex and black race as compared to creatinine (56). Similar to cystatin C, inflammatory conditions, high dose glucocorticoid therapy as well as lymphoproliferative diseases have been described as non-GFR determinants (57). Apart from its use as a renal marker, B2M has also been used as a tumor marker in lymphoproliferative disease with higher levels of B2M associated with tumor burden (58,59). Also, in pediatric patients diagnosed with malignancies or with inflammatory conditions serum levels of B2M have been found to be elevated (60,61). Besides the above mentioned conditions, Liu *et al.* recently presented a summary of non-GFR determinants of low molecular weight serum protein filtration markers in CKD patients (56) whereas Foster *et al.* investigated them in an elderly population (62). In general, both studies showed partly similar and partly diverging non-GFR determinants, both supporting the hypothesis that combined GFR estimating equations have the potential to minimize bias and imprecision and optimize the accuracy of GFR estimates (14,15).

When estimating GFR with B2M it is important to note that due to its characteristics as an acute phase reactant, highly correlating with inflammatory and infectious disorders, its potential as a single-marker for GFR estimation is limited (60) and its use as such has even been abandoned (63). To date, only Inker *et al.* have developed a combined BTP- and B2M-based GFR estimating equation (8). Their internal and external validation did not demonstrate an improvement over the currently available combined creatinine- and cystatin C-based equations (8) in any population including children (64) and older adults (49,65).

### *Kidney metabolomics*

Over the last years, mass spectrometry and associated chromatography methods have become more easily available and affordable which has led to a significant increase of studies investigating metabolomics aiming for new biomarkers for optimizing the diagnosis of various diseases. Also, in the field of GFR estimation new studies have investigated the potential benefit of developing a panel of filtration markers (panel eGFR) from a single blood draw for a less biased and more accurate estimate of measured GFR, the gold standard of GFR assessment (66). The idea behind it is that estimating GFR from multiple non-correlated markers would minimize the impact of non-GFR determinants of each marker and lessen the need for demographics and clinical characteristics as surrogates resulting in an optimized precision as the number of markers increases (10). At present, there have been two groups identified of candidate filtration markers for inclusion in a "panel eGFR": low molecular weight serum proteins and metabolites. For the first time, in 2012, Goek and colleagues performed a large-scale targeted metabolomics cross-sectional study in two independent samples: the KORA F4 study for metabolite discovery and the TwinsUK study for metabolite validation (67). One year later they published the first targeted longitudinal metabolomics study and provided a number of metabolites and metabolite ratios that were associated with kidney function change over the course of seven years (68). In 2016, the same group published the first large-scale non-targeted metabolome-wide association study of kidney function and disease in the general population. With their study they provided a comprehensive list of kidney function-associated metabolites and concluded that these potentially

 

novel filtration markers may help to improve the estimation of GFR (9). Also, Coresh and colleagues published a cross-sectional study in two cohorts with measured GFR in 2018 and identified a panel of multiple metabolites that provided an accurate estimate of GFR with and without including creatinine or demographics (69). Whether this new metabolite panel is going to be robust for GFR estimation and can be applied in the general population has to be confirmed in future studies.

Although there have been interesting new discoveries in the field of kidney metabolomics the topic of standardized laboratory methods for their analysis is far from being resolved. Finally, confirmatory studies quantifying the degree of benefit (and cost-effectiveness) of these novel biomarker-panels over the current practice of GFR assessment with well-known endogenous or exogenous filtration markers are still lacking.

## Measurement of renal biomarkers and standardization issues

When evaluating the significance of estimating GFR with filtration markers, it is of great importance to keep in mind that a standardized measurement method is a key element for precise, accurate and unbiased kidney function assessment. There is a large body of literature that describes the negative impact of non-standardized laboratory methods resulting in a high inter- and intra-laboratory variability for creatinine and/or cystatin C analysis (70-75). As a consequence, the International Federation of Clinical Chemistry (IFCC), the National Kidney Disease Education Program (NKDEP) and the European Communities Confederation of Clinical Chemistry (EC4) launched the Creatinine Standardization Program calling for a standardization of the creatinine assay calibration (7). By now, the majority of clinical laboratories use creatinine assays from manufacturers that assure their assay-calibration is traceable to the isotope dilution mass spectrometry (IDMS) which is the gold standard of reference methods (76). IDMS-traceability can be obtained for both, Jaffe and enzymatic methods, although comparison studies show that the results with the Jaffe or compensated Jaffe assays were inferior as compared to the enzymatic assays (39). To which extent clinical laboratories report creatinine results that have standardized calibration, as required by international guidelines, is not fully understood. Recently, an international survey of creatinine assay kits with English language product information revealed insufficient assay calibration traceability due to incoherent calibrator use and insufficient information provided to assess the assay calibration traceability (77,78).

For cystatin C, in 2010 the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) working group collaborated with the Institute for Reference Methods and Materials of the European Commission and took an important step for the standardization of cystatin C assays when the international certified reference material ERM-DA471/IFCC was made available to assay manufactures (38,79). Consequently, manufactures have been introducing standardized measurement procedures for cystatin C analysis that are commercially available now. In his recent study, where a new cystatin C-based eGFR equation was generated by use of seven standardized assays, Grubb and colleagues showed that the variability of different cystatin C assays could be reduced by using standardized calibration (40). However, the use of standardized reference material by manufacturers is still inconsistent, leading to doubts about the accuracy of several cystatin C assays available on the market (74,80-83).

In daily practice the nephelometry (PENIA) and turbidimetry (PETIA) assays are the two common methods to quantify cystatin C. Recently, Ebert and colleagues found that in Europe, comparing standardized cystatin C assays from Roche and Siemens, the analytical differences between these two PENIA and PETIA cystatin C assays were relatively small in a population of older adults (84). This led to the conclusion that calibration of cystatin C assays had an important impact on their inter-changeability, even if the disagreement about the consistency of these standardization processes still remains.

For BTP, there are currently two assays commercially available: the Cayman Chemicals which is an immunometric ELISA test that uses monoclonal murine antibodies and the particle enhanced nephelometric immunoassay (PENIA) by Siemens which uses polyclonal rabbit antibodies against human urinary BTP (85). In contrast to the assays available for creatinine and cystatin C, there are no reference materials available for BTP which would allow a standardized analysis of this small molecule weight protein. White *et al.* showed in their comparison study of the Cayman and the Siemens assays that analytic performance of BTP measurement procedures is far from ideal. They found only a poor agreement between both assays which resulted in significant differences in BTP-calculated eGFRs. Also, they found differences in the Siemens assay between two laboratories and even in the same laboratory over

 

the course of two years suggesting that the individual lots of the Siemens assay components, calibrators or control materials, may differ over time. They concluded that for usage in clinical routine a robust reference system is needed to harmonize the clinical measurement of BTP across different procedures and to reduce inter- as well as intra-assay variability (85).

B2M has been established as a routine clinical parameter due to its use as tumor marker for multiple myeloma and can be measured in serum using PENIA, PETIA or immunoassay (86-88) but similar to BTP, the current assays for B2M lack a common standardized calibration procedure which leads to a large variability between the different analysis methods (89,90).

## The concept of rescaling renal biomarkers

In 2016, Pottel and colleagues developed the creatinine-based full-age spectrum (FAS)-equation to estimate GFR in healthy and kidney-diseased individuals along the entire lifespan (from 2 years to 100 years) (29). The construction of the FAS equation is based on data from individuals of different nationalities and ages demonstrating that average GFR in individuals aged 2 to 40 years is equal to a value of approximately 107 mL/min/1.73 $m^2$ and that the age-dependent decline of GFR begins at an age of around 40 years. For this FAS concept, Pottel and colleagues developed the principle of "rescaling" serum creatinine to estimate GFR as precise as possible. With the principle of "rescaling" serum creatinine values it becomes possible to minimize the influence of age (e.g., the differing creatinine-generation during young age due to a constant change in body height/composition) and sex (due to different muscle mass of women compared to men) on the renal biomarker (91). When rescaling serum creatinine values they become normally distributed around the mean of "1", resulting in a reference interval of 0.67 to 1.33 corresponding to the 2.5th and 97.5th percentile of the distribution of biomarker values of a healthy person. Rescaled values of creatinine above "1.33" correspond to an increased serum biomarker concentration and indicate kidney disease. In general, rescaling of creatinine can be achieved by dividing an individual's serum creatinine value by the mean serum creatinine concentration of an age and sex specific healthy population, the so-called "rescaling factor" or "$Q_{cr}$" value. For adults and older adults, serum creatinine can be rescaled with the $Q_{cr}$ value of 0.70 mg/dL for (white) females and 0.90 mg/dL for (white) males (92). For infants, children and adolescents the rescaling procedure is a bit more complex due to the differing impact of age and body height on creatinine generation (93).

In 2017, Pottel and colleagues showed that the FAS approach could also be applied to cystatin C and published cystatin C-specific "$Q_{cys}$" values of 0.82 mg/dL for men and women until the age of 70 and 0.95 mg/dL for individuals above the age of 70 years (41). The principle of rescaling renal biomarkers could further be extended to BTP resulting in a $Q_{BTP}$ value of 0.60 mg/L for individuals above the age of 70. Currently, it remains to be proven whether the BTP-specific Q-value of 0.60 mg/L can also be applied to younger individuals and whether an adaptation of Q values is necessary when used in non-Caucasian populations (94).

## Summary and conclusion

Estimating GFR from endogenous filtration markers is a well-established and a vital part in the evaluation of kidney function. Over the last decades, there have been marked improvements in the identification of potentially new renal markers and the harmonization of clinical laboratory methods by implementing standardization of calibration procedures. As a consequence, accuracy and precision of GFR estimation could be improved resulting in a more refined clinical evaluation of an individual's kidney function. Particularly, the better understanding of non-GFR determinants of serum creatinine has led to a rigorous search for alternative markers. In this respect, cystatin C has shown to improve the accuracy of GFR estimation most notably in children, adolescents and older adults. The clinical implication of cystatin C for routine kidney function assessment has gained further importance and demand since a standardized calibration procedure was made available for cystatin C assay manufactures.

Also, BTP and B2M are currently promising candidates as additional filtration markers and new GFR estimating equations have been recently developed for their usage. For now, BTP has not contributed additional benefit to the combination of creatinine and cystatin C when estimating GFR in children, adults or older adults. Due to its characteristics as acute phase reactant, highly correlating with inflammatory and infectious disorders, B2M's potential as a single-marker for GFR estimation was found to be limited: Whether it has the potential to shad additional light on GFR assessment when combined with other markers is still unclear. There have been promising

 

results in the field of kidney metabolomics. However, their discovery and validation is still in a relatively early stage and confirmatory studies quantifying the degree of benefit (and cost-effectiveness) of these novel biomarker-panels for GFR assessment are still lacking. Recently, the concept of "rescaling" renal biomarkers has been introduced for creatinine, cystatin C and BTP allowing the use of biomarker values for quick and age/sex-independent evaluation of kidney function and GFR calculation with the FAS equations.

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