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Aimo Harmoinen · Elisa Ylinen · Marja Ala-Houhala
Martti Janas · Minna Kaila · Timo Kouri

Reference intervals for cystatin C in pre- and full-term infants and children

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Abstract Cystatin C is a non-glycated, 13-kDa basic protein produced by all nucleated cells. Recent studies have indicated that the plasma concentration of cystatin C is a better marker for glomerular filtration rate (GFR) than plasma creatinine, which is most commonly used for this purpose. We established reference values for plasma cystatin C in pre- or full-term infants and children. For comparison we also measured the creatinine concentration in the same samples. Cystatin C was measured by a commercially available immunoturbidimetric method with a Hitachi 704 analyzer in sera obtained from 58 pre-term infants, 50 full-term infants and 299 older children (132 girls, 167 boys, median age 4.17 years, range 8 days to 16 years). No sex differences were found. The pre-term infants had higher cystatin C concentrations (mean 1.88 mg/l, SD 0.36 mg/l) than the full-term (mean 1.70 mg/l, SD 0.26 mg/l, P=0.0145). The reference interval for pre-term infants calculated non-parametrically was 1.34–2.57 mg/l and for full-term infants 1.36–2.23 mg/l. The cystatin C concentration decreased rapidly after birth, and above 3 years of age did not depend on age. The reference interval for children 3–16 years of age calculated non-parametrically was 0.51–1.31 mg/l. Younger children (<1 year: 0.75–1.87 mg/l; 1–3 years: 0.68 –1.60 mg/l) had slightly, but significantly, higher plasma cystatin C levels.

Key words Cystatin C · Creatinine · Glomerular filtration rate · Reference intervals · Renal function tests

A. Harmoinen (✉) · T. Kouri
Centre for Laboratory Medicine, Tampere University Hospital,
PO Box 2000, 33521 Tampere, Finland
e-mail: aimo.harmoinen@tays.fi
Tel.: +358-3-247-6533, Fax: +358-3-247-5554

E. Ylinen
Medical School, University of Tampere, Tampere, Finland

M. Ala-Houhala · M. Janas · M. Kaila
Department of Pediatrics, Tampere University Hospital,
Tampere, Finland

Introduction

Measurements of the glomerular filtration rate (GFR) are needed both for the diagnosis of renal diseases and in the prescription of the correct doses of many therapeutic drugs. However, the most accurate means of measuring GFR, e.g. inulin- or ^{51}Cr-ethylenediaminetetraacetic acid (EDTA)-infusion methods, are laborious, time-consuming and uncomfortable for the patient, for which reason the plasma or serum creatinine concentration is often used to estimate renal function in children. Unfortunately, the plasma creatinine concentration also reflects creatinine production, which is proportional to muscle mass [1]. Creatinine is also insensitive in slightly reduced renal function [2]. Many endogenous and exogenous substances, moreover, interfere with the traditional (picrate or Jaffé) methods of creatinine determination [3, 4]. There is thus a practical need for an easily automated alternative to plasma creatinine, which would be more specific, sensitive and reliable from the analytical and clinical viewpoint.

Human cystatin C is a 13-kDa basic (pI=9.3) protein produced by all nucleated cells, and its production is unaltered in inflammatory diseases. As a small basic protein it easily passes the glomerular basement membrane and is then catabolized by the renal tubular cells. Since it is eliminated from the circulation almost exclusively by glomerular filtration [5], its plasma clearance is virtually identical to that of ^{51}Cr-EDTA [6]. The plasma concentration of cystatin C has been shown to be a significantly better marker for GFR than the plasma creatinine concentration both in adults [7, 8] and in children [9–11].

The aim of the present study was to determine the reference intervals for plasma cystatin C in infants and children. Special care was taken to select children with no diseases affecting renal function and to achieve statistically representative groups [12].

Materials and methods

Methods

Cystatin C concentrations were measured using the latex-enhanced immunoturbidimetric method previously described [7, 11]. The interassay coefficient of variation was 6.6% at 1.5 mg/l and 3.2% at a level of 5.8 mg/l [11]. Creatinine determinations were made by the traditional picrate method. To eliminate bilirubin interference, potassium ferricyanide was added to the reaction mixture [13]. At a creatinine level of 100 µmol/l, the intra-assay CV was 2.05% and the interassay CV 3.17% [11]. All these measurements were made with a Hitachi 704 Analyzer.

Reference sample group

Cystatin C and creatinine were measured in lithium-heparin plasma samples obtained for routine monitoring purposes from 58 pre-term (gestation week: median 32, range 25–37) and 50 full-term infants in our neonatal intensive care unit and in our delivery unit. Patients with any anamnestic, clinical or laboratory evidence of renal disease or urinary tract infection were excluded. None of the pre-term infants was asphyxiated at birth, defined as Apgar points ≤5 (mean 7.9) at 5 min and none was known clinically to have a renal abnormality. Eight were exposed to indomethacin, 5 were treated medically because of hypotension and 14 had received netilmicin. As none of the medically treated groups differed statistically from the non-treated group, we pooled the subgroups for calculation of the reference interval. Older children were selected from patients attending the asthma and allergy unit of Tampere University Hospital. This latter group comprised 132 girls and 167 boys (median age 4.17 years, range 8 days to 16 years). Patients with any anamnestic, clinical or laboratory evidence of renal disease or urinary tract infection were excluded. After centrifugation, plasma was separated and stored at +4°C until assessed within a week.

The study was approved by the Ethics Committee of Tampere University Hospital in accordance with the ethical standards of the Helsinki declaration of 1975; parents' oral consent was obtained in each case.

Statistics

The dependency of the cystatin C concentration on age or sex was tested by the Kruskal-Wallis analysis of variance or the Mann-Whitney U test (STATISTICA for Windows program, Statsoft Inc., Tulsa, OK). If the subgroups differed statistically ($P<0.05$ including Bonferoni's correction), they were treated separately. After regrouping similar subpopulations, non-parametric 95% reference intervals were calculated using a GraphROC program [14].

Results

Plasma cystatin C concentrations of boys and girls did not differ from each other ($P=0.926$) and the respective values were therefore combined. The influence of age on cystatin C concentration was tested by comparison among the different age groups. A significant difference in cystatin C concentrations was observed between pre-term infants, full-term infants, children under 1 year old, from 1 to 2 years old, from 2 to 3 years old and between 3 and 16 years old (chi-square = 203, $df=5$, $P=0.000$, Kruskal-Wallis test). The highest concentrations were found just after birth, with a rapid decrease over the following months. The pre-term infants had significantly higher cystatin C concentrations (mean 1.88 mg/l, SD 0.36 mg/l, range 1.07–2.86 mg/) than the full-term infants (mean 1.70 mg/l, SD 0.26 mg/l, range 1.24–2.32 mg/l; $P=0.0145$, Mann-Whitney U test). The non-parametric 95% reference interval for pre-term infants was 1.34–2.57 mg/l and for full-term infants it was 1.36–2.23 mg/l. In children under 1 year old cystatin C concentration in plasma was slightly, but significantly dependent on age (Fig. 1). Above 3 years of age, the cystatin C concentration was independent of age (mean 0.98 mg/l, SD 0.20), but also in the 2nd and 3rd years of life the values in plasma were slightly higher than subsequently (Fig. 2, Table 1). As no significant difference was found in cystatin C concentrations between children 1 and 2 years old ($P=0.4125$), we pooled these data for the reference interval calculation.

In comparison, the plasma creatinine concentration was lowest very soon after birth, but increased gradually with age until adulthood ($r=0.816$, $n=299$, excluding infants under 8 days of age).

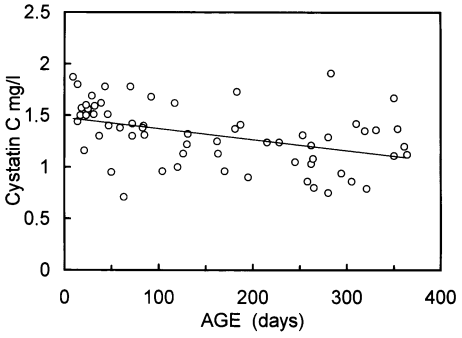

Fig. 1 Plasma cystatin C concentrations between 8 days and 1 year were slightly dependent of age: $y=-0.0011x+1.48$, $r=0.42$, $P=0.000$, $n=65$

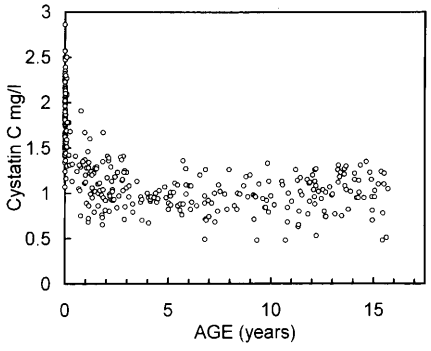

Fig. 2 Plasma cystatin C concentrations at different ages. After the 2nd year of age the concentrations were independent of age: cystatin C mg/l $=0.0081x+0.895$, $r=0.161$, $n=162$

Discussion

In the present study we sought to avoid causing the children unnecessary pain and inconvenience by determining the reference interval using plasma samples obtained for

Table 1 Non-parametric 95% reference intervals for cystatin C in different age groups

	n	Reference interva	90% confidence limits		*P*[a]
			Lower limit	Upper limit	
Pre-term infants	58	1.34–2.57	1.07–1.42	2.47–2.86	0.000
Full-term infants	50	1.36–2.23	1.24–1.44	2.03–2.32	0.000
>8 days – 1 year	65	0.75–1.87	0.71–0.86	1.78–1.91	0.000
>1–3 years	72	0.68–1.60	0.65–0.79	1.39–1.67	0.011
>3 –16 years	162	0.51–1.31	0.48–0.68	1.26–1.35	

[a]Statistical significance vs the oldest group (including Bonferoni's correction factor 5)

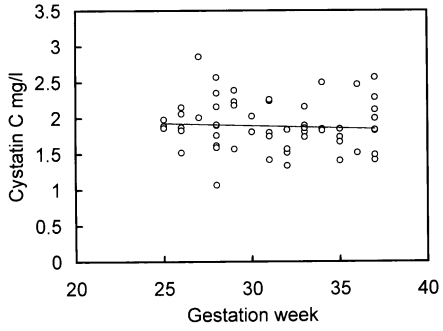

Fig. 3 Plasma cystatin C concentration of the pre-term infants at birth was independent of gestational age: y=–0.006x+2.09, r=0.071, P=0.591, n=58

routine blood tests. Only plasmas from children without anamnestic, clinical or laboratory evidence of renal disease were used. For comparison we also measured plasma creatinine in the same samples as the standard marker of the glomerular filtration rate. The selection of the patients on the criteria mentioned above seemed to be sufficient, since no value was excluded by mathematical rules for outliers [14].

In pre-term infants, we found no significant relation between gestational age and cystatin C concentration (Fig. 3) and thus we pooled all values for the calculation of the reference intervals. Recently, Finney et al. also reported that they found no relationship between gestational age and the cystatin C concentration at birth [15]. During the 1st year of life the decrease in the plasma cystatin C correlated significantly with age (Fig. 1) [16, 19]. This decrease still continued, but more slowly during the next 2 years, reflecting the maturation of the kidneys [10].

The upper 97.5% reference limit (1.31 mg/l) for children over 3 years of age was the same as our cut-off limit for the best diagnostic accuracy for cystatin C recently reported [11]. Calculation of this cut-off limit was based on the ^{51}Cr-EDTA clearance [17] of our pediatric renal patients. Our reference intervals also agree well with those of previous studies [9, 18, 19], as the plasma values for cystatin C are about 5% lower than those in serum [20]. Earlier studies have reported cystatin C values to be constant after the 1st year of life. Our data indicate that also children between 1 and 3 years in age have slightly, but statistically significantly (P=0.0022), higher cystatin C values than older children.

Randers and co-workers have recently published reference values for children using a nephelometric method [21]. Their reference interval of 0.51–0.95 mg/l for children over 1 year of age is clearly lower than that here or elsewhere [9, 18, 15, 19]. This discrepancy is probably attributable to differences in methods [22, 23].

Plasma creatinine is a poor marker of GFR, when renal function is normal or only slightly reduced [24–26]. Its age dependency further reduces its value as a marker for GFR in children. Adjustment for height [27] may improve the usefulness of creatinine [10, 20], but not necessarily so [11, 18]. Although a clear difference has been found between female and male creatinine concentrations in adults [4, 14], in children values were identical in both sexes (boys 52±16 µmol/l, girls 54±16 µmol/l; means ± SD). Plasma creatinine might be useful for detecting temporal changes in renal function in individuals with established renal disease [26].

We conclude that the highest cystatin C concentrations are found in the neonate and decrease during the first 3 years of life, probably reflecting maturation of renal function [10]. The cystatin C reference interval is independent of age beyond the 2nd year of life. The constant reference interval facilitates the identification of renal insufficiency especially in prepubertal children with cystatin C measurement better than with creatinine, whose concentration is dependent on body weight.

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