Evaluation and predictive factors of renal function progression using cystatin C and creatinine in neonates born with CAKUT

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Abstract. Background: Congenital anomalies of the kidney and urinary tract (CAKUT) is a main cause leading to end-stage renal disease (ESRD) during childhood occurring at a frequency of 1 in every 500 pregnancies. No early predictive markers of long-term renal function (RF) are validated in these neonates. The aim of this study was to compare CysC and creatinine (creat) as markers of RF from birth to 2 years and to identify factors of RF progression. Methods: The 56 patients included in this study were followed for a median of 235 days (137 – 739). Repeated measures of CysC and creat were during 2 years of RF evaluation were taken in 28 patients. Changes in RF with age were analyzed. Potential risk factors for RF progression were analyzed for: type of kidney disease (KD), bilateralism of KD, prenatal pelvic dilatation, reflux and initial relative RF (RRF) asymmetry obtained by scan. Results: With age, a rapid decrease of CysC (16.3%, p < 0.001), and creat (68.6%, p < 0.001) was observed at 1 month. Between 1 month and 1 year, CysC decreased 4% per month (p < 0.001) and creatinine stabilized (+ 1.9%/m, p = 0.11). After 1 year, both CysC and creat stabilized. In the multivariate model, CysC significantly increased in patients with bilateralism (p = 0.004) or asymmetric RRF (p = 0.03). Creat was not significant. Conclusion: CysC was a better marker than creat to follow RF in neonates with CAKUT. Using CysC, bilateralism, and RRF asymmetry were significantly associated with RF progression.

Methods

The study protocol (CER 05-111) was approved by the Ethics Committee of Geneva University.

Patients

In our study at Geneva University Hospital, between 2008 and 2011, we consecu-
tively included 56 patients pre-natally diagnosed with congenital kidney anomalies. For patients presenting with prenatal pelvic dilatation, we considered a measure of anterior-posterior diameter ≥ 10 mm during the 3rd trimester of pregnancy as inclusion criteria.

Patients were recruited during an organized prenatal visit to explain renal pathology to the parents. During this visit, we obtained written consent from the parents to draw CysC on cord blood at birth and to include their baby in a database to establish a long-term follow-up.

56 patients were included and the median follow-up was 235 days (137 – 739). Blood CysC was drawn on cord blood at birth. Among the patients who had severe pelvic dilatation, associated or not with severe vesical reflux (VUR), and who needed scintigraphy scans, 28 had repeated CysC and creatinine measurements to evaluate renal function follow-up.

The repartition of renal anomalies is described in Table 1 for the 56 patients and for the 28 patients who had renal function follow-up.

In the database we recorded: diagnosis, prenatal pelvic dilatation classified by the Society for Fetal Urology (SFU) grade [7], VUR graded from 1 to 4 according to the International Grading Reflux System [8], percentage of relative renal function (RRF) obtained with renal scintigraphy (DMSA or MAG3), type of interventions, and urinary tract infections. For the scintigraphy, we considered significant asymmetry in RRF if the percentage difference was greater than 10%.

For the 28 patients with renal function follow-up, the repeated measures of CysC and creatinine as well as and time of measurement were recorded.

At birth, all neonates presenting pelvic dilatation with an antero-posterior diameter ≥ 10 mm received prophylaxis with amoxicillin (10 mg/kg twice a day) until results from the cystography were received. All 56 neonates were investigated for reflux. Prophylaxis was stopped if the cystography confirmed the absence of VUR. 31 patients underwent a scintigraphy (DMSA or MAG3).

**Cystatin C analyses**

CysC was measured by particle-enhanced nephelometric immunoassay on IMMAGE® BECKMAN analyzer (BECKMAN COULTER, Brea, Canada). The assay used for the reaction is from DakoCytomation (DAKO, Glostrup, Denmark). The samples were analyzed in the 4 hours following the time they were drawn, at birth. The samples did not need any dilution, and the results were available within minutes. Only 2 mL of cord blood was required for this analysis. The coefficient of variation for CysC (inter-assay precision) was 4.95% at 1.13 mg/L and 3.30% at 4.91 mg/L (n = 130). The coefficient of variation (intra-assay precision) was 2.0% at 1.71 mg/L and 2.3% at 5.37 mg/L (n = 80). Total measurement of uncertainty was 0.097 mg/L at 1.14 mg/L.

**Patient characteristics**

The characteristics of patients were described by frequencies and percentages (categorical variables) or by the median, minimum and maximum values (continuous variables). Details are reported in Table 2.

**Renal function follow-up**

To assess the progression of renal function in these neonates (n = 28), we analyzed repeated measures of CysC and creatinine over 2 years. These patients had at least two CysC and creatinine measurements during the follow-up period.
Statistical analyses

The changes in CysC and creatinine values with age were analyzed by using a linear regression model with piecewise constant parameters; the decrease or increase of CysC and creatinine was assessed at 1 month, between the 1st and 12th month, and after 1 year. A logarithm transformation was applied on CysC and creatinine to improve the goodness-of-fit of the models. The results of the regression models expressed an increase or decrease of CysC or creatinine per month. As the measures were repeated, a mixed model was performed. Factors potentially associated to CysC and creatinine were added in the linear mixed model. Due to the number of patients, the factors were tested one by one. The significance level was 0.05 for all analyses.

Results

Patients’ characteristics

The female to male ratio in the 56 patients was 17 : 39; 70% were boys. The repartition of renal anomalies is presented in Table 1. In Table 2, the detailed characteristics of the 56 patients and those of the 28 neonates who had CysC and creatinine follow-up are displayed. 22 patients presented with bilateral kidney malformation (BKM) and 34 with unilateral kidney malformation (UKM). 45 out of 56 (80%) neonates presented a pelvic dilatation repartee as follows: 5 SFU grade 1, 16 SFU grade 2, 15 SFU grade 3, and 9 SFU grade 4. In patients with renal function follow-up, 70% presented SFU ≥ 3. Reflux was present in only 23% of the patients. Refluxes were
Renal function progression in neonates born with CAKUT was reported as follows: 2 low grade VUR (I, II) and 11 high grade (III, IV; 85%).

In the group with renal function evaluation, 9 out of 28 had VUR; all were high grade (100%). Asymmetry of RRF was found in 10 out of 31 (38.5%) patients; all patients with RRF asymmetry had renal function follow-up. Eight (14%) patients had a bladder anomaly, and, among these, 4 presented posterior urethral valves and had renal function follow-up.

Fourteen out of 56 (25%) patients needed a total 25 interventions: circumcision (11), vesicostomy (5), PUV section (2), nephroureterectomy (1), endoscopic treatment of ureterocele (2), pyeloplasty (1), ureter reimplantation (1), urachal resection (1), and vesicostomy closure (1). For the analysis, we did not consider circumcisions as an intervention, thus the percentage of neonates needing intervention was 10 out of 56 (17.9%) and 10 out of 28 (35.7%) in the group with renal follow-up (Table 2).

Twelve out of 56 patients (20%) presented urinary tract infection with *Escherichia coli* (44%), *Pseudomonas aeruginosa* (19%), *Enterococcus faecalis* (12%), Klebsiella oxytoca (12%), and miscellaneous (13%).

### CysC and creatinine follow-up analyses

In the 28 patients who had measures for renal function follow-up at birth, the median CysC (mg/L) value was 1.97 (IQR) (1.73; 2.54) and 57.5 (34.8; 144.5) for creatinine (µmol/L). On average, patients had five measurements of CysC during follow-up. The regression model indicated that CysC decreased strongly the 1st month (−16.3%, p < 0.001), continued to decrease between the 1st and 12th month but slower (−4.0% per month, p < 0.001), and was stable thereafter (−0.6% per month, p = 0.81). The fitted regression model is represented in Figure 1a.

On average, patients had 3.7 measurements of creatinine during follow-up. Similar to CysC, a decrease of −68.6% was observed for creatinine in the 1st month (p < 0.001). Changes in creatinine were +1.9% per month between the 1st and 12th month (p = 0.11) and −2.5% per month thereafter (p = 0.63). The fitted regression model is represented in Figure 1b.

The multivariate analyses to identify factors susceptible to influence renal function...
progression measured with CysC or creatinine is detailed in Table 3. Multivariate analyses showed that BKM had an impact on CysC follow-up; the mean value of CysC was 22.4% greater in BKM than in UKM (p = 0.004). Using CysC, RRF asymmetry was found to also be significant in decreasing renal function (p = 0.03).

There were no risk factors identified with creatinine. The patients with BKM had a mean value of creatinine greater than patients with UKM, but it was not significant (+ 49.3%, p = 0.06).

Discussion

CAKUT is the most common abnormality detected by antenatal screening and the main cause of renal failure in children severe enough to warrant long-term dialysis and kidney transplantation [9, 10, 11, 12]. Prenatal ultrasound for complete fetal anatomy is now routinely offered to parents with the possible detection of a kidney anomaly. Therefore, we are being increasingly confronted with discussing the significance and prognosis of these malformations, although we seriously lack knowledge on long-term follow-up to specify the outcome and improve counseling [13]. Lewis et al. published a registry of over 10 years reporting diagnosis related to established end-stage renal failure; the first cause was renal dysplasia with 32% and 14.7% of the patients having obstructive uropathies. In another registry (Italkid), the first cause was obstructive uropathies (21%) [5, 14]. This was consistent with other registries (NAPRTC, Pediatric registries) that reported up to 29 – 51.2% of the proportion of CAKUT in young patients below 21 years presenting ESRD [15].

An early management of these malformations has decreased the mortality and improved long-term renal function. Melo et al. [16] reported an incidence of CAKUT in 17.7 per 1,000 live births, and the overall mortality was 24%, with an increased independent risk in cases of oligoamnios, low birth weight, prematurity, and first pregnancy. Only a few cases have studied long-term outcomes in congenital kidney anomalies [12, 17, 18]. A retrospective follow-up study over 20 years, including 822 children prenatally diagnosed with CAKUT, reported that 6% of patients developed chronic kidney disease at the age of 10, and the mortality rate was 1.5%. The incidence of end-stage renal failure was increased in boys compared to girls, and the post-natal diagnosis was principally posterior urethral valves (61%). The probability of end-stage renal failure was 15% in complicated pelvic dilatation and 2% in isolated fetal pelvic dilatation. The rate of neonates who needed surgical procedures was 28% [18].

Table 3. Multivariate analyses on CysC and creatinine follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Cystatin C (mg/L)</th>
<th>Creatinine (µmol/L)</th>
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<tbody>
<tr>
<td></td>
<td>Estimates</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>CysC per month</td>
<td></td>
</tr>
<tr>
<td>BKM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UKM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BKM</td>
<td>0.217 (0.069)</td>
<td>0.004</td>
</tr>
<tr>
<td>VUR (III, IV)</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.102 (0.084)</td>
<td>0.24</td>
</tr>
<tr>
<td>SFU (III,IV)</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>–0.026 (0.082)</td>
<td>0.75</td>
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<tr>
<td>RRF asymmetry</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.166 (0.071)</td>
<td>0.03</td>
</tr>
<tr>
<td>Bladder anomaly</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>–0.001 (0.088)</td>
<td>0.99</td>
</tr>
<tr>
<td>Intervention</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.080 (0.083)</td>
<td>0.34</td>
</tr>
<tr>
<td>UTI</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.080 (0.086)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Only BKM and RRF asymmetry were significantly associated to CysC level (p = 0.004 and p = 0.03). None of the parameters were significant using creat. BKM = bilateral kidney malformation; UKM = unilateral kidney malformation; VUR = vesico ureteral reflux; SFU = society of fetal urology; RRF = relative renal function; UTI = urinary tract infection.
Sanna-Cherchi et al. [19] published interesting results on the risk of progress to end-stage renal failure in 312 patients with different congenital kidney anomalies, using a model of dialysis-free survival from birth according to the CAKUT category. Survival data showed a significantly weaker outcome for patients carrying bilateral renal dysplasia, solitary kidney, and posterior urethral valves.

There is little knowledge of early determinants of long-term renal function. Therefore, it is difficult to discriminate among neonates born with congenital kidney diseases, those who will need close follow-up, and those with milder anomalies who will present a good outcome [12, 20]. The measure of creatinine at birth has been proposed as a marker of long-term renal function; however, this marker presents some limitations because the mother’s creatinine value interferes with the measurement [6, 12].

In our 56 neonates diagnosed with CAKUT 70% were boys. The rate of VUR was low (23%), but 85% were high grade VUR. When excluding circumcisions, 18% of the patients needed to have one or more interventions. The rate of VUR and intervention in our study was consistent with the literature [17, 21, 22].

The patients followed for renal function presented more severe prenatal pelvic dilatation (SFU III/IV; 70%), and 1/3 had VUR, all of high grade. In a previous publication, we showed a significant increase of CysC at birth in neonates with BKM compared to those with a unilateral anomaly [3]. This was equally observed during renal function follow-up with a mean CysC value increment of 22.4% in neonates with bilateral kidney anomalies compared to those with unilateral malformation (p = 0.004) (Table 3). This highlights that particular attention needs to be taken in neonates with bilateral diseases because they may evolve with a more rapid and severe decrease in renal function.

Comparing the variation of repeated measures of CysC and creatinine obtained over 2 years by a regression model, a strong decrease of CysC was observed in the first month, the decrease weakened but was still significant up until 1 year, then the values of CysC stabilized after 1 year (Figure 1a). To follow renal function after one year, a unique reference interval value of CysC (0.7 – 1.38 mg/L) may be used and is independent of gender, age, and muscle mass contrary to creatinine [2, 23]. We used multivariate analysis to define potential predictive factors for renal function progression from birth up to 2 years. One of the patients with posterior urethral valves was excluded from the analyses because he presented ESRD at birth and peritoneal dialysis was started at 7 months. As illustrated in Figure 1a and Table 3, CysC was superior to creatinine in identifying neonates with a more severe prognosis in renal function.

These factors were BKM at birth and an asymmetric relative renal function obtained by scintigraphy DMSA or MAG3 (Table 3). In patients presenting high grade VUR (III, IV) and in neonates who had surgery, CysC was increased; however, it was not significant. Surprisingly, neonates with a bladder anomaly did not present a decrease in renal function during the first years of life. These patients initially had abnormal renal function, but then stabilized over 2 years after early surgical intervention by vesicostomy or posterior urethral valves. In the case of bladder malformation, Hensle et al. [24] reported that before the first year of life, renal function is the most important prognosis factor in these neonates. Renal function follow-up measured by creatinine was less accurate in our population in identifying markers of renal function progression most likely due to its variability with age, gender, and muscle mass.

**Conclusion**

CysC was superior to creatinine to evaluate renal function progression from birth to 2 years in neonates diagnosed with CAKUT.

In addition, we here demonstrate the superiority of CysC in discriminating predictive factors of renal progression. The conditions associated with a significant decrease in renal function were the bilateralism of the kidney anomaly at birth and the asymmetry of RRF in the initial scan. Creatinine was not sensitive enough to identify factors of renal progression.
Conflict of interest

There is no potential, perceived, or real conflict of interest, and there have been no financial arrangements regarding this paper.

References


