Fabry meets Markov: evaluating biochemistry, disease course and costs in support of health care policy
Rombach, S.M.

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The value of estimated GFR in comparison to measured GFR for the assessment of renal function in adult patients with Fabry disease

Saskia M.Rombach, MD¹* and Marije C.Baas, MD²*, Ineke J.M. ten Berge, MD, PhD², Raymond T. Krediet, MD, PhD², Frederike J.Bemelman, MD, PhD², Carla E. Hollak, MD, PhD¹

¹Division of Internal Medicine, Department of Endocrinology and Metabolism, ²Division of Internal Medicine, Department of Nephrology; Academic Medical Center, Amsterdam, the Netherlands

* Both authors equally contributed to this manuscript

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Abstract

Background. Renal disease is one of the major complications in Fabry disease, an X linked lysosomal storage disease due to deficiency of the enzyme α-galactosidase A. The aim of our study was to determine the value of creatinine-, cystatin C- and beta-trace- based formulas for the estimation of glomerular filtration rate (eGFR) in Fabry patients. For comparison, the gold standard method ¹²⁵I-labelled iothalamate/ ¹³¹I-labelled hippuran (mGFR) was used.

Methods. GFR was estimated by using eleven different formulas based on creatinine, cystatin C and beta-trace protein. Accuracy and precision, detection of early decline of renal function and follow-up of renal function by eGFR was compared to mGFR.

Results. 136 GFR measurements and plasma samples were available from 36 (20 male) Fabry patients, treated with agalsidase α or β with a median follow-up of 3.1 (range 1.5 – 5.2) years. Median mGFR was 97.3 (15.5-148.6) ml/min/1.73 m² in males and 84.4 (23.0-131.0) ml/min/1.73 m² in females at start of follow-up.

Conclusions. Although none of the investigated endogenous markers proved to be an equivalent substitute for mGFR in Fabry patients, the Stevens equation, a creatinine and cystatin C based formula, most closely approximated the mGFR. When a creatinine based formula is preferred, the aMDRD and the recently developed CKD-EPI had the best performance. In male Fabry patients, the aMDRD may overestimate GFR, especially in the higher ranges. In these cases, CKD-EPI may perform better.
Introduction

Renal disease is one of the major complications in Fabry disease, an X linked lysosomal storage disease due to deficiency of the enzyme α-galactosidase A $^{1,2}$. Accumulation of several glycosphingolipids, the main compound being globotriaosylceramide (Gb3), occurs in various cell types. This results in complications of mainly vascular origin, of which progressive renal insufficiency, cardiac hypertrophy and cerebral infarctions are the most severe $^3$. Due to the X-linked nature of the disease, males are most severely affected but females can express symptoms as well. In the kidney, extensive storage has been identified in glomerular, tubular, vascular and interstitial cells $^4$. Renal involvement can already be present during childhood. Before the era of enzyme replacement, the mean age of onset of clinical nephropathy (i.e. proteinuria or chronic kidney disease (CKD) ) has been reported for male Fabry patients to occur at the age of 27 years $^5$. Thereafter, the mean rate of GFR decline was 12.2 ml/min/year. Overall, 13 - 23% of male Fabry patients and approximately 3% of female patients developed ESRD $^6$-$^8$. Before decline of renal function, hyperfiltration can be present early in the course of the disease $^9$. Enzyme replacement therapy (ERT) is available since 2001 and improvement of cardiac disease and stability of renal disease is better achieved in patients with a preserved renal function $^{10,11}$. Early detection of renal impairment is therefore important. Unfortunately, an easy and accurate method to assess renal function is lacking. GFR can be measured as the clearance of exogenous or endogenous filtration markers. The use of exogenous marker methods such as inulin clearance or nuclear methods are considered to be the gold standard for the assessment of renal function in Fabry patients $^{12}$. However, these methods are expensive and time consuming. Creatinine is the most commonly used marker. Apart from the fact that creatinine is influenced by muscle mass, age, sex and race, loss of renal function may already have occurred without a significant rise in plasma creatinine. The MDRD and abbreviated MDRD (aMDRD) $^{13,14}$, based on creatinine, are commonly used. A recent study showed that by using the MDRD formula in Fabry patients, GFR is overestimated more specifically in male Fabry patients at the higher end of GFR range $^{15}$. This hampers early detection of a decline in renal function. Recently a new formula, the CKD-EPI has been published, developed to assess GFR more accurately, especially in the higher ranges of GFR $^{16}$. This formula may therefore be of additional value in Fabry disease.

Cystatin C (cysC) is another endogenous marker. It is a low molecular weight protein (13.3 kD), produced at a constant rate by all nucleated cells and is freely filtered by the glomerulus and completely reabsorbed and broken down in the proximal tubule $^{17,18}$. Its production is not influenced by sex, age, bodyweight or muscle mass $^{19,20}$, although it can be influenced by thyroid dysfunction $^{21-23}$ and large dosages of glucocorticoids $^{24-26}$. CysC appears to be a more sensitive marker than creatinine to detect a decline in renal function and is reported to detect changes in GFR below 80 ml/min $^{27}$. To our knowledge, besides one review $^{12}$, only one study has been published investigating cysC in Fabry nephropathy $^{28}$, concluding...
that cysC is a sensitive and reliable marker. However, a reference method in that study was lacking.

Beta-trace protein (βTP) is also advocated as an early marker for deterioration of renal function in the so-called “creatinine blind range” \(^{29-32}\). βTP, also known as lipocalin prostaglandin D2-synthase, is a low molecular weight protein (22-26 kD) \(^{29}\), that is mainly produced in the central nervous system (leptomeninges, choroid plexus epithelium and oligodendrocytes). Furthermore, it is found in serum, urine, ascites and seminal plasma. Although a comparison between eGFRs using cysC and βTP in paediatric renal transplant patients showed no benefit of βTP over cysC \(^{33}\), so far no studies have investigated the value of βTP as a parameter of renal function in patients with Fabry disease.

The aim of the present study was to determine the value of creatinine-, cysC- and βTP-based formulas for the determination of GFR and the follow-up of renal function in Fabry patients in comparison to a gold standard method, consisting of a continuous infusion of \(^{125}\)I-iothalamate in combination with \(^{131}\)I-hippuran \(^{34-36}\).

Subjects and methods

Patients

The Academic Medical Center in Amsterdam is a tertiary referral center for lysosomal storage diseases in the Netherlands since 1999.

Currently 100 Fabry adult patients are closely monitored in our outpatient clinic. Yearly, GFR measurement with \(^{125}\)I-labelled iothalamate/ \(^{131}\)I-labelled hippuran is routinely performed in patients on ERT. All Fabry patients in whom 3 or more GFR measurements were available for follow-up (n = 36), were included.

The diagnosis of Fabry disease was made by demonstrating decreased enzyme activity in α-galactosidase A in leukocytes in males as well as genotyping in both males and females. Patients were either treated with agalsidase-α (Replagal, 0.2 mg/kg/ 2 weeks) or algalsidase β (Fabrazyme 0.2 mg/kg/ 2 weeks or 1.0 mg/kg/ 2 weeks). Thirty one patients participated in earlier ERT trials \(^{37,38}\). Patients provided informed consent for reanalysis of samples.

Measurements

Plasma samples, frozen at -20 °Celsius, collected during follow-up of treatment were used for this analysis. Creatinine, cysC and βTP measurements were performed in samples from one year after start of treatment due to lack of availability of samples before start of treatment. Samples for measurements of creatinine, cysC and βTP were obtained at the same day. Plasma samples were taken with a median of 1 day (range -83 to + 97 days, with one exception of 175 days) before or after GFR measurement. Urine samples were taken one day before the plasma sample was taken.
Creatinine was measured with an enzymatic PAP+ (phenol/4-aminoantipyrine) assay on a Roche Modular analyser (Roche, Almere, the Netherlands). The creatinine determinations were calibrated according to the IDMS traceable creatinine standard.

CysC was measured in heparinized plasma samples with the N Latex Cystatin C test kit, a particle-enhanced immunonephelometric method, on a BN ProSpec analyser (Siemens, Breda, the Netherlands).

βTP was measured in heparinized plasma samples with the N Latex β-Trace Protein test kit on a BN ProSpec analyser (Siemens, Breda, the Netherlands).

**Estimated GFR (eGFR)**

We chose formulas to estimate GFR which had been constructed in adult patient populations with various causes of renal failure. For βTP, only formulas constructed from data of renal transplant recipients with decreased renal function (mean mGFR< 60 ml/min/1.73 m²) were available. The formulas used to estimate GFR are given in Table 1.

All results are presented in ml/min/1.73m². The Cockcroft and Gault formula, 24h<sub>urine</sub> creatinine clearance and the eGFR using the Larsson formula were corrected for body surface area (BSA) using the duBois formula.

**Gold standard GFR measurement**

A method with continuous infusion of <sup>125</sup>I-iothalamate and <sup>131</sup>I-hippuran was used to determine GFR. With this method, GFR is calculated as the mean urinary clearance of <sup>125</sup>I iothalamate of two 2h periods after a 2h equilibration period. Corrections are made for incomplete urinary collections by using <sup>131</sup>I-hippuran and for fluctuations in plasma concentrations. GFR was then corrected for BSA using the duBois formula.

**Statistical analysis**

Statistical analyses were performed using SPSS16. Demographic data were expressed as median (range). The Wilcoxon signed ranks test was used to compare differences in GFR course over time. For comparison between males and females, we used the Mann-Whitney U test. A p < 0.05 was considered statistically significant. For comparison of the various eGFR methods with mGFR, we used Bland and Altman analysis. Accuracy was defined as the mean difference between mGFR and eGFR. Limits of agreement were defined as mean difference -2SD and mean difference +2SD and precision as ± 1 SD. To correct for repeated measurements, we used a correction factor proposed by Bland and Altman. To compare the accuracies of the various formulas we used Student’s t-test. To investigate whether cysC and/or βTP would be superior to creatinine to detect decreased renal function, receiver operating characteristic (ROC) curves were used. A decreased renal function was defined as a GFR< 90 ml/min/1.73 m². MedCalc statistical software was used to compare AUC of the ROC curves.
## Results

### Patients

Thirty-six patients (20 (56%) male, 16 female) underwent 136 GFR measurements.
The median number of GFR measurements per patient was 4 (range 3 – 5); in males 4 (range 3-5), in females 3.5 (range 3-5). Median follow-up was 3.1 years (range 1.5–5.2) in males 3.1 (1.8-5.2), in females 2.2 (1.5-4.0) year. At the time of the first renal assessment,

### Table 1. Formulas based on creatinine, beta trace and cystatin C for estimation of GFR.

**Creatinine-based:**

1. MDRD formula:
   \[
   GFR = 170 \times \left(\frac{P_{\text{creatinine}}}{88.4}\right)^{0.999} \times \text{age}^{0.176} \times (P_{\text{urea}} \times 2.8)^{0.170} \times (P_{\text{albumin}}/10)^{0.318} 
   \]
   (Female: multiply result by 0.762, if black multiply result by 1.180)

2. Abbreviated MDRD:
   \[
   GFR = 175 \times \left(\frac{P_{\text{creatinine}}}{88.4}\right)^{-1.154} \times \text{age}^{-0.203} 
   \]
   (Female: multiply result by 0.742, if African-American multiply result by 1.210)

3. CKD-EPI:
   - Female and \( P_{\text{creatinine}} \leq 62 \text{ umol/l} \): \( GFR = 144 \times \left(\frac{P_{\text{creatinine}}}{88.4}\right) / 0.7 \) \(^{0.329} \times (0.993)^{\text{age}} \)
   - Female and \( P_{\text{creatinine}} > 62 \text{ umol/l} \): \( GFR = 144 \times \left(\frac{P_{\text{creatinine}}}{88.4}\right) / 0.7 \) \(^{1.209} \times (0.993)^{\text{age}} \)
   - Male and \( P_{\text{creatinine}} \leq 80 \text{ umol/l} \): \( GFR = 141 \times \left(\frac{P_{\text{creatinine}}}{88.4}\right) / 0.9 \) \(^{0.411} \times (0.993)^{\text{age}} \)
   - Male and \( P_{\text{creatinine}} > 80 \text{ umol/l} \): \( GFR = 141 \times \left(\frac{P_{\text{creatinine}}}{88.4}\right) / 0.9 \) \(^{1.209} \times (0.993)^{\text{age}} \)

4. Cockcroft and Gault formula:
   \[
   \text{Creatinine clearance} = \frac{(140 - \text{age}) \times \text{weight (kg)}}{P_{\text{creatinine}}} 
   \]
   (Male: multiply result by 1.23, if female: multiply result by 1.05)

5. 24hurine creatinine clearance:
   \[
   \text{creatinine clearance} = \frac{U_{\text{creatinine}} \times V}{P_{\text{creatinine}}} 
   \]

**Cystatin C-based:**

6. Hoek formula:
   \[
   GFR = -4.23 + 80.35/cysC 
   \]

7. Larsson formula:
   \[
   GFR = 77.24 \times \text{cysC}^{-1.2623} 
   \]

8. Rule formula:
   \[
   GFR = 66.8 \times \text{cysC}^{-1.30} 
   \]

**Beta-trace protein based:**

9. White formula:
   \[
   GFR = 112.108 \times \beta\text{TP}^{-0.662} \times \text{urea}^{-0.280} 
   \]
   (Female: multiply result by 0.88)

10. Pöge formula:
    \[
    GFR = 89.85 \times \beta\text{TP}^{-0.5541} \times \text{urea}^{-0.3018} 
    \]

**Creatinine- and cystatin C-based:**

11. Stevens formula:
    \[
    GFR = 177.6 \times \left(\frac{P_{\text{creatinine}}}{88.4}\right)^{0.65} \times \text{cysC}^{0.57} \times \text{age}^{-0.20} 
    \]
    (female: multiply result by 0.82, black: multiply result by 1.11)
30 patients were treated for one year with enzyme replacement therapies and 6 patients for 2 to 5 years: 10 with agalsidase α at 0.2 mg/kg, 13 with agalsidase β at 0.2 mg/kg and 13 with agalsidase β at 1.0 mg/kg. BMI, weight and height are given in table 2. Table 3 shows the distribution of patients and number of measurements according to mGFR.

Twenty seven and thirty one patients participated in the previous ERT trials 37, 38. There was a significant decline in GFR between the first and last measurement from 89.0 (15.5-148.6) ml/min/1.73m² towards 83.4 (11.5-146.6) ml/min/1.73m² (p = 0.004). This decline can be completely attributed to the decline in GFR in males (from 97.3 (15.5-148.6) to 79.3 (11.5-146.6) ml/min/1.73m², p = 0.002 in males vs 84.4 (23.0-131.0) to 88.3 (18.2-...

Table 2. Demographics at start of follow-up. Data are expressed as median (range).

<table>
<thead>
<tr>
<th>N</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BSA</th>
<th>BMI (kg/m²)</th>
<th>GFR (ml/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>20</td>
<td>41.9</td>
<td>72.5</td>
<td>179</td>
<td>1.93</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18.7-63.0)</td>
<td>(60.0-97.0)</td>
<td>(164-191)</td>
<td>(1.66-2.23)</td>
<td>(18.8-29.8)</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>49.5</td>
<td>7.5</td>
<td>169</td>
<td>1.86</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17.1-72.5)</td>
<td>(58.0-100.0)</td>
<td>(153-178)</td>
<td>(1.58-2.15)</td>
<td>(20.3-35.0)</td>
</tr>
<tr>
<td>All</td>
<td>36</td>
<td>46.5</td>
<td>74.5</td>
<td>172</td>
<td>1.88</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17.1-72.5)</td>
<td>(58.0-100.0)</td>
<td>(153-191)</td>
<td>(1.58-2.23)</td>
<td>(18.8-35.0)</td>
</tr>
</tbody>
</table>

Table 3. Distribution of patients and number of measurements according to mGFR.

<table>
<thead>
<tr>
<th>GFR (ml/min/1.73 m²)</th>
<th>All</th>
<th>Male</th>
<th>Female</th>
<th>Number of measurements (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 90</td>
<td>18</td>
<td>11</td>
<td>7</td>
<td>62 (45 %)</td>
</tr>
<tr>
<td>60-89</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>34 (25%)</td>
</tr>
<tr>
<td>30-59</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>24 (18%)</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>16 (12%)</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>20</td>
<td>16</td>
<td>136 (100%)</td>
</tr>
</tbody>
</table>

30 patients were treated for one year with enzyme replacement therapies and 6 patients for 2 to 5 years: 10 with agalsidase α at 0.2 mg/kg, 13 with agalsidase β at 0.2 mg/kg and 13 with agalsidase β at 1.0 mg/kg. BMI, weight and height are given in table 2. Table 3 shows the distribution of patients and number of measurements according to mGFR.

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The median decline of GFR per year was 1.9 (range – 4.0 to 15.2) ml/min/1.73m² in males and -0.26 (range -7.2 to 16.7) ml/min/1.73m² in females. Six patients (4 males) showed hyperfiltration (> 125 ml/min/1.73m²).

Receiver operating characteristic (ROC) curve

To discover the most sensitive and specific non-invasive method for detection of a decline in GFR < 90 ml/min/1.73m², ROC curves were plotted for creatinine, cysC and βTP. The area under the curve (AUC) was largest for CysC (0.877), compared to creatinine (0.839) and βTP (0.791). The cutoff value to detect a GFR < 90 ml/min/1.73m² was 69.5 μmol/L (sens 81%, spec 76%) for creatinine, 0.90 mg/L (sensitivity 75%, specificity 90 %,) for cysC and 1.04 mg/L (sensitivity 68%, specificity, 77%) for βTP.

All the respective formulas performed better than the AUC of plasma creatinine, cysC and βTP alone, all with an AUC ≥ 0.838. The Stevens formula had the highest AUC (0.926), Cockcroft and Gault (0.918), 24h_\text{urine} creatinine clearance (0.916), CKD-EPI (0.894), aMDRD (0.889), Hoek (0.884) and Rule (0.884) had the best AUC to detect a GFR < 90 ml/min/1.73m². Figure 1 shows the ROC curves of the best performing formula of each biomarker: in case of creatinine and cysC combined the Stevens formula, in case of creatinine the Cockcroft and Gault formula, for cysC the Hoek formula and for βTP the White formula.

Accuracy and limits of agreement of different methods to estimate the GFR

Bland and Altman analysis of the MDRD-, aMDRD-, CKD-EPI, 24h_\text{urine} creatinine clearance-, Cockcroft and Gault-, Larsson-, Hoek-, Rule, White- Pöge- and Stevens equations are shown in Figure 2. Table 4 shows the level of performance of each formula to accurately estimate GFR within 10%, 20%, 30% and 50% of the mGFR, respectively. The Stevens formula approximated mGFR most closely with 41.9% of cases within 10% range of mGFR and 81.6 % within 30% of mGFR. However, the Rule formula had 87.5% of cases within 30% range of mGFR but only 36.8% of cases within 10% range of mGFR. The aMDRD, the Larsson- and the Hoek formulas showed the best overall accuracy (Figure 2) and were within 30% range of mGFR in 77.9 %, 77.9% and 80.1 % of all cases, respectively (Table 4). 24h_\text{urine} creatinine clearance and Cockcroft and Gault systematically overestimated GFR in both males and females; MDRD, aMDRD, CKD-EPI and Stevens systematically overestimated GFR in males but not in females. CKD-EPI performed better in the higher range as compared to the MDRD and aMDRD. Hoek and Larsson overestimated GFR in females. In males, however, the Hoek, Larsson and Rule underestimated GFR in the higher ranges. The White and Pöge formula underestimated GFR in both males and females. The limits of agreement of all formulas were large, but smallest using the Stevens, CKD-EPI, MDRD and aMDRD formulas.

Subgroup analysis showed that when GFR was < 60 ml/min/1.73m² (40 measurements), the performance (i.e. accuracy and limits of agreement) of the βTP-based formulas...
Figure 2. Bland and Altman analysis of the MDRD (A), aMDRD (B), CKD-EPI (C), 24h\textsubscript{urine} creatinine clearance (D), Cockcroft and Gault (E), Larsson (F), Hoek (G), Rule (H), White (I), Pöge (J) and Stevens (K) versus gold standard (GFR) using isotope GFR (\textsuperscript{125}I-iothalamate/\textsuperscript{131}I-hippuran) formula. Males are represented as ●, females as △.
Table 4. Percentage of estimated GFR measurements using the different formulas which falls within 10%, 20%, 30% and 50% of gold standard GFR using 125I-iothalamaat/131I-hippuran.

<table>
<thead>
<tr>
<th>Formula</th>
<th>% within 10% of GFR</th>
<th>% within 20% of GFR</th>
<th>% within 30% of GFR</th>
<th>% within 50% of GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Creatinine-based</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDRD</td>
<td>27.8</td>
<td>55.6</td>
<td>73.7</td>
<td>89.5</td>
</tr>
<tr>
<td>aMDRD</td>
<td>35.3</td>
<td>61.0</td>
<td>77.9</td>
<td>95.6</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>35.3</td>
<td>64.4</td>
<td>74.3</td>
<td>91.2</td>
</tr>
<tr>
<td>24h urine creatinine clearance</td>
<td>25.7</td>
<td>53.7</td>
<td>74.3</td>
<td>91.9</td>
</tr>
<tr>
<td>CG</td>
<td>27.2</td>
<td>56.6</td>
<td>69.1</td>
<td>84.6</td>
</tr>
<tr>
<td><strong>Cystatin C-based</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larsson</td>
<td>30.9</td>
<td>63.2</td>
<td>77.9</td>
<td>97.1</td>
</tr>
<tr>
<td>Hoek</td>
<td>28.7</td>
<td>60.3</td>
<td>80.1</td>
<td>96.3</td>
</tr>
<tr>
<td>Rule</td>
<td>36.8</td>
<td>71.3</td>
<td>87.5</td>
<td>98.5</td>
</tr>
<tr>
<td><strong>BTP-based</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>21.1</td>
<td>45.1</td>
<td>70.7</td>
<td>96.2</td>
</tr>
<tr>
<td>Pöge</td>
<td>15.0</td>
<td>24.8</td>
<td>42.1</td>
<td>88.7</td>
</tr>
<tr>
<td><strong>Creatinine/cystatin C-based</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stevens</td>
<td>41.9</td>
<td>69.1</td>
<td>81.6</td>
<td>97.8</td>
</tr>
</tbody>
</table>

exceeded that of the creatinine- and cysC-based equations. Age did not influence the performance of the formulas. Overall, accuracy and limits of agreement were smaller in females compared to males.

**Follow-up**

Figure 3 shows the follow-up of renal function by the mGFR and the creatinine-based (aMDRD and CKD-EPI), cysC-based (Hoek) and combined formula (Stevens). The latter had the best limits of agreement as shown by the Bland and Altman analysis (figure 2). Only the first three consecutive measurements (i.e. two years of follow-up) are shown, since the number of patients who had more than three sequential GFR measurements, was too small (number of patients after 3, 4 and 5 years of follow-up was 23, 7 and 2 patients, respectively).

The Hoek formula using cysC did not demonstrate a decline in renal function for males and females combined, where the mGFR, aMDRD, CKD-EPI- and Stevens formula did.

**Discussion**

In this study, we assessed the value of known formulas for estimation of GFR using plasma creatinine, cystatin C and βTP in patients with Fabry disease. The creatinine/cystatin C combined formula (Stevens) showed the highest AUC to detect a decline in renal function
Figure 3. Course of renal function at start of follow-up (n = 36, 20 males), 1 year (n = 33, 17 males) and 2 years (n = 33, 17 males) of follow-up, measured by isotope GFR (\(^{125}\text{I}-\text{iothalamate}^{131}\text{I}-\text{hippuran}\)) (mGFR) and estimated by the aMDRD-, CKD-EPI, Hoek- and Stevens formula. All (A), males (B) and females (C).
below a GFR 90 ml/min/1.73m². Moreover, the mGFR values during yearly follow-up of renal function were most closely represented by this formula as well. Together with the aMDRD and CKD-EPI, the Stevens formula had the smallest limits of agreement. Even for these best performing formulas, a small proportion of measurements deviated more than 30% from the gold standard measurement of GFR. The aMDRD and CKD-EPI approached the mGFR nearly as well as the Stevens formula but, like the Stevens formula in general overestimated renal function in males. The CKD-EPI formula was constructed to be more accurate at a higher GFR 16. Indeed in case of hyperfiltration (n = 14 measurements in 6 patients), the CKD-EPI approximated the mGFR value more closely than the aMDRD (Figure 2). At ranges below < 90 ml/min/1.73 m², aMDRD performed slightly better, but both showing overestimation in males. These results are important, since there is clearly a need for a reliable estimate of early kidney failure in Fabry disease patients, especially males. It is hoped that early installation of ERT will prevent deterioration of kidney function and thus adequate monitoring is mandatory. In this respect the Stevens formula would be the preferred method if measured GFR is not feasible. When a cystatin C assay is not available, among the creatinine based formulas, the CKD-EPI provides the most optimal results in the higher range of GFR as compared to the aMDRD, and is therefore the method of choice. Recently the Stevens formula also showed the best performance in a cohort of autosomal dominant polycystic disease patients compared to the MDRD, Cockcroft and Gault formula and CK-EPI formula49.

Further validation studies showed the CKD-EPI was more accurate than the aMDRD in patients with diabetes or a renal transplant 50, 51. In many studies it has been shown that the MDRD and aMDRD may not be accurate enough in non Fabry patients with normal or near normal renal function. Interestingly, in most patients, the aMDRD results in an underestimation of GFR 52, 53. Aakre et al found that in Fabry patients there is an overestimation of GFR by using the aMDRD in subjects with only slightly impaired renal function 15. We have observed the same phenomenon in our study, which indicated that probably in this population the aMDRD behaves differently. The reasons for this are incompletely understood. Aakre et al. postulated that a BMI < 20 mg/kg² in males could contribute to this overestimation 15. In our study however, most males had a normal BMI at start that did not decrease during follow-up.

For the detection of a decline in renal function over time, the same formulas were most accurate: apart from the gold standard mGFR, the Stevens as well as the aMDRD and CKD-EPI formula showed a decrease of GFR in males over 2 years of follow-up. The CysC-based Hoek-formula could only demonstrate a decrease in the first year of follow-up. One other study investigated the value of cysC (Hoek formula) in Fabry disease and found that it was an early marker for decline of GFR 28. In this study no gold standard (mGFR) was used. However, this finding can be interpreted as supportive of our findings that cysC within a formula can add to the precision of eGFR in Fabry disease. For follow-up, formulas based on cysC alone were not superior to creatinine-based or creatinine/
cysC combined formulas, in part due to severe underestimation of GFR in the higher range of renal function (mGFR > 125 ml/min/1.73m², see figure 3). If these measurements (10 in males and 4 in females) were excluded, the accuracy and limits of agreement of the cysC based formulas would improve substantially. Limitations of the use of CysC as a marker for GFR are well known. Since corticosteroids and thyroid function are known to affect CysC, we analyzed this in our cohort. None of the patients were on daily corticosteroids which could have influenced CysC levels. Only 4 patients had a slightly raised TSH with normal fT4; we could not detect a correlation with plasma CysC. We did not assess the possible influence of inflammation on CysC in our cohort. Another aspect of CysC which limits its use is the lack of standardization and higher costs compared to creatinine measurement.

βTP is reported as a marker of renal function in the so-called ‘creatinine blind range’. However, in our study we did not find an advantage of βTP-based formulas over creatinine and/or cysC formulas. βTP-based formulas only resembled mGFR more closely when mGFR was < 60 ml/min/1.73m² (data not shown). Since the mean GFR in the Pöge- and White study was < 60 ml/min/1.73m² (40.1 ± 17 and 59 ± 22 ml/min/1.73m², respectively), their formula was most probably specifically valuable for this population with already severely impaired renal function. In general, we believe that formulas that are based on cysC or beta trace protein alone severely underestimated GFR in the higher GFR ranges, which make them unsuitable for early detection of renal failure in Fabry patients.

A limitation of our study is that blood samples were not taken on the same day but were collected with a median of 1 day (range from -83 to + 97 days, with one exception of 175 days), before or after GFR measurement. Although in theory renal function could have changed during this period of time, only in two female patients, in whom the time between mGFR and the blood sample was 9 weeks, the mGFR had declined slightly. Therefore this is not likely to affect our conclusions.

In summary, although none of the formulas based on cysC, βTP or creatinine proved to be an equivalent substitute for mGFR in Fabry patients, we advise to use the Stevens formula for the estimation of GFR since it showed the best performance over the whole range of mGFR, even when hyperfiltration is present. For creatinine based formulas, the aMDRD or CKD-EPI is advised as both performed nearly as well as the Stevens equation. However, one should be aware of slight overestimation of GFR in males. In patients with a higher range GFR, the most accurate estimation could be obtained using the CKD-EPI.

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Reference List


