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ORIGINAL ARTICLE

Hematology reference intervals in 6–12-year-old children: the health-oriented pedagogical project (HOPP)

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ABSTRACT

Reference intervals are essential for correct interpretation of laboratory test results, supporting clinicians in distinguishing between healthy and sick individuals. The present study aims to establish pediatric reference intervals for hematological parameters based on a large population of healthy schoolchildren. Blood samples were obtained from 1351 children 6–12 years of age participating in the Health-Oriented Pedagogical Project (HOPP). Reference intervals for hematological parameters were estimated by the non-parametric method following the CLSI C28-A3 guidelines. Reference intervals were estimated as 2.5th and 97.5th percentiles with corresponding 90% confidence intervals. While hematocrit and MCV required age and sex partitioning, hemoglobin and erythrocytes were partitioned for age. The remaining parameters, MCH, MCHC, platelets and white blood cell counts did not require partitioning. While red blood cell parameters exhibited an increasing trend with age, there was a slight decrease in leukocytes, lymphocytes, basophils and platelets with age. The remaining parameters were stable across our age span.

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Reference values; hematology; child; pediatrics; Norway

Introduction

Reference intervals are important decision-making tools that aid clinicians in distinguishing between health and disease. Complete blood count and hemoglobin concentration are among the most ordered tests for assessment of health status and essential in the diagnosis of diseases of the blood, including infections, leukemia, and anemia.

Reference intervals should optimally be derived from the distribution of values in a healthy and relevant population. As direct methods are more resource demanding, guidelines have been established for estimation of reference intervals using indirect methods [1]. Clinical analysis of blood parameters is usually only performed in children for diagnosis based on a suspicion of disease, and hence large number of measurements from healthy children are not easily obtained. Because establishing reference intervals in a pediatric population is challenging [2], laboratories often rely on reference intervals from the literature that may not be representative of the local population or laboratory setting. The Clinical and Laboratory Standards institute (CLSI) and the International Federation for Clinical Chemistry (IFCC) [3] recommend that each laboratory should establish or verify reference values in a relevant healthy population. Hematology reference intervals for children have been established in different countries, as in the studies by Adeli et al. in Canada [4], Aldrimer et al. in Sweden [5], and Zierk et al. in Germany [6].

In the present study, the objective was to establish hematology reference intervals from 1351 healthy Norwegian school children in the age span of 6–12 years old.

Methods

Study population

The Health Oriented Pedagogical Project (HOPP) is a controlled longitudinal school-based physical activity intervention program and is described in detail by Fredriksen et al. [7]. In short, children from nine primary schools in south-east Norway were invited to participate. A total of 2297 children (82%) participated in the first year. Blood samples were taken annually, and children were given an opportunity to opt out of the blood sample test while remaining in the study. Children who had had infections during the previous week were asked not to donate a blood sample. At baseline (2015), 1351 children (657 girls) provided blood samples (59.0% of participants) where hematological parameters were measured. Age was defined as age at the day of venepuncture. The ethnicity of the children was not registered; however, the majority of the children were of Caucasian origin. Reference intervals for iron status analytes (ferritin, iron, transferrin and transferrin saturation) in this cohort have been previously published [8].

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Specimen collection and analysis

Blood samples were collected in the nonfasting state between 8:00 a.m. and 1:30 p.m without strenuous exercise prior to sample collection. Blood was drawn from the antecubical vein in 4-mL K2EDTA tubes (Vacuette®, Greiner Bio-One, Austria) by a trained phlebotomist. Samples were collected in the respective schools, and the children had not participated in strenuous exercise prior to sample collection. At the end of each collection session, samples were transported to the central laboratory at Vestfold Hospital Trust and immediately analyzed according to standard procedures. The laboratory is accredited in accordance with NS-EN ISO 15189. All presented analytes were individually accredited and participated in external proficiency testing schemes from NOKLUS [9]. The hematology analyses were performed on Sysmex XE 2100 (Sysmex Corporation, Kobe, Japan) with reagents from the supplier. Of the 1351 blood samples, 1267 were analyzed for all hematological parameters included in this study. The analysis was incomplete for 84 samples, where 84 lacked measurements of mean corpuscular volume (MCH) and MCH concentration (MCHC), 81 lacked differential count and platelets, and 32 lacked measurement of erythrocyte volume fraction (EVF).

Statistics

The statistical analysis was conducted according to the CLSI C28-A3c guideline [3]. Scatter plots and histograms were made using the R software (version 3.6.1) [10] and used for visual inspection of the data. In addition, the data distribution was evaluated using the package fitdistrplus [11] in R. Age and gender partitioning was evaluated formally according to the criteria introduced by Lahti et al. [12]. Partitioning is recommended if <0.9% or >4.1% of the subpopulations are outside the reference limits of the combined population. As the number of children in the two age subgroups was unequal, the multiplication method was applied to adjust the age group 6–8 years [13]. Reference limits (2.5^{th} and 97.5^{th} percentiles) were estimated using the nonparametric method using the referenceIntervals package in R [14]. 90% confidence intervals (CI) were calculated by bootstrapping, $R = 5000$. Outliers were detected using the Horn's algorithm. After individual consideration, all outliers were included in the analysis of reference limits. Scatter plots for figures were made using ggplot2 in R [15], using the geom_smooth function to make linear regression lines with 0.95 confidence intervals. For red cell analytes, samples with results below the present reference interval in our laboratory (MCH < 25 pg and or ferritin < 15 µg/L) were excluded when estimating reference limits.

Ethics and consent to participate

The study is registered in as a clinical trial (ClinicalTrials.gov Identifier: NCT02495714), and the Regional Committees for Medical and Health Research Ethics approved the study protocol (2014/2064/REK sør-øst). All children included in the study provided written parental consent, and participants were able to withdraw from the study at any stage of data collection without explanation. The children could also opt out on parts of the data collection.

Results

The descriptive data of the study population ($n = 1351$) is presented in Table 1. The study participants were between 6 and 12 years old, and there was a larger number of participants in the older age group (9–12). Reference intervals for all hematological analytes are presented in Table 2 as 2.5^{th} and 97.5^{th} percentiles with a 90% confidence interval (CI) for the lower and upper limits. A visual inspection of the data and simple linear regression showed a small, but significant effect of age for most of the analytes measured (Table 3 and Figures 1–2). Because no breaking point was evident, the population was arbitrary split in two (6–8 vs. 9–12 years). The Lahti test [12] revealed that hemoglobin (Hb) and erythrocytes should be partitioned for age, while hematocrit and mean corpuscular volume (MCV) should be partitioned for age and sex. The remaining analytes did not require partitioning, but as platelets have been partitioned by age in other studies, we include both unpartitioned and partitioned data for this analyte. Scatter plots for red blood cell related parameters are displayed in Figure 1, while white blood cell parameters and platelets are displayed in Figure 2.

Red blood cell parameters exhibited an increasing trend with age. Most pronounced for Hb, hematocrit, erythrocyte count and MCH while negligible for MCHC. No difference between girls and boys was observed for any of the analytes studied. For white blood cells, there was a trend of decreasing values for leukocytes, lymphocytes, and basophils with age. Monocytes, neutrophils, and eosinophils remained stable across age for our age span. Platelets decrease slightly with age, and there are no clear sex differences.

Table 1. Descriptive data of the study population ($n = 1351$).

	Unit		
Girls,	n (%)	657	(48.6)
Age 6–8	n (%)	478	(35.4)
Age 9–12	n (%)	873	(64.6)
Weight (age 6–8)	kg	26.5	(5.3)
Weight (age 9–12)	kg	37.6	(8.5)
Height (age 6–8)	cm	128.3	(7.4)
Height (age 9–12)	cm	145.5	(8.8)
BMI (age 6–8)	kg/m^2	16.0	(2.1)
BMI (age 9–12)	kg/m^2	17.6	(2.8)
Hb	g/dL	13.321	(0.663)
Hematocrit (EVF)	%	39.6	(2.1)
Erythrocyte count	$10^{12}/L$	4.8	(0.28)
MCV	fL	83.4	(3.704)
MCH	pg	28.0	(1.411)
MCHC	g/dL	33.6	(0.912)
Leukocyte count	$10^9/L$	6.47	(1.759)
Lymphocyte count	$10^9/L$	2.62	(0.651)
Monocyte count	$10^9/L$	0.53	(0.165)
Neutrophil count	$10^9/L$	3.07	(1.312)
Eosinophil count	$10^9/L$	0.28	(0.290)
Basophil count	$10^9/L$	0.03	(0.019)
Platelet count	$10^9/L$	301.9	(58.12)

Results are presented as mean (SD) if not stated otherwise. EVF: Erythrocyte volume fraction; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; IQR: Interquartile range.

Table 2. Age- and gender-specific pediatric reference intervals for hematology analytes.

	Unit	*n* (adjusted)	Age	Sex	2.5th percentile (90% CI)		97.5th percentile (90% CI)		Outliers
Hemoglobin	g/dL	440 (880)	6–8	FM	11.9	(11.8–12.1)	14.3	(14.2–14.4)	21
		808	9–12	FM	12.2	(12.1–12.3)	14.8	(14.7–14.9)	
Hematocrit	%	204 (408)	6–8	F	36	(36–37)	42	(41–42)	5
(EVF)		379	9–12	F	37	(37–38)	44	(43.5–44.0)	
		218 (436)	6–8	M	35	(35–35)	42	(42–42)	
		417	9–12	M	36	(35.6–36)	44	(43.5–44.5)	
Erythrocytes	10^{12}/L	440 (880)	6–8	FM	4.2	(4.2–4.2)	5.3	(5.2–5.4)	49
		808	9–12	FM	4.2	(4.3–4.4)	5.3	(5.2–5.3)	
MCV	fL	201 (402)	6–8	F	77.0	(77.0–77.9)	88.9	(88.9–90.9)	22
		357	9–12	F	79	(78–80)	92	(92–93)	
		213 (426)	6–8	M	76	(75–77)	88	(88–89)	
		399	9–12	M	78	(78–79)	89	(88–89)	
MCH	pg	1248 (1688)	6–12	FM	26	(26–26)	30	(30–30)	10
MCHC	g/dL	1170 (1584)	6–12	FM	32	(NA)	35	(NA)	22
Leukocytes	10^9/L	1351 (1829)	6–12	FM	3.86	(3.81–3.92)	10.73	(10.15–11.13)	4
Lymphocytes	10^9/L	1270 (1721)	6–12	FM	1.53	(1.49–1.56)	4.17	(4.05–4.28)	17
Monocytes	10^9/L	1270 (1721)	6–12	FM	0.29	(0.29–0.30)	0.91	(0.90–0.92)	6
Neutrophils	10^9/L	1270 (1721)	6–12	FM	1.29	(1.24–1.32)	6.53	(5.93–6.95)	
Eosinophils	10^9/L	1267 (1717)	6–12	FM	0.05	(0.05–0.06)	1.02	(0.99–1.10)	9
Basophils	10^9/L	1267 (1717)	6–12	FM	0.01	(NA)	0.08	(0.07–0.08)	10
Platelets	10^9/L	1270 (1721)	6–12	FM	204	(201–209)	427	(414–435)	16
		451 (902)	6–8	FM	200	(190–204)	435	(425–453)	
		819	9–12	FM	206	(203–211)	423	(406–431)	

EVF: Erythrocyte volume fraction; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; NA: unable to calculate in referenceInterval, outliers were detected by Horn's algorithm.

Table 3. Linear regression results for hematological analytes with age as predictor.

Analyte	B-coefficient	(95% CI)	Standard error	*t*	*p*-value	R^{2*}
Hemoglobin	0.122	(0.101 to 0.143)	0.011	11.33	<0.001	0.0862
Hematocrit (EVF)	0.390	(0.329 to 0.452)	0.031	12.42	<0.001	0.1041
Erythrocytes	0.022	(0.014 to 0.030)	0.004	5.18	<0.001	0.0188
MCV	0.428	(0.314 to 0.541)	0.058	7.39	<0.001	0.0407
MCH	0.122	(0.079 to 0.164)	0.022	5.64	<0.001	0.0223
MCHC	−0.026	(−0.054 to 0.003)	0.015	−1.76	0.079	0.0016
Leukocytes	−0.092	(−0.145 to −0.039)	0.027	−3.39	<0.001	0.0077
Lymphocytes	−0.050	(−0.070 to −0.030)	0.010	−4.89	<0.001	0.0177
Monocytes	−0.003	(−0.008 to 0.003)	0.003	−0.98	0.327	<0.0001
Neutrophils	−0.021	(−0.062 to 0.016)	0.021	−1.23	0.31	<0.0001
Eosinophils	−0.014	(−0.023 to −0.005)	0.005	−3.10	0.002	0.0067
Basophils	−0.001	(−0.002 to −0.001)	<0.001	−4.04	<0.001	0.0119
Platelets	−3.727	(−5.532 to −1.921)	0.920	−4.05	<0.001	0.0120

EVF: Erythrocyte volume fraction; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration, *adjusted R^2.

Discussion

Establishing reference intervals that are relevant for the local population is an important responsibility for clinical laboratories. However, collecting samples from a large number of healthy children is expensive and difficult to accomplish. Due to rapid changes in children with increasing age, it is essential to obtain enough samples per age group. Here, we present reference intervals for hematological parameters, based on blood samples from 1351 healthy school children in the HOPP study in Norway. Age- and sex partitioning was performed based on criteria by Lahti et al. [12].

The increasing trend over age for Hb, hematocrit, erythrocyte count and MCV is similar to previous studies [4–6,16,17]. The reference limits for Hb found in this study are similar to previous studies [4,5]. The age partitioning required for Hb is largely dependent on the age span included [4,5]. There were no sex differences for Hb in our age span, and while others have found sex differences, these are after the age of 12 [5,6]. The reference limits for hematocrit, erythrocyte count and MCV were similar to previous studies [4,5]. While erythrocyte count required partitioning for age, both hematocrit and MCV required age and sex partitioning. While others have found age differences, the sex differences for hematocrit and MCV are more evident from 12 years of age [4–6]. MCH is only slightly increasing for 6–12 years old's, while the trend for MCHC is flat, similar to previous findings [4,6]. There are some differences between the reference limits in this study and the studies by others, and this is likely due to the age span of the children included. Most studies include children from a much younger age in the lowest age partitioning. For instance, the lower Hb limit of 10.7 g/dL found by Aldrimer et al. in Sweden [5] is for the age partition of 6 months–7 years old. In a recent publication from the CALIPER study, Tahmasebi et al. reported a lower limit of 11.4 g/dL in 248

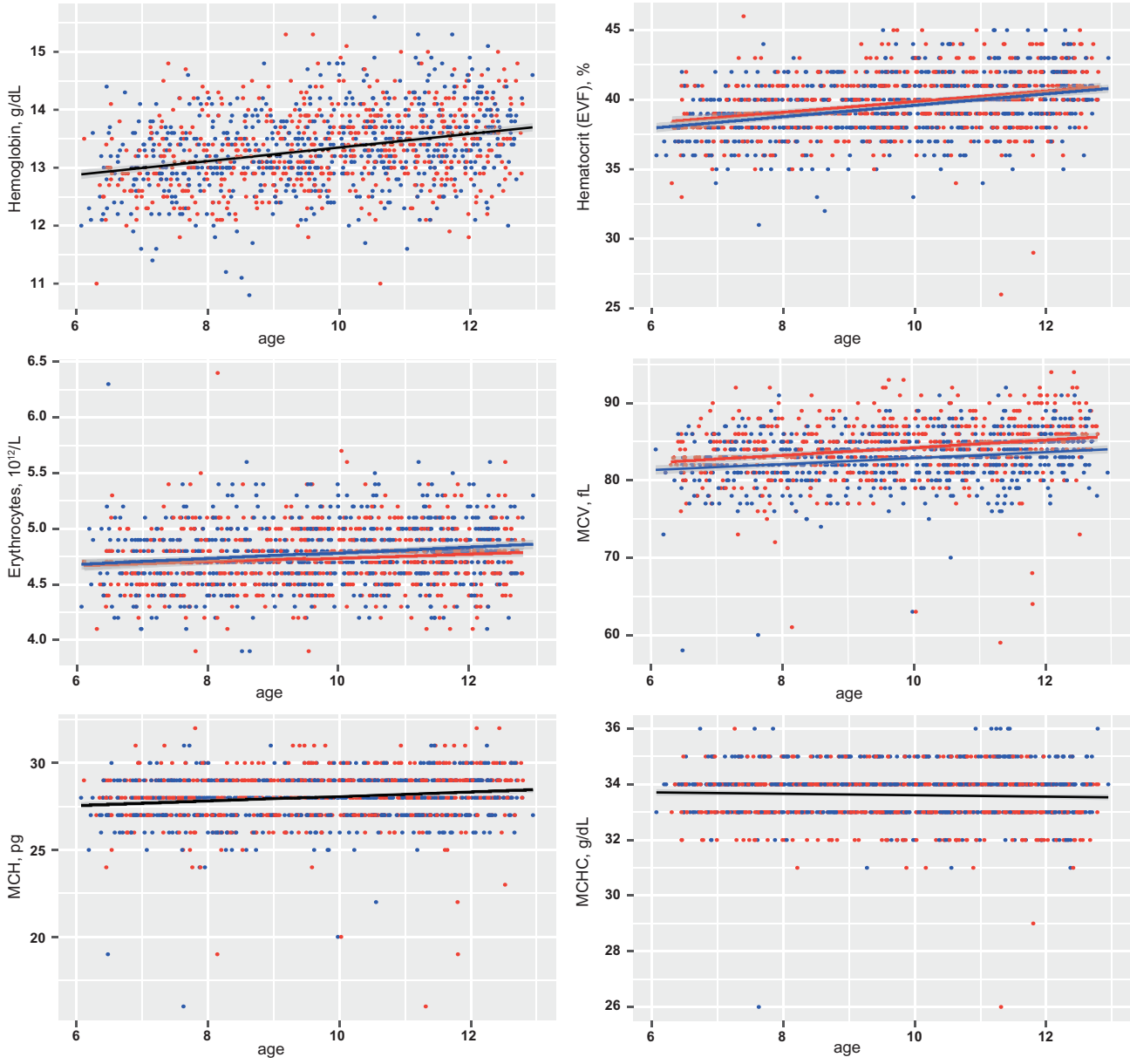

Figure 1. Age-dependent scatterplots of hemoglobin, hematocrit (EVF), erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration by sex. Dots are red for females, and blue for males. The linear regression line is red for females and blue for males for hematocrit, erythrocytes and MCV, and black for both sexes for the remaining parameters, with 95% confidence intervals in dark grey.

children age 4 to < 14 [18]. In the present study, the lower limit was 11.9 g/dL (6–8 years, $n = 440$) and 12.2 g/dL (9–12 years, $n = 808$).

While leukocytes, lymphocytes and basophils decrease slightly from 6–12 years old, the trends for monocytes, neutrophiles and eosinophiles are flat. Others have reported similar trends for leukocytes and lymphocytes [4–6,17]. The reference limits identified in this study are similar to previous results, and while none of the differential count parameters or leukocytes required age or sex partitioning, some of these parameters have been partitioned for age by others [4,5]. Platelet count decreases over age, as found in other studies [4–6,17]. While platelets did not require partitioning according to the Lahti criteria, we included age partitioned data in addition to the non-partitioned data, to facilitate comparison with published studies [4,5].

A major strength of our study is the recruitment of a large number of participants in good health, not seeking medical care, representing different socioeconomic conditions. Selection bias is mitigated through the recruitment of all children from the participating schools in the Horten municipality and the high participation rate (82%). Our study also has limitations. Some of the children included may have unknown underlying diseases that may influence the results. However, due to the large number of participants, it is less likely that this has introduced any significant bias. Hematological parameters are influenced by the time of day and by inflammation [19,20], and while samples were collected within the same time interval, the interval varied from 8:00 a.m. to 1:30 p.m, and variations in time of sampling may have influenced the result.

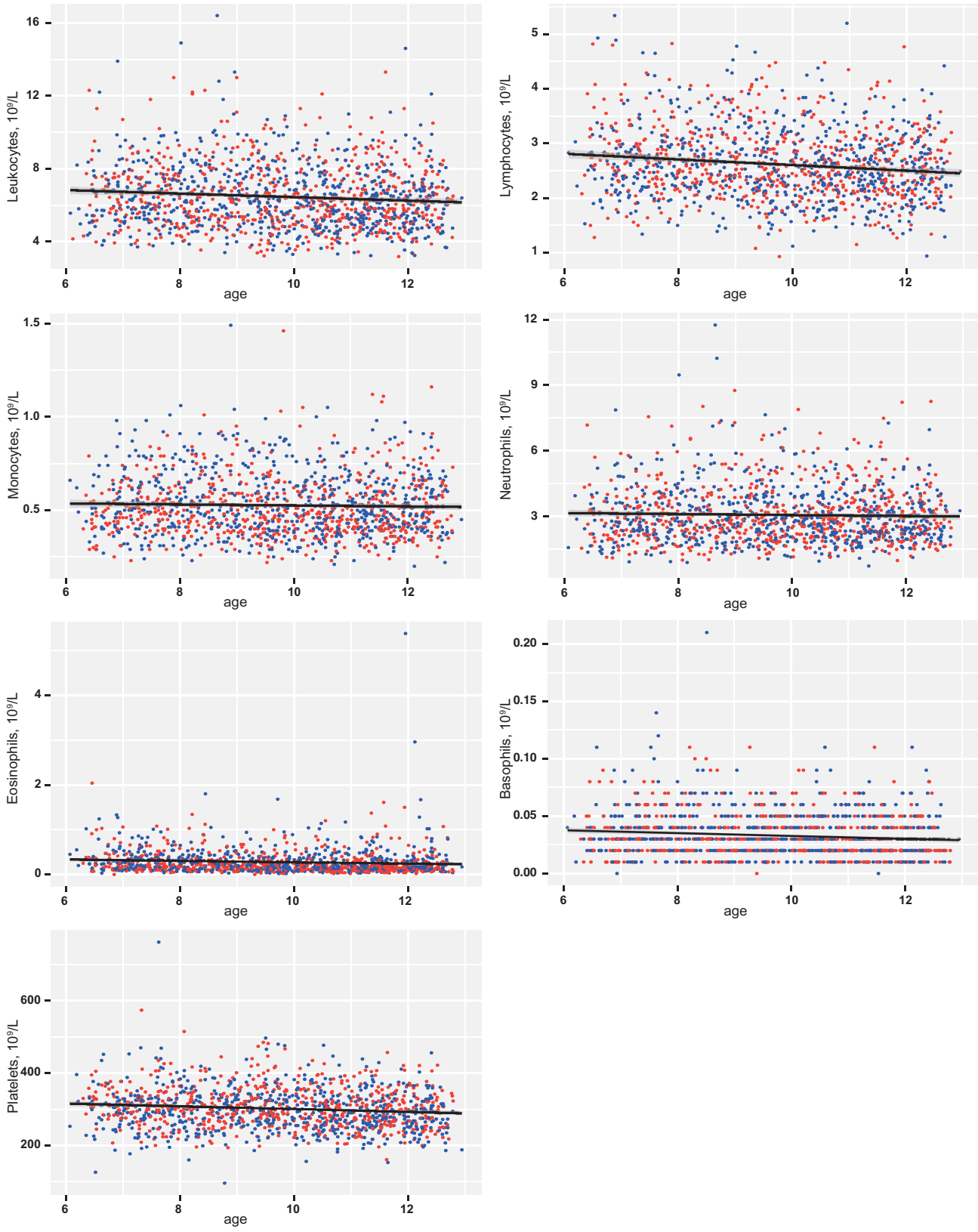

Figure 2. Age-dependent scatterplots of leukocytes, lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelets by sex. Dots are red for females, and blue for males. The linear regression line is black for both sexes with 95% confidence intervals in dark grey.

Conclusion

In this paper, we present up to date age- and sex-specific reference intervals for Norwegian children in the age span of 6–12 years old analyzed on a modern analytical platform. The reported data are based on a large cohort of healthy nonhospitalized children and will contribute to the clinical interpretation of hematological results in children from similar populations.

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Disclosure statement

The authors report there are no competing interests to declare.

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References

[1] Jones GRD, Haeckel R, Loh TP, IFCC Committee on Reference Intervals and Decision Limits, et al. Indirect methods for reference interval determination – review and recommendations. Clin Chem Lab Med. 2018;57(1):20–29.

[2] Pediatric reference intervals: critical gap analysis and establishment of a national initiative. Clin Biochem. 2006;39:559–560.

[3] Wayne PA. Clinical and Laboratory Standards Institute. Defining, establishing and verifying reference intervals in the clinical laboratory(approved guideline- 3rd edition C28-A3C). Clinical and Laboratory Standards Institute. 2008.

[4] Adeli K, Raizman JE, Chen Y, et al. Complex biological profile of hematologic markers across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian health measures survey. Clin Chem. 2015;61(8):1075–1086.

[5] Aldrimer M, Ridefelt P, Rödöö P, et al. Population-based pediatric reference intervals for hematology, iron and transferrin. Scand J Clin Lab Invest. 2013;73(3):253–261.

[6] Zierk J, Hirschmann J, Toddenroth D, et al. Next-generation reference intervals for pediatric hematology. Clin Chem Lab Med. 2019;57(10):1595–1607.

[7] Fredriksen PM, Hjelle OP, Mamen A, et al. The health oriented pedagogical project (HOPP) – a controlled longitudinal school-based physical activity intervention program. BMC Public Health. 2017;17(1):370.

[8] Eie AMØ, Strand MF, Fredriksen PM, et al. Population-based reference intervals for ferritin, iron, transferrin and transferrin saturation and prevalence of iron deficiency in 6-12-year-old children: the health oriented pedagogical project (HOPP). Scand J Clin Lab Invest. 2021;81(3):208–212.

[9] NOKLUS. The Norwegian Organization for Quality Improvement of Laboratory Examinations. [Internet]. [cited 2021 Jul 2]. Available from: https://www.noklus.no/.

[10] R Core Team 2021. R: a language and environment for statistical computing. R foundation for statistical computing., Vienna, Austria. [Internet]. Available from: http://www.R-project.org/.

[11] Delignette-Muller ML, Dutang C. Fitdistrplus: an R package for fitting distributions. J. Stat. Soft. 2015;64(4):1–34.

[12] Lahti A, Hyltoft Petersen P, Boyd JC, et al. Objective criteria for partitioning gaussian-distributed reference values into subgroups. Clin Chem. 2002;48(2):338–352.

[13] Lahti A, Petersen PH, Boyd J, et al. Partitioning of nongaussian-distributed biochemical reference data into subgroups. Clin Chem. 2004;50(5):891–900. doi: 10.1373/clinchem.2003.027953.

[14] Finnegan D. referenceIntervals: Reference Intervals. [Internet]. 2014. Available from: https://CRAN.R-project.org/package=referenceIntervals.

[15] Wickham H. ggplot2: Elegant Graphics for Data Analysis [Internet]. Springer-Verlag: New York2016. Available from: https://ggplot2.tidyverse.org.

[16] Dallman PR, Siimes MA. Percentile curves for hemoglobin and red cell volume in infancy and childhood. J Pediatr. 1979;94(1):26–31.

[17] Taylor MR, Holland CV, Spencer R, et al. Haematological reference ranges for schoolchildren. Clin Lab Haematol. 1997;19(1):1–15.

[18] Tahmasebi H, Higgins V, Bohn MK, et al. CALIPER hematology reference standards (I). Am J Clin Pathol. 2020;154(3):330–341.

[19] Hilderink JM, Klinkenberg LJJ, Aakre KM, et al. Within-day biological variation and hour-to-hour reference change values for hematological parameters. Clin Chem Lab Med. 2017;55(7):1013–1024.

[20] Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. Blood. 2019;133(1):40–50.