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Pitfalls in the measurements and assesment of glomerular filtration rate and how to escape them

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List of abbreviations

BMI	Body mass index
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
GFR	Glomerular filtration rate
IFCC	International Federation of Clinical Chemistry
K/DOQI	Kidney Disease Outcome Quality Initiative
MDRD	Modification of Diet in Renal Disease
PENIA	Particle enhanced nephelometric immunoassay
PETIA	Particle enhanced turbidimetric immunoassay

Introduction

The basic prerequisite of normal kidney function is the filtration of adequate amount of fluid in glomeruli. This is ensured through constant kidney blood flow in a broad range of mean arterial pressures. In physiological conditions the amount of filtered fluid is 180 litres in a day (120 ml/min; 2 ml/s). Although water, electrolyte and acidobase homeostasis is mainly maintained through tubular mechanisms, in case of insufficient supply of starting substance the function of the whole system is endangered. Therefore the glomerular filtration rate (GFR) is the basic marker of kidney function both in health and disease.

Measurement of GFR

The measurement of GFR is based on the clearance principle. Clearance is the virtual amount of plasma cleared from a given substance in a certain time unit. If the substance is freely filtrated from plasma to the ultrafiltrate and is not resorbed back or secreted in the tubuli, its clearance is equal to GFR (Insert 1).
Exact calculation of GFR is possible through measurement the plasma concentration and urinary excretion of exogenous substances fulfilling the above-mentioned criteria (Tab 1). On the other side these exact methods are expensive, cumbersome and invasive and therefore not suitable for everyday clinical practice (1).
The idea of GFR measurement from serum creatinine concentration and its urinary excretion is a logical one because it is an endogenous substance present in the blood and the body gets rid of it almost entirely through the kidneys. However this method is far from perfect. Its basic imperfection is a physiological one – creatinine is not only filtered in the glomeruli but also secreted in

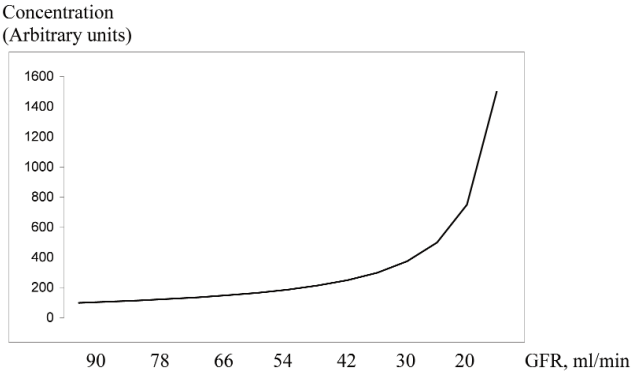

Figure 1
The increase of serum concentration of an „ideal" compound associated with gfr decrease.

Table 1
Measurement of GFR from clearance of exogenous substances

A. Substances fulfilling the criteria

Inulin
Iohexol
I-iothamalate and 125I-iothalamate
^{51}Cr-EDTA (ethylendiaminotetraacetic acid)
^{99m}Tc-DTPA (diethyltriaminopentaacetic acid)

B. Methodical approaches and problems

The substances are applied in infusion or as a bolus. In the case of infusion a period of stable concentration is achieved after some time and the calculation of GFR is without problems. After bolus dose the serum concetration of the substance is decreasing. In the first phase the compound is diffunding into its distribution space. Later a quasy steady state is achieved and the GFR can be calculated from frequent blood and urine sampling and chemical analysis of the substance concentration or through scintigraphy. Scintigraphy provides also a possibility to measure GFR of right and left kidney separately but the results are less accurate as the results of direct biochemical assays. The calculations of GFR after bolus dose are rather complicated but information technology can solve also this problem.

the tubuli. Its concentration in the urine [U] is therefore higher than it could be anticipated from the filtration alone and the calculation according to the clearance formula gives a higher GFR value as compared with the true GFR (2).
The other sources of error are methodological ones. The picric acid („Jaffé") creatinine assay is non-specific and the admixture of other chromogens can be as high as 20 %. Methodical improvements of the assay don't eliminate this error fully. The non-specific chromogens increase both the values of [U] and [P] and therefore these errors are partially compensated. Partially, because the proportion of non-specifically reacting substances can be different in serum and in urine. The correction of the Jaffé results to true creatinine is of illusory value because concentration of these non-specific substances can depend on numerous physiological and pathological factors (3). The last error is originating from the fact that the collection of 24-hour urine is principally a simple task but the practice is mostly very different and relatively small errors in the „V" value have a substantial impact on the results.

Assessment of GFR through calculation from serum creatiníne

GFR can be estimated also from the concentration of creatinine in serum. This approach is also based on physiological principles because the concentration of any substance, which is excreted only through kidneys and is not degraded in any other way, is increasing in kidney failure. If the given substance is appearing in the blood at a constant rate, between its concentration and the GFR is a simple exponential relationship (the product of its serum concentration and the GFR is constant – insert 2; graph 1). Unfortunately no such ideal substance exists in the human body but there are at least two, which are not very far from this presumption.
Serum creatinine is the most commonly used substance not only for the measurement but also to the assessment of GFR in clinical practice. In this approach one problem is eliminated – namely the error from imprecise collection of urine.
Tubular secretion can bias the results but the effect is the opposite as compared with the clearance-based measurement because tubular secretion decreases the concentration of serum creatinine independently on its diminished filtration. Non-specific chromogens distort the results in a similar way as in the case of the calculation from clearence. This error is possible to eliminate only through use of enzymatic creatinine assays.
A more serious problem of the GFR assessment based on serum creatinine is that the presumption of its constant flow from the cells into the blood plasma is not fulfilled. Creatinine formation in the body is a function of muscle mass (4-6). This is taken into consideration also in different reference values for men and women (Tab. 2). The real situation is more complicated because muscle mass in both sexes is very variable and also strongly age dependent. Creatine formation (and its serum concentration) is increased also in different conditions as muscle catabolism, extreme physical activity, traumatic muscle damage, haemolysis and in people on protein-rich diet. Low values of serum creatinine can occur in muscle atrophy and dystrophy, in myasthenia gravis and in malnutrition. Even more marked age-related and nutrition dependent differences in serum creatinine concentration and its urinary excretion are present in children under 18 years of age (4,7).

The strong influence of muscle mass and age on serum creatinine makes the assessment of GFR simply from the theoretical inverse association between creatinine concentration and GFR impossible. Involvement of correction factors is inevitable to achieve reliable results. One should however bear in mind that these factors are purely empirical albeit there were estimated in carefully designed studies comparing serum creatinine values with some of the gold standard method based on clearance of inulin or radionuclides in a high number of probands (table 3, part A, references 8-10).
Unfortunately but no unexpectedly each study provided different results and different equations. The correction according to body

Table 2
Reference ranges of serum creatinine in adult healthy subjects and the calculation of gfr according to the short mdrd equation

	Serum creatinine µmol/l	GFR, ml/min Age 20 years	GFR, ml/min Age 65 years
Men, age 20 – 65 years	64 - 106	138,3 – 77,3	108,9 – 60,8
Women, age 20 – 65 years	52 - 85	99,7 – 74,0	102,6 – 58,2

Note: There is a serious contradiction between the reference values and the nephrologists' concept of fitration! Older people with "normal creatinine" around the upper reference limit have decreased GFR according to K/DQOI criteria.

Table 3
Equations for GFR assesment from serum creatinine and cystatine concetration

A. CREATININE-BASED EQUATIONS

1. **Cockcroft and Gault** (8) (age, body mass, sex)
   GFR = (140 - age)*body mass/48,8*[S_{cre}]
   Correction for women: multiple by 0,85

2. **MDRD equations; Levey** (9,10)
   2.1 (age, urea and albumin)
   GFR = 170*[S_{cre} *0,0113]$^{-0,999}$*age$^{-0,176}$*[Surea*2,8]$^{-0,17}$ *[Salbumin/10]^{0,318}
   Correction for women: multiple by 0,762
   2.2 (age and urea)
   GFR = 270*[S_{cre} *0,0113]$^{-1,007}$*age$^{-0,18}$*[S_{urea} *2,8]$^{-0,169}$
   Correction for women: multiple by 0,762
   2.3 (short form – only age and sex)
   GFR = 186*[S_{cre} *0,0113]$^{-1,154}$*age$^{-0,203}$
   Correction for women: 0,742
   2.4 (short form for standardized creatinine – $MDRD_4$)
   GFR = 175*[$S_{cre\text{-}stand}$*0,0113]$^{-1,154}$*age$^{-0,203}$
   Correction for women: 0,742

3. **CKD-EPI equation system; Levey** (11) (age, sex, ethnicity and creatinine concetratrion)
   GFR = 141*[S_{cre}/κ]$^{\alpha}$*age$^{-0,993}$*X
   The constants „κ", „$^{\alpha}$" and „X" are sex and ethinicity dependent and are explained in Insert 3

The MDRD and CKD-EPI equations are also indexed to body surface area and the above equations are valid for body surface 1,73 m2. The topic of indexing is analysed in the text.

In addition to the mentioned there are also other equations as of Walser (age, body mass and sex); Jeliffe (age); Nankiwell (age, body mass and height and urea). Special equations are for children (6)

B. CYSTATIN C-BASED EQUATIONS

1. **Grubb** (34) (sex and age in children)
   GFR = 84,69*[S_{cys}]$^{-1,68}$
   Correction for women: 0,948; for children under 14 years: 1,384

2. **Stevens** (35)
   2.1 $Stevens_1$ (without any correction)
   GFR = 76,7*[S_{cys}]-1,19
   2.2 Stevens2 (age, sex, ethnicity)
   GFR = 127,7*[S_{cys}]-1,17*age$^{-0,13}$
   Correction for women: 0,91, for people with black skin: 1,06

In addition there are also other equations for cystatin C-based assesment of GFR as of Bönenkamp (linear, without correction), Filler and Corao (logarithmic, without corections) and Zapitelli1 (without corrections). For details see (7)

C. COMBINED EQUATIONS
   2.3 Stevens3 (weighted calculation from cystatin and creatinine, Correction for age a ethnicity)
   GFR = 177,6*[S_{cre}]$^{-0,65}$*[S_{cys}]$^{-0,57}$*age$^{-0,2}$
   Correction for women: 0,82, for people with black skin: 1,11

Other combined equations: Bouvet and Zapitelli2, also with corrections according to age, body mass and conditions as renal transplant and spina bifida. For details see (7)

mass in most of them has an important flaw also from physiological point of view. Increased body mass in most probands is not caused by extra muscle mass but by fat not producing as much creatinine as the muscle cells. In case of oedema this problem is even more pronounced. In some equations there is an attempt to increase their accuracy through involvement of urea or albumin concentration into the equation.

Despite the lack of the pathophysiological foundation of these correction factors their use in general clinical practice is fully warranted with the exception of the yet commonly used Cockroft & Gault equation, which is today considered out of date. In tables 4 and 5 there are some model calculations and the differences between the Cockroft & Gault and MDRD results are not negligible. The most important problems occur in patients with mildly decreased GFR because their categorization into the wrong K/DOQI stage can have serious consequences from therapeutical point of view.

The needs of general practice were behind the last amendment of the MDRD system. The results of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI, ref. 11) study represent a substantial step torwards equations with minimal possible bias in the assessment of GFR in general population. As a matter of fact it is not one equation but a system of equation (insert 3) with correction factors according to age, sex and ethnicity as in MDRD. In addition another an entirely new correction factor is introduced

Table 4
Examples of GFR results calculated according to cockroft and gault (C&G) and accroding to the short MDRD equation

[Screa] µmol/l	Age years	Body Mass kg	GFR ml/min C&G M / W	GFR ml/min/1,73 m² MDRD M / W	Different categorization K/DOQI
100	20		103,3 / 87,8	82.6 / 61,3	Men I⇒II
	50		77,5 / 65,8	68,6 / 50.9	Women II⇒III
	80		51,6 / 43,9	62.3 / 46,3	Men III⇒II
		70			
200	20		51.6 / 43,9	31,7 / 27.6	Women III⇒IV
	50		38,7 / 32,9	30,8 / 22,9	Women III⇒IV
	80		25,8 / 21,9	28,0 / 20,9	
100	20		155,9 /131,7	82,6 / 61,3	Both I⇒ II
	50		116.1 /98,7	68,6 / 50,9	Men I⇒II Women I⇒III
	80		77,5 / 65,8	62,3 / 46,3	Women II⇒III
		105			
200	20		77,5 / 65,8	31,7/27,6	Men II⇒III WomenII⇒IV
	50		58,1 / 49.4	30,8 / 22,9	Women III⇒IV
	80		38,8 / 32,9	28,0 / 20,9	Both III⇒IV

Calculations from the same creatinine concentration and different age, sex and body mass in most cases leads to different categorization. In some cases the difference is more than one stage and although the MDRD in general gives lower GFR values as C&G in one case (80 years old lean men with [S_{crea}] 100 µmol/l) the opposite is true.

Table 5
Differences in creatinine concentration at the borderline value between K/DOQI stage I and II calculated according to Cockroft & Gault and the MDRD4 equations

	[Screa], µmol/l GFR = 90 ml/min		Difference, ml/min
MODEL	C&G	MDRD	
Man, 20 years; 70kg	114,8	92.8	22,0
Woman, 20 years; 70kg	97,5	71.6	25,9
Man, 50 years; 70 kg	86,0	79.0	7,0
Woman, 50 years; 70 kg	73,1	61,1	12,0
Man, 80 years; 70 kg	57,3	72,7	-15.4(!)
Woman, 80 years; 70 kg	56,2	48,7	7.5
Man, 50 years; 105kg	129,0	79.0	50,0
Woman; 50 years; 105kg	109.7	61,1	48,6

Insert 3
The CKD-EPI system of equations

Caucasian# male, $[S_{crea}] \leq 80$ µmol/l	$GFR = 141*([S_{crea}]/0,9)^{-0,411}*0,993^{age}$
Caucasian# male, $[S_{crea}] > 80$ µmol/l	$GFR = 141*([S_{crea}]/0,9)^{-1,209}*0,993^{age}$
Caucasian# female, $[S_{crea}] \leq 62$ µmol/l	$GFR = 144*([S_{crea}]/0,7)^{-0,329}*0,993^{age}$
Caucasian# female, $[S_{crea}] > 62$ µmol/l	$GFR = 144*([S_{crea}]/0,7)^{-1,209}*0,993^{age}$
Afro-American male, $[S_{crea}] \leq 80$ µmol/l	$GFR = 163*([S_{crea}]/0,9)^{-0,411}*0,993^{age}$
Afro-American male, $[S_{crea}] > 80$ µmol/l	$GFR = 163*([S_{crea}]/0,9)^{-1,209}*0,993^{age}$
Afro-American male, $[S_{crea}] \leq 62$ µmol/l	$GFR = 166*([S_{crea}]/0,7)^{-0,329}*0,993^{age}$
Afro-American male, $[S_{crea}] > 62$ µmol/l	$GFR = 166*([S_{crea}]/0,7)^{-1209}*0,993^{age}$
#or other, not Afro-American	
All results as ml/min/1,73 m^2 body surface area	

Note: In contrast to the authors we don't use the term "race" because the whole mankind is one race.

– the calculation is different for probands with low (≤ 80 or 62 µmol/l in men and women, respectively) and high (> 80 or 62 µmol/l) serum creatinine. This is an interesting improvement because it can solve the problem of errors both in low and in high creatinine range inherent in older equations.
The system was approved with enthusiasm by the community of clinical chemists and nephrologists (12 - 20). However at least two important questions should be answered subsequently:

1. Is the CKD-EPI calculation system superior to cystatin C-based equations and if it is (or not) in which range of serum creatinine levels, GFR values, specific groups of probands and different pathological conditions?
2. How to introduce the system into primary care? General practitioners are not so enthusiastic to swallow and adopt changes in any topic initiated by clincal chemistry regardless of their firm scientific evidence. They just switched from Cockroft and Gault to MDRD and now they should switch once more to something else.

Another specific and neglected topic in GFR assessment is its indexing to body surface (21). Although this seems to be a rational step to get more reliable results in probands with different body shape, it doesn't bear the scrutiny of rational analysis (22) because the equations to calculate body surface are not accurate enough and in general population obesity is a strong confounding factor. The same 1,65 m high person has a calculated body surface 1,53 m^2 if his weight is 65 kg (BMI = 18,4 kg/m^2) and 1,87 m^2 if he is becoming obese and weights 85 kg (BMI = 29,4 kg/m^2) but we don't think that at a given creatinine his GFR differs by 22%.
A second argument against indexing is that in primary care probably (almost) nobody uses it.

Assesment of GFR from serum concentration of cystatín C

GFR can be estimated from the serum concentration of other substances formed in the body at a constant rate, secreted into the blood and filtered freely in the glomeruli. Cystatin C, a 13,3 kDa molecular weight cystein protease inhibitor fulfills both these criteria quite well. Its production is constant in all nucleated cells, it is present in all body fluids and after filtration in the kidneys is fully degraded in the tubuli (23 - 26).
The normal reference interval of serum cystatin C is broader as compared with the serum creatinine values. The reference rande is also dependent on the assay used (PENIA or PETIA Dade-Behring, PETIA Roche – Table 6) and others; table but this problem should be solved by IFCC standardization (27) The broad normal range of cystatine C is however in a contradiction to the statement about its constant synthesis in the cells and stable excretion in the extracellular space (28). As a matter of fact numerous nonrenal factors influence its concentration in serum (Insert 4). On the other side body mass is not among these factors which is a remarkable advantage against creatinine. An interesting novel possibility is the use of cystatin C serum levels as a cardiovascular risk factor (33). This association can be explained in two ways: it can be connected with the well-known accelerated atherosclerosis due to diminished kidney function or cystatin C as a protease inhibitor can play some independent role in cardiovascular pathology.
Assesment of GFR from serum cystatine C concetration is possible according to equations based on comparative studies as in the case of creatinine (Table 3 part B, references 34 – 35). The Grubb equation is commonly used, however in low range of cystatin C concentration it gives unrealistic high values of GFR. The Stevens equation perform in this range better (Table 7).

Table 6
Cystatin C reference ranges according to different manufacturers (A) and their comparison with the preliminary results of hepameta study (B)

A

Manufacturer	Reference range, µg/ml
Roche	0,47 – 1,09
Randox	0,58 – 1,05
Dade Behring	0,53 – 0,95

B

Group, ethnicity	N	Mean and SD	Median	Range
Men, Roma	158	0,62 ± 0,14	0,61	0,34 – 1,02
Men, other	182	0,64 ± 0,16	0,62	0,30 – 1,68
Women, Roma	284	0,58 ± 0,16	0,57	0,25 – 1,35
Women, other	109	0,55 ±0,16	0,53	0,23 – 1,01

The Hepameta study is aimed at the general health status of marginalized (Roma and other) but apparently healthy social groups in Slovakia. The age of the probands was between 18 and 55 years without any difference among the groups. Cystatin assay: Imu La Test Erba-Lachema.

Insert 4
Nonrenal factors affecting cystatin c serum concetration

Inflammation – association with CRP (29)

Corticoid treatment – increase (30)

Thyroid dysfunction – low in hypo-, high in hyperfunction (31)

Decompensated diabetes with ketonuria (32)

Note: In some of the above mentioned conditions the association with small changes in GFR are not excluded

TABLE 7
Differences in cystatine c concentration at the borderline values between diffetent k/doqi stages calculated according to grubb and stevens equations

Stage, GFR	Cystatin C Grubb	Cystatin C Stevens
I/II – 90 ml/min	0,95	0,88
II/III – 60 ml/min	1,35	1,23
III/IV – 30 ml/min	2,43	2,20

Similar contradiction between the reference values (Table 6) and cutoff values between stage I and II K/DOQI as in the case of creatinine. The upper limits of reference ranges correspond to decreased GFR.

Creatinine or cystatin C?

In laboratory medicine such catherogical questions are usually wrong and therefore it is not possible to answer them adequately. This was confirmed also in the metaanalysis of Andersen et al. (7) and also in other studies (36). The authors were not able to confirm neither the superiority nor the inferiority of cystatine based equations.aginst the creatinine equations. The existence of different equations is also a proof of the uncertainities in this topic.
Despite all these uncertainities some useful recommendations is possible according to present-state situation:

- The use of creatinine-based MDRD or CKD-EPI equations as a first step in adult general practice is fully warranted. Measurement of serum creatinine is involved in the basic panel of laboratory assays and therefore its value is at hand in most probands. Enzymatic measurements of creatinine are strongly preferred.
- In some conditions listed below GFR assesment from cystatine C is recommended not instead of creatinine-based prediction but in addition to it (Insert 5). The use of combined equations from the serum concentration of both compounds is possible but not recommended because it can conceal errors from one of them or from both.
- If there is a contradiction between the creatinine-based and cystatine-based result, GFR measurement with an exact method is inevitable.

Insert 5
Recommended use of cystatin C-based GFR assesment

A. In general

In all probands with suspected kidney disease and creatinine-based GFR value near the cutoff value between stage I and stage II according to K/DOQI classification.

B. Special groups of probands and special conditions

Children under age 4
Old age
Diabetes mellitus
Hypertension
Kidney transplants
Oncological patients
In the assessment of residual renal function, patients on hemodialysis and peritoneal dialysis (37)

Note: These recommendation are not official, they reflect the opinion of the authors based on their experience in clinical chemistry, pathological physiology and internal medicine.

A different model („the lund model“) is recommended by (38) where cystatine is measured routinely in the first step together with creatinine and the diagnostic decision is made after the comparison of the results.
The interpretation of the results of GFR assessment is not only a question of the measurement accuracy and the use of proper equations. Basic knowledge on the physiology of kidney function, on biochemistry behind creatinine and cystatine C and on the possible sources of error in the assessment of GFR are the prerequisites of a correct diagnosis and proper therapy of patients with kidney diseases.
As a conclusion it is possible to state, that the assessment of GFR from cystatine C is an open chapter of clinical chemistry with bright perspectives after solving the open questions mentioned in this paper.
From both the clinicians' and the biochemists' point of view it is important to bear in mind that "creatinine" is not a snake oil and „cystatin" is not a panacea.

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