High prevalence of renal dysfunction after liver transplantation: non-invasive diagnosis using a cystatin C-based equation

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Abstract

Background. Chronic kidney disease (CKD) has been increasingly shown to be a negative prognostic factor after liver transplantation (Ltx). Creatinine-based glomerular filtration rate (GFR) formulas are notoriously insensitive. In children, non-invasive determination of GFR by measurement of serum cystatin C is feasible and repeatedly correlated to the gold standards of GFR measurements.

The aim of our study was to determine GFR using cystatin C (GFR\textsubscript{cys}) in comparison with conventional calculated creatinine clearance (GFR\textsubscript{crea}) in the long-term follow-up after paediatric liver transplantation (pLtx) in a large number of patients.

Methods. GFR of 168 children following liver transplantation was determined using cystatin C (GFR\textsubscript{cys}) and the Schwartz formula (GFR\textsubscript{crea}). In order to evaluate risk fac-

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tors for CKD, a logistic regression analysis was performed. A multivariate model was applied to assess the impact of immunosuppressive treatment.

**Results.** The mean follow-up after transplantation was 7.8 (0.44–15.72) years. Due to a high overestimation of GFR as demonstrated in a Bland–Altman plot, only three patients with CKD stages 2–3 were detected with GFR<sub>cys</sub> compared with 34 with GFR<sub>cys</sub> (P < 0.001). Thus, prevalence of CKD with GFR<sub>cys</sub> < 90 mL/min/1.73 m<sup>2</sup> was 30.4%, 7.6% and 27% in patients with 5, 10 and >10 years of follow-up, respectively. Patients on cyclosporine showed a significantly lower GFR than patients on tacrolimus. Logistic regression analysis did not show any significant risk factor for the development of CKD.

**Conclusions.** The cystatin C equation is a non-invasive and sensitive diagnostic tool to detect renal dysfunction in children after Ltx at an early stage. The choice of first-line calcineurin inhibitor has an important impact on the development of CKD.

**Keywords:** children; chronic kidney disease; cystatin C; liver transplantation; outcome

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### Introduction

Recent advances in immunosuppression and operative technique for paediatric liver transplantation (Ltx) have led to a dramatic increase in organ and patient survival. However, data on the long-term morbidity are incomplete. Thus, long-term risks of transplantation need to be analysed in more detail. One of the most important long-term complications of transplantation is chronic renal failure [1,2]. The two main reasons for chronic renal failure after liver transplantation are the nephrotoxicity of the calcineurin inhibitors cyclosporine (CSA) and tacrolimus (TAC) [3–5] and a potential primary renal involvement of the diagnosis leading to Ltx [6]. In adult patients, chronic kidney disease (CKD) is one of the most important predictive factors for long-term survival [2]. In paediatric patients, there are several different studies showing CKD ranging from 0% to 32% [6–9]. These differences are attributed to the individual immunosuppressive regimes of the different centres and, more importantly, to the varying methods of the studies to determine renal function.

The gold standard to measure kidney function is the inulin clearance. In practice, most centres do not use inulin clearance due to the great effort and the cost involved. Usually, a calculated glomerular filtration rate (GFR) based on serum creatinine is used. Unfortunately, many different studies showed that these methods are inaccurate, especially in children [9–11]. Due to this inaccuracy, some centres use radioactive markers such as 51Cr-ethylenediaminetetra acetic acid (Cr-51-EDTA) [8] or technetium-99m-diethylene triamine penta acetate (Tc-99m-DTPA) [7]. However, these methods require much effort, and usually, multiple drug tests need to be taken. Due to the invasiveness of the procedure, it is not practised widely.

In recent years, cystatin C has been established to determine kidney function even in children [12–15]. Filler et al. proposed an easy formula to calculate a correct GFR in millilitre per minute per 1.73 m<sup>2</sup> from serum cystatin C values [16]. Recently, a meta-analysis demonstrated good agreement between measured and cystatin C-calculated GFR (r = 0.816) [17]. In adult patients, cystatin C is a reliable marker of renal function, especially after liver transplantation [18]. Recent studies after paediatric liver transplantation showed a decrease of GFR in the first months after Ltx [9] and a slow deterioration of kidney function in the long-term follow-up [8]. But even in patients with mildly reduced kidney function, the calculated GFR from serum creatinine is inaccurate and mostly overestimates renal function [19]. Therefore, a reliable method, which can detect an early decrease of renal function, is needed. For these patients, cystatin C offers an alternative method to detect chronic renal failure and a chance to switch the immunosuppressive regime to less renal toxic drugs.

The purpose of our study was to evaluate kidney function non-invasively in the long-term follow-up of 168 children following liver transplantation using a cystatin C-based equation of glomerular filtration rate and compare the results to the widely used Schwartz formula.

### Materials and methods

**Study design**

We performed a retrospective analysis of all liver-transplanted children admitted to our centre for the routine follow-up in 2007 and 2008. In all patients, clinical (age, sex, height, weight and diagnosis) and laboratory data were collected simultaneously. Immunosuppressive medication with trough levels was recorded. From the cystatin C levels, glomerular filtration rate was calculated according to the formula<br>

\[
\text{GFR} = \frac{186}{\text{cystatin C}} \times 1.123
\]

proposed by Filler et al. [16], expressed in millilitre per minute per 1.73 m<sup>2</sup>. From the creatinine level and the body height, the GFR was calculated using the Schwartz formula [20]. Creatinine was measured using a Jaffe reaction-based method. As Schwartz et al. proposed, factor k was used age and gender-dependent as 0.45, 0.55 and 0.7. Chronic kidney disease stages are provided according to the National Kidney Foundation [21].

**Immunosuppression**

Since the year 2000, children have received two single doses of basiliximab (Simulect®, Novartis Pharma GmbH, Basel, Switzerland) on Day 1 and Day 4 post-transplant as induction therapy. All children received cyclosporine A (CSA, Sandimmun® optimal, Novartis Pharma GmbH, Basel, Switzerland) twice daily (initial trough levels 170–200 μg/L; trough levels for maintenance immunosuppression after 1 year 80–100 μg/L, after 5 years 40–70 μg/L) and prednisolone (starting dose 15 mg/m<sup>2</sup>/d) as primary immunosuppressive drugs. The corticosteroids were continuously tapered down and were withdrawn in most patients 12 months after Ltx. In the case of corticosteroid-resistant graft rejection, patients were switched to TAC (trough levels for maintenance immunosuppression 6–8 μg/L) or received additionally mycophenolate mofetil (MMF).

**Statistical analysis**

Only patients in a stable clinical condition were considered for analysis. All patients with acute rejection, acute severe infection, dehydration or surgical procedures were excluded from analysis.

We used the Bland–Altman plot to assess the agreement between the two methods determining the glomerular filtration rate [23]. GFR values
Table 1. Details of primary diagnoses of the 168 children analysed in the study

<table>
<thead>
<tr>
<th>Diagnosis leading to pLtx</th>
<th>n = 168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (without potential renal involvement)</td>
<td></td>
</tr>
<tr>
<td>Biliary atresia</td>
<td>88</td>
</tr>
<tr>
<td>Progressive familial intrahepatic cholestasis</td>
<td>14</td>
</tr>
<tr>
<td>Unknown</td>
<td>11</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>6</td>
</tr>
<tr>
<td>Crigler–Najjar syndrome</td>
<td>6</td>
</tr>
<tr>
<td>Neonatal hepatitis</td>
<td>4</td>
</tr>
<tr>
<td>Haemangiendothelioma</td>
<td>2</td>
</tr>
<tr>
<td>Mycotoxicosis</td>
<td>1</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>1</td>
</tr>
<tr>
<td>Haemochromatosis</td>
<td>1</td>
</tr>
<tr>
<td>Sepsis</td>
<td>1</td>
</tr>
<tr>
<td>Neonatal hepatitis B</td>
<td>1</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>1</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>1</td>
</tr>
<tr>
<td>Total (percentage)</td>
<td>138 (82%)</td>
</tr>
<tr>
<td>Group 2 (potential renal involvement)</td>
<td></td>
</tr>
<tr>
<td>Alagille syndrome</td>
<td>15</td>
</tr>
<tr>
<td>Ornithine transcarbamylase deficiency</td>
<td>3</td>
</tr>
<tr>
<td>Post-stem cell transplantation</td>
<td>3</td>
</tr>
<tr>
<td>Alpha-1-antitrypsin deficiency</td>
<td>2</td>
</tr>
<tr>
<td>Wilson disease</td>
<td>2</td>
</tr>
<tr>
<td>Tyrosinaemia</td>
<td>2</td>
</tr>
<tr>
<td>Mitochondriopathy</td>
<td>1</td>
</tr>
<tr>
<td>Citrullinaemia</td>
<td>1</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>1</td>
</tr>
<tr>
<td>Total (percentage)</td>
<td>30 (18%)</td>
</tr>
</tbody>
</table>

Patients are divided into two groups. Group 1 (without primary renal involvement) consists of 138 (82%), and Group 2 (inherited metabolic disease and Alagille syndrome) of 30 patients (18%), respectively.

were log-transformed. Loess regression was used to determine the disagreement as a function of the average glomerular filtration rate [24]. Student's t-test was used to compare continuous variables, whereas chi-square tests were used to compare categorical variables. McNemar's test was used to test the agreement of the diagnosis of CKD between the cystatin C-based equation and the Schwartz formula.

Since primary diagnosis is an important confounding factor affecting kidney function, we divided all patients in the two subgroups as proposed by Harambat et al. [8]. The first group (Group 1) was without potential renal involvement, and the second group was with potential renal involvement (including Alagille syndrome and inborn errors of metabolism, Group 2). Both groups had comparable pre-transplant GFR measured by the Schwartz formula [mean GFR Group 1: 144 mL/min/1.73 m² (range 30–370) and mean GFR Group 2: 156 mL/min/1.73 m² (range 56–316)]. In order to evaluate the differences in renal function between the diagnosis groups of patients, logistic regression analysis was performed, with cystatin C-based GFR ≤89 mL/min/1.73 m² as dependent variable and with adjustment for diagnosis groups, age at transplantation, gender, and immunosuppressive treatment, respectively.

We compared GFR(cys) values associated with pharmacological treatment regimes in a multivariate model adjusted for diagnosis group, age at transplantation and gender. Patients who had been switched from CSA to TAC were analysed in the TAC group if the duration of TAC treatment was >3 years.

Statistical analyses were conducted using SPSS Statistics 16 German (SPSS Inc., Chicago, IL, USA) and SAS version 9.1.3. (SAS Institute, Cary, NC, USA). A P-value <0.05, two-tailed, was considered statistically significant.

Results

A total of 168 patients were included in the analysis (87 males and 81 females). Mean age at the time of transplantation was 2.7 years (range 0.02–14.72 years), and mean follow-up time since Ltx was 7.8 years (range 0.44–15.72 years). The diagnoses leading to Ltx are given in Table 1. The mean GFR calculated with cystatin C (GFR(cys)) was 109 mL/min/1.73 m² (95% confidence intervals: 105–113 mL/min/1.73 m²), and the mean GFR calculated by the Schwartz formula (GFR(crea)) was 157 mL/min/1.73 m² (95% CI: 151–163 mL/min/1.73 m²). Using the cystatin C equation, we found 34 patients (20%) with GFR(cys) of 89 mL/min/1.73 m² or less, 29 patients had CKD stage 2 and 5 patients had CKD stage 3 (GFR between 30 and 59 mL/min/1.73 m²). No patient suffered from end-stage renal disease or required dialysis. The Schwartz formula identified only three patients (1.8%) who had a slightly reduced kidney function with a GFR between 85 and 90 mL/min/1.73 m². Thus, there was an inhomogeneity between both methods, which is highly significant (P < 0.001).

The Bland–Altman plot showed a high overestimation of kidney function by the Schwartz formula (Figure 1). Loess regression (smoothing = 0.7) shows that the discrepancy is increasing with higher values.

The prevalence of CKD with GFR(cys) <90 mL/min/1.73 m² was 30.4%, 7.6% and 27% in patients with 5, 10 and >10 years of follow-up, respectively.

No significant difference was found in GFR(cys) between boys and girls [mean (95% confidence intervals), 106 (101–112) versus 111 (105–118) mL/min/1.73 m²]. No difference was found between the proportion of males and females with and without reduced kidney function.

In a logistic regression model, we analysed whether GFR(cys) values of 89 mL/min/1.73 m² or less are associated with diagnosis group, when we adjusted for age at transplantation, gender, and the type of pharmacological treatment. We found that none of the variables had a significant effect for the development of decreased renal function (Figure 2).

Analysis of the immunosuppressive treatment showed a significant lower adjusted GFR(cys) for patients with a CSA-based immunosuppression versus patients with a TAC-based regime (P = 0.027) (Figure 3). Additionally, patients with a combined immunosuppression had a lower GFR than patients on monotherapy, which is statistically significant for the comparison of CSA versus CSA and MMF (P = 0.003). The mean trough level of CSA was 78 ± 39.5 μg/L, and the mean trough level of TAC was 7.9 ± 2.1 μg/L. The trough level of CSA or TAC was not different in the group with reduced kidney function versus the group with normal GFR.

In the group of patients with CKD (n = 34), six patients (17%) had arterial hypertension, six patients (17%) had a metabolic acidosis and four of them (12%) had both.

Discussion

This retrospective analysis of a large cohort of children confirms the emerging problem of chronic kidney disease after liver transplantation. For the first time, a cystatin C-based equation was used for the assessment of renal function in the long term after paediatric liver transplantation. The data
show that a detection of a decreasing GFR is possible, is superior to a creatinine-based equation and is consistent to other published data using measured GFR [7,8]. The Schwartz formula, overestimating the GFR, would have missed almost all patients with CKD.

The gold standard to evaluate kidney function in children is still the inulin clearance or GFR measurements, e.g. using radioactive tracers. For our own study, we opted not to use these invasive measurements due to the great burden involved. We think that it is justifiable to avoid multiple blood tests and the application of radioactivity if a replacement method with a sufficient accuracy is available. In this respect, cystatin C is currently the best evaluated method [15,16,25–28].

The equation used from Filler et al. [16] was initially developed in children with chronic kidney disease and was retested by Zappitelli in children with various diagnoses [13]. He showed 10% overestimation of GFR when the cystatin C equation from Filler [16] was used. Thus, the risk of underestimation of GFR is low. Importantly, the equation used is independent from age and muscle mass [28]. Samyn et al. [27] showed good agreement between cystatin C levels and measured GFR especially in children after liver transplantation in the short term.

The presented data comprise the largest study after paediatric liver transplantation, and the results are consistent with the recently published series by Harambat et al. [8], who used an inulin clearance to assess kidney function. Both studies show ~30% of chronic renal failure after >10 years of follow-up and demonstrate that a close monitoring, especially in the first year after Ltx and in the long-term follow-up, is important. Additionally, our data present long-term data for 15 years of follow-up with a prevalence of chronic renal failure of again ~30%. The high prevalence of CKD in the first 3 years of follow-up is remarkable. Campbell et al. [7] showed that the GFR at 1 year

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis group (Group 1 vs Group 2)</td>
<td>1.436</td>
<td>(0.549</td>
<td>0.461</td>
</tr>
<tr>
<td>Gender (female vs male)</td>
<td>0.693</td>
<td>(0.314</td>
<td>0.363</td>
</tr>
<tr>
<td>Immunosuppression (TAC vs CSA)</td>
<td>1.223</td>
<td>(0.769</td>
<td>0.391</td>
</tr>
<tr>
<td>Follow-up (years)</td>
<td>0.984</td>
<td>(0.884</td>
<td>0.773</td>
</tr>
<tr>
<td>Age at transplantation (years of age)</td>
<td>1.074</td>
<td>(0.956</td>
<td>0.232</td>
</tr>
</tbody>
</table>

Fig. 1. Bland–Altman plot showing high overestimation of glomerular filtration rate calculated by the Schwartz formula in comparison with cystatin C-based equation. The Loess regression (smoothness = 0.7) shows that the discrepancy is increasing with higher values.

Fig. 2. Forest plot showing the odds ratios and 95% confidence intervals for developing chronic kidney disease using the cystatin C equation adjusted for gender, age at transplantation and the type of immunosuppression.
after the transplantation is strongly associated with the long-term renal outcome. In our series of patients, we found a high number of patients with CKD in the first years after Ltx ($n = 17$, prevalence of 30% in the patients with 0–5 years of follow-up). Especially, these patients have a high risk to develop CKD in the long-term follow-up. Therefore, they need to be closely monitored with a reliable method to determine GFR.

The patients with a follow-up of 5–10 years showed a relatively low prevalence of CKD with 7.6%, confirming other studies [6,11]. This stabilization of kidney function can be attributed to a clinical stabilization after the first years of transplant. In the long-term follow-up, the chronic toxicity of calcineurin inhibitor (CNI), e.g. glomerular sclerosis, seems to play a key role and may lead to a higher prevalence in the very long-term follow-up (>10 years). A second point is the underlying disease with potential renal involvement which might deteriorate kidney function. Therefore, the prevalence increases again in the group of patients with a follow-up of >10 years.

After dividing the patient population into two subgroups, with and without a potential primary renal involvement, we did not find a significantly different renal outcome in the long-term follow-up. The logistic regression model did not find any parameter which could serve as a significant predictor for the development of renal dysfunction. Other studies showed that the diagnosis leading to the transplantation is associated with CKD [8]. Since our study was a cross-sectional analysis, our patient population can be biased (e.g. patients who died shortly after transplantation were not considered), and Harambat et al. [8] analysed only a very few number of patients in the two diagnosis groups in the follow-up of >5 years. Thus, further prospective studies are needed to investigate the potential effects of other risk factors on the development of decreased kidney function.

Nevertheless, especially in the group of patients with a known risk for renal impairment, e.g. Alagille syndrome or inborn errors of metabolism, it will be a challenge to reduce the risk of end-stage renal disease in the following decades. Therefore, clear concepts on how to change the immunosuppression protocols after detection of renal dysfunction are needed. Protocols minimizing the calcineurin inhibitors are desirable, especially in children. The use of different immunosuppressants, such as sirolimus or MMF, has been published, but to date, little data are available [29–31]. More randomized studies are needed to assess these protocols.

The use of calcineurin inhibitors such as CSA and TAC led to a dramatic increase of organ survival after transplantation. Unfortunately, this comes together with nephrotoxicity, and many studies compared both drugs with regard to their renal side effects. The presented data show a significantly lower adjusted GFR for the patients on CSA versus the patients on TAC. These data are consistent with other published studies [7,8,11], but the literature is contradictory. Some other studies found no difference between CSA- and TAC-treated patients [6,32]. Our data are limited due to the fact that some patients were switched from CSA to TAC at an earlier stage and that we cannot refer to the whole CNI exposure over time since transplant; thus, we analysed data with the current medication. Additionally, the molecular pathophysiology of CNI-induced nephrotoxicity does not provide a good explanation for different renal outcomes of the two drugs due to the same intracellular pathway.

Of the patients with renal impairment, 12% suffered from arterial hypertension and metabolic acidosis. It is well known that these are risk factors for atherosclerosis and cardiovascular disease [33]. Arterial hypertension should be monitored by 24-h blood pressure measurement and should be treated in the first line with calcium channel blockers [34,35]. It may be useful to consider angiotensin-converting enzyme (ACE) inhibitors even in the setting of paediatric liver transplantation due to the good control of arterial hypertension and the nephroprotective effect.
References


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