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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

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BACKGROUND: In a collaboration between the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) and the Canadian Health Measures Survey (CHMS), we determined reference value distributions using an a priori approach and created a comprehensive database of age- and sex-stratified reference intervals for clinically relevant hematologic parameters in a large household population of children and adults.

METHODS: The CHMS collected data and blood samples from 11 999 respondents aged 3–79 years. Hematology markers were measured with either the Beckman Coulter HmX or Siemens Sysmex CA-500 Series analyzers. After applying exclusion criteria and removing outliers, we determined statistically relevant age and sex partitions and calculated reference intervals, including 90% CIs, according to CSLI C28-A3 guidelines.

RESULTS: Hematology marker values showed dynamic changes from childhood into adulthood as well as between sexes, necessitating distinct partitions throughout life. Most age partitions were necessary during childhood, reflecting the hematologic changes that occur during growth and development. Hemoglobin, red blood cell count, hematocrit, and indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) increased with age, but females had lower hemoglobin and hematocrit starting at puberty. Platelet count gradually decreased with age and required multiple sex partitions during adolescence and adulthood. White blood cell count remained relatively constant over life, whereas fibrinogen increased slightly, requiring distinct age and sex partitions.

CONCLUSIONS: The robust dataset generated in this study has allowed observation of dynamic biological profiles of several hematology markers and the establishment of comprehensive age- and sex-specific reference intervals that may contribute to accurate monitoring of pediatric, adult, and geriatric patients.

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Complete blood count (CBC)[5] is one of the most commonly ordered tests for assessment of health and disease status. Yet for many parameters, accurately established reference intervals are not available. Reference intervals are often selected from manufacturer information sheets or outdated publications and may not always be representative of the local population or laboratory setting. CBC parameters are well known to vary with age and sex, requiring reference intervals that are specific for the population served *(1–3)*. The use of reference data from hospitalized patients *(4)* and the variation in criteria used to determine health status between different laboratories have further confounded test result interpretation. In addition, appropriate age- and sex-specific reference intervals for hematology are often lacking or incomplete across a broad age range, as studies tend to establish their intervals on select subsets of the population, focusing on either children or adults as distinct entities *(5)*.

The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) is a large, Canada-wide

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[5] Nonstandard abbreviations: CBC, complete blood count; CALIPER, Canadian Laboratory Initiative on Pediatric Reference Intervals; CHMS, Canadian Health Measures Survey; RBC, red blood cell; RDW, RBC distribution width; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; WBC, white blood cell.

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initiative that has been establishing a comprehensive database of pediatric reference intervals. CALIPER has consistently shown that many biomarkers of pediatric disease are subject to age- and sex-specific variability during childhood growth and development *(6–9)*; however, CALIPER has been unable to establish reference intervals for hematology due to the necessity of assaying fresh whole blood in a timely manner, which poses additional obstacles. Another major challenge has been the difficulty of obtaining sufficient population size and sample volumes from small children, which limits the robustness of the dataset *(5)*. Although large adult hematology datasets have been reported *(10)*, few studies have looked at adults >65 years of age *(11, 12)*. The geriatric population has a relatively high prevalence of chronic pathologies and comorbidities and regularly takes prescription medications, making it difficult to find healthy reference individuals. Furthermore, common to many reference interval studies is the assumption that distinct age groups should be treated as isolated entities, when in fact markers of disease constantly change over time and should be regarded as a dynamic continuum from childhood to adulthood and into geriatric ages.

The Canadian Health Measures Survey (CHMS) is an initiative developed to close data gaps and address limitations within Canada's health information system *(13)*. The CHMS was developed by Statistics Canada in partnership with Health Canada and the Public Health Agency of Canada to collect population-representative health information for important public health indicators such as obesity, nutrition, cardiovascular disease, infectious disease, and environmental exposures. Since 2007, >10 000 samples have been collected from the Canadian household population, aged 3–79 years, to create comprehensive national baseline values *(13)*. The CHMS has analyzed several important hematologic markers in Canadians across the country. CALIPER has partnered with Statistics Canada to gain access to these data and establish robust reference intervals for these blood tests. The extensive sample size of the CHMS has enabled us to generate reference intervals that are truly representative of the apparently healthy population. In addition, use of CHMS data has permitted extension of the CALIPER reference interval database to include adult and geriatric values. For the first time, we demonstrate the dynamic changes that occur throughout the life of healthy individuals from early childhood to geriatric age ranges.

Materials and Methods

The protocol for this study was approved by the Institutional Review Board at the Hospital for Sick Children, Toronto, Canada.

CHMS PARTICIPANT RECRUITMENT AND SAMPLE ACQUISITION

Cycle 1 of the CHMS took place from March 2007 through February 2009 and collected information from respondents aged 6–79. Cycle 2 took place from August 2009 through November 2011 and collected data from respondents aged 3–79. Full-time members of the Canadian Forces and people living on reserves or in other Aboriginal settlements, in institutions, and in some remote regions were excluded. More than 96% of the population was represented. Census geography was used to determine 360 potential data collection sites, from which 16 sites for cycle 1 and 18 sites for cycle 2 were randomly selected by use of systematic sampling, with probability of selection proportional to each site's population. The sites were stratified on the basis of 5 regions across Canada: Atlantic, Quebec, Ontario, prairies, and British Columbia. Selected dwellings were asked for a current household member list, which was then used to select 1 or 2 participants per household (randomly by use of a vector with variable selection probabilities by age group or sex). Data for >500 male and >500 female participants were collected in each of the following age groups: 3–5 (cycle 2 only), 6–11, 12–19, 20–39, 40–59, and 60–79 years.

The survey consisted of an initial in-home interview to collect general health information including nutrition, smoking habits, and medical history. This was followed by a visit to a mobile examination center to obtain biological samples and direct physical measures such as height, weight, and blood pressure. Response to the survey was voluntary. Ethics approval was obtained from Health Canada's Research Ethics Board. Details of the survey are available at www.statcan.gc.ca/chms.

SAMPLE ANALYSIS

For CBC parameters, venous whole blood was collected into lavender K2-EDTA Vacutainer tubes by a certified phlebotomist in mobile examination centers. Samples were analyzed immediately after blood draw with onboard Beckman Coulter HmX analyzers, which were maintained and serviced per the manufacturer's user manual (see Supplemental Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol61/issue8, for method details). The 16 hematology parameters analyzed included hemoglobin, hematocrit, red blood cell (RBC) count, RBC distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and mean platelet volume (MPV). Total white blood cell (WBC) count and a 5-part differential were obtained for neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The final analyte measured was fibrinogen, which was collected in citrate plasma, then stored at −20 °C

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(−4 °F) or below for less than a week before being measured off-site with the Siemens Sysmex CA-500 Series analyzer. For QC and proficiency testing data, see online Supplemental Tables 2–5 and Supplemental Fig. 1.

STATISTICAL ANALYSIS AND DETERMINATION OF REFERENCE INTERVALS

We excluded participants from statistical analysis on the basis of pregnancy, serious medical illness, chronic conditions, or use of prescription medication as outlined in online Supplemental Table 2 of Adeli et al. *(14)*. Ethnicity-related partitions could not be computed owing to small sample size. CHMS data were analyzed according to CLSI C28-A3 guidelines. Statistical analysis was performed with SAS and R software. First, exclusion criteria were applied to ensure that only data from healthy participants were included in the study. Scatterplots were generated with R, with each data point on the scatterplot representing the mean of 11 or more closely associated results to ensure participant confidentiality (Statistics Canada policy). Partitions were decided on the basis of trends observed within the scatter and distribution plots and then statistically evaluated with the Harris and Boyd method as previously described *(8)*. The data in each partition was transformed by the Box–Cox transformation method, and Q-Q plots were used to assess the normality of each partition. We used the Tukey test and adjusted Tukey test twice to remove outliers in normally distributed partitions and skewed partitions, respectively. The use of exclusion criteria combined with outlier exclusion adds additional means of eliminating erroneous and abnormal data to allow reference intervals to be robust and representative of analyte levels in healthy individuals only.

We used the nonparametric rank method to calculate the reference intervals for all partitions, since all had sample sizes >120 participants, and calculated 90% CIs for the upper and lower limits of each reference interval with weighted data.

Results

Table 1 shows the reference intervals and 90% CIs for the upper and lower limits of the RBC parameters, platelet count, WBC count differential, and fibrinogen (see also online Supplemental Table 6). Sex differences are also noted. Figs. 1 through 5 show the scatterplot distributions for hemoglobin, hematocrit, MCV, platelet count, and WBC count, respectively, over the 3- to 79-year age range for male and females. Scatterplots for the remaining analytes/parameters are found in online Supplemental Fig. 2.

Multiple age partitions were required for many analytes within childhood and adulthood, reflecting the dynamic physiological changes that occur throughout life. Of the analytes that required multiple age partitions, most occurred within the first 14 years. Sex differences were observed for almost half the analytes but were required only for select age groups. For most analytes, sex differences occurred between puberty to mid-adulthood, but not during early childhood or in geriatrics. An exception was monocyte count, which demonstrated sex differences in the 45- to 79-year period. All analytes except for hemoglobin, hematocrit, and RBC count had parallel age and sex intervals. Interestingly, hemoglobin, hematocrit, and RBC count all demonstrated a similar pattern, requiring 1 or 2 additional age partitions in males compared with females. Hemoglobin required 3 age partitions within the first 10 years of life for both sexes; however, compared with females, males required 2 additional age partitions within puberty and adolescence. For men, a final age partition encompassed all of adulthood from 20 to 79 years of age, whereas for females, hemoglobin concentrations remained constant from 11 to 79 years. For hematocrit and RBC count, no sex differences were observed until early puberty, after which time sex partitions diverged, with males requiring an additional partition at adolescence. For RBC count, this extra age partition encompassed both adolescents and adults ≤49 years old. In contrast, female hematocrit and RBC count required only 1 broad partition that covered adolescence to geriatrics.

Most other RBC indices (with the exception of MCHC) exhibited a similar trend and showed rising levels with age that were more pronounced in males than females. Notably, MCV and MCH showed dramatic changes, demonstrating rapid increases during childhood that required 3 partitions; levels peaked in early adulthood and then remained relatively consistent.

Similarly, platelet count and neutrophils also required ≥4 age-specific partitions for both sexes. Platelet count declined sharply during childhood and required 3 partitions during this time, before leveling off in early adulthood. Neutrophil counts were highest in early childhood, followed by fluctuations in the remaining age range, which required 4 age partitions. Two of these age partitions also required sex partitioning, with higher counts observed in females in childhood and adulthood. Monocyte counts were slightly higher in early childhood, with decreases observed in subsequent years. Sex differences were noted in mid-adulthood to geriatrics, where the counts were higher in males than in females. Fibrinogen required multiple partitions, although this analyte was only measured in participants starting in early puberty (≥12 years of age). Although fibrinogen required 3 age partitions, the first encompassed only the early puberty period. One sex partition was required in the 14- to 39-year period, in which females had higher concentrations than males.

Other parameters including MCHC, RDW, WBC count, and basophils showed considerably less variability

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Table 1. Age- and sex-specific reference intervals for 16 hematologic parameters.

	Male reference interval						Female reference interval					
Analyte	Age range, years	Samples, n[a]	Lower limit	Upper limit	Lower 90% CI	Upper 90% CI	Age range, years	Samples, n[a]	Lower limit	Upper limit	Lower 90% CI	Upper 90% CI
Hemoglobin, g/dL												
	3–5	420	11.4	14.3	11.1–11.6	14.0–14.7	3–5	420	11.4	14.3	11.1–11.6	14.0–14.7
	6–8	670	11.5	14.3	11.3–11.6	14.2–14.4	6–8	670	11.5	14.3	11.3–11.6	14.2–14.4
	9–10	540	11.8	14.7	11.8–11.9	14.5–14.8	9–10	540	11.8	14.7	11.8–11.9	14.5–14.8
	11–14[b]	480	12.4	15.7	12.2–12.6	15.4–15.9	11–79[b]	2450	11.9	14.8	11.8–12.0	14.7–14.9
	15–19[b]	460	13.3	16.9	12.9–13.6	16.7–17.1						
	20–79[b]	1570	13.6	16.9	13.4–13.8	16.7–17.0						
Hematocrit, %												
	3–5	420	34	42	33–35	42–43	3–5	420	34	42	33–35	42–43
	6–7	420	34	42	33–35	41–43	6–7	420	34	42	33–35	41–43
	8–11	1070	35	43	35–35	43–44	8–11	1070	35	43	35–35	43–44
	12–15[b]	440	38	47	37–38	46–47	12–79[b]	2330	35	43	35–36	43–44
	16–79[b]	1950	40	50	39–41	50–50						
RBCs, 10^6/μL												
	3–5	420	4.0	5.1	3.8–4.1	4.9–5.2	3–5	420	4.0	5.1	3.8–4.1	4.9–5.2
	6–10	1200	4.1	5.2	4.0–4.1	5.2–5.3	6–10	1200	4.1	5.2	4.0–4.1	5.2–5.3
	11–14[b]	470	4.2	5.3	4.2–4.3	5.3–5.4	11–14[b]	470	4.1	5.1	4.0–4.1	5.0–5.2
	15–49[b]	1630	4.3	5.7	4.3–4.4	5.7–5.8	15–79[b]	2110	3.8	5.0	3.8–3.9	5.0–5.0
	50–79[b]	410	4.2	5.5	4.1–4.3	5.3–5.6						
RDW, %												
	3–5	400	11.3	13.4	11.2–11.5	13.2–13.5	3–5	400	11.3	13.4	11.2–11.5	13.2–13.5
	6–79	6000	11.4	13.5	11.3–11.4	13.5–13.5	6–79	6000	11.4	13.5	11.3–11.4	13.5–13.5
MCV, fL												
	3–5	410	77.2	89.5	76.7–77.7	88.5–90.4	3–5	410	77.2	89.5	76.7–77.7	88.5–90.4
	6–11	1460	77.8	91.1	77.4–78.2	90.6–91.5	6–11	1460	77.8	91.1	77.4–78.2	90.6–91.5
	12–14	610	79.9	93.0	79.2–80.7	92.3–93.7	12–14	610	79.9	93.0	79.2–80.7	92.3–93.7
	15–79	4070	82.5	98.0	82.2–82.8	97.5–98.5	15–79	4070	82.5	98.0	82.2–82.8	97.5–98.5

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Table 1. Age- and sex-specific reference intervals for 16 hematologic parameters. *(Continued from page 1078)*

Analyte	Male reference interval						Female reference interval					
	Age range, years	Samples, n[a]	Lower limit	Upper limit	Lower 90% CI	Upper 90% CI	Age range, years	Samples, n[a]	Lower limit	Upper limit	Lower 90% CI	Upper 90% CI
MCH, pg												
	3–5	400	26.1	30.7	25.8–26.3	30.4–31.0	3–5	400	26.1	30.7	25.8–26.3	30.4–31.0
	6–15	2250	26.3	31.7	26.2–26.5	31.6–31.8	6–15	2250	26.3	31.7	26.2–26.5	31.6–31.8
	16–79	3820	27.6	33.3	27.5–27.8	33.1–33.4	16–79	3820	27.6	33.3	27.5–27.8	33.1–33.4
MCHC, g/dL												
	3–5	410	32.4	34.9	32.1–32.7	34.7–35.1	3–5	410	32.4	34.9	32.1–32.7	34.7–35.1
	6–79	6270	32.5	35.2	32.4–32.5	35.1–35.3	6–79	6270	32.5	35.2	32.4–32.5	35.1–35.3
Platelets, 10^3/μL												
	3–5	420	187.4	444.6	170.9–203.9	429.7–459.4	3–5	420	187.4	444.6	170.9–203.9	429.7–459.4
	6–9	910	186.7	400.4	179.2–194.2	387.0–413.9	6–9	910	186.7	400.4	179.2–194.2	387.0–413.9
	10–13	1010	176.9	381.3	168.5–185.2	370.7–391.9	10–13	1010	176.9	381.3	168.5–185.2	370.7–391.9
	14–26[b]	770	138.7	319.6	128.0–149.3	303.2–336.0	14–26[b]	680	158.1	361.6	153.1–163.2	347.6–375.5
	27–79[b]	1390	151.8	324	144.2–159.4	315.0–333.0	27–79[b]	1490	153.2	361.3	137.9–168.5	348.1–374.5
MPV, fL												
	3–5	420	6.4	9.5	6.2–6.6	9.2–9.7	3–5	420	6.4	9.5	6.2–6.6	9.2–9.7
	6–11	1490	6.6	9.8	6.5–6.7	9.6–9.9	6–11	1490	6.6	9.8	6.5–6.7	9.6–9.9
	12–79	4730	7.0	10.3	6.9–7.1	10.2–10.4	12–79	4730	7.0	10.3	6.9–7.1	10.2–10.4
WBCs, 10^3/μL												
	3–5	420	4.4	12.9	3.9–4.9	11.5–14.2	3–5	420	4.4	12.9	3.9–4.9	11.5–14.2
	6–79	6280	3.8	10.4	3.8–3.9	10.0–10.7	6–79	6280	3.8	10.4	3.8–3.9	10.0–10.7
Neutrophils, 10^3/μL												
	3–5	420	1.6	7.8	1.3–1.9	6.7–8.9	3–5	420	1.6	7.8	1.3–1.9	6.7–8.9
	6–16[b]	1310	1.4	6.1	1.3–1.5	5.9–6.3	6–14[b]	1000	1.5	6.5	1.4–1.6	6.2–6.7
	17–50[b]	1420	1.8	7.2	1.7–1.9	6.7–7.6	15–50[b]	1660	2.0	7.4	1.9–2.1	6.9–7.9
	51–79	820	2.0	6.4	1.9–2.1	6.1–6.7	51–79	820	2.0	6.4	1.9–2.1	6.1–6.7

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Table 1. Age- and sex-specific reference intervals for 16 hematologic parameters. *(Continued from page 1079)*

Analyte	Male reference interval						Female reference interval					
	Age range, years	Samples, n[a]	Lower limit	Upper limit	Lower 90% CI	Upper 90% CI	Age range, years	Samples, n[a]	Lower limit	Upper limit	Lower 90% CI	Upper 90% CI
Lymphocytes, 10^3/μL												
	3–5	410	1.6	5.3	1.5–1.8	4.9–5.7	3–5	410	1.6	5.3	1.5–1.8	4.9–5.7
	6–11	1460	1.4	3.9	1.3–1.4	3.8–4.0	6–11	1460	1.4	3.9	1.3–1.4	3.8–4.0
	12–79	4710	1.0	3.2	1.0–1.1	3.1–3.4	12–79	4710	1.0	3.2	1.0–1.1	3.1–3.4
Monocytes, 10^3/μL												
	3–5	400	0.3	0.9	0.2–0.3	0.8–0.9	3–5	400	0.3	0.9	0.2–0.3	0.8–0.9
	6–44	4920	0.2	0.8	0.2–0.2	0.8–0.8	6–44	4920	0.2	0.8	0.2–0.2	0.8–0.8
	45–79[b]	610	0.3	0.9	0.3–0.3	0.9–0.9	45–79[b]	660	0.2	0.8	0.2–0.2	0.7–0.8
Eosinophils, 10^3/μL												
	3–5	400	0.0	0.5	0.0–0.0	0.5–0.5	3–5	400	0.0	0.5	0.0–0.0	0.5–0.5
	6–11	1400	0.0	0.5	0.0–0.0	0.5–0.5	6–11	1400	0.0	0.5	0.0–0.0	0.5–0.5
	12–79	3450	0.1	0.2	0.1–0.1	0.2–0.2	12–79	3450	0.1	0.2	0.1–0.1	0.2–0.2
Basophils, 10^3/μL												
	3–5	420	0.0	0.1	0.0–0.0	0.1–0.1	3–5	420	0.0	0.1	0.0–0.0	0.1–0.1
	6–79	6280	0.0	0.1	0.0–0.0	0.1–0.1	6–79	6280	0.0	0.1	0.0–0.0	0.1–0.1
Fibrinogen, mg/dL												
	12–13	430	180	350	180–180	340–360	12–13	430	180	350	180–180	340–360
	14–39[b]	1170	210	370	210–210	360–370	14–39[b]	1240	200	420	190–210	410–430
	40–79	1690	200	420	180–210	410–420	40–79	1690	200	420	180–210	410–420

[a] As a result of different numbers of outliers excluded in each partition, the net sample size was slightly different for each analyte/parameter.
[b] Sex-specific reference interval.

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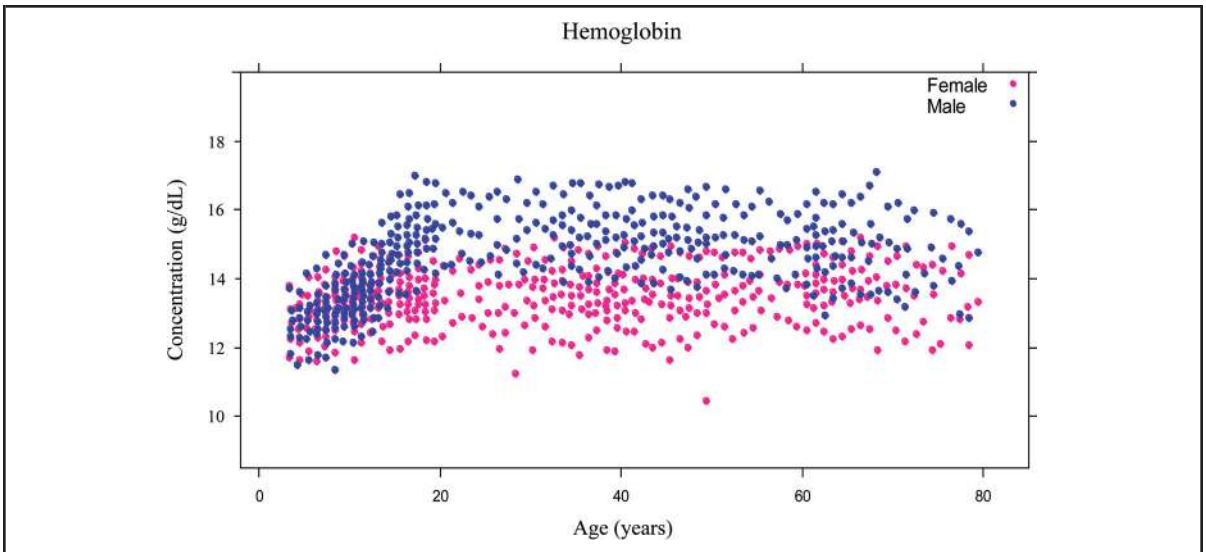

Fig. 1. Scatterplot distributions for hemoglobin over the 3- to 79-year age range demonstrating rapid changes from childhood into adulthood and sex differences among adolescents and adults.

Each data point represents the mean of 11 or more closely associated results to ensure participant confidentiality (on the basis of Statistics Canada policy). Scatterplots were visually inspected and outliers were removed before reference intervals were calculated. Males are denoted in blue and females in pink.

and required only 2 partitions across the entire age range examined. These intervals spanned a tight partition in early childhood before broadening into a larger partition for the remaining age range. RDW and MCHC were relatively constant with age but exhibited slight increases during childhood. Similarly, MPV rose slightly in childhood before peaking in early adulthood and remaining stable thereafter. Overall, WBC count declined slightly early in life and subsequently plateaued for the rest of childhood and into adulthood. A similar trend was ob-

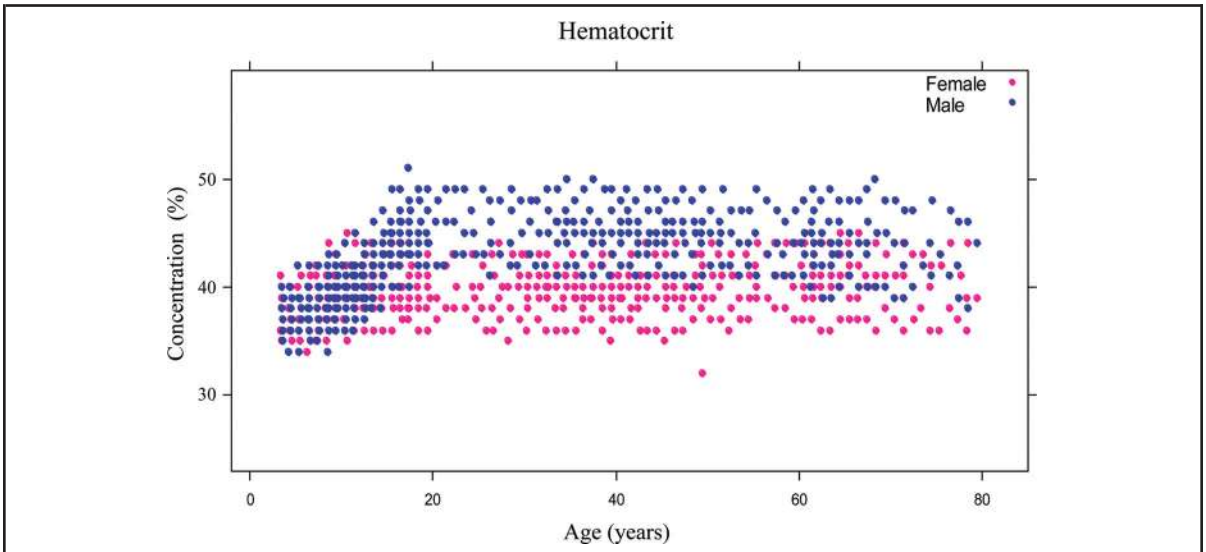

Fig. 2. Scatterplot distributions for hematocrit over the 3- to 79-year age range demonstrating similar trends to hemoglobin.

Each data point represents the mean of 11 or more closely associated results to ensure participant confidentiality. Scatterplots were visually inspected and outliers were removed before reference intervals were calculated. Males are denoted in blue and females in pink.

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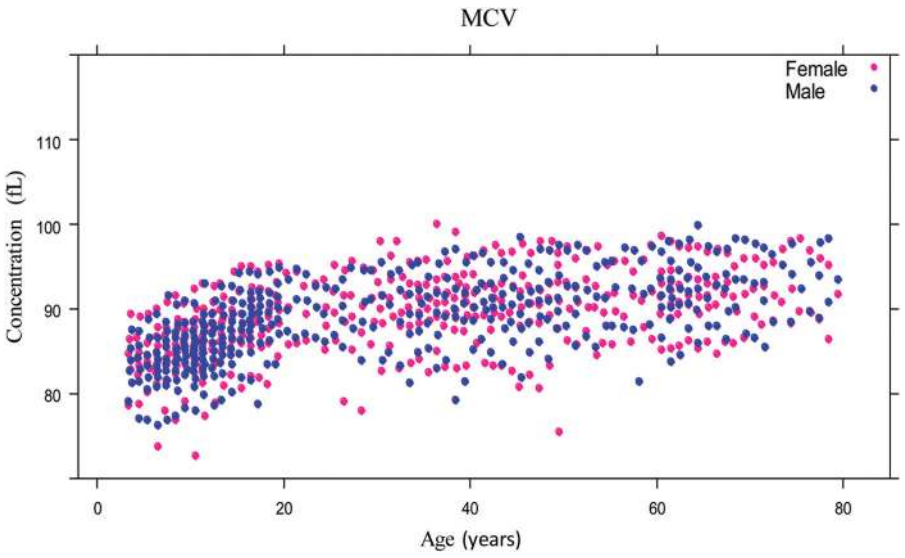

Fig. 3. Scatterplot distributions for MCV over the 3- to 79-year age range.

Levels increased slightly throughout life without any sex difference. Each data point represents the mean of 11 or more closely associated results to ensure participant confidentiality. Scatterplots were visually inspected and outliers were removed before reference intervals were calculated. Males are denoted in blue and females in pink.

served in lymphocyte concentrations. Eosinophil and basophil concentrations were low, as these markers were not detected in many of the participants. Upper limits, however, were highest in early life.

Discussion

The aim of this study was to establish hematology reference intervals from healthy individuals, representative of

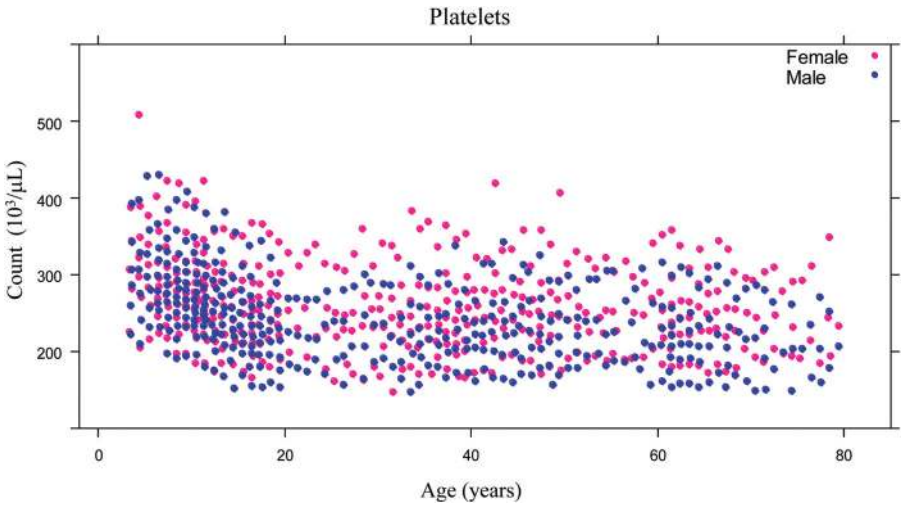

Fig. 4. Scatterplot distributions for platelet count showing highest levels in childhood that fall into adulthood and then again into the geriatric population.

Each data point represents the mean of 11 or more closely associated results to ensure participant confidentiality. Scatterplots were visually inspected and outliers were removed before reference intervals were calculated. Males are denoted in blue and females in pink.

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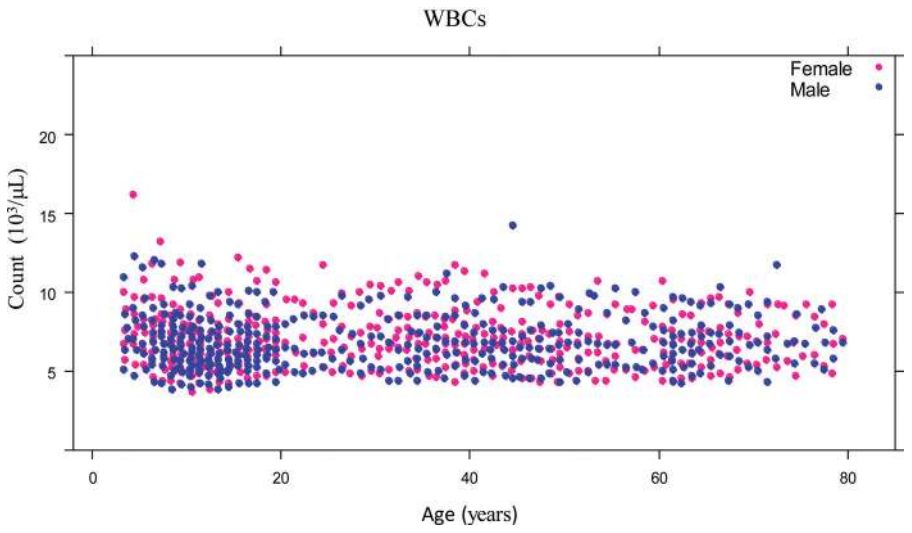

Fig. 5. Scatterplot distributions for white blood cell (WBC) count over the 3- to 79-year age range.
Levels remained relatively constant throughout life requiring only two age partitions. Each data point represents the mean of 11 or more closely associated results to ensure participant confidentiality. Scatterplots were visually inspected and outliers were removed before reference intervals were calculated. Males are denoted in blue and females in pink.

the Canadian population. These updated reference intervals span from childhood to geriatric age and provide insight into the hematologic changes that occur through life. Results were based on data from the CHMS, an extensive survey that collected health information and blood samples from the Canadian household population. Exclusion criteria were used to identify healthy participants before application of a rigorous statistical approach to ensure robust calculation of reference intervals. Excluding outliers from the dataset enabled unusual observations to be eliminated before reference interval calculation. The large sample size has also ensured a high degree of statistical power in determining age- and sex-specific partitions. The door-to-door interviewing approach used by the CHMS to generate this robust dataset ensured that samples were collected from families within the community setting and were reflective of a healthy population. This is in contrast to other large hematology studies, which have previously relied on patient hospital data *(4)*. Not only is patient data nonrepresentative of healthy individuals, but it also confines the use of intervals to a particular population subset, limiting broader utility.

Hemoglobin concentration, hematocrit, and the other RBC indices reflect the same biological parameters and showed similar variability throughout life. Male and female levels were similar in early childhood and increased slowly until 10 years of age before sex differences were observed. The sharp rise in levels during puberty and adolescence may be due to the effect of testosterone, which activates erythropoiesis by stimulating erythropoietin production *(15)*. The 2 age-specific partitions required during puberty and adolescence are consistent with a previous CALIPER study demonstrating rapidly rising total testosterone concentrations during this same period *(9)*. The increased erythropoiesis observed during childhood likely occurs to keep pace with the rapid metabolic demand during growth and development, reflective of the rising hemoglobin levels. Females also required a unique age partition from late childhood to geriatrics (11–80 years), but as expected, levels were much lower into adulthood. The decreased metabolic demand in females, decreased muscle mass, and lower iron stores due to menstruation are factors well known to contribute to lower hemoglobin in females compared with males. This is in line with studies that showed low serum ferritin in healthy asymptomatic menopausal females *(16, 17)*. The WHO hemoglobin cutoff for mild anemia is 11.1–11.9 g/dL in nonpregnant adult females and 11.1–12.9 g/dL in adult males *(18–20)* and agrees well with the lower limits generated in this study. However, differences in RBC indices between recent studies from various cohorts in Malaysia *(2)*, the US *(10)*, Germany *(21)*, the UK *(22)*, and Africa *(3)* likely reflect differences in diet, environment, and testing conditions, underscoring the importance of population-based reference intervals for these important measurements. The lower levels of hemoglobin and MCV found in children compared with adult counterparts were accompanied by expected ferritin concentrations with lower-limit values of >10 ng/mL *(23)*,

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suggesting healthy iron status in our pediatric population. Because of reactive elevations in conditions of inflammatory disease, ferritin should be interpreted with serum iron and total iron binding capacity to calculate percentage iron saturation. A limitation of this study is that serum iron and total iron binding capacity were not measured in the CHMS population and the data are not available.

Similar to other studies, we found that platelet count decreased with age during childhood *(24)* and that adult women had slightly higher counts than men, but levels tended to decrease with age in both sexes *(2, 25)*. Thrombopoietin, a hormone that regulates production of platelets, has been found to peak shortly after birth and gradually decline to adult concentrations *(26)*, which could explain the higher platelet counts observed in early childhood. Although it is not clear why there are sex differences during adolescence and adulthood, they may be related to hormonal changes and genetic variability *(27)*. Interestingly, Biino et al. found that thrombocytopenia is more common in men than women and tends to be more frequent among the elderly *(25)*, consistent with our findings. MPV required partitioning within the first 11 years of life, with only 1 broad partition for the remaining age group, a trend that correlates with the multiple age partitions required for platelet count. However, MPV displayed an inverse correlation with platelet count, showing an increase with age that was most pronounced in early childhood, consistent with previous studies *(1, 24)*. Moreover, genomewide association studies have found that regulation of platelet count and volume in healthy individuals may occur as a result of highly heritable traits between individuals due to single nucleotide polymorphisms *(28)* in several genes that regulate megakaryopoiesis and proplatelet formation *(29)*. Indeed, this genetic variability could explain the dynamic fluctuations in platelets across age groups in Canada, which is a diverse multiethnic population.

The intervals for WBC count resemble previous studies, demonstrating higher counts in early childhood, which then declined in older children and adults *(1)*. The higher counts in early childhood could reflect the early development of the immune system. We speculate that the compensation for reduced immunity early in life is to drive production of immune-regulatory processes to protect against invading pathogens, thus explaining the high WBC counts in young children. The lower counts observed later in childhood and into adulthood further reflect the development of the acquired and adaptive immune response as the immune system gains exposure to pathogens and nonself-antigens in the environment. This was particularly evident for neutrophils and lymphocytes, which exhibited the highest counts in early childhood, requiring 2 unique partitions before onset of adulthood (<18 years of age). Our data agree well with previous reports showing that males have fewer neutrophils than females *(2)*; however, this difference was observed only for 2 age partitions between 6 and 50 years of age. In a pediatric population study by Taylor et al. *(24)*, higher neutrophil counts in girls were also observed in the 7- to 19-year age group, which agrees well with our study. There is evidence that sexual development has an impact on development of autoimmunity *(30)*. Estrogen has been shown to stimulate the level of immunological response, whereas some androgens, such as testosterone, suppress the response to infection *(30)*, which could help explain the higher levels of neutrophils observed in females during puberty and adulthood. For monocytes, sex differences were observed only in mid-adulthood to geriatrics (45–79 years). Although it is unclear why males had slightly higher counts, these sex differences have not been previously observed. Many studies that have previously established hematology reference intervals have been based on small sample sizes. For example, Roshan et al. used 110 adults for calculation of monocyte count reference intervals, and although sex differences were observed, none were statistically significant *(31)*. In the present study, >1000 adults were included, which invariably improves the study power and thus enables determination of statistical differences between even very subtle sex- or age-related changes. The low basophil and eosinophil counts in both sexes are consistent with previously calculated intervals *(32)*, likely because of the low prevalence of parasitic infection in the Canadian population and the narrow range of environmental antigens. This is in contrast to other regional studies, such as in Africa, which tend to have higher basophil and eosinophil counts due to a higher prevalence of parasitic infections *(3)*.

The final interval established in this study was fibrinogen. Concentrations increased during childhood, consistent with the development of the synthetic function of the liver. Sex differences were observed in adolescence to mid-adulthood (14–39 years), with females having higher concentrations than males. This has been previously noted in a number of studies *(33, 34)*. A temporary rise in fibrinogen concentration has been known to be induced by the use of oral contraceptives and pregnancy *(35, 36)*, suggesting the role of female sex hormones in modulating coagulation factors.

The advantage of the CHMS-CALIPER collaboration is the potential to harmonize reference intervals across Canada. This approach is warranted, because in contrast to chemistry and immunoassay analytes, hematology largely suffers from lack of standardization. Hemoglobin is the only hematology parameter with an international reference material, whereas other parameters such as RBC, hematocrit, MCV, MCH, MCHC, and platelets have reference methods only. For these analytes, results between instruments in healthy individuals agree

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closely. However, for other indices such as MPV, reference standards and methods are lacking. Because vendors use different statistical models to derive cell count and their indices, results are not directly commutable between platforms, and therefore it is important to keep in mind the platform dependence of these measurands when deriving reference intervals.

To the best of our knowledge, this is the first study showing a robust dataset of routine hematology markers across a broad range of ages. Nationally representative data from the CHMS, a large sample size, and the rigorous approach to calculate the intervals on the basis of exclusion criteria and outlier removal have allowed insight into dynamic hematologic changes from early childhood to elderly adults. Reference intervals established in this study will be directly applicable to many Canadian laboratories currently using the Beckman and Siemens analyzers. After transference and validation studies, these reference intervals will also be applicable to users of other analytical systems, thereby facilitating the broad application of CALIPER-CHMS reference intervals at pediatric and adult centers globally. The rich dataset collected also allows for data mining by use of a systems biology approach and, therefore, has the potential to uncover relationships between hematology biomarker values, clinical outcomes, and risk factors for many disease states.

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