Defining Normal Healthy Term Newborn Automated Hematologic Reference Intervals at 24 Hours of Life

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• ***Context.***—**Automated analyzers have advanced the field of clinical hematology, mandating updated complete blood count (CBC) reference intervals (RIs) to be clinically useful. Contemporary newborn CBC RI publications are mostly retrospective, which some authors have cited as one of their cardinal limitations and recommended future prospective studies.**

Objective.—**To prospectively establish accurate hematologic RIs for normal healthy term newborns at 24 hours of life given the limitations of the current medical literature.**

Design.—**This prospective study was conducted at an academic tertiary care center, and hematology samples were collected from 120 participants deemed to be normal healthy term newborns. Distributions were assessed for normality and tested for outliers. Reference intervals were values between the 2.5th percentile and 97.5th percentile.**

Results.—**The novel RIs obtained for this study population are as follows: absolute immature granulocyte count, 80/μL to 1700/μL; immature granulocyte percentage, 0.6% to 6.1%; reticulocyte hemoglobin equivalent, 31.7 to 38.4 pg; immature reticulocyte fraction, 35.9% to 52.8%; immature platelet count, 4.73×10^3/μL to 19.72×10^3/μL; and immature platelet fraction, 1.7% to 9.8%.**

Conclusions.—**This prospective study has defined hematologic RIs for this newborn population, including new advanced clinical parameters from the Sysmex XN-1000 Automated Hematology Analyzer. These RIs are proposed as the new standard and can serve as a strong foundation for continued research to further explore their value in diagnosing and managing morbidities such as sepsis, anemia, and thrombocytopenia.**

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Laboratory tests leverage 60% to 70% of medical decisions and one of the most often ordered and impactful tests is the complete blood count (CBC).[1,2] These decisions require that clinicians have a standard for interpreting laboratory test results for their patients. For that reason, reference values interchangeably known as reference ranges or reference intervals (RIs) serve as critical tools. Reference intervals for a specific test usually represent a range of values between which 95% of reference individuals in a target population will fall.[1] Target populations for RIs optimally consist of healthy individuals selected in advance and defined by specific reference criteria. This selection process is termed *a priori* and is the recommended method.[3]

The a priori approach, however, is not feasible for populations where recruitment of healthy individuals is limited. Ethical constraints exist for performing an unnecessary needle stick and collecting nonclinically indicated blood samples from this vulnerable newborn population. Many newborn CBC RI publications have been retrospective and some authors cite this as one of their cardinal limitations. They employed an *a posteriori* selection process using existing clinically detailed large databases.[3] Retrospective data extraction was completed from newborns who had a CBC ordered for a clinical indication. Those identified with conditions that may affect the CBC were then deselected and their values excluded from the RI calculations.[4] Researchers have acknowledged that their values were not obtained from normal healthy newborns but that some pathologic concern existed, requiring an evaluation with a CBC.[5] Wiland et al[6] concluded that these population types are not ideal for determining a true RI and also reported that adult and child values for certain CBC parameters do not apply to newborns. They have recom-

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mended a future prospective study to determine those RIs in healthy newborns who otherwise have no indication for a CBC.[6]

Conducting a prospective study on newborns not requiring a clinically indicated CBC was perceived to be difficult. However, for this study design we seized the opportunity provided by Arizona's requirement that the nationally mandated blood spot newborn screen (NBS) be obtained between 24 and 72 hours of life (HOL).[7] Specifically at the study site, the Arizona NBS tests are collected at approximately 24 HOL. This timetable appeared optimal for this study, as many inherent stressors related to the delivery process, which could have affected the CBC, should have been resolved by this point. Since the newborn had a heel capillary blood sample (CBS) obtained for the NBS, the study did not require an additional procedure. The amount of blood collected was 0.5 mL, which is clinically insignificant in the patient population of normal healthy term newborns, who would not be adversely affected by the removal of this small volume.

New automated hematology analyzers continue to evolve, using fluorescent flow cytometry. This technology allows laboratories to obtain a more reproducible CBC, as well as the new advanced clinical parameters. Continued advancements mandate that the newborn CBC RIs be updated.

Therefore, there was a need for this prospective study, the purpose of which was to define RIs for the normal healthy term newborn at 24 HOL. In addition to the standard CBC test, the goal is to establish RIs for the advanced hematology parameters that include absolute immature granulocyte (IG) count, immature granulocyte percentage (IG%), reticulocyte hemoglobin equivalent (RET-H*e*), immature reticulocyte fraction (IRF), immature platelet count (IPC), and immature platelet fraction (IPF).

METHODS

The study protocol approval and human protection oversight was provided by the Banner Health Institutional Review Board. Informed parental consent was obtained, and STROBE and RECORD reporting guidelines were followed.[8] This prospective, single center, cross-sectional study was designed to establish automated hematologic RIs for normal healthy term newborns at approximately 24 HOL. Study enrollment occurred between May 24, 2016, and November 1, 2017. The research was conducted at Banner – University Medical Center Phoenix (BUMCP) (Phoenix, Arizona), an academic tertiary care center where approximately 5800 newborns are delivered annually.

The study subjects included were inborn at BUMCP and deemed to be normal healthy term newborns. The patients enrolled in this study were determined to be both normal and healthy newborns, which may be perceived as redundant terminology. *Normal* is defined as "the usual, typical, or expected" condition, in this case referring to a newborn's physical examination.[9] Newborns who were determined to have any examination deviations such as dysmorphisms consistent with a chromosomal syndrome, or congenital anatomical abnormality as simple as polydactyly, were excluded. *Healthy* refers to the body's relative well-being and absence of clinical signs of disease or infection.[10] Newborns who developed any sign or symptom that required additional medical evaluation as simple as jaundice were excluded. This study population was also derived by enrolling patients whose history was void of maternal and newborn conditions that could affect the newborn's CBC results. Maternal exclusionary conditions were as follows: hypertension; preeclampsia; antibiotic/antiviral treatment; hormonal, hematologic, or autoimmune disorders; intrauterine growth restriction; twin to twin transfusion; exposure to steroids or erythropoietin. Newborn exclusionary conditions were as follows: gestational age (GA) less than 37 0/7 weeks or greater than 41 6/7 weeks; birth weight less than 2800 g or greater than 4000 g; intrauterine drug exposure to prescribed medications (other than those required for obstetrical management and that were not excluded on the basis of maternal criteria) or illicit and legal drugs including alcohol and tobacco; performance of any diagnostic evaluations (eg, CBC, glucose, chest radiographs) with the exception of the mandated Arizona NBS or the standardly obtained blood type and direct antiglobulin test when indicated; associated birth stressors (eg, shoulder dystocia, nuchal cord, fracture, meconium, 5-minute Apgar score less than 7); chromosomal disorders (eg, trisomy 21); or congenital anomalies (eg, cleft lip).

The study sample was obtained by CBS after local warming with DeNovo Products Gel Infant Heel Warmer (product No. 356, Ft. Myers, Florida) for at least 2 minutes and appropriate cleansing of the site. After piercing the skin with the Cardinal Health's GentleHeel incision device (product No. GHN4X250, Waukegan, Illinois) and removing the first drop, 0.5 mL of blood was collected in a Beckton, Dickinson and Company's Microtainer Blood Collection Tube with K2EDTA (product No. BD 365974-1, Franklin Lakes, New Jersey), followed by rolling and inverting the tube multiple times to prevent clotting. The de-identified sample was analyzed within 2 hours of collection.

A single Sysmex XN-1000 Automated Hematology Analyzer (Sysmex XN-1000, Sysmex America, Lincolnshire, Illinois) was used to generate a CBC with white blood cell (WBC) differential for each newborn, including the advanced hematology parameters. A total of 170 blood specimens were sent to the laboratory. All samples underwent a visual specimen integrity check to determine the presence of clots and, if absent, were deemed acceptable to run on the analyzer. Forty-eight samples were rejected secondary to clotting and 2 for insufficient quantity of blood, yielding 120 evaluable samples. No samples were excluded as a result of the peripheral blood smear review. Daily quality control procedures were performed according to the manufacturer's recommendations. A peripheral blood smear slide was scanned in the event of an instrument flag. All flags were addressed, and if necessary, the samples were rerun per laboratory policy. A sample size was chosen per the recommendations of the Clinical and Laboratory Standards Institute that states "the best means to establish a RI is to collect samples from a sufficient number of qualified reference individuals to yield a minimum of 120 samples for analysis."[11]

Descriptive statistics were reported on all values measured with the Sysmex XN-1000, using mean and standard deviation for normally distributed variables and median and interquartile range (IQR) for those not normally distributed. Distributions were assessed for normality by using Kolmogorov-Smirnov tests, and transformed as needed by using the Box-Cox method.[12] Data were tested for outliers by using the method of Dixon.[13] Briefly, the IQR was calculated, and 1.5 times IQR was added to the 75th percentile value and subtracted from the 25th percentile value. Values more extreme than these limits were excluded from further analysis. Reference intervals for each variable were then designated as those between the 2.5th percentile and 97.5th percentile.

RESULTS

Parental consent for 200 potential participants was obtained. There were 30 instances in which patients were no longer eligible at the time of sample collection because, in the interim, a CBC, bilirubin, or a blood glucose test was performed for a clinical indication. The CBS was collected with a mean time of 24 hours and 13 minutes and a standard deviation of 35 minutes from the same procedure used to obtain the Arizona NBS. The samples were assigned a study number and sent without patient identifiers to the laboratory for analysis.

Demographic information, shown in Table 1, was collected for each newborn and included GA, birth weight, sex, and route of birth. There was a higher representation of

Table 1. Demographics (n = 120)		
	Mean	Standard Deviation
Gestational age, wk	39 4/7	0.96
Birth weight, g	3398	277
	Fraction	Percentage
Female	68/120	56.7
Male	52/120	43.3
Vaginal deliveries	90/120	75.0

females (68 of 120 [57%]) and vaginal births (90 of 120 [75%]) in the population, with a mean GA of 39 weeks and 4 days and a mean birth weight of 3398 g.

The RIs for the hematologic parameters are listed in Tables 2 through 4. In addition to the usually reported CBC parameters, RIs are reported for these novel measurements: absolute IG count, IG%, RET-H*e*, IRF, IPC, and IPF.

DISCUSSION

Factors That Can Affect CBC Reference Intervals

Analyzing RI studies can be very difficult, specifically in the newborn population, because multiple factors can impact the results. Gestational age and weight at birth, postnatal age down to the actual HOL when the study specimen was drawn, the laboratory collection method, the study type and design including how data are interpreted and represented, the type of analyzer used, and an evolving obstetrical intervention called *delayed umbilical cord clamping* can all affect newborn CBC values. Hemoglobin and hematocrit increase with GA, while the mean corpuscular volume and reticulocyte counts decrease.[14] Yet, GA has a negligible impact on RET-H*e* levels.[15] Platelet counts mildly increase with GA and the IPF decreases.[16–18] Reviewing the work of Mouzinho et al[19] and Manroe et al[20] with healthy newborns revealed that neutrophil counts in low-birth-weight preterm newborns weighing less than 1500 g were lower and had a wider range of distribution than in larger, term newborns. Comparatively, 95% of the low-birth-weight preterm newborns in the study of Mouzinho et al[19] would have been classified as neutropenic with the data reported by Manroe et al[20] on term newborns.

Hematologic parameters change in the first hours, days, weeks, and months of postnatal life. In term newborns, mean hemoglobin values decreased approximately 5 g/dL between birth and 28 days of life and the mean platelet count increased $140 \times 10^3/\mu L$ by 90 days of life.[17] Lofving et al[21] reported that RET-H*e* decreases after birth and levels off at 4 months. Neutrophil counts are low at delivery, but increase after birth, with a peak value between 6 and 14 HOL, then decreasing and leveling off at approximately 72 hours.[20,22] Immature granulocytes are higher during the first 2 days of life with MacQueen et al[18] reporting the 95th percentile for IG% as 6.2% from 0 to 48 HOL, decreasing to 4.2% after 48 hours.

There is a difference in CBC results dependent on whether a specimen was obtained as a CBS or venous blood sample. A recent review of 27 studies comparing hemoglobin concentration between capillary and venous blood samples found that 20 studies had reported the hemoglobin levels from CBS to be higher, 4 studies found venous blood samples to be higher, and 3 found no significant difference.[23] Two studies compared CBC results on healthy, term newborns during the first day of life who had capillary and venous blood samples drawn at the same time. Both studies found the hemoglobin level to be higher in the CBS.[24,25] One of these studies also found capillary samples to have lower platelet counts than the venous samples.[25]

The study type, retrospective versus prospective, can impact the RI results. Many RI studies are retrospective, where newborns had a clinical indication requiring a CBC to be performed, which was an exclusionary criterion of our study. Depending on the study design and population, RI cutoffs ranged from excluding the upper and lower 2.5th percentile, 5th percentile, or 10th percentile of results, or used 1 or 2 standard deviations. How RI data is analyzed and reported is inconsistent, making it difficult to determine where the upper and lower cutoffs should be placed. Additionally, in some publications, data comparisons are difficult because results were reported graphically, and the readers needed to estimate laboratory values from the curves.

Table 2. White Blood Cell Reference Intervals at 24 Hours of Life				
Measurand	2.5th Percentile	Median	97.5th Percentile	Outliers, N
White blood cell count/μL ($\times 10^9$/L)	11 500 (11.5)	20 400 (20.4)	29 400 (29.4)	1
Absolute neutrophil count/μL ($\times 10^9$/L)	6300 (6.3)	12 400 (12.4)	18 700 (18.7)	8
Neutrophil percent, % (proportion of 1.0)	51.6 (0.52)	61.1 (0.61)	73.0 (0.73)	6
Absolute immature granulocyte count/μL ($\times 10^9$/L)	80 (0.08)	410 (0.41)	1700 (1.70)	0
Immature granulocyte percent, % (proportion of 1.0)	0.6 (0.01)	2.0 (0.02)	6.1 (0.06)	0
Absolute lymphocyte count/μL ($\times 10^9$/L)	2800 (2.8)	4700 (4.7)	7000 (7.0)	7
Lymphocyte percent, % (proportion of 1.0)	14.8 (0.15)	23.8 (0.24)	36.4 (0.36)	2
Absolute monocyte count/μL ($\times 10^9$/L)	820 (0.82)	1790 (1.79)	3300 (3.30)	0
Monocyte percent, % (proportion of 1.0)	5.6 (0.06)	9.4 (0.09)	12.5 (0.13)	8
Absolute eosinophil count/μL ($\times 10^9$/L)	80 (0.08)	470 (0.47)	1250 (1.25)	1
Eosinophil percent, % (proportion of 1.0)	0.7 (0.01)	2.4 (0.02)	7.5 (0.08)	3
Basophil count/μL ($\times 10^9$/L)	40 (0.04)	110 (0.11)	240 (0.24)	5
Basophil percent, % (proportion of 1.0)	0.3 (0.00)	0.6 (0.01)	1.0 (0.01)	6

Note: Système International (SI) units in parentheses.

To convert microliters (μL) to 10^9/L, multiply by 0.001.

To convert a percentage (%) to a proportion of 1.0, multiply by 0.01.

Table 3. Red Blood Cell Reference Intervals at 24 Hours of Life				
Measurand	**2.5th Percentile**	**Median**	**97.5th Percentile**	**Outliers, N**
Red blood cell count, × 10^6/μL (× 10^{12}/L)	3.88 (3.88)	4.70 (4.70)	5.74 (5.74)	0
Hemoglobin, g/dL (g/L)	13.6 (136)	16.8 (168)	19.8 (198)	0
Hematocrit, % (proportion of 1.0)	39.2 (0.39)	47.1 (0.47)	56.5 (0.57)	6
Mean corpuscular volume, μm^3 (fL)	90.5 (90.5)	99.6 (99.6)	106.8 (106.8)	7
Mean corpuscular hemoglobin, pg	32.3	35.3	37.6	8
Mean corpuscular hemoglobin concentration, g/dL	34.1	35.4	36.7	6
Red cell distribution width – standard deviation, μm^3 (fL)	51.6 (51.6)	57.6 (57.6)	68.1 (68.1)	0
Red cell distribution width – coefficient of variation, %	15.0	16.5	18.8	4
Nucleated red blood cells, %	0.1	0.4	2.2	0
Reticulocyte count, × 10^3/μL (× 10^9/L)	0.16 (0.16)	0.21 (0.21)	0.29 (0.29)	0
Reticulocyte percent, % (proportion of RBCs)	3.2 (0.03)	4.5 (0.05)	6.0 (0.06)	4
Immature reticulocyte fraction, %	35.9	44.7	52.8	0
Reticulocyte hemoglobin equivalent, pg	31.7	35.6	38.4	2

Abbreviation: RBCs, red blood cells.

Note: Système International (SI) units in parentheses.

To convert 10^6/μL to 10^{12}/L, multiply by 1.

To convert a percentage (%) to a proportion of 1.0 or RBCs, multiply by 0.01.

To convert grams per deciliter (g/dL) to grams per liter (g/L), multiply by 10.

To convert cubic micrometer (μm^3) to femtoliter (fL), multiply by 1.

To convert 10^3/μL to 10^9/L, multiply by 1.

Newer automated hematology analyzers do not all share exactly the same technology. Most have improved cell classification such that many of the manual peripheral blood smear differential counts are no longer required. Older instruments could not separate nucleated red blood cells (NRBCs) from lymphocytes, falsely elevating the WBC count. In patients with circulating NRBCs, a peripheral blood smear with a manual differential had to be performed so that a corrected WBC count could be calculated and reported. As NRBCs are present in healthy newborns in their first few days of life, older instruments required that a manual differential be performed.[26] With newer instruments, the NRBCs are identified and the WBC count and automated NRBC count can be reported directly from the analyzer. Sysmex instruments also identify and quantify immature granulocytes, which include metamyelocytes, myelocytes, and promyelocytes. Previously these cells could only be identified and reported by a manual differential performed on a peripheral blood smear.

Delayed umbilical cord clamping has been shown to increase hemoglobin levels in newborns; however, the procedural aspects of delayed cord clamping with a focus on the length of time prior to cord clamping remains variable in the literature and in practice. The most common time increment recommended is 1 minute, which aligns with the World Health Organization.[27] The American Academy of Pediatrics and American College of Obstetricians and Gynecologists recommend at least 30 to 60 seconds, which our institution supports.[27] Our institution supports a 30- to 60-second delay. At the time of this study, and currently, umbilical cord clamping was not uniformly performed, resulting in the decision to not record those data. Most reported neonatal RIs also do not include cord clamping time in their results.

Comparison to Other Studies

Our results, and comparison with other studies reporting hemoglobin, RET-H*e*, platelet count, IPF, and IG% RI in healthy term newborns, are summarized in Table 5. Our study suggests that hemoglobin levels below 13.6 g/dL should be considered anemic in term newborns at 24 HOL. However, as multiple factors can affect the hemoglobin results, this value may not be applicable to a preterm infant, or a term newborn at other postnatal ages. Our platelet counts are higher than those of other studies, including those using venous blood, which was not expected. This may be due to differences in the analyzer used, methods of data reporting, and/or postnatal age. There are a few studies reporting neonatal RIs for the newer advanced clinical parameters of IG%, IRF, Ret-H*e*, and IPF. One goal of the study was to define RIs for these tests and highlight how they can be used in the newborn population.

Table 4. Platelet Reference Intervals at 24 Hours of Life				
Measurand	**2.5th Percentile**	**Median**	**97.5th Percentile**	**Outliers, N**
Platelet count, × 10^3/μL (× 10^9/L)	160 (160)	277 (277)	406 (406)	7
Immature platelet fraction, %	1.7	3.5	9.8	2
Immature platelet count, × 10^3/μL (× 10^9/L)	4.73 (4.73)	10.06 (10.06)	19.72 (19.72)	3
Mean platelet volume, μm^3 (fL)	9.0 (9.0)	10.3 (10.3)	11.6 (11.6)	3

Note: Système International (SI) units in parentheses.

To convert 10^3/μL to 10^9/L, multiply by 1.

To convert cubic micrometer (μm^3) to femtoliter (fL), multiply by 1.

Table 5. Comparison of Reference Intervals for Select Complete Blood Count (CBC) Tests With Information on Patient Selection and Study Design

Source, y	Lower Reference Interval	Percentile (%-ile) or SD ()	Mean[a]	Upper Reference Interval	Percentile (%-ile) or SD ()	Prospective (P) or Retrospective (R)	Single Institution (S) or Multiple Institutions (M)	Analyzer	Gestational Age, wk	Postnatal Age	Sample Size	Sample Type
Hemoglobin, g/dL												
Current study, 2020	13.6	2.5%-ile	16.8	19.8	97.5%-ile	P	S	Sysmex XN-1000	37–42	23–25 h	120	Capillary blood
Jopling et al,[51] 2009	14.0	5%-ile	17.0	22.0	95%-ile	R	M	Beckman Coulter	37–42	0-6 h	24 416	Not specified
Henry & Christensen,[17] 2015	14.0	5%-ile	17.0	21.8	95%-ile	R	M	Not specified	35–42	Day of birth	Thousands	Not specified
Christensen et al,[28] 2016	15.0	10%-ile	17.5	21.5	90%-ile	R	M	Sysmex XE-5000 and XT-4000i	37.6 (2.4)	First day of life	6632	Not specified
Lorenz et al,[15] 2017	14.7	−SD (2.3)	17.0	19.3	+SD (2.3)	R	S	Sysmex XE-2100	37–42	0–24 h	216	Venous blood
Özbek et al,[24] 2000	16.9	−SD (2.4)	19.3	21.7	+SD (2.4)	P	S	Coulter STKS	38–41	6–20 h	95	Capillary blood
(capillary versus venous blood)	15.3	−SD (1.9)	17.2	19.1	+SD (1.9)							Venous blood
Eslami et al,[25] 2012	13.6	−SD (5.8)	19.6	25.4	+SD (5.8)	P	M	Advia 120	38–42	First day of life	1558	Capillary blood
(capillary versus venous blood)	9.2	−SD (7.8)	17.0	24.8	+SD (7.8)							Venous blood
Andersson et al,[52] 2011	15.6	−SD (1.9)	17.5	19.4	+SD (1.9)	P	S	Sysmex XE-2100	37–42	48–72 h	160	Venous blood; early cord clamping
(early versus late cord clamping)	17.2	−SD (1.7)	18.9	20.6	+SD (1.7)						162	Venous blood; delayed cord clamping
Reticulocyte hemoglobin equivalent, pg												
Current study, 2020	31.7	2.5%-ile	35.6	38.4	97.5%-ile	P	S	Sysmex XN-1000	37–42	23–25 h	120	Capillary blood
Christensen et al,[28] 2016	30.0	10%-ile	34.0	37.0	90%-ile	R	M	Sysmex XE-5000 and XT-4000i	37.6 (2.4)	First day of life	6632	Not specified
Lorenz et al,[15] 2017	25.5	2.5%-ile	32.0	37.6	95%-ile	R	S	Sysmex XE-2100	37–42	0–24 h	216	Venous blood
Lofving et al,[21] 2018	28.1	−2SD	32.9	37.7	+2SD	P	S	Sysmex XE-2100	39.8 (1.1)	48–72 h	253	Not specified
Andersson et al,[52] 2011	30.1	−SD (2.4)	32.5	34.9	+SD (2.4)	P	S	Sysmex XE-2100	37–42	48–72 h	160	Venous blood; early cord clamping
(early versus late cord clamping)	30.1	−SD (2.6)	32.7	35.3	+SD (2.6)						162	Venous blood; delayed cord clamping
Platelet count, × 10^3/μL												
Current study, 2020	160	2.5%-ile	277	406	97.5%-ile	P	S	Sysmex XN-1000	37–42	23–25 h	120	Capillary blood
Wiedmeier et al,[53] 2009	130	5%-ile	250	375	95%-ile	R	M	Beckman Coulter	37–42	0–3 d	22 887	Not specified
Henry & Christensen,[17] 2015	110	5%-ile	240	350	95%-ile	R	M	Not specified	37–42	Day of birth	Thousands	Not specified
Özbek et al,[24] 2000	111	−SD (68)	179	247	+SD (68)	P	S	Coulter STKS	38–41	6–20 h	95	Capillary blood
(capillary versus venous blood)	136	−SD (77)	213	290	+SD (77)							Venous blood
Immature platelet fraction, %												
Current study, 2020	1.7	2.5%-ile	3.5	9.8	97.5%-ile	P	S	Sysmex XN-1000	37–42	23–25 h	120	Capillary blood
Cremer et al,[54] 2009	0.7	2.5%-ile	4.3	7.9	97.5%-ile	P	S	Sysmex XE-2100	36.3 (3.7)	0-7 d	456	Not specified
Yuko et al,[16] 2013	1.5	−SD (1.3)	2.8	4.1	+SD (1.3)	R	S	Sysmex XE-2100	37–41	0–24 h	45	Not specified
MacQueen et al,[32] 2017	2.0	5%-ile	4.0[a]	8.0	95%-ile	R	M	Sysmex XE-5000 and XT-4000i	37–42	Day of birth	8969	Not specified

Table 5. Continued

Source, y	Lower Reference Interval	Percentile (%-ile) or SD ()	Mean[a]	Upper Reference Interval	Percentile (%-ile) or SD ()	Prospective (P) or Retrospective (R)	Single Institution (S) or Multiple Institutions (M)	Analyzer	Gestational Age, wk	Postnatal Age	Sample Size	Sample Type
Immature granulocyte, %												
Current study, 2020	0.6	2.5%-ile	2.0	6.1	97.5%-ile	P	S	Sysmex XN-1000	37–42	23–25 h	120	Capillary blood
Wiland et al,[6] 2014	—	—	—	5.2	95%-ile	R	M	Sysmex-XE	≥35	0–48 h	215	Not specified
MacQueen et al, 2016[18]	0.5	5%-ile	2.0[a]	6.2	95%-ile	R	M	Sysmex XE-5000 and XT-4000i	37.3 (2.4)	0–48 h	4808	Not specified
Patient selection and exclusions												
Current study, 2020	Healthy term newborns without fetal or maternal indications for clinical laboratory tests. Maternal exclusions: hypertension; preeclampsia; antibiotic/antiviral treatment; hormonal, hematologic, or autoimmune disorders; intrauterine growth restriction; twin to twin transfusion; exposure to steroids or erythropoietin. Neonatal exclusions: birth weight < 2800 g or > 4000 g; intrauterine drug exposure, or diagnostic evaluations performed											
Jopling et al,[51] 2009	Data from archived electronic records on neonates born between January 2002 and June 2008 during the neonatal period (28 days). Maternal exclusions: placenta previa or abruptio placenta. Neonatal exclusions: RBC transfusion, neonatal anemia. Data extrapolated from a graph											
Henry & Christensen,[17] 2015	CBC results on neonates 0 to 28 days old between 2005 and 2014. Exclusions: International Classification of Disease, 9th revision (ICD9 code) for chromosomal abnormality or RBC transfusion before CBC was drawn. Data extrapolated from a graph											
Christensen et al,[28] 2016	Clinically ordered CBC results from infants 0 to 90 days old, between May 2014 and May 2015. RET-He performed as a convenience sample on the CBCs and not reported. Exclusions: RBC transfusion or a diagnosis of anemia. Data extrapolated from a graph											
Lorenz et al,[15] 2017	CBCs from infants with a clinically indicated blood sample obtained within 24 hours after birth from December 2012 to January 2014. Exclusions: hematologic diseases, metabolic disorders, congenital viral infection, and congenital anomalies. Composed of singletons (73.2%), twins (22.7%), and triplets (4.1%)											
Özbek et al,[24] 2000	Comparison of CBC values from capillary blood and venous blood samples that were obtained simultaneously. Healthy term newborns from normotensive, nondiabetic mothers following uneventful, singleton pregnancies. Exclusions: inappropriate development for gestational age or abnormal physical examination findings											
Eslami et al,[25] 2012	Comparison of CBC values from capillary blood and venous blood samples that were obtained simultaneously. Maternal exclusions: placenta previa, abruptio placenta, chronic hypertension, or diabetes. Neonatal exclusions: anemia, intrauterine growth retardation, or small for gestational age											
Andersson et al,[52] 2011	Comparison of CBC results with early cord clamping (less than 10 seconds after delivery) and delayed cord clamping (greater than 180 seconds after delivery). Data collected from April 2008 to September 2009. Nonsmoking, healthy women after normal pregnancy, singleton, term pregnancy, expected vaginal birth with cephalic presentation. Exclusions: serious congenital malformations, syndromes, or other congenital diseases that could affect the outcome measures											
Lofving et al,[21] 2018	Term neonates with normal birth weights, nonsmoking mothers, and uneventful pregnancies. Data collected from April 2008 to May 2009 and from June 2010 to February 2017. Exclusions: anemia, iron deficiency, or presence of inflammation											
Wiedmeier et al,[53] 2009	De-identified limited data set from archived electronic medical records of inpatients and outpatients with at least 1 platelet count obtained between January 2001 and September 2007 who were between 0 and 90 days old. All of the eligible records contained in the database were used for the analysis. Data extrapolated from a graph											
Cremer et al,[54] 2009	All neonates admitted to the neonatal intensive care unit during a 6-month period. Exclusions: platelet count less than 150 × 10^3/μL (150 × 10^9/L) or platelet transfusion											
Yuko et al,[16] 2013	Newborns admitted to the neonatal intensive care unit from January to October 2011. Exclusions: chromosomal abnormalities, perinatal infection, early-onset sepsis, disseminated intravascular coagulation, neonatal thrombocytopenia, or neonates admitted at night or on the holidays											
MacQueen et al,[32] 2017	De-identified data from neonatal intensive care unit patients, collected from February 2013 through January 2016. Exclusions: platelet counts less than 120 × 10^3/μL (120 × 10^9/L) or platelet transfusion. Data extrapolated from a graph											
Wiland et al,[6] 2014	Retrospective chart review from December 2010 to June 2011 of newborns who had an automated CBC with differential performed in the first 48 hours of life. Neonates with a negative blood culture who received antibiotics for <72 hours were not excluded. Exclusions: <35 weeks, birth at an outside hospital, treatment with antibiotics before a blood culture was drawn, a positive blood culture, presumption of sepsis and/or meningitis by the attending physician, treatment with medications known to interfere with CBC (such as antiretroviral medications), ABO incompatibility with positive direct antibody test, or major congenital anomaly											
MacQueen et al,[18] 2016	CBCs obtained between May 2014 and September 2015. Reference intervals were developed for IGs after excluding from the database values from neonates likely to have an infection. Exclusions: positive culture of a usually sterile body fluid (specifically, blood, urine, or spinal fluid), or negative culture but a diagnosis of "clinical sepsis" defined by antibiotic administration for greater than 5 days because of the diagnosis of pneumonia or clinical sepsis charted by the attending physician											

Abbreviations: IG, immature granulocyte; RBC, red blood cell; RET-He, reticulocyte hemoglobin equivalent.

Note: Système International (SI) units in parenthesis.

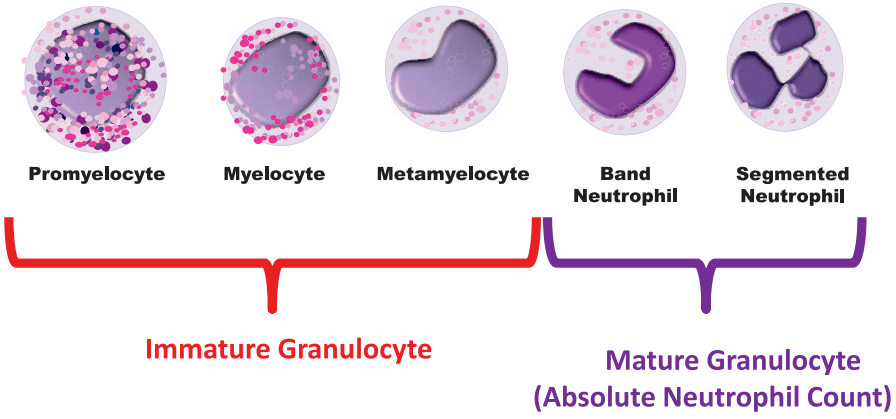
To convert grams per deciliter (g/dL) to grams per liter (g/L), multiply by 10.

To convert 10^3/μL to 10^9/L, multiply by 1.

To convert a percentage (%) to a proportion of 1.0, multiply by 0.01.

[a] MacQueen and colleagues reported median values. The rest of the studies reported mean values.

Stages of neutrophil maturation.



Immature Granulocytes.—The automated hematology analyzer separates neutrophil maturation into 2 groups as illustrated in the Figure. Mature granulocytes consist of segmented neutrophils and bands, and are also reported as the absolute neutrophil count. Immature granulocytes include metamyelocytes, myelocytes, and promyelocytes. Historically, bands have been considered less mature and were correlated with a shift toward immature cells (left shift); however, bands are fully functional phagocytes and based on their ability to fight infection, are classified as mature neutrophils.

Several publications have looked at immature granulocytes as a predictor of newborn sepsis and as a potential surrogate for the immature to total neutrophil ratio.[6,28–31] Since a left shift can be present at birth, it is difficult to determine what is abnormal in the absence of a well-established normal RI for IG% and absolute IG count. To develop RIs that are representative of the normal newborn population, Wiland et al[6], Nigro et al,[29] and MacQueen et al[32] looked independently at samples of newborns considered noninfected by excluding those with positive blood cultures or "clinical sepsis charted by the physician." From these presumptions, Wiland et al[6] established the IG% at 5.2% and MacQueen[32] established the upper limit for IG% at 6.2% during the first 48 HOL. Our study identifies an IG% of up to 6.1% at 24 HOL as normal, which correlates with the study of MacQueen and colleagues.[32]

Reticulocytes and Immature Reticulocytes.—Red blood cells (RBCs) mature from committed stem cells in the bone marrow by progressing from NRBCs, to reticulocytes, to mature RBCs. After the nucleus is extruded, the reticulated RBC contains residual RNA that is lost during the first 24 hours after it has been released into the peripheral blood. The reticulocytes are quantified from the presence of RNA. The IRF indicates the youngest reticulocytes with the highest RNA and is reported as a percentage of the total reticulocytes.

A recent large, retrospective study[28] reported the IRF as 30% to 40% on the first day of life. The IRF can be used to help determine erythropoietic activity. An increase in the IRF may predate the increase in the absolute reticulocyte count.[33] The IRF can be used along with the reticulocyte count to help assess the causes of anemia. With ineffective erythropoiesis (eg, myelodysplasia and dyserythropoietic anemia), IRF is increased, while the reticulocyte count is reduced or normal.[34] Increased erythropoietic activity is supported when IRF and reticulocyte count are both elevated.[35] Our study identified the RI for IRF as 35.9% to 52.8%.

Reticulocyte Hemoglobin Equivalent.—RET-H*e* is a measurement of hemoglobin in reticulocytes. Since reticulocytes represent young RBCs in the peripheral blood, which become mature during a 24-hour period, the information from these cells is a snapshot of the available iron in the bone marrow. A low RET-H*e* suggests iron deficiency.[36] Other tests for iron deficiency, including serum iron, ferritin, and total iron-binding capacity, can be affected by inflammation and uremia, limiting the usefulness of these tests when evaluating a patient with an infection or renal disease. The RET-H*e* is not affected by inflammation or uremia, giving accurate results for patients with these conditions.[37] The classic findings of microcytic, hypochromic RBCs from iron deficiency anemia are late changes. RET-H*e* can detect functional iron deficiency in the bone marrow before a patient has morphologic RBC changes and even before the onset of anemia.[38,39] For RET-H*e*, our study identified the 2.5%th percentile as 31.7 pg. Other studies identified the lower cutoff for RET-H*e* as ranging from 25.5 pg to 30.1 pg (Table 5).

Immature Platelets.—The IPF is a measurement of the percentage of reticulated platelets that have been recently released from the bone marrow. The IPC represents the absolute value of the immature platelets and is the newest platelet parameter reportable by the next-generation analyzers.

The IPF provides information on the rate of thrombopoiesis and can help determine the pathophysiological mechanism of thrombocytopenia.[40] It is well accepted that a higher IPF is usually found in either consumptive or recovering thrombocytopenic disorders, owing to increased platelet production compensating for the peripheral destruction.[40,41] A low or normal IPF is characteristic of decreased bone marrow production.[42] The IPF can also be used to avoid unnecessary platelet transfusion for certain pediatric patients following bone marrow recovery.[40,43] Zucker et al[44] found that the IPF recovered on average 3.1 days before the platelet count. Obtaining serial IPF levels is recommended in patients with thrombocytopenia.[45] Our study found the upper cutoff for IPF to be 9.8%, and in other studies it ranges from 4.1% to 8.0% (Table 5).

STRENGTHS AND LIMITATIONS

This is the first prospective study reporting hematologic RIs, including the new advanced parameters for normal healthy term newborns at 24 HOL, from a globally used

modern hematology analyzer. Of all CBC parameters, the neutrophil count is the main reason this test is ordered in the newborn period. It is most commonly used for those newborns at risk and/or having symptoms that could be indicative of infection. These RIs are aimed at providing clinicians with a critical threshold to initiate investigation, and if needed, treatment. As cited above, a complicating factor is that hematologic parameters, specifically neutrophils, even in heathy newborns, show variations as postnatal age progresses.

There are also unique challenges in newborn medicine that create obstacles to conducting a prospective research study. Foremost, this population is deemed vulnerable, which results in ethical constraints. Understandably, obtaining parental consent is difficult, even if their newborn is considered to be normal and healthy. Time was the other critical constraint in accomplishing this study. The postpartum LOS (length of stay) had already diminished greatly by the 1970s with the evolvement of early discharge programs.[46] In the United States, the average LOS following an uncomplicated vaginal birth had dropped from 4 days in the 1970s to 2 days by the mid 1990s, adjusting minimally since.[46–48] Postpartum LOS on average is currently 36 to 48 hours with some stays as short as 12 to 24 hours, which severely curtailed the time available to perform this study.[46] The above obstacles were cited as the paramount reasons that other studies on this subject matter were performed retrospectively.

To overcome both major challenges, a study design had to be crafted that could accommodate the limited LOS and that would decrease the burden of the decision process for the parents giving participation consent. This led to a design that paired the study's CBS with one already scheduled for the state-mandated NBS. This would not require an additional procedure, potentiating parents' consideration to enroll their baby. This created an inherent limitation, which was that the RIs were all obtained at a single time point and therefore the clinical usefulness may be questioned. However, the mitigating aspects of obtaining the study specimen at 24 hours in this study design allowed for the following: consistency in the timing and the source of the blood collection, time for any potential effect of the birthing process on hematologic parameters to defervesce, neutrophil counts to trend downward following their 6 to 14 HOL peak, and generally sufficient time for well-appearing newborns to show signs and symptoms of a clinical issue. Given the limited time normal healthy term newborns remain hospitalized, the merits of these single time point RIs may prove advantageous.

Newborn providers understand that the proper interpretation of laboratory data mandates knowledge not only of the gestational and postnatal age, but also of the blood sampling technique used. Repeated arterial and venous blood sampling is not always feasible in certain populations. In newborn medicine the preferred method of obtaining small volume samples (<1 mL) for laboratory analysis is CBS. The ease and safety of the procedure are among its advantages, but it is imperative to understanding the comparability of CBS results obtained from other blood sampling techniques.[49] Generally when compared to venous blood sampling, the CBS has higher hemoglobin, hematocrit, RBC, WBC, and lymphocyte counts but lower platelet counts.[50]

As with any research, a subject that was outside of the study's inclusion criteria was not represented. Therefore, the results do not transfer to those who are not normal healthy term newborns as defined in the study. Other limitations included the absence of a long-term follow-up confirmation that the normal healthy term newborn classification was maintained, lack of data collection on demographics of race and ethnicity, and the fact that the study was conducted at a single site with varying degrees of lifestyle and blends of ethnicity, which may lead to modification of these results. All study laboratory samples were processed on a single research designated Sysmex analyzer, which may be viewed as a study limitation. However, this ensured strict quality control, allowing the RIs to be consistent and limiting study variability, though these results may not be generalizable to other types of automated hematology analyzers that use differing technologies.

CONCLUSIONS

Reference intervals for normal healthy term newborns at approximately 24 HOL were prospectively established for CBC, absolute IG count, IG%, IRF, Ret-H*e*, IPF, and IPC. These RIs are proposed as the new standard and can serve as a strong foundation for continued research to further explore their value in diagnosing and managing morbidities such as sepsis, anemia, and thrombocytopenia.

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