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Cystatin C: A more reliable biomarker of renal function in young infants? A longitudinal cohort study

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Abstract

Aim: We carried out a longitudinal cohort study to measure serial CysC (Cystatin C) in a cohort of neonates born preterm until the age of 2 years. We hypothesised that CysC levels are independent of body weight and would not vary with gestational age.

Methods: This prospective cohort study was conducted from August 2014 until October 2016, and follow-up was completed in October 2018. Preterm infants at less than 28 weeks of gestation (extremely preterm infants) were recruited and followed up until the age of 24 months. Blood samples for measurement of CysC were collected at regular intervals.

Results: We recruited 58 preterm neonates with mean gestation was 26.2 (1.5) weeks, and a mean birth weight was 917 (140) g. One-way analysis of variance (ANOVA) did not show any significant difference in CysC levels between 28, 32 and 37 weeks' gestation ($P = .09$) despite a significant increase in body weight ($P < .001$). The mean CysC level was higher in the neonatal period and subsequently plateaued by 24 months.

Conclusion: Serum CysC level is independent of body weight and not influenced by postnatal age nor by gender.

KEYWORDS
biomaker, Cystatin C, preterm infant, renal

1 | INTRODUCTION

Nephrogenesis in humans is completed by 36 weeks of gestation and can be interrupted or altered by prematurity, medication and poor intrauterine growth.[1] The total glomerular filtration rate is the product of single-nephron filtration multiplied by total nephron number. Serum creatinine (SCr) measurements remain the most commonly used method of assessing renal function in neonates.[2] Measurement of serum creatinine is based on a technique first described by Professor Max Jaffe in 1886.[3] In 1926, Rehberg proposed that due to its clearance properties, SCr could be used to determine glomerular filtration rate (GFR).[4] Despite subsequent studies showing that SCr has several limitations, SCr measurements continue to be used because of cost and convenience. SCr measured soon after birth reflects maternal creatinine.[5] Furthermore, there is a passive reabsorption of creatinine in the

renal tubules, rendering GFR estimate inaccurate.[6] This is to a certain extent compensated for by the Jaffe method which is known to falsely elevate SCr as a result of non-specific protein and other interferences such as Bilirubin, which is physiologically elevated during the newborn period.[6] Finally, it can be influenced by muscle mass and muscle wasting. Also, SCr levels are extremely low in young infants (due to low muscle mass); thus, measures of creatinine will always lack the analytical sensitivity and are of limited value in helping clinicians to determine accurately renal function, especially in young infants.[7] Alternative enzymatic methods for determining creatinine have been suggested because they are highly sensitive, and background interferences are reduced.[8] However, these are not routinely available.

CysC, a product of cell metabolism, is increasingly being recognised as a more reliable biomarker of renal function.[6] It is a low-molecular-mass (13 kDa) basic protein that belongs to the CysC superfamily of reversible inhibitors of cysteine proteases. This biomarker is produced at a constant rate by all nucleated cells, freely filtered by the glomerulus and not reabsorbed in renal tubules, making it an ideal biomarker measure of GFR. CysC is also independent of muscle mass, and in the newborn, it does not correlate with maternal CysC levels.[5] A systematic review by Nakhjavan et al[9] concluded that CysC can be a suitable alternative to traditional diagnostic measures and provides an acceptable prognostic value for the prediction of AKI in children. However, data on CysC measurements in neonates and infants are rather limited.[7] Therefore, we carried out a longitudinal cohort study to measure CysC in a cohort of neonates born preterm and to follow them up with serial measurements until the age of 2 years. We postulated that CysC would be independent of body weight and would not vary with gestational age.

2 | METHODS

This longitudinal cohort study was conducted in the Department of Neonatology, Townsville Hospital, Queensland, Australia. The recruitment was conducted from August 2014 until October 2016, and follow-up was completed in October 2018. The study population consists of preterm babies >23 weeks gestation admitted to the neonatal unit. Preterm infants at less than 28 weeks of gestation (extremely preterm infants), with birth weights between the 10th and 90th centile (appropriate for gestational age (AGA)), admitted to the neonatal department during the study period were eligible to participate in this study. Gestational age as determined by first trimester ultrasound scan was used. These infants were then followed from term corrected (37 postmenstrual age (PMA)) upon discharge from the neonatal unit at ages of 6, 12 and 24 months. Infants with antenatally diagnosed renal abnormalities, growth restriction (weight < 10th centile) and no parental consent were excluded from this study. Data from a cohort of term infants (gestation > 37 weeks) admitted during the same period for minor neonatal conditions such as neonatal jaundice, risk of sepsis and poor feeding were recruited

> **Key Notes**
> - Serum Cystatin C level is independent of body weight and not influenced by postnatal age nor by gender.
> - There is a gradual decline in Cystatin C level from the corrected term period until the age of two when it plateaus.
> - Cystatin C has the potential to become the primary biomarker to assess renal function in neonates

with parents' consent as control. The Townsville Health District Human Research Ethics Committee approved this study, which was conducted following the tenets of the Declaration of Helsinki. Written consent was obtained from parents of all infants who participated in this study. STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) check list was utilised in reporting this study.[10]

CysC concentrations were measured in serum samples using an immunoturbidimetric assay designed for use on the Beckman AU480 automated analyser platform (Gentian, Australia). The Gentian CysC calibrator was standardised against the international standard ERM-DA471/IFCC. The range of the assay is 0.34-7.95 mg/L. The intra- and inter-assay precision for samples with concentrations in this range is <10% (Beckman), and this was confirmed under our experimental conditions (our coefficient of variation was 7%).

Serum and plasma creatinine concentrations were measured using a kinetic modification of the Jaffe procedure on the Beckman DXCi biochemistry analyser (Beckman Coulter, Australia) using a commercial calibrator which is traceable to an isotope dilution mass spectrometry (IDMS) reference method using the National Institutes of Standards and Technology (NIST) Standard Reference Material 967. (Beckman Coulter DxCi Creatinine Package Insert).

2.1 | Recruitment and statistical analysis

Convenience sampling method was used to recruit patients. All eligible neonates with parental consent were recruited. The normality of the variables was determined by the D'Agostino-Pearson test.[11] The results are expressed as the means and (standard deviation [SD]) for continuous normally distributed data and as median (interquartile range [IQR]) for continuous non-normally distributed data. One-way analysis of variance (ANOVA) will be used to test the difference between the means of several subgroups of a variable. Pearson's correlation coefficient (r) will be used to measure of the strength of the association between the two variables. Comparisons of means of normally distributed data were made using t tests, and P value < .05 was considered statistically significant. Statistical analyses were performed using MedCalc for Windows, version 16.4.3 (MedCalc Software).

3 | RESULTS

A total of 131 premature neonates <28 weeks gestation were admitted to the neonatal unit. We recruited 58 neonates with gestation <28 weeks, and there were nine deaths. Data from 45 patients were available at 28 weeks corrected for analysis, with none being diagnosed as having Acute kidney injury. The mean gestation was 26.2 (1.5) weeks, and the mean birth weight was 917(140) g. One-way analysis of variance (ANOVA) did not show any significant difference in CysC level between 28, 32 and 37 weeks of age ($P = .09$) although a significance increases body weight ($P < .001$) (Figure 1). We also recruited 29 term neonates (gestation > 37 weeks), and there was no significant difference in CysC level between preterm infants (at 37 PMA) and term infants (1.61(0.3) vs. 1.62(0.27) mg/L; $P = .84$), respectively.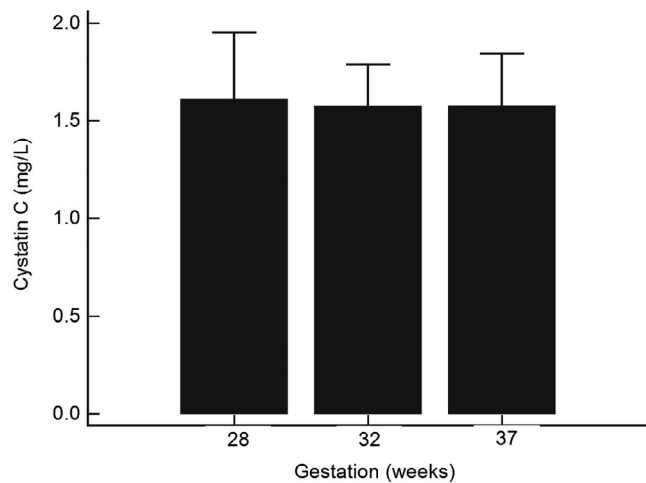 There was significant correlation between body weight and Scr level in premature neonates from 28 to 37 weeks ($r = −.61$, $P < .001$).

The mean CysC level was higher in the neonatal period and had plateaued by 24 months (Figure 2). There was no correlation between body weight and CysC levels at the respective age groups, as shown in Table 1. There was no difference between male and female neonates. Serum creatinine levels were 48.6(18.2), 21.2(6.2) and 16.2(6.3) umol/L at 28, 32 and 37 weeks, respectively.

4 | DISCUSSION

Our data show that CysC levels remain constant between 28 and 37 weeks of gestation. Subsequently, there is a decline in the CysC level in the first two years of life. There was no difference in CysC levels between preterm (at 37 weeks PMA) and term neonates. CysC was also independent of body weight. We believe these properties as well as the fact that it is freely filtered at the glomerulus and does not undergo tubular reabsorption make CysC a more reliable biomarker of glomerular filtration rate than SCr.[6] We propose that clinicians should routinely use CysC to determine glomerular filtration rate in infants. CysC can be used to calculate estimated GFR (eGFR).[12]

Nakashima et al published a longitudinal study which involved 261 preterm infants, which were divided into three groups based on the gestational age (27-30 weeks, 31-33 weeks and 34-36 weeks) and followed these infants up for the first year of their lives.[13] Serum CysC levels were measured at 6-30 days, 3-5 months, 7-9 months and 12-14 months after birth. The investigators reported that there was no difference in the CysC level between the three groups, and CysC level decreased in the first year of life, similar to our results. In another study, Bardallo et al investigated a cohort of 109 preterm neonates.[14] The infants were divided into three groups: Group A

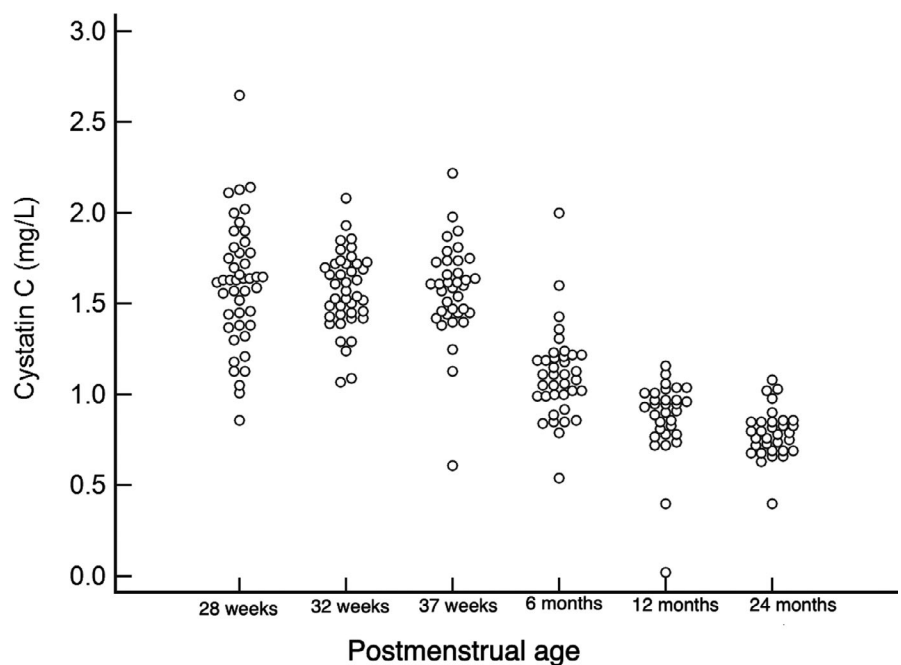**FIGURE 1** Cystatin C levels (mg/L) at postnatal age of 28, 32 and 37 wk

FIGURE 2 Cystatin C levels (mg/L) in the first 2 y of life after discharge from the neonatal unit

Age	Number of infants	Mean CysC (mg/L)	Body weight (kg)	Correlation coefficient	P value
28 wk	45	1.61 (0.3)	0.9 (0.1)	.16	.316
32 wk	41	1.57 (0.2)	1.4 (0.2)	.06	.723
37 wk	36	1.57 (0.3)	2.4 (0.3)	−.23	.210
6 mo	37	1.11 (0.2)	6.6 (1.0)	−.18	.280
12 mo	31	0.87 (0.2)	8.7 (1.3)	−.22	.245
24 mo	32	0.79 (0.1)	11.2 (1.5)	−.24	.10

TABLE 1 The lack of significant correlation between Cystatin C levels (mg/L) and body weight (kg) at different ages

(24-27 weeks), Group B (28-33 weeks) and Group C (34-36 weeks). Blood samples were collected at birth, within 48-72 h and after 7 days of life. Serum CysC decreased within 48-72 h of life, and after 7 days in all three groups, and there was no difference in CysC level between the different gestational age groups, quite similar to our findings.

Cystatin C is measured using an immunoassay, either a traditional enzyme-linked immunoassay (ELIZA) or an automated method using enhanced particle detection with either turbidometry (PETIA), nephelometry (PENIA) or immunofluorescence. Some studies are suggesting that the particle-enhanced nephelometric assays (PENIA) are more accurate than the immunoturbidometric assays (PETIA).[15] However, inter-assay variability has been reduced through a harmonisation project, and the introduction of an International Federation of Clinical Chemistry and Laboratory Medicine (IFCC recommended) calibrator (ERM- DA471/IFCC).[15] Serum Cystatin C is a better indicator of AKI than eCCl (estimated creatinine clearance) within the first 24 hours.[15] There a few hurdles that prevent CysC from being used more readily by clinicians. CysC is not readily available for clinicians, unlike SCr measurements and the costs are higher. Recently, point care of testing for CysC (Eurolyser Diagnostica GmbH, Salzburg, Austria) has become available, which makes it more likely that clinicians will use this test of renal function.

The main limitation of this study is its relatively small sample size. Approximately 50% of the neonates that fulfilled the recruitment criteria were recruited. A significant proportion of the neonates admitted to the unit for regional area, and these neonates are often transferred back to their regional hospital for ongoing management once they are slightly more mature. Some parents were unable to commit to two years follow-up due to various reasons, hence the attrition seen in the follow-up cohort. We were unable to determine whether growth restricted and large for gestational age neonates have a different CysC level. We were also unable to provide both SCr measurements for the whole cohort as after discharge from the neonatal unit, we opted for a less invasive blood collection method for older infant, using finger prick. This is to reduce discomfort the older child. However, limitations of this approach included the limited amount of specimen collected each time, sufficient for CysC measurement only and the higher chance of haemolysis with this technique due to squeezing, and that would interfere with the Jaffe method used for SCr measurements.

5 | CONCLUSION

Our study shows that serum CysC levels are independent of body weight and postnatal age and gender. There is a gradual decline in CysC levels from those measured at corrected term age period onwards until the age of two. CysC has features that make it a more reliable biomarker for the clinician.

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