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ORIGINAL ARTICLE

The Estimation of Glomerular Filtration Rate Based on the Serum Cystatin C and Creatinine Values

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SUMMARY

Background: **Renal insufficiency is one of the most serious renal diseases. Early diagnosis plays an essential role. Serum creatinine as a marker has had priority so far. The use of cystatin C for estimation of glomerular filtration rate has been recommended recently. There is a set of formulae which consolidates serum values of both creatinine and cystatin C. The study focuses on different formulae and their estimation for the purpose of better, faster, and more accurate diagnosis of renal insufficiency.**
Methods: **The sample consists of 75 examinees which are divided into three groups. The first group is made of patients with the glomerular filtration rate (GFR) 90 - 120 mL/minute/1.73m². The second group consists of patients with GFR 60 - 89 mL/minute/1.73m² and the third with GFR 30 - 59 mL/minute/1.73m². The exclusion criteria are the following: children and adolescents under the age of 20, pregnant women, patients on corticosteroid therapy and blockers of the distal tubular creatinine secretion. The five equations (GFR1-5) used in this research are: a Cockcroft-Gault equation - GFR1; a MDRD equation - GFR2; CKD-EPI - an equation based on cystatin C - GFR3; CKD-EPI - an equation based on cystatin C, adjusted according to the gender and age - GFR4; and CKD-EPI - a combined equation based on cystatin C and creatitine, adjusted according to the age, gender, and race - GFR5. Upon acquiring the results, it is followed up with the statistical data analysis followed by graphs and tables.**
Results: **After data analysis, it is established that data distribution does not show normal distribution in each case, which leads to the use of nonparametric statistics. Depending on the stage of kidney injury there are different results regarding the difference in statistics of the used formulae. The highest sensitivity is recorded with the formulae GFR4 and GFR5. Then the increase in cystatin C levels increases sensitivity with the formulae GFR3 and GFR4.**
Conclusions: **As a result of this study, it is to be established that the formula of choice is the GFR3 - CKD-EPI formula based on serum cystatin C values, without adjustments. Its sensitivity, specificity, price, and feasibility are to be observed as parameters. Besides that, the increase in serum cystatin levels leads to the increase of sensitivity of the GFR3 formula, which could be an additional factor in the selection of formulae.**
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KEY WORDS

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INTRODUCTION

Acute kidney failure (AKF) is a rapid loss of renal function which leads to a progressive increase of nitrogenous components in the blood in people whose kidneys were healthy prior to the failure. AKF is always a

consequence of another underlying harmful factor, another disease or injury, and it is never a disease in its own right [1,2].

Chronic kidney disease (CKD) is a syndrome which is caused by the gradual, progressive, and irreversible decrease in glomerular filtration until the final stage of uremia. It is characterized by the retention of the uremic toxins, changes in the volume and system of the bodily fluids and electrolytes, and the imbalance of many hormones.

For the purpose of improving early diagnosis of renal diseases, the "National Kidney Foundation" has issued a standardized clinical reference guide which recommends the glomerular filtration rate as the primary indicator of the renal function [3]. The ideal solution for the estimation of GFR should be physiologically inert, have a stable plasma concentration, be filtered at the glomerulus, not be secreted, synthesized, and reabsorbed by the kidney tubules, and be filtered from the blood just with glomerular filtration [4]. The increase in concentration of biomarkers should occur in an early stage of disorders. It should enable the monitoring of the therapeutic effect, while the decrease in concentration should occur upon the termination of a disorder. Such a biomarker which is easily measured and prognostically relevant should be determined with a simple non-invasive method [5].

Creatinine as a marker

The estimation of glomerular filtration rate based on creatinine is dependent on many factors such as gender, diet, muscle mass, and tubular secretion [6]. Despite the GFR decrease by 20 - 30%, there are no changes in the concentration of serum creatinine in an early stage of renal insufficiency, while in the later stages the considerable increase in the concentration of serum creatinine is followed by the slower GFR decrease. Such a relation is a consequence of compensatory hypertrophy and hyperfiltration of the undamaged nephrons. For these reasons, the GFR and the concentration of serum creatinine stay within the reference intervals in the beginning of a disorder. In later stages, the tubular secretion of creatinine is elevated by more than 25% compared to people with a normal GFR, which considerably adds to the uncertainty in determining the concentration of serum creatinine as a GFR biomarker [7]. Nevertheless, creatinine is the most widely used test for estimating GFR [5].

Cystatin C as a marker

Cystatin C is synthesized in all cells with a nucleus and it is present in all human body fluids. The concentration of cystatin C is not affected by inflammation, muscular mass, gender, and age. It is eliminated from blood circulation exclusively by glomerular filtration and reabsorbed and catabolized in the proximal tubules. It is important to emphasize that the cystatin C production is constant. Only few conditions which can affect its production are identified. Large doses of glucocorticoids can increase the production of cystatin C, while the small and medium doses have no effect whatsoever. Thyroid dysfunction has a huge effect [8]. For the stated reasons, cystatin C is a very reliable test for estimating glomerular filtration rate, especially in cases when the renal function starts to weaken. Its use is largely applicable to people with a moderately decreased glomerular filtration (between 80 and 40 mL/minute) and children whose clearance is difficult to determine due to the problem of collecting a urine sample and the change of body surface over the years.

This study has several aims. The main objective is to evaluate the equations for estimating glomerular filtration rate based on serum cystatin C values, serum creatinine values, and the combined equations based on the given diagnosis and to establish whether there is a significant difference in estimating glomerular filtration rate among the used formulae.

After that, the next goal is to analyze the changes in the values of serum cystatin C and to establish whether the increase of those values leads to the changes in sensitivity of the formulae used in the estimation of glomerular filtration rate. This factor would be one of the factors on the basis of which to chose the most appropriate formula for calculating GFR and, thus, to establish a more accurate profile of the therapy.

In the end, the main aim is to evaluate which method for the estimation of GFR would be the most appropriate regarding all factors together: sensitivity, complexity, and cost of the method.

MATERIALS AND METHODS

The choice of patients

The retrospective clinical study was conducted at the Clinical Center of the University of Sarajevo at the Department of Chemistry and Biochemistry. The study was comprised of seventy-five examinees which met the inclusion criteria. Medical records were used, i.e., case history which provided us with information about laboratory, clinical, and demographical data.

We used the existing data of the serum levels of cystatin C and creatinine, as well as the data of body mass, gender, age, and clinical condition, i.e., diagnosis which was not restricted by the gender and body mass but was restricted by the age. Mainly, adult patients were analyzed without including children into the study. The examinees were not necessarily diagnosed with chronic kidney disease.

The sample was divided into two groups, a control group and an experimental group. The first group was the control group with GFR values within a reference interval. In this group diagnosed diseases such as diabetes, nephrolithiasis and others were not recorded. The group consisted of 25 examinees. The experimental group was divided into two divisions each consisting of 25 examinees. The division was carried out based on the established GFR threshold. The first division of the experimental group consisted of the patients with a mildly

decreased GFR, and the second one included patients with a moderately decreased GFR. Cases of renal and endocrinological diseases were recorded in these patients. The duration of the disease and therapy were monitored in these cases.
The exclusion criteria were the following: children, and adolescents under the age of 20, pregnant women, patients on corticosteroid therapy and blockers of the distal tubular creatinine secretion such as cimetidine, trimethoprim, cefoxitin, and persons with the GFR < 29 mL/minute/1.73m².

Materials and methods of research
Blood samples from the cubital vein were centrifuged at 4000 rpm up to five minutes. Human serum or plasma was used in the research. The samples were frozen or fresh.
An *in vitro* diagnostic kit which contains reagents for quantitative determination of cystatin C with a particle-enhanced *immunonephelometry* (Dade Boehring) was used for determination of cystatin. The reference interval for cystatin C is 0.53 - 0.95 mg/L.
The serum creatinine concentration was determined by the *Jaffe method*. Reference interval for serum creatinin is 0.6 - 1.0 mg/dL for women and 0.8 - 1.3 mg/dL for men.

Equations used in the research:
- **Cockcroft-Gault equation**:
  GFR1 = [(140-age) x kg/SCr] x constant
  Constant is 1.23 for males and 1.04 for females
- **MDRD equation** [5]:
  $GFR2 = 32788 \times Scr^{-1.154} \times age^{-0.203} \times constant$
  Constant is 0.742 for females
- **CKD-EPI:** an equation based on cystatin C which was not adjusted according to gender and age:
  $GFR3 = 76.7 \times CysC^{-1.19}$
- **CKD-EPI:** an equation based on cystatin C which was adjusted according to gender and age:
  $GFR4 = 127.7 \times CysC^{-1.17} \times age^{-0.13} \times constant$
  Constant is 0.91 for females
- **CKD-EPI:** a combined equation based on cystatin C and creatitine which was adjusted according to age, gender, and race [8]:

$GFR5 = 177.6 \times SCr^{-0.65} \times CisC^{-0.57} \times age^{-0.2} \times constant$
Constant 1 is 0.80 for females; constant 2 is 1.11 for African Americans.

Statistical methods
Data preparation and storage for the statistical analysis were done in Microsoft Excel. Software package used for data processing was IBM SPSS Statistics 20.0. The obtained results are represented by tables and column charts that display quantitative data - histograms. The following statistical procedures are used in the study:
- The Kolmogorov-Smirnov and the Shapiro-Wilk tests to test normal distribution.
- Dependent *t*-test.
- The Wilcoxon test for the non-parametric statistics.
- Pearson's correlation coefficient for measuring correlation values between the results of the formulae.
- Formulae for calculating sensitivity, specificity, proportions of positive and proportions of negative results.
- ROC curves and AUC dose calculation.

RESULTS

We can see ranges of values for cystatin C in Table 1. We have compared all three groups.

Testing statistical differences among formulae
Data distribution was done with the help of two tests: the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Shapiro-Wilk test is used only if the value of Kolmogorov-Smirnov test is at the lower limit of significance. That can happen in the case of small samples or otherwise distorted data.
Since we noticed that our distribution results were not significantly different from the normal distribution, we did a correlation and *t*-test for each group.
Same procedures were done for all three groups. We used non-parametric statistics, more precisely the Willcoxon test, in cases where the data distribution was not normal.
The group which had examinees with the referential values, i.e., GFR > 90 mL/minute/1.73m², confirmed that the statistically significant differences among the used formulae exist.
The division of examinees with a moderately decreased GFR showed such results as well.
Observing the division with a mildly decreased glomerular filtration rate as a separate one, the results do not show a significant difference among the used formulae.

Testing of sensitivity among formulae
Sensitivity among the used formulae was examined using GFR = 90 mL/minute/1.73m² as a limit value. People with glomerular filtration rate above 90 mL/minute/1.73m² are considered to have no renal diseases, while GFR values below 90 mL/minute/1.73m² denote the existence of a certain disorder. Statistical calculations are done for all five formulae. The overall results are shown in Table 2.
The highest sensitivity is shown by the CKD-EPI formulae based on cystatin C, adjusted according to the age and gender (GFR4), and the second one is a CKD-EPI combined formula based on cystatin C and creatinine which is also adjusted for age and gender (GFR5). The results are presented with the ROC curve.
The figure and table show that the ROC curve for the equations GFR3, GFR4, and GFR5 have the steepest slope and pass near the top left corner, which indicates very satisfactory results in terms of the importance of these equations in the detection of renal disease. The area under the curve is highest in GFR3 and amounts to

Table 1. Ranges of values for cystatin C.

Groups of examinees	Control group	Mildly decreased GFR	Moderately decreased GFR
Cystatin C (mg/L)	0.59 - 0.87	0.86 - 1.24	1.27 - 2.88

Table 2. Presentation of results for sensitivity and specificity.

	Sensitivity	Specificity	Proportion - positive	Proportion - negative
GFR1	≈ 84%	75%	≈ 87%	≈ 69%
GFR2	≈ 86%	≈ 37%	≈ 74%	≈ 56%
GFR3	≈ 96%	100,00%	100%	≈ 92%
GFR4	≈ 98%	≈ 96%	≈ 98%	≈ 96%
GFR5	≈ 98%	≈ 71%	≈ 87%	≈ 94%

Table 2 shows: equation GFR4 detect that a patient has a disease in 98% of cases, GFR3 in 96%. GFR3 equation identified those patients who did not have a disease in 100% of cases, GFR4 in 96%.

Table 3. Area under the curve.

	Size areas	Standard error [a]	Significance [b]	Interval of confidence	
				Lower limit	Upper limit
GFR1	0.898	0.037	0.000	0.827	0.970
GFR2	0.866	0.042	0.000	0.783	0.948
GFR3	0.998	0.003	0.000	0.992	1.000
GFR4	0.996	0.004	0.000	0.988	1.000
GFR5	0.976	0.013	0.000	0.950	1.000

[a] - under nonparametric suppositions, [b] - null hypothesis: true area = 0.5.

Table 4. Presentation of results for sensitivity and specificity.

	Sensitivity	Specificity	Proportion - positive	Proportion - negative
GFR1	≈ 56%	87%	≈ 82%	≈ 65%
GFR2	≈ 76%	≈ 62%	≈ 68%	≈ 71%
GFR3	≈ 100%	≈ 96%	≈ 95%	100%
GFR4	100%	≈ 83%	≈ 86%	100%
GFR5	≈ 96%	≈ 71%	≈ 77%	≈ 94%

Table 5. Area under the curve.

	Size areas	Standard error [a]	Significance [b]	Interval of confidence	
				Lower limit	Upper limit
GFR1	0.794	0.066	0.000	0.664	0.923
GFR2	0.790	0.066	0.000	0.660	0.921
GFR3	0.979	0.021	0.000	0.938	1.000
GFR4	0.990	0.009	0.000	0.973	1.000
GFR5	0.942	0.031	0.000	0.882	1.000

a - under nonparametric suppositions, b - null hypothesis: true area = 0.5.

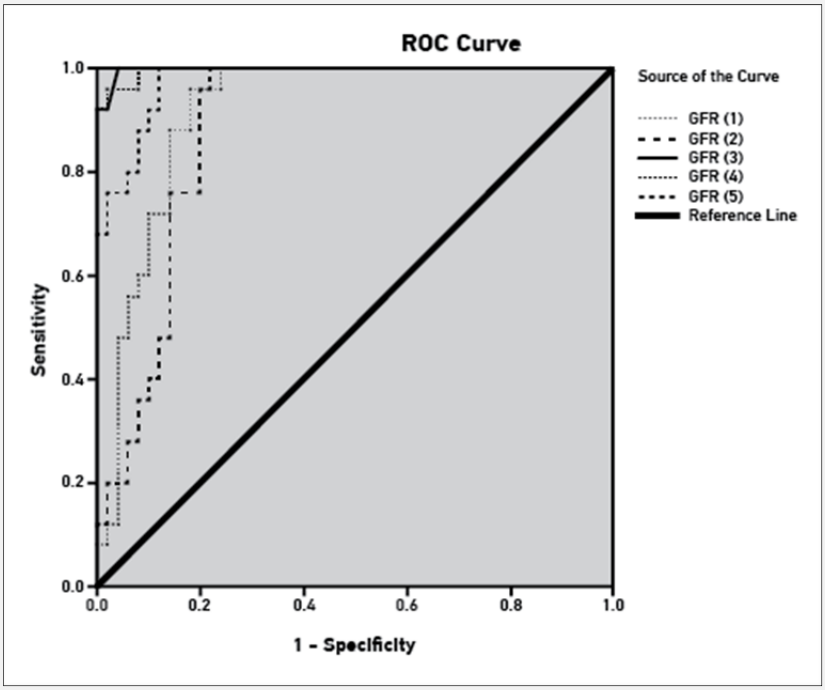

Figure 1. ROC curve.

0.998.

Testing of cystatin C-impact at the sensitivity of formulae

Our next goal was to prove whether the increase of cystatin C level changed the sensitivity of the formulae used. For this reason, in this part of study, we used only the last two groups, those with mildly and moderately decreased glomerular filtration rate. For comparison we used values obtained from previous measurements. Since "sensitivity" is one of the parameters that we use to select the most appropriate formula, confirmation of this thesis would have significance in that part and in practice, as well. In clinical practice we could choose a more accurate formula for patients with advanced-stage of renal insuficiency. Therefore, it would be important for the therapeutical profile.

The analysis was based on the turning point, which was 60 mL/minute/1.73m². The table 4 shows results for all five formulae.

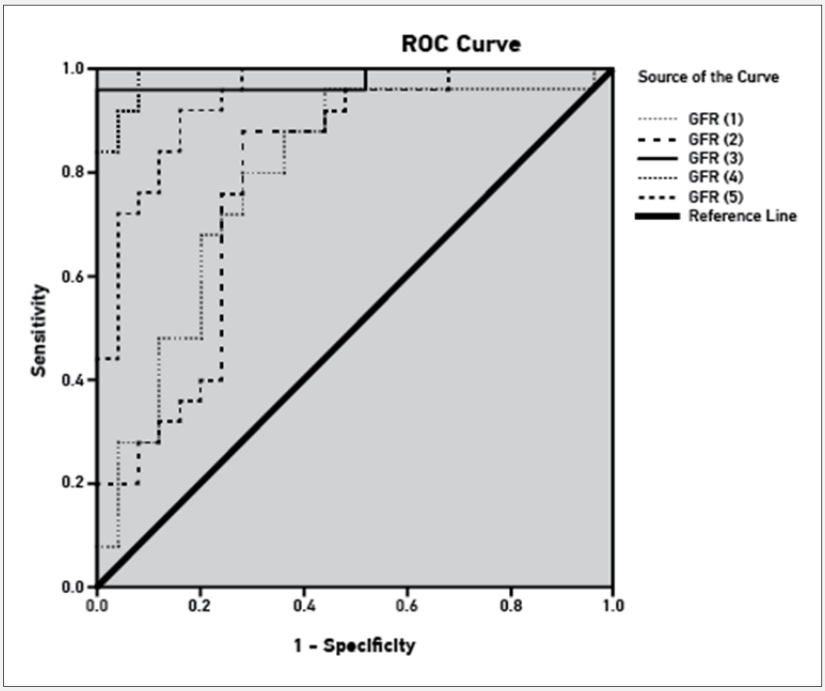

Figure 2. ROC curve.

Formulae GFR3 and GFR4 show the highest level of sensitivity which is 100%. Sensitivity for these formulae is increased compared to the previous part. We must emphasize that the higher increase among these two formulae is the CKD-EPI formula based on cystatin C without adjustments. The increase of 4% is recorded. For the GFR4 formula the increase is 2%. Other formulae do not show a corresponding increase in sensitivity (Table 4).

All five equations have a very high value of area under the curve and can be used to detect GFR. However the best results are shown with GFR3, GFR4, and GFR5.

DISCUSSION

Six hundred million people around the world suffer from chronic kidney disease and the prevalence is rising [10]. It is considered that 30% of older population suffers from some kind of chronic kidney disease. Apart from age, diabetes, cardiovascular diseases, and hypertension are related to the increased risk of CKD [11]. In July 2013, the researchers estimated that 10% of world population is affected by CKD. CKD is also recognized as an independent risk factor for cardiovascular diseases and mortality [12].

It is necessary to detect CKD as early as possible for the purpose of discovering early disorders. It would enable timely action and prevent irreversible changes in renal function.

New research has established that the use of serum cystatin C, alone or combined with the serum creatinine, shows a stronger connection between kidney diseases and the risk of death and the risk of final stages of renal disorders. The results published in the study by Shlipak M et al. indicate that the use of this biomarker can lead to a better CKD risk classification [13].

A certain number of studies show that the differences in using the mentioned formulae indeed exist. Studies by Grubb et al. [14] state that serum cystatin-based formulae have a greater precision in estimating GFR compared to a C&G formula. They used formulae without adjusting them according to age and gender and they demonstrated high precision and small bias. When they included a formula with adjustments into the research the results were even better. In our research those formulae are GFR3 and GFR4. They have showed very good results in estimating GFR in our research as well. The C&G formula and CKD-EPI cystatin-based formula with adjustments have shown the greatest difference in our study in a group with mildly decreased GFR. The difference between the C&G formula and CKD-

EPI cystatin-based formula, with or without adjustments, was shown in the group with moderately decreased GFR. Also, the MDRD formula has shown the difference compared to cystatin-based formulae, except for one which is CKD-EPI cystatin formula, without adjustments [14].

Hoek et al. reached the conclusion about the higher precision of cystatin C formula in comparison to other formulae. The conclusion shows cystatin C as the most important endogenous marker for the estimation of GFR in all the data sets. The results based on cystatin C signal disorders in their early stages. At that stage, the results for creatinine are still within the referential interval. In our group with mildly decreased GFR there is indeed a statistically significant difference between the C&G creatinine-based formulae and other formulae. However, a certain relation which does not show that difference occurs and it is between GFR3, i.e., CKD-EPI cystatin-based formula without adjustments, and the C&G formula. This relates to one drawback of our study, the inability to use a gold standard, inulin or 125I io thalamate [15].

In the second part of our study, it can be said that the combined formula, GFR5, certainly gives the highest sensitivity together with formula GFR4. The results for GFR3 are not far behind. Specificity for the GFR3 formula is actually 100%, while for the combined formula is only 71%. Also, one more parameter which indicates that the cystatin C formula without adjustments obtains the best results is the proportion of positive and negative results. Then, we have to say as well that in daily practice it is easier and more economical to work with a single parameter.

Considering the fact that our aim was "to evaluate which method for the estimation of GFR would be the most appropriate regarding sensitivity, complexity and cost", we have concluded that CKD-EPI cystatin C-based formula without adjustment is the most appropriate for the estimation of GFR.

In the third part we calculated the sensitivity and specificity for all formulae for patients with mildly and moderately reduced glomerular filtration rate. The goal was to prove that there is a change in the percentage of sensitivity of formulae with an increase in cystatin C level. That would show which formula should be the formula of choice for patients with advanced stage of the disease. These two groups have higher serum levels of the monitored parameter compared to the control group. For this reason, we examined these two groups and compared them with the results of the previous section. Formulae GFR3 and GFR4 showed the greatest degree of sensitivity of 100%. The sensitivity of this formula is increased compared to the previous part. Larger increases between these two formulae are shown with the formula based on cystatin C, without adjustment, GFR3. Increased sensitivity in formulae based on creatinine was not recorded.

Behring and Wagner in their study talk about creatinine as a marker which is far from being perfect in relation to sensitivity in early stages of this disease. Those ranges are called "creatinine-blind ranges". Such ranges do not exist for cystatin C [16].

The study which focuses on older population, uses a simple cystatin C formula and compares it with other sophisticated formulae, confirming the conclusion that we have also reached [17].

In our study, as mentioned before, focusing on the sensitivity, the cystatin-based formulae are the formulae of our choice. Creatinine-based formulae do not show changes in sensitivity levels even in cases with more serious stages of disease. Even the CKD-EPI combined formula shows only a slight decrease in the percentage of the sensitivity.

CONCLUSION

- The introduction of cystatin C as a marker into clinical use facilitates diagnosis, classification, and progression monitoring of chronic kidney disease.
- The study shows that statistically significant differences among formulae used for glomerular filtration rate detection are evident.
- The results of the study have shown that cystatin C is a biomarker with the most potential in early CKD detection and is detectable in the "creatinine blind range" which adds to the reliability of the early diagnosis of this disease.
- Cystatin C is a highly sensitive and specific parameter for CKD which has been proven by all the used formulae.
- According to the results of the study and taking into account a vast number of parameters, a cystatin C equation, without adjustments (GFR3) is the most appropriate equation to be used in clinical practice.
- The increase in serum cystatin levels leads to the increase of sensitivity of the formulae based exclusively on cystatin, and the highest increase is recorded by the GFR3 formula, which could be an additional factor in the selection of formulae.

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Declaration of Interest:
The author declares that there is no conflict of interest.

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