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LETTER TO THE EDITORS

Giovanni Montini · Loredana Cosmo
Gianpaolo Amici · Michele Mussap
Graziella Zacchello

Plasma cystatin C values and inulin clearances in premature neonates

Keywords Cystatin C · Reference intervals · Control populations

Sirs,
We read with interest the recent paper by Harmoinen et al.[1] in *Pediatric Nephrology*. We would like to focus on the plasma reference values that the authors established for cystatin C concentration in preterm neonates. In sera obtained from 58 preterm infants the mean cystatin C concentration was 1.88 mg/l (range 1.07–2.86 mg/l) and the reference interval calculated non-parametrically was 1.34–2.57 mg/l. No significant relationship between gestational age and cystatin C concentration was found.

In this study, children were selected if anamnestic, clinical, or laboratory (probably plasma creatinine levels) evidence of renal disease was absent. None of these infants was asphyxiated at birth (Apgar score ≥5 at 5 min), but 27 received either indomethacin, netilmicin, or were medically treated because of hypotension. As the aim of the study was to determine reference intervals, we believe that a gold standard was necessary in a population at high risk for renal dysfunction.

G. Montini · G. Zacchello
Pediatric Department,
Azienda Ospedaliera and Università degli Studi Padova,
Padua, Italy

L. Cosmo
Pediatric Department, Ospedale Civile di Mestre, Venice, Italy

G. Amici
Nephrology Unit, Ospedale di Treviso, Treviso, Italy

M. Mussap
Department of Laboratory Medicine,
Azienda Ospedaliera and Università degli Studi Padova,
Padua, Italy

G. Montini (✉)
Dipartimento di Pediatria, Università degli Studi,
Via Giustiniani 3, 35128 Padua, Italy
e-mail: montini@child.pedi.unipd.it
Tel.: +39-049-8213505, Fax: +39-049-8213509

We would like to report our experience on 20 premature neonates (9 males) with gestational age ranging between 28 and 34 weeks (median 33 weeks) and birth weight between 910 and 2,250 g (median 1,645 g) at a postnatal age of 4–7 days. Seven neonates were intubated and ventilated. None had clinical evidence of renal dysfunction**.** We evaluated glomerular filtration rate (GFR) through:

1. single-shot inulin clearance: a single dose (250 mg/kg) of inulin (10% inulin, Jacopo Monico, Mestre-Venezia, Italy) was injected over 3–6 min through a central catheter, previously placed for clinical reasons. Blood samples (100 µl) were obtained at approximately 0, 15, 60, 120, 480, and 1,440 min after the beginning of the infusion. Inulin was measured in the blood by high-performance liquid chromatography (HPLC), as previously described [2]. Inulin clearance was calculated by applying the least-squares iterative non-linear fitting according to Gauss-Newton-Raphson (JMP v. 3.2.2, SAS Institute, Cary, N.C., USA) on the inulin plasma values, using the biexponential model and standard pharmacokinetic calculations.
2. Plasma concentrations of creatinine were measured with the HPLC reference method and the routine Jaffé method.
3. Cystatin C was determined by a fully automated particle-enhanced turbidimetric immunoassay (PETIA, Dako, Milan, Italy). Two serum samples for creatinine and cystatin C were obtained at the beginning (t=0) and the end (t=1,440) of the 24-h plasma inulin clearance. The results are given as a mean of these two samples.

Informed consent was obtained from the parents and the study had the approval of the Pediatric Department Ethics Committee.

Mean results of the evaluation of glomerular function are summarized in Table 1, where patients are also evaluated on the basis of inulin clearance ≤ or >0.5 ml/min per kg, which may be considered the gold standard for any comparison with other methods of evaluation [3]. In our study population, inulin clearance shows a statisti-

Table 1 Mean results of the evaluation of glomerular filtration rate (*HPLC* high-performance liquid chromatography)

Median and range	Group[a]		
	A (*n*=20)	B (*n*=6)	C (*n*=14)
Creatinine HPLC (μmol/l)[b]	83.6 (31–159)	106 (77–159)	70.7 (31–149)
Cystatin C (mg/l)[b]	1.88 (1.2–2.3)	2.11 (1.94–2.3)	1.8 (1.2–2.1)
Biexponential inulin clearance (ml/min per kg)	0.68(0.14–1.44)	0.26 (0.14–0.47)	0.80 (0.52–1.44)
Biexponential inulin clearance (ml/min per 70 kg)	44.1 (11.4–101.1)	18.2 (11.4–33.0)	56.2 (36.4–101.1)

[a] Group A, all the premature infants; group B, 6 premature infants with inulin clearance ≤0.5 ml/kg; group C, 14 premature infants with inulin clearance >0.5 ml/kg
[b] Mean of the two samples obtained at *t*=0 and *t*=1,440 min of the 24-h curve of plasma inulin disappearance

cally significant correlation with either 1/creatinine by HPLC, 1/creatinine by Jaffé reaction, and 1/cystatin C (r=0.852, 0.850, and 0.766, respectively, with P<0.001 for all). All cystatin C plasma concentrations, except 1, were above 2 mg/l in the group of premature infants with inulin clearance <0.5 ml/min per kg and, conversely, all values were <2 mg/l (except 1) in the premature infants with inulin clearance >0.5 ml/min per kg.

As the reference interval calculated non-parametrically reported by Harmoinen et al.[1] was 1.34–2.57 mg/l, it appears that in this category there will be a group of patients with abnormal glomerular function, especially when plasma cystatin values are above 2 mg/l. This is of major importance, since in premature infants glomerular function is significant for the individual adaptation of water and electrolyte balance and the prescription of drugs excreted by the kidney.

As it is well known that in preterm infants there is a positive linear relationship between GFR and gestational age [4], the most-premature infants should have the lowest GFR. The absence of a significant relationship between gestational age and cystatin C concentration in this study needs explanation and, we believe, does not allow pooling all the values for the calculation of the reference interval.

Only a larger study, with the use of inulin clearance as a gold standard, will allow clarification of whether cystatin C is of particular value in identifying situations of altered renal function when creatinine is within the normal range.

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LITERATURE ABSTRACT

B. Kirschbaum

Spurious metabolic acidosis in hemodialysis patients

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Metabolic acidosis with an increased anion gap (AG) is frequently seen among patients with end-stage renal failure that is corrected to a variable degree by chronic hemodialysis. The degree of acidosis is generally interpreted from the concentration of total carbon dioxide (tCO(2)) in blood drawn from the vascular access used for dialysis. As with many dialysis units in the United States, our laboratory studies for outpatients are performed in a central laboratory several hundred miles away and must be shipped there by air freight. We observed a consistent and clinically important difference between the tCO(2) content of samples reported from the central laboratory compared with results reported from a local university hospital chemistry laboratory. The central laboratory readings were always lower, resulting in an increase in the AG. Delays in centrifugation of the blood to separate the clot from the serum and in the initiation of analysis led to an increase in the lactate content of the samples. That increase, however, was insufficient to explain the difference in tCO(2) levels. These data suggest that something happens to the samples in transit to cause an artifactual reduction of the tCO(2) level. For many dialysis patients, the severity of their acidosis may be falsely represented by the tCO(2) content of blood samples reported from central laboratories.