

PITFALLS IN THE MEASUREMENTS AND ASSESMENT OF GLOMERULAR FILTRATION RATE AND HOW TO ESCAPE THEM

Oliver Rácz^{1,2,4}, Ján Lepej³, Bertalan Fodor², Katarína Lepejová⁴, Peter Jarčuška⁵, Anna Kováčová⁶ and the Hepameta Study Group

¹Šafárik University, Medical School, Department of Pathological Physiology, Košice, Slovakia

²Miskolc University, Faculty of Healthcare, Department of Nanobiotechnology and Regenerative Medicine, Hungary

³Institute of Nuclear and Molecular medicine, Košice, Slovakia

⁴Cumulus Ltd., Laboratory of Clinical Chemistry, Košice, Slovakia

⁵1st Department of Internal Medicine, Šafárik University, Medical School, Košice, Slovakia; Coordinator of the Hepameta Study Group

⁶Labmed Ltd, (Medirex Group), Laboratory of Clinical Chemistry, Košice, Slovakia

Corresponding Author:

Prof. Oliver Rácz, MD, PhD.

Šafárik University, Medical School

Department of Pathological Physiology

1 SNP Avenue, SK 04066 Košice - Slovakia

Tel.: 00421 55 6423350

e-mail: olliracz@gmail.com

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

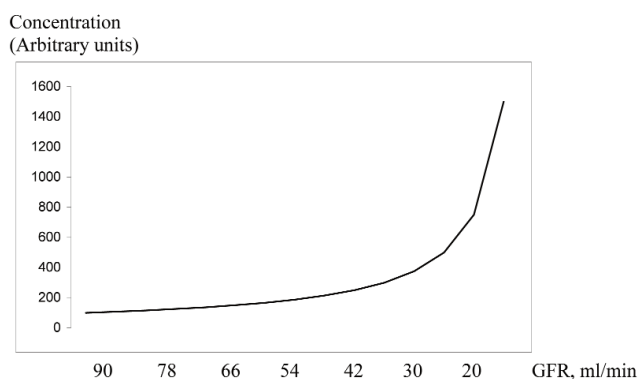


Figure 1
The increase of serum concentration of an „ideal” compound associated with gfr decrease.

| Table 1 Measurement of GFR from clearance of exogenous substances |
|--|
| A. Substances fulfilling the criteria Inulin Iohexol I-iothalamate and 125I-iothalamate ⁵¹ Cr-EDTA (ethylenediaminetetraacetic 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 concentration of the substance is decreasing. In the first phase the compound is diffusing 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 CREATININE

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 clearance. 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

| | 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 filtration! Older people with "normal creatinine" around the upper reference limit have decreased GFR according to K/DQOI criteria.

| |
|---|
| <p>A. CREATININE-BASED EQUATIONS</p> <p>1. Cockcroft and Gault (8) (age, body mass, sex) $GFR = (140 - \text{age}) * \text{body mass} / 48,8 * [S_{cre}]$ Correction for women: multiple by 0,85</p> <p>2. MDRD equations; Levey (9,10)</p> <p>2.1 (age, urea and albumin) $GFR = 170 * [S_{cre} * 0,0113]^{-0,999} * \text{age}^{-0,176} * [S_{urea} * 2,8]^{-0,17} * [S_{albumin}/10]^{0,318}$ Correction for women: multiple by 0,762</p> <p>2.2 (age and urea) $GFR = 270 * [S_{cre} * 0,0113]^{-1,007} * \text{age}^{-0,18} * [S_{urea} * 2,8]^{-0,169}$ Correction for women: multiple by 0,762</p> <p>2.3 (short form – only age and sex) $GFR = 186 * [S_{cre} * 0,0113]^{-1,154} * \text{age}^{-0,203}$ Correction for women: 0,742</p> <p>2.4 (short form for standardized creatinine – MDRD₄) $GFR = 175 * [S_{cre-stand} * 0,0113]^{-1,154} * \text{age}^{-0,203}$ Correction for women: 0,742</p> <p>3. CKD-EPI equation system; Levey (11) (age, sex, ethnicity and creatinine concetration) $GFR = 141 * [S_{cre}/\kappa]^{\alpha} * \text{age}^{-0,993} * X$ The constants „κ”, „α” and „X” are sex and ethnicity dependent and are explained in Insert 3</p> <p>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.</p> <p>In addition to the mentioned there are also other equations as of Walser (age, body mass and sex); Jelliffe (age); Nankiwell (age, body mass and height and urea). Special equations are for children (6)</p> |
| <p>B. CYSTATIN C-BASED EQUATIONS</p> <p>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</p> <p>2. Stevens (35)</p> <p>2.1 Stevens₁ (without any correction) $GFR = 76,7 * [S_{cys}]^{-1,19}$</p> <p>2.2 Stevens₂ (age, sex, ethnicity) $GFR = 127,7 * [S_{cys}]^{-1,17} * \text{age}^{-0,13}$ Correction for women: 0,91, for people with black skin: 1,06</p> <p>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)</p> |
| <p>C. COMBINED EQUATIONS</p> <p>2.3 Stevens₃ (weighted calculation from cystatin and creatinine, Correction for age a ethnicity) $GFR = 177,6 * [S_{cre}]^{-0,65} * [S_{cys}]^{-0,57} * \text{age}^{-0,2}$ Correction for women: 0,82, for people with black skin: 1,11</p> <p>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)</p> |

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 Cockcroft & Gault equation, which is today considered out of date. In tables 4 and 5 there are some model calculations and the differences between the Cockcroft & 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 towards 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

| [S _{crea}] μmol/l | Age years | Body Mass kg | GFR ml/min C&G M / W | GFR ml/min/1,73 m ² MDRD M / W | Different categorization K/DOQI |
|-----------------------------|-----------|--------------|----------------------|---|---------------------------------|
| 100 | 20 | 70 | 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 |
| 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 | 105 | 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 |
| 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.

| | [S _{crea}], 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}] ≤ 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}] ≤ 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}] ≤ 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}] ≤ 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) ^{-1,209} *0,993 ^{age} |
| #or other, not Afro-American | |
| All results as ml/min/1,73 m ² body surface area | |
| <i>Note: In contrast to the authors we don't use the term "race" because the whole mankind is one race.</i> | |

– 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 clinical chemistry regardless of their firm scientific evidence. They just switched from Cockcroft 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 m² if his weight is 65 kg (BMI = 18,4 kg/m²) and 1,87 m² if he is becoming obese and weights 85 kg (BMI = 29,4 kg/m²) 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.

ASSESSMENT 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 range 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 |
| <i>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.</i> | | | | |

| Insert 4 |
|---|
| Nonrenal factors affecting cystatin c serum concentration |
| Inflammation – association with CRP (29) |
| Corticoid treatment – increase (30) |
| Thyroid dysfunction – low in hypo-, high in hyperfunction (31) |
| Decompensated diabetes with ketonuria (32) |
| <i>Note: In some of the above mentioned conditions the association with small changes in GFR are not excluded</i> |

| TABLE 7 | | |
|---|------------------|--------------------|
| Differences in cystatine c concentration at the borderline values between different 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 |
| <i>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.</i> | | |

CREATININE OR CYSTATIN C?

In laboratory medicine such catheterical 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 against the creatinine equations. The existence of different equations is also a proof of the uncertainties in this topic.

Despite all these uncertainties 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.

References

1. Piepsz A, Colarinha P, Gordon I et al. Guidelines for glomerular filtration rate determination in children. Eur J Nucl Med 2001; 28; BP31-BP36.
2. Peronne RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: New insights into old concepts. Clin Chem 1992; 38; 1933-1953.
3. Panteghini M: Enzymatic assays for creatinine: time for action. Clin Chem Lab Med 2008; 46, 567-572.
4. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children and adolescents. Pediatr Clin North Am 1987; 34, 571-590.
5. Schück O, Engliš M, Horáčková M, Tesař V. Vyšetřovací metody (Methods of investigation). In: Tesař V et al. (Eds): Klinická nefrologie (Clinical Nephrology). Grada Avicenum Praha, 2006, 63-104. In Czech.
6. Remer T, Neubert A, Maser-Gluth C. Anthropometry based reference values for 24-h urinary creatinine excretion during growth and their use in endocrine and nutritional research. Am J Clin Nutr 2002; 75; 561-569.
7. Andersen TB, Eskild-Jensen A, Frøkiær J, Brøchner-Mortensen J. Measuring glomerular filtration rate in children: can cystatin C replace established methods? Pediatr Nephrol 2009; 24, 929-941.
8. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16, 31-41.
9. Levey AS, Bosch JP, Lewis JB et al. A more accurate method to estimate glomerular filtration from serum creatinine: a new prediction equation. Ann Intern Med 1999; 130, 461-470.
10. Levey AS, Coresh J, Greene T, et al. Expressing the Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate with Standardized Serum Creatinine Values. Clin Chem 2007; 53, 766-772.
11. Levey AS, Stevens LA, Schmid CH et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150, 604-612.
12. Miller WG. Estimating glomerular filtration rate. Clin Chem Lab Med 2009; 49. 1017-1019.
13. Cepoi V, Onofriescu M, Segall, Covic A. The prevalence of chronic kidney disease in the general population in Romania: a study of 60,000 persons. Int Urol Nephrol 2012; 44, 213-220.
14. Iliadis F, Didangelos T, Ntemka A et al. Glomerular filtration rate estimation in patients with type 2 diabetes: creatinine- or cystatin C-based equations? Diabetologia 2011; 54, 2987-2994.
15. Du X, Liu L, Hu B et al. Is the chronic kidney disease epidemiology collaboration four-level race equation better than the cystatin-C equation? Nephrology 2012; 17, 407-414.
16. Juutilainen A, Kastarinen H, Antikainen R et al. Trends in estimated kidney function: the FINRISK surveys. Eur J Epidemiol 2012; 27, 305-313.
17. Korhonen PE, Kautiainen H, Järvenpää, S, Kivelä SL. Time to change the glomerular filtration rate estimating formula in primary care? Eur J Int Med 2012; 23, 355-357.

18. Matsushita Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *JAMA* 2012; 307: 1941-1951.
19. Arora P, Rajagopalan S, Patel N et al. The MDRD equation underestimates the prevalence of CKD among blacks and overestimates the prevalence of CKD among whites compared to the CKD-EPI equation: a retrospective cohort study. *BMC Nephrology* 2012; 13, <http://www.biomedcentral.com/1471-2369/13/4>.
20. O'Callaghan C, Shine B, Lasserson DS. Chronic kidney disease: a large-scale population-based study of the effects of introducing the CKD-EPI formula for eGFR reporting. *BMJ Open* 2011; 1:e000308. doi:10.1136/bmjopen-2011-000308.
21. Delanaye P, Mariat C, Cavalier E, Krzesinski JM. Errors induced by indexing glomerular filtration rate for body surface area: reductio ad absurdum. *Nephrol Dial Transplant* 2009; 24, 3593-3596.
22. Milic R, Colombini A, Lombardi G et al. Estimation of glomerular filtration rate by MDRD equation in athletes: role of body surface area. *Eur J Appl Physiol* 2012; 112, 201-206.
23. Grubb A, Lofberg H. Human gamma-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis. *Proc Natl Acad Sci USA* 1982; 79, 3024-3027.
24. Simonsen O, Grubb A, Thysell H. The blood serum concentration of cystatin C (gamma-trace) as a measure of the glomerular filtration rate. *Scand J Clin Lab Invest* 1985; 45, 97-101.
25. Abrahamson M, Olafsson I, Palsdottir A et al. Structure and expression of the human cystatin C gene. *Biochem J* 1990; 268, 287-294.
26. Jacobsson B, Lignelid H, Bergerheim US. Transthyretin and cystatin C are catabolized in proximal tubular epithelial cells and the proteins are not useful as markers for renal cell carcinomas. *Histopathology* 1995; 26; 559-564 .
27. Grubb A, Blirup-Jensen S, Lindström V et al. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. *Clin Chem Lab Med* 2010; 48, 1619-1621.
28. Podracka L, Feber J, Lepage N, Filler G. Intra-individual variation of cystatin C and creatinine in pediatric solid organ transplant recipients. *Pediatr Transplant* 2005; 9, 28-32.
29. Knight EL, Verhave JC, Spiegelman D et al. Factors influencing serum cystatin C levels other than renal function and the impact of renal function measurement. *Kidney Internat* 2004; 65, 1416-1421.
30. Bjarnadottir M, Grubb A, Olafsson I. Promoter-mediated, dexamethasone-induced increase in cystatin C production by HeLa cells. *Scand J Clin Lab Invest* 1995; 55, 617-623.
31. Manetti L, Pardini E, Genovesi M et al. Thyroid function differently affects serum cystatin C and creatinine concentrations. *J Endocrinol Invest* 2005; 28, 346-349.
32. Holmqvist P, Torfvitt O, Sjoblad S. Metabolic status in diabetes mellitus affects markers for glomerular filtration rate. *Pediatr Nephrol* 2003; 18, 536-540.
33. Taglieri N, Koenig W, Kaski JC. Cystatin C and cardiovascular risk. *Clin Chem* 2009; 55, 1932-1942.
34. Grubb A, Nyman U, Björk J, Lindström V, Rippe B, Sterner G, Christensson A: Simple cystatin C-based prediction equations for glomerular filtration rate compared with the Modification of Diet in Renal Disease Prediction Equation for adults and the Schwartz and the Counahan-Barratt Prediction Equations for children. *Clin Chem* 2005; 51,1420-1431.
35. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, Rossert J, Van Lente F, Bruce RD III, Zhang Y, Greene T, Levey AS: Estimating GFR using cystatin C alone and in combination with serum creatinine: A pooled analysis of 3418 individuals with CKD. *Am J Kidney Dis* 2008; 51, 395-406.
36. Eriksen BO, Mathisen UD, Melsom T et al. The role of cystatin C in improving GFR estimation in the general population. *Am J Kidney Dis* 2012; 59, 32-40.
37. Filler G, Huang SHS, Lindsay RM. Residual renal function assessment with cystatine C. *Pediatr Nephrol* 2011; 26, 333-335.
38. Grubb A. Non-invasive estimation of glomerular filtration rate (GFR). The lund model: Simultaneous use of cystatin C- and creatinine-based GFR-prediction equations, clinical data and an internal quality check. *Scand J Clin Lab Invest* 2010; 70, 65-70.