Are Cystatin C and β2-Microglobulin Better Markers than Serum Creatinine for Prediction of a Normal Glomerular Filtration Rate in Pediatric Subjects? Guido Filler, Iris Witt, Friederich Priem, Jochen H.H. Ehrich, and Klaus Jung (1Dept. of Paediatr. Nephrol., 2Clin. Biochem., and 3Urol., Charité Hosp., Humboldt Univ., Schumannstr. 20–21, D-10098 Berlin, Germany; *author for correspondence: fax +49 30 2802 8844, e-mail ehrich@rz.charite.hu-berlin.de)

Serum creatinine is the marker most widely used to predict glomerular filtration rate (GFR). In childhood, there is age and muscle mass dependency of serum creatinine, and assessing a normal GFR accurately even with the use of body length/creatinine ratios remains difficult [1, 2]. Recently, cystatin C (Cys-C), a 13-kDa protein, was found to correlate closely with GFR in adults [3–5]. β2-Microglobulin (β2-MG) serum concentrations also correlate with GFR, and have been shown to be age independent in infants [6]. Here, we report reference limits of Cys-C and β2-MG in children between ages 0.8 and 18 years and demonstrate age independency of these parameters as an essential advantage compared with the conventionally used serum creatinine values.

We studied serum Cys-C and β2-MG in 216 urological pediatric patients (age range 0.8 to 18 years, mean 11.0 ± 6.2 years) with normal GFR, using leftover serum after routine serum chemistry measurements, including serum creatinine. The study is in accordance with the ethical standards of the Helsinki declaration of 1975 (revised in 1983), and parents’ (in the case of 18-year-olds: patients) oral consent was obtained in each case. 51Cr-EDTA clearance studies with a modified method of Chantler and Barratt [7] served as a gold standard for GFR assessment; values between 90 and 150 mL/min per 1.73 m² were defined as normal GFR. Cys-C was measured with the particle-enhanced turbidimetric assay (Dako, Glostrup, Denmark) in the Hitachi 717 analyzer. β2-MG was measured with the microparticle enzyme immunoassay (Abbott, Wiesbaden, Germany), and serum creatinine was determined enzymatically (PAP; Boehringer Mannheim, Mannheim, Germany).

We calculated correlation coefficients according to Spearman and regression coefficients between age and serum concentrations of creatinine, Cys-C, and β2-MG, and a strong correlation between creatinine concentration and age could be demonstrated (Fig. 1). Cys-C and β2-MG showed slopes of the regression lines not significantly different from zero and there was no correlation between their concentrations and age. Thus, age-independent reference values for both low-molecular-mass proteins can be considered.

We calculated the central 95% reference intervals according to the IFCC guidelines by using the nonparametric method [8]. The upper 97.5 percentiles amounted to 1.38 mg/L for Cys-C and 2.27 mg/L for β2-MG. Likewise, the lower 2.5 percentiles amounted to 0.18 mg/L for Cys-C and 0.361 mg/L for β2-MG. Those data correspond to values found in adults [4, 5]. In subjects with normal GFR (>80 mL/min per 1.73 m²), a preliminary reference interval between 0.61 and 1.21 mg/L was defined [4]. Newman et al. [5] used 1.25 mg/L as the upper reference value. However, all these references values were established in a very limited number of subjects (n = 27) [4]. Using an ELISA method, we previously found higher values [3]. These differences are very likely explained by...
the different calibrator material used in the different studies. In the present study, the same calibrator material (recombinant Cys-C) was used as in the studies of Kyhse-Anderson et al. [4] and Newman et al. [5], whereas we calibrated the ELISA method with a urinary protein calibrator of Cys-C (Behring, Marburg, Germany). To date, there are no published Cys-C reference limits for children. Our β2-MG reference values match well with the published reference values [9] for healthy children (95th percentile 2.20 mg/L).

Measurement of serum creatinine can be performed by various methods. The results can be influenced by numerous factors, and each method will produce different normal values for adults and children [10]. In addition to GFR, muscle mass and physical activity also influence the creatinine concentration [10], and thus impairment of GFR may easily be overlooked in young children. Here, Cys-C or β2-MG may serve as a useful alternative. Our data clearly demonstrate age independency for serum concentrations of both Cys-C and β2-MG. Cys-C concentrations >1.4 mg/L and β2-MG concentrations >2.3 mg/L suggest an impaired GFR irrespective of age; hence, measurement of Cys-C or β2-MG concentrations may be advantageous compared with measurements of serum creatinine concentration for the detection of an impaired GFR.

References


Interference of Methylene Blue with CO-Oximetry of Hemoglobin Derivatives, Hervé Gourlain," François Bu"eneaux," Stephen W. Borron," Bernard Gouet," and Pierre Levillain (1 Lab. de Toxicol, and 2 Réanimation Toxicol, Hôpital Fernand Widal, 200 rue du Fg-St-Denis, 75475 Paris Cedex 10, France; 3 C.N.E.H., 9 rue Antoine Chantin, 75014 Paris, France; *author for correspondence: fax +33 1 40 05 48 78) Methylene blue (MB) is frequently used as an antidote in treating methemoglobinemia [1] because it facilitates the reducing activity of the NADPH-dependent methemoglobin reductase system in erythrocytes [2]. However, MB absorbs strongly between 550 and 700 nm (Fig. 1), the same spectrophotometric region as that of the various hemoglobin derivatives: oxyhemoglobin (O2Hb), deoxyhemoglobin (HHb), methemoglobin (MetHb), and carboxyhemoglobin (COHb). To evaluate the potential magnitude and direction of errors linked to the presence of MB for the results for total hemoglobin (THb) and its derivatives, we evaluated six CO-Oximeters. The wavelengths used by each instrument for these determinations are as follows: IL 482 (Instrumentation Laboratory, Lexington, MA), 535, 585.2, 594.5, and 626.6 nm; CCD 270 (Chiron Diagnostics, Medfield, MA), 557, 577, 597, 605, 624, 635, and 650 nm; CCD 835 (Chiron; wavelengths not communicated); OSM3 (Radiometer, Copenhagen, Denmark), 535, 560, 577, 622, 636, and 670 nm; ABL 520 (Radiometer; same wavelengths as OSM3); AVL 912 (AVL Scientific Corp., Roswell, GA), 530, 536, 542, 548, 554, 560, 566, 572, 578, 584, 590, 604, 612, 622, 630, 640, and 648 nm.

Blood was collected from five healthy volunteers with informed consent. Because the study involved only blood sampling, Institutional Ethics Committee Review was not required in France. The five samples were combined to obtain 120 mL of pooled blood, which were then separated into three 40-mL fractions:

- Fraction N, which had no enrichment in CO or MetHb.
- Fraction CO, which was enriched in CO by tonometry with use of an IL 237 tonometer (Instrumentation Laboratory) and CO in nitrogen, 10 mL/L (Société Cosma, Igy 94130, France). Because of the time required to analyze a large number of samples, the tonometry was carried out separately on four 10-mL specimens just before analysis.
- Fraction Met, which was treated with 4 mg of hydroquinone (Prolabo, Paris, France) to obtain samples enriched in MetHb. Hydroquinone, a known inducer of MetHb, was selected for its lack of absorbance in the spectral range of hemoglobin. Again, this enrichment step was carried out on four 10-mL specimens just before analysis.

Each fraction was separated into aliquots. Four aliquots were adulterated with MB by dilution with a stock 10 g/L solution (Pharmacie Centrale des Hôpitaux de Paris, France) to obtain final concentrations of 0.1, 0.25, 0.50, and 1 g/L. We then added 1.0 mL of one of these solutions to 9.0 mL of blood to obtain a blood MB concentration of 10, 25, 50, or 100 mg/L. These MB concentrations were chosen to correspond to the plasma concentrations clinically anticipated when MB is slowly injected intravenously as 5–25 mL of a 10 g/L solution (the 100 mg/L concentration is rarely attained). Four control aliquots were prepared for each fraction as well, the MB being replaced with NaCl 9 g/L. Each adulterated sample was compared with its own control, and measurement was performed immediately after treatment of the blood with either MB or NaCl. Measurements were performed in triplicate with all six CO-Oximeters.

The THb concentrations of controls measured by CO-Oximetry were ~146 g/L. The COHb and MetHb percent-