Reference Interval for Serum Cystatin C in Children, Else Randers,1* Søren Krue,2 Erland J. Erlandsen,3 Henning Danielsen,1 and Lars G. Hansen2 (Departments of 1Internal Medicine, 2Pediatrics, and 3Clinical Biochemistry, Viborg Hospital, DK-8800 Viborg, Denmark; * address for correspondence: Department of Internal Medicine, Viborg Hospital, Helbergs Allé 4, DK-8800 Viborg, Denmark; fax 45-8927-3484, e-mail elseranders@sol.dk)

Serum creatinine and creatinine clearance are widely used as measures of glomerular filtration rate (GFR) in clinical medicine. Unfortunately, serum creatinine concentrations are not determined only by glomerular filtration (1). Alteration in renal handling and metabolism of creatinine and methodological interferences in its measurement may influence the concentrations of serum creatinine (2). Creatinine production is proportional to muscle mass (2). In children, the muscle mass increases significantly with linear growth, and serum creatinine concentrations have to be adjusted for body height and body size to reflect the renal function (3, 4).

Cystatin C, a nonglycosylated low molecular weight protein (M, 13,359) (5), is a proteinase inhibitor involved in the intracellular catabolism of proteins (6). Unlike creatinine, cystatin C is produced in all investigated nucleated cells at a constant rate, freely filtered in the renal glomeruli and almost completely reabsorbed and catabolized in the renal proximal tubular cells (7, 8). Recent studies have indicated that serum cystatin C can be used as an endogenous marker of GFR in adults (9, 10) and is a promising marker in children (11-13). The aim of the study was to establish a reference interval for serum cystatin C in children without evidence of kidney disease.

One hundred and thirty-seven children (79 boys and 58 girls) of ages between 7 days and 14.1 years (3.2 ± 3.5 years; mean ± SD) with body weight >1.5 kg and without clinical evidence of kidney diseases were included in the study (Table 1). Twenty-nine children had pneumonia, 25 viral infections, 32 other infectious diseases, 23 bronchial asthma, and 28 other noninfectious diseases. The children’s parents gave their informed consents according to the Declaration of Helsinki, and the study was approved by the local committee of ethics. During a period of 5 months, blood samples were collected randomly from children acutely hospitalized in the Department of Pediatrics or visiting the outpatient pediatric clinic, Viborg Hospital. Leftover serum after routine chemistry measurements was used to analyze cystatin C and creatinine.

Serum cystatin C was analyzed using the N Latex Cystatin C assay on the Dade Behring Nephelometer II (BN II; Dade Behring Diagnostics). The BN II was programed and calibrated according to the instructions from the manufacturer. By using the BN II pediatric sample rack, the assay could be performed with a total sample volume of 70 μL (instrument dead volume, 30 μL; sample volume, 40 μL). The measuring range was 0.25–7.90 mg/L, with the default dilution 1:100 of the sample. The detection limit was 0.05 mg/L, corresponding to a minimum dilution 1:20 of the sample. The coefficient of variation for within-run and between-run imprecision studies was between 1.1% and 1.8% in serum pools with cystatin C concentrations between 0.87 and 4.63 mg/L (14). No interferences from hemoglobin, bilirubin, and lipemic samples have been demonstrated (14).

Serum creatinine was analyzed using the Vitros CREA slide, an enzymatic method, on the Vitros 950 Chemistry System (Ortho-Clinical Diagnostics). The CV for within-run and between-run imprecision studies was between 0.4% and 1.0% using the Vitros Performance Verifier I and II as controls (15). The measuring range was 4–1238 μmol/L. Estimated GFR was calculated using the Morris formula (4) in children >2 years: GFR (mL/min per 1.73 m²) = [40 × height (cm)]/serum creatinine (μmol/L).

All data are expressed as mean ± SD. Statistical analysis was performed using GraphPad Prism Ver. 2.01 for Windows NT (GraphPad Software, Inc.). Nonparametric reference intervals were calculated using GraphROC™ for Windows Ver. 2.0 (developed by Veilo Kairisto, Turku, Finland and Allan Poola, Tallin, Estonia). P < 0.05 was considered significant.

The serum concentrations of cystatin C were highest after birth, followed by a decrease over the following weeks (Fig. 1a). In the group of children younger than 1 month, the serum concentrations of cystatin C were 1.63 ± 0.26 mg/L (mean ± SD), and in the group of

| Table 1. Patient characteristics in different age groups in 137 children.a |
|-----------------|-----------------|-----------------|
|                 | <1 month         | 1–12 months     | >12 months      |
| n               | 12              | 29              | 96              |
| Sex, girls/boys| 6/6             | 7/22            | 45/51           |
| Height, m       | 0.48 ± 0.06     | 0.70 ± 0.10     | 1.03 ± 0.25     |
| Weight, kg      | 2.72 ± 0.88     | 8.58 ± 2.72     | 18.57 ± 10.28   |
| BMI, kg/m²      | 11.71 ± 1.82    | 16.84 ± 3.98    | 16.37 ± 1.98    |
| Surface area, m²| 0.18 ± 0.04     | 0.39 ± 0.09     | 0.72 ± 0.29     |
| Serum cystatin C, mg/L | 1.63 ± 0.26 | 0.95 ± 0.22     | 0.72 ± 0.12     |
| Serum creatinine, μmol/L | 51 ± 16       | 34 ± 9          | 44 ± 12         |
| Estimated GFR, mL/min per 1.73 m² | 96.1 ± 15.2 |               |                 |

a All values are mean ± SD.

b Body mass index.

c Estimated GFR was calculated using the Morris formula (4) in children >2 years (n = 62).
children of ages between 1 and 12 months, the serum concentrations of cystatin C were 0.95 ± 0.22 mg/L (mean ± SD; Table 1). After the first year of life, serum cystatin C became constant, with serum concentrations of 0.72 ± 0.12 mg/L (mean ± SD), and the nonparametric reference interval for serum cystatin C was calculated to be 0.51–0.95 mg/L (2.5 and 97.5 percentiles); median, 0.71 mg/L; and range, 0.46–1.00 mg/L. The 90% confidence limits for the lower limit were 0.46–0.55 mg/L, and for the upper limit, 0.91–1.00 mg/L. The Mann–Whitney U-test revealed no gender-related differences of serum cystatin C (P = 0.23). There was a linear relationship between age and serum cystatin C in children older than 1 year [y = 0.73–0.002x; S_{\mu x} = 0.12; r = 0.07; n = 96] and the slope -0.002 (-0.009 to +0.004) (95% confidence intervals) did not differ significantly from zero (P = 0.51).

The concentrations of serum creatinine had high concentrations at the time of birth, followed by a rapid decrease over the following weeks, and were replaced by a steady increase with age until adulthood (Fig. 1b). Linearity was found between age and serum creatinine (y = 33 + 2.59x; S_{\mu x} = 7.58; r = 0.78; n = 96) and the slope 2.60 [2.17–3.02 (95% confidence intervals)] differed significantly from zero (P < 0.0001).

In children older than 2 years, serum creatinine concentrations have been adjusted to height using the Morris formula and linearity between age and estimated GFR (y = 93.6 + 0.41x; S_{\mu x} = 15.24; r = 0.10; n = 62) was found. The slope 0.41 [-0.67 to 1.49 (95% confidence intervals)] did not differ significantly from zero (P = 0.45).

No significant difference was found between the subgroups of the reference population (Fig. 1c). The Mann–Whitney U-test revealed no differences between the group having infectious diseases (n = 62) and the group having noninfectious diseases (n = 34; P = 0.43).

In the present study serum cystatin C concentrations were measured in a group of pediatric patients without overt evidence of kidney diseases. Serum creatinine concentrations were measured as a marker of GFR, being a noninvasive method, and normal values of serum creatinine in relation to age were found. Our reference population consisted of children mainly having infectious diseases. No significant differences were found between the subgroups of the reference population or between the children having infectious and noninfectious diseases. Thus, we find it unlikely that the results of the present study are influenced by infection.

The highest serum cystatin C concentrations were found after birth, followed by a rapid decrease over the following weeks in accordance with data published recently (16). Plebani et al. (17) demonstrated that serum cystatin C does not cross the placental barrier, and the high values of serum cystatin C after birth probably reflect the degree of maturation of the glomerular filtration capacity (16).

The concentrations of serum cystatin C were constant in children >1 year, and the nonparametric reference interval was calculated to be 0.51–0.95 mg/L. The present reference interval is slightly lower than in studies re-
ported previously (11, 12, 16). The difference is probably attributable to the use of a particle-enhanced nephelometric immunoassay (14) in the present study in contrast to previous studies (11, 12, 16) using a particle-enhanced turbidimetric immunoassay (10). Method calibration is not yet established internationally but is required to allow direct comparison between methods (18).

In children >1 year, serum creatinine increases with age until adulthood. Serum creatinine concentrations were adjusted to height and body size using the Morris formula, and a better correlation was found. As a single-sample measurement for estimation of GFR, serum cystatin C concentrations do not require adjustments to height and body size, which assigns serum cystatin C measurements advantages for use in clinical practice.

Gender did not alter the serum concentrations of cystatin C in agreement with previous studies (12, 13) contrary to serum creatinine. No interferences from hemoglobin, bilirubin, and lipemia have been demonstrated using the particle-enhanced nephelometric immunoassay method, making the assay suitable for measurements of pediatric samples (14).

In conclusion, the results demonstrate that serum concentrations of cystatin C in children without evidence of kidney diseases are constant after the first year of life, contrary to serum creatinine. The reference interval for serum cystatin C in children >1 year was calculated to be 0.51–0.95 mg/L. After the first year of life, serum cystatin C possesses the advantage of being independent of age, gender, and muscle mass, which facilitates the recognition of abnormal renal function.

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References

Diurnal Rhythm of CrossLaps in Human Serum, Maria Wichers,* Elke Schmidt, Frank Bidlingmaier, and Dietrich Klingmüller (Department of Clinical Biochemistry, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany; *author for correspondence: fax 49-228/287-5028)

Measurements of molecules of bone resorption or formation can be used for estimating the rate of bone turnover. Bone turnover, as assessed by several bone metabolic markers, has been reported to undergo a diurnal rhythm. Thus far, urinary pyridinoline and deoxypyridinoline, serum osteocalcin, bone specific alkaline phosphatase, serum type I collagen cross-linked N-telopeptides (NTx), the C-terminal pyridinoline cross-linked telopeptide of type I collagen, and urinary excretion of NTx were

Fig. 1. Individual serum CTx concentration profiles in a 24-h time period in six healthy male volunteers.