New combined serum creatinine and cystatin C Quadratic formula for glomerular filtration rate assessment in children

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Materials and methods

Population

This study has been approved by the local research ethics board. Two hundred and forty-three sinistrin clearances (iGFRs) performed between January 2012 and April 2013 for 243 children were evaluated. Indications for clearance performance were left at the discretion of the referring physician (pediatric nephrologists or urologists). All patients aged between 2 and 18.5 years old, referred to our laboratory unit for glomerular filtration rate (GFR) measurement were included. Children with bladder dysfunction, unable to void spontaneously, and in whom bladder catheterization failed, were excluded. Proper emptying of the bladder was also evaluated by comparing the urine output with its osmolality. A decreasing diuresis with a concomitant decreasing urine osmolality was an indication of poor bladder emptying, and the child was then excluded from the study for technical purpose. Our study did not include transplant patients, already enrolled in another study. Body weight (BW) and height (Ht) were expressed in absolute values and in percentiles (P) according to Swiss pediatric growth charts (1). Body mass index (BMI) was calculated as a person's weight in kilograms divided by the square of his height in meters (kg/m^2). Growth retardation was

defined by a Ht and/or a BW below P10. Overweight was defined by a BMI between 25 and 29.9 kg/m^2 and obesity was defined by a BMI of 30 kg/m^2 or higher. Patients' renal disorders and chronic kidney disease (CKD) classification are presented in table 1.

Analytical analysis

Serum creatinine (SCreat) calibration:

SCreat was measured using the kinetic colorimetric compensated Jaffe method as reported by the manufacturer Roche Modular P system (Roche Diagnostics, Mannheim, Germany) that was standardized to the isotope-dilution mass spectrometry (IDMS) reference. The method was calibrated with the calibrator and procedures were described by the Roche Diagnostics. The IDMS traceability involves two points calibration (target values at 0 µmol/l and 360-390 µmol/l depending on the calibrator lot) and the subtraction of 26 µmol/l from the results in order to compensate for the non specific chromogens. The inter-assay coefficients of variation (CVs) obtained in the laboratory with the internal quality controls were 3.9% at 45.7 µmol/l and 2.4% at 108 µmol/l. The intra-assay CVs were 3.3 % at 44.5 µmol/l and 0.7% at 148 µmol/l. The laboratory is participating in an external quality assessment scheme.

Cystatin C (CysC) calibration:

CysC was measured by particle-enhanced nephelometric immunoassay (PENIA) on BN ProSpec analyser (Siemens Healthcare Diagnostics). The results were multiplied by 1.174 as indicated in the Siemens customer bulletin to adjust to the values obtained with the assay standardized to the new traceable International Reference Preparation (IRP)- ERM®-DA471/IFCC as recommended by the KDIGO (Kidney Disease Improve Global Outcomes) (2). Normal reference range of CysC was 0.55 to 1.06 mg/l. The inter-assay CVs were 4.95% at 1.13 mg/l and 3.30% at 4.91 mg/l. The intra-assay CVs were 2.0% at 1.71 mg/l and 2.3% at 5.37 mg/l. Total measurement imprecision was 0.097 mg/l at 1.14 mg/l.

GFR measurement:

All patients fasted for an overnight before the day of investigation, and drugs interfering with the sinistrin measurement were omitted before and during the test. Sinistrin clearance (iGFR) was obtained as follows: Two intravenous catheters were inserted on admission, one in each arm and a loading dose of sinistrin 25% was administrated according to the (Inutest SPC - Fresenius Kabi Pharma Austria GmbH) protocol. The loading dose was calculated in order to obtain a required plasma concentration of 200-250 mg/l, as follow: Loading sinistrin dose = Required plasma concentration x Estimated sinistrin distribution volume. The estimated sinistrin distribution volume corresponds to the extracellular volume and amounts to 15% of the body weight. Subsequently, sinistrin was continuously infused over 90 minutes at a rate given by the required inulin plasma concentration (200-250 mg/l) and the estimated GFR as follow: Infusion rate in mg/mn = (Required plasma concentration / 1000) x Estimated GFR in ml/mn. Water diuresis was induced by oral administration of 20 ml/kg of water (maximum 1200ml) in the first hour followed by 3 ml/kg/h of water. This was combined with an intravenous infusion of 0.9% sodium chloride (maximum 300 ml) every 30 minutes. After a 90-minutes equilibration period, 3 timed-urine samples were collected every 30 minutes, according to the manufacturer's protocol (Inutest SPC - Fresenius Kabi Pharma Austria GmbH), with a blood test in the middle of each urine collection. Sinistrin was measured using the anthrone method by the manufacturer Wright's automatic Wright and Gann (3), using an Autoanalyzer 3 system (High resolution digital colorimeter of SEAL; BRAN+LUEBBE, Norderstedt, Germany). The method was calibrated with five points calibrations (targets values at 10 mg/100 ml, 20mg/100ml, 30 mg/100ml, 40 mg/100ml, 50 mg/100ml) with coefficients correlations of 0.9993±0.0005. The procedure is automatized with software (Bran+ Luebbe, AACE 6.03) which automatically includes corrections for baseline, carryover, sensitivity drift and dilution factor. For the serum, the intra-assay coefficients of

variation (CVs) obtained in our laboratory with the internal quality controls were 2.44% at 10mg/100ml, 1.47% at 30mg/100ml and 0.94% at 40 mg/100ml, while for urine the intra-assay CVs were 1.71% at 10mg/100ml, 1.22% at 30mg/100ml and 1.07% at 50 mg/100ml. The inter-assay CVs obtained in the internal laboratory standards were 2.35% at 10mg/100ml, 2.23% at 30mg/100ml and 0.87% at 50 mg/100ml.

iGFR was calculated as the mean of the three clearance periods. When sinistrin clearance difference between 2 periods exceeded 20%, that period was excluded, and iGFR was calculated as the mean of the 2 valid periods.

Statistical analysis

Statistical analysis was performed using R software, version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria). Population demographics were summarized with median (interquartile range), minimal and maximal values for continuous characteristics and percentages for categorical characteristics. Linear regression techniques (function "lm" in R software) were used to fit the different equations to estimated GFR (eGFR). We considered as predictors parameters for iGFR, the SCreat, the CysC, the age and the sex. In total, 9 models adjusted for age and sex with iGFR as dependant variable and CysC or SCreat as independent variable were evaluated. To assess the performance of the different models, we calculated for each model the Cohen's Kappa (κ) coefficient that measures agreement between two measurements, using values below and above 90 ml/mn per 1.73m^2 to categorize iGFR and eGFR values. To compare κ coefficient between two models, we used a permutation procedure by randomly permuting the estimated values from both models. We also calculated the accuracy, i.e. the % of estimated values within 10% and 30% of observed values. Type of correlation between iGFR and CysC was also assessed using a graphical representation (function lowess). Likelihood ratio tests were performed to compare the fit of the combined SCreat and CysC based logarithmic Schwartz model and the combined SCreat and CysC

based quadratic model. Significance was defined as $p \leq 0.05$. The cut-off of the applicability of the new combined Schwartz formula was determined by applying the circular binary segmentation (CBS) method (4, 5, 6) to formula residuals, as follows: If we consider R1, R2,… Rn the residuals (iGFR-eGFR) of iGFR with iGFR sorted in ascending order and let Si = R1 + R2 +… Ri, $1 \leq i \leq n$, be the partial sums, the likelihood ratio statistic for testing the null hypothesis that there is no change against the alternative that there is exactly one change at unknown location I is given by $ZB = \max 1 \leq i < n \mid Zi \mid$, where $Zi = \{1/i+1/(n-i)\} - 0.5 \{Si /I – (Sn- Si)/(n-i)\}$. The null hypothesis of no change is rejected if the statistic exceeds the upper αth quantile of the null distribution of ZB and the location of the change-point is estimated to be i such that $ZB = \mid Zi \mid$. The CBS method allows to segment data through change-point detection using a maximal t-test.

To test for normality of model residuals, we used two approaches: the first one was to perform D'Agostino and Pearson omnibus normality test (package fBasics, R software) and the second one to graphically assess the normality assumption by plotting residuals on a Q-Q plot. The Q-Q plot shows the quantile of the distribution of the new quadratic formula residuals (y-axis) versus the quantile of a Gaussian distribution (x-axis) with mean 0 and standard deviation 1.

To check for internal validity of our models estimates, we performed a Cross-validation technique called repeated random sub-sampling validation, also known as Monte Carlo Cross-validation (7). This involves dividing randomly the data into two samples: the training set (2/3 of the sample), on which the model was fitted, and the testing set (1/3 of the sample), on which the model was evaluated. The cross-validation was done with 1000 bootstrap replications. For each replication, the model was fitted to the training data, and the root mean square error (RMSE) was calculated using this fitted model for the training set, and then for the testing set. We therefore developed for each replication our estimating equation from the

training data and validated it on the validation data. The distribution (mean, median, first quartile, third quartile, minimum and maximum) of RMSE values was reported.

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Table 1:

Etiologies:	CKD stage I	CKD stage II	CKD stage III	CKD stage IV and V
Obstructive or reflux uropathy	66	56	6	0
Congenital and acquired single kidney	20	25	4	1
Polycystic kidney disease	7	6	2	1
Glomerulopathies	6	5	0	1
Haemolytic and uremic syndrome	1	1	1	1
Metabolic disease	3	0	3	0
Post-chemotherapy	1	4	3	0
Other	2	8	4	0
Total=238	106	105	23	4

Legend: Patients' renal disorders and CKD classification. CKD: chronic kidney disease, CKD stage I, II, III, IV and V denote GFR $\geq$ 90, 60-89, 30-59, 15-29 and < 15 ml/mn per 1.73 m^2, respectively.