

## THE DIAGNOSTIC VALUE OF RESCALED RENAL BIOMARKERS SERUM CREATININE AND SERUM CYSTATIN C AND THEIR RELATION WITH MEASURED GLOMERULAR FILTRATION RATE

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### ABSTRACT

**Background:** Serum creatinine (Scr) is the major contributing variable in glomerular filtration rate (GFR) estimating equations. Serum cystatin C (ScysC) based GFR estimating (eGFR)-equations have also been developed. The present study investigates the relation between 'rescaled' levels of these renal biomarkers (with reference interval of [0.67-1.33]) and measured GFR (mGFR).

**Methods:** We evaluated the diagnostic ability to detect impaired kidney function of the rescaled renal biomarkers in 8584 subjects from 12 cohorts with measured GFR, standardized Scr and ScysC. We calculated sensitivity and specificity of the rescaled biomarkers to identify kidney disease, with reference to a fixed (60mL/min/1.73m<sup>2</sup>) as well as an age-dependent threshold for mGFR.

**Results:** The upper reference limit of 1.33 for rescaled renal biomarkers is closely related to the age-dependent threshold for defining kidney status by mGFR with sensitivity and specificity for the rescaled biomarkers close to 90% for all ages. If the fixed threshold of 60mL/min/1.73m<sup>2</sup> for mGFR is used, then lower specificity in children and sensitivity in older adults are observed.

**Conclusions:** Impaired kidney function can be diagnosed by rescaled renal biomarkers instead of eGFR-equations using the fixed threshold of 1.33 for all ages, consistent with an age-dependent threshold of mGFR.

## 1. INTRODUCTION

Serum creatinine (Scr) is the most widely used renal biomarker and can be measured using assays that are specific (e.g. enzymatic assays) and calibrated against the reference method of isotope dilution mass spectrometry (IDMS). The test is relatively cheap and commonly ordered as part of a basic investigation panel in clinical biochemistry. However, Scr has some limitations as a renal marker: Scr is influenced by non-GFR determinants, e.g. muscle mass, and part of renal clearance of creatinine is due to tubular secretion [1]. Moreover, Scr remains within the reference interval in a substantial proportion of patients despite a reduced ( $< 60\text{mL}/\text{min}/1.73\text{m}^2$ ) glomerular filtration rate (GFR) [2-3] leading to doubts about Scr's ability to detect impaired kidney function [4-9]. Also, there are age and gender differences in creatinine generation which complicates the interpretation of Scr (as well as Scr-based eGFR) when assessing kidney function. For Caucasians, the determination of reference intervals has been the subject of detailed studies [10-11]. After birth, Scr rapidly decreases to a value of approximately  $22\mu\text{mol}/\text{L}$  ( $0.25\text{mg}/\text{dL}$ ) during the first month of life and then starts to increase linearly with age. Scr concentration remains constant, on average, for the healthy subject between 20 and 70 years of age, with a mean of  $80\mu\text{mol}/\text{L}$  ( $0.90\text{mg}/\text{dL}$ ) and reference interval  $55.7\text{-}102.5\mu\text{mol}/\text{L}$  ( $0.63\text{-}1.16\text{mg}/\text{dL}$ ) for men and  $62\mu\text{mol}/\text{L}$  ( $0.70\text{mg}/\text{dL}$ ) and reference interval  $42.4\text{-}82.2\mu\text{mol}/\text{L}$  ( $0.48\text{-}0.93\text{mg}/\text{dL}$ ) for women. Above the age of 70, Scr starts to slowly increase again in both genders [12].

To simplify the interpretation of Scr, Pottel et al [13-19] proposed to 'normalize' Scr. Normalization is a statistical term that means adjusting values measured on different scales to a notionally common scale. Indeed, dividing Scr with the mean Scr-value (called  $Q_{\text{crea}}$ ) of the age and sex specific healthy population rescales the reference intervals to a common interval as it makes  $\text{Scr}/Q_{\text{crea}}$  independent of age/sex. Consequently the rescaled biomarker  $\text{Scr}/Q_{\text{crea}}$  may serve as a renal biomarker incorporating its limitations due to muscle mass variation, related to age and sex differences. Due to the 'rescaling/normalizing' action, the values of  $\text{Scr}/Q_{\text{crea}}$  become normally distributed around the mean of '1' (a consequence of the definition of  $Q_{\text{crea}}$ ) with 2.5<sup>th</sup> percentile (Pct) =0.67 and 97.5<sup>th</sup> Pct=1.33, (corresponding to a standard deviation of 0.1683). This holds equally true for children, adolescents and adults [16].

Serum cystatin C (ScysC) is considered a valid alternative to Scr, especially now that a certified reference material is available [20]. ScysC can be rescaled more easily than Scr, as ScysC is almost independent of age and sex [21-22]. Only in older adults we have seen a gradual but significant increase in ScysC-concentration with age [19], most probably due to a decline in mGFR and additionally influenced by non-GFR related determinants, such as cardiovascular disease risk factors and inflammation [23-25]. Rescaling ScysC with  $Q_{\text{cysc}}=0.82\text{mg}/\text{L}$  for children and adults up to 70 years and with  $Q_{\text{cysc}}=0.95\text{mg}/\text{L}$  beyond that age brings the rescaled  $\text{ScysC}/Q_{\text{cysc}}$  to the same common scale as  $\text{Scr}/Q_{\text{crea}}$  [19].

In this study we show that rescaled renal biomarkers contain important diagnostic information about kidney function that can easily be interpreted in terms of 'normal' (healthy) or 'abnormal' (diseased) using the upper limit of 1.33 (=97.5<sup>th</sup>Pct). We also wanted to investigate how mGFR evolved with age in the subgroup defined by renal biomarkers  $<1.33$ . Finally, we wanted to check the consistency between this fixed reference interval [0.67-1.33] to diagnose kidney function in terms of normal/abnormal and the use of a fixed or age-dependent threshold for mGFR.

## 2. METHODS AND PARTICIPANTS

### 2.1. Participants

The current retrospective analysis collected data from 12 cohort studies. Among the 12 cohorts five [25-29] prospectively investigated the use of Scr and ScysC-based eGFR-equations for diagnosing chronic kidney disease. Eleven of the 12 cohorts, including  $n=6132$  subjects, have been presented in detail elsewhere [19]. An additional cohort of healthy and kidney diseased subjects from Slovenia ( $n=134$ ), has been included to increase the sample size for the [20-40] year age-range. The paediatric data from Lyon ( $n=259$  children with 695 measurements) and adult data from the CRIC cohort ( $n=674$  adults with 1534 measurements) were also included in the current analysis. An additional number of adult patients from Paris and from Lyon were included to increase the sample size, especially for the [20-40] year age range. The data were anonymized and centralized for this study, which received IRB approval from Leuven University Hospital, Belgium. In total  $n=8584$  participants, with data for mGFR,

standardized Scr and ScysC, age and gender were included.

## 2.2. Measurement methods

A summary of the methods used in the different collaborating centres is given in Table 1 and have been previously described (except for Slovenia) in detail [19]. Direct GFR measurement was performed with different reference methods, as described before [18-19]. Scr was measured with assays traceable to IDMS, re-calculated to IDMS (CRIC study), or directly with IDMS [27] in all studies. ScysC was measured with assays that were calibrated against the certified ERM-DA471/IFCC standard or the results were recalculated to the certified reference standard [30]. The rescaling values for ScysC have been introduced in a previous study [19] and are further justified reference [31].

## 2.3. Statistical analysis

The data are presented as means and standard deviations or with 95% confidence intervals. Sensitivity (S) and specificity (Sp) are defined as:

$$S = \frac{TP}{TP+FN} \quad \text{and} \quad S_p = \frac{TN}{TN+FP}$$

With TP = True Positive, TN = True Negative, FP = False Positive and FN = False Negative.

To define TP, TN, FP and FN, the healthy and diseased groups have to be defined, as well as the positivity and negativity of the test result. For this analysis, we used the total cohort of 8584 subjects, subdivided in age-decades. We here present three different scenarios:

In the first scenario, measured GFR is used to define the kidney status in terms of 'healthy' or 'diseased' based on the currently recommended fixed cutoff ( $CO_F$ ) of 60mL/min/1.73m<sup>2</sup>. In this scenario, a true positive (TP) test result is obtained when  $(Scr/Q_{crea}+ScysC/Q_{cysC})/2 > 1.33$  in the subgroup with  $mGFR < CO_F = 60\text{mL/min/1.73m}^2$ . A true negative (TN) test result is obtained when  $(Scr/Q_{crea}+ScysC/Q_{cysC})/2 < 1.33$  in the subgroup with  $mGFR > CO_F = 60\text{mL/min/1.73m}^2$ . We here combine the rescaled biomarkers by taking the mean of both into one criterion. We can do this, as both rescaled biomarkers are evaluated on the same common "normalized" scale. It has been shown recently that using the combination of biomarkers increases the precision of the eGFR-equation significantly [18-19, 26, 32-33]. Therefore, diagnosing kidney disease based on the combined rescaled biomarkers is likely to be more accurate. Additionally, we have repeated this analysis for the single rescaled biomarkers in reference [31].

In the second scenario, measured GFR is used to define the kidney status in terms of 'healthy' or 'diseased' based on an age-dependent threshold or cut-off ( $CO_{AD}$ ), which we here define as:  
 $CO_{AD} = 107.3/1.33 [ \times 0.988^{(Age-40)} \text{ when Age} > 40 \text{ years} ]$

This threshold equals  $107.3/1.33 = 80.7\text{mL/min/1.73 m}^2$  for ages <40 years, and then the threshold gradually declines with age (e.g. at the age of 65 years, the threshold becomes  $59.7 \approx 60\text{mL/min/1.73m}^2$ ; at the age of 80 years, the threshold becomes  $49.8 \approx 50\text{mL/min/1.73m}^2$ ). The decrease in GFR with age has been well described even in healthy subjects [34-36]. The age-dependent threshold, presented here, is based on the FAS-equation with  $Scr/Q_{crea} = ScysC/Q_{cysC} = 1.33$ , the upper reference limit for the rescaled renal biomarkers [18-19] and has been introduced as an age-dependent threshold for GFR in a recent publication [37].

TP is here defined as  $(Scr/Q_{crea}+ScysC/Q_{cysC})/2 > 1.33$  in the subgroup with  $mGFR < CO_{AD}$ . TN is defined as  $(Scr/Q_{crea}+ScysC/Q_{cysC})/2 < 1.33$  in the subgroup with  $mGFR \geq CO_{AD}$ .

In the third scenario, we define kidney status in terms of 'healthy' or 'diseased' based on the rescaled renal biomarkers, which is the setting closest to clinical routine, as mGFR is usually not available. The kidney function is considered 'normal', or 'healthy', when the renal biomarkers are within the reference interval, or, when the average of both rescaled renal biomarkers is  $< 1.33$ . Thus, when  $(Scr/Q_{crea}+ScysC/Q_{cysC})/2 > 1.33$ , the kidney function is considered 'abnormal' or 'diseased'. To define TP and TN we now use mGFR (which is here available for the purpose of the study) as the reconfirming test result. As in the first and second scenario, we can use the fixed threshold of 60mL/min/1.73m<sup>2</sup> and the age-dependent threshold  $CO_{AD}$ . In other words, in the third scenario we turn things around by changing the roles of mGFR and the biomarkers in the definitions of TP, TN, FP and FN. Hereby we want to test the hypothesis whether biomarkers and mGFR can be used

interchangeably to diagnose and define kidney status. Therefore, we evaluate S and Sp versus age for the scenarios 1 compared to scenario 3 (with fixed cutoff for mGFR), and for scenario 2 versus scenario 3 (with age-dependent cutoff for mGFR). All analysis were done in SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

### 3. RESULTS

#### 3.1. Patient characteristics

Mean ( $\pm$ SD) for age, mGFR, rescaled Scr and ScysC are summarized for all individuals of the twelve contributing cohorts in Table 2.

#### 3.2. Rescaled renal biomarkers within the reference interval and their relation with mean measured GFR

There were  $n=4749$  subjects exhibiting both rescaled biomarkers within the common reference interval [0.67-1.33]. They had a mean age of 55.0 years (SD=17.8) and mean mGFR of 88.6 (SD=19.6) mL/min/1.73m<sup>2</sup>. Table 3 presents an overview of the results per age-decade for this subgroup of patients. We also observe overall mean rescaled biomarker values close to '1.00', in each age-group, corresponding to the mean rescaled biomarker value for healthy people. Interestingly, the mean mGFRs in the different age-decades are very similar to the results described in the meta-analysis in healthy potential kidney donors [38].

In the next part of the results section we test the possibility to interchangeably use the renal biomarkers with fixed threshold of 1.33 and measured GFR with fixed threshold of 60mL/min/1.73m<sup>2</sup> or with the previously defined age-dependent threshold.

#### 3.3. Disease status defined by fixed mGFR threshold of 60mL/min/1.73m<sup>2</sup>

We here combine the first scenario (=fixed threshold for mGFR) with the third scenario (=fixed threshold for rescaled renal biomarkers). We present the frequency of observations with renal biomarkers below and above 1.33 in the subgroups defined by mGFR < and >60mL/min/1.73m<sup>2</sup> (Table 4 and Figure 1).

For children and older adults, sensitivity and specificity deviate largely depending on the definition of kidney status (either based on the rescaled renal biomarkers or on the fixed threshold of mGFR). For example, for children aged between 2 and 10 years, all patients with mGFR <60 have biomarker values >1.33 (S=28/28=100%), but only about 159 out of 218 (Sp = 73%) children with mGFR  $\geq$ 60 have biomarker values <1.33. Conversely, in older adults, aged 80-90, we observe that 182/(94+182) (S=66%) of the patients with mGFR <60 have biomarker values >1.33 and 153/(153+4) (Sp=97.5%) with mGFR  $\geq$ 60 having biomarker values  $\leq$ 1.33. This analysis shows the lack of consistency or agreement in defining or diagnosing kidney disease based on the rescaled renal biomarker reference intervals and measured GFR combined with the fixed threshold of 60mL/min/1.73m<sup>2</sup>.

#### 3.4. Disease status defined by an age-dependent threshold of mGFR

Here, we combine the second scenario (=age-dependent threshold for mGFR) with the third scenario (=fixed threshold for the normalized renal biomarkers). We present the frequency of observations with normal and abnormal renal biomarkers in the subgroups defined by mGFR < and  $\geq$ CO<sub>AD</sub> (Table 4). Sensitivity and specificity are nearly equal for all age-categories and close to 90% (Figure 2). This observation shows that when an age-dependent mGFR threshold is used to define the renal status as healthy or diseased, rescaled renal biomarkers and measured GFR give consistent results.

### 4. DISCUSSION

In this study we have evaluated the diagnostic value of Scr and ScysC as single renal biomarkers (see also the reference [31]) and by combining them. We used reference intervals of both renal biomarkers to diagnose kidney function as 'healthy' or 'diseased'. This was done by rescaling the biomarkers with appropriate rescaling factors: Q<sub>crea</sub> for Scr and Q<sub>cysC</sub> for ScysC. Rescaling renal biomarkers reveals several interesting and important properties, which are valid for both Scr/Q<sub>crea</sub> and ScysC/Q<sub>cysC</sub>. The rescaled biomarkers are independent of age and sex, and can thus be used for children, adolescents, adults and older adults. The rescaled biomarker is normally distributed with a mean of '1'. Therefore, the rescaled biomarker can, on average, be interpreted as follows: the further the value deviates from '1' the more the patient's kidney function deviates from the kidney function of the average healthy

subject. Outside the reference interval [0.67-1.33] the rescaled biomarker values are abnormal. Our analysis shows that in the subgroup of patients with rescaled biomarkers within the range of [0.67-1.33], the mean measured GFR remains constant until the age of 40 years and then starts to decline with age. The identical phenomenon, that measured GFR remains constant until 40 years of age, and then starts to decline, was also observed in several studies with mGFR in healthy living kidney donors [39-43] and summarized in a recent meta-analysis on mGFR of living kidney donors, without information on renal biomarkers [38]. The fact that these 'healthy' mGFR-age profiles, are identical in the current study and in the previous meta-analysis, shows that 'healthy' kidney function can be diagnosed by rescaled biomarkers within the reference range. Interestingly, the rescaled biomarkers in the 'healthy' subgroup of the current analysis do not show an age-dependency, but remain approximately constant and, on average, equal to '1' during the complete lifetime. The present data therefore reconfirm the hypothesis that rescaled biomarkers are independent of age and gender (see also reference [31] for more detailed information on rescaled renal biomarkers and their relation with measured GFR).

Measured GFR is the reference method to define kidney function. However, there is controversy about the thresholds to define 'healthy' or 'diseased' status of the kidney [34-36]. A necessary requirement of the current recommendation for CKD Stage 3 is that mGFR is  $< 60\text{mL}/\text{min}/1.73\text{m}^2$ . In clinical practice, measured GFR is mostly unknown, as measuring GFR is not routinely performed. On the other hand, biomarkers are more easily obtained as this requires only one blood sample of the patient. As reference intervals of these rescaled biomarkers are available ([0.67-1.33]) it could be expected that these would correlate well with reference intervals for measured GFR. In the present study, we have confirmed that this is indeed the case, on condition that reference intervals for measured GFR are age-dependent. The present analysis shows that individuals above the age of 40 years, with biomarkers within the reference range of [0.67 - 1.33], have measured GFR that declines with age.

Using biomarkers instead of mGFR to detect impaired kidney function would imply that sensitivity (S) and specificity (Sp), based on disease status as defined by the renal biomarkers, or as defined by measured GFR, are similar on average, when both definitions are used interchangeably. Ideally, S and Sp should be 100% and it should not make a difference which criterion (biomarkers or mGFR) is used to define kidney function (healthy or diseased) and which criterion is used to define the test result (positive or negative, based on a threshold). We have here demonstrated in a population of 8584 individuals aged 1 to 95 that this interchangeability is true, with S and Sp close to 90% for all age-groups, when an age-dependent threshold for mGFR is used. We also evaluated the correspondence between the renal biomarker threshold of 1.33 and the measured GFR threshold of  $60\text{mL}/\text{min}/1.73\text{m}^2$ . We observed large deviations between S (or Sp) for children and for older adults. This confirms that the use of renal biomarkers and mGFR do not correspond well when a fixed mGFR threshold for the definition of kidney disease is used, in agreement with earlier suggestions for an age-calibrated definition of mGFR-categories [34,44]. When the here defined age-dependent threshold for mGFR is used, the S and Sp remain close to 90% for all ages, including children and older adults. The fact that S and Sp do not equal 100% is most likely due to variability in the measured GFR results (different methods), variability in the biomarker assay results (different tests, intra-test variability) and non-GFR determinants.

The major strengths of our study are the large sample size and wide age-range, allowing statistical analysis per age-decade, the wide variety of measured GFR methods, and the use of calibrated assays for both biomarkers. Several limitations deserve attention. First, the participating study cohorts included only white participants. Second, we were not able to detect the influence of non-GFR determinants on the rescaled biomarkers' performance to diagnose renal impairment on an individual basis. Third, in contrast to measured GFR, rescaled/normalized biomarkers are not able to detect renal hyperfiltration. Fourth, in special populations like patients with abnormal muscle mass, chronic inflammation, under long-term steroid therapy, with oncologic diseases that go along with high cell turn-over, rescaled biomarkers have the same downside like traditional (non-rescaled) biomarkers or estimating equations and mGFR should be considered.

This multi-cohort study allows to determine general trends but physicians should always consider the individual patient's characteristics when making a final decision about his/her renal function [45]. We would also like to emphasize that our current work is focusing on 'detection' of impaired renal function, not on estimating GFR.

## 5. CONCLUSION

There is a consistent correspondence between rescaled biomarkers within the reference interval and the course of mean mGFR with age in healthy subjects, highlighting the fact that from the age of 40

years onwards mean mGFR in healthy subjects declines with age. The rescaled renal biomarkers show excellent diagnostic performance to detect impaired kidney function, across all ages, corresponding with an age-dependent threshold for mGFR .

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## CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

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## TABLES AND FIGURES

**Table 1:** Overview of the methods for mGFR, Scr and ScysC in the 12 cohorts included for analysis

Origin	mGFR	Scr	SCysC
Leuven, Belgium	<sup>51</sup> Cr-EDTA (4 points)	Creatinine Plus, Roche enzym.	Roche PETIA (Tina quant ® Gen2)
Lyon, France (2 cohorts)	Inulin* iohexol (3 points)	Creatinine Plus, Roche enzym.	Siemens PENIA
Maribor, Slovenia	<sup>51</sup> Cr-EDTA (4 points)	Creatinine Plus, Roche enzym.	Siemens PENIA
Saint-Etienne, France	iohexol (2 points)	Enzymatic, Orthoclinical Diagn.	Siemens PENIA [29]
Paris, France	<sup>51</sup> Cr-EDTA*	Enzymatic, Siemens	Siemens PENIA
CRIC, USA	<sup>125</sup> I-iothalamate*	Calculated back to Creatinine Plus, Roche enzym.	Calculated back to Siemens PENIA
Troms0, Norway	iohexol (1 point)	Creatinine Plus, Roche enzym	Gentian PETIA [28]
Rochester, USA (2 cohorts)	iothalamate*	Creatinine Plus, Roche enzym.	Siemens PENIA [25]
Berlin, Germany	iohexol (8 points)	Creatinine Plus, Roche enzym.	Siemens N-Latex® PENIA [26]
Kent, UK	iohexol (3 points)	IDMS	Siemens PENIA [27]

For mGFR, \* = urinary and plasma clearance, all other methods are plasma clearance only. mGFR = measured glomerular filtration rate; Scr = serum creatinine; ScysC = serum cystatin C

**Table 2:** summary of patient characteristics of the 12 cohorts (Mean±SD) ordered by mean age

Data Origin	N	Age (years)	mGFR (mL/min/1.73m <sup>2</sup> )	Scr/Q <sub>crea</sub>	ScysC/Q <sub>cysc</sub>
Leuven (Belgium)	114	8.8 ±5.5	89.2 ±21.5	1.17 ±0.57	1.22 ±0.42
Lyon (France)	695	12.7 ±3.8	84.7 ±32.7	1.37 ±0.60	1.52 ±0.62
Maribor (Slovenia)	134	30.5 ±6.9	75.8 ±46.5	2.40 ±2.13	1.91 ±4.41
Saint-Etienne (France)	203	48.7 ± 10.3	94.7 ± 24.4	1.01 ±0.22	1.09 ±0.28
Paris (France)	1371	51.9 ± 14.4	65.5 ±27.4	1.62 ±0.90	1.65 ±0.90
Lyon (France)	852	51.8 ±14.4	77.5 ±29.3	1.29 ±0.61	1.37 ±0.64
CRIC (USA)	1534	58.6 ± 12.3	49.9 ±22.2	2.02 ±0.76	1.71 ±0.67
Troms0 (Norway)	1627	58.1 ±3.8	91.7 ±14.4	0.95 ±0.14	0.90 ±0.14
Rochester CKD (USA)	687	64.8 ± 8.8	80.4 ±21.3	1.08 ±0.26	1.01 ±0.26
Rochester KFC (USA)	406	65.9 ±9.2	79.5 ±20.7	1.05 ±0.19	0.97 ±0.20
Berlin (Germany)	567	78.5 ±6.2	60.3 ±16.4	1.22 ±0.43	1.21 ±1.11
Kent (UK)	394	80.4 ±4.6	51.5 ±18.8	1.62 ±0.75	1.52 ±0.64
<b>Total</b>	<b>8584</b>				

N = total number of participants; mGFR = measured glomerular filtration rate; Scr = serum creatinine; (Q<sub>crea</sub> = normalization factor for Scr; ScysC = serum cystatin C; Q<sub>cysc</sub> = normalization factor for ScysC

**Table 3:** overview of the mean mGFR, mean rescaled Scr and mean rescaled ScysC (with 95% CI) for the subjects with both biomarker values within the reference interval.

n	n	Age	Mean	Scr/Q <sub>crea</sub>	ScysC/Q <sub>cysc</sub>	mGFR	Meta-analysis [38]
Total	"normal"	(years)	Age			(mL/min/1.73m <sup>2</sup> )	mGFR(mL/min/1.73 m <sup>2</sup> )
		(years)	(years)				



246	135	[2-10[	6.5	0.99	[0.96-1.02]	1.07	[1.05-1.10]	107.0	[103.2-110.9]		
599	245	[10-20[	14.7	0.99	[0.97-1.01]	1.07	[1.05-1.09]	104.6	[101.7-107.5]		
227	113	[20-30[	25.6	1.00	[0.97-1.04]	1.00	[0.97-1.03]	100.3	[96.4-104.2]	106.7	[104.6-108.9]
539	277	[30-40[	35.0	1.01	[0.99-1.02]	0.95	[0.94-0.97]	102.4	[100.2-104.6]	104.9	[102.8-107.0]
801	407	[40-50[	44.9	1.01	[0.99-1.03]	0.97	[0.96-0.99]	95.6	[93.9-97.3]	99.0	[96.5-101.6]
2131	1474	[50-60[	55.3	0.97	[0.96-0.98]	0.92	[0.91-0.93]	91.8	[91.0-92.6]	91.1	[88.2-94.1]
2271	1255	[60-70[	63.4	0.99	[0.98-1.00]	0.97	[0.96-0.97]	85.8	[84.9-86.7]	84.0	[79.5-88.5]
1285	618	[70-80[	74.6	1.04	[1.03-1.05]	0.97	[0.96-0.98]	72.1	[71.0-73.1]	69.4	[66.1-72.7]
433	211	[80-90[	83.1	1.05	[1.03-1.07]	1.05	[1.03-1.07]	65.6	[63.9-67.2]		
52	14	>90	91.9	1.07	[0.98-1.16]	1.18	[1.12-1.23]	52.7	[47.6-57.7]		
8584	4749										

n "normal" = number of subjects with both rescaled biomarkers within the common reference interval of [0.67 - 1.33]. mGFR = measured glomerular filtration rate; Scr = serum creatinine; (Q<sub>crea</sub> = normalization factor for Scr; ScysC = serum cystatin C; Q<sub>cysC</sub> = normalization factor for ScysC;

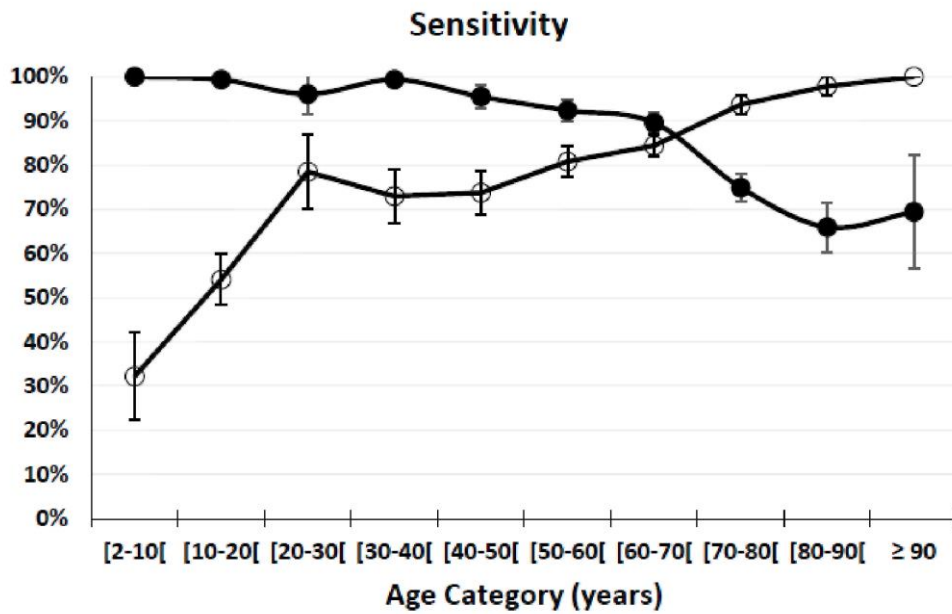
**Table 4:** Frequency of patients with rescaled biomarkers below and above 1.33 in the subgroups defined by mGFR (fixed and age-dependent threshold CO<sub>AD</sub>)

Age Group	(Scr/Q <sub>crea</sub> +ScysC/Q <sub>cysC</sub> )/2 <1.33					(Scr/Q <sub>crea</sub> +ScysC/Q <sub>cysC</sub> )/2 >1.33					Grand Total
	mGFR <60	mGFR >60	mGFR <CO <sub>AD</sub>	mGFR ≥CO <sub>AD</sub>	Total	mGFR <60	mGFR >60	mGFR <CO <sub>AD</sub>	mGFR ≥CO <sub>AD</sub>	Total	
[2-10[	0	159	14	145	159	28	59	67	20	87	246
[10-20[	1	317	38	280	318	152	129	245	36	281	599
[20-30[	3	131	21	113	134	73	20	83	10	93	227
[30-40[	1	331	41	291	332	151	56	191	16	207	539
[40-50[	11	474	69	416	485	233	83	298	18	316	801
[50-60[	34	1587	81	1540	1621	412	98	465	45	510	2131
[60-70[	82	1355	92	1345	1437	704	130	700	134	834	2271
[70-80[	174	557	65	666	731	519	35	472	82	554	1285
[80-90[	94	153	16	231	247	182	4	160	26	186	433
>90	15	3	2	16	18	34	0	30	4	34	52
Total	415	5067	439	5043	5482	2488	614	2711	391	3102	8584

mGFR = measured glomerular filtration rate; Scr = serum creatinine; Q<sub>crea</sub> = normalization factor for Scr; ScysC = serum cystatin C; Q<sub>cysC</sub> = normalization factor for ScysC; CO<sub>AD</sub> = age-dependent cutoff for mGFR

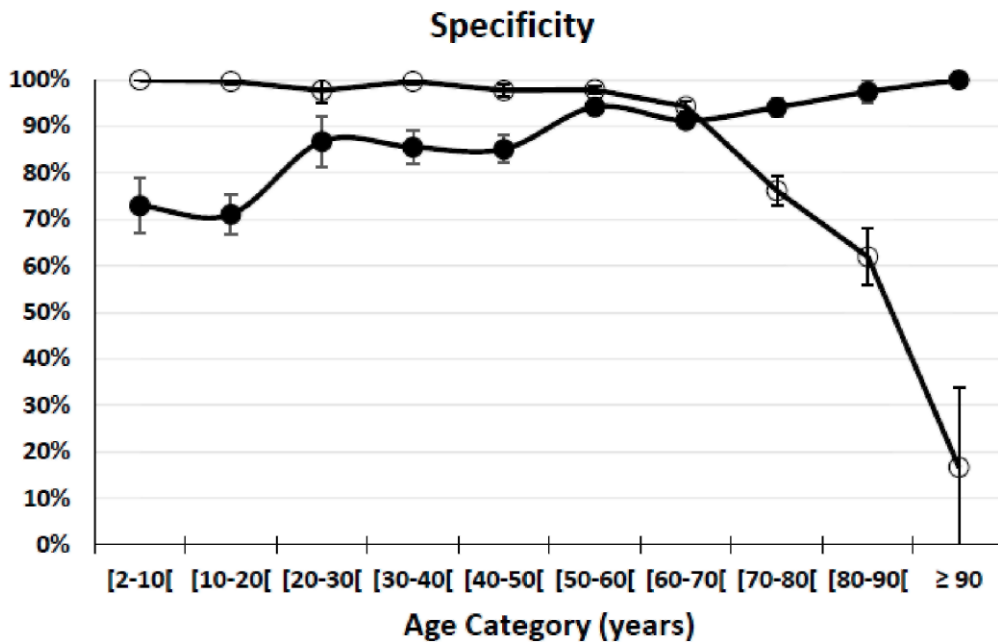
**Figure 1a:** Sensitivity is presented by age-category (Table 4). Solid circles represent the first scenario in which a true positive test result is defined as (Scr/Q<sub>crea</sub>+ScysC/Q<sub>cysC</sub>)/2>1.33 in the subgroup with mGFR<60mL/min/1.73m<sup>2</sup>. The open circles represent the third scenario in which a true positive test result is defined as mGFR<60mL/min/1.73m<sup>2</sup> in the subgroup with (Scr/Q<sub>crea</sub>+ScysC/Q<sub>cysC</sub>)/2>1.33.

Error bars are defined as  $\pm 1.96x \sqrt{\frac{S(1-S)}{n}}$ .



**Figure 1b:** Specificity is presented by age-category (Table 4). Solid circles represent the first scenario in which a true negative test result is defined as  $(Scr/Q_{crea}+ScysC/Q_{cysc})/2 \leq 1.33$  in the subgroup with  $mGFR \geq 60 mL/min/1.73m^2$ . The open circles represent the third scenario in which a true negative test result is defined as  $mGFR \geq 60 mL/min/1.73m^2$  in the subgroup with  $(Scr/Q_{crea}+ScysC/Q_{cysc})/2 \leq 1.33$ .

Error bars are defined as  $\pm 1.96x \sqrt{\frac{Sp(1-Sp)}{n}}$ .



**Figure 2a:** Sensitivity is presented by age-category (Table 4). Solid circles represent the second scenario in which a true positive test result is defined as  $(Scr/Q_{crea}+ScysC/Q_{cysc})/2 > 1.33$  in the subgroup with  $mGFR < GFR_{CO}$ . The open circles represent the third scenario in which a true positive test result is defined as  $mGFR < GFR_{CO}$  in the subgroup with  $(Scr/Q_{crea}+ScysC/Q_{cysc})/2 > 1.33$ . Error bars

are defined as  $\pm 1.96x \sqrt{\frac{S(1-S)}{n}}$ .

**Figure 2b:** Specificity is presented by age-category (Table 4). Solid circles represent the second scenario in which a true negative test result is defined as  $(Scr/Q_{crea}+ScysC/Q_{cysc})/2 \leq 1.33$  in the subgroup with  $mGFR \geq GFR_{co}$ . The open circles represent the third scenario in which a true negative test result is defined as  $mGFR \geq GFR_{co}$  in the subgroup with  $(Scr/Q_{crea}+ScysC/Q_{cysc})/2 < 1.33$ . Error

bars are defined as  $\pm 1.96x \sqrt{\frac{Sp(1-Sp)}{n}}$ .

