Indirect determination of pediatric blood count reference intervals

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1a Abstract

Background: Determination of pediatric reference intervals (RIs) for laboratory quantities, including hematological quantities, is complex. The measured quantities vary by age, and obtaining samples from healthy children is difficult. Many widely used RIs are derived from small sample numbers and are split into arbitrary discrete age intervals. Use of intra-laboratory RIs specific to the examined population and analytical device used is not yet fully established. Indirect methods address these issues by deriving RIs from clinical laboratory databases which contain large datasets of both healthy and pathological samples.

Methods: A refined indirect approach was used to create continuous age-dependent RIs for blood count quantities and sodium from birth to adulthood. The dataset for each quantity consisted of 60,000 individual samples from our clinical laboratory. Patient samples were separated according to age, and a density function of the proportion of healthy samples was estimated for each age group. The resulting RIs were merged to obtain continuous RIs from birth to adulthood.

Results: The obtained RIs were compared to RIs generated by identical laboratory instruments, and to population-specific RIs created using conventional methods. This comparison showed a high concordance of reference limits and their age-dependent dynamics.

Conclusions: The indirect approach reported here is wellsuited to create continuous, intra-laboratory RIs from clinical laboratory databases and showed that the RIs generated are comparable to those created using established methods. The procedure can be transferred to other laboratory quantities and can be used as an alternative method for RI determination where conventional approaches are limited.

1b Zusammenfassung

Hintergrund: Die Bestimmung pädiatrischer Referenzintervalle (RIs) für Laborgrößen einschließlich hämatologischer Größen ist komplex. Die gemessenen Werte sind altersabhängig und die Probengewinnung von gesunden Kindern ist problematisch. Viele verbreitete RIs basieren auf kleinen Stichprobenpopulationen und sind in arbiträre und diskrete Altersintervalle aufgeteilt. Die Verwendung von RIs, die zugleich labor-, analysenmethoden- und populationsspezifisch sind, ist oft nicht möglich. Mit der Verwendung indirekter Methoden können RIs aus klinischen Labordatenbanken bestimmt werden, die große Datensätze pathologischer und nicht-pathologischer Messwerte enthalten.

Methoden: Mit einer weiterentwickelten indirekten Methode wurden kontinuierliche altersabhängige RIs für Parameter des Blutbilds und Natrium von Geburt bis zum Erwachsenenalter bestimmt. Für jede Messgröße standen mehr als 60.000 Messwerte aus unserem Routinelabor zur Verfügung. Die Patientenmesswerte wurden altersabhängig aufgeteilt und der Anteil nicht-pathologischer Messwerte mittels einer Dichtefunktion für jede Altersgruppe geschätzt. Die RIs wurden zu kontinuierlichen altersabhängigen RIs von Geburt bis Erwachsenenalter fusioniert.

Ergebnisse: Die ermittelten RIs wurden mit Analysemethoden-spezifischen RIs sowie mit populationsspezifischen RIs verglichen, die mit konventionellen Methoden generiert wurden. Dieser Vergleich zeigte eine hohe Übereinstimmung.

Schlussfolgerungen: Der entwickelte indirekte Ansatz ist für die Bestimmung kontinuierlicher, labor- und populationsspezifischer RIs aus Labordatenbanken gut geeignet. Die generierten RIs sind vergleichbar mit konventionell bestimmten. Die Methode kann auf andere Laborwerte übertragen werden und ist eine Alternative zu konventionellen Methoden, insbesondere, wenn deren Anwendbarkeit eingeschränkt ist.

2a Introduction

Determination of reference intervals (RIs) poses particular challenges in the pediatric population [1]. The dynamic anatomical and physiological development, particularly in the first years of life and during puberty, accounts for a high variability for many measured quantities with age. To accurately represent the age-dependent characteristics of such quantities, relatively large reference cohorts are required for the generation of RIs with an adequate ageresolution. The classical approach of sampling a healthy reference population and using the 2.5th and 97.5th percentiles as the lower or upper reference limit [2-4] can often not be applied to children. Reference populations such as healthy blood donors, often referred to when determining RIs for the adult population, are not readily available and ethical considerations would restrict blood sampling from healthy children for the purpose of reference interval generation [5].

Only few studies have embedded blood sampling in the context of a more comprehensive study objective, e.g., the Canadian CALIPER study (Canadian Laboratory Initiative on PEdiatric Reference Intervals) [6-8] and the German Health Interview and Examination Survey for Children and Adolescents (KiGGS), which collected 14,076 blood samples from a representative German cohort of children aged from 1 to 18 years [9]. The considerable effort associated with this study design makes it impossible, however, to apply this approach to any possible quantity and age group. Although clinically most relevant in neonatal medicine, the critical age group of newborns and young infants had been excluded by the KiGGS survey and other studies.

Therefore, in many laboratories, RIs have been adopted from values provided by the literature or the analyzer ' s manufacturer irrespective of the underlying ethnical background of the reference population or additional influence factors due to methodological differences [10]. Even these RIs are often derived from very small sample sizes, well below the International Federation of Clinical Chemistry and Laboratory Medicine ' s (IFCC) recommendation [10, 11].

Alternative approaches evading several of the above mentioned obstacles, apply algorithms to calculate RIs from laboratory values containing a mixture of healthy and pathological samples [12-17]. These methods are based on the assumption that the input dataset consists of a mixture of parametrically distributed samples from healthy individuals, and random pathologic samples. When applied to a sufficiently large dataset, these two sample sets can be reliably separated and the pathological samples have no significant impact on the healthy population ' s RIs. The underlying

parametric distribution of healthy samples is estimated by statistical methods, and its 2.5th and 97.5th percentile defines the RI.

Another shortcoming of conventional pediatric RIs is that age ranges are often arbitrarily chosen irrespective of developmental stages. In analogy to other developmental quantities, routinely specified in relation to age (e.g., height-for-age charts, blood pressure percentiles), a continuous description would also seem to be an appropriate approach for laboratory quantities.

The aim of the study was to augment previously developed algorithms for indirect RI calculation to create continuous RIs for pediatric laboratory quantities. The correctness of the generated RIs for hematologic quantities and sodium was tested by comparing them to RIs determined by established methods.

2b Einleitung

Die Bestimmung von Referenzintervallen (RIs) ist komplex, dies gilt insbesondere in der Pädiatrie. Die dynamische anatomische und physiologische Entwicklung führt vor allem in den ersten Lebensjahren und in der Pubertät zu einer hohen altersabhängigen Variabilität vieler Messgrößen. Um diese Dynamik adäquat und genau abzubilden sind relativ große Referenzkohorten erforderlich. Die konventionelle Methode, eine gesunde Referenzpopulation zu messen und die 2,5. und 97,5. Perzentile als unteren bzw. oberen Referenzwert zu verwenden kann im Kindesalter oft nicht angewandt werden. In der Erwachsenenmedizin häufig verwandte Referenzpopulationen wie z.B. gesunde Blutspender sind nicht verfügbar und die Verwendung von Blutproben gesunder Kinder zum Zweck der Normwertgenerierung ist aus ethischen Gründen eingeschränkt.

In einigen wenigen Studien, wie z.B. der kanadischen CALIPER-Studie (Canadian Laboratory Initiative on Pediatric Reference Intervals) und der deutschen KiGGS-Studie (Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland) wurden im Rahmen einer umfassenderen Fragestellung Blutabnahmen durchgeführt. In der KiGGS-Studie wurden 14.076 Blutproben von Kindern zwischen 1 und 18 Jahren aus einer repräsentativen deutschen Stichprobe gewonnen. Der mit diesen Studien verbundene Aufwand verhindert jedoch, diese Methode auf jede Laborgröße und Altersgruppe zu übertragen. Und obwohl die Altersgruppe der Neugeborenen und Säuglinge klinisch von höchster Relevanz ist, wurde diese Gruppe aus der KiGGS-Studie und anderen Studien ausgeschlossen.

Viele Laboratorien verwenden daher entweder publizierte Referenzintervalle oder vom Hersteller des verwendeten Analysegeräts zur Verfügung gestellte Angaben. Der ethnische Hintergrund der analysierten Population oder methodische Unterschiede können dabei nicht immer berücksichtigt werden. Selbst diese Referenzintervalle basieren jedoch oftmals auf Stichproben, die kleiner sind als von der IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) empfohlen.

Um diese Probleme zu umgehen, wurden alternative Methoden entwickelt, die durch die Anwendung mathematischer Algorithmen die Ableitung von RIs aus Labordatenbanken erlauben. Diese Datenbanken enthalten sowohl pathologische Messwerte auch normale Messwerte, die zusammen eine Gesamtverteilung bilden. Sogenannte indirekte Methoden der Normwertgenerierung basieren auf der Annahme, dass diese Gesamtverteilung aus der Summe einer parametrischen (mathematisch beschreibbaren) Verteilung gesunder Messwerte und zufällig

verteilten pathologischen Messwerten besteht. Bei Verwendung eines ausreichend großen Datensatzes können diese zwei Verteilungen mittels statistischer Methoden zuverlässig getrennt werden und das RI durch Berechnung der 2,5. und 97,5. Perzentile der Verteilung gesunder Messwerte berechnet werden.

Die Altersabhängigkeit pädiatrischer RIs wird meist durch die Einteilung in diskrete Altersgruppen abgebildet, diese Einteilung widerspricht der Vorstellung von Entwicklung als kontinuierlichem Prozess. Analog zu anthropometrischen Messgrößen (z.B. Perzentilekurven für altersbezogenes Körpergewicht und -größe) erscheint die Verwendung kontinuierlicher RIs auch für Labormessgrößen adäquat.

In dieser Studie wurden Algorithmen zur indirekten Bestimmung von RIs weiterentwickelt um kontinuierliche, indirekte pädiatrische RIs zu berechnen. Die entwickelten RIs für hämatologische Messgrößen und Plasma-Natrium wurden durch einen Vergleich mit konventionell bestimmten RIs validiert.

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Abstract

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Keywords: blood count; hematology; indirect determination of reference intervals; pediatric reference intervals; reference range.

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Introduction

Determination of reference intervals (RIs) poses particular challenges in the pediatric population [1]. The dynamic anatomical and physiological development, particularly in the first years of life and during puberty, accounts for a high variability for many measured quantities with age. To accurately represent the age-dependent characteristics of such quantities, relatively large reference cohorts are required for the generation of RIs with an adequate age-resolution. The classical approach of sampling a healthy reference population and using the 2.5th and 97.5th percentiles as the lower or upper reference limit [2–4] can often not be applied to children. Reference populations such as healthy blood donors, often referred to when determining RIs for the adult population, are not readily available and ethical considerations would restrict blood sampling from healthy children for the purpose of reference interval generation [5].

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infants had been excluded by the KiGGS survey and other studies.

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Another shortcoming of conventional pediatric RIs is that age ranges are often arbitrarily chosen irrespective of developmental stages. In analogy to other developmental quantities, routinely specified in relation to age (e.g., height-for-age charts, blood pressure percentiles), a continuous description would also seem to be an appropriate approach for laboratory quantities.

The aim of the study was to augment previously developed algorithms for indirect RI calculation to create continuous RIs for pediatric laboratory quantities. The correctness of the generated RIs for hematologic quantities and sodium was tested by comparing them to RIs determined by established methods.

Materials and methods

Data source

All blood counts performed during a 29-month period at the University Children's Hospital Erlangen – a tertiary care center covering the entire spectrum of pediatrics – were retrieved from the laboratory's database. For each quantity, between 56,253 and 60,394 measurements were available (see Supplemental Table 1, which accompanies the article at <http://www.degruyter.com/view/j/cclm.2013.51.issue-7/issue-files/cclm.2013.51.issue-7.xml>). Stratification according to ethnicity was not performed; distinct RIs for different ethnic groups are not established in Germany. The composition of the population in

Germany is mainly influenced by migration of individuals of Caucasian origin.

Blood counts were performed on a Sysmex XE-2100 hematology analyzer (Sysmex Europe). The quantities examined were hemoglobin, hematocrit, red cell count, mean red cell volume (MCV), mean red cell hemoglobin (MCH), mean red cell hemoglobin concentration (MCHC), platelet count, red cell distribution width variation coefficient (RDWCV).

Data was also collected for plasma sodium. Sodium was chosen for comparison as it is a quantity which is largely stable during development, and its measurement is well-standardized. Plasma sodium measurements were performed on a COBAS Integra 800 (Roche Diagnostics).

Measurement accuracy and precision data is available in Supplemental Table 2. The stability of each quantity over time during the study period is demonstrated by stable monthly median values (Supplemental Table 3).

Two different data sets were created and compared in order to explore the stability of the method regarding the influence of pathologic samples: one containing the complete data set and another in which samples from patients in intensive care units and oncological units were excluded (41,517–42,848 samples).

Calculation of continuous reference intervals

The calculation of continuous RIs was performed in three steps: First, the data was split into overlapping time frames. Discrete RIs for each time frame were generated using an algorithm published earlier by Arzideh et al. [12, 13]. These calculated RIs were plotted in time/value graphs and continuous splines were fitted.

Timeline generation

For each quantity, all the samples were collated and split into overlapping 'time frames', each containing 750 samples (Figure 1B). When multiple samples were available from a single patient, only one sample was included in each time frame (Supplemental Figure 1). A sample count of 750 was chosen because pilot tests showed that 750 samples were the optimum compromise for accuracy and resolution (data not shown). This resulted in 122–127 time frames for each quantity, and 114–117 time frames when patients from intensive care units and oncology units were excluded (Supplemental Table 1). Due to the age distribution of our hospital population, time frames contain measurements for age groups spanning different time ranges, depending on the number of patients within a certain age interval (e.g., the hemoglobin frame for age=1 day contains only values for age=1 day while the frame for age=12.1 years contains values from 11.7 to 12.5 years).

Calculation of RIs

For the analysis of the data of single time frames, an expanded version of the algorithm developed by Arzideh et al. was used [12, 13]. Briefly, to obtain a non-parametrical function of the sample value distribution, a smoothed kernel density function is estimated

Figure 1 Algorithm for the creation of continuous RIs.

(A) Typical analysis of the samples of a single time frame. This figure shows the calculation of the hemoglobin RI for male patients between age=86 days and age=212 days. The black line shows the kernel density estimation (kde) of hemoglobin value incidences. Into this kde the optimal Box-Cox distribution was fitted and the 2.5th and 97.5th percentiles for this distribution were calculated (gray line). (B) Timeline generation: For each time frame generated, separate RIs were calculated.

Data are presented in conventional units; factors for converting to SI units are as follows: hemoglobin, 1 mg/dL=0.6206 mmol/L.

(Figure 1A). The resulting data set is assumed to consist of three subgroups, the non-diseased ('healthy'), and two subgroups with values lower and higher than those of the non-diseased subgroup. The subset of non-diseased samples is modeled using a Box-Cox transformed truncated normal distribution. The Box-Cox transformation is used to transform 'skewed' distributions into symmetrical distributions and is defined as follows:

$$Y = \log X \quad (X > 0, \lambda = 0)$$

$$Y = 1/\lambda \times (X^\lambda - 1) \quad (X > 0, \lambda \neq 0)$$

The two subgroups with lower and higher values are assumed to be non-parametrical functions. The parameters for the Box-Cox transformation and the truncated normal distribution are estimated using an optimization method (maximum likelihood method). This results in a parametrical distribution of the non-diseased samples. The distribution's 2.5th and 97.5th percentiles are calculated to obtain RIs [13]. This step is performed for each quantity and for each time frame (Figure 1).

Creation of continuous splines

For each quantity, parametric curves for the 2.5th and 97.5th percentile were generated, one for the first 180 days of life and one for the remaining time span from 180 days to 18 years (Figure 2A and B). The R software's 'smooth.spline' method was used to convert the RI points into a smooth spline [18]. This method was chosen as no assumptions are made about the model behind the data, and the upper and lower reference ranges are drawn independently.

Results

Continuous indirect RIs (iRIs) from birth to adulthood were created for hemoglobin concentration, hematocrit, red cell

count, erythrocyte indices, RDWCV, platelet count and plasma sodium. The iRIs were analyzed for the influence of sample selection, were compared to instrument-specific RIs and to RIs determined using a validated approach.

Influence of sample selection

Generation of indirect RIs is based on the assumption that the portion of pathological samples has no significant influence on the generated RIs [13, 14]. Within a pediatric hospital's population, patients from intensive care units and oncological departments are expected to contain the largest proportion of pathological samples, particularly concerning blood cell count and electrolytes. Two different datasets were created, one containing all samples and one in which samples from patients in intensive care units and oncological units were removed to test the influence of sample selection.

Figure 2B shows a comparison of the resulting iRIs for hemoglobin, Figures for all other quantities are available in the Supplemental Data (Supplemental Figures 2–9). There is no difference in the iRIs after 180 days, whether samples from intensive care units and oncological units are removed, or not. All further analyses were therefore performed on the unfiltered data set.

In the first 180 days of life there is an apparent difference, which is especially pronounced in the first 100 days of life for the hemoglobin, red cell count and RDWCV RIs. This difference is attributable to the comparison of two different populations (preterm infants and term infants) which are not usually differentiated in clinical laboratory practice.

Figure 2 Calculation of hemoglobin RIs.

(A) Fitting a continuous spline (continuous line) to the discrete Hemoglobin RIs (crosses). (B) Comparison of calculated hemoglobin RIs. (Continuous line=All samples; Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated Hemoglobin RIs (continuous line) versus RIs provided by the manufacturer (dashed line) [11]. (D) Calculated Hemoglobin RIs (continuous line) versus RIs determined in the KiGGS study (crosses) [9]. Data are presented in conventional units; factors for converting to SI units are as follows: hemoglobin, 1 mg/dL=0.6206 mmol/L. KiGGS, German Health Interview and Examination Survey for Children and Adolescents.

Comparison with instrument-specific RIs

Comparison of different RIs is hampered by differences in methodology. RIs generated on the same analyzer system provided by the manufacturer were compared to the indirect RIs from our cohort. Figure 3 shows all iRIs, Figure 2C shows the hemoglobin iRIs in comparison with those provided by the analyzer's manufacturer [11]. Figures for all quantities are available in the Supplemental Data (Supplemental Figures 2–9).

The calculated iRIs show curves similar to the RIs supplied by the manufacturer but differ in terms of exact placement on the y-axis. This displacement is especially pronounced in the first 180 days of life, resulting from two main factors. As discussed above, the differences are to be expected, as different populations are studied. In addition, the sample sizes underlying the instrument-specific RIs are very low ($n=37$ – 116 for age groups younger than 60 days, $n=44$ – 1955 for older children) [11] in comparison to the sample size from our cohort ($n \geq 750$).

Comparison with RIs from the KiGGS study

Indirect methods for creating RIs have been criticized due to the lack of a precise definition of the reference population, the frequent failure to create RIs for non-normal distributions and being not applicable to laboratories other than the originating one [19]. A comparison with results determined using validated methods is therefore essential to assess the reliability of the indirect method applied in this study. For this comparison, the results of the KiGGS study were chosen, in which 14,076 blood samples were drawn

from a representative German cohort, and percentile charts generated for children aged from 1 to 18 years; a stratification according to the ethnic background of the children was not performed [9]. (RIs for children under the age of 1 year were not included in the study, platelets, RDWCV and plasma sodium were not measured. Blood samples were taken either sitting or lying throughout the day and children were not required to be fasting. Analysis of hemoglobin concentration, red cell count, red cell indices and hematocrit was performed on an Abbott Cell-Dyn 3500).

Due to the large amount of data available we could also create RIs for the first 180 days of life; data for this age group are missing in the KiGGS study.

Figure 2D shows the hemoglobin iRIs in comparison to the RIs determined in the KiGGS study (the 3rd and 97th percentile were used for iRIs and KiGGS RIs, Figures for the other comparisons are available in the Supplemental Data, Figures 2–9). The computed differences between the iRIs and the KiGGS RIs are shown in Table 1.

The hemoglobin, hematocrit and red cell count RIs are visually parallel, the lower RIs are nearly equivalent, the upper RIs diverge by about 0.6 g/dL (0.37 mmol/L), 1.9%, $0.2 \times 10^6/\mu\text{L}$ ($0.2 \times 10^{12}/\text{L}$), respectively. The MCV RIs also run parallel, although the upper iRIs tend to be higher, especially for female children. Also, the RIs generated by the KiGGS study show an ascending slope for the MCV RIs in the first 6 years of life, which is less pronounced in the iRIs. Except for an ascending slope for male MCH lower KiGGS RIs in the first 4 years of life, which has no representation in our iRIs, the MCH iRIs are congruent to the KiGGS RIs. The upper iRI curve for MCHC is congruent to the KiGGS RI curve, the lower iRI curve is shifted by about 1–2 g/dL.

	Male				Female			
	3rd percentile		97th percentile		3rd percentile		97th percentile	
	Mean difference	Maximum difference	Mean difference	Maximum difference	Mean difference	Maximum difference	Mean difference	Maximum difference
Hemoglobin, g/dL	1.4%	−4.7%	4.1%	+5.7%	0.9%	−2.2%	4.8%	+6.0%
MCH, pg	2.2%	+8.3%	0.5%	+1.3%	2.1%	+2.9%	0.7%	+1.5%
MCHC, g/dL	3.0%	+4.0%	0.9%	+1.4%	3.7%	+5.2%	0.2%	−0.7%
MCV, fL	1.2%	+6.1%	1.5%	+4.8%	1.9%	+4.6%	2.9%	+4.6%
Red cell count, $10^6/\mu\text{L}$	2.1%	+5.5%	3.7%	+5.3%	1.3%	+4.3%	4.0%	+5.1%
Hematocrit, %	1.7%	−3.3%	4.0%	+6.3%	3.3%	+4.6%	5.1%	+6.2%

Table 1 Relative differences between the calculated RIs and the RIs from the KiGGS study.

The relative difference of the RIs calculated using our indirect method and the RIs published in the KiGGS study [9] was calculated. For each quantity, the difference between our RIs and KiGGS RIs was calculated for age=1.5 years to age=17.5 years (in steps of 0.5 years). The mean (without sign) and maximum difference (with sign) of 3rd and 97th percentile is depicted above. KiGGS, German Health Interview and Examination Survey for Children and Adolescents; MCH, mean red cell hemoglobin; MCHC, mean red cell hemoglobin concentration; MCV, mean red cell volume.

Figure 3 Calculated RIs.

Continuous lines: male RIs; dashed lines: female RIs. Data are presented in conventional units; factors for converting to SI units are as follows: hemoglobin, 1 mg/dL=0.6206 mmol/L.

Differences between the calculated iRIs and the RIs generated using validated methods are in the range of allowed measurement uncertainty (Table 1), i.e., the guidelines of the German Medical Association for laboratory quality control [20] allow a measurement inaccuracy for hemoglobin, hematocrit, red cell count and sodium of 4.0%, 5.0%, 4.0% and 3.0%, respectively. Overall, the two different approaches show a near-exact match.

Comparison with RIs calculated using a healthy control group

To enable the comparison to population- and method-specific RIs we calculated RIs using the conventional approach of sampling a group of healthy children. Out-patient children presenting to our hospital for elective electroencephalography and endocrinology or neuropsychiatric evaluation (8 and 16 years) and out-patient children presenting to our hospital for elective electroencephalography, endocrinology or neuropsychiatric evaluation and elective surgery (160 days) were sampled. The 2.5th and 97.5th percentile of the resulting distribution after the elimination of outliers (Tukey's method) and the associated 95% confidence interval were calculated and compared to the indirect RIs (Table 2). The comparison shows that the great majority of calculated RIs are within the expected bounds.

Continuity

Most RIs currently used are valid for discrete intervals. This approach is reasonable, as it is impractical to create RIs using established methods for every possible age. The extension of the indirect method for RI determination allows the creation of continuous RIs for each quantity, especially important in pediatrics. (Supplemental Table 4 contains a summary of the calculated RIs, tables containing distinct upper and lower reference limits for each day of life from birth to 18 years are available as a downloadable Supplemental Data file, Supplemental Data Table (Reference Intervals).zip).

Discussion

For clinical decision making, the availability of reliable RIs is crucial. While technical advances have improved the precision of available laboratory tests and enabled

their widespread employment, the task of generating RIs remains challenging. Applying the conventional approach of establishing reference ranges by sampling a healthy reference population to pediatric laboratory quantities is laborious, ethically challenging and often not possible. There are therefore few age-specific RIs for the pediatric population and especially for neonates. Those pediatric RIs available are often heterogeneous and created decades ago using small reference populations. Standard textbooks [21, 22] and current guidelines for the diagnosis of childhood anemia [23] refer to hemoglobin RIs created in 1978 using a Finnish cohort of as few as 52 children in certain age groups [24]. In this context, the development of in-house, instrument-specific pediatric RIs with sufficient age-resolution using conventional methods seems especially challenging.

These problems have led to the development of alternative approaches for RI creation. Indirect methods based on hospital databases, containing both pathological and non-pathological samples, have been proposed and used [12–17]. Arzideh et al. have applied an indirect method to create valid RIs for creatinine and electrolytes [12, 13]. In these instances a homogenous population was examined and the problem of age-dependent RIs did not have to be considered. The feasibility of generating continuous hemoglobin concentration RIs based on a hospital population was demonstrated by Virtanen et al. [25], although in this case the separation of healthy and pathological samples was performed manually.

We augmented an indirect method to create continuous RIs from birth to adulthood. As proof of concept, we generated RIs for blood count quantities and compared them to existing RIs. The pediatric blood count was chosen, as it is one of the most commonly performed laboratory tests and its quantities are very important in clinical decision making. It is subject to significant and rapid change during development, making fine-grained age-resolution essential. Yet despite its importance in clinical decision making and its pervasiveness, available RIs are heterogeneous.

The results of the German KiGGS study were chosen for comparison. Those RIs were determined by direct methods, as recommended by the IFCC, using a sufficiently large sample size of 14,076 blood samples and represent the best available RIs for German children. Comparison of the RIs created using our approach and those generated in the KiGGS study shows only minor differences. Despite the considerable effort invested in this study, RIs for neonates and infants up to 12 months could not be established. Using our indirect approach, it was possible to create continuous RIs from birth to 18 years with sufficient resolution in the first weeks of life.

	n	2.5th percentile		97.5th percentile	
		Indirect method	Control group (95% CI)	Indirect method	Control group (95% CI)
Male, 160 days					
RDWCV, CV	86	11.8	12.1 (11.6–12.5)	15.3	15.5 (15.0–16.1)
Platelets, 1000/μL	91	174	191 (167–265)	565	515 (473–557)
Hematocrit, %	91	29.1	30.6175 (28.5–32.0)	40.1	43.0 (41.4–43.6)
MCH, pg	91	23	23 (23–25)	30	30 (29–30)
Hemoglobin, g/dL	91	9.8	10.0 (9.9–10.5)	13.5	13.9 (13.2–14.3)
MCV, fL	91	72	73 (70–74)	89	91 (88–92)
MCHC, g/dL	91	30	31 (31–32)	35	35 (34–35)
Red blood cells, 10 ⁶ /μL	91	3.5	3.6 (3.5–3.8)	5.3	5.3 (5.1–5.5)
Female, 160 days					
RDWCV, CV	83	11.2	11.9 (11.5–12.1)	14.8	15.4 (14.9–16.0)
Platelets, 1000/μL	84	200	134 (132–252)	605	568 (495–603)
Hematocrit, %	84	29.4	30.6 (28.7–31.6)	39.7	41.5 (39.3–42.2)
MCH, pg	84	24	25 (23–25)	30	30 (30–31)
Hemoglobin, g/dL	84	10.0	9.9 (9.4–10.2)	13.4	13.6 (13.1–15.0)
MCV, fL	84	73	76 (72–77)	91	92 (91–93)
MCHC, g/dL	84	30	30 (30–31)	35	35 (34–35)
Red blood cells 10 ⁶ /μL	84	3.4	3.5 (3.4–3.8)	5.1	5.0 (4.9–5.2)
Male, 8 years					
RDWCV, CV	228	12.1	12.3 (12.2–12.3)	14.0	14.3 (14.2–14.4)
Platelets, 1000/μL	236	177	191 (172–210)	394	379 (367–399)
Hematocrit, %	236	34.0	34.0 (33.8–34.7)	43.2	43.4 (42.7–43.8)
MCH, pg	236	25	25 (24–25)	30	30 (30–31)
Hemoglobin, g/dL	236	11.4	11.2 (10.9–11.8)	14.8	14.8 (14.5–15.5)
MCV, fL	236	75	75 (73–76)	88	90 (88–91)
MCHC, g/dL	236	32	32 (32–32)	36	36 (36–37)
Red blood cells, 10 ⁶ /μL	236	4.1	4.1 (4.0–4.2)	5.4	5.4 (5.3–5.6)
Female, 8 years					
RDWCV, CV	224	12.1	12.1 (11.8–12.1)	13.6	14.2 (14.0–14.4)
Platelets, 1000/μL	232	182	194.3 (176–212)	393	439 (416–447)
Hematocrit, %	232	34.5	34.6 (34.1–35.7)	43.5	42.9 (42.3–44.7)
MCH, pg	232	26	26 (26–26)	30	30 (30–31)
Hemoglobin, g/dL	232	11.4	11.5 (11.3–11.7)	14.9	14.4 (14.2–14.6)
MCV, fL	232	77	77 (76–78)	90	90 (90–91)
MCHC, g/dL	232	31	32 (32–32)	36	35 (35–35)
Red blood cells, 10 ⁶ /μL	232	4.1	4.0 (4.0–4.1)	5.3	5.3 (5.2–5.4)
Male, 16 years					
RDWCV, CV	167	12.0	12.2 (12.0–12.4)	13.9	14.7 (14.2–14.9)
Platelets, 1000/μL	169	149	153 (136–164)	320	344.7 (319–373)
Hematocrit, %	169	37.5	36.3 (35.0–37.3)	49.8	50.2 (48.9–51.6)
MCH, pg	169	27	26 (25–26)	32	31 (31–32)
Hemoglobin, g/dL	169	12.4	12.3 (11.4–12.6)	17.3	17.0 (16.4–17.4)
MCV, fL	169	78	76 (74–77)	94	95 (93–98)
MCHC, g/dL	169	32	31 (30–32)	36	36 (35–36)
Red blood cells, 10 ⁶ /μL	169	4.3	4.3 (4.2–4.6)	5.9	5.8 (5.6–5.9)
Female, 16 years					
RDWCV, CV	122	11.7	12 (11.6–12.2)	14.0	14.9 (14.6–15.0)
Platelets, 1000/μL	122	151	126 (114–158)	351	390 (349–400)
Hematocrit, %	122	35.4	35.2 (34.6–37.1)	44.9	45.6 (44.7–46.7)
MCH, pg	122	26	26 (26–27)	32	32 (31–32)
Hemoglobin, g/dL	122	11.5	11.5 (11.4–12.1)	15.3	15.2 (14.8–15.7)
MCV, fL	122	79	80 (78–81)	97	96 (94–99)

(Table 2 Continued)

	n	2.5th percentile		97.5th percentile	
		Indirect method	Control group (95% CI)	Indirect method	Control group (95% CI)
MCHC, g/dL	122	31	31 (31–32)	35	35 (35–35)
Red blood cells, 10 ⁶ /μL	122	3.9	3.9 (3.9–4.2)	5.3	5.2 (5.1–5.3)

Table 2 Comparison of indirect RIs with RIs calculated using a healthy control group.

The RIs calculated using the presented indirect method were compared to RIs determined using the conventional approach of sampling a healthy group of children. Out-patient children presenting for elective electroencephalography and endocrinology or neuropsychiatric evaluation (8 and 16 years) and out-patient children presenting for elective electroencephalography, endocrinology or neuropsychiatric evaluation and elective surgery (160 days) were sampled. The 2.5th and 97.5th percentile of the resulting distribution after the elimination of outliers (Tukey's method) and the associated 95% confidence interval are shown (n denotes the number of samples in the healthy control group). MCH, mean red cell hemoglobin; MCHC, mean red cell hemoglobin concentration; MCV, mean red cell volume; RDWCV, red cell distribution width variation coefficient.

The high concordance to RIs created using established methods is also reflected by the precise overlap of physiological landmarks, such as the Physiologic Anemia of Infancy between weeks 7 and 14 and the gender-specific diversion of, e.g., hemoglobin concentration after the age of 10 years. Also, the indirect RIs show the steep increase in platelets during the first 1–2 months after birth and the following decline. The decrease in fetal red blood cells is shown by a corresponding drop in MCH and MCV until about 5–6 months. The ability of our method to capture these biologic events is a strong indicator of its correctness. Plasma sodium representing less age-dependent quantities shows the expected constant RIs.

Unlike most common RIs for laboratory quantities – which are defined for discrete age intervals – the RIs developed using our approach are continuous from birth to adulthood. This seems sensible, as the underlying physiological changes which influence laboratory quantities are also continuous states rather than abrupt events.

We conclude that the approach presented generates valid RIs and can be used as an alternative, when conventional methods cannot be applied. Possible applications are the generation of RIs where none exist or when those available are of poor quality. Furthermore, the generation of RIs for defined subgroups such as ethnic populations, or children with chronic diseases is possible. With additional information the development of RIs stratified by gestational age is also possible.

Albeit less pronounced than during the first two decades of life, most laboratory quantities are subject to age-dependent variation from birth to old age. The use of age-dependent RIs should therefore not be restricted to pediatrics, but be expanded to adult medical specialties, particularly with respect to the increasing elderly patient population resulting from demographic change.

Conclusions

An extended indirect method was developed to generate continuous RIs from birth to adulthood with an excellent concordance to conventionally created RIs. This approach enables the establishment of RIs for a wider range of quantities and population groups where the conventional concept of RI creation is limited.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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5 Abkürzungsverzeichnis

RI, -s	Referenzintervall, -e
CALIPER	Canadian Laboratory Initiative on PEdiatric Reference Intervals
KiGGS	Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland
	German Health Interview and Examination Survey for Children and Adolescents
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
MCV	Mittleres Erythrozyteneinzelvolumen Mean red cell volume
MCH	Mittleres korpuskuläres Hämoglobin des Einzelerythrozyten Mean red cell hemoglobin
MCHC	Mittlere Hämoglobinkonzentration des Einzelerythrozyten Mean red cell hemoglobin concentration
RDWCV	Erythrozytenverteilungsbreite Red cell distribution width coefficient of variation
iRIs	Indirekte Referenzintervalle

Supplemental data

Quantity	No. of samples		No. of time frames	
	Total	w/o ICU, ONC	Total	w/o ICU, ONC
Hemoglobin	58,065	42,845	127	117
MCV	58,070	42,847	127	117
MCH	58,067	42,844	127	117
MCHC	58,066	42,844	127	117
Platelets	58,040	42,836	127	117
Red blood cells	58,071	42,848	127	117
Hematocrit	58,069	42,846	127	117
RDWCV	56,253	41,517	122	114
Plasma sodium	60,394	45,126	125	114

Supplemental Table 1 Quantities for which RIs were calculated.

Sample counts differ since not all measurements could be performed on all blood samples, RDWCV measurement was not performed until 02/2008.

ICU, intensive care unit; MCH, mean red cell hemoglobin; MCHC, mean red cell hemoglobin concentration; MCV, mean red cell volume; ONC, oncological unit; RDWCV, red cell distribution width variation coefficient.

	Control sample target value (low)	Inaccuracy, bias	Imprecision, CV	QMMA	n	Control sample target value (high)	Inaccuracy, bias	Imprecision, CV	QMMA	n
Hemoglobin	5.6 g/dL	0.3%	0.70%	0.75%	34	12.3 g/dL	0.0%	0.47%	0.46%	32
MCV	74.2 fL	1.1%	0.49%	0.85%	34	80.8 fL	1.5%	0.41%	1.28%	32
MCH	24.5 pg	1.7%	1.09%	0.48%	34	27.8 pg	1.2%	0.57%	0.36%	32
MCHC	32.9 g/dL	0.2%	1.07%	0.35%	34	34.5 g/dL	0.1%	0.74%	0.25%	32
Platelets	$52 \times 10^3 / \mu\text{L}$	0.2%	3.53%	3.44%	34	$204 \times 10^3 / \mu\text{L}$	1.0%	1.42%	2.15%	32
Red blood cells	$2.29 \times 10^6 / \mu\text{L}$	1.8%	0.71%	1.90%	34	$4.42 \times 10^6 / \mu\text{L}$	1.3%	0.48%	1.38%	32
Hematocrit	17%	0.6%	0.79%	1.01%	34	35.7%	0.2%	0.66%	0.68%	32
Plasma sodium	115 mmol/L	0.0%	0.99%	1.44%	36	140 mmol/L	0.0%	0.59%	0.80%	33

Supplemental Table 2 Measurement accuracy and precision data.

Accuracy and precision data for selected quantities in a single month. The results of measurements of control samples (with defined target values) as part of our routine quality control are shown. QMMA denotes quadratic mean of error of measurement and is a measurement of

both accuracy and precision, it is defined as follows: $QMMA = \sqrt{\frac{1}{n} \sum_{i=1}^n (x_i - x_0)^2}$ (x_i – sample value; x_0 – target value).

CV, coefficient of variation; MCH, mean red cell hemoglobin; MCHC, mean red cell hemoglobin concentration; MCV, mean red cell volume; RDWCV, red cell distribution width variation coefficient.

Month	Hemoglobin, g/dL	MCV, fL	MCH, pg	MCHC, g/dL	Platelets, 1000/ μ L	Red blood cells, 10 ⁶ / μ L	Hematocrit, %	RDWCV, CV	Plasma sodium, mmol/L
2008/01	12.5	87	29	33	262	4.41	38.0	14.3	136
2008/02	12.3	86	29	33	256	4.36	37.4	14.4	136
2008/03	12.3	86	29	33	251	4.34	37.5	14.5	136
2008/04	12.5	87	29	33	252	4.37	37.9	14.6	136
2008/05	12.6	87	29	33	251	4.36	37.9	14.6	136
2008/06	12.4	85	29	34	257	4.34	37.2	14.1	136
2008/07	12.3	85	29	34	240	4.34	36.6	13.8	136
2008/08	12.2	86	29	34	253	4.28	36.6	13.8	136
2008/09	12.8	87	29	34	254	4.43	38.2	14.1	136
2008/10	12.4	87	29	33	265	4.36	37.4	14.2	136
2008/11	12.4	87	29	33	260	4.39	37.9	14.2	136
2008/12	12.5	87	29	33	256	4.43	38.0	14.4	136
2009/01	12.5	87	29	33	251	4.42	38.2	14.3	137
2009/02	12.3	86	28	33	259	4.37	37.7	14.2	136
2009/03	12.3	87	29	33	254	4.35	37.3	14.7	136
2009/04	12.2	86	29	33	256	4.32	37.0	14.4	136
2009/05	12.3	87	29	33	247	4.32	37.6	14.3	136
2009/06	12.4	86	29	33	241	4.36	37.7	14.2	136
2009/07	12.3	86	29	34	247	4.40	37.0	14.2	135
2009/08	12.3	85	29	34	248	4.36	36.7	14.5	136
2009/09	12.4	86	29	33	243	4.38	37.4	14.3	137
2009/10	12.4	87	29	33	255	4.41	38.0	14.2	137
2009/11	12.3	87	29	33	249	4.40	37.8	14.2	137
2009/12	12.2	87	28	33	254	4.34	37.3	14.2	137
2010/01	12.3	87	29	33	248	4.38	37.8	14.5	137
2010/02	12.6	86	28	33	253	4.45	38.5	14.2	137
2010/03	12.6	87	29	33	249	4.45	38.5	14.4	137
2010/04	12.5	87	29	33	243	4.43	38.3	14.6	137
2010/05	12.6	87	29	33	237	4.46	38.7	14.7	137

Supplemental Table 3 Median values of quantities over time.

Monthly median values for all quantities examined. MCH, mean red cell hemoglobin; MCHC, mean red cell hemoglobin concentration; MCV, mean red cell volume; RDWCV, red cell distribution width variation coefficient.

Supplemental Figure 1 Timeline generation algorithm.

Supplemental Figure 2 Calculation of red cell count RIs.

(A) Fitting a continuous spline (continuous line) to the discrete red cell count RIs (crosses). (B) Comparison of calculated red cell count RIs. (Continuous line=All samples. Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated Red cell count RIs (continuous line) versus RIs provided by the manufacturer (dashed line) [11]. (D) Calculated red cell count RIs (continuous line) versus RIs determined in the KiGGS study (crosses) [9].

KiGGS, German Health Interview and Examination Survey for Children and Adolescents.

Supplemental Figure 3 Calculation of hematocrit RIs.

(A) Fitting a continuous spline (continuous line) to the discrete hematocrit RIs (crosses). (B) Comparison of calculated hematocrit RIs. (Continuous line=All samples. Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated hematocrit RIs (continuous line) versus RIs provided by the manufacturer (dashed line) [11]. (D) Calculated hematocrit RIs (continuous line) versus RIs determined in the KiGGS study (crosses) [9].

KiGGS, German Health Interview and Examination Survey for Children and Adolescents.

Supplemental Figure 4 Calculation of MCH RIs.

(A) Fitting a continuous spline (continuous line) to the discrete MCH RIs (crosses). (B) Comparison of calculated MCH RIs. (Continuous line=All samples. Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated MCH RIs (continuous line) versus RIs provided by the manufacturer (dashed line) [11]. (D) Calculated MCH RIs (continuous line) versus RIs determined in the KiGGS study (crosses) [9].

KiGGS, German Health Interview and Examination Survey for Children and Adolescents; MCH, mean red cell hemoglobin.

Supplemental Figure 5 Calculation of MCHC RIs.

(A) Fitting a continuous spline (continuous line) to the discrete MCHC RIs (crosses). (B) Comparison of calculated MCHC RIs. (Continuous line=All samples. Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated MCHC RIs (continuous line) versus RIs provided by the manufacturer (dashed line) [11]. (D) Calculated MCHC RIs (continuous line) versus RIs determined in the KiGGS study (crosses) [9].

KiGGS, German Health Interview and Examination Survey for Children and Adolescents; MCHC, mean red cell hemoglobin concentration.

Supplemental Figure 6 Calculation of MCV RIs.

(A) Fitting a continuous spline (continuous line) to the discrete MCV RIs (crosses). (B) Comparison of calculated MCV RIs. (Continuous line=All samples. Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated MCV RIs (continuous line) versus RIs provided by the manufacturer (dashed line) [11]. (D) Calculated MCV RIs (continuous line) versus RIs determined in the KiGGS study (crosses) [9].

KiGGS, German Health Interview and Examination Survey for Children and Adolescents; MCV, mean red cell volume.

Supplemental Figure 7 Calculation of RDWCV RIs.

(A) Fitting a continuous spline (continuous line) to the discrete RDWCV RIs (crosses). (B) Comparison of calculated RDWCV RIs. (Continuous line=All samples. Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated RDWCV RIs (continuous line) versus RIs provided by the manufacturer (dashed line) [11].

RDWCV, red cell distribution width variation coefficient.

Supplemental Figure 8 Calculation of platelet count RIs.

(A) Fitting a continuous spline (continuous line) to the discrete platelet count RIs (crosses). (B) Comparison of calculated platelet count RIs. (Continuous line=All samples. Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated platelet count RIs (continuous line) versus RIs provided by the manufacturer (dashed line) [11].

Supplemental Figure 9 Calculation of plasma sodium RIs.

(A) Fitting a continuous spline (continuous line) to the discrete plasma sodium RIs (crosses). (B) Comparison of calculated plasma sodium RIs. (Continuous line=All samples. Dashed black line=Samples from intensive care units/oncological units removed). (C) Calculated plasma sodium RIs (continuous line) versus literature RIs (dashed line) [11].

Age	Hemoglobin, g/dL				MCV, fL				MCH, pg			
	Male		Female		Male		Female		Male		Female	
	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th
0 day	12.6	20.2	12.8	20.5	95	116	97	118	32	39	32	39
1 day	12.3	20.1	12.5	20.3	95	116	97	118	32	39	32	39
2 days	12.0	20.0	12.2	20.2	95	116	96	117	32	39	32	39
3 days	11.7	19.9	11.9	20.0	95	116	96	117	32	39	32	39
4 days	11.4	19.8	11.6	19.9	94	115	96	117	32	39	32	39
5 days	11.1	19.7	11.4	19.7	94	115	96	117	32	39	32	39
6 days	10.9	19.6	11.1	19.6	94	115	95	117	32	39	32	39
1 week	10.6	19.5	10.8	19.4	94	115	95	116	32	39	32	39
2 weeks	9.2	19.3	9.4	18.8	92	113	93	115	31	38	31	38
3 weeks	8.2	18.8	8.3	18.2	90	112	91	114	30	38	31	38
1 month	7.6	17.4	7.2	17.0	88	110	89	112	29	37	30	37
2 months	7.6	14.1	7.3	13.8	81	104	82	106	27	35	27	34
3 months	8.2	13.3	8.5	13.2	76	99	77	100	25	33	26	32
4 months	9.3	13.5	9.5	13.3	73	93	74	95	24	31	25	31
5 months	9.7	13.5	9.9	13.4	72	89	73	91	23	30	24	30
6 months	9.8	13.5	9.9	13.5	72	88	73	90	23	30	24	30
8 months	9.8	13.5	9.9	13.5	72	88	73	90	23	30	24	30
10 months	9.8	13.6	9.9	13.6	72	88	73	90	23	30	24	30
1 year	9.9	13.6	10.0	13.6	72	88	73	90	23	30	24	30
2 years	10.2	13.8	10.2	13.9	73	88	74	89	24	29	24	30
3 years	10.4	13.9	10.5	14.1	73	87	74	89	24	29	24	30
4 years	10.6	14.1	10.7	14.2	74	87	75	88	24	30	25	30
5 years	10.8	14.3	10.8	14.4	74	87	75	88	25	30	25	30
6 years	11.0	14.4	11.0	14.5	74	87	76	89	25	30	25	30
8 years	11.4	14.7	11.3	14.8	75	88	77	89	25	30	26	30
10 years	11.6	15.0	11.6	15.0	76	88	77	91	26	30	26	31
12 years	11.9	15.5	11.6	15.3	77	90	78	92	26	31	26	31
14 years	12.1	16.3	11.6	15.4	77	91	79	94	26	31	26	32
16 years	12.3	17.1	11.6	15.4	78	93	79	96	27	32	26	32

Age	MCHC, g/dL				Platelets, 1000/ μ L				Red blood cells, 10 ⁶ / μ L			
	Male		Female		Male		Female		Male		Female	
	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th
0 day	31	36	31	36	97	439	116	474	3.17	5.97	3.20	5.89
1 day	31	36	31	36	99	447	117	483	3.15	5.96	3.18	5.87
2 days	31	36	31	36	101	456	119	491	3.14	5.94	3.17	5.85
3 days	31	36	31	36	103	464	120	499	3.12	5.93	3.15	5.84
4 days	31	36	31	36	105	472	121	507	3.10	5.92	3.13	5.82
5 days	31	36	31	36	107	481	122	516	3.09	5.90	3.12	5.81
6 days	31	36	31	36	109	489	124	524	3.07	5.89	3.10	5.79
1 week	31	36	31	36	111	497	125	532	3.06	5.88	3.09	5.78
2 weeks	31	36	31	36	125	549	134	583	2.95	5.78	2.98	5.67
3 weeks	31	36	31	36	140	592	144	623	2.85	5.69	2.88	5.56
1 month	31	36	31	36	161	634	159	662	2.73	5.56	2.76	5.41
2 months	30	36	30	35	202	690	195	702	2.57	5.28	2.60	5.08
3 months	30	35	30	35	220	673	213	689	2.69	5.18	2.72	4.96
4 months	30	35	30	35	207	617	212	653	3.07	5.21	3.07	5.00
5 months	30	35	30	35	176	568	201	608	3.46	5.27	3.41	5.10
6 months	30	35	30	35	168	552	196	592	3.58	5.28	3.53	5.16
8 months	30	35	30	35	168	549	195	587	3.60	5.28	3.55	5.17
10 months	30	35	30	35	167	544	193	581	3.63	5.28	3.57	5.19
1 year	30	35	30	35	167	537	192	573	3.65	5.28	3.60	5.21

(Supplemental Table 4 Continued)

Age	MCHC, g/dL				Platelets, 1000/ μ L				Red blood cells, 10 ⁶ / μ L			
	Male		Female		Male		Female		Male		Female	
	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th
2 years	31	35	31	35	167	496	185	522	3.79	5.28	3.76	5.26
3 years	31	36	31	35	168	472	183	491	3.86	5.29	3.84	5.28
4 years	31	36	31	35	170	452	183	465	3.93	5.29	3.91	5.29
5 years	31	36	31	35	173	436	183	444	3.98	5.30	3.96	5.30
6 years	31	36	31	35	175	422	184	426	4.03	5.31	4.01	5.31
8 years	32	36	31	36	177	399	183	398	4.09	5.35	4.07	5.32
10 years	32	36	31	36	174	379	177	377	4.14	5.43	4.09	5.33
12 years	32	36	31	36	167	365	169	360	4.19	5.53	4.07	5.34
14 years	32	36	31	35	159	349	162	352	4.25	5.66	4.02	5.32
16 years	32	36	31	35	151	326	154	351	4.31	5.83	3.93	5.30

Age	Hematocrit, %				RDWCV, CV				Plasma sodium, mmol/L			
	Male		Female		Male		Female		Male		Female	
	2.5th	97.5th	2.5th	2.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th
0 day	34.2	61.5	36.1	62.1	14.6	19.0	14.1	19.2	128	144	129	143
1 day	34.0	61.2	35.9	61.8	14.5	18.9	14.1	19.1	128	144	129	142
2 days	33.8	61.0	35.6	61.6	14.5	18.9	14.1	19.1	128	144	129	142
3 days	33.5	60.7	35.4	61.3	14.5	18.9	14.0	19.1	128	143	129	142
4 days	33.3	60.5	35.2	61.0	14.4	18.9	14.0	19.0	128	143	129	142
5 days	33.1	60.2	35.0	60.7	14.4	18.8	13.9	19.0	128	143	129	142
6 days	32.8	60.0	34.7	60.4	14.3	18.8	13.9	19.0	128	143	129	142
1 week	32.6	59.7	34.5	60.2	14.3	18.8	13.9	18.9	128	143	129	142
2 weeks	31.0	57.9	33.0	58.2	14.1	18.6	13.6	18.7	129	142	129	141
3 weeks	29.5	56.1	31.5	56.3	13.8	18.3	13.3	18.5	129	141	130	141
1 month	27.6	53.7	29.7	53.6	13.5	18.1	13.0	18.2	130	140	130	140
2 months	24.5	47.0	26.4	46.4	12.6	17.3	12.1	17.2	131	139	131	139
3 months	24.5	42.7	25.8	41.8	12.0	16.5	11.4	16.3	132	139	131	140
4 months	26.6	40.5	27.4	39.7	11.7	15.7	11.1	15.3	132	140	132	140
5 months	28.9	40.1	29.3	39.7	11.8	15.3	11.2	14.9	131	140	132	140
6 months	29.8	40.3	30.0	40.2	11.9	15.3	11.3	14.9	131	140	132	140
8 months	29.9	40.4	30.1	40.4	11.9	15.3	11.4	14.9	131	140	131	140
10 months	30.1	40.5	30.3	40.6	12.0	15.3	11.4	15.0	131	140	131	140
1 year	30.3	40.6	30.5	40.8	12.0	15.3	11.5	15.0	131	140	131	140
2 years	31.2	41.0	31.4	41.7	12.1	15.1	11.8	14.9	131	141	131	141
3 years	31.7	41.3	32.0	42.0	12.1	14.8	11.9	14.6	131	141	132	141
4 years	32.1	41.6	32.5	42.2	12.1	14.6	12.0	14.4	132	141	132	141
5 years	32.5	41.9	33.0	42.4	12.2	14.4	12.1	14.2	132	141	132	141
6 years	32.9	42.3	33.4	42.7	12.2	14.3	12.1	14.0	132	141	132	141
8 years	33.8	43.0	34.3	43.3	12.1	14.0	12.1	13.7	133	141	133	142
10 years	34.6	44.0	34.9	44.1	12.1	14.0	12.0	13.6	133	141	133	142
12 years	35.4	45.3	35.4	44.7	12.0	14.0	12.0	13.6	133	141	133	142
14 years	36.3	47.1	35.5	45.1	12.0	14.0	11.9	13.7	133	142	133	141
16 years	37.3	49.3	35.5	45.0	12.0	13.9	11.8	13.9	133	142	133	141

Supplemental Table 4 Calculated indirect reference intervals.
 Calculated age-dependent percentiles for each quantity.

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