
Available from: http://dx.doi.org/10.1097/PCC.0b013e318271f4a5

This is a non-final version of an article published in final form in Pediatric Critical Care Medicine Vol. 14, Issue 3, p. 318-322

Accessed from: http://hdl.handle.net/1959.13/1036882
Measuring Cystatin C to Determine Renal Function in Neonates

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Financial support: 1. The Royal Australasian College of Physicians
2. ANZ trustee Queensland Pediatric Medical Research Grant

Keywords: creatinine, cystatin C, acute kidney injury, neonates, preterm, renal failure
Abstract

Objective: The incidence of acute kidney injury (AKI) in neonates is high and associated with up to a 50% mortality rate. The purpose of this review was to determine the feasibility of using serum cystatin C (CysC) measurements to assist clinicians in making early and accurate diagnoses of AKI in neonates.

Data source: We searched for the following 7 key words within the PubMed database and The Cochrane Database of Systematic Reviews: Cystatin C, neonates, newborn, preterm, premature, kidney failure, and kidney injury.

Study selection: The selected studies included neonates within their study populations and were published in English. We reviewed literature published between January 1990 and May 2012.

Data extraction: Ten studies had conducted serum CysC measurements in neonates.

Data synthesis: The CysC level in neonates is not influenced by the maternal level and is highest at birth. In most studies, CysC levels on day 1 of life ranged between 1 and 2 mg/L, gradually declining during the first year, and then remained relatively stable thereafter. CysC levels did not differ between male and female infants, and no significant gestational age-dependent differences were found. CysC level were increased in cases of sepsis, AKI, and congenital renal abnormalities.

Conclusions: CysC has all of the theoretical properties needed to be an ideal marker of renal function. It can be used to determine base line renal function on day 1 and is increasingly being used to determine renal function in sick neonates. In the majority of studies, the day 1 CysC level ranged between 1 to 2 mg/L, which gradually declined in the first year of life. However, the number of available studies evaluating CysC in sick neonates is currently limited, and there are also no studies linking CysC levels in sick babies with short term and long-term outcomes.
Introduction

Acute kidney injury (AKI) is a complex clinical condition ranging from mild kidney dysfunction to complete renal failure with anuria (1). It is characterized by a decrease in the glomerular filtration rate (GFR), increase in the serum concentration of creatinine and nitrogenous waste products, and the inability of the kidney to maintain fluid and electrolyte homeostasis (2). AKI and elevated serum creatinine (SCr) levels are independent risk factors for mortality (3). Moreover, neonates with AKI are believed to be at a higher risk of developing chronic kidney disease (1). The incidence of AKI in neonates varies according to the underlying pathology. In one prospective study involving 229 very low birth weight babies, 18% of the cohort developed AKI, with a mortality rate of 42% (4). The incidence of AKI in neonates undergoing cardiac surgery is higher, with one study showing that approximately half of the patients developed AKI with a mortality rate that was 4 times higher than those without AKI (5). Similarly, a very high incidence rate of AKI (41.7%) has been reported in neonates with perinatal asphyxia (6). Importantly, our inability to recognize AKI has prevented early intervention and improved outcomes.

Measuring renal function in neonates

Creatinine clearance and SCr levels are routinely used to monitor adult renal function. However, the use of SCr to measure the GFR of neonates has never been completely investigated. Van Anker et al. evaluated 144 preterm infants (gestational age, 23.4-36.9 weeks), where they measured GFR as the clearance of inulin, which was determined from the infusion rate and SCr level (7). They observed that the level of SCr was inversely proportional to the clearance of insulin and concluded that the reciprocal of the SCr value provides an accurate measurement of glomerular filtration (7). However, using SCr levels to determine GFR has many limitations. For example, assays that measure SCr levels are subject to negative interference by bilirubin and hemoglobin and positive interference by cephalosporin and ketones (7). In addition, other limitations include (i) SCr levels are not altered until there is a loss of 25-50% of kidney function; (ii) SCr levels overestimate renal function due to tubular secretion of creatinine at lower GFRs; and (iii) SCr levels are influenced by an infant’s muscle bulk, hydration status, and gestational age (1). At birth, SCr concentrations reflect both infant and maternal levels (8). It was previously believed that the SCr concentration in newborns steadily declines after birth. However, recent data have shown that the SCr concentration increases during the first 48 hours of...
extrauterine life, subsequently reaches a peak, and then declines thereafter as the GFR rises (8). Because of incomplete nephrogenesis, the GFR in pre-term neonates is significantly lower than that in full-term infants and matures more slowly as well. In a full-term newborn, the GFR at birth is approximately 20 mL·min⁻¹/1.73 m², which doubles within the first 2 postnatal weeks (9). However, a delayed rise in SCr after the onset of renal injury makes it an unreliable marker; thus, the search for early AKI biomarkers has been a prominent research focus (10).

CysC—composed of 122 amino acids—is a cysteine proteinase inhibitor with a relative molecular mass of 13,250 Da (11). It is produced by a housekeeping gene expressed in all nucleated cells at a constant rate and is freely filtered at the glomerulus with no tubular secretion. Moreover, CysC is completely catabolized by the renal tubules, and therefore its plasma level is only dependent on the GFR (11). Meta-analyses have shown that CysC is superior to SCr for the estimation of the GFR in children (12, 13). Andersen et al. (13) evaluated the usefulness of CysC as marker for pediatric renal function based on literature on children from various age groups. They concluded that the sensitivity of serum CysC for detecting impaired GFR in the pediatric population is superior to that of plasma creatinine. However, the investigators did not make any comments or conclusions on the value of CysC as a marker in neonates. Therefore, this review focuses on the current literature on CysC measurements in neonates and explores the possibility that this marker could substitute for creatinine measurements in clinical practice.

**Methods**

We performed a review of the literature from PubMed, US National Library of Medicine, and The Cochrane Database of Systematic Reviews using the following keywords: Cystatin C, neonates, newborn, preterm, premature, kidney failure, and kidney injury. The keywords were searched alone or in combination with other keywords. The search was restricted to articles that had neonates in their study population and those that were published in English. We reviewed literature published between January 1990 and May 2012.

**Results**

To date, 10 studies on CysC measurements in neonates have been published (Tables 1 and 2) (14-23). Previous studies focused on determining the normal CysC reference range in term neonates and
compared the values to maternal levels (14) and to older infants and children (15, 17). Subsequent publications compared CysC levels between premature and term babies (16, 18-20). More recent publications considered CysC as a marker for AKI in ill neonates (21-23). The measurements have been presented in various ways, including as a mean with 95% confidence interval, median and interquartile range (IQR), mean with one standard deviation (SD), and mean with 2 SD. The most recent studies have indicated that the CysC level in neonates does not have a normal Gaussian distribution, and have reported the measurements as a median with an IQR (20, 22, 23).

**Method of analysis**

Particle-enhanced immunonephelometry (PENIA) and particle-enhanced immunoturbimetry (PETIA) are the two most common methods used to measure serum CysC levels. The use of PETIA results in up to 30% higher CysC measurements (13), but recent studies have explored the use of the PENIA method. The International Federation of Clinical Chemistry and Laboratory Medicine/Institute for Reference Materials and Measurements Working Group for Standardization of CysC (24) are currently establishing a uniform calibrator for both techniques. This will facilitate a direct comparison of measurements obtained from both assays.

**Normal CysC values**

One of the earliest studies assessing CysC levels compared the levels in healthy women with their full-term neonates (14). Seventy-eight women with uncomplicated pregnancy and their newborns babies (43 males and 35 females) were enrolled in the study. The investigators evaluated the relationship between maternal and neonatal serum CysC levels and SCr levels. The gestational age ranged from 37 to 43 weeks and the birth weight ranged from 2.50 to 4.15 kg. Serum levels of CysC, creatinine, and urea were measured in all women immediately before delivery and in their newborns at the time of birth as well as 72 and 96 h after birth. The investigators found that the maternal serum CysC levels ranged from 0.64 to 2.30 mg/L at term gestation. At birth, the neonatal serum CysC values ranged from 1.17 to 3.06 mg/L, and decreased significantly after 3-5 days of life. No correlation between maternal and neonatal serum CysC values was observed. The authors concluded that neonatal serum CysC originates exclusively in the neonate.
Rander et al. measured CysC levels in a mixed cohort of 12 neonates (age range, 7 days to 1 month old) and 137 children (15). CysC levels were highest in the neonatal period and gradually declined from 1.63 ± 0.26 mg/L to a steady state of 0.51-0.95 mg/L for children aged at least 1 year. Two subsequent studies also compared CysC measurements between full-term infants and older children (16, 17), which corroborated the earlier finding of declining CysC levels during the first year of life. These studies also showed that CysC measurements do not differ between male and female infants. Subsequent studies showed that there were no gestational age-dependent differences in CysC measurements (16, 17, 20). Table 3 shows the comparison between SCr and CysC levels in neonates.

Cystatin C in ill neonates

The number of studies that have evaluated CysC as a marker for AKI in sick neonates is limited, and earlier studies have focused on determining CysC normal values. In the last 12 months, three studies that evaluated serum CysC measurements in different neonatal conditions have been published (21-23). Sarafidis et al. compared asphyxiated neonates (n = 13) to healthy neonates (n = 22) in a cohort study. In that study, a subgroup analysis was performed among babies with asphyxia where they were separated based on the presence or absence of AKI (n = 8 and n = 5, respectively)(23). A standardized definition was used to diagnose perinatal asphyxia and classify the severity according to Sarnat and Sarnat (25). AKI was defined as persistently increased SCr (≥ 1.5 mg/dL) for at least 24 h. In the asphyxiated group, serum CysC measurements were significantly higher, and SCr levels were also elevated. The CysC level on day one in babies with asphyxia was higher (3.03 vs. 2.86 mg/L, P < 0.05), and the level was reduced on the following days, which was attributed to improvement in renal function.

Maruniak-Chudek et al. compared serum CysC with SCr as a marker for renal function in 32 septic neonates (gestational age, 34–40 weeks) admitted to neonatal intensive care during the first 2 weeks of life (21). The cohort was divided into 3 groups: sepsis (n = 9), severe sepsis (n = 14), and septic shock (n = 9). AKI was determined using SCr and urinary output changes (26). Both CysC and SCr levels were higher in neonates with sepsis and severe sepsis. However, despite a difference in SCr between the 3 groups, there was no difference in CysC levels, and it was concluded that CysC does not offer any advantage over SCr in assessing renal function. The CysC level in sepsis, severe sepsis, and septic shock were 1.23, 1.47, and 1.50 mg/L, respectively, with a normal range of 0.81-2.6 mg/L.
However, the authors cautioned that they had used a different measurement technique (ELISA) compared to the 2 commonly methods, and we were unable to determine how this method differs from PENIA and PETIA. Moreover, the main limitation of this study is the absence of control group.

Parvex et al. compared CysC levels between normal neonates and neonates with kidney failure secondary to congenital anomalies in order to determine residual renal function at birth for babies with congenital renal anomalies (22). Because maternal creatinine crosses the placenta, SCr is an unreliable marker for renal function at birth. Umbilical cord blood was collected from 100 term infants, and the median level with IQR was determined (median = 2.02 mg/L, IQR = 1.86-2.23). The investigators compared this level with that obtained from 33 term infants diagnosed antenatally with kidney anomalies, and this cohort was divided into 2 groups based on whether the anomaly was unilateral or bilateral. CysC was significantly increased by 24.5% (P < 0.001) in the cohort with bilateral kidney malformations compared to controls, independent of gender, weight, and size. Based on these findings, the investigators suggested that a high level of CysC in neonates with bilateral kidney malformation may reflect the low renal endowment in this cohort.

**Conclusion**

AKI is associated with an increased risk of morbidity and mortality in neonates, especially in very low-birth weight infants, infants with congenital heart disease who undergo cardiopulmonary bypass, and those with perinatal asphyxia. To improve outcome, AKI needs to be identified and managed early. Serum creatinine measurements have many inherent limitations, and there is sufficient evidence to suggest that SCr as a marker of renal injury results in a late and under diagnosis of AKI. In contrast, CysC has all of the theoretical properties to be an ideal marker of renal function. Measurements can be easily taken through umbilical cord blood analysis, and there is sufficient information to predict the changes in the level that occur in the first year of life. Earlier studies on CysC focused on determining the normal values in neonates. The number of studies evaluating CysC in sick neonates is limited at the moment, and there are no studies linking CysC levels in sick babies with short-term and long-term outcomes. In the majority of studies, the day 1 CysC level ranges between 1 and 2 mg/L, and this level gradually declines during the first year of life. Currently, there are insufficient data to determine the best level at which treatment should be initiated. Therefore, there is a need for more studies, especially
in sick neonates with renal impairment. A few studies evaluating CysC in ill neonates have emerged more recently, which provides an encouraging trend. We postulate that there could be an increasing awareness among clinicians that CysC may have a role in the early detection of AKI. Therefore, we are hopeful that more studies will become available in the near future that can assist clinicians in the early diagnosis and treatment of AKI.

Acknowledgements: Prof. Dr. Eugenie R Lumbers

Reference


Table 1. Summary of studies of neonates and premature infants to determine the normal serum levels of CysC.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of neonates</th>
<th>Gestational age (weeks)</th>
<th>Cystatin C level (Day 1) mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randers et al. (1999)</td>
<td>12</td>
<td>Term</td>
<td>1.63 ± 0.26 #</td>
</tr>
<tr>
<td>Finney et al. (2000)</td>
<td>16</td>
<td>24-28</td>
<td>1.48 (0.65–3.37) *</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>29-36</td>
<td>1.65 (0.62–4.42) *</td>
</tr>
<tr>
<td>Harmoinen et al. (2000)</td>
<td>58</td>
<td>25-37</td>
<td>1.88 ± 0.36 #</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Term</td>
<td>1.7 ± 0.26 #</td>
</tr>
<tr>
<td>Treiber et al. (2006)</td>
<td>75</td>
<td>34-41</td>
<td>2.0 [1.38-3.23] ^</td>
</tr>
<tr>
<td>Armangil et al. (2008)</td>
<td>108</td>
<td>29.9-35.1</td>
<td>1.80 ± 0.3 #</td>
</tr>
<tr>
<td>Bariciak et al. (2011)</td>
<td>25</td>
<td>24-28</td>
<td>1.63 (1.17–2.24) ^</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>28-32</td>
<td>1.79 (1.05–2.41) ^</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>32-36</td>
<td>1.89 (0.58–2.93) ^</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>&gt; 36</td>
<td>1.84 (1.32–2.63) ^</td>
</tr>
</tbody>
</table>

Note: * = mean with 95% confidence interval; ^ = median and interquartile range (IQR); # = mean (SD); " = mean (2SD).
Table 2. Summary of studies measuring Cystatin C level in ill neonates.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of neonates</th>
<th>Gestational age (weeks)</th>
<th>Cystatin C level (Day 1) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maruniak-Chudek et al. (2012)</td>
<td>32</td>
<td>34-40</td>
<td>1.35 [1.20-1.49] *</td>
</tr>
<tr>
<td>Parvex et al. (2012)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (UKM)</td>
<td>37-42</td>
<td>1.88 [1.76–2.01]^</td>
<td></td>
</tr>
<tr>
<td>13 (BKM)</td>
<td>37-42</td>
<td>2.52 [2.16–2.71]^</td>
<td></td>
</tr>
<tr>
<td>100 (control)</td>
<td>40-41</td>
<td>2.02 [1.86-2.23] ^</td>
<td></td>
</tr>
<tr>
<td>Sarafidis et al. (2012)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (AKI)</td>
<td>38.2 ± 1.7</td>
<td>1.52 ± 0.43 #</td>
<td></td>
</tr>
<tr>
<td>5 (no AKI)</td>
<td>37.2 ± 1.3</td>
<td>1.0 ± 0.18 #</td>
<td></td>
</tr>
<tr>
<td>22 (control)</td>
<td>38.6 ± 1.1</td>
<td>1.02 ± 0.26 #</td>
<td></td>
</tr>
</tbody>
</table>

Note: * = mean with 95% confidence interval; ^ = median and interquartile range (IQR); # = mean (SD); BKM = bilateral kidney malformation; UKM = unilateral kidney malformation; AKI = acute kidney injury.
Table 3. Comparison between Creatinine and Cystatin C levels in neonates.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Creatinine</th>
<th>Cystatin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influence from maternal plasma level</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Level affected by gestational age</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Level in the first week of life</td>
<td>Fluctuating</td>
<td>Gradual decline</td>
</tr>
<tr>
<td>Excretion by kidney</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Reabsorption/secretion by renal tubules</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Affected by infant muscle mass</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>