



Gestation-wise Reference Ranges of Neutrophil Counts in Indian Newborns

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ABSTRACT

Objectives: Blood counts are commonly performed tests in neonatal intensive care units with the results having various clinical ramifications. Interpreting blood counts as normal or abnormal requires reference ranges as per gestation. Studies on reference ranges for neonatal neutrophil counts are already scarce, and data is lacking in the Asian context. We sought to formulate gestation-wise reference ranges of neutrophil counts in an Indian setting. **Methods:** Healthy, newborn babies of either gender, aged between 30 to 41 weeks gestation were included in the study. Gestational age was corroborated through first trimester dating scan and postnatally by the New Ballard Score. Single venous blood samples were drawn on day three and day five for estimation of total leukocyte count, differential count (neutrophils, lymphocytes), and peripheral blood smear examination. **Results:** We evaluated the data of 420 newborns. The normative values were compiled week-wise for gestational ages of 30 to 41 weeks at birth. We observed a clustering of neutrophil count values below 8000 cells/ μ L on day three and below 5000 cells/ μ L on day five. No gender-based differences in counts were observed. We were able to generate reference range curves for neutrophil counts as per gestational age. **Conclusions:** The absolute neutrophil counts of term and preterm Indian newborns are higher than the values depicted in the standard reference chart used currently. This indicates that a different standard chart as per gestation should be used in Indo-Asian countries to differentiate 'normal' from 'abnormal'.

Complete blood count is a commonly performed test in the neonatal intensive care unit. The results have various clinical ramifications (e.g., neutropenia would be prone to sepsis, candidiasis, etc).¹ However, interpreting counts is more difficult in children, and particularly in neonates, owing to the scarcity of normative data.¹

The landmark publication by Manroe et al,² from the University of Texas South Western Medical School at Dallas, USA, published in 1979, established a method for determining whether a neonate's neutrophil count should be considered neutrophilic, normal, or neutropenic. In 1994, the same group led by Mouzinho et al,³ published new information on neutrophil counts in very low birth weight neonates. The pioneering observations of this group in defining the normal ranges for blood neutrophil counts of term and preterm neonates, famous as Manroe and Mouzinho's

charts, are widely used reference data in the field of neonatal hematology.⁴

The reference ranges obtained from western populations have been used in developing countries owing to lack of indigenous normative data. However, neutrophil count patterns may be affected by ethnicity, gender and environmental factors, altitude, maternal hypertension, and maternal fever before delivery.⁵⁻¹⁰ Hence, routine application of western data to Indian context is questionable. Furthermore, there could be variations within the umbrella term 'pediatric' or 'neonatal' reference ranges. Hematologic values in neonates differ significantly from those in older children. Neonatal results may show quantitative and qualitative differences as a reflection of the developmental changes during fetal hematopoiesis, and values at birth may vary with gestational age.⁵ As an added confounding factor, Schmutz et al,⁴ revisited the Manroe and Mouzinho's charts and suggested a

higher value for the upper limit of neutrophil counts than indicated in the traditional charts. This was attributed to the use of modern technology for blood count estimation and variations in altitude.

In the face of all these sources of variation, it is imperative that blood count data generated by studying newborn babies in an Indian setting should be used in compiling normative data for Indian newborns. Such data could then be used as reference ranges for interpreting neutrophil counts in Indo-Asian newborns. Therefore, we conducted an observational study to fulfill this objective.

METHODS

We conducted an observational study of newborns admitted to the neonatal unit of the department of pediatric medicine in a large tertiary care teaching hospital in Kolkata, India over 14 months (May 2016 to June 2017). The institutional ethics committee approved the study protocol and written informed consent was obtained from either parent or accompanying grandparent of the recruited child.

Healthy newborns of either gender were recruited through purposive sampling to ensure at least 30 newborns represented every week between the gestational ages of 30 to 41 weeks. Gestational age was estimated by first trimester dating scan or, in the absence of that, as per last menstrual period (LMP) provided mother's cycles were regular and corroborated postnatally by New Ballard Score (NBS). If there was a discrepancy exceeding two weeks between gestation age calculated using LMP and by NBS, the latter was followed. The antenatal record was checked to exclude babies with a history of intrauterine growth retardation and babies born to mothers with a history of maternal fever. Birth records were also considered to ensure that only newborns with a five-minute Apgar score > 7 were included. Sick newborns requiring mechanical ventilator support for more than 24 hours, or taking antibiotics or inotropes were excluded.

On the third and fifth postnatal day, 2 mL of venous blood were collected from each newborn into EDTA tubes. The collection was done in the daytime from a peripheral vein using a disposable 24 gauge (0.55 mm × 25 mm) hypodermic needle (Becton Dickinson, Haryana India Pvt Ltd). An automated cell counter system Sysmex XN-1000TM (Sysmex, Kobe, Japan) was used to analyze the blood samples.

If the newborn became sick between the third and fifth day of life, the second blood sample was not collected, and data of these subjects were excluded.

No formal sample size calculation was done for this study, but an effort was made to include at least 30 babies per gestational week between 30 to 41 weeks. The raw data were entered into a Microsoft Excel spreadsheet and subsequently analyzed by Statistica version 6 (Tulsa, Oklahoma: StatSoft Inc., 2001) and MedCalc version 11.6 (Mariakerke, Belgium: MedCalc Software 2011) software. Birth weight and neutrophil count data were skewed by Kolmogorov-Smirnov goodness-of-fit test. Normative data summary, therefore, includes the median, percentile values, and mean ± standard deviation. Counts were compared between day three and day five by Wilcoxon matched pairs signed rank test, and between genders by Mann-Whitney U test. A *p*-value < 0.050 was considered statistically significant. Associations between counts were quantified by calculating Spearman's rank correlation coefficients.

RESULTS

Of the 434 newborns recruited in the study, complete data were available for analysis from 420. Table 1 depicts the descriptive summary stratified by gender for the whole cohort. There were no statistically significant differences in neutrophil counts between male and female newborns (Mann-Whitney U test). The mean neutrophil count on day three was 6295.0 ± 3423.0, 6390.0 ± 3790.0, and 6208.0 ± 3053.0 for the whole cohort, males, and females, respectively, and on day five was 3836.0 ± 1914.0, 3807.0 ± 1980.0, and 3837.0 ± 1856.0 for the whole cohort, males, and females, respectively.

Figure 1 depicts the dispersion of neutrophil counts in the whole study cohort. The distributions are obviously skewed, and there are several outlier values. With neutrophil count, there is clustering of values below 8000 cells/μL on day three and below 5000 cells/μL on day five. The dispersions were wider on day three, and the median and interquartile range (IQR) values were lower on day five. The decline from day three was statistically significant (*p* < 0.001) for neutrophil counts (Wilcoxon matched pairs signed rank test). Table 2 shows the neutrophil count in newborns as per gestational age from 30 to 41

Table 1: Summary profile of neutrophil counts in healthy Indian newborns.

Parameters	Overall, n = 420	Males, n = 201	Females, n = 219	p-value
Birth weight, g				
Range	840–3900	840–3860	850–3900	0.164
Mean ± SD	2000.0 ± 692.3	2054.0 ± 716.2	1950.0 ± 667.4	
Median (IQR)	1853 (1400–2604)	1975 (1400–2670)	1795 (1400–2590)	
10th to 90th percentile	1195–2958	1200–3100	1165–2800	
ANC, cells/μL – day 3				
Range	1000–28900	1000–28900	1270–20000	0.936
Mean ± SD	6295.0 ± 3423.0	6390.0 ± 3790.0	6208.0 ± 3053.0	
Median (IQR)	5600 (4100–7600)	5800 (3900–7600)	5600 (4300–7600)	
10th to 90th percentile	3000–10150	2400–10200	3300–10100	
ANC, cells/μL – day 5				
Range	1010–11100	1080–11100	1010–9700	0.430
Mean ± SD	3836.0 ± 1914.0	3807.0 ± 1980.0	3837.0 ± 1856.0	
Median (IQR)	3275 (2530–4580)	3200 (2400–4600)	3400 (2600–4500)	
10th to 90th percentile	2100–6600	1900–6700	2100–6500	

ANC: absolute neutrophil count; IQR: interquartile range; SD: standard deviation.

Table 2: Normative data of absolute neutrophil count in Indian newborns as per gestational age (n = 420).

Gestation week	n	Day	Mean ± SD	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile
30	37	D3	6241.0 ± 4018.0	2600	3600	5100	8100	13400
		D5	3342.0 ± 1545.0	1600	2100	2800	4300	5700
31	31	D3	4161.0 ± 2345.0	2400	3100	3600	4700	6100
		D5	2649.0 ± 939.0	1890	2100	2400	3100	3200
32	32	D3	6372.0 ± 3995.0	3800	4300	5400	6950	8800
		D5	3371.0 ± 1676.0	1400	2400	3200	3600	5400
33	31	D3	4550.0 ± 2256.0	2000	3200	4300	5600	6700
		D5	2829.0 ± 1393.0	1300	1800	2760	3200	4100
34	30	D3	6857.0 ± 4650.0	3400	4600	6250	8400	8850
		D5	3807.0 ± 1613.0	2100	2700	3300	5300	5850
35	39	D3	5911.0 ± 3639.0	1900	3500	5600	7200	10100
		D5	3777.0 ± 2046.0	1780	2300	3200	4500	7200
36	50	D3	6390.0 ± 2641.0	3200	4600	6400	7600	9450
		D5	4054.0 ± 1879.0	2350	2700	3475	5000	6750
37	44	D3	7116.0 ± 3200.0	3600	4550	6700	9150	11100
		D5	4620.0 ± 2317.0	2300	2950	3950	6000	8200
38	37	D3	7863.0 ± 3257.0	3900	5600	7600	9100	11500
		D5	4853.0 ± 2140.0	2300	3400	4312	6300	7800
39	30	D3	6802.0 ± 3122.0	3950	5600	6400	7000	10350
		D5	4357.0 ± 2026.0	2600	3200	3800	4560	8450
40	29	D3	7182.0 ± 3574.0	3900	5600	6000	7700	13900
		D5	4312.0 ± 2222.0	2360	2700	3600	5500	8400
41	30	D3	5562.0 ± 2368.0	3685	4390	5030	6300	8500
		D5	3567.0 ± 1205.0	2530	2750	3200	3700	5800

SD: standard deviation.

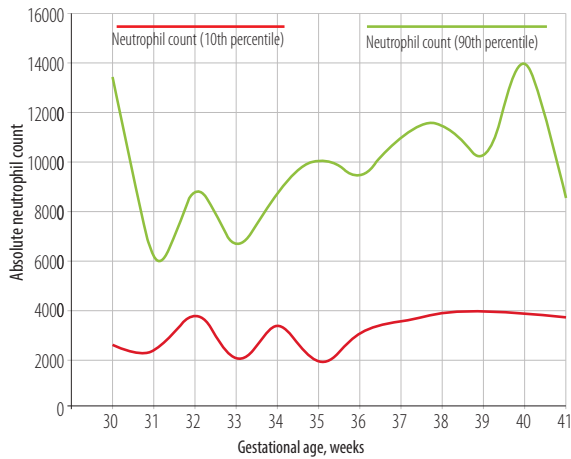


Figure 1: Day three neutrophil count percentile chart in Indian newborns as per gestational age.

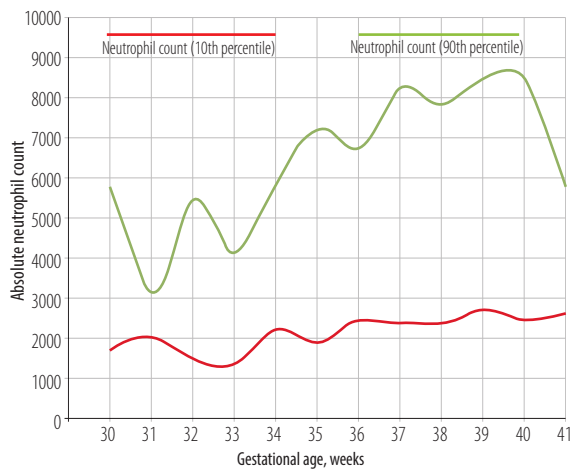


Figure 2: Day five neutrophil count percentile chart in Indian newborns as per gestational age.

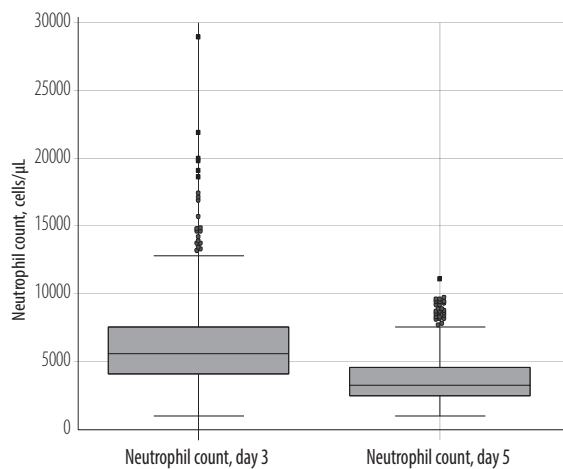


Figure 3: Box and whisker plots of neutrophil counts in the whole study cohort ($n = 420$).

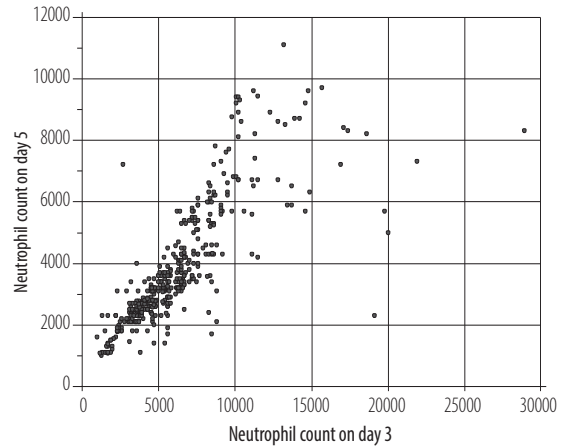


Figure 4: Scatter plot to show correlation (correlation coefficient rho 0.871) between absolute neutrophil counts in newborns on day three and five.

weeks ($n = 420$). The percentile chart of neutrophil generated are graphically depicted in Figure 1 (day 3) and Figure 2 (day 5). The figures can be used for checking normality of absolute neutrophil count (ANC) in neonatology departments, especially in India.

We observed skewed distributions of neutrophil counts in the whole study cohort with the median value on day five significantly lower ($p < 0.001$) when compared to day three [Figure 3].

Figure 4 depicts the association between neutrophil counts on day three and day five. Although values decline on day five, there is a strong association between the two sets of values (Spearman's rank correlation coefficient rho 0.871, $p < 0.050$).

DISCUSSION

We have quantified normative data of blood counts stratified by gestational age from 420 newborns. Neutrophil counts declined significantly from day three to five while maintaining good correlation. The values indicate that the ANC in healthy term Indian newborns is higher than indicated in Manroe and Mouzinho's charts. Further, in preterm babies, neutrophil count does not fall as low as 1500 cells/ μ L.

In our study, we focused on determining gestation-wise ANC in our population context. The high neutrophil count at birth mostly arises from bone marrow mobilization of the pre-existing neutrophil pool owing to stress during birth.⁶ Beyond

48 hours of life most neonates show a decline of total leukocyte count and differential count with an almost equal proportion of neutrophils.⁷ Therefore, we decided to do the first blood sampling on day three. Repeat blood sampling was also done on day five as we presumed the count remains stable from then onwards. Hematologic parameters differ from different samples (i.e., cord blood, arterial blood, or venous blood). Neutrophil counts are better reflected in peripheral venous blood, which was used in our study.⁸

The Manroe et al,² publication documented the expected range of neutrophil concentrations during the first 60 hours after delivery using data obtained from 108 term neonates hospitalized between 1974 and 1976. In 1994, Mouzinho et al,³ published new information on neutrophil counts from healthy, very low birth weight neonates clearly showing a difference in neutrophil dynamics among term and preterm babies. According to their study, ANC ranged from 500 to 2200 cells/ μ L 18 to 20 hours after birth.³ Values between 61 hours and 28 days had upper and lower boundaries of 6000 and 1100 cells/ μ L, respectively, unlike our results. Possible explanations for the difference included the timing of blood sampling (third day), ethnicity of the population studied, and the use of modern equipment in the index study. These boundaries of ANC were substantially beneath the low boundaries of the counts in larger, term newborns as reported earlier.² Thus, if gestational age is not considered, a vast majority of premature newborns will be considered neutropenic. Therefore, reference ranges for newborns need to be matched to gestational age. Other perinatal factors that may alter neutrophil dynamics include maternal hypertension, maternal fever before delivery, and mode of delivery.^{9,10}

Studies in the early 1990s suggested that at high altitude a much higher upper limit of neutrophil count occurs.^{11,12} Hence, at high altitude, Manroe and Mouzinho charts are inappropriate to diagnose neutrophilia or neutropenia. Relatively recently, Schmutz et al,⁴ described the expected ranges for ANC over the first 10 days of life among neonates aged 23 to 42 weeks gestation at high altitude taking advantage of modern methods of neutrophil quantification (electronic cell counter). Their findings are more in accordance with those reported by Carballo et al, and Maynard et al.^{11,12} Mean ANC

was $7.7 \pm 3.0 \times 10^9/L$ in term babies from rural Sindh in samples from cord blood, which is somewhat comparable to our result.¹³ Whereas another similar study from Iraq showed the mean white cell count of cord blood in term neonates was $10.12 \pm 2.8 \times 10^9/L$ (range 3.1–21.6), unlike our result in term babies.¹⁴

Some studies have reported sex differences in ANC in neonates.¹⁵ A review study suggested that newborn females have neutrophil counts averaging 2000 cells/ μ L higher than males.¹⁰ However, we did not detect any sex differences in neutrophil counts on day three or five.

Studies on the distribution of hematological values in newborns are limited in the Indian context. A study conducted in Chandigarh over 25 years ago investigated routine hematological parameters in 240 term normal neonates – 40 neonates in the first week of life and 49 infants between three and six months old.¹⁶ The authors reported a wide variation in total and differential leukocyte counts and highlighted the difficulty in interpreting white cell counts in the newborn period. A more recent study from Chennai analyzed umbilical cord blood at birth from 120 full-term newborns of normal birth weight born out of uneventful pregnancy to mothers aged between 21 to 45 years with hemoglobin above 10 g/dL.¹⁷ They reported ANC of 5700 ± 900 cells/ μ L, which is comparable to our study without any sex differences.

Our study was limited by its small sample size, use of hospital-based data, and lack of serial neutrophil count from day one to day seven. However, it is the first study of gestation-wise reference ranges of neutrophil counts from an Indo-Asian context. Thus, the gestation-wise reference ranges for neutrophil counts in the Indo-Asian context we have produced have the potential to be used as a standard for comparing counts for the precision of diagnosis of neonatal sepsis with a long-term goal of reducing neonatal mortality.

CONCLUSION

Normative values of neutrophil counts in Indo-Asia newborns vary from those stated in the standard chart framed by Manroe and Mouzniho for western populations. Our study has established reference ranges of neutrophil count stratified by gestational age for the Indian population, which can help in the precise diagnosis of neonatal sepsis.

Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

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