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Mining the Gap: Deriving Pregnancy Reference Intervals for Hematology Parameters Using Clinical Datasets

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BACKGROUND: Physiological changes during pregnancy invalidate use of general population reference intervals (RIs) for pregnant people. The complete blood count (CBC) is commonly ordered during pregnancy, but few studies have established pregnancy RIs suitable for contemporary Canadian mothers. Prospective RI studies are challenging to perform during pregnancy while retrospective techniques fall short as pregnancy and health status are not readily available in the laboratory information system (LIS). This study derived pregnancy RIs retrospectively using LIS data linked to provincial perinatal registry data.

METHODS: A 5-year healthy pregnancy cohort was defined from the British Columbia Perinatal Data Registry and linked to laboratory data from two laboratories. CBC and differential RIs were calculated using direct and indirect approaches. Impacts of maternal and pregnancy characteristics, such as age, body mass index, and ethnicity, on laboratory values were also assessed.

RESULTS: The cohort contained 143 106 unique term singleton pregnancies, linked to >972 000 CBC results. RIs were calculated by trimester and gestational week. Result trends throughout gestation aligned with previous reports in the literature, although differences in exact RI limits were seen for many tests. Trimester-specific bins may not be appropriate for several CBC parameters that change rapidly within trimesters, including red blood cells (RBCs), some leukocyte parameters, and platelet counts.

CONCLUSIONS: Combining information from comprehensive clinical databases with LIS data provides a robust and reliable means for deriving pregnancy RIs. The present analysis also illustrates limitations of using conventional trimester bins during pregnancy, supporting use of gestational age or empirically derived bins for defining CBC normal values during pregnancy.

Background

Clinical laboratory testing supports diagnosis, prognosis, and monitoring of maternal health and complications (1). Blood volume, hormonal status, and hemostasis change during pregnancy. Plasma volume and iron requirements increase. Despite increased erythropoietin synthesis, red blood cell (RBC) count, hemoglobin, and hematocrit decrease (2, 3). Immune regulation changes to support fetal development and protect against infection, which increases white blood cell (WBC) count and neutrophils (4). Increased spleen size and plasma volume contribute to mild thrombocytopenia (5). Distinguishing between physiological hematological changes and pregnancy complications is challenging due to pregnancy-associated adaptions. Use of gestational age-specific reference intervals (RIs) would therefore improve pregnancy and perinatal care.

Few studies have established gestational age-specific RIs for hematology parameters (6–13). Klajnbard et al. derived trimester-specific RIs for complete blood count (CBC) indices using blood samples collected longitudinally from apparently healthy White pregnant individuals in Denmark (6). More recently, Jin et al. completed a

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longitudinal study of 565 healthy pregnant Chinese women during early (8–12 weeks) and late (28–37 weeks) gestation (7). Additional longitudinal (8–11) and cross-sectional studies (12, 13) of varying sample sizes have reported trimester-specific CBC RIs using analytical assays from Sysmex (8, 11), Abbott (9, 12), Mindray (13), and Beckman Coulter (10). Studies consistently demonstrate dynamic variation in CBC indices throughout gestation illustrating the need for pregnancy-specific RIs for result interpretation (6–13). However, additional studies in larger populations are needed to investigate the influence of covariates such as maternal age, ethnicity, and body mass index (BMI) on hematology indices in pregnancy. In addition, all published studies have binned RIs by trimester only. Assessment of the appropriateness of using trimester-specific bins as opposed to a continuous or weekly approach for hematology test interpretation is needed.

This study sought to establish gestational-specific RIs for hematology parameters in a large Canadian pregnancy cohort using retrospective data mining techniques. Covariates beyond gestational age that may influence hematology parameters (e.g., maternal age, ethnicity, BMI) were also assessed. Computational modeling was utilized to determine the rate of change in RBC, WBC, and platelet indices throughout gestation.

Methods

DATA EXTRACTS AND COHORT DEFINITION

Retrospective data were extracted from laboratory and provincial databases. A 5-year pregnancy cohort was defined by extracting all live births in British Columbia recorded in the British Columbia Perinatal Data Registry (BCPDR) between January 1, 2010 and December 31, 2014. The BCPDR has collected health data on nearly every birth in BC since 2000 and contains records for nearly 1 000 000 births and deliveries, serving as a rich resource for research, surveillance, program delivery, and evaluation (14). Clinical information from the BCPDR was used to exclude high-risk pregnancies—those with complications, preterm delivery, or documented maternal comorbidities—to define a cohort of healthy mothers and healthy term singleton pregnancies to include in RI calculations (Supplemental File 1 in the online Data Supplement details the exclusion criteria). Records without final gestation, date of collection, and/or delivery date were excluded from analyses. Retrospective laboratory data, including CBC, were extracted from BC Children's and Women's Hospital (CW) and LifeLabs BC laboratory information systems (LIS). CW is a tertiary maternity care hospital serving mothers living in the hospital catchment area as well as all BC mothers and neonates requiring higher levels of perinatal care. The CW laboratory used a Sysmex XE-2100 platform for CBC analysis during the study period. LifeLabs laboratory in BC is the largest provider of outpatient laboratory services in the province. The LifeLabs LIS contains tests performed at LifeLabs as well as the former BCBio Laboratories (acquired by LifeLabs in 2013). These laboratories used Sysmex XE-2100 (LifeLabs and BCBio) as well as Sysmex XT-2000i, XS-1000, and PoCHi 100i (select LifeLabs locations) CBC analyzers. Quantitative laboratory test results were identified through review of the laboratory catalogs. Results and affiliated information were extracted. For this analysis, laboratory data were restricted to the CBC with and without differential, as well as some tests used as proxies for possible hematological conditions that can affect CBC parameters (additional exclusion criteria, Supplemental File 1). Self-reported ethnicity information was obtained from the provincial prenatal screening laboratory database. Self-reported ethnicity was chosen from a list of options included on the BC maternal serum screening requisition, and included the following selections: Caucasian, First Nations, Black, East Asian (e.g., Chinese, Japanese, Filipino, Vietnamese, Korean), South Asian (e.g., Indian, Pakistani, Sri Lankan), and Other/mixed race (with a request to specify using a blank space provided).

Data extracts were requested through and prepared by Population Data BC (PopData, https://www.popdata.bc.ca/about). Extracts were de-identified and assigned unique patient-specific study identifiers. Laboratory data was linked to demographic and clinical data from the BCPDR for each pregnancy. Gestational age at the time of each laboratory test was calculated as the difference between the date of delivery and the date of blood collection in relation to the final gestational age reported. Data linkage and exclusions are diagrammed in Supplemental File 1, using the WBC analysis for illustration.

STATISTICAL ANALYSIS

Covariate analysis. The impact of pregnancy characteristics including maternal age, pre-pregnancy BMI, smoking status, maternal group B Streptococcus status, ethnicity, and use of in vitro fertilization (IVF), were assessed using mixed-effects linear regression, adjusting for the effect of gestational age, multiple CBC results within a pregnancy, and testing laboratory. The effect of laboratory on CBC results was assessed graphically, by comparing data distributions from each testing site and by mixed-effects linear regression, adjusting only for multiple measures and gestational age at sample collection. Boxplots were generated and result mean (95% confidence intervals) and standard error were calculated for all categorical variables. All covariate analysis was completed prior to further outlier removal (see next).

Reference interval analysis. After clinical exclusion criteria were applied, statistical outlier detection and removal was performed for each week of gestation using the Tukey or adjusted Tukey method for normal and skewed distributions, respectively, determined by quantile-quantile plots (15, 16). RIs (2.5th and 97.5th percentiles) were calculated using the direct nonparametric rank method as per CLSI EP-28A3c (17). Several partitioning strategies were evaluated. First, RIs were calculated for each trimester separately (T1: 0 to 13 weeks; T2: 14–27 weeks; T3: 28–41 weeks) and differences between trimester partitions were assessed using the method of Harris and Boyd (17, 18). Second, as trimester bins are broad and physiological changes in pregnancy are continuous, RIs were also estimated for each week of gestation. To further investigate changes in laboratory results with gestational age, piecewise linear regression was used. The model was adjusted for maternal age and testing laboratory, and set to estimate 2 gestational age breakpoints to determine when the relationship between CBC result and gestational age changes. The model used the borders of each trimester bin (i.e., 14 and 28 weeks) as initial starting points.

The derived RIs were compared with those calculated using the truncated maximum likelihood and refineR methods of indirect RI establishment using patient datasets (19, 20). Methods were applied to the initial cohort, without applying clinical or laboratory-based exclusion criteria from Supplemental File 1. Results were compared to the nonparametric RI estimates to determine whether the clinical exclusion criteria applied was sufficient to justify direct RI calculations.

Data cleaning, dataset merging and subsetting, and RI calculations were performed using R (v.3.6.1). Cohort demographic summaries and regression analyses were performed using SAS v.9.4 TS Level 1M5 (SAS Institute, Cary, NC).

This study was approved by the UBC Research Ethics Board (H17-00849). Access to data provided by the Data Steward(s) is subject to approval, but can be requested for research projects through the Data Steward(s) or their designated service providers. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s).

Results

COHORT DESCRIPTION

A total of 216 503 live births were included in the 5-year BCPDR extract. After applying exclusion criteria, 121 469 healthy mothers, representing 143 106 unique term singleton pregnancies remained (Table 1). Average maternal age was 30.7 years. Mothers from all 5 geographical health authorities in BC are represented in proportions similar to those of the general population (21). Self-reported ethnicity was available for 28.3% of the cohort. Of these, 60% of mothers self-reported Caucasian ethnicity, 22% East Asian, and 10% South Asian (Table 1), similar to the ethnic distributions of female residents in BC per recent Canadian census data (22). Of the pregnancies, 45% represented the mother's first live birth. More than 972 000 CBC results were available. Most results were from LifeLabs BC and BCBio laboratories (approximately 98%) and spanned gestation (0–41 + 6 weeks), with higher density at 5–12 and 25–30 weeks. Roughly 1%–2% of CBC results were from the CW laboratory where data contribution was more consistent throughout gestation and proportionally higher in the third trimester (Supplemental File 1).

COVARIATE AND REFERENCE INTERVAL ANALYSIS

Trimester-specific RIs were calculated for 12 CBC parameters; most warranted trimester-based partitioning using the Harris and Boyd method (Table 2) (18). Use of indirect or direct statistical methods for calculating RI limits produced similar results (Supplemental File 1), therefore we only report results from direct methods here. Figures 1–3 illustrate the distribution of test results across gestational age for each CBC parameter. Covariate analysis, segmented regression, and RIs estimated per week of gestation are provided in Supplemental Files 2–13 for each parameter.

RIs for RBC count, hemoglobin, hematocrit, mean corpuscular volume (MCV), and RBC distribution width (RDW) are summarized in Figs. 1 and 3. First trimester values were significantly different from second and third trimesters, warranting partitioning (Table 2). However, average RBC, hemoglobin, and hematocrit values decreased steadily during the first and second trimesters, then remained relatively constant until delivery, raising the question of whether trimester binning is appropriate. Estimated regression breakpoints were similar for RBC count, hemoglobin, and hematocrit at approximately 20- and 30-weeks gestation. Concentrations changed most markedly from 0 to 20 weeks with RBC count and hemoglobin decreasing 0.03×10^{12}/L and 0.76 g/L, respectively, per week of gestation. Results then remained relatively constant until 30 weeks after which values increased at a similar rate until delivery (Supplemental Files 2 and 3). MCV RI upper limit increased, widening the RIs (Fig. 1, Table 2).

Significant associations between several covariates and RBC parameters were detected. These associations were very small, and unlikely to be clinically significant (Supplemental Files 2–6).

Table 1. Maternal and pregnancy characteristics of the BC pregnancy cohort.

Variable	Mean (SD) or N (%)
Number of pregnancies	143 106 (100%)
Unique mothers	121 469 (84.9%)
Maternal age (years)	30.7 (5.2)
Final gestational age at delivery (weeks)	39.1 (1.2)
Parity	
Nulliparous (N)	64 267 (44.9%)
Multiparous (N)	78 837 (55.1%)
Conceived by IVF/AR	2570 (1.8%)
Unknown	13 408 (9.4%)
Pre-pregnancy BMI	
Underweight (<18.50)	6435 (4.5%)
Normal (18.5–24.99)	67 556 (47.2%)
Overweight (25.00–29.99)	20 864 (14.6%)
Obese (≥30.00)	10 988 (7.7%)
Unknown	37 263 (26.0%)
Smoking status	
Nonsmoking	47 586 (33.3%)
Former	13 066 (9.1%)
Currently smoking	8472 (5.9%)
Unknown	73 982 (51.7%)
Group B Streptococcus status	
Positive	29 549 (20.7%)
Unknown	16 155 (11.3%)
Health authority of residence	
Interior Health	21 435 (15.0%)
Fraser Health	54 572 (38.1%)
Vancouver Coastal	34 483 (24.1%)
Vancouver Island Health	20 561 (14.4%)
Northern Health	11 238 (7.9%)
Unknown or out of BC	817 (0.6%)
Method of labor induction	
Spontaneous	106 317 (74.3%)
Induced	19 031 (13.3%)
No labor	17 754 (12.4%)
Mode of delivery	
Vaginal	105 246 (73.5%)
Cesarean section	37 860 (26.5%)
Self-reported ethnicity[a]	40 795 (28.5%)
0.7% Black, 60.3% Caucasian, 21.7% East Asian, 10.2% South Asian, 1.8% Indigenous, 4.4% Other	

[a]Proportions of each ethnicity category are calculated based only on the total number of pregnancies for which ethnicity information was available.

Some data are incomplete in the BCPDR and have been categorized as "unknown." Where cell counts were <5, counts are not reported. Percentages are calculated based on total number of pregnancies in the entire healthy cohort, except for ethnicity in which proportions reported are limited to the number of pregnancies that had linked ethnicity data.

Table 2. Trimester-specific RIs for 12 hematology parameters after outlier removal.

Analyte (unit)	Trimester	Mean	Lower limit	Upper limit	*N*	Lower limit 90% CI	Upper limit 90% CI
RBC count (10^{12}/L)	0–13 weeks	4.31	3.75	4.92	75 289	(3.75, 3.75)	(4.92, 4.93)
	14–27 weeks[a]	3.89	3.34	4.50	57 999	(3.33, 3.34)	(4.50, 4.51)
	28–41 weeks[a]	3.94	3.36	4.58	33 970	(3.35, 3.36)	(4.57, 4.59)
Hemoglobin (g/L)	0–13 weeks	128	112	143	75 917	(112, 112)	(143, 143)
	14–27 weeks[a]	117	101	132	59 312	(100, 101)	(131, 132)
	28–41 weeks[a]	118	99	135	34 632	(98, 99)	(135, 135)
Hematocrit (L/L)	0–13 weeks	0.38	0.34	0.41	64 579	(0.34, 0.34)	(0.41, 0.41)
	14–27 weeks[a]	0.35	0.31	0.38	50 606	(0.31, 0.31)	(0.38, 0.38)
	28–41 weeks[a]	0.35	0.31	0.39	29 292	(0.31, 0.31)	(0.39, 0.39)
MCV (fL)	0–13 weeks	88	80	94	73 701	(80, 80)	(94, 94)
	14–27 weeks[a]	90	81	97	56 913	(81, 81)	(97, 97)
	28–41 weeks[a]	89	79	97	33 596	(79, 79)	(97, 97)
RDW (%)	0–13 weeks	13.1	12.0	14.8	40 758	(12.0, 12.0)	(14.8, 14.8)
	14–27 weeks[a]	13.4	12.4	14.8	27 640	(12.4, 12.4)	(14.8, 14.8)
	28–41 weeks[a]	13.5	12.4	15.0	17 626	(12.4, 12.4)	(14.9, 15.0)
WBC count (10^9/L)	0–13 weeks	8.1	4.8	12.3	77 233	(4.8, 4.8)	(12.3, 12.3)
	14–27 weeks[a]	9.4	5.9	13.8	59 626	(5.8. 5.9)	(13.7, 13.8)
	28–41 weeks[a]	9.5	6.0	14.1	34 665	(6.0, 6.0)	(14.0, 14.2)
Neutrophil count (10^9/L)	0–13 weeks	5.5	2.7	9.0	77 116	(2.7, 2.7)	(8.9, 9.0)
	14–27 weeks[a]	6.8	4.0	10.6	59 586	(4.0, 4.0)	(10.5, 10.6)
	28–41 weeks[a]	6.9	4.0	10.8	38 018	(4.0, 4.0)	(10.8, 10.9)
Lymphocyte count (10^9/L)	0–13 weeks	1.9	1.1	3.0	76 945	(1.1, 1.1)	(3.0, 3.0)
	14–27 weeks	1.7	1.0	2.7	59 688	(1.0, 1.0)	(2.7, 2.7)
	28–41 weeks	1.7	1.0	2.7	34 574	(1.0, 1.0)	(2.7, 2.7)
Monocyte count (10^9/L)	0–13 weeks	0.6	0.3	0.9	76 370	(0.3, 0.3)	(0.9, 0.9)
	14–27 weeks[a]	0.6	0.3	1.0	58 348	(0.3, 0.3)	(1.0, 1.0)
	28–41 weeks	0.7	0.4	1.1	31 741	(0.4, 0.4)	(1.1, 1.1)
Eosinophil count (10^9/L)	0–13 weeks	0.2	0.1	0.4	68 383	(0.1, 0.1)	(0.4, 0.4)
	14–27 weeks	0.2	0.1	0.4	55 063	(0.1, 0.1)	(0.4, 0.4)
	28–41 weeks	0.2	0.1	0.4	30 542	(0.1, 0.1)	(0.4, 0.4)
Basophil count (10^9/L)	0–13 weeks[b]	0.1	0	0.3	77 661	NA	NA
	14–27 weeks[b]	0.3	0	0.6	59 785	NA	NA
	28–41 weeks[b]	0.1	0	0.3	34 698	NA	NA
Platelet count (10^9/L)	0–13 weeks	252	165	362	71 176	(165, 166)	(362, 363)
	14–27 weeks	232	149	339	54 377	(149, 150)	(337, 340)
	28–41 weeks	220	133	335	34 266	(133, 134)	(333, 336)

[a]Significant difference as per Harris and Boyd relative to first trimester (18).
[b]RI could not be estimated, minimum and maximum provided. NA.

Leukocytes. RIs were established for WBC count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils (Figs. 2 and 3). WBCs and neutrophils increased each trimester justifying RI binning (Table 2). Piecewise regression determined breakpoints of 6 and 26 weeks for WBCs and neutrophils. Values increased most markedly earlier in gestation (0–6 weeks) (Supplemental Files 7 and 8). For monocytes, trimester-

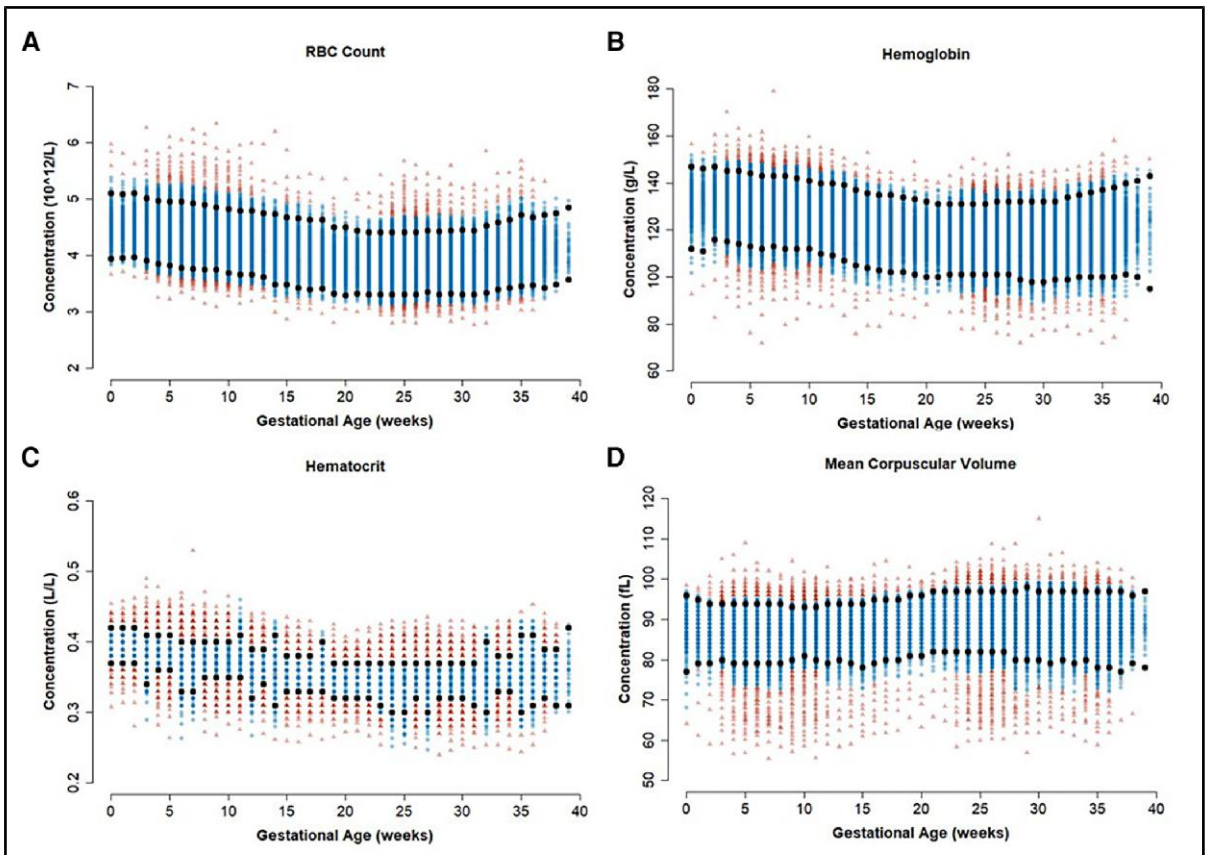

Fig. 1. Gestational age-specific scatterplots for (A) RBC count, (B) hemoglobin, (C) hematocrit, and (D) MCV. Circles indicate data included in final RI analysis. Triangles indicate data excluded as outliers. Lower and upper reference limits estimated for each week of gestation are marked in bold. The nearest 5 values per week of gestation were averaged for graphing purposes.

based partitioning was only significant between the first and second trimester, although changes in RI limits were only 0.1×10^9/L across trimesters and piecewise regression did not demonstrate marked quantitative changes in result values. Trimester bins were not indicated for lymphocytes, eosinophils, and basophils. Breakpoint regression demonstrated decreasing lymphocyte counts from 0 to 12 weeks and constant values between weeks 12 and 30, then slightly increasing (Supplemental File 9). These changes affected weekly RIs very minimally, with negligible differences when comparing RIs of week 7 to week 40 (Supplemental File 9). For basophils and eosinophils, RI limits at each week of gestation were identical (Fig. 3), and additional analyses could not be performed.

Covariate analysis for leukocyte parameters revealed statistically significant associations with several covariates (Supplemental Files 7–10) but could not be completed for basophil and eosinophil counts due to categorical data distribution (Supplemental Files 11 and 12).

Platelets. Trimester RIs for platelet count were significantly different between the first trimester compared to second and third trimesters (Fig. 3A). RI limits decreased by approximately 30 $\times 10^9$/L across gestation. In contrast to other parameters, the breakpoint regression model did not suit the data well ($R^2 = 0.066$) due to continuous and similar rate of decrease in platelet count throughout gestation (Supplemental File 13).

Significant associations between several covariates and platelet counts were also detected (e.g., age, prepregnancy BMI, smoking status, and use of IVF) (Supplemental File 13).

Discussion

In the current study, we defined a cohort of roughly 140 000 healthy Canadian women with uncomplicated pregnancies and leveraged existing clinical laboratory data to establish hematology RIs for each trimester

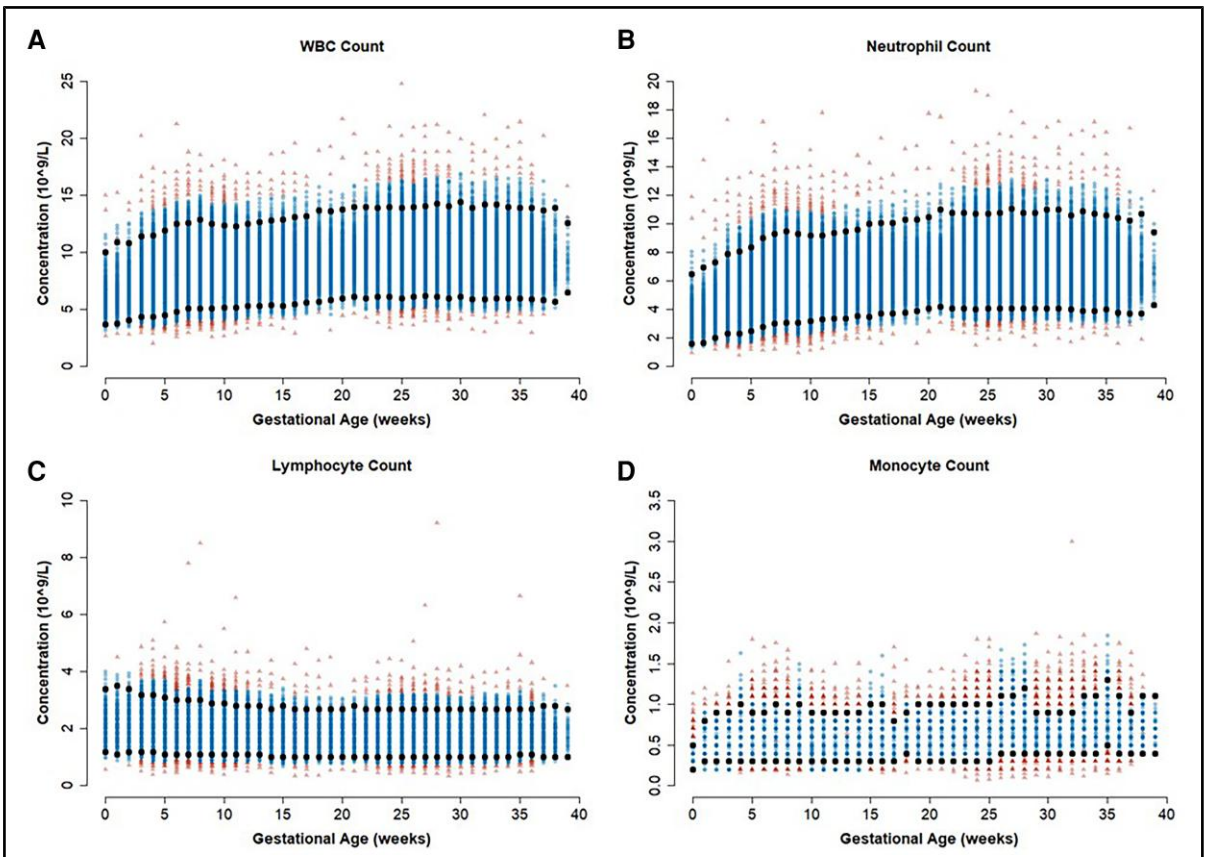

Fig. 2. Gestational age-specific scatterplots for (A) WBC count, (B) neutrophil count, (C) lymphocyte count, and (D) monocyte count. Circles indicate data included in final RI analysis. Triangles indicate data excluded as outliers. Lower and upper reference limits estimated for each week of gestation are marked in bold. The nearest 5 values per week of gestation were averaged for graphing purposes.

and week of gestation. Effectiveness of the clinical exclusion criteria applied was confirmed by the similar RI estimations produced using direct and indirect methods, and by comparing our healthy cohort to cohorts defined using other methods which identify complicated pregnancies (i.e., Obstetric Comorbidity Index and Severe Maternal Morbidity index) (23, 24). The derived RIs show that erythrocytes, leukocytes, and platelets change throughout pregnancy and highlight the need for pregnancy-specific test interpretation.

Analysis of RBC indices reaffirm dynamic changes in RBC count, hemoglobin, and hematocrit during pregnancy (6–13, 25). Segmented regression analysis revealed a U-shape pattern, with breakpoints at 20 and 30 weeks. The World Health Organization defines anemia in pregnancy as a hemoglobin level of <110 g/L (26). UK guidelines use a lower cutoff of 105 g/L in the second and third trimester (27). Our data indicate hemoglobin levels in pregnant women may be as low as 100 g/L in the second or third trimester. Future work evaluating pregnancy and infant outcomes in relation to maternal laboratory values and pregnancy RIs is needed to determine whether the definition of anemia in pregnancy requires revision. Previous studies have established RIs using trimester-specific bins (12, 13) or at specific time-points only (6, 9, 10). Our findings suggest trimester-specific RI bins for RBCs, hemoglobin, and hematocrit mask GA-associated changes, limiting result interpretation. Interestingly, recent dynamic modeling of pre-, post-, and pregnancy laboratory values in a large dataset in Israel support this conclusion as well (25).

MCV and RDW increased slightly across gestation. Trimester-specific increases in MCV were previously reported (9) and are consistent with physiological macrocytosis in pregnancy. While MCV varied by trimester, values were within 80–100 fL, a commonly used nonpregnant RI, and differences are unlikely to be clinically

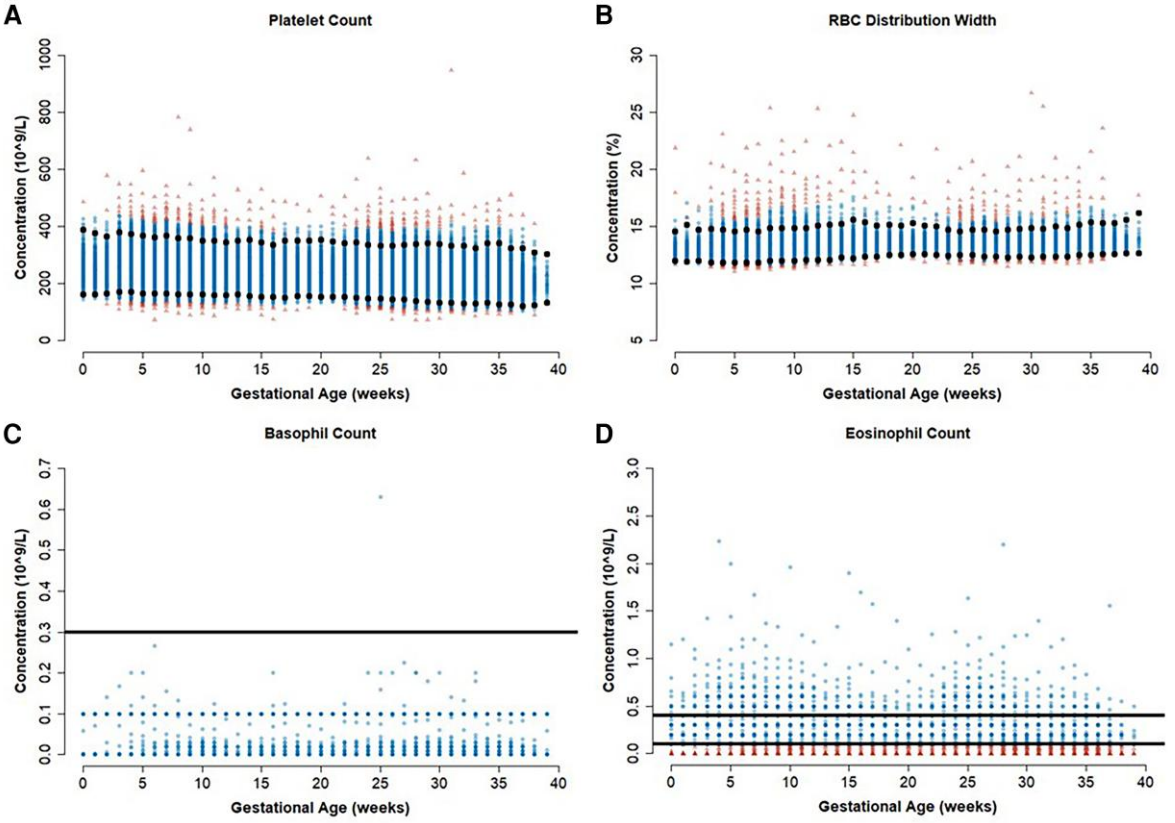

Fig. 3. Gestational age-specific scatterplots for (A) platelet count, (B) RDW, (C) basophil count, and (D) eosinophil count. Circles indicate data included in final RI analysis. Triangles indicate data excluded as outliers. Lower and upper reference limits estimated for each week of gestation are marked in bold. The nearest 5 values per week of gestation were averaged for graphing purposes.

significant (28). A recent systematic review of 28 studies summarizing RDW values in pregnancy reported differences between nonpregnant and pregnant RDW values in addition to increasing values with gestation similar to our findings (29).

Platelet counts decreased steadily throughout gestation in our cohort. Gestational thrombocytopenia ($<150 \times 10^9$/L), particularly in the third trimester, is estimated to occur in 5%–10% of normal pregnancies (30). A recent systematic review identified 46 studies where platelet counts were reported in $N \geq 30$ women with uncomplicated pregnancies (30). Pooled data showed decreasing platelets with combined trimester means of 251×10^9/L, 238×10^9/L, and 224×10^9/L (30). However, of the 21 studies that directly compared changes within pregnancy, 11 reported a significant decrease and 10 reported no change (30). Reese et al. evaluated platelet counts in 4568 uncomplicated pregnancies using retrospective data (5). In line with our findings, they reported a decline in platelet counts beginning in the first trimester and continuing throughout pregnancy (5). Bar et al. report the same trend (25). Platelets form a part of the diagnosis of preeclampsia and HELLP syndrome (hemolysis elevated liver enzymes and low platelet count), where a cutoff of $<100 \times 10^9$/L is used to define thrombocytopenia (31). In the present study, we report a third trimester platelet RI of 133–335×10^9/L. Reese et al. similarly reported that 97% of pregnant women had platelet counts $>125 \times 10^9$/L (5). Our findings thus support the recommended thrombocytopenia cutoff of $<100 \times 10^9$/L, as even in the third trimester, values <130 would be rare in uncomplicated pregnancy (31).

Of the leukocyte indices evaluated, WBC and neutrophil counts changed most across gestation, increasing markedly. Previous studies also reported increasing WBCs across trimesters, predominantly granulocytes (6–13). However, upper limits of WBC count vary across studies, ranging from 9.7×10^9/L to 15×10^9/L in the third trimester. The upper limits in our study

were 13.8×10^9/L and 14.1×10^9/L in the second and third trimester, respectively. The largest increases occurred early in gestation (0–6 weeks) at a rate of 0.28×10^9/L and 0.31×10^9/L per week for WBC and neutrophil count, respectively. No significant RI differences were observed across trimesters for lymphocyte, basophil, and eosinophil counts, similar to other reports (6, 11). Monocyte counts demonstrated a statistically significant difference between the first and second trimester; however, RIs only changed by 0.1×10^9/L. Pregnancy-specific and possibly GA-specific RIs are needed to interpret WBC and neutrophil counts. This is particularly important given that infections contribute significantly to maternal mortality and use of a nonpregnancy RI is not reliable given these rapid changes during pregnancy (32).

An additional outcome of this study is the evaluation of perinatal covariates on hematology indices, including maternal age, pre-pregnancy BMI, use of IVF, group B Streptococcus status, and ethnicity. The association with maternal age was significant, with lower RBCs, hemoglobin, hematocrit, platelets, and WBCs seen with increasing maternal age when adjusted for GA. A RI study of >12 000 healthy Canadian adults did not detect age-specific differences in hematology parameters between 20 and 40 years, with the exception of platelets (partitioned at 14–26 years and 27–79 years) (33). Our findings may suggest age interacts differently with CBC parameters in pregnancy as compared to nonpregnant states, or perhaps that normal pregnancy-related changes occur to a lesser degree in older mothers.

A positive association between pre-pregnancy BMI and RBC count, hemoglobin, hematocrit, platelet count, and leukocyte parameters was observed. BMI correlated negatively with MCV. In nonpregnant individuals, the effect of BMI on RBC indices is debated. A meta-analysis of 26 studies reported that overweight or obese nonpregnant individuals had increased risk of iron deficiency (34). Others report increased RBC and hemoglobin levels in obese individuals (35). Minimal evidence is available in pregnancy, with some studies reporting contrasting findings and suggesting the relationship could change in a trimester-specific manner (36). The observed positive association between pre-pregnancy BMI and leukocytes is expected and may be explained by elevated subclinical inflammatory parameters such as IL-6 and their role in bone marrow granulopoiesis (37).

We also evaluated whether CBC parameters vary with method of conception. In our study, lower RBC count, hemoglobin, RDW, and higher platelet and WBC counts were observed in individuals who had used assisted reproductive techniques relative to those who had not. While statistically significant, the difference in results between groups were minimal (e.g., RBC count: -0.07×10^{12}/L, platelet count: 3.59×10^9/L, WBC count: 0.27×10^9/L).

The influence of self-reported ethnicity was also assessed. Studies in nonpregnant populations have reported variation in hematology indices, particularly platelets and hemoglobin, across ethnicities (38). In our cohort, hemoglobin was observed to be lower in individuals identifying as Black, East Asian, and South Asian, and First Nation, relative to Caucasian. However, the difference in means was only 1–2 g/L. Platelets were lower in Caucasian populations (mean: 236×10^9/L) relative to individuals of Black (240×10^9/L), East Asian (240×10^9/L), South Asian (249×10^9/L), and First Nations ethnicity (291×10^9/L). It is unclear whether these differences need to be taken into consideration. Further work in this area is needed.

Last, given that data was extracted from 3 different laboratories, differences between laboratories were also assessed and found to be statistically significant (Supplemental Files 2–13). However, the magnitude of these differences was very small, not exceeding allowable performance limits specified by the Institute for Quality Management in Healthcare (a proficiency testing and laboratory accreditation body in Canada), which commonly defines allowable performance limits as 3 times the median method CV. Minimal differences between laboratories was expected since most data was derived from hematology systems from the same manufacturer.

The current findings are derived from a very large and comprehensive cohort of contemporary women and represent the most up-to-date pregnancy RI information applicable to Canadian mothers. Limitations of the current approach include that the BCPDR does not include data on all pregnancies in British Columbia, and while the cohort was restricted to live births only, because the BCPDR does not include data for unattended or home deliveries attended by an unregistered healthcare provider, some pregnancies were not represented. Based on BC Vital Statistics data, 220 737 births were recorded in the province over the same time period, suggesting 98% coverage by the BCPDR (Supplemental File 1). Information regarding iron or other supplement use that may affect CBC values was unavailable, although it is reasonable to assume a majority were taking prenatal vitamins. Geographic characteristics such as altitude that may affect CBC parameters could not be accounted for. Laboratory data were also not comprehensive. While we estimate that up to 80% of laboratory testing during pregnancy may be completed at LifeLabs, laboratory testing performed at other public or private laboratories was not included, and may cause a geographical or other location-based population bias in our cohort. Finally, while a priori clinical exclusions as well as statistical methods for

excluding distinct result distributions from a central healthy RI distribution were employed, no additional consideration was made to exclude or otherwise evaluate individuals who had repeated testing or an unusual frequency of testing performed during pregnancy.

The present study focused on approaches for deriving RIs for pregnant Canadians, and implementation of these findings remains an important future direction faced with significant hurdles such as a lack of GA information in the LIS, challenges integrating granular RI bins in laboratory reports, and illustrating normal vs abnormal dynamics of laboratory values throughout pregnancy.

Conclusion

Physiological changes of pregnancy impact laboratory values, including the CBC, complicating laboratory test interpretation. Use of existing laboratory and clinical data from comprehensive and well-documented sources provides an alternative and robust means of defining healthy pregnancy cohorts in a retrospective manner to derive RIs and improve laboratory test interpretation and inform future pregnancy RI studies. Individual CBC parameters change differently during pregnancy, and the ability to distinguish normal from abnormal changes requires RI granularity at the level of gestational week, rather than conventional trimester bins.

Supplemental Material

Supplemental material is available at Clinical Chemistry online.

Nonstandard Abbreviations: RIs, reference intervals; CBC, complete blood count; LIS, laboratory information systems; RBC, red blood cell; WBC, white blood cell; BMI, body mass index; BCPDR, British Columbia Perinatal Data Registry; CW, BC Children's and Women's Hospital; IVF, *in vitro* fertilization; MCV, mean corpuscular volume; RDW, RBC distribution width.

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