

A Study of Peripheral Smears in Neonates

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Abstract: The hematological parameters in a newborn are distinctly different from the normal adult values. NRBCs are immature RBCs normally seen in the peripheral blood of neonates upto 5th day of life. At birth 3 to 10 NRBCs per 100 WBCs are present. Premature birth and foetal hypoxia can cause the number to increase. NRBC count in umbilical venous blood of neonates has been reported as a possible marker of perinatal asphyxia. Leukocytosis refers to an increase in the total number of WBCs due to any cause. Thrombocytopenia can be a marker of an underlying disease as well as an obvious risk factor for hemorrhage.

Keywords: Neonates, NRBC's, Peripheral smear, Thrombocytopenia

I. Introduction

A peripheral blood smear is a glass microscope slide coated on one side with a thin layer of venous blood. The slide is stained with Leishmans, and examined under a microscope. Microscopic examination of the peripheral blood is used to supplement the information provided by automated cell counter. However precise classification of abnormal cells requires a trained hematologist, a well-made peripheral blood smear, and a light microscope with good optical characteristics.

Peripheral blood smear examination provides information that cannot be obtained from automated cell counting. Hematologic values in neonates differ significantly from those in older children and adults. Quantitative and qualitative differences are present as a reflection of the developmental changes during fetal hematopoiesis and, so, correlate with gestational age. At birth, the hemoglobin, mean corpuscular volume, and WBC counts of term newborns are significantly higher than those of older children and adults, and in preterm neonates the differences are even more pronounced. This review explores these differences and the major factors that account for them from the hematology laboratory standpoint. Early diagnosis of neonatal septicemia is a vexing problem because of its nonspecific clinical picture. Bacterial infection in the newborn still account for a considerable morbidity and mortality. This is because the newborn especially the premature are prone to serious infections by organisms and partly because the signs of these infections may be absent or minimal and hard to detect. Thus fatal septicemia may occur with little warning. Hence the timely diagnosis of sepsis in neonates is critical as the illness can be rapidly progressive and in some instances fatal.

II. Objectives

1. To study the variations in RBC, WBC, and platelets in peripheral smear of neonates.
2. To correlate with the clinical findings

III. Methodology

Source of Data: Peripheral smear slides of all term neonates born in GGH, Vijayawada. 300 cases will be included in the study.

Method of collection of data:

This is a prospective study done from January 2012 to September 2013. 1ml of venous blood will be collected from newborns in EDTA vacutainers. Peripheral smear will be studied according to standard operating procedure with special emphasis on RBC, WBC and platelet counts. A proforma containing the details of the patient and informed consent from the parents of the patient for the present study is maintained.

Inclusion criteria: All term neonates

Exclusion criteria:

1. Preterm neonates
2. Those born with any congenital anomalies

Preparation of slides:

Blood for examination is obtained by venepuncture and collected in EDTA vacutainers. Ethylene diamine tetra acetic acid (EDTA) is the anticoagulant preferred.

Staining of Thin Blood Films:

For the current study Leishmans stain has been made use of due to easy feasibility.

Preparation- Dissolve 0.2g of powered Leishmans dye in 100ml of acetone-free methyl alcohol in a conical flask.Warm it to 50° C for half an hour with occasional shaking.Cool it and filter it.

Procedure: Pour Leishmans stain drop wise on the slide and wait for 2 minutes. This allows fixation of blood film in methyl alcohol. Add double the quantity of buffered water over the slide. Mix by rocking for 8 min. Wash in water for 1 to 2 minutes. Dry in air and examine under oil immersion lens of the microscope.

Statistical analysis: A qualitative analysis of the data was done using Pearsons Chi square test, and Fischers Exact test.

IV. Results

Age Of The Study Group: The age of the patients in our study group ranged from Day 1 of life to Day 28.76% of newborns were <3 days old.18% of newborns were between 4 to 10 days old and 16% of newborns were between 11 to 28 days old as shown in the table and chart below.

Table 3 .Age distribution

Age(days)	Counts	Percentages
Less than 3	230	76.7%
4 to 10	54	18%
11 to 28	16	16%

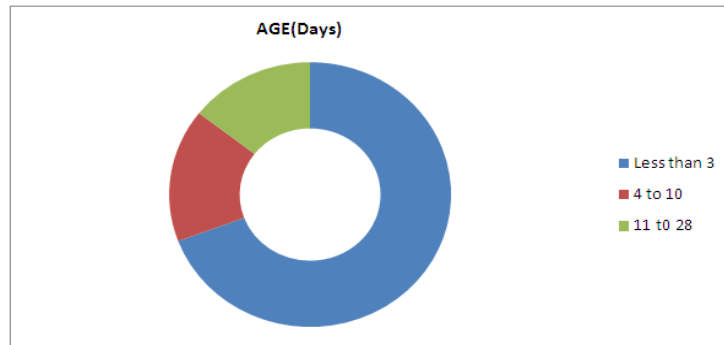


Figure 8. Doughnut chart showing age distribution.

The mean age of the study group was day 1 of life as shown in the table below.

Table 4 .Mean age of study group

N	Minimum	Maximum	Mean	SD
300	1	3	1.29	.559

Sex Distribution: The bulk of the newborns in this study were females. Below is the table and chart showing the data.

Table 5. Sex distribution

Sex	Counts	Percentage
Male	97	32.3%
Female	203	67.7%

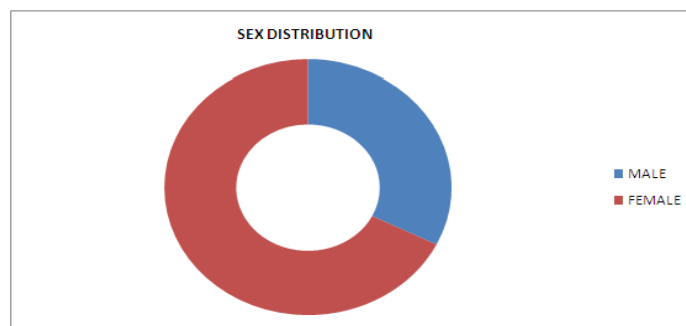


Figure 9. Doughnut chart showing sex distribution.

Age And Sex Correlation

Among the 230 newborns who were less than <3 days old,166 were females and 64 were males and those between 4 to 10 days old,31 were males and 23 were females. Out of the 16 newborns between 11 to 28 days,6 were females and 10 were males. The age and sex cross tabulation encountered in our study was as follows.

Table 6. Age and sex correlation

SEX			Male	Female	Total
AGE(Days)	Less than 3	Count	64	166	230
		% of total	21.3%	55.3%	76.7%
	4 to 10	Count	23	31	54
		% of total	7.7%	10.3%	18.0%
	11 to 28	Count	10	6	16
		% of total	3.3%	2.0%	5.3%
TOTAL		Count	97	203	300
		% of total	32.3%	67.7%	100.0%

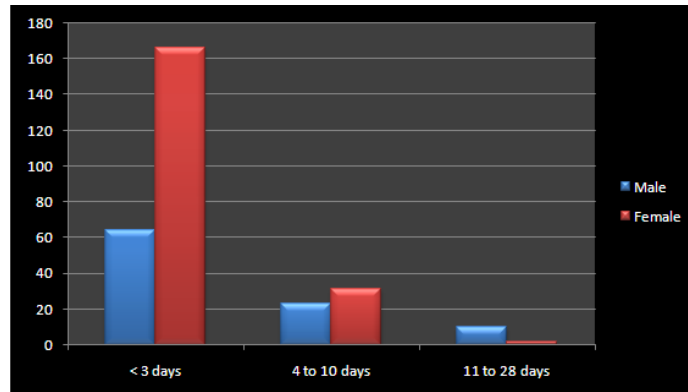


Figure 10. Bar graph representing age and sex cross tabulation.

Rbc Morphology: A thorough peripheral smear examination was done and morphology of RBCs were studied. Of the 300 newborns, 283 of them had normocytic normochromic RBCs and 17 of them had RBCs which were microcytic and hypochromic. The data has been given in the table and chart below.

Table 7. RBC morphology

	Count	Percentage
NN	283	94.3
MH	17	5.7

*NN-Normocytic Normochromic, MH- Microcytic hypochromic.

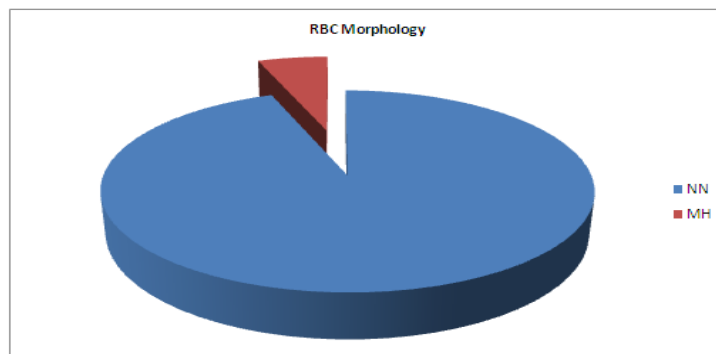


Figure 11. RBC morphology.

Mean Hb Value

Of the total 300 cases, Hb levels varied between 6g/dl to 22g/dl. The mean value of Hb in our study is given in the table below.

Table 8. Mean Hb value

Total(N)	Minimum	Maximum	Mean	Std deviation
300	6	22	14.70	2.035

Total Count (Tc): Out of 300 cases, 273 cases had a total count <25000 and 27 cases had total count >25000. Data obtained is given in the table and chart below.

Table 9. Total count (WBCs)

TC	Frequency	Percentage
Less than 25000	273	91%
More than 25000	27	9%

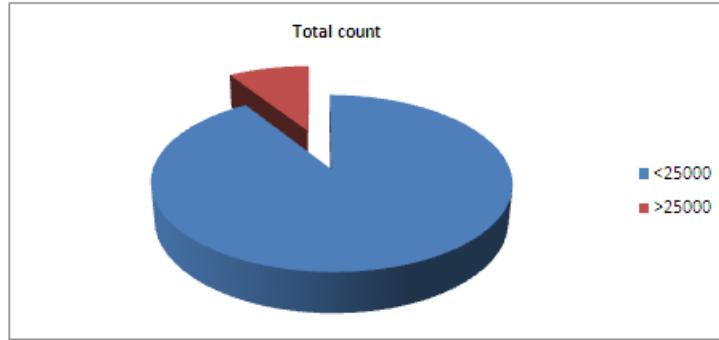


Figure 12. Pie chart showing total count.

Estimation Of Total Count In Neonates To Diagnose Sepsis By Hss

The HSS scores obtained in our study were 0, 1, 4 and 5. 257 cases in our study had an HSS score 0 of which 253 cases had a TC <25000 and 4 cases had a TC >25000

16 cases had an HSS score of 1. All of them had TC <25000

24 cases had an HSS score of 4, of which 21 cases had a TC >25000 and 3 cases had a TC <25000

3 cases had an HSS score of 5, of which 2 cases had TC >25000 and 1 case had a TC <25000

Table 10. TC and HSS correlation

TC		HSS				TOTAL
		0	1	4	5	
<25000	count	234	35	3	1	273
	%	78%	11.6%	1.0%	3%	91.0%
>25000	count	4	0	21	2	27
	%	1.3%	0%	7.0%	7%	9.0%
Total	count	238	35	24	3	300
	%	79.3%	11.6%	8.0%	1.0%	100.0%

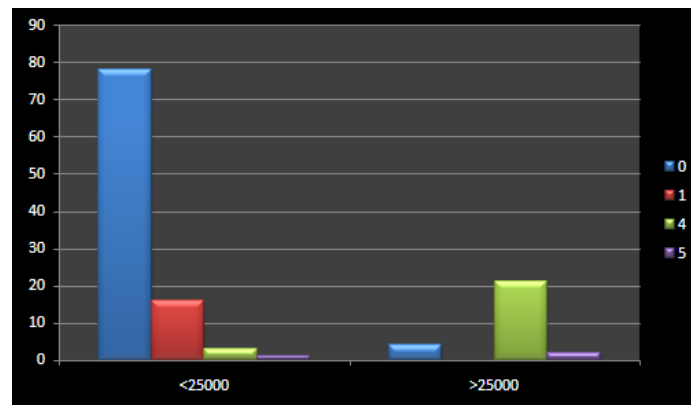


Figure 13. Bar diagram showing TC and HSS correlation.

The mean value of NRBCs is shown in table below.

Table 11. Mean NRBC value

N	Minimum	Maximum	Mean	Std Deviation
300	5	18	9.56	1.739

In a normal neonate, NRBCs are rapidly cleared from the blood stream after birth. In healthy term neonates, virtually no NRBC will be found after third or fourth day of life, although they may be present in small number upto 1 week in preterm babies. In our study we got a mean value of 9.56 NRBCs per 100 WBCs.

The mean value of band forms of neutrophils is as follows.

Table 12 .Mean value of band forms of neutrophils

N	Minimum	Maximum	Mean	Std Deviation
300	0	25	0.66	2.424

Elevation of band forms of neutrophils is not specific for infection, but may be secondary to inflammatory processes, tissue damage or necrosis, neoplasia, intoxication, metabolic abnormalities, haemorrhage, hemolysis or drugs.

Numerous studies have investigated the clinical utility of band forms of neutrophils in the discrimination between patients with and without bacterial infection. It is widely used to diagnose bacterial infection in newborns and infants less than 3 months of age.

The mean value of platelets is shown in the table below.

Table 13. Mean value of platelets

N	Minimum	Maximum	Mean	Std Deviation
300	14000	600000	221800.00	92457.927

Evaluation Of Platelet Count In Neonates To Diagnose Sepsis By Hss

The platelet count increases linearly with gestation from a mean of 187000 at 15 weeks and 274000 at 40 weeks. Thus, at any age, a platelet count less than 150000 is abnormally low.

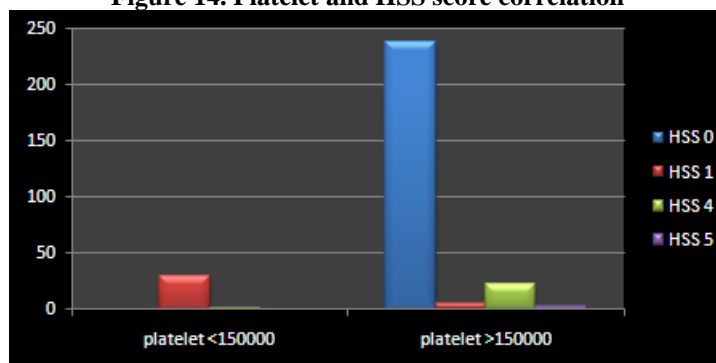
Our study showed that out of 31 cases who had a platelet count <150000, 30 cases had an HSS score of 1, and 1 case had an HSS score of 4.

The remaining 269 cases who had a platelet count >150000, 5 cases had an HSS score of 1, 23 cases had an HSS score of 4 and 3 had an HSS score of 5. The details are shown in the table and chart below.

Table 14. Platelet and HSS cross tabulation

Platelet count		HSS				Total
		0	1	4	5	
<150000	Count	0	30	1	0	31
	%	0%	10%	3%	0%	10.3%
>150000	Count	238	5	23	3	269
	%	79.3%	1.7%	7.7%	1.0%	89.7%
Total		238	35	24	3	300
		79.3%	11.6%	8.0%	1.0%	100%

Figure 14. Platelet and HSS score correlation



In our study, out of 27 cases of probable sepsis, 1 case had an increase in I:T ratio. Similarly, 1 case had an increased I:M ratio also.

V. Discussion

Microscopic examination of peripheral blood is used to supplement information provided by automated cell counters. Examination of peripheral smear is an inexpensive but powerful diagnostic tool in childrens and adults. It provides rapid, reliable access to information about a variety of hematological disorders. However, the precise classification of abnormal cells requires a trained hematologist, a well-made peripheral blood smear and a light microscope with good optical characteristics.

Comparison of Hb level:

Our study included 300 term neonates. Normal Hb levels in term neonates <1 month old ranges from 10 to 20g/dl with a mean Hb value of 13.9g/dl. This was compared with other studies as shown in table below: -

Table 15: Comparison of mean Hb value

Study	Marwaha et al (1992)	Charles et al (2005)	Present study (2013)
Mean Hb value	14.0g/dl	13.9g/dl	14.7g/dl

Assessment of RBC morphology:

In our study, 283 cases had RBCs which were normocytic normochromic and 17 cases showed RBCs which were microcytic hypochromic with a decreased Hb level. This is compared with other studies as given

Table 16: Comparison of RBC morphology

Study	Kim et al (1996)	Gupta et al (1994)	Kilbride et al (1999)	Present study (2013)
RBC morphology	NN-178 MH-22 Total-200	NN-276 MH-24 Total-300	NN- 166 MH-34 Total-200	NN-283 MH-17 Total-300
Total cases	200	300	200	300

The high prevalence of anemia during pregnancy compromises the health of mothers. Also women tend to have subsequent pregnancies with an inadequate interval to replenish nutritional iron stores. Their infants also appear to be at increased risk of developing iron deficiency anemia, undetected at birth.

Among the 17 cases of anemia, 16 cases were due to fetal blood loss, bleeding from obstetric complications and internal haemorrhage associated with birth trauma. The only physical finding is pallor of skin and mucous membrane. Peripheral smear shows mild normocytic normochromic anemia to a more severe microcytic hypochromic anemia. 1 case showed features of hemolytic anemia secondary to ABO incompatibility.

Correlation of TC with HSS score in neonatal sepsis

Sepsis neonatorum continues to be the major cause of morbidity and mortality in developing countries, but is treatable if diagnosed on time.⁵⁹ One to eight cases of neonatal septicemia are reported in all live births.⁶⁰ The infection can be contracted from the mother via transplacental route, ascending infection, during passage through an infected birth canal, or exposure to infected blood at delivery.⁶¹ The newborn babies are more susceptible to bacterial invasion than older children or adults, due to their weaker immune system.⁶² The major concern of clinicians is its non-specific presentation, sometimes rapid progression of sepsis and unavailability of tests with a high positive predictive value. Therefore early detection of neonatal sepsis is a vexing problem. A definite diagnosis is made by blood culture. However, the procedure is time consuming, yield is low and facilities for the test might not be available in all laboratories. HSS is an important tool for early diagnosis of sepsis.

Maximum cases presented on 1st day of life, with majority in less than 12 hours.⁶³ Our study showed majority of neonates were less than 24 hours old. This correlated well with the study done by Monroe et al.

The normal WBC count in a term baby at birth is between 18±8 cells/cubic mm, at day 3- 15±8 cells/cubic mm and by 1 month 12±8 cells/cubic mm. HSS score of 4 indicates there is chance of sepsis and an HSS score of 5 indicates sepsis is very likely.

An increase in TC was seen in 27 cases in our study. We had 21 cases with TC > 25000 and HSS score of 4 and 2 cases with TC > 25000 and HSS score of 5. This correlated well with the study done by Manisha et al and Marcocha et al.

Table 17: Comparison of total count and HSS score

Study	Manisha et al (1999)	Marcocha et al (2001)	Present study (2013)
TC	>25000	>25000	>25000
HSS score	5	5	5
Total cases	3	5	2

Also we found that higher the score, more are the chances of sepsis and vice versa. This was in correlation with the study done by Ghosh et al and Narasimha et al. Simplification and standardization of interpretation of this global test is still required.⁶⁶

Comparison of mean NRBC value:

In the year 1924, reference value for the number of NRBC found in neonates on the first day of life have been published. In healthy neonates, the NRBC normally disappear from peripheral blood within the first week of life. Even in premature babies and neonates, knowing the exact number of NRBC allows drawing

conclusions on their condition of health. Thus, NRBC count greater than the indicated reference values may, for instance, refer to chronic or postnatal hypoxia, anemia, maternal diabetes or acute stress. Extremely increased values upto 500 NRBC/100 WBC may even indicate an infectious disease such as congenital syphilis.⁶⁷

Table 18: Comparison of mean NRBC value

Study	Green et al (1999)	Buonocore et al (1996)	Naeye et al (1998)	Present study (2013)
Mean NRBC	10.2	9.2	10	9.56

Studies done by Green et al, Buonocore et al and Naeye et al showed mean NRBC value of 10, in term, healthy neonates which correlated well with our study.

Although in 1-2% of the cases extremely increased NRBC concentrations might be idiopathic^{68,69}, they normally require rapid investigations of their cause in order to start a potentially necessary treatment of the newborns as soon as possible.

Correlation between platelet count and HSS score:

Thrombocytopenia was frequently associated with sepsis and indicated poor prognosis. This is thought to be due to increased platelet destruction, sequestration secondary to infection, failure in platelet production due to reduced megakaryocytes or damaging effects of endotoxin.

In the neonatal hematological parameter, the association of low platelet count with neonatal systemic infection is significant.

Table 19: Comparison of platelet count and HSS score

Study	Speer et al (1999)	Rodwell et al (2001)	Philip et al (2005)	Present study (2013)
Platelet count	<150000	<150000	< 150000	< 150000
HSS score	1	1	1	1
Total cases	20	25	28	30

Our study showed that out of 31 cases who had a platelet count <150000, 30 cases had an HSS score of 1, and 1 case had an HSS score of 4. This correlated with studies done by Speer et al, Rodwell et al, Philip et al and Basu et al.^{64,65}

Limitations of our study:

1. Blood culture is considered as the gold standard for diagnosis of septicemia which we have not included in our study.
2. Also estimation of CRP levels is not done in our study, which is also an early indicator of neonatal sepsis.

VI. Summary And Conclusion

1. The present study included 300 cases.
2. Mean age of the study group was 1.29 days.
3. Female babies were predominant constituting 68% cases.
4. RBC morphology was normal in 94.3% (283) cases.
5. Anemia amounted to 5.7% (17) cases.
6. WBC count was normal in 91% (273) cases.
7. Leukocytosis was seen in 9% (27) cases.
8. HSS score was 4 for 8% (24) cases, of which 7% (21) cases had leukocytosis and 1% (3) cases had normal WBC count.
9. HSS score was 5 for 10% (3) cases, of which 7% (2) cases had leukocytosis and 3% (1) case had normal WBC count.
10. Mean value of NRBC was 9.56.
11. Mean value of platelets was 221000.
12. Normal platelet count was seen in 89.7% (269) cases.
13. Thrombocytopenia was seen in 10.3% (31) cases.

To conclude our study, the peripheral blood film remains a very useful diagnostic tool in hematological assessment. Attention to details in slide preparation, staining and standard criteria for all cell estimates, morphology and differential reporting, enhance the value of results provided to the clinician. Also assessing the HSS score is important. The feasibility and the cost effectiveness of the system increases the usefulness of this test. HSS is a useful test to distinguish the infected from the non infected newborn. It has high sensitivity and specificity, the certainty of sepsis increasing with higher scores. This helps the clinicians to reach a probable

diagnosis, decreasing the death toll and institute a rational approach towards the patient medication, ie; avoiding unnecessary instillation of antibiotics and preventing the development of resistance to these drugs.

Colour Plates

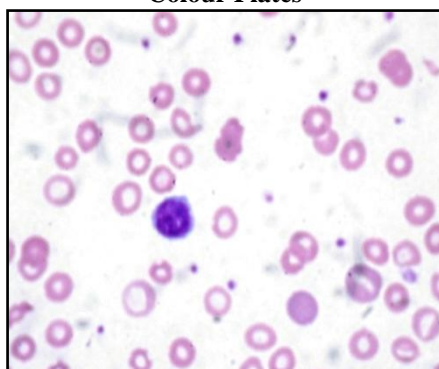


Figure 15: Iron deficiency anemia.

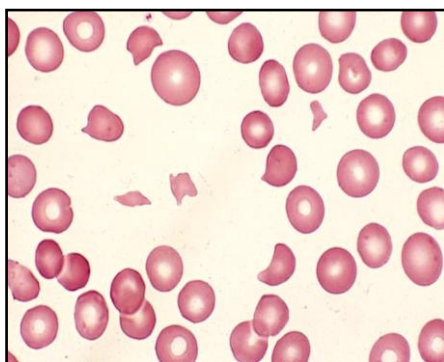


Figure 16: Hemolytic anemia.

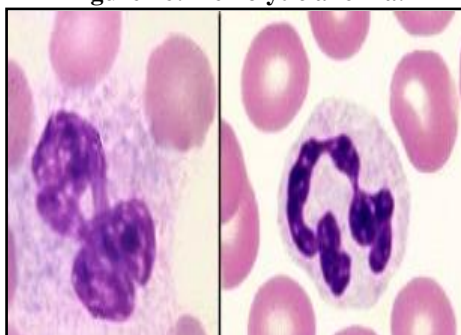


Figure 17: Neutrophils with toxic granules.

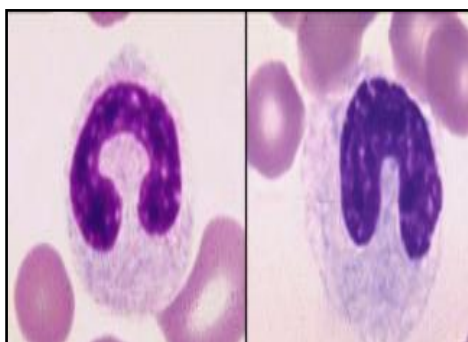


Figure 18: Band forms of neutrophils.

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