Tamara van Donge*, Eveline Staub, Andrew Atkinson, Verena Gotta, John van den Anker, Lorenz Risch, Tatjana Welzel and Marc Pfister

Age appropriate reference intervals for eight kidney function and injury markers in infants, children and adolescents

https://doi.org/10.1515/cclm-2020-0781
Received April 24, 2020; accepted July 24, 2020; published online August 6, 2020

Abstract

Objectives: The use of kidney function and injury markers for early detection of drug-related glomerular or tubular kidney injury in infants, children and adolescents requires age-specific data on reference intervals in a pediatric healthy population. This study characterizes serum values for eight kidney function and injury markers in healthy infants, children and adolescents.

Methods: A single center prospective observational study was conducted between December 2018 and June 2019. Serum samples from 142 healthy infants, children and adolescents aged between 0 and ≤15 years were collected. Statistical analyses for eight markers (albumin (ALB), β_2-microglobulin (B2M), β-trace protein (BTP), creatinine (SCR), cystatin C (CYSC), kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), uromodulin (URO)) were performed to obtain reference intervals and associations with age, sex and weight were investigated (Pearson correlation, linear and piecewise regression).

Results: ALB and SCR increased with age ($p<0.01$), whereas B2M, BTP and KIM-1 values decreased with advancing age ($p<0.05$) in this healthy pediatric study population. CYSC showed dependency on sex (lower concentration in females) and decreased with age until reaching approximately 1.8 years; thereafter an increase with age was seen. NGAL and URO did not show any age-dependency.

Conclusions: This study provides age appropriate reference intervals for key serum kidney function and injury markers determined in healthy infants, children and adolescents. Such reference intervals facilitate the interpretation of changes in kidney function and injury markers in daily practice, and allow early detection of glomerular and tubular injury in infancy, childhood and adolescence.

Keywords: age-dependency; kidney biomarker; kidney injury; pediatrics; reference intervals.

Tamara van Donge and Eveline Staub contributed equally to this work.

Tatjana Welzel and Marc Pfister contributed equally to this work.

***Corresponding author: Tamara van Donge** Pediatric Pharmacology and Pharmacometrics Research, Universitäts-Kinderspital beider Basel (UKBB) Spitalstr. 33, 4031, Basel, Switzerland, Phone: +41 61 704 12 12, E-mail: tamara.vandonge@ukbb.ch, https://orcid.org/0000-0003-4607-3179

Eveline Staub, Department of Neonatology, Royal North Shore Hospital, St Leonards, Australia

Andrew Atkinson, Verena Gotta, Tatjana Welzel and Marc Pfister, Pediatric Pharmacology and Pharmacometrics, University Children's Hospital Basel (UKBB), University of Basel, Basel, Switzerland

John van den Anker, Pediatric Pharmacology and Pharmacometrics, University Children's Hospital Basel (UKBB), University of Basel, Basel, Switzerland; Division of Clinical Pharmacology, Children's National Health Hospital, Washington, DC, USA; and Intensive Care and Department of Pediatric Surgery, Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands

Lorenz Risch, Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; Labormedizinisches Zentrum Dr. Risch, Vaduz, Liechtenstein; and University Institute of Clinical Chemistry, University of Bern, Bern, Switzerland

Introduction

Early detection of (drug-related) glomerular and tubular kidney injury in infants, children and adolescents is crucial for clinical monitoring and therapeutic decision-making. Currently, there is a lack of validated diagnostic approaches to detect early (drug-related) kidney injury in pediatric patients. This can result in adverse long-term kidney function outcomes, particularly in patients treated with potential nephrotoxic drugs (e.g. chemotherapy or antibacterial drugs). Simple extrapolation of kidney function and injury markers, often established in adults, to pediatric populations have the limitation that (i) adult and pediatric kidney diseases are different and, more importantly, (ii) maturational and physiological changes cannot be neglected [1]. Therefore, it is important to account for

developmental changes when interpreting kidney function and injury marker concentrations in the pediatric population.

Serum creatinine (SCR) and (estimated) glomerular filtration rate (GFR) are commonly used clinically to evaluate kidney function, although these are not ideal markers for early detection and monitoring renal and particularly tubular injury in pediatrics [2, 3]. The most accurate assessment of GFR using inulin or iohexol is cumbersome, invasive and not suitable for regular monitoring of kidney function [4]. This is particularly true for the pediatric population. For this reason, estimations of GFR by applying calculations relying on endogenous markers (e.g. SCR) are commonly used. Such endogenous markers are thought to imitate the exogenous markers as closely as possible. The (updated bedside) Schwartz formula estimates kidney function based on the children's height and SCR [5, 6]. Despite the standardization of creatinine assays, the estimated GFR (eGFR) has considerable imprecision due to non-GFR related variation of SCR, e.g. rise in SCR levels after meat consumption [7]. Furthermore, disease related muscle mass variations and nutritional changes can result in overestimation of eGFR leading to potential overdosing of nephrotoxic drugs [8].

Investigations of alternative kidney function and injury markers have been the subject of increased interest over the last decades, especially for the pediatric population [2]. Most low molecular weight proteins, such as β_2-microglobulin (B2M), β-trace protein (BTP), neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (CYSC), are filtered by the glomerulus and reabsorbed by the proximal tubules (Table 1). While increased serum concentrations of these markers indicate glomerular damage, an increase in urinary concentrations implies tubular injury [3, 9]. Uromodulin (URO) is a glycoprotein, which is exclusively produced by the cells in the thick ascending limb, and lower serum levels indicate a function decrease of these epithelial cells [10, 11]. It has been demonstrated that urinary NGAL, urinary kidney injury molecule-1 (KIM-1) and serum CYSC show a decreasing trend from preterm neonates to infants [2, 12–20].

The majority of pediatric studies focused on the comparison of kidney function and injury markers between critically ill patients or between patients with kidney diseases and their control subgroups [16–18, 21, 22]. Since other disease conditions influence the kidney function, these control groups cannot be viewed as true controls for establishing a reference or normative interval [2]. Consequently, there is a paucity of data on age-dependency of serum kidney function and injury markers in the healthy pediatric population. The establishment of age-dependent reference intervals for kidney function and injury markers in a healthy pediatric population is important because it will allow earlier detection of kidney injury in pediatric patients who are ill and/or treated with nephrotoxic drugs [23]. Therefore, it is necessary to examine whether these markers have different values with advancing age, since that will help establish reference intervals for different pediatric age groups that are currently lacking.

The aim of this study was to establish age-dependent reference intervals in a healthy pediatric population for a set of kidney function and injury markers (BTP, B2M, CYSC, KIM-1, NGAL and URO) that are currently not part of routine clinical care, as well as two markers (SCR and albumin [ALB]) that are commonly used clinically. Additionally, the relationship between these markers and demographic characteristics such as weight and/or sex was investigated.

Table 1: Kidney function and injury markers and their characteristics.

Biomarker	Features	Molecular weight	Sample medium	Specific kidney localization	Refs.
β_2-Microglobulin	Component of MHC class I	11 kDa	Serum Urine	Glomerulus Tubules	[3, 36]
β-Trace protein	Expressed in brain, retina and kidneys	23–29 kDa	Serum Urine	Glomerulus Tubules	[3, 37]
Cystatin C	Expressed in all tissues and body fluids	13.3 kDa	Serum Urine	Glomerulus Tubules	[3, 34]
NGAL	Expressed mainly in neutrophils, as well in kidney, prostate and respiratory tracts	22–25 kDa	Serum Urine	Glomerulus Tubules	[38]
KIM-1	Expressed on surface of tubular epithelial cells	85 kDa	Serum Urine	Glomerulus Tubules	[34]
Uromodulin	Produced in kidney (thick ascending limb)	105 kDa	Serum Urine	Distal tubule Distal tubule	[10, 39]

NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1.

Materials and methods

Study design

This single center prospective observational study of consecutive healthy children was conducted at the University Children's Hospital Basel, Switzerland between December 2018 and June 2019. Infants, children and adolescents aged between 0 and ≤15 years, including patients up to 15 years and 11 months were eligible for study participation if they were healthy, required a venous line for elective surgery/anesthesia during their stay at the short stay unit or ward, allowing one blood draw without additional venipuncture. Patients with chronic or congenital diseases were excluded, with exception of adolescents with mild to moderate acne vulgaris. Study protocol was approved by the local Ethics Committee (EKNZ BASEC 2016-00884). Parents were informed about the study verbally and in writing, and written informed consent was obtained. In addition to parental consent, children aged 8–14 years could sign voluntary. Adolescents aged ≥14 years were eligible to sign the informed consent themselves for studies of low risk (risk category A study). The study was performed in accordance with Good Clinical Practice and the principles of the Declaration of Helsinki. Participants were included based on stratification into four age groups; 0–2 years, >2–5 years, >5–10 years and >10–≤15 years, in order to ensure a balanced age distribution. Demographic and clinical data and exposure to medication were collected on the day of study enrollment and at the day of surgery.

Sample collection and lab assessment

Blood samples were collected prior to (elective) surgery in lithium-heparin tubes. Samples were centrifuged (2500 g for 10 min at 20 °C) within 2 h after blood draw and serum was frozen at −20 °C for a maximum of 24 h, and subsequently stored at −80 °C until analysis. Samples were stored for a maximum of eight months (stability was showed for a period of 12 months). Unique biomarkers were quantitated on the same day.

Samples were processed at an ISO-17025 accredited medical laboratory, where eight serum kidney function and injury markers (ALB, BTP, B2M, SCR, CYSC, KIM-1, NGAL and URO) were measured. A minimum volume of 300 µL serum was required for analysis.

ALB (Tina-quant® Albumin 2nd generation, turbidimetric, standardized to BCR470/CRM470), SCR (IDMS-standardized), CYSC (Tina-quant® cystatin C 2nd generation, turbidmetric, standardized to ERM-DA471/IFCC) and NGAL (particle-enhanced turbidimetric immunoassay, Bioporto diagnostics, Hellerup, Denmark, kit specific standardization), were measured with reagents from Roche Diagnostics (Rotkreuz, Switzerland) on a Cobas 6000 instrument (Roche Diagnostics, Rotkreuz, Switzerland). B2M (Tina-quant® β2-Microglobulin Roche, Rotkreuz, Switzerland) was determined on a Cobas 8000 instrument (Roche Diagnostics, Rotkreuz, Switzerland). URO (ELISA, Euroimmun, Luzern, Switzerland, kit specific standardization) and KIM-1 (ELISA, Cohesion Biosciences, London, UK, kit specific standardization) were assayed on a DSX instrument (Dynex technologies, Denkendorf, Germany). Finally, BTP (N Latex BTP, nephelometry, Siemens Diagnostics, Zurich, Switzerland, kit specific standardization) was measured on a BN2 instrument (Siemens Diagnostics, Zurich, Switzerland). Liquid control material was used for CREA (Biorad Laboratories, Crissier, Switzerland), BTP (Siemens Diagnostics, Zurich, Switzerland), NGAL (Bioporto diagnostics, Hellerup, Denmark), URO (Euroimmun, Luzern, Switzerland) and ALB (Biorad Laboratories, Crissier, Switzerland). For CYSC (Biorad Laboratories, Crissier, Switzerland), B2M (Biorad Laboratories, Crissier, Switzerland) and KIM-1 (Cohesion Boosciences, London, UK) lyophilized control material was used. Inter-assay coefficients of variation, as assessed with commercially available control materials, were the following, with at least two control level concentrations: <3.2% for ALB, <7.7% for B2M, <8.4% for BTP, <2.7% CREA enzymatic, <2.9% for CYSC, <9.1% for KIM-1 , <3.5% for NGAL, <5.5% for URO.

Statistical analysis

Kidney function and injury marker values are presented as mean values with standard deviation (SD), median values with interquartile ranges (IQR) and minimum and maximum values. Categorical variables are presented as numbers with percentages. The eGFR is calculated according to the bedside Schwartz formula and additionally corrected for actual body surface area [5, 6]. Measured SCR concentrations were compared to previously published age-specific SCR reference ranges retrieved from a healthy pediatric population [24, 25].

The study population is descriptively analyzed for age, sex, weight, height, prematurity, drug exposure two weeks and 48 h before surgery, and type of elective surgery. Sample size was defined to detect an 18% difference in URO concentrations between adjacent age groups (0–2 years, 2–5 years, 5–10 years and 10–≤15 years) with significance levels of 5 and 80% power (Supplemental Material). Prior to statistical analysis, outlying observations were removed in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI), as these extreme values potentially affect the estimation of reference intervals [26]. Outliers were identified by Tukey's method where values within the distribution that are either less than $Q1 - 1.5 \times IQR$ or greater than $Q3 + 1.5 \times IQR$, are defined as extreme values [23, 27]. Values of markers which were not normally distributed were log transformed before analysis. The primary analysis for the study was an analysis of variance (ANOVA) comparing URO concentrations of the age groups. As secondary endpoints, the relationship between each marker and age was investigated using Pearson's correlation coefficient. A linear regression model was fitted to determine associations between the individual markers and age. For the summary statistics, and in addition to ANOVA, the Kruskal-Wallis test was also used to determine if there were differences in (untransformed) kidney function and injury marker values between age groups. Sex and weight associations were assessed using standard ANOVA. In case of multiple associations between the marker and demographic characteristics, a multivariate linear regression model was fitted. When a linear regression model did not capture the trend of the data, more complex models such as nonlinear and piecewise regression models were investigated. The Akaike Information Criterion (AIC) was used to guide model selection. Subsequently, the predicted means and 95% prediction intervals of all markers were computed based on the fitted regression model to determine the reference intervals. A correlation matrix was used to visualize the correlation among the different markers. R (version 3.5.1; R Development Core Team, Vienna, Austria, http://r-project.org) was used for data analysis and visual graphics.

Results

One hundred and 58 children were enrolled of which 16 participants (10%) had to be excluded (withdrawn consent or no blood sampling prior to anesthesia, Supplemental Table 1). In total, reference intervals for eight kidney function and injury markers from 142 pediatric subjects were determined.

Study population

The demographic and clinical characteristics of study participants are shown in Table 2. Median weight and height per age group were 9.8 kg and 76 cm (0–2 years), 16.5 kg and 103 cm (>2–5 years), 23 kg and 125 cm (>5–10 years) and 50 kg and 157 cm (>10–≤15 years). 37.3% were female, 92.3% were Caucasian and 9.2% were born preterm (mean gestational age of 35 weeks). The distribution for types of surgery changed for specific age groups (Supplemental Table 2). In total, 20.4% of participants received medication or nutritional supplements in the 48 h before surgery, of these 37.9% were treated with vitamin D and 55.2% of participants received their last dose one day prior the surgery (Supplemental Table 3). Only 2% of participants were treated with drugs with potential nephrotoxic side effects (ibuprofen or isotretinoin, Supplemental Table 3).

Statistical analysis

The mean (SD) values of for each marker of the total studied pediatric population were; ALB 38.2 (4.9) g/L, B2M 1.7 (0.4) mg/L, BTP 0.7 (0.2) mg/L, CYSC 0.9 (0.2) mg/L, KIM-1 2460.1 (3163.6) ng/L, NGAL 40.0 (19.7) µg/L, SCR 39.5 (16.0) µmol/L, and URO 152.7 (75.9) µg/L (Figure 1).

For the primary analysis, no significant differences were observed when comparing URO values between the different age groups using ANOVA (Supplemental Table 4). There were similarly no differences for KIM-1 and NGAL, and these results were the same using the non-parametric Kruskall-Wallis test for the untransformed measurements (Table 3).

Table 2: Demographic characteristics of study participants. eGFR calculated by bedside Schwartz formula (0.41 × height (cm)/serum creatinine (mg/dL)) and body surface area calculated by Mosteller formula [5, 40].

	0–2 years (n=20)	2–5 years (n=22)	5–10 years (n=35)	10–15 years (n=65)	Total (n=142)
Age, years					
Mean (SD)	1.06 (0.5)	3.73 (0.9)	7.13 (1.4)	12.90 (1.8)	8.39 (4.8)
Median (Q1, Q3)	1.14 (0.8, 1.4)	3.92 (2.9, 4.5)	7.01 (6.1, 8.1)	12.93 (11.4, 14.2)	8.75 (4.5, 12.6)
Weight, kg					
Mean (SD)	9.4 (2.2)	16.6 (3.2)	25.1 (7)	51.8 (15.5)	33.8 (20.5)
Median (Q1, Q3)	9.8 (8.1, 11.2)	16.4 (14.8, 19.7)	23 (20, 26.9)	50 (40, 65)	27.5 (18, 48.8)
Height, cm					
Mean (SD)	75.3 (9.2)	103.6 (9.7)	125 (9.5)	156.9 (12.2)	128.4 (31.6)
Median (Q1, Q3)	75.5 (71.5, 83)	103 (97.2, 113)	125 (117, 129)	157 (149, 166)	133 (104, 155)
eGFR, mL/min/1.73m^2					
Mean (SD)	134.5 (19.4)	144.2 (40.1)	133.8 (22.8)	115.57 (33.2)	127.2 (32.4)
Median (Q1, Q3)	137.2 (120.4, 144.8)	138.3 (119.8, 151.4)	130.2 (122.1, 143.9)	110.8 (100.4, 124.5)	123.52 (108.2, 139.8)
eGFR, mL/min					
Mean (SD)	34.8 (9.5)	56.9 (13.9)	72.5 (18.7)	99.2 (34.1)	76.8 (34.8)
Median (Q1, Q3)	35.5 (28.4, 42.2)	56.2 (52.2, 65.3)	68.4 (61.7, 77.9)	96.1 (84.0, 110.4)	73.9 (54.6, 96.3)
Sex					
Female	1 (5%)	10 (45.5%)	15 (42.9%)	27 (41.5%)	53 (37.3%)
Male	19 (95%)	12 (54.5%)	20 (57.1%)	38 (58.5%)	89 (62.7%)
Ethnicity					
Caucasian	18 (90%)	21 (95.5%)	31 (88.6%)	61 (93.8%)	131 (92.3%)
Born preterm					
No	18 (90%)	20 (90.9%)	34 (97.1%)	57 (87.7%)	129 (90.8%)
Yes	2 (10%)	2 (9.1%)	1 (2.9%)	8 (12.3%)	13 (9.2%)
Medication 48 h prior surgery					
No	14 (70%)	17 (77.3%)	30 (85.7%)	52 (80.0%)	113 (79.6%)
Yes	6 (30%)	5 (22.7%)	5 (14.3%)	13 (20.0%)	29 (20.4%)

eGFR, estimated glomerular filtration rate.

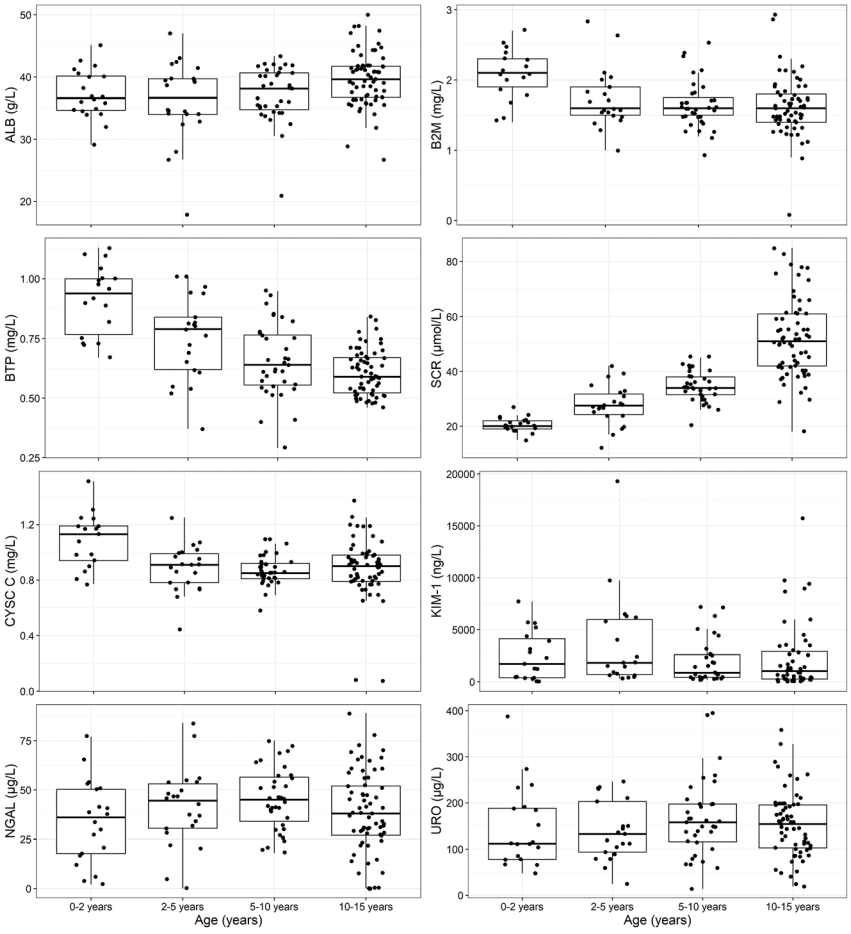

Figure 1: Indiviual serum biomarker values represented per age group.
Median values and interquartile ranges are presented by boxplots, and dots represent individual values for each biomarker. ALB: albumin, B2M: β_2-microglobulin, BTP: β-trace protein, SCR: creatinine, CYSC: cystatin C, KIM-1: kidney injury molecule-1, NGAL: neutrophil gelatinase-associated lipocalin, URO: uromodulin.

After excluding outlying observations according to Tukey's method (Supplemental Table 1) and log transforming SCR and KIM-1 due to non-normality, Pearson's correlation test was performed between the marker values and age as a continuous variable (Supplemental Table 5). The strongest positive correlation with age as a continuous variable (no categories) was observed for SCR ($r=0.864$ and $p<0.001$), and the strongest negative correlation for BTP ($r=-0.544$ and $p<0.001$). ALB showed a mild positive correlation ($r=0.261$ and $p<0.01$), and both B2M and KIM-1 showed a negative ($r=-0.276$ and $p<0.01$, $r=-0.217$ and $p<0.05$) correlation with continuous age, respectively. The other evaluated markers did not show any significant correlation with age as a continuous variable ($p>0.05$).

Five kidney function and injury markers ALB, B2M, BTP, SCR and KIM-1, showed an association with age (Figure 2). Based on the multivariable linear model (Supplemental Table 6), the mean and 95% prediction intervals for those five markers were calculated for four different age groups (Table 4). The predicted B2M concentrations from the fitted model (Eq. (1)) for a child of 2, 5, 10 or 15 years were 1.9 mg/L (95% CI (1.3–2.5)), 1.7 mg/L (95% CI (1.1–2.3)), 1.6 mg/L (95% CI (1.0–2.1)) or 1.7 mg/L (95% CI (1.1–2.3)), respectively (Figure 2). BTP showed a trend to decrease with age ($p<0.001$), and the predicted concentrations were estimated at 0.9 mg/L (95% CI (0.6–1.1)), 0.7 mg/L (95% CI (0.6–1.0)), 0.6 mg/L (95% CI (0.4–0.9)) or 0.6 mg/L (95% CI (0.4–0.9)) for a 2, 5, 10 or 15 year-old child (Eq. (2), Figure 2). SCR showed a strong positive association with age and increased from 22.7 µmol/L (95% CI (15.2–33.9)) for a 2-year old infant to 58.9 µmol/L (95% CI (39.4–88.1)) for a 15-year old adolescent (Eq. (3), Table 4). KIM-1 decreased with age ($p<0.05$), and showed a 50% decrease between a two-year-old child and a 15-year old adolescent (Table 4).

CYSC was significantly associated with sex, showing lower concentrations in female pediatric population as compared to male participants ($p<0.01$, Supplemental Table 6). Piecewise regression with a breakpoint at approximately 1.8 years was a better fit compared to linear regression for CYSC in terms of AIC criteria (Supplemental Table 6). In the period before 1.8 years, CYSC appears to show a decrease, and after this breakpoint a slight increase in CYSC is observed until the age of 15 years (Eq. (6a) and (6b)). Overall, girls show a lower CYSC concentration (median 0.86 mg/L (IQR 0.78–0.94)) compared to boys

Table 3: Values for eight kidney biomarkers (mean (SD), median (IQR) and minimum and maximum values). Group differences for untransformed measurements tested using Kruskal-Wallis test.

	0-2 years (n=20)	2-5 years (n=22)	5-10 years (n=35)	10-15 years (n=65)	Total (n=142)
Albumin, g/L					p-value 0.011
Mean (SD)	37.2 (3.9)	36.2 (6.4)	37.2 (4.4)	39.7 (4.4)	38.2 (4.9)
Median (Q1, Q3)	36.6 (34.7, 40.1)	36.7 (34.0, 39.7)	38.1 (34.8, 40.7)	39.6 (36.7, 41.7)	38.6 (35.3, 41.3)
Min – max	29.1–45.1	17.8–47.0	20.9–43.3	26.7–50.0	17.8–50.0
β_2-Microglobulin, mg/L					p-value <0.001
Mean (SD)	2.1 (0.4)	1.7 (0.4)	1.7 (0.3)	1.6 (0.4)	1.7 (0.4)
Median (Q1, Q3)	2.1 (1.9, 2.3)	1.6 (1.5, 1.9)	1.6 (1.50, 1.8)	1.6 (1.4, 1.8)	1.6 (1.5, 1.9)
Min – max	1.4–2.7	1.0–2.8	0.9–2.5	0.1–2.9	0.1–2.9
β-Trace protein, mg/L					p-value <0.001
Mean (SD)	0.9 (0.2)	0.8 (0.2)	0.7 (0.2)	0.6 (0.1)	0.7 (0.2)
Median (Q1, Q3)	0.9 (0.8, 1.0)	0.8 (0.6, 0.8)	0.6 (0.6, 0.8)	0.6 (0.5, 0. 7)	0.7 (0.6, 0.8)
Min – max	0.7–1.1	0.5–1.0	0.4–0.9	0.5–0.8	0.5–1.0
Creatinine enzymatic, µmol/L					p-value <0.001
Mean (SD)	20.5 (2.7)	27.8 (7.2)	34.6 (5.6)	51.9 (14.2)	39.5 (16.0)
Median (Q1, Q3)	20.0 (19.0, 22.0)	27.5 (24.3, 31.8)	34.0 (31.5, 38.0)	51.0 (42.0, 61.0)	37.5 (28.0, 50.0)
Min – max	15.0–27.0	12.0–42.0	20.0–45.0	18.0–85.0	12.0–85.0
Cystatin C, mg/L					p-value 0.002
Mean (SD)	1.1 (0.20)	0.9 (0.2)	0.9 (0.1)	0.9 (0.2)	0.9 (0.2)
Median (Q1, Q3)	1.1 (0.9, 1.2)	0.9 (0.8, 1.0)	0.8 (0.8, 0.9)	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)
Min – max	0.8–1.5	0.4–1.3	0.6–1.1	0.1–1.4	0.1–1.5
Kidney injury molecule-1, ng/L					p-value 0.225
Mean (SD)	2465.3 (2344.0)	3729.7 (4659.3)	1984.9 (2155.5)	2264.8 (3216.3)	2460.1 (3163.6)
Median (Q1, Q3)	1713.4 (401.9, 4125.0)	1824.6 (695.7, 5974.1)	871.2 (429.1, 2608.7)	1029.4 (271.6, 2901.8)	1228.9 (391.9, 3440.2)
Min – max	21.1–7710.7	319.9–19290.0	161.6–7169.0	24.4–15716.0	21.1–19290.0
NGAL, µg/L					p-value 0.198
Mean (SD)	34.0 (20.8)	41.2 (20.0)	45.4 (15.7)	38.6 (20.9)	40.0 (19.7)
Median (Q1, Q3)	36.0 (17.8, 50.3)	44.5 (30.5, 53.0)	45.0 (34.0, 56.5)	38.0 (27.0, 52.0)	41.0 (28.0, 53.8)
Min – max	2.0–77.0	0.0–84.0	18.0–75.0	0.0–89.0	0.0–89.0
Uromodulin, µg/L					p-value 0.494
Mean (SD)	143.4 (88.01)	139.8 (63.8)	166.2 (84.8)	152.4 (70.7)	152.7 (75.9)
Median (Q1, Q3)	111.9 (77.9, 188.3)	132.4 (93.5, 202.8)	157.7 (115.8, 197.4)	154.1 (102.6, 195.6)	147.5 (100.2, 196.7)
Min – max	47.4–387.4	24.6–246.1	13.4–394.7	18.7–358.1	13.4–394.7

NGAL, neutrophil gelatinase-associated lipocalin.

(median 0.92 mg/L (IQR 0.81–1.04, p=0.01)), with the predicted CYSC concentration from the fitted model at 1.5 years of age being 0.96 mg/L (95% CI (0.71–1.22)) for boys and 0.91 mg/L (95% CI (0.66–1.17)) for girls, respectively (Table 4).

$$\mathrm{B2M}_i\left[\frac{\mathrm{mg}}{\mathrm{L}}\right] = 2.11 - 0.114 \times \mathrm{age}_i[\text{in years}] + 0.00578 \times \mathrm{age}_i^2[\text{in years}] \quad (1)$$

$$\mathrm{BTP}_i\left[\frac{\mathrm{mg}}{\mathrm{L}}\right] = 0.958 - 0.0604 \times \mathrm{age}_i[\text{in years}] + 0.00252 \times age^2{}_i[\text{in years}] \quad (2)$$

$$\mathrm{SCR}_i\,[\mu\mathrm{mol/L}] = \exp(2.98 + 0.0735 \times \mathrm{age}_i[\text{in years}]) \quad (3)$$

$$\mathrm{KIM1}_i[\mathrm{ng/L}] = \exp(7.31 - 0.0633 \times \mathrm{age}_i[\text{in years}]) \quad (4)$$

$$\mathrm{ALB}_i[\mathrm{g/L}] = 36.4 + 0.236 \times \mathrm{age}_i[\text{in years}] \quad (5)$$

$$\mathrm{CYSC}_{i\,[<1.76\,\text{years}]}\left[\frac{\mathrm{mg}}{\mathrm{L}}\right] = -0.27 \times \mathrm{age}_i[\text{in years}] + 0.044 \times \mathrm{sex}_i \quad [0:\text{male},\ 1:\text{female}] \quad (6a)$$

$$\mathrm{CYSC}_{i\,[>1.76\,\text{years}]}\left[\frac{\mathrm{mg}}{\mathrm{L}}\right] = 0.003 \times \mathrm{age}_i[\text{in years}] + 0.044 \times \mathrm{sex}_i \quad [0:\text{male},\ 1:\text{female}] \quad (6b)$$

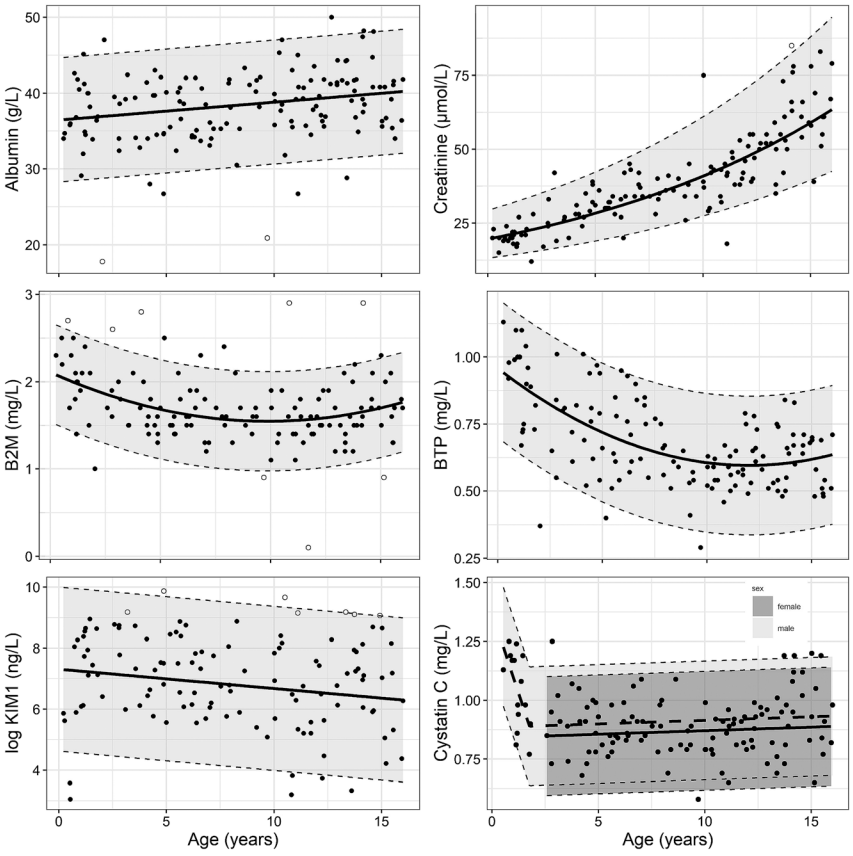

Figure 2: Prediction reference intervals for age dependent kidney biomarkers. Lines represent the predicted mean concentration and shaded areas illustrate the 95% prediction reference intervals. Filled circles represent values included in statistical analysis, open circles represent outlier values (not included in statistical analysis).

Both NGAL and URO did not show any significant association between age, sex or weight. Predicted mean and 95% reference interval for NGAL were 40.0 µg/L (95% CI (1.4–78.6)) for all ages. For URO, predicted mean and 95% reference interval were 145.9 µg/L (95% CI (17.4–274.4)) for all ages (Table 4).

We found a positive correlation between CYSC and ALB, B2M and BTP ($p<0.001$, Supplemental Figure S1). A positive correlation between SCR and ALB and CYSC was observed ($p<0.05$) and a negative correlation between SCR and KIM-1 and BTP was observed (Supplemental Figure S1). Positive correlation between BTP and B2M was detected ($p<0.01$). There were no significant correlations otherwise.

Discussion

This prospective study provides references intervals for eight endogenous serum kidney function and injury markers, reflecting normal kidney function in 142 healthy infants, children and adolescents. Six markers showed age-dependency (ALB, SCR, B2M, BTP, CYSC and KIM-1), whereas the other two markers (NGAL and URO) did not show any association with age.

In terms of defining reference intervals for the whole pediatric age range (0–≤15 years), the sample size for each marker, except KIM-1, is in accordance with the recommendations of the CLSI, which recommends a minimum of 120 individuals [23, 26]. No relevant nephrotoxic drug exposure was reported, only 2% of participants were exposed to medication (ibuprofen, isotretinoin) potentially associated with renal side effects. Ibuprofen was discontinued the day before surgery, and as the half-life of ibuprofen is approximately 2 h, near complete elimination of ibuprofen can be assumed after 8–10 h [28]. SCR concentrations were comparable with previous published reference values for healthy pediatric populations [24, 25]. The calculated median SCR values according to the reported age groups did not differ by more than 20% (8% on average) with the 2.5 and 97.5% percentiles from data presented in previous studies (Supplemental Table 7) [24, 25]. Our study population represents a healthy pediatric population, as we have applied strict inclusion and exclusion criteria. This is in contrast to previous studies, where subgroups of a diseased patient group serve as controls and do not guarantee the absence of other conditions influencing kidney function. Establishing reference intervals of kidney function and injury markers in a healthy pediatric population is a necessary component for

Table 4: Predicted mean values and 95% confidence intervals based on the fitted linear or piecewise regression models for eight kidney biomarkers, with age treated as a continuous variable.

	2 years[a]	5 years	10 years	15 years	All ages (0–15 years)
Age dependent kidney biomarkers					
ALB, g/L	36.9 [28.7–45.1]	37.6 [29.4–45.8]	38.8 [30.6–47.0]	40.0 [31.8–48.2]	na
B2M, mg/L	1.9 [1.3–2.5]	1.7 [1.1–2.3]	1.6 [1.0–2.1]	1.7 [1.1–2.3]	na
BTP, mg/L	0.9 [0.6–1.1]	0.7 [0.5–1.0]	0.6 [0.4–0.9]	0.6 [0.4–0.9]	na
SCR, µmol/L	22.7 [15.2–33.9]	28.3 [18.9–42.3]	40.8 [27.3–61.0]	58.9 [39.4–88.1]	na
KIM-1, ng/L	1319.4 [88.4–19684]	1091.2 [73.1–16278]	795.1 [53.3–11861]	579.3 [38.8–8642]	na
CYSC, mg/L					
Boys	1.06 [0.71–1.22]	0.89 [0.64––1.16]	0.91 [0.65–1.17]	0.93 [0.67–1.19]	na
Girls	0.91 [0.66–1.17]	0.85 [0.60–1.11]	0.87 [0.61–1.13]	0.89 [0.63–1.14]	na
Age independent kidney biomarkers					
NGAL, µg/L	na	na	na	na	40.0 [1.4–78.6]
URO, µg/L	na	na	na	na	146.0 [17.4–274.4]

na, not applicable; ALB, albuim; B2M, β_2-microglobulin; BTP, β-trace protein; SCR, serum creatinine; KIM-1, kidney injury molecule-1; CYSC, cystatin C; NGAL, neutrophil gelatinase-associated lipocalin; URO, uromodulin. [a]For cystatin C, a breakpoint of 1.5 years was used.

demonstrating the clinical utility of these markers and will allow their interpretation in ill pediatric patients. Furthermore, the use of several markers in addition to SCR, ALB and eGFR in clinical routine will support the interpretation of physiological and pathological conditions.

B2M concentrations appeared to be higher for infants (1.9 mg/L [1.3–2.5]) and remain constant in children and adolescents. B2M concentrations are strongly associated with cell turnover, which is a physiological state in early infancy, as well as various infectious and hematological diseases [3, 22]. During infectious diseases an increase in B2M concentrations is observed [29, 30]. BTP) shows a similar profile with highest concentrations in infants (0.9 mg/L [0.6–1.1]). Serum BTP is a small molecular weight protein which is freely filtered through the glomerulus. It has been previously shown that serum BTP concentrations in pediatric patients with impaired kidney function (eGFR <90 mL/min/1.73 m^2) are decreased (mean of 0.68 mg/L) [31]. In this study, a small, although statistically significant, positive relationship between ALB concentrations and age has been observed. This ALB increase with age has also been illustrated by several other studies [32, 33]. Clinical significance of this age-dependency is unclear, given that the 95% predicted reference intervals of ALB for our cohort were similar across all age groups. Our results confirmed the age-dependence of serum SCR concentrations [24, 25]. After the first couple of months of life, SCR concentrations increase as a result of increasing muscle mass. KIM-1 is removed from tubular epithelial cells into the urine in response to injury; therefore an increase in urinary KIM-1 concentrations could indicate kidney damage [34]. In this study, a small, although statistically significant, negative association of KIM-1 with age was detected. Urinary KIM-1 concentrations in healthy pediatric populations has been shown to increase with age (3.8%/year), although up to now, no published data could be found for serum KIM-1 concentrations in healthy pediatric population. This study showed that CYSC concentrations decrease until the age of approximately 2 years is reached, after which CYSC concentrations showed an increase until adolescence. Previous studies showed a similar inverse age correlation with CYSC during the first year of life and a lower CYSC concentration in pre-pubertal children (4–12 year) as compared to adolescents (12–17 years) [35]. In a previous study, significant differences have also been observed between sexes, with generally lower concentrations for girls [35]. Whether these discrepancies between sexes are of clinical relevance, remains to be investigated. The predicted reference intervals for serum NGAL and serum URO were found to be independent of age in our study. In healthy individuals, serum NGAL is expressed at low concentrations, filtered by the glomerulus and reabsorbed by the proximal tubule [34]. Pediatric patients with acute kidney injury showed an increase in serum NGAL concentrations (median of 355 µg/L) [20]. Our data showed significant correlations between some, but not all kidney markers (Figure S1). This suggests that partly different information on renal function may be obtained, although this needs further investigation and might be of particular interest in diseased populations.

There are several limitations to this study. An association between CYSC concentrations and sex was observed over the entire study population, but it needs to be acknowledged that the female population was underrepresented in the youngest age group. We emphasize that a study

with a larger sample size is required to draw more definitive conclusions on this potential sex effect. Furthermore, due to the rapid physiological changes between neonatal age and infanthood due to maturation, the sample size in the youngest age group with a relatively wide age range from 0 to 2 years might not be large enough to detect age-associated changes within this particular group. While our study examines kidney markers across a broad age range, others have focused their data on infants younger than 2 months of age and found similar results [19, 21]. Additionally, we recognize that prediction interval point estimates of the different age groups overlap for all markers. Studies with larger sample sizes are required to confirm our results. It is important to distinguish between statistically significant and clinically relevant results. For instance, the effect of age on albumin concentrations, which was statistically significant, can be questioned in terms of clinical relevance. Several biomarkers in the pediatric population (e.g. hormone levels or whole blood values, such as hemoglobin) show age-dependency, whereas other laboratory parameters (e.g. C-reactive protein or erythrocyte sedimentation rate) do not show an age effect. Our findings are relevant for pediatric health care providers for the interpretation of kidney function and injury markers to help to distinguish between physiological effects or pathological states.

In conclusion, this study provides age appropriate reference intervals for eight kidney function and injury markers in healthy pediatric population from 2 months until 15 years. In addition to the more commonly used markers such as ALB and SCR; B2M, BTP and KIM-1 show a decrease with age in the healthy pediatric population. CYSC was lower in girls, decreased with age until approximately two years of age and increased thereafter. For healthy pediatric infants, children and adolescents, no significant age-dependency could be identified for NGAL and URO. These results highlight the importance for further evaluation of these kidney function and injury markers in early detection, and enhanced monitoring of kidney injury related to underlying diseases or nephrotoxic drugs in pediatric patients.

Acknowledgments: We like to thank all patients and parents, physicians, nurses and other medical staff from the short stay units and the hospital wards together with the Departments of Surgery, Anesthesia, ENT and Orthopedics for facilitating blood sample collections and participant recruitment. Thomas Erlanger (Clinical Trial Unit, Department of Clinical Research) is acknowledged for his assistance concerning the sample size calculation. Additionally, we want to thank Mrs. Hillmann for her assistance regarding the lab measurements. We also like to thank the study nurses from the Ambulatory Study Center for their assistance with participant recruitment and sample preparation.

Research funding: This project has been supported by the Eckenstein-Geigy Foundation and was part of the national SwissPedNet and SwissPedPha consortia. This funding organizations played no role in the study design; in the collection, analysis and interpretation of the data or in the writing of the manuscript.

Author contributions: TVD, ES, JVDA, TW and MP conceived and designed the study. TVD and TW collected the data and were responsible for data management. LR provided the means for the analysis for the samples. TVD, VG and AA performed the statistical analysis. TVD, ES, VG, JVDA, TW and MP contributed to the interpretation of the results. TVD and TW wrote the draft of the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Study protocol was approved by the local Ethics Committee (EKNZ BASEC 2016-00884).

References

1. van den Anker JN, Schwab M, Kearns GL. Developmental pharmacokinetics. Handb Exp Pharmacol 2011;205:51–75.
2. van Donge T, Welzel T, Atkinson A, van den Anker J, Pfister M. Age-dependent changes of kidney injury biomarkers in pediatrics. J Clin Pharmacol 2019;59:S21–32.
3. den Bakker E, Gemke RJ, Bökenkamp A. Endogenous markers for kidney function in children: a review. Crit Rev Cl Lab Sci 2018;55: 163–83.
4. van den Anker JN, de Groot R, Broerse HM, Sauer PJ, van der Heijden BJ, Hop WC, et al. Assessment of glomerular filtration rate in preterm infants by serum creatinine: comparison with inulin clearance. Pediatrics 1995;96:1156–8.
5. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate gfr in children with ckd. Clin J Am Soc Nephrol 2009;20:629–37.
6. Schwartz GJ, Haycock GB, Edelmann CM, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 1976;58:259–63.
7. Hilderink JM, van der Linden N, Kimenai DM, Litjens EJ, Klinkenberg LJ, Aref BM, et al. Biological variation of creatinine, cystatin c, and egfr over 24 hours. Clin Chem 2018;64:851–60.
8. Sharbaf FG, Farhangi H, Assadi F. Prevention of chemotherapy-induced nephrotoxicity in children with cancer. Int J Prev Med 2017; 8:76.
9. Donadio C, Bozzoli L. Urinary β-trace protein: a unique biomarker to screen early glomerular filtration rate impairment. Medicine 2016;95:e5553.

10. Risch L, Lhotta K, Meier D, Medina-Escobar P, Nydegger UE, Risch M. The serum uromodulin level is associated with kidney function. Clin Chem Lab Med 2014;52:1755–61.
11. Rindler MJ, Naik S, Li N, Hoops T, Peraldi M. Uromodulin (tamm-horsfall glycoprotein/uromucoid) is a phosphatidylinositol-linked membrane protein. J Biol 1990;265:20784–9.
12. Saeidi B, Koralkar R, Griffin RL, Halloran B, Ambalavanan N, Askenazi DJ. Impact of gestational age, sex, and postnatal age on urine biomarkers in premature neonates. Pediatr Nephrol 2015; 30:2037–44.
13. Armangil D, Yurdakök M, Canpolat FE, Korkmaz A, Yiğit Ş, Tekinalp G. Determination of reference values for plasma cystatin c and comparison with creatinine in premature infants. Pediatr Nephrol 2008;23:2081.
14. Finney H, Newman D, Thakkar H, Fell J, Price C. Reference ranges for plasma cystatin c and creatinine measurements in premature infants, neonates, and older children. Arch Dis Child 2000;82: 71–5.
15. Kornhauser C, Dubey L-A, Garay M-E, Pérez-Luque E-L, Malacara J-M, Vargas-Origel A. Serum and urinary insulin-like growth factor-1 and tumor necrosis factor in neonates with and without acute renal failure. Pediatr Nephrol 2002;17:332–6.
16. Bennett MR, Nehus E, Haffner C, Ma Q, Devarajan P. Pediatric reference ranges for acute kidney injury biomarkers. Pediatr Nephrol 2015;30:677–85.
17. Cangemi G, Storti S, Cantinotti M, Fortunato A, Emdin M, Bruschettini M, et al. Reference values for urinary neutrophil gelatinase-associated lipocalin (ngal) in pediatric age measured with a fully automated chemiluminescent platform. Clin Chem Lab Med 2013;51:1101–5.
18. McWilliam SJ, Antoine DJ, Sabbisetti V, Pearce RE, Jorgensen AL, Lin Y, et al. Reference intervals for urinary renal injury biomarkers kim-1 and ngal in healthy children. Biomark Med 2014;8: 1189–97.
19. Zwiers AJ, de Wildt SN, de Rijke YB, Willemsen SP, Abdullahi NS, Tibboel D, et al. Reference intervals for renal injury biomarkers neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 in young infants. Clin Chem Lab Med 2015;53: 1279–89.
20. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N, et al. Serum neutrophil gelatinase-associated lipocalin (ngal) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 2008;36:1297.
21. Zwiers AJ, Cransberg K, de Rijke YB, Willemsen SP, Amerik C, Tibboel D, et al. Reference ranges for serum β-trace protein in neonates and children younger than 1 year of age. Clin Chem Lab Med 2014;52:1815–21.
22. Ikezumi Y, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, et al. Establishment of a normal reference value for serum β_2 microglobulin in Japanese children: reevaluation of its clinical usefulness. Clin Exp Nephrol 2013;17:99–105.
23. Shaw JLV, Binesh Marvasti T, Colantonio D, Adeli K. Pediatric reference intervals: challenges and recent initiatives. Crit Rev Cl Lab Sci 2013;50:37–50.
24. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, et al. Age, gender, and body length effects on reference serum creatinine levels determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol 2011;15:694–9.
25. Schlebusch H, Liappis N, Kalina E, Klein C. High sensitive crp and creatinine: reference intervals from infancy to childhood: hochsensitives crp und kreatinin: referenzbereiche für neugeborene und kinder. Laboratoriums Medizin 2002;26: 341–6.
26. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline, 3rd ed. Clinical Laboratory Standards Institute Document C28-A3; 2008.
27. Tukey JW. Exploratory data analysis. Reading, MA: Adison-Wesley; 1977.
28. Rainsford KD. Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology 2009;17:275–342.
29. Bataille R, Durie BG, Grenierj J. Serum beta2 microglobulin and survival duration in multiple myeloma: a simple reliable marker for staging. Br J Haematol 1983;55:439–47.
30. Li L, Dong M, Wang X-G. The implication and significance of beta 2 microglobulin: a conservative multifunctional regulator. Chin Med J 2016;129:448.
31. Filler G, Priem F, Lepage N, Sinha P, Vollmer I, Clark H, et al. B-trace protein, cystatin c, β2-microglobulin, and creatinine compared for detecting impaired glomerular filtration rates in children. Clin Chem 2002;48:729–36.
32. Rödöö P, Ridefelt P, Aldrimer M, Niklasson F, Gustafsson J, Hellberg D. Population-based pediatric reference intervals for hba1c, bilirubin, albumin, crp, myoglobin and serum enzymes. Scan J Clin Lab Inv 2013;73:361–7.
33. Weaving G, Batstone GF, Jones RG. Age and sex variation in serum albumin concentration: an observational study. Ann Clin Biochem 2016;53:106–11.
34. Aulbach A, Amuzie C. A comprehensive guide to toxicology in nonclinical drug development. Academic Press, Elsevier; 2017. pp. 447–71.
35. Marmarinos A, Garoufi A, Panagoulia A, Dimou S, Drakatos A, Paraskakis I, et al. Cystatin-c levels in healthy children and adolescents: influence of age, gender, body mass index and blood pressure. Clin Biochem 2016;49:150–3.
36. Argyropoulos CP, Chen SS, Ng Y-H, Roumelioti M-E, Shaffi K, Singh PP, et al. Rediscovering beta-2 microglobulin as a biomarker across the spectrum of kidney diseases. Front Med 2017;4:73.
37. Donadio C, Lucchesi A, Ardini M, Donadio E, Giordani R. Serum levels of beta-trace protein and glomerular filtration rate—preliminary results. J Pharm Biomed 2003;32: 1099–104.
38. Kjeldsen L, Johnsen AH, Sengeløv H, Borregaard N. Isolation and primary structure of ngal, a novel protein associated with human neutrophil gelatinase. J Biol 1993;268:10425–32.
39. Rampoldi L, Scolari F, Amoroso A, Ghiggeri G, Devuyst O. The rediscovery of uromodulin (tamm–horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease. Kidney Int 2011;80:338–47.
40. Mosteller R. Simplified calculation of body-surface area. N Engl J Med 1987;317:1098.

Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2020-0781).