

Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children

H Finney, D J Newman, H Thakkar, J M E Fell, C P Price

Abstract

Aim—To establish a reference range in the paediatric population for the new glomerular filtration rate (GFR) marker, cystatin C, and to compare it with that of creatinine.

Methods—Cystatin C and creatinine were measured by particle enhanced nephelometric immunoassay (PENIA) and fixed interval Jaffé methods, respectively, in 291 children aged 1 day to 17 years, including 30 premature infants with gestational ages ranging from 24 to 36 weeks.

Results—In the premature infants, concentrations of both cystatin C and creatinine were significantly raised compared with term infants, with cystatin C concentrations being between 1.10 and 2.06 mg/litre and creatinine between 32 and 135 $\mu\text{mol/litre}$. In premature infants, there was no significant relation between gestational age and cystatin C or creatinine concentration. Creatinine concentrations fell to a nadir at 4 months of age, rising gradually to adult values by about 15–17 years of age, in contrast to cystatin C, which fell to a mean concentration of 0.80 mg/litre by the 1st year of life, and remained constant throughout adulthood up to the age of 50 years. Neither analyte showed any influence of sex.

Conclusion—The measurement of cystatin C, rather than creatinine, is more practical for monitoring GFR changes in the paediatric population.

(Arch Dis Child 2000;82:71–75)

Keywords: reference ranges; glomerular filtration rate; cystatin C; creatinine

The measurement of the glomerular filtration rate (GFR) is an important part of the clinical evaluation of renal function.^{1,2} Although the measurement of serum creatinine concentrations is the most common method for estimating GFR, it often fails to identify those patients with moderately reduced renal function. The investigation of renal function in children is complicated by the superimposition of continuing renal development overlaying any possible renal damage. Furthermore, the changing muscle mass of the infant influences the circulating creatinine concentration, and the analytical interferences of bilirubin and haemoglobin are important in this population, because of the problems of neonatal jaundice and the in vitro haemolysis that occurs with the collection of

small paediatric samples.² Thus, it would be an advantage if an alternative marker of GFR were available that was unaffected by changing muscle mass, which could be measured by a technique that was free from these analytical problems.

Cystatin C, a 13 600 Da cysteine protease inhibitor constitutively synthesised by all nucleated cells, has been shown by ourselves and others to be a more specific and sensitive marker of GFR in adults.^{3–5} Fully automated immunoassays for cystatin C are now available using the same analysers that are used for creatinine measurement.^{6–8} These immunoassays are free from bilirubin, ketone, and haemoglobin interference and need only a few microlitres of serum or plasma. Furthermore, in adult studies, the serum concentration of cystatin C shows no relation to muscle mass and is not affected by inflammatory stimuli.^{3,4} Although cystatin C concentrations in children are poorly described, it seemed likely that measurement of cystatin C might provide a considerable improvement over creatinine measurement as a marker for changes in GFR. Although there is a range of algorithms available for the calculation of predicted creatinine clearance for use in children, they are not suitable for immediate clinical application. A simple serum or plasma concentration is always easier to interpret, and the measurement of cystatin C seems to offer this benefit.

There are limited data available on reference ranges for cystatin C. Norlund and colleagues⁹ have established an adult reference range, which we have confirmed recently.¹⁰ An extension of this study was to establish a paediatric reference range for which very few data have been collected,^{11–13} and to extend the range to premature infants and neonates for which no data have been recorded.

Here, we have set out to establish a paediatric reference range for cystatin C and for creatinine including preterm and term neonates.

Materials and methods

CREATININE

We measured serum and plasma creatinine concentrations using a fixed interval Jaffé method performed on the Monarch 2000 (Instrumentation Laboratory, Warrington, UK) with reagents supplied by the manufacturer. The method was calibrated using ReferrIL A Calibrator (catalogue number 35261), an aqueous solution for creatinine calibration, on the Monarch 2000. We used Nycomed (Oslo, Norway) low, medium, and

Department of Clinical Biochemistry, St Bartholomew's and The Royal London, School of Medicine and Dentistry, Turner Street, London E1 2AD, UK
H Finney
C P Price

South West Thames Institute for Renal Research, St Helier NHS Trust, Wrythe Lane, Carshalton, Surrey SM5 1AA, UK
D J Newman

Clinical Pathology, SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Hertfordshire AL6 9AR, UK
H Thakkar

Department of Paediatric Gastroenterology, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, UK
J M E Fell

Correspondence to:
Ms Finney
email: h.finney@
mds.qmw.ac.uk

Accepted 14 September 1999

high control materials for quality control. We obtained published age related reference ranges for creatinine via a literature search.¹⁴⁻²⁴

CYSTATIN C

We measured serum and plasma cystatin C concentrations using latex particle enhanced immunoassays; for the term neonates and the remaining children's samples we performed the assay on the BNA (Behring Nephelometer Analyser; Dade Behring Marburg GmbH, Marburg, Germany).⁸ However, for the premature infants, we used an in house assay validated as described previously⁷ because these samples were analysed before the BNA assay became available. The results of the in house assay have been shown to be directly

comparable to the Dade Behring method.⁸ We calibrated both methods using a standard supplied by Dade Behring, made with purified cystatin C from human urine, and the value assigned by amino acid analysis. We used lyophilised cystatin C control sera from Dade Behring as the quality control material.

PATIENTS

Paediatric serum samples were collected from the routine biochemistry laboratory of the Royal London Hospital after the requested tests had been completed. All samples had been refrigerated by the laboratory within six hours of being collected. Upon collection for this study, all samples were immediately aliquoted into 1.5 ml polypropylene tubes and frozen to -70°C . The criteria for collecting the samples were that the patients were below the age of 19 years, that their electrolytes and renal function test results (creatinine and urea) were normal, and that they were in hospital because of an accident or minor surgery. Plasma samples, which were collected daily and stored at 4°C before being sent, were also received from the Birmingham Children's Hospital and immediately frozen upon receipt. Altogether 244 patient samples were collected; there were 112 female patients and 132 male patients, with ages ranging from 2 days to 17 years.

A further 47 plasma samples were collected from newborn infants who were in the neonatal unit or postnatal wards at the Royal London Hospital. Seventeen of these infants were 7 days old having been born at term. Thirty babies were 1 day old premature infants, 16 had been born at 24–28 weeks' gestation, and 14 at 29–36 weeks' gestation. Most infants born at 24–28 weeks' gestation required ventilatory support, but were otherwise healthy. None was asphyxiated at birth, defined as an Apgar ≤ 5 at five minutes or a cord pH ≤ 7.2 at delivery, or had received nephrotoxic medication, and none was known clinically to have a specific renal abnormality. The gestational age was calculated from the first day of the mother's last period, confirmed by ultrasound scans. Our study was approved by the ethics committee of the Royal London Hospital Trust. In total, there were 291 patient samples collected for our study.

STATISTICAL ANALYSIS

We performed all statistical analyses using the "Astute" statistical package (Diagnostic Development Unit, University of Leeds, UK). We performed tests of normality by means of the Kolmogorov-Smirnov and Anderson Darling tests, taking $p < 0.05$ as a significant result. The Mann-Whitney U test was used, with $p < 0.05$ taken as a significant result.

Reference intervals

The reference intervals for cystatin C were calculated using the mean (2 SD) of the log transformed data and for creatinine by using the 95th centiles of the untransformed data.

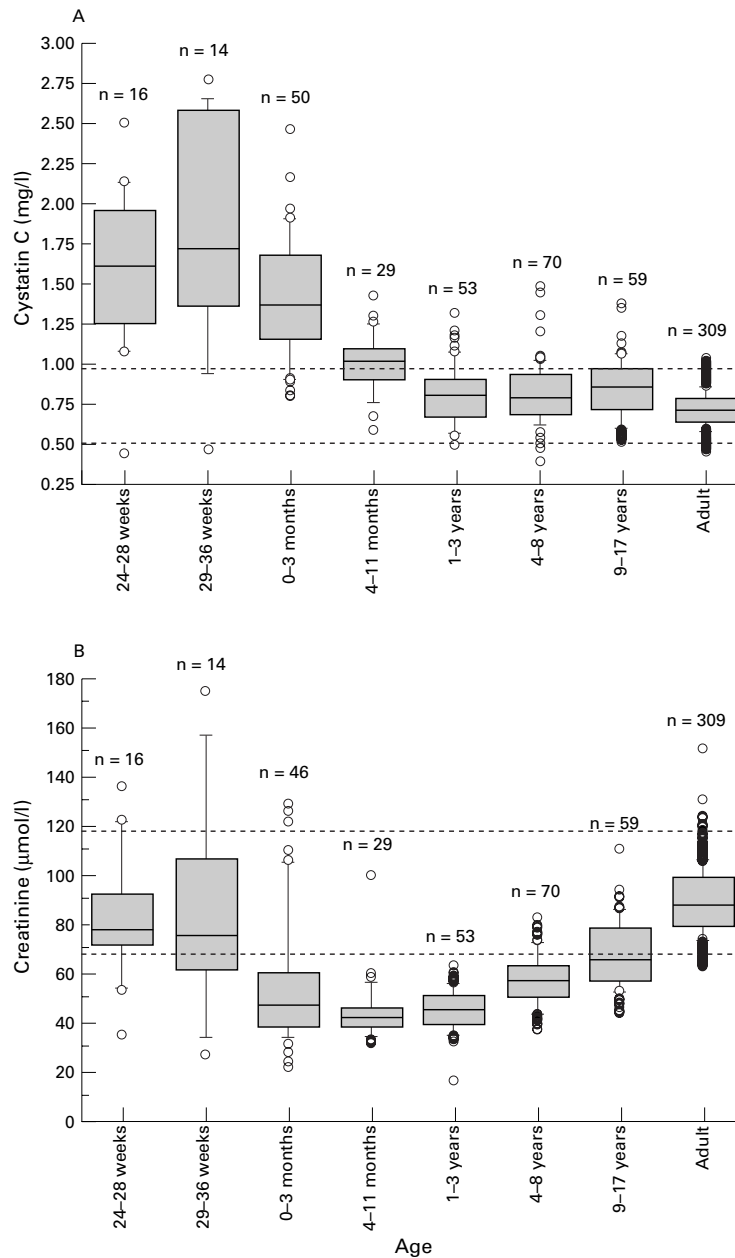


Figure 1 Box plot distributions showing (A) cystatin C and (B) creatinine values (10th, 25th, 50th, 75th, and 90th centiles) across the age groups. The categories of 24–28 and 29–36 weeks refer to the gestational ages of preterm babies. Dotted lines indicate 95% confidence interval of adult range.¹⁰ Preterm babies born between 24–36 weeks' gestation were 1 day old when samples were taken.

Table 1 Summary of reference range data obtained

Age	All		Girls		Boys	
	n	Mean/median (range)	n	Mean/median (range)	n	Mean/median (range)
<i>Cystatin C (mg/litre)</i>						
24–28 weeks*	16	1.48 (0.65–3.37)	–	–	–	–
29–36 weeks*	14	1.65 (0.62–4.42)	–	–	–	–
0–3 months	50†	1.37 (0.81–2.32)	14	1.28 (0.73–2.26)	19	1.37 (0.85–2.22)
4–11 months	29	0.98 (0.65–1.49)	12	0.94 (0.64–1.35)	17	1.00 (0.63–1.57)
1–3 years	53	0.79 (0.50–1.25)	27	0.76 (0.47–1.21)	26	0.83 (0.57–1.21)
4–8 years	70	0.80 (0.49–1.29)	32	0.74 (0.49–1.11)	38	0.86 (0.52–1.41)
9–17 years	59	0.82 (0.53–1.29)	27	0.83 (0.49–1.45)	32	0.80 (0.56–1.14)
24–36 weeks*	30	1.56 (0.43–2.77)	–	–	–	–
< 1 year	79†	1.20 (0.59–1.97)	34	1.17 (0.67–2.05)	38	1.20 (0.69–2.08)
1–17 years	182	0.80 (0.50–1.27)	87	0.77 (0.48–1.24)	95	0.83 (0.54–1.29)
<i>Creatinine (µmol/litre)</i>						
24–28 weeks*	16	78 (35–136)	–	–	–	–
29–36 weeks*	14	75 (27–175)	–	–	–	–
0–3 months	46†	47 (23–127)	11	47 (36–131)	18	50 (35–111)
4–11 months	29	42 (32–100)	12	40 (32–59)	17	42 (34–100)
1–3 years	53	45 (33–60)	27	43 (17–60)	26	48 (34–63)
4–8 years	70	57 (40–82)	32	54 (38–79)	38	59 (40–83)
9–17 years	59	66 (46–94)	27	69 (46–92)	32	66 (43–111)
24–36 weeks*	30	78 (27–175)	–	–	–	–
< 1 year	79†	44 (33–127)	30	47 (32–132)	36	45 (34–119)
1–17 years	182	56 (35–88)	87	54 (33–85)	95	58 (35–91)

Cystatin C reference intervals are parametric (mean (2 SD)) after log transformation.

Creatinine reference intervals are non-parametric (median) with 2.5 to 97.5 centile range.

*Gestational age.

†Includes term neonates whose sex was not categorised.

Results

The between batch coefficient of variation ($n = 10$) of the quality controls (QC) for each method were as follows: Jaffé creatinine ($\mu\text{mol/litre}$): low QC, 10.1% (mean, 50.1; SD, 5.3); medium QC, 1.3% (mean, 196.9; SD, 2.6); and high QC, 1.3% (mean, 485.1; SD, 6.1); PENIA cystatin C (mg/litre): QC, 2.6% (mean, 1.28; SD, 0.034).

The frequency distributions for cystatin C and creatinine were both non-Gaussian, even when separated by sex. Only cystatin C values reverted to a Gaussian distribution after log transformation. Neither of the analytes showed any significant sex differences using the Mann-Whitney U test. Figure 1 shows box plot distributions of analyte concentrations across the age groups. Cystatin C concentrations were highest in premature and full term neonates, and gradually declined with increasing age until about 1 year, when adult values were reached (fig 1A). Creatinine concentrations were also highest in the premature and full term neonates during the 1st week of life, then dropped to approximately $40 \mu\text{mol/litre}$, resulting in a very wide variation in values, followed by a progressive increase to adult concentrations during adolescence (fig 1B). The largest creatinine increases occurred at 9–17 years of age with the onset of puberty. Female adult creatinine values were reached at about 18 years, but male adolescent values were lower than adult values. Creatinine values for each age group were all significantly different from each other ($p < 0.0001$), except between 4–11 months and 1–3 years, whereas only those groups below 1 year had significantly higher ($p < 0.0001$) cystatin C concentrations.

For the premature babies, the cystatin C values were less variable than the creatinine values. Cystatin C values at birth, whether pre-term or full term, were higher than adult values and fell over the age of 1–2 months. Table 1 shows the reference intervals for each analyte

for different age groups. For cystatin C, we calculated three ranges: premature infants ($n = 30$), 0.43–2.77 mg/litre (mean, 1.56); over 1 year ($n = 79$), 0.59–1.97 mg/litre (mean, 1.20); and 1–17 years ($n = 182$), 0.50–1.27 mg/litre (mean, 0.82). For creatinine, we calculated the following ranges: premature infants ($n = 30$), 27–175 $\mu\text{mol/litre}$ (median, 78); younger than 1 year ($n = 79$), 33–127 $\mu\text{mol/litre}$ (median, 44); and 1–17 years ($n = 182$), 35–88 $\mu\text{mol/litre}$ (median, 56).

Discussion

This is one of the first studies to assess kidney function in premature infants and children using the new GFR marker, cystatin C. Although serum creatinine is the most widely used marker of renal function, it is insensitive to small changes in renal function and is proportional to muscle mass and body weight, which increase with growth. Cystatin C concentrations are unaffected by these physiological variables and might therefore reflect GFR more closely in the paediatric population.

We have compared creatinine and cystatin C values in children and adolescents, ranging from 24 weeks premature to 17 years of age. The creatinine concentrations across the age groups (fig 1B) confirmed the results of others, with high creatinine values at birth and during the 1st week of life, which then decreased to approximately $40 \mu\text{mol/litre}$. A study by Feldman and Guignard¹⁷ showed that there was a wide range of creatinine values during the first 5 days of life in infants born at 30–40 weeks. These high creatinine concentrations at birth, possibly of maternal origin, declined dramatically during the 1st month of life, greatly reducing the value of creatinine as an index of GFR in infants. Creatinine concentrations remained constant until 2 years of age, at which point they were seen to rise to adolescent values, in agreement with the findings of Schwartz *et al.*¹⁴ Newborn infants have a

muscle mass that is 24% of body weight, and growth in infancy is not associated with a major change in the proportion of muscle mass.²⁵ During childhood, accretion of muscle mass exceeds the increase in body weight so that by 11 to 13 years, 39% of body weight is muscle, approaching the value of 43% in male adults.

GFR measurement, by ⁵¹Cr-EDTA clearance, has been shown to reach adult values of 114 ml/min/1.73 m² by 18 months, and then to be constant up to 17 years.²⁶ This makes the measurement of serum creatinine unreliable when its concentration rises continuously because of an increase in muscle mass. The cystatin C values in our study (fig 1A) mirror the reported GFR, falling to within the adult range by 1 year. Cystatin C is raised preterm until birth; the maternal contribution of cystatin C is unknown.

It has been shown that GFR increases with postconceptional age at a rate that accelerates with maturation.^{15 16 27} From 20 weeks of gestation, kidney weight and body weight have a linear relation to gestational age and body surface area.²⁸ Although there are studies that have measured the changes of GFR occurring in the last weeks of gestation the results are not uniform. A study by Aperia *et al.*,²⁹ measuring creatinine clearance, showed that preterm infants, born before 34 weeks' gestation, had significantly lower GFRs than full term infants, and that this difference persisted for up to 3–5 weeks postnatally. During the 1st week of life, the GFR increased significantly more in full term than preterm infants. Between 1 and 5 weeks of age the GFR increased at a slower rate in both sets of infants. Brion and colleagues³⁰ have also shown that GFR measurement, using inulin and creatinine clearance, increases with gestational and postnatal age, whether expressed as absolute value (for each unit surface area) or for each kg body weight.

Creatinine concentrations were significantly different between the premature and term infants. This could be because of the different postnatal sampling points: the term samples were collected at day 2 versus day 1 for the premature infants.¹⁹ Although there are indications that creatinine reabsorption is increased in the preterm kidney,³¹ this is likely to be passive diffusion down a concentration gradient, a process unlikely to occur with a protein like cystatin C. Once filtered, cystatin C would normally be reabsorbed and degraded by the proximal tubular epithelial cells. In the neonate, the tubular length is less than in the adult so the proportion reabsorbed is likely to be much less, but the protein will still be removed and pass into the urine. The much smaller and statistically insignificant differences between the premature and term cystatin C concentrations suggests a smaller maternal contribution to the neonatal circulatory pool. Although a longer half life of cystatin C in the circulation might contribute to the sustained level at day 7 in the term infants, our own (unpublished, 1995) studies during adult donor nephrectomies suggest that it is, in fact, not significantly different to that of creatinine. There is evidence indicating that the longer

Key messages

- Cystatin C is a better marker than creatinine of glomerular filtration rate (GFR) in preterm infants
- A single reference range for plasma cystatin C can be used, regardless of sex, from 1 year of age
- Cystatin C offers a more specific and practical measure for monitoring GFR in the paediatric population than does creatinine

half life of dextrans might be the result of differences in the permeability of the neonatal glomerulus³²; however, it is possible that this could be explained by the reduced GFR that is also present, rather than permeability differences. In previous work, looking at the glomerular permeability and tubular reabsorption of proteins in neonates, it was concluded that there was insufficient evidence to establish whether the increase in albumin excretion was the result of an increase in permeability or a decrease in tubular reabsorption.³³ However, our data and that of others suggest that a protein with the molecular weight of cystatin C is unlikely to be retained to any great degree by the neonatal kidney. Whereas in the adult kidney, the passage of cystatin C through the glomerular barrier is less free than the much smaller creatinine molecule, it will be closer to that of creatinine in the neonate because of the greater overall permeability of the neonatal barrier.

In summary, we have shown that cystatin C is a better marker of GFR than creatinine in the paediatric population because it appears to mirror what is known about the maturation of renal function more closely. In premature infants, cystatin C is significantly raised at all gestational ages. Cystatin C concentrations in children reach adult values by the age of 1 year (range, 0.50–1.27 mg/litre; adult range, 0.51–0.98); therefore, a separate reference range is not required. Below the age of 1 year, cystatin C values are higher, reflecting the immaturity of the kidneys (< 1 year, 0.59–1.97 mg/litre). Creatinine does not show these trends and is influenced mainly by the increase in muscle mass during growth. At birth, the kidneys are immature and cystatin C concentration changes suggest it takes 12 months to attain maturity (in accord with reference GFR measurements), whereas serum creatinine concentrations are influenced by increasing muscle mass and do not reach adult values until after puberty. Cystatin C concentrations are effectively constant from 1 year of age upwards. This suggests that cystatin C might offer a considerable advantage to paediatric nephrologists in the measurement of GFR.

HF was supported by a grant from Dade Behring Marburg GmbH, Marburg, Germany. We are grateful to colleagues at the Birmingham Children's Hospital for collection of some of the paediatric samples.

1 Chantler C. The measurement of renal function in children: a review. *Guy's Hospital Reports* 1973;122:25–41.

- 2 Schwartz GJ, Brion LP. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children and adolescents. *Pediatr Clin North Am* 1987;34:571-90.
- 3 Simonsen O, Grubb A, Thysell H. The blood serum concentration of cystatin C (γ -trace) as a measure of the glomerular filtration rate. *Scand J Clin Lab Invest* 1985;45:97-101.
- 4 Grubb A, Simonsen O, Sturfelt G, Truedsson L, Thysell H. Serum concentration of cystatin C, factor D and B₂-microglobulin as a measure of glomerular filtration rate. *Acta Med Scand* 1985;218:499-503.
- 5 Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin Nephrol* 1992;38:S20-7.
- 6 Kyhse-Andersen J, Schmidt C, Nordin G, et al. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. *Clin Chem* 1994;40:1921-6.
- 7 Newman DJ, Thakkar H, Edwards RG, et al. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. *Kid Int* 1995;47:312-18.
- 8 Finney H, Newman DJ, Gruber W, Merle P, Price CP. Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems (BNA, BN II). *Clin Chem* 1997;43:1016-22.
- 9 Norlund L, Fex G, Lanke J, et al. Reference intervals for the glomerular filtration rate and cell-proliferation markers: serum cystatin C and serum B₂-microglobulin/cystatin C-ratio. *Scand J Clin Lab Invest* 1997;57:463-70.
- 10 Finney H, Newman DJ, Price CP. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. *Ann Clin Biochem*. [In press.]
- 11 Filler G, Witt I, Priem F, Ehrlich JHH, Jung K. Technical briefs: are cystatin C and B₂-microglobulin better markers than serum creatinine for prediction of a normal glomerular filtration rate in pediatric subjects? *Clin Chem* 1997;43:1077-8.
- 12 Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Brodehl J. Reference values for cystatin C serum concentrations in children. *Pediatr Nephrol* 1998;12:125-9.
- 13 Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C—a new marker of glomerular filtration rate in children independent of age and height. *Pediatrics* 1998;101:875-81.
- 14 Schwartz GJ, Feld LG, Langford DJ. A simple estimate of glomerular filtration rate in full term infants during the first year of life. *J Pediatr* 1984;104:849-54.
- 15 Gordjani N, Burghard R, Leitis JU, Brandis M. Serum creatinine and creatinine clearance in healthy neonates and pretermatures during the first 10 days of life. *Eur J Pediatr* 1988;148:143-5.
- 16 Sonntag J, Prankel B, Waltz S. Serum creatinine concentration, urinary creatinine excretion and creatinine clearance during the first 9 weeks in preterm infants with a birth weight below 1500 g. *Eur J Pediatr* 1996;155:815-19.
- 17 Feldman H, Guignard JP. Plasma creatinine in the first month of life. *Arch Dis Child* 1982;57:123-6.
- 18 van den Anker JN, de Groot R, Broerse HM, et al. Assessment of glomerular filtration rate in preterm infants by serum creatinine: comparison with inulin clearance. *Pediatrics* 1995;96:1156-8.
- 19 Savory DJ. Reference ranges for serum creatinine in infants, children and adolescents. *Ann Clin Biochem* 1990;27:99-101.
- 20 Donckerwolcke RAMG, Sander PC, van Stekelenburg GJ, Stoop JW, Tiddens HAWM. Serum creatinine values in healthy children. *Acta Paediatr Scand* 1970;59:399-402.
- 21 Laskarzewski P, Kelly KA, Mellies MJ, et al. Clinical chemistry determinations for a biracial cohort of 1,605 normal and hyperlipidemic schoolchildren aged 6 to 17. *Am J Clin Pathol* 1980;74:371-80.
- 22 Cherian AG, Hill JG. Percentile estimates of reference values for fourteen chemical constituents in sera of children and adolescents. *Am J Clin Pathol* 1978;69:24-31.
- 23 Schwartz GJ, Haycock GB, Spitzer A. Plasma creatinine and urea concentration in children: normal values for age and sex. *J Pediatr* 1976;88:828-30.
- 24 Burritt MF, Slockbower JM, Forsman RW, Bergstrahl-J, Smithson WA. Pediatric reference intervals for 19 biologic variables in healthy children. *Mayo Clin Proc* 1990;65:329-36.
- 25 Fawer C-L, Torrado, Guignard J-P. Maturation of renal function in full-term and premature neonates. *Helv Paediatr Acta* 1979;34:11-21.
- 26 Piepsz A, Pintelon H, Ham HR. Estimation of normal chromium-51 ethylene diamine tetra-acetic acid clearance in children. *Eur J Nucl Med* 1994;21:12-16.
- 27 Schultz DM, Giordano DA, Schultz DH. Weights of organs of fetuses and infants. *Arch Pathol* 1962;74:244.
- 28 Heijden AJ vd, Grose WFA, Ambagtsheer JJ Provoost AP, Wolff ED, Sauer PJJ. Glomerular filtration rate in the pre-term infant: the relation to gestational and postnatal age. *Eur J Pediatr* 1988;148:24-8.
- 29 Aperia A, Broberger O, Elinder G, Herin P, Zetterström R. Postnatal development of renal function in pre-term and full-term infants. *Acta Paediatr Scand* 1981;70:183-7.
- 30 Brion LP, Fleischmann AR, McCarton C, Schwartz GJ. A simple estimate of glomerular filtration rate in low weight infants during the first year of life: noninvasive assessment of body composition and growth. *J Pediatr* 1986;109:698-707.
- 31 Guignard JP, Drukker A. Why do newborn infants have a high plasma creatinine? *Pediatrics* 1999;103:e49.
- 32 Emmrich P, Baumann W, Stechele U. Studies on the kinetics and renal excretion of low and high molecular weight dextrans in preterm babies, newborns and young infants. *Eur J Pediatr* 1977;125:181-90.
- 33 Fell JM, Thakkar H, Newman DJ, Price CP. Measurement of albumin and low molecular weight proteins in the urine of newborn infants using a cotton wool ball collection method. *Acta Paediatr* 1997;86:518-22.