

Cystatin C serum levels in healthy children are related to age, gender and pubertal stage

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Niels Ziegelasch

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Leipzig

Betreuung:

Prof. Dr. med. Wieland Kiess

Dr. med. Katalin Dittrich

Dr. Mandy Vogel

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*Dedicated with great gratitude to my mother with all her affectionate care
and my father for his anticipation on my way.*

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2 Introduction

GFR measurement

Measuring renal function in pediatric patients is a necessity due to numerous infectious and non-infectious diseases that may result in renal dysfunction. As many therapies include pharmaceuticals with renal elimination, the renal function must also be evaluated for the dosage of these pharmaceuticals. Thus overall, a reliable as well as simple and fast renal diagnostic is of great value for a general practitioner as well as specialists in pediatric nephrology, cardiology or oncology among others.

The glomerular filtration rate (GFR) serves as a reliable parameter of the renal function. It is measured in milliliters of blood plasma filtered by the glomeruli per minute and adjusted to the body surface area (ml/min/1.73m²). The GFR may be evaluated by the urinary clearance of a substance “x” and is measured in [ml/min].¹ It indicates the plasma volume that is theoretically cleared from a certain substance “x” in one minute and is estimated by the functional relationship:

$$C_x = U_x \cdot V / P_x$$

where C_x is the clearance, U_x the concentration of x in the urine, V the milliliters of urine per minute, and P_x the concentration of x in the plasma.

Technically advanced methods

The inulin clearance is one gold standard established in renal diagnostic. Inulin is freely filtered in the glomeruli and undergoes neither tubular nor extra-renal secretion.² However, current recommendations suggest three sampling times, the first one no earlier than 90 minutes after inulin infusion. Especially in patients with renal function impairment, a delayed sampling is necessary, as precipitate samples will lead to GFR overestimation.^{3,4} Thus, this diagnostic is very time-consuming, impractical especially in clinical settings, and it strains the patience particularly of small children.

An equivalent alternative is the measurement of GFR using radioisotopes. Renal function is evaluated through imagery diagnostics. Radiation exposure limits the repetitive use of this method.³

Furthermore, radioisotopes are bound by plasma proteins leading to GFR underestimation of about ten percent compared to inulin.⁵

Finally, computer tomography may be used to determine renal function. The administration of the iod-compound iothalamate only requires one injection and no further blood nor urine samples. Nevertheless, radiation once more limits this method. Additionally, iothalamate undergoes tubular secretion leading to an overestimation of GFR.⁶

Serum creatinine

Methods using this endogenous marker are standards in daily renal diagnostics. Besides the MDRD formula used particularly in adults, the Schwartz formula is most often applied to pediatric patients. It considers the serum concentration of creatinine (sCrea) as well as the body height.⁷ The height is taken into account because of the inter- and intra-patient variability of sCrea related to varying body stature and muscle mass.⁸

$$\text{GFR} = 0.413 \times \text{height/sCrea(mg/dl)}^9$$

To make up for the gain of muscle mass in puberty, equations including gender-depending factors during puberty are necessary. Additionally, several GFR equations consider the body surface area as denominator to diminish that effect.¹⁰ Furthermore, in patients with spina bifida, neuromuscular diseases, liver cirrhosis or anorexia nervosa sCrea is unable to be used as the muscle mass is decreased (especially in patients bound to a wheelchair).¹¹ Besides its glomerular filtration, creatinine is also secreted by the proximal tubules, leading to an over-estimation of the GFR, especially in patients with mild renal impairment.¹²

Serum Cystatin C

A promising new endogenous marker for renal function is Cystatin C (CysC), a cysteine protease inhibitor and low molecular weight protein.¹³ As a product of a housekeeping gene, it is produced by all human nucleated cells at a stable production rate.¹⁴ Therefore, CysC seems to be unaffected by body muscle mass and growth. CysC is freely filtered in the glomeruli and shows 94-99% of the plasma clearance measured by the GFR-marker ⁵¹Cr-EDTA.^{15,16} At least 99% of the glomerular filtered CysC is then metabolized by tubular cells, resulting in less than 0.5% of the filtered CysC

appearing in the urine.¹⁶ More features of CysC including the physicochemical properties were reviewed by Filler, Bokenkamp et al.¹: Its molecular mass accounts to 13,343 Da, although 50% of the CysC proteins carry an additional hydroxylated proline residue adding to the molecular mass of then totally 13,359 Da. Its isoelectric point is 9.3; therefore it is positively charged in almost all body fluids. CysC levels are measured using the rapid and precise particle-enhanced turbidimetric or nephelometric immunoassays (PETIA and PENIA).^{17,18}

In adults, CysC is already well established in renal diagnostic routine. It generally correlates higher with gold standard methods compared to sCrea: a systematic review in 27 population groups (n=2 007)¹⁹ as well as a meta-analysis of 46 articles published until 2001 show that overall CysC is superior to sCrea.²⁰ Galteau et al. (n= 1 223) showed that CysC levels are 0.74 ± 0.100 mg/l for males and 0.65 ± 0.085 mg/l for females aged 20–59 years, independent from intake of contraceptives, menopause or hormonal replacement therapy.²¹ In individuals aged 60 years or older, CysC reference values were estimated as 0.83 ± 0.103 mg/l. Those results were confirmed on an international conference held in 2002.¹

Current state of research

Reference values of Cystatin C

In 2009, Andersen et al. reviewed several studies and compared CysC levels resulting from different applied methods. One year later Andersen stated that CysC was independent of age (n=30, age range 2-14 years, p=0.11).²² Ridefelt et al. were able to evaluate the data of a larger group of children (n=692). Using the Abbott Architect ci8200, they proposed reference intervals of 0.77-1.09 for 6-12-month-old and 0.63-1.08 for 1-18-year-old children.²³

Overall, newborns have higher CysC levels. Various studies confirmed physiological concentrations of 1.70 ± 0.26 mg/l (PETIA) or 1.97 ± 0.36 mg/l (PENIA).²⁴⁻²⁷ Pre-term infants have higher CysC concentrations of 1.88 mg/l ± 0.36 mg/l (PETIA).²⁸ The concentrations significantly decrease two days after birth (1.61 ± 0.37 mg/L on day 3)²⁹ and are neither associated with gestational age, birth weight nor maternal renal function.^{26,29,30} So far, most studies showed that CysC concentrations reach steady levels after one to three years of life.^{25-27,28} CysC levels of 0.72 ± 0.12 mg/L (PENIA) or 0.98 ± 0.20 mg/l (PETIA) respectively were reported independent from age and gender.^{25,28} It remained constant up to the age of 14-16 years, according to some studies even up to adulthood.²⁵ Most studies found no effect of gender or age during childhood and adolescence.^{25,26,31} Furthermore, Galteau et al. could not find an association to the hormonal status in women or alcohol intake.²¹

Nevertheless, Yata et al. were able to collect data from a much larger group of 1128 children. They could show that CysC levels decreased at the age of 15-16 years and were higher in males compared to females at that same age (see Figure 1 and Figure 2 below).²⁷ Groesbeck et al. confirmed that especially in females aged 12-19 years, cystatin C levels decrease (n=719).³²

Therefore, we assume that age and gender affect CysC levels.

Cystatin C in healthy test persons

In contrast to serum Creatinine (sCrea), neonatal CysC concentrations are independent from maternal blood levels^{30,33} and inversely correlate with the GFR of gold standard methods three days after birth. Both seem to be unaffected by sex, gestational age, birth weight, bilirubin levels and hydration state, but are associated with cord blood pH and hemoglobin level.³³ Bokenkamp et al. collected data of 258 children without evidence of kidney disease (age range one day to 18 years). They support the thesis that CysC remains constant after the first year of life, while sCrea steadily increases until adulthood due to the gain of muscle mass.³⁴ Comparing sCrea and CysC, the reciprocal of sCrea correlated less with the inulin clearance as the gold standard ($r=0.72$) than CysC ($r=0.88$). Furthermore, the body height was a covariate for sCrea. Further, female gender as well as dystrophy were linked to an underestimation of GFR.³⁵

Validity of Cystatin C for kidney diseases

In patients with renal diseases, CysC increases the diagnostic sensitivity in contrast to sCrea.³⁶ Furthermore, when correlated with the creatinine clearance, CysC shows a stronger correlation coefficient ($r=0.64$) than sCrea ($r=0.55$), but still a lower correlation coefficient than the height/creatinine ratio ($r=0.73$).³⁷ Another study concluded that the creatinine clearance seems to be the best estimate of GFR, followed by the Haycock-Schwartz formula followed by CysC and finally sCrea (all significantly different from one another).³⁸ Due to the changing muscle mass during growth and development, in pediatric patients, the inpatient variability of sCrea is significantly higher when compared to CysC.^{39,40} Besides, sCrea tends to overestimate renal function most notably in females as well as in patients with dystrophy or glomerular diseases.^{12,35} In order to detect especially initial renal failure, CysC is the more sensitive parameter with a furthermore higher negative predictive value.⁴¹ Overall, this suggests supremacy of CysC over sCrea due to its higher diagnostic accuracy.

CysC is also superior to Creatinine in examining specific kidney diseases. Mussap et al. measured amniotic fluid of 95 pregnant women. They found out that CysC levels were elevated in women with uropathic fetuses, whereas Creatinine levels showed no significant difference when compared to women with healthy fetuses.⁴² In infants with bilateral kidney hypoplasia and cystic dysplasia, both

cystatin C as well as β 2-microglobulin were significantly elevated.⁴³ Because of small sample sizes, data on pediatric patients with polycystic kidney diseases are nonconclusive and controversial.⁴³

Post-transplant validity of Cystatin C

Several studies reviewed the diagnostic value of CysC in post-transplant pediatric patients. When correlated with the iothalamate-GFR as the gold standard, CysC showed a higher correlation coefficient in post-transplant patients ($r=0.83$) than sCrea ($r=0.67$) or the creatinine clearance ($r=0.57$). Nevertheless, its diagnostic accuracy was the same as that of the creatinine clearance. At a cut-off value of 1.64 mg/l Risch et al. calculated a positive predictive value of 93%, a specificity of 89% and a sensitivity of 70%. Only the sensitivity of the creatinine clearance scored higher with 85%.^{44,45} However, Aufricht et al. were not able to confirm CysC as the superior marker.⁴⁶ Research in adult post-transplant patients may help to end this controversy: compared to radiolabeled DTPA measuring as the gold standard, CysC equations (especially the equations of Filler and Le Bricon) showed less bias and highest accuracy in contrast to sCrea equations. That effect remained the same in patients with GFR > 60ml per minute per 1.73m² (87-89% within 30% of measured GFR) whereas only 53-80% of Creatinine estimates were within 30% of measured GFR.⁴⁷

Overall, considering the high standard deviation of the creatinine clearance of 17.8%, CysC as well as CysC-based equations (currently only validated for a GFR between 15-75 ml/min/1.73m²) seem to be more suitable markers in post-transplant pediatric patients.⁴⁸

Validity of Cystatin C in extra-renal diseases

In diabetic patients with a stable metabolic status, CysC was significantly decreased compared to patients with ketonuria and the superior marker over sCrea when compared to the iothalamate clearance. The creatinine clearance was furthermore an unreliable marker of GFR.⁴⁹ CysC was also the most accurate diagnostic marker in patients with renal diseases as a result of uropathy due to a spina bifida ($r=0.45$), whereas sCrea and the Schwartz formula showed no correlation with GFR.¹¹ A similar effect was found in pediatric cancer patients, where the diagnostic value of CysC was superior over that of sCrea.⁵⁰

GFR-equations

In 1976, the first Schwartz equation considered only body height and sCrea (**$0.55 \times \text{Length} / \text{sCrea}$**), but is still the most commonly used formula in clinical practice with a single modification in 2009 ($\text{GFR} = 0.413 \times \text{height} / \text{sCrea}(\text{mg/dl})$).^{7,9} It showed an excellent agreement with creatinine ($r=0.935$) and inulin clearance ($r=0.905$).⁷ The four most common CysC equations were developed by Filler, Grubb, Zappitelli and Schwartz.

Besides CysC concentration, Grubb included a factor for prepubertal children (younger than 14 years). The equation assessed the GFR at least as well as the creatinine-based Schwartz formula. Its prediction performance was apart from the factor for prepubertal children unaffected by age.⁵¹

Grubb: $\text{GFR} = 84.69 \times [\text{CysC}]^{1.680} \times 1.384(\text{if a child } < 14 \text{ years})$

Filler et Lepage compared a solely on CysC based equation with the creatinine-based Schwartz formula (in contrast to ^{99m}Tc DTPA as gold standard). The children (aged 1–18 years, $n=536$) tested had various renal pathologies. The Schwartz formula tended to overestimate the GFR. That effect was not found in the CysC-based equation, not even in patients with a lower GFR.⁵² Two years later, Filler et al. confirmed in a similar study design that CysC-based equations resulted in the least error.⁵³

Filler: $\log(\text{GFR}) = 1.962 + [1.123 \times \log(1/\text{CysC})]$

Zappitelli derived two CysC equations (thereof one combined with sCrea) and compared them to iothalamate as the gold standard. Both equations were superior over the Schwartz formula and showed less bias and greater precision in transplant patients due to a transplant-factor included in each formula.⁵⁴

Zappitelli 1: $\text{GFR} = 75.94 / [\text{CysC}]^{1.17} \times 1.2(\text{if renal transplant})$

Zappitelli 2: $\text{GFR} = (507.76 \times e^{0.003 \times \text{height}}) / (\text{CysC}^{0.635} \times \text{sCrea}^{0.547}) \times 1.165(\text{if renal transplant})$

Not only Zappitelli, but also Schwartz tried to combine CysC and sCrea in one formula to achieve better diagnostic accuracy. The equation based on turbidimetrically measured CysC (2009) yielded to 87.7% within 30% of the iothalamate GFR whereas the second one based on nephelometrically measured CysC (2012) yielded to 91% within 30% of iGFR.^{9,55} Both formulas include blood urea

nitrogen (BUN) as an additional nephrodiagnostic parameter and are the only equations validated for pediatric patients with a GFR between 15 and 75 ml/min/1.73m².

Schwartz (turbidimetric): $GFR = 39.1 \times (\text{height}/\text{Scr})^{0.516} \times (1.8/\text{CysC})^{0.294} \times (30/\text{BUN})^{0.169}$
 $\times 1.099$ (if male) $\times (\text{height}/1.4)^{0.188}$

Schwartz (nephelometric): $GFR = 39.8 \times (\text{height}/\text{Scr})^{0.456} \times (1.8/\text{CysC})^{0.418} \times (30/\text{BUN})^{0.079}$
 $\times 1.076$ (if male) $\times (\text{height}/1.4)^{0.179}$

Finally, Andersen et al. established and validated a formula based on CysC, sCrea as well as body cell mass (BCM) and body surface area (BSA). They assumed that concentration of CysC depends on BCM as it is produced by all nucleated cells. Indeed, the formula's predictive value is higher than those of any other equation (98% within $\pm 30\%$ of GFR and 66% within $\pm 10\%$). Furthermore, the weight-based (instead of BCM) equation performed almost as well.⁵⁶

Andersen (BCM-based): $GFR = 0.542 \times (\text{BCM}/\text{CysC})^{0.40} \times (\text{height} \times \text{BSA}/\text{sCrea})^{0.65}$

Andersen (weight-based): $GFR = 0.426 \times (\text{weight}/\text{CysC})^{0.39} \times (\text{height} \times \text{BSA}/\text{sCrea})^{0.64}$

Confounders

A large cross-sectional analysis of 3418 adults including clinical trial participants as well as patients with chronic kidney diseases compared CysC and sCrea levels with the urinary clearance of iothalamate and creatinine. CysC levels were 4.3% lower for every 20 years of age and 9.2% lower in females. The impact of age and gender were even higher on sCrea than on CysC (Figure 3).⁵⁷ Another large study with more than 8000 adults showed that age, gender, weight and height as well as current cigarette smoking were solely associated with CysC levels.⁵⁸ No similar associations have been found specifically in children yet.

The hormone balance seems to be linked to CysC serum concentration. Diabetes, for example, is associated with ~8.5% higher CysC concentrations, whereas sCrea has ~3.9% lower levels (Figure 3).⁵⁷ Besides, CysC levels were significantly elevated in children treated with glucocorticoids for malignancy or renal disease, although levels of other low molecular weight proteins were more affected.⁵⁹ That may lead to an underestimation of GFR in for example renal transplant patients

treated with glucocorticoids.⁶⁰ Additionally, in patients with thyroid dysfunction, CysC levels moderately but significantly raise along with thyroxin blood levels.⁶¹

Finally, patients with proteinuria seem to have elevated urinary levels of CysC.⁶² Higher levels of CRP and white blood cells as well as lower serum albumin concentrations are associated with elevated concentrations of CysC, but lower levels of sCrea.^{58,59,63} After adjustment for creatinine clearance, CysC was furthermore shown to be higher with older age, male gender, higher weight, greater height, and current cigarette smoking (n=8058 adults aged 28-75 years).⁵⁸

Relevance of the topic

Urinary and renal infections are very frequent diseases.⁶⁴ Besides, the overall prevalence of renal anomalies accounts up to 0.1%.⁶⁵ This already points out the importance of reliable as well as fast and convenient renal diagnostic methods.

sCrea partly satisfies these requirements, but additional information such as height need to be considered (as mentioned above). When compared to for example iothalamate clearance as the gold standard, sCrea and the Schwartz formula overestimate GFR, especially in renally impaired patients.⁶⁶ Furthermore, sCrea is affected by gender, malnutrition and other diseases such as lupus erythematoses, dystrophy, spina bifida and neuromuscular diseases, and correlates with the gestational age of newborns.^{11,35,67–69}

Besides, children with renal dysfunctions, the therapy of pediatric patients suffering from cardiac diseases and cancer requires a repetitive evaluation of GFR. As many pharmaceuticals are renally eliminated, their dosage needs to be adjusted to renal functioning. Thus, accurate markers of GFR are needed.⁷⁰

CysC appears to be independent of gender, birth weight, and gestational age.⁶⁷ Several studies already tried to analyze the diagnostic accuracy of CysC and different CysC-based GFR equations, but they also proposed further research in large multicenter studies.^{26,71} To current knowledge, as the product of a housekeeping gene, CysC levels are affected by only a few confounders, e.g., high levels of glucocorticoids.¹⁴ Still, even in transplant patients with glucocorticoid therapy, studies showed that CysC remains a reliable and accurate marker.⁶⁰

Renal dysfunctions require early diagnostic and therapy. Only then, renally eliminated medications may adequately be adjusted considering GFR. Reliable endogenous markers such as CysC are technically simple and permit fast as well as repetitive diagnostics, whereas GFR-measurements with exogenous markers such as inulin or radioisotopes are time-consuming, extensive and inappropriate to be used for constant monitoring.

Among others, Filler stated, "The best approach towards a better formula for worldwide use would be the pooling of data to generate more robust formulae with appropriate validation cohorts. [...] of

all endogenous markers, cystatin C appears to be the best surrogate for GFR and it is hoped that serum creatinine be combined or replaced for the estimation of GFR in children."²²

Hypothesis

Renal diseases such as urinary tract infections and congenital anomalies are frequent.^{64,65} Therefore, reliable as well as fast and convenient renal diagnostic methods are needed in clinical routine. **Cystatin C is a suitable and valuable serum marker for renal function and perfusion. In infants and adolescents, it is related to age and gender.** Nevertheless, as it varies neither depending on muscle mass, infectious diseases, shock nor dehydration, it is a more reliable parameter in pediatric renal diagnostic than serum creatinine. Furthermore, CysC is constantly produced at a stable rate and freely filtered in the glomerulum.^{14–16}

We derive the following questions and hypotheses from the background information given above:

How do serum blood levels of CysC vary depending on age and gender?

In newborns as well as in puberty children's parameters of CysC alter and depend on gender.

How does serum CysC compare to sCrea?

While sCrea varies depending on muscle mass despite age and gender, CysC is a more reliable and accurate parameter.

What factors influence CysC parameters?

As proposed by earlier studies, CysC serum concentrations are associated with pubertal stage and are different for children of different gender and age.

3 Publication – manuscript

Cystatin C serum levels in healthy children are related to age, gender, and pubertal stage

Niels Ziegelasch, Mandy Vogel, Eva Müller, Nadin Tremel, Anne Jurkutat, Markus Löffler, Nicolas Terliesner, Joachim Thiery, Anja Willenberg, Wieland Kiess, Katalin Dittrich

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Niels Ziegelasch¹ · Mandy Vogel^{1,2} · Eva Müller¹ · Nadin Tremel¹ · Anne Jurkutat¹ · Markus Löffler³ · Nicolas Terliesner⁴ · Joachim Thiery⁵ · Anja Willenberg⁵ · Wieland Kiess^{1,2,4} · Katalin Ditttrich^{2,4}

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Abstract

Background This study aims to establish age- and gender-specific cystatin C (CysC) reference values for healthy infants, children, and adolescents and to relate them to pubertal stage, height, weight, and body mass index (BMI).

Methods Serum CysC and creatinine levels of 6217 fasting, morning venous blood samples from 2803 healthy participants of the LIFE Child study (age 3 months to 18 years) were analyzed by an immunoassay. Recruitment started in 2011; 1636 participants provided at least one follow-up measurement. Percentiles for CysC were calculated. Age- and gender-related effects of height, weight, BMI, and puberty status were assessed through linear regression models.

Results Over the first 2 years of life, median CysC levels decrease depending on height ($\beta = -0.010$ mg/l/cm, $p < 0.001$) and weight ($\beta = -0.033$ mg/l/kg, $p < 0.001$) from 1.06 to 0.88 mg/l for males and from 1.04 to 0.87 mg/l for females. Following the second year of age, the levels remain stable for eight years. From 11 to 14 years of age, there is an increase of median CysC levels in males to 0.98 mg/l and a decrease in females to 0.86 mg/l. The change is associated with puberty ($\beta = 0.105$ mg/l/Tanner stage, $p < 0.001$ in males and $\beta = -0.093$ mg/l/Tanner stage, $p < 0.01$ in females) and in males with height ($\beta = 0.003$ mg/l/cm, $p < 0.001$).

Conclusions CysC levels depend on age, gender, and height, especially during infancy and puberty. We recommend the use of age- and gender-specific reference values for CysC serum levels for estimating kidney function in clinical practice.

Keywords Children · Adolescents · Cystatin C · Serum creatinine · Reference values

Introduction

Cystatin C (CysC), a cysteine protease inhibitor and low molecular weight protein, is an endogenous marker for glomerular filtration rate (GFR). Thus, the kidney function may be estimated based on CysC [1]. It is produced by all human nucleated cells at a stable rate, as it is the product of a house-keeping gene [2]. In contrast, creatinine is produced by the

muscle tissue [3]. Therefore, the currently used GFR estimation formula ($0.413 \times \text{height/sCrea}(\text{mg/dl})$) must consider serum creatinine (sCrea) levels as well as the body height [4] due to varying body composition (especially muscle mass) causing inter- and intra-patient variability in sCrea levels [5–8]. Besides its glomerular filtration, creatinine is also secreted by the proximal tubules, leading to an overestimation of GFR, especially in patients with mild renal impairment [9].

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✉ Katalin Ditttrich
katalin.ditttrich@medizin.uni-leipzig.de

¹ LIFE Leipzig Research Center for Civilization Diseases, University of Leipzig, Philipp-Rosenthal-Strasse 27, 04103 Leipzig, Germany

² Center of Paediatric Research (CPL), University of Leipzig, 04103 Leipzig, Germany

³ Institute for Medical Informatics, Statistics and Epidemiology (IMISE), University of Leipzig, 04107 Leipzig, Germany

⁴ Hospital for Children and Adolescents, University of Leipzig, Liebigstraße 20a, 04103 Leipzig, Germany

⁵ Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics (ILM), University Hospital Leipzig, 04103 Leipzig, Germany

CysC, however, is freely filtered in the glomeruli and demonstrates a high correlation with GFR determined by the gold standard such as CrEDTA [6, 10–12]. CysC levels are measured using the rapid and precise particle enhanced turbidimetric or nephelometric immunoassays (PETIA and PENIA) [13, 14].

Previously published data suggest that CysC allows the assessment of the renal function independently from age and gender, so a universal reference range of 0.63–1.08 for 1–18-year-old children was proposed (of note: before 2012/13, different calibrators were used in commercial assays having led to slightly different reference ranges) [15–18]. Nevertheless, newborns and infants show higher CysC levels [15–17, 19, 20] and reach steady levels after 1 to 3 years of age [7, 16, 19, 21]. Apparently independent of age and gender, the CysC levels remain constant up to the age of 14 to 16 years, according to some studies even up to adulthood [17]. Yata et al. ($n = 1128$) as well as Groesbeck et al. ($n = 719$) published the first larger pediatric cohort studies showing that CysC levels decrease in adolescents aged 15–16 years and are elevated in males compared to females at that same age [19, 22]. Miliku et al. found that GFR estimation equations using CysC were negatively associated with body mass index (BMI) and body surface area (BSA), but not lean or fat mass percentage [23]. An effect of age, gender, height, and weight on CysC was also found in adults [24].

This study aims to establish age- and gender-specific CysC reference values for generally healthy infants, children, and adolescents. Furthermore, we aim to analyze the effect of pubertal stage, height, weight, and BMI on CysC serum levels.

Methods

Design and study population

This article is structured according to the STROBE Checklist (Strengthening the Reporting of Observational Studies in Epidemiology) [25]. As part of the Leipzig Research Centre for Civilization Diseases (LIFE), the population-based cohort study LIFE Child has started recruiting urban, primarily healthy infants, children, and adolescents in Leipzig (Germany) in 2011. This large population-based cohort has already been used to establish reference intervals for serum lipids [26], liver enzymes [27], and iron-related blood parameters [28] in children. The examinations take place in the LIFE Child study center and are carried out by trained medical staff using highly standardized procedures [29, 30]. LIFE Child pursues the Declaration of Helsinki [31] and has been approved by the Ethics Committee of the University of Leipzig (Reg. No. 264-10-19042010). It is registered under the NCT trial number 02550236. All data were appropriately anonymized to comply to the German data protection law.

More information including the recruitment process and repetitive examinations can be found in Poulain et al. and Quante et al. [29, 30].

In this study, all participants of the LIFE Child cohort having valid CysC measurements taken between 2011 and 2017 (2926 participants) were included. Children with an age of 0–16 years can participate in the study and receive invitations for follow-up examinations until the age of 18 years. Furthermore, during the first year of life, there are visits at the age of 3, 6, and 12 months. Thus, participants provided data on one to six follow-up visits. We excluded all participants with renal anomalies, nephrolithiasis, or febrile urinary tract infections (118 participants). This information was obtained through computer-assisted personal interview and sonography diagnostic. Furthermore, we identified and excluded four remaining isolated extreme values of CysC (< 0.4 mg/l) as well as one participant with implausible anthropometric data. Thus, a total of 6217 observations of 1337 females and 1466 males (age 0–18 years) are included in this study (Fig. 1).

Laboratory assessment

We examined the CysC levels depending on age and gender. Furthermore, we examined sCrea levels in order to analyze

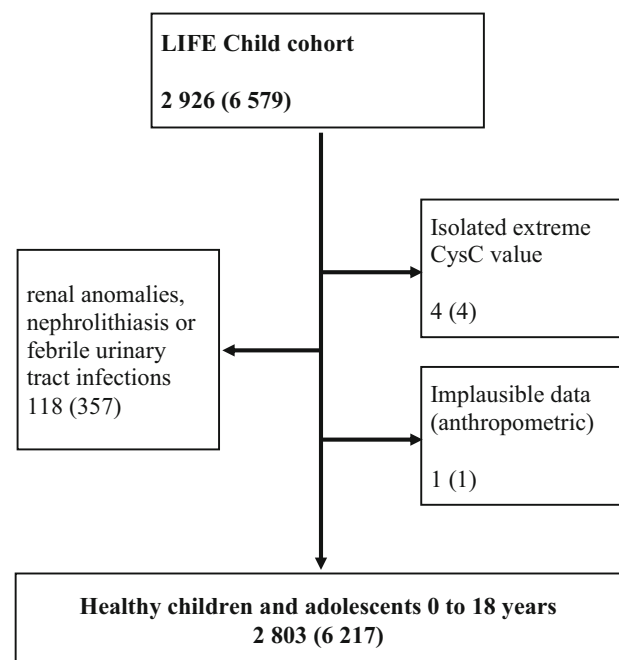


Fig. 1 Flow chart for the participants of this study. Numbers of participants (observations). 2926 participants were observed at the age of 3, 6, and 12 months and thereafter up to once a year. We excluded participants with renal anomalies, nephrolithiasis, or febrile urinary tract infections. One participant was excluded due to implausible anthropometric data. Furthermore, isolated extreme values were excluded. In summary, 6217 observations of 2803 participants were available for analysis

whether or not our data is comparable to sCrea cohorts of earlier studies. For CysC and sCrea, morning venous blood was drawn from each participant by venipuncture using serum monovettes (Sarstedt AG&Co, Nümbrecht, Germany). The analyses were performed by the Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics (ILM), University Hospital Leipzig. Serum samples were analyzed on an automated laboratory analyzer, Cobas8000 (Roche Diagnostics, Mannheim Germany), according to manufacturer's protocol. sCrea was measured with an enzymatic assay (Roche Diagnostics). Measurement of serum CysC was performed using the turbidimetric immunoassay (PETIA) Tina-quant® Cystatin C (Roche Diagnostics). The primary measurement range is 0.4–8.0 mg/l. Traceability of the method was standardized against a Roche in-house reference preparation of recombinant human CysC. In April 2015, the Tina-quant® Cystatin C-assay was advanced to the second generation ($n = 2070$ during second versus $n = 4401$ observations during the first generation), now standardized against the international reference material ERM-DA471/IFCC [30]. Between October 2011 and April 2015, the variation coefficient of control level 1 varied between 1.3 and 6.4% (mean 3.0%), control level 2 varied between 0.9 and 4.5% (mean 2.0%). The primary measurement range of the Tina-quant® Cystatin C 2nd generation is 0.4–6.8 mg/l. Comparative measurement of 143 serum samples was performed between Tina-

quant® Cystatin C and Tina-quant® Cystatin C 2nd generation. Using the MedCalc (MedCalc Software bvba, Belgium), a Passing-Bablok-regression [32] and Bland-Altman-plot [33] were calculated (Online Resources 2 and 3). The comparison showed a good conformity between the first and second generation of the immunoassay Tina-quant®. The mean bias accounts for 0.03 mg/l. The CysC reference values were not corrected for this clinically not relevant bias, which is also comparable to the usual batch effects.

Anthropometric assessment

Height, weight, BMI, and puberty status were taken into account as potential confounding variables. BMI was calculated using height and weight measured by instructed and qualified personnel applying standardized procedures and regularly calibrated devices (a stadiometer with a measurement accuracy of 0.1 cm and a Seca 701 scale with a measurement accuracy of 50 g). The puberty status was examined by means of Tanner stages and assessed by trained staff members [34, 35].

Statistics

The percentiles were estimated applying generalized additive models for location, shape, and scale as implemented in the gamlss package combined with a resampling method using

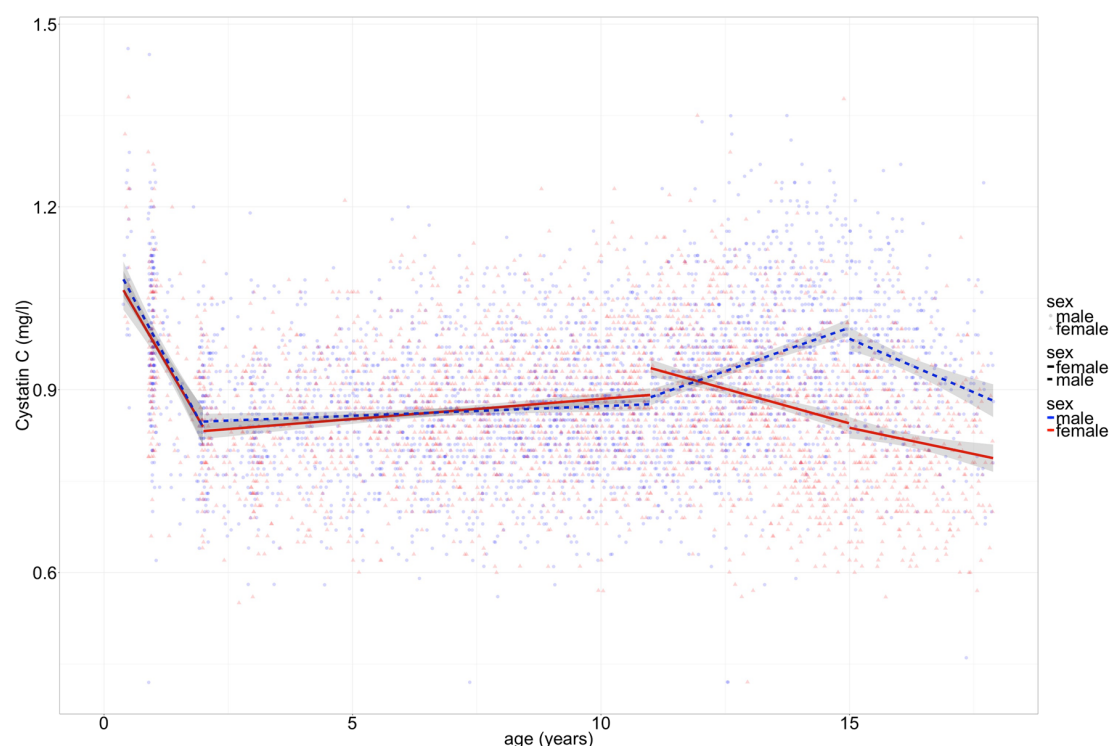


Fig. 2 Cystatin C depends on age in infant and adolescent participants of the LIFE Child cohort. Solid line = female, dashed line = male participants. Note that at the age of 12 years, the curves diverge and

show different patterns for males and females thereafter. $n = 6217$ observations of 2803 participants (0–18 years old)

the ChildSDS packages as described by Vogel et al. [36, 37]. All statistical analyses and visualization were done using the R-Software (version 3.3.2) [38]. To examine the influence of anthropometric measures on CysC levels, we stratified the data into four age intervals of linear course identified through visual inspection and local non-parametric regression (infancy 0–2 years, childhood 2–11 years, and adolescence with 11–15 and 15–18 years; see also Fig. 2 created with ggplot) [39]. Linear modeling was favored due to better interpretability. Hierarchical linear regression analyses (backward deletion) were applied to determine the effects of the independent variables on CysC levels (lmer-function of the R-package lme4) [40]. To account for repetitive measurements in follow-up participants, the subject was added as random effect on the intercept. *T* tests were used to compare mean CysC and sCrea levels of boys and girls (Table 1).

Results

A total of 2803 participants with 6217 observations (Fig. 1 and Table 1) were included. The distributions of pubertal stages and BMI in the LIFE Child cohort are summarized in Table 2.

Serum creatinine distribution

The percentiles of sCrea levels for girls and boys are provided in the Online Resources 1 and 4. First, we evaluated the sCrea distribution to show that the LIFE Child cohort is comparable to other studies and, therefore, a representative sample of the population; sCrea levels rise continuously until the age of 12.5 years (β -slope = 2.744 $\mu\text{mol/l/a}$ = 0.031 mg/dl/a; a = period of 1 year of life) for both boys and girls. At that age, median sCrea levels are 53 $\mu\text{mol/l}$ = 0.60 mg/dl in girls and 55 $\mu\text{mol/l}$ = 0.62 mg/dl in boys. sCrea levels increase more rapidly in 12.5- to 18-year-old boys (β = 5.905 $\mu\text{mol/l/a}$ = 0.067 mg/dl/a). In contrast, the slope (β) in 12.5- to 18-year-old girls is constant at around 2.8 $\mu\text{mol/l/a}$ = 0.032 mg/dl/a. From the age of 13 years, boys exhibit significantly ($p < 0.01$) higher sCrea levels than girls.

Serum cystatin C distribution

The distribution of CysC levels in the LIFE Child cohort, the percentiles and the degree of freedom spread, skewness, location, and kurtosis parameters are shown in Fig. 3 and Table 3. Measurements were elevated for children between the age of 3 months and 18 years. The median CysC serum

Table 1 Numbers of participants, observations, cystatin C (CysC) mean, intercept, and β -slope of the LIFE Child cohort sorted in age intervals

		Infancy	Childhood	Adolescence		All
Age in years		0–2	2–11	11–15	15–18	0–18
Observations, <i>n</i> (%)	Males	213 (6)	1712 (52)	1002 (30)	363 (11)	3290 (100)
	Females	169 (6)	1433 (49)	895 (31)	430 (15)	2927 (100)
Participants	Males	180	903	540	245	1466
	Females	146	771	490	278	1337
CysC mean (\pm SD) in mg/l	Males	0.964 (0.149)	0.863 (0.106)	0.940 (0.128)	0.940 (0.117)	0.901 (0.124)
	Females	0.950 (0.134)	0.864 (0.105)	0.886 (0.123)	0.813 (0.108)	0.868 (0.117)
	<i>t</i> test	$p > 0.05$	$p > 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$
CysC intercept in mg/l	Males	1.152	0.846	0.886	0.973	0.860
	Females	1.127	0.828	0.933	0.830	0.902
	<i>p</i> value	$p > 0.05$	$p > 0.05$	$p < 0.001$	$p < 0.01$	$p < 0.001$
CysC β -slope in mg/l/a	Males	− 0.159	0.003	0.028	− 0.033	0.005
	Females	− 0.148	0.007	− 0.023	− 0.017	− 0.003
	<i>p</i> value	$p > 0.05$	$p < 0.05$	$p < 0.001$	$p > 0.05$	$p < 0.001$
Height mean (\pm SD) in cm	Males	78.2 (5.77)	124.9 (18.0)	161.4 (11.5)	176.6 (7.1)	138.7 (29.4)
	Females	76.3 (5.9)	123.5 (18.3)	159.7 (8.6)	166.5 (6.3)	138.2 (27.9)
Weight mean (\pm SD) in kg	Males	10.19 (1.67)	27.03 (10.91)	54.21 (16.10)	68.47 (16.78)	38.79 (21.59)
	Females	9.45 (1.68)	26.51 (11.21)	53.93 (15.27)	62.74 (15.41)	39.20 (21.11)
BMI mean (\pm SD) in kg/m ²	Males	16.6 (1.2)	16.7 (3.0)	20.5 (4.6)	21.9 (4.8)	18.4 (4.3)
	Females	16.0 (1.1)	16.7 (3.1)	21.0 (5.0)	22.6 (5.3)	18.8 (4.8)

Age intervals were determined by visual inspection for better interpretation with linear regression models (see Fig. 2). Note that participants may have undergone various observations that belong to different age intervals. Intercepts and β -slopes (in mg/l per year of life) were calculated with the lmer-function in R. The intercept represents the mean CysC concentration of the first observations of each age interval

n number of observations, *SD* standard deviation, *BMI* body mass index

Table 2 Distribution of puberty status and BMI in the LIFE Child cohort (0–18 years)

	Puberty status (Tanner stage)					BMI			
	1	2	3	4	5	All	Underweight	Normal	Overweight
Males, <i>n</i> (%)	1541 (67)	332 (14)	121 (5)	172 (7)	149 (6)	2315 (100)	244 (7)	2529 (77)	203 (6)
Males age mean (\pm SD) in years	6.5 (3.1)	11.6 (1.2)	13.0 (1.2)	14.2 (1.2)	15.9 (1.6)	9.5 (4.4)			
Females, <i>n</i> (%)	1324 (50)	302 (11)	269 (10)	292 (11)	441 (17)	2628 (100)	214 (7)	2194 (76)	199 (7)
Females age mean (\pm SD) in years	5.9 (2.9)	10.9 (1.2)	12.4 (1.3)	14.2 (1.6)	15.9 (1.7)	9.9 (4.6)			

Puberty status: pre-pubertal (Tanner stage 1), pubertal (Tanner stages 2–4), post-pubertal (Tanner stage 5). BMI groups: underweight < 10th percentile, overweight > 90th percentile, obese > 97th percentile. As the puberty status and BMI were not examined in all participants, the total numbers of all observations and participants in this table differ from the numbers presented in Fig. 1

n number of observations, *BMI* body mass index

concentrations are highest in toddlers (males 1.06 mg/l, females 1.04 mg/l). They decrease during the first 2 years of life ($\beta = -0.154$ mg/l/a) to slightly but significantly lower levels ($p < 0.001$; males: 0.88 mg/l; females: 0.87 mg/l) and remain constant during childhood until the age of 11 years. The mean CysC values in girls and boys do not differ significantly at this age ($p > 0.05$). While the serum levels of female adolescents start to decrease at 11 years ($\beta = -0.023$ mg/l/a), those for male adolescents increase ($\beta = 0.028$ mg/l/a). Thus, at the age of 13 years, CysC levels differ significantly between males and females ($p < 0.001$). After reaching 15 years of age and median levels of 0.97 mg/l in males and 0.84 mg/l in females, CysC levels of male participants drop again ($\beta = -0.033$ mg/l/a). In our study cohort, we found that CysC levels in males and females remained significantly different until the age of 18 ($p < 0.05$) with the highest and most significant difference at the age of 15 years ($p < 0.001$, mean CysC levels 0.97 mg/l in males and 0.84 mg/l in females).

Among all participants of the LIFE Child cohort, the scale remains constant as indicated by the sigma-value of 0.12–0.14 mg/l (Table 3).

Effects of height, weight, BMI, puberty, and age on cystatin C

To identify potential influential factors for the changes in CysC levels during infancy and adolescence, we correlated height, weight, BMI, the Tanner stage, and age with the CysC concentrations for boys and girls separately. For better interpretability, linear regression models were applied to four different intervals of linear course identified through visual inspection (infancy 0–2 years, childhood 2–11 years, adolescence with 11–15 and 15–18 years; Fig. 2 and Table 1). All effects are corrected for age (except age itself) and repetitive measurement in follow-up participants.

In a simple linear regression analysis of the participants aged 0–2 years, age, height, and weight were shown to be negatively associated with CysC serum concentrations ($p < 0.001$), whereas BMI does not show a significant effect. In hierarchical regression analyses, CysC levels were negatively correlated with height ($\beta = -0.010$ mg/l/cm, $p < 0.001$) as well as weight ($\beta = -0.033$ mg/l/kg, $p < 0.001$).

Using stepwise multiple regression models including age, height, weight, BMI, and Tanner stages CysC levels of male participants between the age of 11 and 14 years showed a strong dependency on puberty status ($p < 0.001$). Even after adjustment for age, CysC concentrations are significantly higher in pubertal boys (especially in Tanner stages three and four with $\beta \approx 0.1$ mg/l/Tanner stage, $p < 0.001$), compared to prepubertal boys. In females at the same age, pubertal stage is also the strongest predictor of CysC levels ($p < 0.001$), but in contrast to males negatively associated with CysC levels ($\beta = -0.093$ mg/l/Tanner stage, $p < 0.01$). We observe a peak

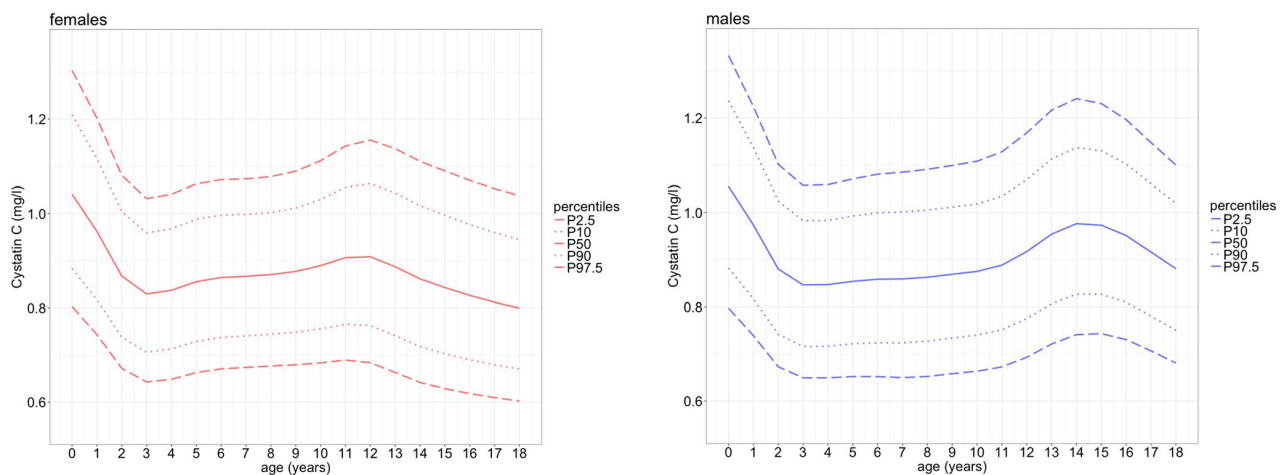


Fig. 3 Percentiles of cystatin C and its effector variable age for 0- to 18-year-old children of the LIFE Child cohort. Solid line = 50th percentile, dotted line = 10th and 90th percentile, dashed line = 2.5th and 97.5th percentile. P percentile. The percentiles were calculated using the

ChildSDS package [37]. Note that just before the age of 12 years, the curves diverge and show different patterns for males and females thereafter. $n = 6217$ observations in 2803 participants

of CysC concentrations in female adolescents during pubertal stage two that however is not significantly different from pre-pubertal CysC concentrations. Weight and BMI show no significant effect on CysC levels in adolescents. Height is another predictor of serum CysC concentrations in pubertal males ($\beta = 0.003$ mg/l/cm, $p < 0.001$).

Discussion

This study aimed to propose CysC reference values for healthy infants, children, and adolescents. We have shown that our study cohort is a representative sample as sCrea levels are distributed similar to published results from earlier population-based studies [16, 41–43]. CysC levels depend on height, weight, age, and puberty. In newborns, CysC levels are higher than at later ages. They decrease rapidly during the first 2 years of life, being negatively associated with height and weight. In 11- to 14-year-old adolescents, the puberty status is the strongest predictor of CysC serum concentrations with an increase of CysC levels in males during early puberty and a decrease in females during late puberty.

Miliku et al. recently published the results of a study in healthy 6-year-olds in Rotterdam (Netherlands) using the same Roche kit for CysC analysis. They did not find significant associations of sex and CysC levels nor the eGFR (calculated using CysC levels with the Zappitelli formula) and sex. Puberty was not considered as they analyzed values of an age-homogeneous population without newborns and pubertal adolescents [23].

The strength of our study is a broad age range from 0 to 18 years and a large number of observations ($n = 6217$) of healthy participants and a standardized assessment. To our knowledge, LIFE Child is the first European study to present

data from such a large cohort including very young infants from the age of 3 months. Nevertheless, the results are based on the social distribution in Leipzig [29, 30]. Therefore, cohort studies in other geographical areas such as Marmarinos et al. may be necessary in order to take regional variability into account [44].

Although earlier studies used different measuring methods for CysC, the course of the percentiles can be compared: The given percentiles for CysC levels are concordant to those proposed by earlier studies (0.63–1.08 mg/l) [15–18, 45], but do not support the thesis of age- and gender-independent reference values. We found that infants exhibited higher CysC levels up to the age of 2 years, thereby confirming the results of other studies such as Andersen et al., Ridefelt et al., Randers et al., and Filler et al. [15–17, 19–21]. A possible explanation is the maturation of kidney function: only the juxtamedullary glomeruli filter blood in newborns, while all other nephrons—although already terminally differentiated—are recruited up to the age of 18–24 months [46, 47]. In 11- to 14-year-old male adolescents, the median CysC concentrations increase to about 0.98 mg/l and thereafter constantly drop to mean values of 0.88 mg/l. In female adolescents, these parameters are up to 0.13 mg/dl lower. This partly confirms the percentiles described by Yata et al. (Japan), Groesbeck et al. (USA), and Marmarinos et al., who were the first to conduct larger pediatric cohort studies ($n = 1128$, 719, and 536, respectively) and showed that CysC levels depend on age and gender during adolescence [19, 22, 44].

In contrary to the results of Marmarinos et al., the BMI shows no significant effect on CysC that cannot be explained by the single variables height or weight. The low correlation coefficient of $r^2 = 0.003$ ($p < 0.001$) may explain why no correlation with lean or fat mass percentage was found by Miliku et al. in 6-year-old children [23, 44]. The estimation

Table 3 (a) Percentiles of cystatin C (mg/l) as a function of age based on the LIFE Child cohort with 0- to 18-year-old boys. (b) Percentiles of cystatin C (mg/l) as a function of age based on the LIFE Child cohort with 0- to 18-year-old girls

Age	<i>n</i>	P2.5	P5	P10	P50	P90	P95	P97.5	Mu	Sigma	Nu	Tau
(a)												
0	106	0.80	0.84	0.88	1.06	1.24	1.29	1.33	1.06	0.13	0.73	2.12
1	107	0.74	0.77	0.82	0.97	1.14	1.18	1.22	0.97	0.13	0.73	2.09
2	135	0.67	0.70	0.74	0.88	1.02	1.07	1.10	0.88	0.12	0.72	2.05
3	132	0.65	0.68	0.72	0.85	0.98	1.02	1.06	0.85	0.12	0.71	2.00
4	141	0.65	0.68	0.72	0.85	0.98	1.02	1.06	0.85	0.12	0.71	1.96
5	156	0.65	0.68	0.72	0.85	0.99	1.03	1.07	0.85	0.12	0.70	1.90
6	198	0.65	0.69	0.72	0.86	1.00	1.04	1.08	0.86	0.13	0.70	1.85
7	197	0.65	0.68	0.72	0.86	1.00	1.05	1.09	0.86	0.13	0.69	1.79
8	251	0.65	0.69	0.73	0.86	1.01	1.05	1.09	0.86	0.13	0.67	1.74
9	260	0.66	0.69	0.73	0.87	1.01	1.06	1.10	0.87	0.13	0.64	1.69
10	242	0.66	0.70	0.74	0.87	1.02	1.07	1.11	0.87	0.13	0.61	1.65
11	265	0.67	0.71	0.75	0.89	1.03	1.08	1.13	0.89	0.13	0.58	1.61
12	259	0.69	0.73	0.78	0.92	1.07	1.12	1.17	0.92	0.13	0.55	1.59
13	253	0.72	0.76	0.81	0.95	1.11	1.17	1.22	0.95	0.13	0.54	1.59
14	225	0.74	0.78	0.83	0.98	1.14	1.19	1.24	0.98	0.13	0.53	1.61
15	190	0.74	0.78	0.83	0.97	1.13	1.18	1.23	0.97	0.12	0.54	1.65
16	110	0.73	0.77	0.81	0.95	1.10	1.15	1.20	0.95	0.12	0.56	1.69
17	63	0.71	0.74	0.78	0.92	1.06	1.11	1.15	0.92	0.12	0.58	1.76
(b)												
0	86	0.80	0.84	0.88	1.04	1.21	1.26	1.30	1.04	0.12	0.59	1.97
1	83	0.74	0.78	0.82	0.96	1.12	1.16	1.20	0.96	0.12	0.61	1.96
2	115	0.67	0.70	0.74	0.87	1.00	1.04	1.08	0.87	0.12	0.63	1.95
3	110	0.64	0.67	0.71	0.83	0.96	1.00	1.03	0.83	0.12	0.65	1.94
4	119	0.65	0.68	0.71	0.84	0.97	1.01	1.04	0.84	0.12	0.67	1.93
5	139	0.66	0.69	0.73	0.86	0.99	1.03	1.06	0.86	0.12	0.69	1.91
6	183	0.67	0.70	0.74	0.86	1.00	1.04	1.07	0.86	0.12	0.70	1.89
7	181	0.67	0.70	0.74	0.87	1.00	1.04	1.07	0.87	0.12	0.70	1.87
8	181	0.68	0.71	0.74	0.87	1.00	1.04	1.08	0.87	0.12	0.70	1.85
9	201	0.68	0.71	0.75	0.88	1.01	1.05	1.09	0.88	0.12	0.70	1.83
10	204	0.68	0.72	0.76	0.89	1.03	1.07	1.11	0.89	0.12	0.68	1.82
11	214	0.69	0.72	0.77	0.91	1.06	1.10	1.14	0.91	0.13	0.66	1.80
12	235	0.68	0.72	0.76	0.91	1.06	1.11	1.16	0.91	0.13	0.63	1.80
13	224	0.66	0.70	0.74	0.89	1.04	1.09	1.14	0.89	0.13	0.58	1.79
14	222	0.64	0.68	0.72	0.86	1.02	1.07	1.11	0.86	0.14	0.53	1.78
15	188	0.63	0.66	0.70	0.84	1.00	1.05	1.09	0.84	0.14	0.48	1.77
16	151	0.62	0.65	0.69	0.83	0.98	1.03	1.07	0.83	0.14	0.42	1.76
17	91	0.61	0.64	0.68	0.81	0.96	1.01	1.05	0.81	0.14	0.36	1.74

The 2.5th, 10th, 90th, and 97.5th percentiles as well as the median are given

n participants, *P* percentile, *Mu* location parameter, *Sigma* spread parameter, *Nu* skewness parameter, *Tau* kurtosis parameter

of the GFR based on sCrea must also consider the body height (Schwartz et al.) [4] during entire childhood and adolescence. CysC shows small variance due to height in 0- to 2-year-old infants ($\beta = -0.010$ mg/l/cm) and 11- to 14-year-old male adolescents ($\beta = 0.003$ mg/l/cm). Thus, body growth may affect CysC concentrations as supposed by its association with height in infancy and male adolescents during puberty. The

hypothesis is that during body growth more body cells exist and so more housekeeping protein CysC will be produced. That leads to a rise in CysC concentrations, which appears especially applicable to pubertal boys due to a higher body growth compared to pubertal girls.

We found an increase of CysC levels in male and a decrease in female adolescents associated with pubertal development.

There is no explanation so far, why pubertal development has a reverse effect on CysC serum concentrations in male and female adolescents. Similar to the association described by Groesbeck et al., CysC levels of females showed a peak in Tanner stage two whereas those of male participants had a peak in Tanner stage four [22]. At the age of 13 years, the CysC levels start to be significantly different for males and females. At the age of 15 years, this difference amounts to 0.13 mg/l (15.5% higher in males compared to females, Table 1) and is similar to that of sCrea levels at the same age (15.2% higher in males compared to females). We consider this difference as clinically relevant.

In clinical practice, kidney injury is diagnosed by loss of estimated GFR or increase in sCrea by 25%, which depends highly on muscle mass [48]. Any known gender- or age-related changes in parameters of normal kidney function are necessary for the recognition of renal damage. This especially applies to formerly unknown patients at the time of admission for example onto a pediatric intensive care unit. Therefore, when using CysC parameters, we suggest the use of age- and gender-related CysC reference values to evaluate renal function in pediatric patients.

Overall, growth rate, serum levels of sexual hormones, blood glucose, smoking or alcohol consumption may affect CysC serum concentrations. As we continue our research, we aim to include the socioeconomic status among the other potential effector variables and confounders in subsequent studies.

Nevertheless, the percentiles of this study suggest that CysC serum concentration is a stable parameter with narrow ranges, but with a notable variation in infancy and adolescence related to age, gender, and puberty.

Conclusion

Our study provides CysC reference values derived from a large pediatric cohort in a homogeneous Caucasian population (6217 observations of 2803 participants). The results of this population-based cohort indicate that serum CysC levels do vary significantly according to age, gender, and pubertal status. Therefore, we suggest the use of age and gender-specific reference ranges for the assessment of kidney function in newborns, children, and adolescents.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards and was approved by the Ethics Committee of the University of Leipzig (Reg. No. 264-10-19042010). It is registered under the NCT trial number 02550236. All data were appropriately anonymized to comply with German data protection law.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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4 Summary and interpretation

Dissertation to obtain the academic degree Dr. med.

Cystatin C serum levels in healthy children are related to age, gender and pubertal stage

Submitted by: Niels Ziegelasch

Written at: University of Leipzig/ Center for Pediatric Research Leipzig (CPL),
Hospital for Children & Adolescents

Supervised by: Prof. Dr. med. Wieland Kiess

Dr. med. Katalin Dittrich

Dr. Mandy Vogel

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The study aimed to check the hypothesis that CysC is related to age and gender in infants and adolescents. We were able to show that CysC does not only depend on age and gender, but furthermore is associated with pubertal status. These findings oppose the thesis of one uniform CysC reference range for infants and adolescents, which only applies to children from the age of two up to eleven years. We suggest the application of percentiles for laboratory assessment. The percentiles presented in this study (derived from a large Caucasian pediatric cohort with 6 217 observations) will soon be adopted in clinical routine by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics (University of Leipzig).

The difference of CysC between girls and boys at the age of 15 years amounts to 15.5% and therefore is similar to that of sCrea at the same age. Nevertheless, the usage of CysC serum concentrations in renal diagnostics has several benefits: First, it appears to be more sensitive for renal impairment in patients with a GFR above 60ml/min/1.73m². Second, CysC shows a stronger correlation with gold standard methods and higher diagnostic accuracy as described earlier in this dissertation.^{35–38,41}

Furthermore, compared to sCrea CysC showed a higher correlation with gold standard methods in post-transplant patients^{44,45,47}, in diabetic patients with a stable metabolic status⁴⁹, in patients with renal diseases as a result of urine reflux due to a spina bifida¹¹ and in pediatric cancer patients.⁵⁰ These findings support the hypothesis of CysC as a reliable and accurate renal parameter in clinical routine diagnostics. Finally, CysC equations for estimating the GFR seem to be at least as accurate as equations relying on sCrea. Besides, these equations can be applied without additional information like height and therefore enable a fast and convenient GFR estimation.^{51–54} Combined formulas considering also height and blood urea nitrogen like the Schwartz formulas yield an even better result. These are furthermore the only CysC equations validated for children so far, although they are only valid for a GFR between 15 and 75 ml/min/1.73m².^{9,55} However, the equation proposed by Andersen et al. also adjusts for body cell mass and body surface area (besides height), and predicts the GFR most accurate.⁵⁶

Nevertheless, a major benefit of sCrea needs to be considered: The costs of one laboratory assessment at our Institute of Laboratory Medicine in Leipzig is 2€ compared to 15€ for CysC according to the medical fee schedule (“Gebührenordnung für Ärzte”).

When additionally examining data of the LIFE Adult cohort (n=254 observations in 228 18- to 26-year-old adults), from the age of 20 years, we found no significant differences of CysC serum concentrations for male and female participants anymore. They approximate one another and reach 0.81 mg/l for male and 0.75 mg/l for female 26-year-old adults (Figure 4). These findings confirm results presented earlier by Galteau et al.²¹ Along with the data published by Filler et al. they showed CysC serum concentrations of 0.83±0.103 mg/l^{1,21,72} in elderly subjects (60 years of

age and older). CysC levels as well as CysC formulas in elderly – similarly to children – are more sensitive compared to sCrea, although no significant difference in females and males was described so far.^{72,73} Higher serum concentrations of CysC are positively correlated with BMI, nephritis, hypertension and leptin, and negatively with neoplasm.^{74,75} Furthermore, Odden et al. found an association of higher CysC with poorer physical function such as slower or not completed 400-meter walk.⁷⁶ Therefore, we assume an effect of body composition: as CysC is produced by all nucleated cells, a higher body cell mass may result in higher serum concentrations of CysC. This hypothesis is also consistent with the fact that the Andersen equation including body cell mass and body surface is the most accurate in estimating the GFR as mentioned above.⁵⁶ Therefore, a changing body cell composition due to growth and development may explain the significantly higher levels of CysC in newborns, pubertal boys and – to a less extent – pubertal girls. During puberty, lutein hormone and follicle stimulating hormone are secreted and stimulate the production of testosterone and estrogen and thus body growth and the development of secondary sexual characteristics. Especially the body growth is stimulated by a further secretion of thyroxin and insulin-like growth factor 1 (IGF1), which may explain the association of CysC and thyroxin found by Wiesli et al..⁶¹ This applies to girls during early puberty (for pubertal group stratification see Table 1) and to an even greater extent to boys during late puberty, as their growth spurt occurs later compared to girls. Therefore, girls show a peak of CysC at the age of 12 years, whereas the boys' peak occurs at the age of 14 years. This is in line with Groesbeck et al..³² The higher peak for boys may occur because boys grow taller. Besides, higher blood glucose due to increased levels of IGF1 may be associated with increased CysC, as it is also found in patients with diabetes or glucocorticoid therapy.^{57,59,60} Overall, an interaction of the hormone system and CysC seems obvious.

Limits of this study are data only representing a local, homogenous Caucasian cohort.^{77,78} Nevertheless, studies in Japan and the U.S. show similar results.^{27,32} Still, we recommend the examination of CysC serum concentrations in children in other geographical areas. Additionally, although we were able to show that CysC depends on pubertal stage beyond the effect of age and gender, the underlying physiological and biochemical mechanisms remain unknown. Earlier studies suggest that in newborns only the juxtamedullary glomeruli

already filtrate blood, whereas the other glomeruli are recruited over the first two years of life.^{52,79} During puberty, growth, thyroxin, testosterone, and insulin-like growth factor 1 among others may affect CysC levels as described above. For a better understanding, further research should examine potential associations of CysC with hormones (such as follicle stimulating and lutein hormone, testosterone, IGF1, glucocorticoids as well as parathyroid hormone), growth velocity and blood glucose.

Studies already showed that cigarette smoking was associated with higher serum levels of CysC in adults.⁵⁸ Furthermore, patients with proteinuria had elevated urinary levels of CysC⁶², whereas higher levels of CRP and white blood cells and lower serum albumin concentrations were associated with elevated serum concentrations of CysC.^{57–59} Infectious diseases, especially of the urinary organ system, may explain these effects. Nevertheless, so far, no studies investigated these effects in children. As urinary and renal infections are very frequent⁶⁴, a reliable and accurate as well as fast and non-invasive renal diagnostic with endogenous markers is desirable. CysC seems to be more accurate and reliable compared to sCrea. Besides, CysC shows a better correlation with the GFR above 60ml/min/1.73m² than sCrea. Therefore, even the dosage adjustment for pharmaceuticals in cardiology or chemotherapeutics in oncological patients may benefit from the application of CysC, as it enables a reliable and continued monitoring of the GFR.⁷⁰

As proposed by Filler, we calculated percentiles for CysC by assessing a large pool of data.² Due to the large number of observations (n=6 217) of healthy participants as well as the standardized assessment, the data and percentiles presented in this study are of high accuracy. To our knowledge, LIFE Child is the first European study including very young infants from the age of three months as well as children up to 18 years of age. Overall, we strongly recommend the usage of age and gender specific percentiles for CysC in pediatric renal diagnostic.

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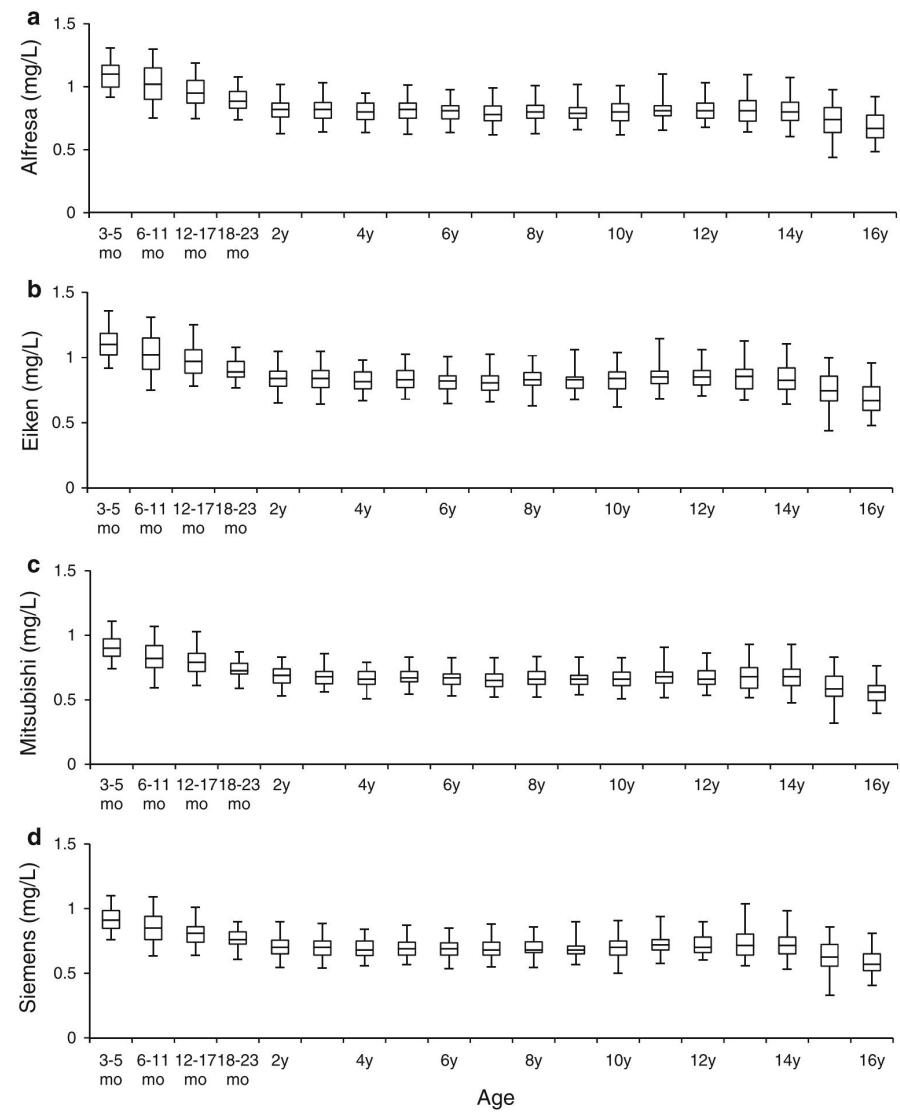
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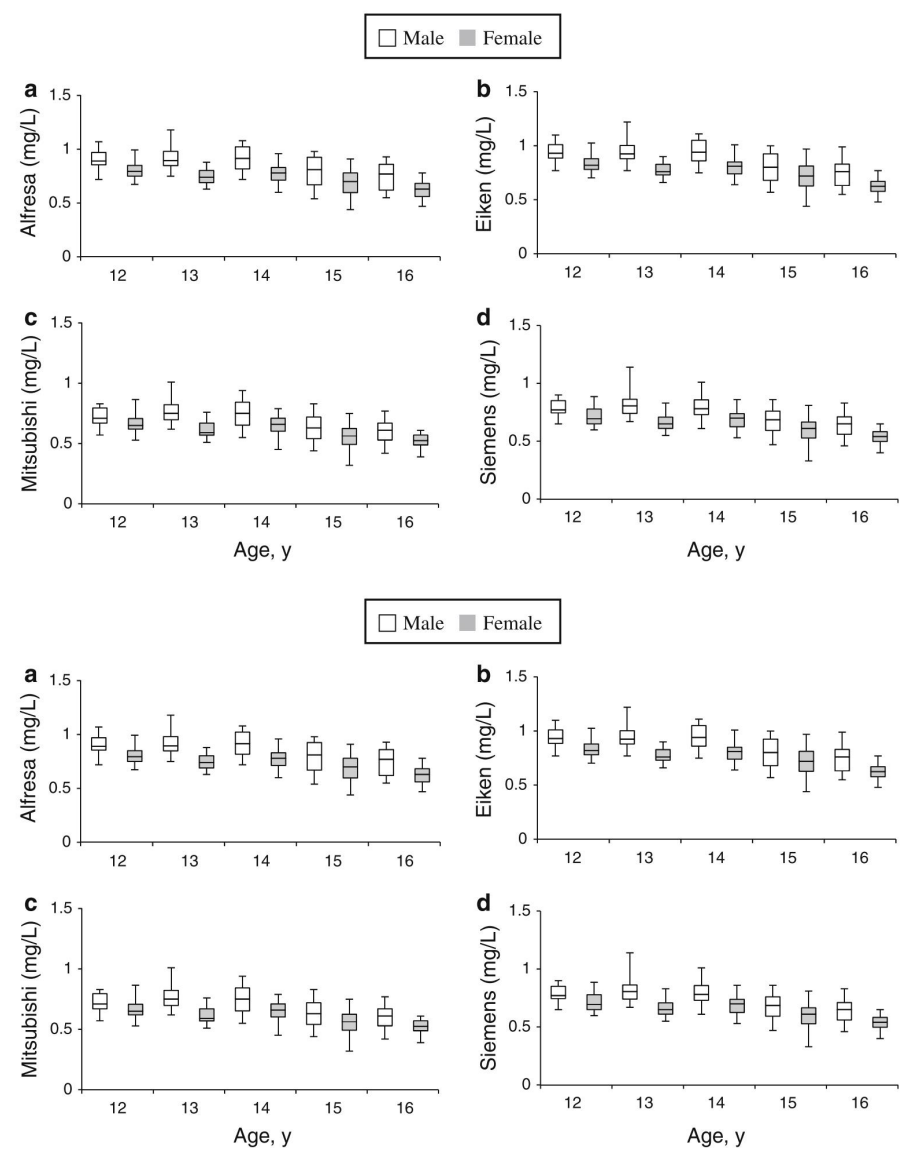
6 Appendix

Figure 1. Serum CysC in children aged 3 months to 16 years according to Yata et al..²⁷



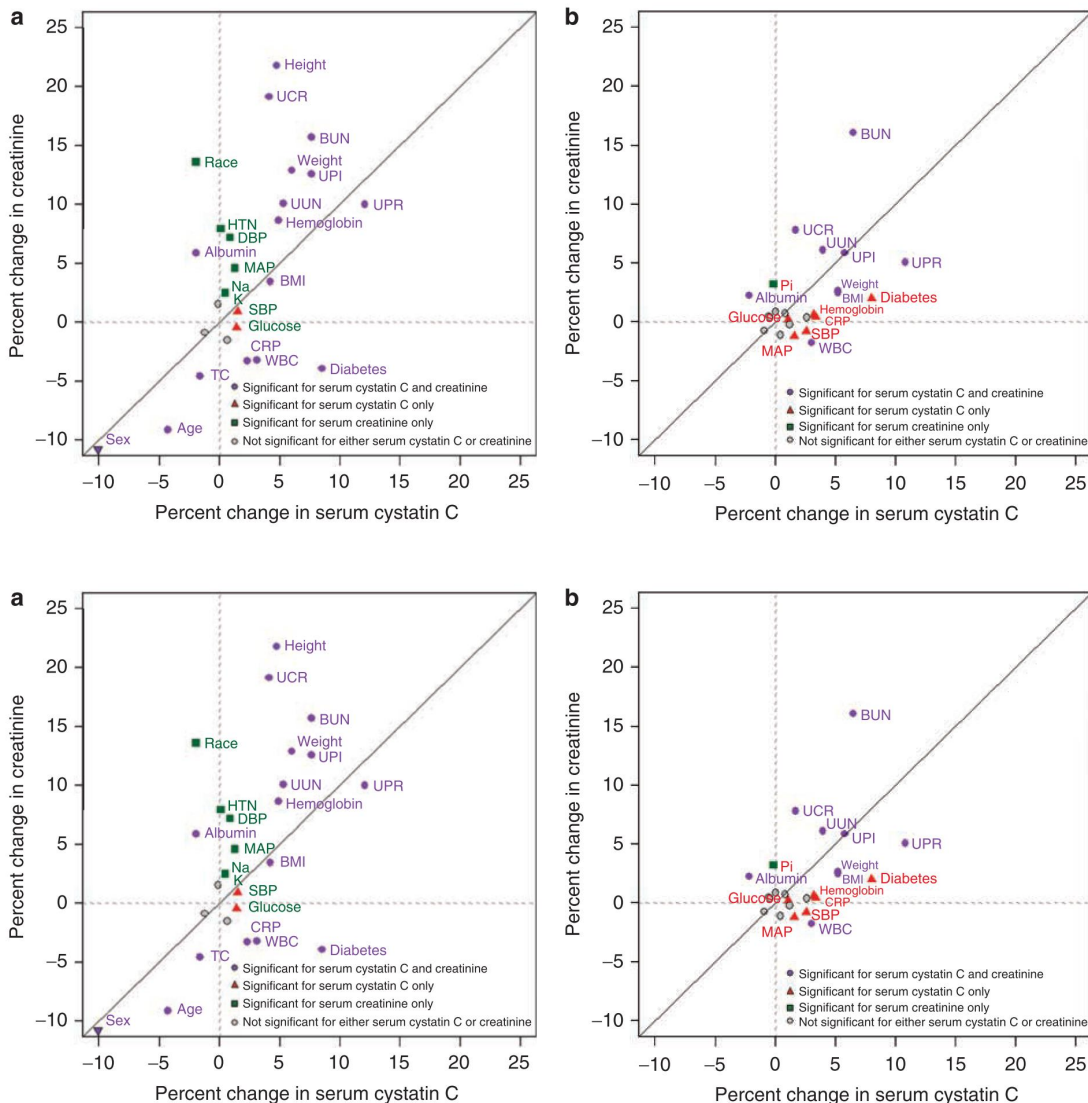
The box plot extends from the 25th percentile to the 75th percentile, with the horizontal line at the median, and the whiskers show the central 95 % of the data for Alfresa (a), Eiken (b), Mitsubishi (c), and Siemens assays (d).²⁷

Figure 2. Serum CysC in male and female children according to Yata et al.²⁷



The box plot extends from the 25th percentile to the 75th percentile, with the horizontal line at the median, and the whiskers show the central 95 % of the data for Alfresa (a), Eiken (b), Mitsubishi (c), and Siemens assays (d).²⁷

Figure 3. Effector variables of CysC and sCrea according to Stevens et al. 2009.⁵⁷



Comparison of coefficients of variables predicting log cystatin and log creatinine. Solid diagonal line is the line of identity. (...) HTN, hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; WBC, white blood cell count; Na, sodium; K, potassium; Pi, phosphate; Ca, calcium; HCO₃, bicarbonate; TC, total cholesterol; alb, albumin; gluc, glucose; UUN, urine urea nitrogen; UCR, urine creatinine; UPI, urine phosphate; UPR, urine protein (...).⁵⁷

Figure 4. The curves of CysC serum concentrations in males and females approach one another after adolescents and are not significantly different in adults.

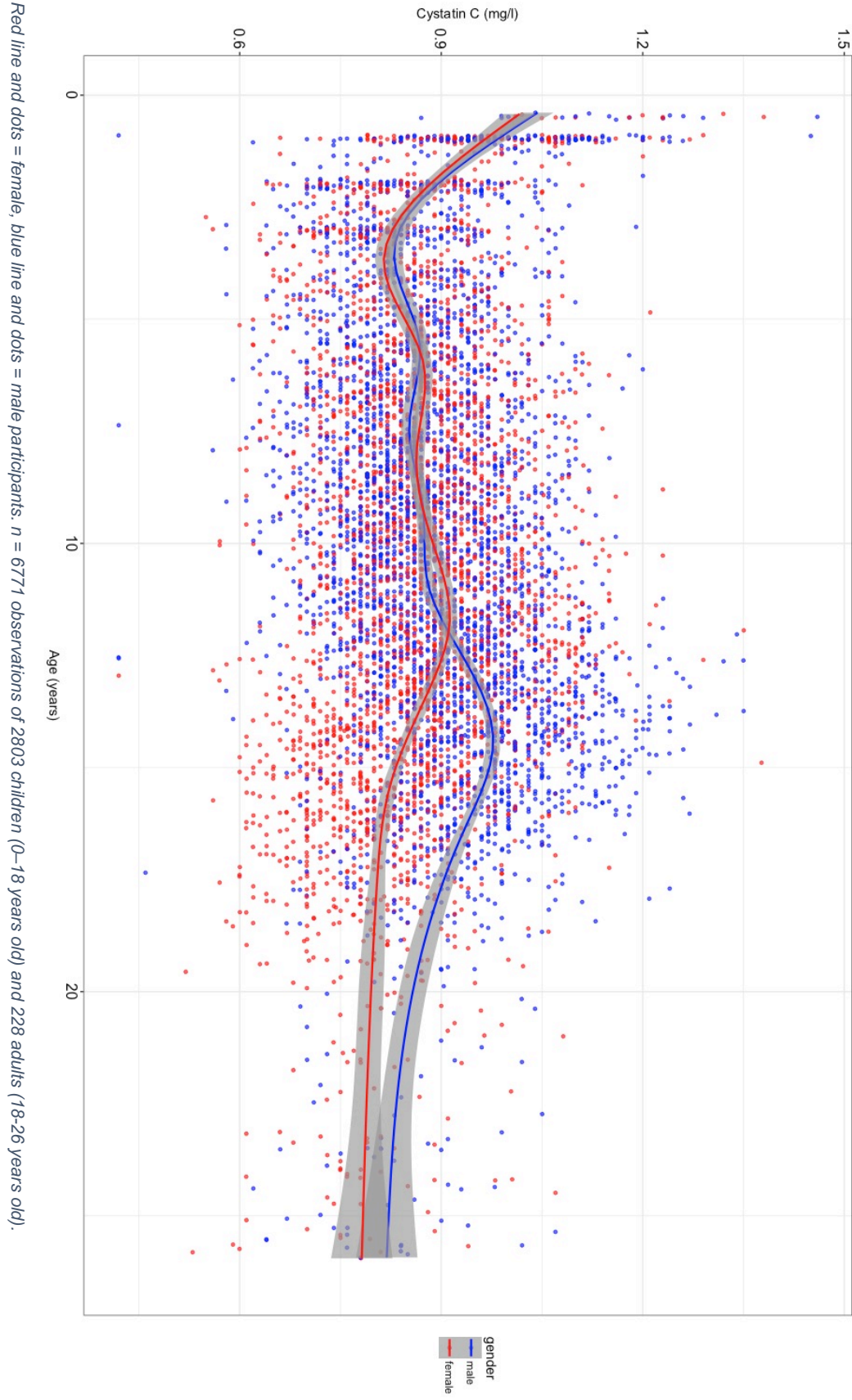


Table 1. Criteria for Pubertal Group Stratification

Stage 1	Girls	PH=1, B=1, <13,5years
	Boys	PH=1, TV≤3, <14years
Stage 2	Girls	PH≤3, B=2, ≥8years
	Boys	PH≤2, 4≤TV≤10, ≥9years
Stage 3	Girls	2≤PH≤4, B=3
	Boys	PH=3, TV≥4, ≥9years
Stage 4	Girls	3≤PH≤5, B=4, ≥8years
	Boys	PH=4, TV≥4, ≥9years
Stage 5	Girls	4≤PH≤5, B=5
	Boys	PH≥5, TV≥7, ≥9years

Abbreviations: PH = Pubic Hair, B = Breast, TV = Testicular Volume; according to Tanner Stages.

7 Description of the own contributions

Niels Ziegelasch revised, summarized and interpreted the data using the statistical R-software. Along with the literature research, he drafted as well as revised the manuscript excluding the laboratory assessment and part of the discussion. Furthermore, he worked two months in the Leipzig LIFE Child study center to examine further probands in the ongoing research project.

Dr. Mandy Vogel

Dr. Eva Müller

Dr. Nadin Tremel

Anne Jurkutat

Prof. Dr. Markus Löffler

Dr. Nicolas Terliesner

Prof. Dr. Joachim Thiery

Dr. Anja Willenberg

Prof. Dr. Wieland Kiess

Dr. Katalin Dittrich

8 Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

Niels Ziegelasch

Leipzig, 04.03.2019

9 Curriculum vitae

Niels Ziegelasch

born August 4th, 1993 in Schwerin

Education

2003-2012	graduation from GutsMuths-Gymnasium Quedlinburg (final grade 1.0)
2012-2014	medical school (Ludwig-Maximilians-University Munich)
Sep 2014	first medical examination (final grade 1.5)
2014	certificate as trainer of intercultural workshops (YFU)
Since 2014	medical school at the University of Leipzig
Since 2016	dissertation at the University of Leipzig (LIFE Child)
Apr 2018	second medical examination (final grade 2.0)

International experiences

Oct 2006	exchange program with a French high school (one week)
Mar 2008	exchange program with a Latvian music school (one week)
2009-2010	high school year in Michigan, USA with YFU
Apr 2012	second exchange program with the Latvian music school
Aug 2013	internship at the University Hospital Linköping, Sweden
Sep 2015	second internship at the University Hospital Linköping, Sweden
Jan-Apr 2019	internship at the University Hospital Geneva, Switzerland

Practical trainings and employment

2013	tutor for Chemistry at the medical school in Munich (LMU)
Sep 2016	internship at the YFU office in Saginaw, USA
Oct/Nov 2016	two months internship at the LIFE Child study center
2017	student assistant at the intensive care unit (heart center Leipzig)

Publication

Nov 2018 Cystatin C serum levels in healthy children are related to age, gender and pubertal stage
Pediatric Nephrology

Scholarships and awards

2004-2012 scholarship for saxophone tuition at the „Kreismusikschule Harz“
2009-2010 partial scholarship for the high school year tuition (YFU)
April 2013 „Deutschlandstipendium“ at the Ludwig-Maximilians-University Munich
April 2014 renewal of the „Deutschlandstipendium“
March 2018 award for the best presentation at the conference of pediatric nephrology

Further commitment and interest

since 2011 volunteering for Youth For Understanding (YFU)
2015-2017 formation and conduction of the student choir „Chorioso“ (University of Leipzig)

Languages

German mother tongue
English fluently
Swedish B1-certificate
French B2-certificate

10 Scientific publications and presentations

- March 2018 award for the best presentation at the conference of pediatric nephrology, Hanover
- Sep 2018 presentation of further results regarding Cystatin C at the conference of nephrology, Berlin
- Nov 2018 Cystatin C serum levels in healthy children are related to age, gender and pubertal stage
Pediatric Nephrology

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