Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Gestational and age-specific cystatin C reference intervals for newborns in China

Chao Tong
Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Yalan Liu
Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Yanqiu Wu
Yuhuangding Hospital of Qingdao University
Qiong Li
Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Yipin Wu
Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Lin Wang
Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Yan Chen (chenyan126926@163.com)
Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
https://orcid.org/0000-0001-8793-4698

Research article

Keywords: Cystatin C, Newborn, Reference, Renal function

Posted Date: December 23rd, 2019

DOI: https://doi.org/10.21203/rs.2.19480/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Backgroud : To establish reference values of serum cystatin C (CysC) in Chinese newborn infants.

Methods: Serum CysC levels were measured in 1919 blood samples from 1044 newborns during their first 28 days of life. CysC levels were analyzed for association between subgroups dichotomized by postnatal age (PA) and gestational age (GA). Reference intervals of serum CysC were determined according to the PA and GA. The association between serum CysC level and other biochemical parameters as well as perinatal factors were also analyzed.

Results: In this study, the mean GA was 35.75±2.90 weeks and birth weight was 2613.77±696.72 g. Reference ranges of serum CysC were determined and a general decreasing trend of CysC levels was observed as GA increased. CysC levels differed significantly among the PA and GA groups (P□0.001). Serum CysC levels were relatively stable throughout GA, except being impacted by white blood cell count within postnatal 24 hours. Moreover, their levels always correlated positively with serum creatinine concentrations (P<0.001).

Conclusion: The reference level of serum CysC should be determined according to PA and GA. In contrast to creatinine, serum CysC is a dependable index for assessing renal function in neonate, for there are rare factors influencing it.

Background

Newborns are a particularly vulnerable population, especially when born prematurely. Monitoring of renal function in neonates is important for determining the safe and accurate dosing of medication and monitoring the effect of various pathologies on the kidney [1].

As we all know, renal function is typically measured by assessment of the glomerular filtration rate (GFR), and GFR is measured by reference procedures that use exogenous markers, including inulin, ^{51}Cr-EDTA and ^{99m}Tc-DTPA, and so on. However, direct assessment of neonatal renal function by GFR is challenging due to the impracticability of frequent blood sampling, urine collection, and constant infusion of exogenous markers [2]. At present, serum creatinine (Cr) is the most commonly measured proxy for renal function. However, variability of muscle mass, age, gender, diet, glomerular and tubular secretion precludes Cr to be a valuable tool for GFR assessment in children [3]. Moreover, Cr is influenced by maternal renal function for at least 72 hours after birth due to placental transfer, and the degree of immaturity [4].

Cystatin C (CysC), a member of family II of cystatins, is a nonglycosylated 13 kDa protein inhibitor of cysteine proteases. CysC was suggested as a marker of GFR, since it is produced at a constant rate by all nucleated body cells, freely filtered in the renal glomeruli and almost completely reabsorbed and catabolized in the proximal tubules [5, 6]. In contrast with Cr, CysC does not cross the placenta [7, 8],{Plebani, 1997, Determination of blood cystatin C in pregnant women during labor and in their newborns} and it is independence of muscle mass and has a better diagnostic sensitivity [9–11]. Therefore, CysC has been evaluated as a promising alternative to Cr. However, there is not an acknowledged CysC reference standard in neonates because of few studies on CysC reference. We carry out the present study for attempting to identify reference values of CysC.

Methods

This observational study was conducted at 2 neonatal intensive care unit (NICU), the Union Hospital, Tongji Medical College (Wuhan, China) and Yuhuangding Hospital of Qingdao University (Yantai, China). Approval for the study was obtained from the Ethics Committee of Huazhong University of Science and Technology (NO.2016-S251). The study was carried out on consecutive babies, born to women followed antenatal at the two above hospitals, between August 2015 and July 2019. In order to obtain a reference standard representative for "healthy" newborn infants, the following exclusion criteria were applied: (1) any congenital anomaly, (2) any renal pathology, including oliguria of <1.0 ml/kg/h lasting for >6 h, (3) severe systemic illness, (4) mothers had any chronic or systemic disease [12, 13].

Maternal/prenatal medical history, gestational age (GA), birth weight (BW), sex, Apgar score, and postnatal age (PA) were recorded for each newborn. Data on serum CysC, blood urea nitrogen (BUN), serum Cr, C-Reactive Protein (CRP), hemoglobin (HgB), white blood cell count (WBC), and bilirubin levels were collected from peripheral arteries or veins at the same time when routine blood screening tests were performed. Serum CysC, Cr and BUN levels were measured using an automatic analyser (Abbott ARCHITECT® c1600, USA). Patients were grouped according to GA (≤ 28 weeks, 29–32 weeks, 33–36 weeks, and≥37 weeks) and PA (0–3 days, 4–7 days, 8–14 days, 15–21days and 22–28 days).

Statistical analysis was performed using the SPSS package for Windows, version 15.0 (SPSS Inc, Chicago, IL).Results are expressed as mean ± standard deviation (SD). Shapiro-Wilk test and normal probability plot were used to test for normality of data. Data were analyzed statistically using ANOVA or Kruskal–Wallis test. The reference intervals of serum CysC levels were calculated as mean± 1.96*SD. Associations between variables were assessed with Pearson's correlation coefficient. Significance was accepted at $P<0.05$.

Results

This study included 1044 newborns and 1919 blood samples. The mean GA was 35.75±2.90 weeks. And the mean BW was 2613.77±696.72g. Demographic characteristics of the study group were shown in *Table 1.*

Reference intervals of serum CysC levels are presented in *Table 2.* Significant differences were found in all of the GA groups when mean serum CysC was compared among the five postnatal subgroups in each GA group (P 0.001). In addition, when the mean serum CysC levels of the GA groups was compared between the four GA subgroups in each PA group, all of the patients had significantly different mean CysC levels, except those of PA 22–28 days (22–28 days, P = 0.224) [*Supplementary table 1]*..

We further assessed the correlation of CysC or Cr with other biochemical parameters and perinatal factors. The level of serum Cr was found to be positively correlated with BW, GA, total bilirubin (TB), direct bilirubin (DB), WBC, CRP and BUN within postnatal 24 hours. But, they are correlated negatively on postnatal 3 days except for BUN. We found no remarkable correlation between CysC with these factors, except for WBC within postnatal 24 hours. Serum CysC always correlated positively with Cr. (Data was shown in *Table 3* and *Table4)*

Discussion

Neonates, and particularly preterm newborns, are at increased risk for acute kidney injury (AKI) due to immature kidney function. It was reported 6.5% newborn infants admitted to the NICU developed acute kidney injury [14]. CysC has been shown to be useful in diagnosing AKI and predicting its outcomes in children [15]. To determine

the reference levels of serum CysC for newborn population will allow physicians to diagnose the disease accurately and timely. Different countries or regions usually establish corresponding RIs for their local populations. Here, we determined the reference levels of serum CysC for newborns in China. Most clinical studies have shown that neonatal serum CysC levels are mainly affected by PA [16–19]. However, those studies were limited as they enrolled small numbers of subjects. Our study had a larger sample size, and there were total of 1919 samples from 1044 patients. In our results, when the mean serum CysC levels of five postnatal subgroups in each GA group were compared, significant differences were found in all of the PA groups (P<0.001). And we found a trend toward decreasing serum CysC levels, as PA increases during the first months. (Show in *Table 2)*

Previous researches have shown that CysC is superior to serum Cr for the estimation of the GFR in children [15, 20, 21]. Therefore, we further assessed the correlation of CysC or Cr with other biochemical parameters and perinatal factors. In the first days of life, neonatal serum Cr levels reflect maternal renal function and are increased in premature infants. It was reported serum Cr values reach stable levels by 1 to 2 weeks of age in term neonates, and inversely correlated with BW and GA in young infants [22, 23]. Consistent with these researches, we found BW and GA inversely correlated with Cr on postnatal age 3 in this study (*Table 4)*.. However, they were correlated positively within postnatal 24 hours, which further showed it is unreliable to assess renal function using Cr for newborn especially in the first days of life (*Table 3)*..

The available literatures have shown that CysC is independent of birth weight and gestational age [3, 24, 25, 19]. In this study, with larger samples, we further confirmed CysC is independent of BW and GA in newborn, at least within 3 days after birth (P>0.01).

There are 2 assay methods for clinical laboratory use to measure serum Cr: the Jaffe and enzymatic methods, and serum Cr were measured by the Jaffe method in this study. The Jaffe method is known to affect seum Cr level as a result of non-specific protein interference [23]. Bilirubin is physiologically elevated in the newborn period. It was reported bilirubin can affect serum Cr measurement by the Jaffe method [26]. In this study, the results showed that there was a statistically significant correlation between bilirubin levels and Cr level (P <0.001).

However, in our research, CysC was independent of bilirubin levels, which was similar to Treiber's research [12]. In this study, the correlation between HgB levels and serum CysC or Cr levels on the day 0 and day 3 were analyzed. We found no statistically significant correlation between serum CysC and HgB levels, which was similar to Armangil's report [27].

Initial studies suggest that CysC does not vary from non-renal factors, however further investigation has shown some biological variability [28]. Such as thyroid diseases, glucocorticoids treatment, and inflammation may affect CysC concentrations independent of kidney function [29–32]. Evangelopoulos AA ect al. have a research in 490 adults who underwent a typical health examination. The results of this study demonstrated that peripheral monocyte blood count and WBC, were independently related with CysC concentrations [33]. In this study, we also found there was a statistically significant correlation between WBC and CysC levels within 24 hours after birth. These findings with ours suggest these biological variability factors must be considered when evaluating CysC levels in clinical practice. As most research demonstrated statistically significant correlation also were found between serum CysC with both the Cr and BUN levels in this study (P<0.001) [13].

Conclusion

In summary, we present reference intervals of the serum CysC in newborn according to the GA and PA. In contrast to Cr, only few non-renal factors influence serum CysC, which suggests CysC is more stable to assess the kidney function of neonatal.

Several limitations exist in the present study. First, it was difficult to enroll “healthy” subjects, especially for preterm need treatment. Second, the number of subjects was still small for calculating completely reliable reference ranges, as shown in the subject numbers of GA lower than 28 weeks groups. Third, larger samples from multicenter study will help improve the precision of reliability of the data.

Abbreviations

CysC, Cystatin C; GFR, Glomerular filtration rate; Cr, Creatinine; GA, Gestational age; BW, Birth weight; PA, Postnatal age; BUN, Blood urea nitrogen; CRP, C-Reactive Protein; TB, Total bilirubin; DB, Direct bilirubin; HgB, Hemoglobin; WBC, White blood cell count; AKI, Acute kidney injury.

Declarations

Ethics approval and consent to participate

Approval for the study was obtained from the Ethics Committee of Huazhong University of Science and Technology (NO.2016-S251). The ethics committee approves the exemption of subject's informed consent, because this study is completely harmless to subjects.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no conflict of interest.

Funding

This work was supported by National Nature Science Foundation of China (no. 81300523, no. 81601324). Both of the funding supported us to accomplish the study and the manuscript. However, the funders have not had any influence over the design, aim or the process in this study.

Authors' contributions

CT, LW and YC are responsible for the conception of the study. CT and YC contributed significantly to analysis and manuscript preparation. CT, YL, YQW, QL and YPW collected patients’ samples and data. CT and YC

performed the data analyses and wrote the manuscript. LW and YC prepared the final version of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1.van den Anker JN, Allegaert K. Pharmacokinetics of Aminoglycosides in the Newborn. Current Pharmaceutical Design. 2012 Jul;18(21):3114–18.

2.Bartelink IH, Rademaker CMA, Schobben AFAM, van den Anker JN. Guidelines on paediatric dosing on the basis of developmental physiology and pharmacokinetic considerations. Clinical Pharmacokinetics. 2006 2006;45(11):1077–97.

3.Demirel G, Celik IH, Canpolat FE, Erdeve O, Biyikli Z, Dilmen U. Reference values of serum cystatin C in very low-birthweight premature infants. Acta Paediatrica. 2013 Jan;102(1):e4-e7.

4.Askenazi DJ, Koralkar R, Levitan EB, Goldstein SL, Devarajan P, Khandrika S, et al. Baseline Values of Candidate Urine Acute Kidney Injury Biomarkers Vary by Gestational Age in Premature Infants. Pediatric Research. 2011 Sep;70(3):302–06.

5.Softic A, Begic L, Halilbasic A, Vizin T, Kos J. The predictive value of cystatin C in monitoring of B non-hodgkin lymphomas: relation to biochemical and clinical parameters. ISRN oncology. 2013 2013;2013:752792–92.

6.Allegaert K, Mekahli D, van den Anker J. Cystatin C in newborns: a promising renal biomarker in search for standardization and validation. Journal of Maternal-Fetal & Neonatal Medicine. 2015 2015;28(15):1833–38.

7.Plebani M, Mussap M, Bertelli L, Moggi G, Ruzzante N, Fanos V, et al. Determination of blood cystatin C in pregnant women during labor and in their newborns. La Pediatria medica e chirurgica: Medical and surgical pediatrics. 1997 1997;19(5):325–9.

8.Cataldi L, Mussap M, Bertelli L, Ruzzante N, Fanos V, Plebani M. Cystatin C in healthy women at term pregnancy and in their infant newborns: Relationship between maternal and neonatal serum levels and reference values. American Journal of Perinatology. 1999 1999;16(6):287–95.

9.Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: A meta-analysis. American Journal of Kidney Diseases. 2002 Aug;40(2):221–26.

10.Pham-Huy A, Leonard M, Lepage N, Halton J, Filler G. Measuring glomerular filtration rate with cystatin C and beta-trace protein in children with spina bifida. Journal of Urology. 2003 Jun;169(6):2312–15.

11.Abitbol CL, Seeherunvong W, Galarza MG, Katsoufis C, Francoeur D, DeFreitas M, et al. Neonatal Kidney Size and Function in Preterm Infants: What Is a True Estimate of Glomerular Filtration Rate? Journal of Pediatrics. 2014 May;164(5):1026-+.

12.Treiber M, Balon BP, Gorenjak M. Cystatin C versus creatinine as a marker of glomerular filtration rate in the newborn. Wiener Klinische Wochenschrift. 2006 May;118(9–10):66–70.

13.Lee J-H, Hahn W-H, Ahn J, Chang J-Y, Bae C-W. Serum cystatin C during 30 postnatal days is dependent on the postconceptional age in neonates. Pediatric Nephrology. 2013 Jul;28(7):1073–78.

14.Timovska SN, Cekovska S, Tosheska-Trajkovska K. Acute Kidney Injury in Newborns. Prilozi (Makedonska akademija na naukite i umetnostite Oddelenie za medicinski nauki). 2015 2015;36(3):83–9.

15.Roos JF, Doust J, Tett SE, Kirkpatrick CMJ. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children- A meta-analysis. Clinical Biochemistry. 2007 Mar;40(5–6):383–91.

16.Cavalcanti Ferreira Novo ACdA, Sadeck LdSR, Okay TS, Leone CR. Longitudinal study of Cystatin C in healthy term newborns. Clinics. 2011 2011;66(2):217–20.

17.Kandasamy Y, Smith R, Wright IMR. Measuring Cystatin C to Determine Renal Function in Neonates. Pediatric Critical Care Medicine. 2013 Mar;14(3):318–22.

18.Bardallo Cruzado L, Perez Gonzalez E, Martinez Martos Z, Bermudo Guitarte C, Granero Asencio M, Luna Lagares S, et al. Serum cystatin C levels in preterm newborns in our setting: Correlation with serum creatinine and preterm pathologies. Nefrologia. 2015 May-Jun;35(3):296–303.

19.Yang Y, Li S-J, Pan J-J, Hu Y-H, Zhou X-Y, Cheng R, et al. Reference values for serum cystatin C in very low-birthweight infants: From two centres of China. Journal of paediatrics and child health. 2017 2017-Sep–28.

20.Andersen TB, Eskild-Jensen A, Frokiaer J, Brochner-Mortensen J. Measuring glomerular filtration rate in children; can cystatin C replace established methods? A review. Pediatric Nephrology. 2009 May;24(5):929–41.

21.Treiber M, Balon BP, Gorenjak M. A new serum cystatin C formula for estimating glomerular filtration rate in newborns. Pediatric Nephrology. 2015 Aug;30(8):1297–305.

22.Drukker A, Guignard JP. Renal aspects of the term and preterm infant: a selective update. Current Opinion in Pediatrics. 2002 Apr;14(2):175–82.

23.Muhari-Stark E, Burckart GJ. Glomerular Filtration Rate Estimation Formulas for Pediatric and Neonatal Use. The journal of pediatric pharmacology and therapeutics: JPPT: the official journal of PPAG. 2018 2018;23(6):424–31.

24.Nakashima T, Inoue H, Fujiyoshi J, Matsumoto N. Longitudinal analysis of serum cystatin C for estimating the glomerular filtration rate in preterm infants. Pediatric Nephrology. 2016 Jun;31(6):983–89.

25.Abdelaal NA, Shalaby SA, Khashana AK, Abdelwahab AM. Serum Cystatin C as an Earlier Predictor of Acute Kidney Injury than Serum Creatinine in Preterm Neonates with Respiratory Distress Syndrome. Saudi Journal of Kidney Diseases and Transplantation. 2017 Sep-Oct;28(5):1003–14.

26.Askenazi DJ, Griffin R, McGwin G, Carlo W, Ambalavanan N. Acute kidney injury is independently associated with mortality in very low birthweight infants: a matched case-control analysis. Pediatric Nephrology. 2009

May;24(5):991–97.

27.Armangil D, Yurdakok M, Canpolat FE, Korkmaz A, Yigit S, Tekinalp G. Determination of reference values for plasma cystatin C and comparison with creatinine in premature infants. Pediatric Nephrology. 2008 Nov;23(11):2081–83.

28.Kar S, Paglialunga S, Islam R. Cystatin C Is a More Reliable Biomarker for Determining eGFR to Support Drug Development Studies. Journal of Clinical Pharmacology. 2018 Oct;58(10):1239–47.

29.Knight EL, Verhave JC, Spiegelman D, Hillege HL, De Zeeuw D, Curhan GC, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney International. 2004 Apr;65(4):1416–21.

30.Herget-Rosenthal S, Bokenkamp A, Hofmann W. How to estimate GFR - serum creatinine, serum cystatin C or equations? Clinical Biochemistry. 2007 Feb;40(3–4):153–61.

31.Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney International. 2009 Mar;75(6):652–60.

32.Taglieri N, Koenig W, Kaski C. Cystatin C and cardiovascular risk. Klinicheskaia laboratornaia diagnostika. 2012 2012-Oct(10):65–72.

33.Evangelopoulos AA, Vallianou NG, Bountziouka V, Katsagoni C, Bathrellou E, Vogiatzakis ED, et al. Association between serum cystatin C, monocytes and other inflammatory markers. Internal Medicine Journal. 2012 May;42(5):517–22.

Tables

Table 1 Demographic characteristics of study groups.

Variables	Preterm	Term	total
Number of patients (n)	608	436	1044
Males/females (n)	350/258	252/184	602/442
Birth weight (mean ± SD, g)	2184.96±495.14	3211.76±454.26	2613.77±696.72
Gestational age (mean± SD, weeks)	33.72±1.91	38.58±1.15	35.75±2.90
Apgar 1 min (median, IQR)	7 (8-7)	8(8-8)	8(8-7)
Apgar 5 min (median, IQR)	8 (9-8)	9(9-9)	8(9-8)
Han Chinese (n, %)	588(96.7%)	423(97%)	1020(97.7%)

Table 2 Reference serum CysC level (mg/L) according to the gestational age (GA).

GA(weeks)[a]	Postnatal age(day)	n[b]	mean±SD	Median	P25	P75	Range	mean 95%CI Lower	mean95%CI Upper	Reference interval	P[c]
≤28(N=15)	0-3	18	2.03±0.20	1.98	1.88	2.18	1.72-2.42	1.93	2.13	1.638-2.422	<0.001
"	4-7	11	2.46±0.09	2.44	2.39	2.51	2.34-2.66	2.40	2.52	2.284-2.636	"
"	8-14	14	2.15±0.42	2.13	1.76	2.52	1.56-3.03	1.91	2.40	1.327-2.973	"
"	15-21	14	1.96±0.20	1.93	1.78	2.15	1.75-2.36	1.84	2.08	1.568-2.352	"
"	22-28	8	1.79±0.23	1.79	1.63	2.01	1.36-2.08	1.59	1.99	1.339-2.241	"
29~32(N=119)	0-3	130	1.94±0.24	1.95	1.76	2.09	1.31-2.56	1.89	1.98	1.470-2.410	0.001
"	4-7	102	1.81±0.24	1.78	1.64	1.98	1.37-2.38	1.77	1.86	1.340-2.280	"
"	8-14	90	1.83±0.25	1.84	1.62	1.96	1.16-2.43	1.78	1.89	1.340-2.320	"
"	15-21	55	1.83±0.22	1.81	1.67	2.02	1.37-2.26	1.77	1.89	1.399-2.261	"
"	22-28	18	1.83±0.19	1.86	1.69	1.95	1.46-2.17	1.74	1.93	1.458-2.202	"
33~36(N=474)	0-3	482	1.97±0.23	1.96	1.81	2.13	1.46-2.56	1.95	1.99	1.519-2.421	<0.001
"	4-7	253	1.81±0.23	1.80	1.63	1.95	1.25-2.41	1.78	1.84	1.359-2.261	"
"	8-14	140	1.83±0.22	1.81	1.66	1.98	1.27-2.38	1.79	1.86	1.399-2.261	"
"	15-21	33	1.74±0.16	1.74	1.64	1.83	1.39-2.03	1.69	1.79	1.426-2.054	"
"	22-28	10	1.73±0.05	1.73	1.70	1.75	1.64-	1.69	1.77	1.632-	"

							1.83			1.828	
≥37(	0-3	295	1.87±0.23	1.88	1.70	2.03	1.26-	1.84	1.90	1.419-	<0.001
N=436)							2.46			2.321	
_	4-7	152	1.79±0.23	1.76	1.61	1.93	1.22-	1.75	1.82	1.339-	_
							2.40			2.241	
_	8-14	64	1.91±0.22	1.90	1.75	2.09	1.30-	1.85	1.96	1.479-	_
							2.44			2.341	
_	15-21	19	1.85±0.23	1.80	1.70	2.01	1.37-	1.74	1.96	1.399-	_
							2.33			2.301	
_	22-28	11	1.72±0.16	1.71	1.59	1.82	1.49-	1.61	1.83	1.406-	_
							2.07			2.034	

[a] Number of patients (N).

[b] Number of blood samples (n); total of 1919 samples from 1044 patients.

[c] Mean serum CysC (mg/L) of subgroups of postnatal days were compared in each gestational age age group by the analysis of variance test.

Table 3 Correlation between serum CysC and creatinine with other variables of the patients within 24hour.

variables	CysC		Cr	
	R	P	R	P
BW (n=757)	-0.007	0.837	0.092	0.011
GA (n=757)	-0.069	0.058	0.296	<0.001
TB (n=757)	-0.030	0.411	0.147	<0.001
DB (n=757)	0.007	0.850	0.214	<0.001
HgB (n=759)	0.016	0.660	0.040	0.276
WBC (n=759)	0.201	<0.001	0.144	<0.001
CRP(n=760)	-0.052	0.149	0.149	<0.001
BUN (n=757)	0.052	0.153	0.606	<0.001
Cr (n=757)	0.147	<0.001	_	_
CysC (n=757)	_	_	0.147	<0.001

Table 4 Correlation between serum CysC and creatinine with other variables of the patients on day 3.

variables	CysC		Cr	
	R	P	R	P
BW(n=90)	-0.287	0.006	-0.675	<0.001
GA(n=90)	-0.357	0.001	-0.666	<0.001
TB(n=90)	-0.198	0.061	-0.552	<0.001
DB(n=90)	-0.015	0.886	-0.220	0.037
HgB(n=89)	-0.063	0.558	-0.132	0.216
WBC(n=89)	0.159	0.137	-0.094	0.381
CRP(n=92)	-0.167	0.111	-0.392	<0.001
BUN(n=90)	0.407	<0.001	0.781	<0.001
Cr(n=90)	0.563	<0.001	_	_
CysC(n=90)	_	_	0.563	<0.001

Supplementary table 1. Reference serum CysC level (mg/L) according to the postnatal age (PA).

PA(day)	Gestational age(weeks)	n	mean±SD	Median	P25	P75	Range	mean 95%CI Lower	mean95%CI Upper	Reference interval	P
0-3	≤28	18	2.03±0.20	1.98	1.88	2.18	1.72-2.42	1.93	2.13	1.638-2.422	<0.001
	29~32	130	1.94±0.24	1.95	1.76	2.09	1.31-2.56	1.89	1.98	1.470-2.410	
	33~36	482	1.97±0.23	1.96	1.81	2.13	1.46-2.56	1.95	1.99	1.519-2.421	
	≥37	295	1.87±0.23	1.88	1.70	2.03	1.26-2.46	1.84	1.90	1.419-2.321	
4-7	≤28	11	2.46±0.09	2.44	2.39	2.51	2.34-2.66	2.40	2.52	2.284-2.636	<0.001
	29~32	102	1.81±0.24	1.78	1.64	1.98	1.37-2.38	1.77	1.86	1.340-2.280	
	33~36	253	1.81±0.23	1.80	1.63	1.95	1.25-2.41	1.78	1.84	1.359-2.261	
	≥37	152	1.79±0.23	1.76	1.61	1.93	1.22-2.40	1.75	1.82	1.339-2.241	
8-14	≤28	14	2.15±0.42	2.13	1.76	2.52	1.56-3.03	1.91	2.40	1.327-2.973	0.004
	29~32	90	1.83±0.25	1.84	1.62	1.96	1.16-2.43	1.78	1.89	1.340-2.320	
	33~36	140	1.83±0.22	1.81	1.66	1.98	1.27-2.38	1.79	1.86	1.399-2.261	
	≥37	64	1.91±0.22	1.90	1.75	2.09	1.30-2.44	1.85	1.96	1.479-2.341	
15-21	≤28	14	1.96±0.20	1.93	1.78	2.15	1.75-2.36	1.84	2.08	1.568-2.352	0.007

	29~32	55	1.83±0.22	1.81	1.67	2.02	1.37-2.26	1.77	1.89	1.399-2.261	
	33~36	33	1.74±0.16	1.74	1.64	1.83	1.39-2.03	1.69	1.79	1.426-2.054	
	≥37	19	1.85±0.23	1.80	1.70	2.01	1.37-2.33	1.74	1.96	1.399-2.301	
22-28	≤28	8	1.79±0.23	1.79	1.63	2.01	1.36-2.08	1.59	1.99	1.339-2.241	0.224
	29~32	18	1.83±0.19	1.86	1.69	1.95	1.46-2.17	1.74	1.93	1.458-2.202	
	33~36	10	1.73±0.05	1.73	1.70	1.75	1.64-1.83	1.69	1.77	1.632-1.828	
	≥37	11	1.72±0.16	1.71	1.59	1.82	1.49-2.07	1.61	1.83	1.406-2.034	