Estimated glomerular filtration rate in the nephrotic syndrome

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Abstract

Background. Plasma creatinine concentration and creatinine-based equations are most commonly used as markers of glomerular filtration rate (GFR). The abbreviated MDRD formula is considered the best available formula. Altered renal handling of creatinine, which may occur in the nephrotic syndrome, will invalidate creatinine-based formulas.

Methods. Data on a cohort of patients with glomerular diseases were available from a large database. We have studied the relationship between estimated GFR (MDRD formula), and plasma cystatin C (CysC) and plasma beta-2-microglobulin (β2m) as markers of GFR.

We have evaluated the abbreviated MDRD formula in a large cohort of patients with proteinuria.
plasma creatinine concentration and GFR will affect the accuracy of the MDRD formula as estimate of GFR. We have previously suggested that renal tubular handling of creatinine is altered in patients with a nephrotic syndrome [2]. In a pilot study with a limited number of patients, we observed that creatinine clearance overestimated GFR (measured by insulin clearance) in patients with hypoalbuminaemia.

In the present study, we have evaluated the abbreviated MDRD formula as well as other formulas in a large cohort of patients with proteinuria. We used plasma cystatin C (CysC) and plasma beta-2-microglobulin (β2m) as markers of GFR. The data suggest that the currently used formulas for eGFR may be less valid in patients with a nephrotic syndrome.

Materials and methods

In our centre, patients with recently diagnosed glomerular diseases are evaluated using a standardized protocol as described [3]. In brief, blood samples and timed urine samples are collected for the measurement of plasma creatinine, electrolytes, albumin, urea, β2m, and urinary excretion of creatinine, total protein, β2m and albumin. In addition, aliquots of plasma, plasma and urine are stored at −70°C. Patient data are collected in a database.

For this study, we have used the data on 180 patients who were entered in the database between 1995 and 2006. In addition, plasma samples of these patients were retrieved, thawed, and used for the measurement of CysC and plasma creatinine. Methods for the determination of albumin, urea and β2m have been described [3]. In our laboratory, the creatinine assay was changed in 2005, and a Jaffe-based technique was replaced by an enzymatic method. Therefore, plasma creatinine concentration was re-analysed in all thawed samples using the Roche enzymatic method (Roche Diagnostics, Creatinine Plus, Indianapolis, IN, USA). CysC was measured using the Dade Behring N latex Cystatin C assay [4]. Although creatinine and CysC can be reliably determined in frozen samples, errors may occur due to effects such as freeze-drying. To avoid such errors, we regularly measure plasma sodium concentrations in thawed samples. Samples with differences in sodium concentration >3% compared with the original measurement are not included for this study.

Calculations and statistics

Estimated GFR (eGFR) was calculated with the re-expressed four-variable MDRD equation (eGFR-MDRD4) = 175 × [standardized plasma creatinine (mg/dL)]^1.154 × [age (year)]^0.203 × 1.212 (if black) × 0.742 (if female) [5]. We also calculated eGFR using the re-expressed six-variable MDRD equation (eGFR-MDRD6) = 161.5 × [standardized plasma creatinine (mg/dL)]^0.999 × [age (year)]^-0.176 × [BUN (mg/dL)]^-0.17 × [plasma albumin (g/dL)]^0.318 × 1.18 (if black) × 0.762 (if female) [5]. We also calculated eGFR by the new CKD-EPI equations using serum creatinine, CysC or both (Table 1) [6,7].

### Table 1. The CKD-EPI equations for estimating GFR in Caucasians [6,7]

<table>
<thead>
<tr>
<th>CKD-EPI-Cr</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCr [mg/dL (μmol/L)]</td>
<td>≤0.7 (58)</td>
<td>≤0.9 (80)</td>
</tr>
<tr>
<td>eGFR</td>
<td>144 × (sCr/0.7)</td>
<td>144 × (sCr/0.7)</td>
</tr>
<tr>
<td>Age</td>
<td>1.209 × (if black)</td>
<td>1.209 × (if black)</td>
</tr>
<tr>
<td>β2m</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>CysC</td>
<td>0.176</td>
<td>0.176</td>
</tr>
<tr>
<td>β2m × CysC</td>
<td>0.129</td>
<td>0.129</td>
</tr>
</tbody>
</table>

CKD-EPI-Cr, creatinine-based CKD-EPI equation for eGFR; eGFR, estimated glomerular filtration rate; sCr, serum creatinine (mg/dL); CKD-EPI-CysC, cystatin C-based CKD-EPI equation for eGFR; CysC, serum cystatin C (mg/L); CKD-EPI-Cr + CysC, CKD-EPI equation for eGFR, based on both serum creatinine and serum cystatin C.
The correlation between two parameters (non-parametric distributions) was analysed by Spearman’s rank coefficient of correlation.

To clarify if the relation between the markers of GFR (CysC and β2m) and eGFR-MDRD4 was influenced by other parameters, we performed univariable and multiple regression analysis. To allow regression analysis, non-parametric parameters were transformed. The dependent variable was the ratio between inversed CysC or β2m, respectively, and eGFR.

The following variables were tested in the regression analysis: age, gender, plasma albumin, plasma urea, proteinuria, weight, BMI and body surface area (BSA). Multiple regression analysis was performed in forward stepwise fashion.

For comparison between multiple variables, we used a non-parametric Kruskal–Wallis test.

All data are presented as means (± SD) or medians (range) when appropriate. All statistics were performed using SPSS software, version 16.0 (Chicago, IL, USA). P < 0.05 was considered significant.

Results

Data of 180 patients with a glomerular disease were available. Sixteen patients (9%) were excluded from analysis because of differences in plasma sodium concentration between original and thawed samples (see Materials and methods section). Ten patients were treated with immunosuppressive agents including steroids, and information on immunosuppressive medication was lacking in 12 patients. Since the use of steroids clearly affects cellular metabolism and lymphocyte survival, and thus will affect production of CysC or β2m, we excluded these patients from the analysis. Baseline characteristics of the remaining patients (n = 142) are shown in Table 2. All patients were Caucasian, and there were no diabetics. Biopsy-proven renal diseases were membranous nephropathy (n = 66), primary focal segmental glomerulosclerosis (n = 22), IgA nephropathy (n = 23), minimal change nephrotic syndrome (n = 11) and others (n = 20). Eighty patients had a nephrotic syndrome (plasma albumin <30 g/L and proteinuria >3.5 g/day), and a few patients (n = 15) had proteinuria <1 g/day. The majority of patients (66%) were treated with ACE inhibitors and/or angiotensin receptor blockers.

As expected, we observed a linear relationship between, respectively, β2m and CysC and plasma creatinine (r = 0.69 and r = 0.86, respectively) and a hyperbolic relationship between β2m and CysC and eGFR-MDRD4. In univariable analysis, plasma urea, plasma albumin and proteinuria significantly predicted the relationship between CysC and eGFR-MDRD4 (respectively, P = <0.001, P = 0.003 and P = 0.049). In multiple regression analysis, both plasma urea and plasma albumin remained significant independent predictors (respectively, P < 0.001 and P = 0.016). At equal levels of CysC, patients with hypoalbuminaemia (plasma albumin <25 g/L) had higher eGFR-MDRD4 (rightward shift of curve, Figure 1).

We used plasma β2m as a second marker of GFR. In univariable analysis, only plasma albumin significantly influenced the relationship between β2m and eGFR-MDRD4 (P = 0.024). In multiple regression analysis, plasma albumin remained an independent predictor (P = 0.027). In the presence of hypoalbuminaemia, there was again a rightward shift of the curve of the relationship between β2m and eGFR-MDRD4 (Figure 2). Thus, these results suggest that

Table 2. Baseline characteristics (n = 142)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (Mean ± SD or Median [Range])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>93/49</td>
</tr>
<tr>
<td>Age (year)</td>
<td>48 ± 15</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 (15.8–42.2)</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.95 ± 0.21</td>
</tr>
<tr>
<td>Plasma creatinine (µmol/L)</td>
<td>101 (42–368)</td>
</tr>
<tr>
<td>Plasma albumin (g/L)</td>
<td>28.0 (10.0–47.0)</td>
</tr>
<tr>
<td>Plasma CysC (mg/L)</td>
<td>1.14 (0.56–4.00)</td>
</tr>
<tr>
<td>Plasma β2m (mg/L)</td>
<td>3.43 (0.7–13.8)</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>6.4 (0.03–37.9)</td>
</tr>
<tr>
<td>eGFR-MDRD4 (mL/min/1.73 m²)</td>
<td>64 (15–165)</td>
</tr>
<tr>
<td>eGFR-MDRD6 (mL/min/1.73 m²)</td>
<td>53 (12–128)</td>
</tr>
<tr>
<td>eGFR-CKD-EPI (mL/min/1.73 m²)</td>
<td>69 (15–141)</td>
</tr>
<tr>
<td>Treatment with ACEI/ARB (%)</td>
<td>94 (66%)</td>
</tr>
</tbody>
</table>

Data are means ± SD or medians (range).

CysC, cystatin C; β2m, beta-2-microglobulin; eGFR-MDRD4, estimated GFR calculated using the abbreviated MDRD formula; eGFR-MDRD6, estimated GFR calculated using the original MDRD formula; eGFR-CKD-EPI, estimated GFR calculated using the CKD-EPI formula; ACEI, ACE inhibitor; ARB, angiotensin receptor blocker.
eGFR-MDRD4 provided higher estimates of GFR in patients with hypoalbuminaemia.

We performed subgroup analyses, limiting the analyses to patients who were or were not treated with ACE inhibitors or angiotensin receptor blockers. Results were similar (data not shown).

The original MDRD formula for estimating GFR (eGFR-MDRD6) included plasma albumin and plasma urea as variables. Obviously, the relationship of eGFR-MDRD6 with eGFR-MDRD4 is thus dependent on plasma albumin concentrations.

We next analysed the relationship between eGFR-MDRD6 and β2m and CysC. In both univariable and multivariable regression, the relationship between β2m and eGFR-MDRD4 was not influenced by any of the parameters, including plasma albumin (P = 0.28, Figure 3). Results with CysC were different, somewhat to our surprise.

We observed a good correlation between CysC and eGFR-MDRD6 (r = 0.87). In univariable analysis, age (P = 0.043), proteinuria (P = 0.002), plasma albumin (P < 0.001) and plasma urea (P < 0.001) significantly influenced the relationship between CysC and eGFR-MDRD6. In multivariable analysis, both plasma albumin (P < 0.001) and plasma urea (P < 0.001) remained independent predictors, although the effect was small. Of note, for plasma albumin, the effect was now in the opposite direction with a leftward shift of the curve (Figure 4).

We next evaluated the relationship between CysC and β2m. As expected for markers of GFR, we observed a high correlation between CysC and β2m (r = 0.87). In univariable analysis, age (P = 0.005), proteinuria (P = 0.002) and plasma albumin (P < 0.001) significantly influenced the relationship between CysC and β2m. In multivariable analysis, both age (P = 0.01) and plasma albumin (P < 0.001) remained independent predictors of this relationship. Variables included in this analysis were similar to those mentioned in the Materials and methods section, complemented with eGFR (both eGFR-MDRD4 and eGFR-MDRD6 were introduced once). At equal levels of β2m, patients with hypoalbuminaemia had lower levels of plasma CysC. The relationship between CysC and β2m is depicted in Figure 5.

In recent years, other formulas for estimating GFR have been proposed. These include formulas based on serum creatinine concentration such as the CKD-EPI formula (CKD-EPI-Cr), as well as formulas based on serum CysC concentration (CKD-EPI-CysC) or a combination thereof (CKD-EPI-CysC-Cr). We evaluated these formulas in our patients. In multivariable analyses, plasma albumin proved an independent predictor of the relationship between both CysC and β2m and eGFR calculated by CKD-EPI-Cr. The relationship between CysC and eGFR calculated by CKD-EPI-CysC is invalid for this analysis. Analysing the relationship between β2m and eGFR calculated by CKD-EPI-CysC, we again found plasma albumin to be an independent predictor.
Table 3. Comparison of the MDRD Study and CKD-EPI equations in patients with plasma albumin ≥30 g/L

<table>
<thead>
<tr>
<th>eGFR a</th>
<th>n</th>
<th>MDRD6 (mL/min/1.73 m²)</th>
<th>MDRD4 (mL/min/1.73 m²)</th>
<th>CKD-EPI-Cr (mL/min/1.73 m²)</th>
<th>CKD-EPI-CysC (mL/min/1.73 m²)</th>
<th>CKD-EPI-Cr + CysC (mL/min/1.73 m²)</th>
<th>K–W test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;90</td>
<td>13</td>
<td>102 (91–128)</td>
<td>106 (86–131)</td>
<td>105 (98–126)</td>
<td>105 (75–133)</td>
<td>108 (94–133)</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>61–90</td>
<td>15</td>
<td>78 (61–90)</td>
<td>79 (56–97)</td>
<td>95 (64–107)</td>
<td>84 (54–123)</td>
<td>88 (68–107)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>46–60</td>
<td>10</td>
<td>53 (46–60)</td>
<td>55 (44–61)</td>
<td>62 (48–67)</td>
<td>61 (53–74)</td>
<td>56 (50–69)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>31–45</td>
<td>11</td>
<td>40 (31–44)</td>
<td>39 (31–44)</td>
<td>42 (33–49)</td>
<td>50 (34–69)</td>
<td>46 (35–55)</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>16–30</td>
<td>12</td>
<td>23 (16–30)</td>
<td>24 (17–35)</td>
<td>25 (17–35)</td>
<td>23 (19–42)</td>
<td>23 (18–34)</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>≤15</td>
<td>4</td>
<td>15 (14–15)</td>
<td>16 (15–17)</td>
<td>16 (15–17)</td>
<td>18 (15–27)</td>
<td>17 (14–19)</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>54 (14–128)</td>
<td>55 (15–131)</td>
<td>63 (15–126)</td>
<td>63 (15–133)</td>
<td>59 (14–133)</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median value (range). Median plasma albumin in this cohort was 40 g/L (range 30–47).

Table 4. Comparison of the MDRD Study and CKD-EPI equations in patients with plasma albumin <30 g/L

<table>
<thead>
<tr>
<th>eGFR a</th>
<th>n</th>
<th>MDRD6 (mL/min/1.73 m²)</th>
<th>MDRD4 (mL/min/1.73 m²)</th>
<th>CKD-EPI-Cr (mL/min/1.73 m²)</th>
<th>CKD-EPI-CysC (mL/min/1.73 m²)</th>
<th>CKD-EPI-Cr + CysC (mL/min/1.73 m²)</th>
<th>K–W test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;90</td>
<td>6</td>
<td>93 (92–128)</td>
<td>118 (99–165)</td>
<td>112 (107–141)</td>
<td>110 (83–138)</td>
<td>120 (112–170)</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>61–90</td>
<td>26</td>
<td>70 (61–83)</td>
<td>89 (67–110)</td>
<td>103 (69–121)</td>
<td>85 (55–148)</td>
<td>95 (63–125)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>46–60</td>
<td>19</td>
<td>51 (46–59)</td>
<td>66 (54–80)</td>
<td>74 (56–96)</td>
<td>75 (42–96)</td>
<td>72 (53–90)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>31–45</td>
<td>13</td>
<td>40 (31–45)</td>
<td>52 (41–64)</td>
<td>53 (46–87)</td>
<td>54 (27–83)</td>
<td>54 (35–73)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>16–30</td>
<td>11</td>
<td>20 (16–27)</td>
<td>25 (19–38)</td>
<td>24 (18–39)</td>
<td>29 (14–42)</td>
<td>26 (16–37)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>≤15</td>
<td>2</td>
<td>13 (12–15)</td>
<td>19 (17–21)</td>
<td>20 (16–23)</td>
<td>22 (19–24)</td>
<td>20 (17–23)</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>53 (12–128)</td>
<td>70 (17–165)</td>
<td>81 (16–141)</td>
<td>72 (14–148)</td>
<td>78 (16–170)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median value (range). Median plasma albumin in this cohort was 20 g/L (range 10–29).

Table 5. Comparison of the MDRD Study and CKD-EPI equations in patients with plasma albumin ≥30 g/L

<table>
<thead>
<tr>
<th>β2m (mg/L)</th>
<th>n</th>
<th>MDRD6 (mL/min/1.73 m²)</th>
<th>MDRD4 (mL/min/1.73 m²)</th>
<th>CKD-EPI-Cr (mL/min/1.73 m²)</th>
<th>CKD-EPI-CysC (mL/min/1.73 m²)</th>
<th>CKD-EPI-Cr + CysC (mL/min/1.73 m²)</th>
<th>K–W test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>13</td>
<td>89 (41–127)</td>
<td>84 (43–127)</td>
<td>96 (49–121)</td>
<td>106 (60–133)</td>
<td>103 (53–129)</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>2–3</td>
<td>18</td>
<td>68 (40–106)</td>
<td>66 (40–111)</td>
<td>71 (42–110)</td>
<td>73 (53–110)</td>
<td>73 (48–108)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>3–4</td>
<td>12</td>
<td>56 (37–128)</td>
<td>58 (33–131)</td>
<td>64 (37–126)</td>
<td>59 (41–112)</td>
<td>58 (38–133)</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>4–6</td>
<td>8</td>
<td>34 (27–88)</td>
<td>35 (26–97)</td>
<td>38 (28–103)</td>
<td>44 (31–88)</td>
<td>38 (29–98)</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>6–10</td>
<td>9</td>
<td>20 (14–36)</td>
<td>20 (15–31)</td>
<td>20 (15–28)</td>
<td>25 (21–28)</td>
<td>22 (18–28)</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>5</td>
<td>16 (14–30)</td>
<td>17 (15–35)</td>
<td>17 (15–35)</td>
<td>19 (15–21)</td>
<td>18 (14–26)</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>54 (14–128)</td>
<td>55 (15–131)</td>
<td>63 (15–126)</td>
<td>63 (15–133)</td>
<td>59 (14–133)</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median value (range). Median plasma albumin in this cohort was 40 g/L (range 30–47).

Table 3 and 4 summarize the results of eGFR estimates using various formulas, for patients with serum albumin levels above and below 30 g/L, respectively. In these tables, patients are grouped according to the level of eGFR calculated using the MDRD6 equation. Similar data were obtained when using classes of GFR based on serum β2m levels (Tables 5 and 6). The tables clearly illustrate that the various formulas provide rather similar results in patients with serum albumin levels >30 g/L, although as expected the eGFR estimated by CKD-EPI formulas was higher in the higher categories of GFR. In contrast, the MDRD4 formula as well as the CKD-EPI formulas provided higher estimates of GFR in patients with serum albumin <30 g/L, when compared with eGFR-MDRD6.

### Discussion

We have evaluated the performance of the MDRD4 and MDRD6 formulas for estimating GFR in patients with proteinuric kidney disease. Our data indicate that these formul-
las are not interchangeable in patients with low serum albumin levels. Notably, we observed that the relationship between eGFR-MDRD4 and the GFR markers CysC or \( \beta_2m \) was influenced by plasma albumin levels. At equal levels of CysC or \( \beta_2m \), eGFR-MDRD4 was higher in patients with hypoalbuminaemia. In contrast, the relationship between eGFR-MDRD6 and \( \beta_2m \) was not dependent on serum albumin levels. These discrepancies in performance between the MDRD4 and MDRD6 formula are clearly illustrated in Tables 3–6, showing that eGFR estimated by the MDRD6 formula is consistently lower than values estimated by the MDRD4 formula in patients with low serum albumin levels. Tables 3 and 4 suggest that the MDRD6 formula is biased in patients with hypoalbuminaemia since the MDRD4 formula provides values that are closest to values derived by CysC-based formulas. Could the MDRD6 formula be invalid in patients with a nephrotic syndrome? Integration of serum albumin levels in the MDRD6 formula provided a means to account for decreased creatinine generation in patients with malnutrition of chronic inflammation. Since hypoalbuminaemia in patients with a nephrotic syndrome is related to urinary albumin loss and not to malnutrition, the MDRD6 formula may be expected to underestimate GFR in patients with a nephrotic syndrome.

However, we suggest that our data indicate that the abbreviated MDRD4 formula overestimates GFR in patients with a nephrotic syndrome, due to altered tubular handling of creatinine. We observed that serum albumin levels influenced the relationship between eGFR-MDRD4 and CysC or \( \beta_2m \). At equal levels of CysC or \( \beta_2m \), eGFR-MDRD4 was higher in patients with hypoalbuminaemia. Both CysC and \( \beta_2m \) are low-molecular-weight proteins that are filtered in the glomerulus without evidence of tubular secretion. Plasma levels of both CysC and \( \beta_2m \) correlate well with GFR, both markers are used as estimates of GFR, and at least, \( \beta_2m \) has been validated in patients with proteinuria [8–13]. Based on these premises, the data indicate that eGFR-MDRD4 overestimates real GFR in patients with a nephrotic syndrome, which must be attributed to altered tubular handling of creatinine in patients with a nephrotic syndrome.

We have evaluated the recently developed CKD-EPI formulas. Our data suggest that also the creatinine-based CKD-EPI formula is less valid in patients with a nephrotic syndrome. This is not unexpected since alterations in the renal tubular handling of creatinine will affect all formulas that are merely based on serum creatinine levels as the sole laboratory parameter.

A closer look at our data and our additional analyses suggest that plasma \( \beta_2m \) levels are a more consistent marker of GFR than levels of CysC.

Firstly, the effect of plasma albumin levels on the relationship between plasma \( \beta_2m \) and eGFR-MDRD4 was quantitatively similar to the effect of plasma albumin levels on the relationship between endogenous creatinine clearance (ECC) and inulin clearance observed in our previous study [2]. In the present data, an eGFR-MDRD4 of 80 mL/min/1.73 m\(^2\) equalled a plasma \( \beta_2m \) level of 2.10 mg/L in a patient with a plasma albumin of 40 g/L and 3.32 mg/L in a patient with a plasma albumin of 20 g/L (percentage overestimation 37%). In comparison, ECC of 80 mL/min equalled an inulin clearance of 57, respectively, 42 mL/min (percentage overestimation 26%) [2].

Secondly, plasma \( \beta_2m \) levels correlated with eGFR-MDRD6, independent from plasma albumin levels. We have previously suggested that eGFR-MDRD6 more accurately reflects inulin clearance in the nephrotic syndrome [2]. Thus far, plasma \( \beta_2m \) has received a little attention as marker of GFR. Older studies observed a very high correlation between plasma \( \beta_2m \) and GFR [12,13]. The performance of \( \beta_2m \) was disputed in other studies [14]. However, the latter study included patients with malignancies and systemic inflammation, conditions which may be associated with increased lymphocyte proliferation and thus generation of \( \beta_2m \). Our data suggest that a renewed interest in plasma \( \beta_2m \) as a marker of GFR seems warranted.

Although CysC has been used more widely as a marker of GFR, its usefulness in clinical practice is debated. In a recent cross-sectional analysis of 3418 patients with kidney disease, Stevens et al. noted that factors such as age, gender, race, BMI, weight, plasma urea, diabetes, proteinuria and blood pressure influenced CysC independent of GFR [15]. Of note, in the latter study, serum albumin was negatively correlated with CysC, in apparent contrast with our findings showing lower CysC levels in patients with hypoalbuminaemia. There are clear differences between the studies. We have evaluated patients with often severe hypoalbumi-

### Table 6. Comparison of the MDRD Study and CKD-EPI equations in patients with plasma albumin <30 g/L

<table>
<thead>
<tr>
<th>( \beta_2m ) (mg/L)</th>
<th>( n )</th>
<th>MDRD6 (mL/min/1.73 m(^2))</th>
<th>MDRD4 (mL/min/1.73 m(^2))</th>
<th>CKD-EPI-Cr (mL/min/1.73 m(^2))</th>
<th>CKD-EPI-CysC (mL/min/1.73 m(^2))</th>
<th>CKD-EPI-Cr + CysC (mL/min/1.73 m(^2))</th>
<th>K-W test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>5</td>
<td>70 (64–92)</td>
<td>89 (80–99)</td>
<td>107 (91–111)</td>
<td>135 (97–138)</td>
<td>116 (96–123)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>2–3</td>
<td>22</td>
<td>70 (45–128)</td>
<td>87 (51–165)</td>
<td>97 (56–141)</td>
<td>87 (57–148)</td>
<td>91 (62–170)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>4–6</td>
<td>16</td>
<td>56 (40–118)</td>
<td>76 (53–121)</td>
<td>69 (45–115)</td>
<td>78 (54–127)</td>
<td>78 (54–127)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>6–10</td>
<td>5</td>
<td>24 (16–38)</td>
<td>34 (19–46)</td>
<td>33 (19–50)</td>
<td>31 (23–35)</td>
<td>33 (21–41)</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>6</td>
<td>17 (12–31)</td>
<td>21 (17–41)</td>
<td>22 (16–46)</td>
<td>19 (14–27)</td>
<td>20 (16–35)</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>53 (12–128)</td>
<td>70 (16–175)</td>
<td>81 (16–141)</td>
<td>72 (14–148)</td>
<td>78 (16–170)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median value (range). Median plasma albumin in this cohort was 20 g/L (range 10–29).

### References

1. Stevens et al. noted that.
2. In the latter study, serum albumin was negatively correlated with CysC, in apparent contrast with our findings showing lower CysC levels in patients with hypoalbuminaemia. There are clear differences between the studies. We have evaluated patients with often severe hypoalbumi-
naemia due to urinary protein losses. In contrast, patients in
the study of Stevens et al. were normoalbuminaemic (serum
albumin 42 g/L). The low serum albumin levels in their
patients could be due to inflammation or malnutrition, fac-
tors that may influence cell metabolism. Furthermore, CysC
generation is influenced by changes in thyroid hor-
mone metabolism [16,17]. Our data also point to changes in
CysC production in patients with hypoalbuminaemia.
The relationship between CysC and eGFR-MDRD6 was
shifted to the left in patients with low albumin levels.
Moreover, at equal levels of plasma β2m, CysC levels
were lower in patients with hypoalbuminaemia. Our recent
observations that subclinical hypothyroidism is frequent in
patients with proteinuria may explain these findings [18].

Theoretically, formulas based on alternative markers such
as CysC might be more advantageous. However, this will
only be true if the production of this alternative marker is
not changed in the nephrotic syndrome. Our observation
of lower CysC levels in patients with hypoalbuminaemia
already suggested that also CysC-based GFR formulas
could be invalid in patients with a nephrotic syndrome.
Analysis of the data confirmed this.

Literature data support our conclusions. Based on a
study of 42 patients with proteinuria, we already suggested
that creatinine was an invalid marker of GFR in patients
with a nephrotic syndrome [2]. In this study, we showed
that endogenous creatinine clearance as well as eGFR-
MDRD4 overestimated GFR as measured by inulin clear-
ance in patients with nephrotic syndrome. The study can
be criticized for its small number of patients. Other studies
provide supportive arguments. Several studies have re-
ported glomerular hypofiltration due to a decreased filtra-
tion coefficient in patients with a nephrotic syndrome,
developing normal levels of serum creatinine [19,20]. Altered
renal handling of creatinine in patients with proteinuria is
supported by an analysis of data from the MDRD study,
which showed an increased tubular secretion of creatinine
in patients with glomerular diseases [21].

Limitations of the study

Our conclusions are based on the assumption that plasma
CysC and β2m are markers of GFR. We have not used inu-
lin clearance or its alternatives to measure GFR. Therefore,
our conclusions must be regarded as hypothesis generating
and need further validation in larger studies in which gold-
en standard techniques for measurement of GFR are used.

In conclusion, in patients with hypoalbuminaemia, we
propose that estimates of GFR using creatinine-based for-
mulas may be invalid. As a result, a clinically significant
decrease of GFR may be overlooked in patients with a
nephrotic syndrome. In daily clinical practice, one should
be aware of the limitations of formulas for estimating GFR
in patients with a nephrotic syndrome.

References

1. Melamed ML, Bauer C, Hostetter TH. eGFR: is it ready for early
2. Branten AJW, Vervoort G, Wetzelis JFM. Serum creatinine is a poor
marker of GFR in nephrotic syndrome. Nephrol Dial Transplant
2005; 20: 707–711
3. Branten AJW, du Buf-Vereijken PW, Klansen IS et al. Urinary excre-
tion of β2-microglobulin and IgG predict prognosis in idiopathic
C measurement by particle-enhanced immunonephelometry on the
Behring nephelometer systems (BNA, BN II). Clin Chem 1997; 43:
1016–1022
5. Levey AS, Coresh J, Greene T et al. Using standardized serum creatin-
ine values in the modification of diet in renal disease study equation
for estimating glomerular filtration rate. Ann Intern Med 2006; 145:
247–254
cystatin C alone and in combination with serum creatinine: a pooled
analysis of 3,418 individuals with CKD. Am J Kidney Dis 2008; 51:
395–406
8. Newman DJ, Thakkar H, Edwards RG et al. Serum cystatin C meas-
ured by automated immunoassay: a more sensitive marker of changes
9. Dhamidharka VR, Kwon C, Stevens G. Serum cystatin C is superior
to serum creatinine as a marker of kidney function: a meta-analysis.
10. Donadio C, Lucchesi A, Ardini M et al. Cystatin C, beta 2-microglo-
bulin, and retinol-binding protein as indicators of glomerular filtra-
tion rate: comparison with plasma creatinine. J Pharm Biomed Anal
2001; 24: 835–842
11. Bianchi C, Donadio C, Tramonti G et al. Reappraisal of serum beta2-
microglobulin as marker of GFR. Ren Fail 2001; 23: 419–429
12. Viberti GC, Keen H, Mackintosh D. Beta 2-microglobulinaemia: a
sensitive index of diminishing renal function in diabetics. Br Med J
1983; 74: 256–264
14. Grubb A, Simonsen O, Sturfelt G et al. Serum concentration of cy-
statin C, factor D and beta 2-microglobulin as a measure of glomeru-
15. Stevens LA, Schmid CH, Greene T et al. Factors other than glomeru-
lar filtration rate affect serum cystatin C levels. Kidney Int 2009; 75:
652–660
17. Wiespi P, Schwegler B, Spinus GA et al. Serum cystatin C is sen-
sitive to small changes in thyroid function. Clin Chim Acta 2003;
338: 87–90
cystatin C, factor D and beta 2-microglobulin as indicators of glomeru-
19. Guasch A, Myers BD. Determinants of glomerular hypofiltration in
nephrotic patients with minimal change nephropathy. J Am Soc Ne-
phrol 1994; 4: 1571–1581
20. Koomans HA, Boer WH, Dorhout Mees EJ. Renal function during
recovery from minimal lesions nephrotic syndrome. Nephron 1987;
47: 173–178
21. Modification of Diet in Renal Disease Study Group. Effects of diet
and antihypertensive therapy on creatinine clearance and serum cre-
atinin concentration in the Modification of Diet in Renal Disease

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