



# Comparison of time frame critical for feed supplementation on haematological indices of azawak cattle breed in semi–arid zone of Nigeria Republic

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## Abstract

The haematological parameters of apparently healthy of Azawak cattle breed consisting of 36 animals (18 males and 18 females) at CERRA, Maradi in semi–arid zone in Niger Republic were studied. Data were analyzed for the effect of the time frame critical for utilization of feed supplement per sex. According a time frame critical for utilization of feed supplementation (male and female) higher and lower values of packed cell volume (PCV) was obtained from T5 (26.32±3.78%) and T1 (23.44±4.26%), there was no significantly lower ( $P>0.05$ ) for Azawak cattle breed in semi–arid zone. Haemoglobin (Hb) values was higher from T5 (10.66±0.57 g/dl) and lower T4 (9.92±1.28 g/dl). Red Blood Cell Count (RBC) was no significantly ( $P>0.05$ ) for Azawak cattle breed. According treatment, the Mean Corpuscular Hemoglobin (MCH) was higher from T1 (18.89±2.28 pg) and lower value was from T6 (17.53±0.53 pg). The mean corpuscular hemoglobin concentration (MCHC) was significantly higher ( $P<0.05$ ) for Azawak cattle breed after feeding. The mean corpuscular volume (MCV) was observed to be higher from T6 (44.55±1.37 fl) while the values were much higher from T4 (45.25±3.05 fl) of female and T1 (44.59±0.87 fl) of male. T3 (10.07±2.73  $\times 10^9/L$ ) for all sex had the highest White Blood Cell Count (WBC). White blood cell differential shows that lymphocytes was no significantly higher ( $P>0.05$ ) Azawak cattle breed. Neutrophils was significantly higher ( $P<0.05$ ) for Azawak cattle breed (male and female). Include Eosinophil, Monocyte and Basophil was observed a higher value from T6 (12.90±1.05%) while the values were much higher from T6 (13.70±0.50%) for female and T2 (13.20±1.97%) for male. Conclusively, the haematological profile level, higher values for leucocytes (white blood cells) and lymphocytes were noticed with increased differences between the investigated categories of animals taken into study.

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**Keywords:** Haematological parameters; Feed supplement; Azawak cattle breed; Semi–arid zone



## Introduction

Blood is a special type of connective tissue composed of formed elements in a fluid matrix. Plasma is the fluid portion called serum when depleted of fibrinogen [1]. The formed elements include erythrocytes (red blood cells), leukocytes (white blood cells) and platelets [2]. Hence, the haematological values during different physiological situations should be known for the diagnosis of various pathological and metabolic disorders which can adversely affect the productive and reproductive performance of cows, leading to heavy economic losses [3]. Many of haematological parameters are influenced by many factors like breed, age, sex, seasonal variations, lactation, pregnancy, health and nutrition status [4-6]. It is acknowledged that for comparisons between individuals and with reference data in a clinical diagnostic situation, it is necessary to consider normal variations due to age, sex and breed in order to increase diagnostic precision [7]. It is recognized that normal values for the various blood cell parameters not only differ from species to species but can vary between the breeds within a species [8]. Red Blood Cells (RBCs) are small, disc-shaped, anucleate cells and the primary function of them is to transport hemoglobin which carries oxygen and carbon dioxide to and from tissues, therefore red blood cells play an important role in pH regulation [9]. White Blood Cells (WBCs) are basic cellular components of the immune system and can be divided into neutrophilic, eosinophilic and basophilic granulocytes, monocytes and lymphocytes. Only a small percentage (0.5 to 3%) of the leukocytes of domestic mammals is basophils. Hence, they are not often found in blood smears. Platelets play an important role in hemostasis [2,9]. Mean Corpuscular Volume (MCV) is more valuable than blood film examination in assessing the true size of erythrocytes. Using automated cell counting systems, a histogram or volume distribution curve of the erythrocyte population can be generated [1].

The aim of this study was comparison of time frame critical for feed supplementation on haematological indices of Azawak cattle breed in semi-arid zone of Niger Republic.

## Materials and methods

### Experimental animals and management

A total number of thirty six (36) Azawak cattle (18 males and 18 females) with an average initial Body Weight (BW) of 184±40 kg aged 3 to 4 years owned by CERRA, Maradi ranch were used for the experiment. The animals were quarantined for 3 weeks and ear tagged for identification. The trial was conducted at CERRA, Maradi. The cattle were grouped into six (6) animals per experimental diet. They were arranged in six different treatments of feed. The experimental animals were allowed to move out for normal grazing within the rangeland twice daily (8:30am – 1:30pm and 2:30pm – 4:30pm) and supplement were given daily. The houses were disinfected with IZAL® solutions and were allowed to dry for one week before the commencement of the experiment. The pens were cleaned fortnightly. The animals were housed in a cage (3m long, 3m wide and 2m high). The cages were enriched with iron platforms and parallel iron bars. The house is located at the border of a gallery forest, in an area of environmental protection. Before the commencement of the experiment, animals were given prophylactic treatment of Ivermectin (0.2 mg/kg sub-cutaneous), Oxytetracycline (5mg/kg intra-muscular) and multivitamin (10ml/kg intra-muscular) injections were administered. The animals were supplemented to provide necessary minerals for 90 days. Water was supplied

*ad-libitum* through the watering place.

### Feed composition of the experimental diet

The composition of experimental diet contained 15, 10, 15 and 1% of cotton seed cake, *Faidhebia albida* pod, wheat bran and salt. Also, added to it were cowpea fodder (13%), urea (1%), phosphorus (5%) and calcium (5%).

### Sample preparation and processing

The proximate composition of feed supplement and experimental diet was carried out according to the method described by [10]. All the samples were properly labeled and analyzed in Animal Feed and Nutrition Laboratory of Faculty of Agriculture, Bayero University, Kano.

### Experimental design

The animals were allocated into six treatment groups of three replicates per sex each containing 6 cattle (3 males and 3 females). There were thus 6 cattle per group in the Completely Randomized Design experiment. The treatments were evaluated by time of supplementation of the diet to the experimental animals. T1: No supplementation (control), T2: Supplementation early (8:00 am) in the morning daily before moving out for grazing, T3: Supplementation in evening (5:00 pm) daily after the afternoon grazing, T4: Supplementation in the morning (8:00 am) and in the evening (5:00 pm) daily, T5: Supplementation once every two days in the morning (8:00 am) before morning out for grazing and T6: Supplementation once every two days in the evening (5:00 pm).

### Blood sample collection

Blood sample was collected from each animal twice; on the first day of experiment and the last day of experiment through the jugular vein, bloodletting was performed from apparently health cattle. Blood samples were collected in test tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) as an anticoagulant. These tubes were placed in an icebox and carried to the laboratory within 4 h of collection. In the laboratory, these samples were centrifuged at 3000 rpm for 10 minutes; the plasma was separated and stored at -4°C for further analysis.

### Haematological parameters

Red Blood Cell (RBC) mass, White Blood Cell (WBC) mass, Hemoglobin (Hb) concentrations, Packed Cell Volume (PCV or hematocrit), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), MCH and platelet mass were determined with methods cell counter set (Coulter T 860, England) and methods that described by Thrall. Statistical analysis was performed using the SPSS version 20.

### Bovine hematological reference ranges

The most appropriate reference range is generated from a group of healthy animals with environmental and physiological characteristics as similar to the patient as possible. As in all species, a certain amount of physiological variability is observed in hematologic profiles of cattle. Variables that contribute to the thresholds and width of reference intervals include age, sex, stress, diet, body condition, reproductive status, recent activity, hydration, ambient temperature, and altitude.

### The comparison of some normal hematological parameters according to a time frame critical for utilization of feed supplement

The comparison of some normal haematological parameters according a time frame critical for utilization of supplement (T1: No supplementation (control), T2: Supplementation early at 8:00 am in the morning daily before moving out for grazing, T3: Supplementation in evening at 5:00 pm daily after the afternoon grazing, T4: Supplementation in the morning at 8:00 am and in the evening at 5:00 pm daily, T5: Supplementation once every two days in the morning at 8:00 am before morning out for grazing and T6: Supplementation once every two days in the evening at 5:00 pm) is shown in Table 2. The comparison of some normal hematological parameters according a time frame critical for utilization of supplement per male is shown in Table 3. The comparison of some normal hematological parameters according a time frame critical for utilization of supplement per female is shown in Table 4.

### Statistical analysis

Differences in mean percentages and concentrations of the variables between to a time frame critical for utilization of supplement were analyzed by one-way analysis of variance and subsequent Duncan's multiple comparisons Test (post-hoc). Differences in mean percentages and concentrations of the variables between male and female animals were determined by Paired-Samples t-test.

### Results and discussion

#### Chemical composition of experimental feed supplementation

The nutritive characteristics of feed supplementation ingredients are shown in Table 1. This dry matter content indicates all constituents excluding water of the ingredients used in the formulation. DM was recorded in feed supplementation as (91.09%). The DM in this study is slightly above (95.4%DM) reported by Mubi *et al.*, [11] but similar to the range of 93.80%-98.70% DM reported by [12]. The crude protein content of the feed was 22.11% this value is slightly lower than what was reported by Addass *et al.* [13] who reported in a similar experiment 10.00% - 11.20% CP range. The crude protein content of the diet is sufficient for ruminants which will provide ammonia required by rumen microorganism to support optimum microbial activity [14]. This differences and variation in crude protein percentage among diets may be due to the type of protein source and its level of inclusions in the rations. This is completely different from what was reported by Onwuka, [15] and Mohammed *et al.* [6], who reported 10.9% to 14.8% CP and 11.0%-13% CP respectively. The differences observed could also be associated with soil nitrogen condition, level of maturity of the crop residue and varietal differences [16]. Crude fibre was obtained at 45.64% CF which agrees reported by Addass *et al.* [13] and also in line with (11-45%) CF reported by Malgwi *et al.* [17]. This feed supplementation was recorded highest crude fibre level. Such high crude fibre content of the diet could be due to the quality and fibrous nature of ingredients used which reduces digestibility rate of the diet as well. Ether extract was obtained at 9.85% EE higher value to the work of Kinfemi *et al.* with 6.13% EE. The mean ash content of feed supplementation was (9.18%) which is a little higher than the value of (6.34%) reported by Dibal [18] for semi-arid browse plants. The ME content in MJ/kg DM was 2086.57kcal/kg DM. However, The NDF content from feed was higher than that reported by Pereira *et al.* [19] and Batajoo and Shaver [20]. The ADF content from diet was higher than that reported by Pereira *et al.* [19] and NRC [21]. Many factors affect chemical composition such as oil extraction process [22]

stage of growth [23] maturity and species or variety [24,25]. Ca was recorded 148.53mg/kg Ca and TP was 45.86 mg/kg TP. The observed differences in mineral composition in these products may be due to genetic factor and added in different level. Decrease in minerals, energy and protein contents also contribute to lower intake, reduced digestibility and consequently losses in weight by grazing animals. The report of Chinast and Mayer [26] was in line with the findings of this study who reported that the crude protein content of tropical forages decreases during the advanced periods of the dry season.

#### Effect of inorganic minerals of feed supplementation on haematological indices profile of Azawak cattle

The results of this study showed that application of feed supplementation contributed to an increased level of selected haematological parameters. In end of treatment, RBC, PCV, PLT, WBC, Hb, MID and MCV increased in both the control and experimental group. However, the observed increase was higher at T5. A reverse association was observed for the WBC, which decreased in the experimental group. Nevertheless, the haematological parameters values obtained in the study fell within the range of normal values [27]. Similar changes in the levels of the aforementioned haematological indicators were observed in calves, which received the herbal mixture in the amount of 3.5% as a concentrate supplement [28]. Packed Cell Volume (PCV) in this study was higher from T5 for all sexes (male and female) and per each sex obtained. In contrast, Patterson *et al.* [29] attributed increase in PCV values in cattle to increase in environmental temperature. The PCV values obtained for the female Azawak cattle were comparable to those obtained for the males. This observation is not in contrast with values obtained for Red Sokoto goats in Nigeria [30] in which female animals have higher values than males. PCV in Angus was significantly lower than Sharuleh [31]. Unlike this study, Jain [32] reported significantly higher RBC count in Brown-swiss cattle than other strains.

Sex and nutritional status of animals could cause differences in values observed for MCHC was differed significantly ( $P < 0.05$ ) from female while PLT, Hb, NEUT and MCV were differed significantly ( $P < 0.05$ ) from male. A significant sex effect was also observed, with males having higher values of PCV and RBC and females have shown higher value on WBC. A significant age effect was observed for MCV and MCHC. Significant sex effect was evident with females having highest value on MCHC while males had higher MCV. A significant sex effect was observed on Hb concentration. Another study conducted by Egbe-Nwiyib *et al.* [33] revealed the influence of age and sex on haematological values of goats and sheep; age and sex had remarkable influence on the RBC counts of goats, age influenced the Hb and PCV values, age and sex greatly influenced the MCV and age influenced MCHC. Age and sex influenced neutrophil and eosinophil counts in sheep. Sex influenced the RBC values of sheep. Sex significantly ( $P < 0.05$ ) influenced the total WBC and monocyte counts (which was higher in males and females).

### Conclusion

In haematological profile level, higher values for leucocytes (white blood cells) and lymphocytes were noticed with increased differences between the investigated categories of animals taken into study. The best time frame critical for utilization of supplement of Azawak cattle in Semi-Arid zone was obtained per treatment with increased difference between the investigated categories of sex taken into study. In hematologic

profile examination, comparing obtained results and appreciation must be carried out taking reference in individuals belonging not only to the same sex but to the same line or breed.

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### Tables

**Table 1:** Chemical composition of experimental diet.

Constituents	Mean±SD of nutritive values
Ash(%)	9.18±0.25
MOISTURE(%)	8.92±0.59
CP(%)	22.11±1.22
CF(%)	45.64±0.54
EE(%)	9.85±0.35
NFE(%)	13.24±2.37
ME (kcal/kg DM)	2086.57±10.16
DM(%)	91.09±0.59
ADF(%)	40.65±0.35
NDF(%)	50.28±0.55
Ca (mg/kg)	148.53±5.35
TP (mg/kg)	45.86±1.36

DM: Dry matter; CP: Crude protein; CF: Crude fiber; EE: Ether extract; NFE: Nitrogen free extract; ADF: Acid detergent fiber; NDF: Nitrogen detergent fiber; ME: Metabolizable energy; Ca: Calcium; TP: Total phosphorus; SD: Standard deviation.

**Table 2:** The comparison of some normal hematological parameters according to a time frame critical for utilization of supplements.

Haematological indices		Treatment						F value	Pr>F
		T1	T2	T3	T4	T5	T6		
RBC (x10 <sup>12</sup> /L)	Mean±SD	5.37±0.79	5.78±0.30	5.59±0.67	5.59±0.66	6.00±0.52	5.72±0.57	0.648	0.422ns
	SEM	0.35	0.12	0.28	0.27	0.23	0.23		
PCV (%)	Mean±SD	23.44±4.26	25.57±1.87	24.18±3.73	24.40±3.53	26.32±3.78	25.50±3.12	0.515	0.348ns
	SEM	1.91	0.76	1.52	1.44	1.69	1.27		
PLT (x10 <sup>9</sup> /L)	Mean±SD	877.78±460.96	850.17±447.72	948.33±424.09	928.17±262.95	736.00±491.03	702.50±449.91	0.322	0.457ns
	SEM	206.15	182.78	173.13	107.35	219.59	183.67		
WBC (x10 <sup>9</sup> /L)	Mean±SD	9.54±1.89	8.83±1.67	10.07±2.73	9.58±1.80	9.24±2.26	8.93±1.82	0.296	0.824ns
	SEM	0.85	0.68	1.12	0.74	1.01	0.74		
Hb (g/dl)	Mean±SD	10.00±0.65	10.25±0.81	10.05±1.04	9.92±1.28	10.66±0.57	10.02±0.99	0.443	0.309ns
	SEM	0.29	0.33	0.42	0.52	0.26	0.40		
LYMP (%)	Mean±SD	62.22±4.50	57.28±7.97	55.14±5.85	51.68±12.78	59.70±6.12	57.67±5.78	1.203	0.015*
	SEM	2.01	3.26	2.39	5.22	2.74	2.36		

NEUT (%)	Mean±SD	26.42±2.63	30.20±7.24	32.12±6.26	36.42±11.13	29.48±4.27	29.43±4.82	1.375	0.004*
	SEM	1.18	2.95	2.56	4.54	1.91	1.97		
MID (%)	Mean±SD	11.36±1.88	12.52±2.32	12.75±2.35	11.90±2.25	10.82±2.81	12.90±1.05	0.795	0.518ns
	SEM	0.84	0.95	0.96	0.92	1.25	0.43		
MCHC (g/dl)	Mean±SD	4.37±0.75	4.02±0.29	4.19±0.30	4.07±0.18	4.09±0.35	3.94±0.15	0.894	0.052*
	SEM	0.34	0.12	0.12	0.07	0.16	0.06		
MCH (pg)	Mean±SD	18.89±2.28	17.71±0.80	18.02±0.68	17.74±0.66	17.82±0.81	17.53±0.53	1.066	0.172ns
	SEM	1.02	0.33	0.28	0.27	0.36	0.22		
MCV (fl)	Mean±SD	43.47±2.36	44.19±1.80	43.14±1.94	43.58±1.65	43.74±2.99	44.55±1.37	0.374	0.385ns
	SEM	1.06	0.74	0.79	0.67	1.34	0.56		

PCV: Packed cell volume; RBC: Red blood cell; Hb: Hemoglobin; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean cell hemoglobin Concentration; WBC: White blood cell; LYM: Lymphocyte; PLT: Platelets; NEUT: Neutrophil; MID: include –eosinophil, monocyte, basophil.

**Table 3:** The comparison of some normal hematological parameters according to a time frame critical for utilization of supplement per male.

Haematological indices		Treatment						F value	Pr>F
		T1	T2	T3	T4	T5	T6		
RBC (10 <sup>12</sup> /L)	Mean±SD	5.72±0.24	5.57±0.26	5.47±0.67	5.27±0.78	5.69±0.16	5.64±0.72	0.227	0.078ns
	SEM	0.17	0.15	0.38	0.45	0.11	0.41		
PCV (%)	Mean±SD	24.95±0.07	24.83±1.54	23.53±3.56	22.77±3.67	23.60±0.57	24.80±3.38	0.281	0.085ns
	SEM	0.05	0.89	2.06	2.12	0.40	1.95		
PLT (10 <sup>9</sup> /L)	Mean±SD	1044.00±12.73	800.33±405.08	1064.67±158.02	979.00±160.12	1071.00±29.70	872.00±558.85	0.327	0.021ns
	SEM	9.00	233.87	91.23	92.45	21.00	322.65		
WBC (10 <sup>9</sup> /L)	Mean±SD	8.85±1.77	7.47±0.70	8.57±2.48	10.63±1.57	9.70±0.71	10.07±1.17	1.577	0.095ns
	SEM	1.25	0.41	1.43	0.91	0.50	0.68		
Hb (g/dl)	Mean±SD	10.40±0.14	9.63±0.57	10.13±1.24	9.27±1.33	10.30±0.14	9.93±0.93	0.531	0.035*
	SEM	0.10	0.33	0.72	0.77	0.10	0.54		
LYMP (%)	Mean±SD	62.45±6.86	56.33±9.00	56.87±4.91	51.43±12.50	63.45±7.85	62.53±1.74	0.943	0.057ns
	SEM	4.85	5.20	2.84	7.22	5.55	1.00		
NEUT (%)	Mean±SD	26.35±3.89	30.47±9.30	30.30±7.50	35.60±10.86	27.25±6.43	25.37±1.31	0.680	0.047*
	SEM	2.75	5.37	4.33	6.27	4.55	0.75		
MID (%)	Mean±SD	11.20±2.97	13.20±1.97	12.93±2.77	12.97±1.86	9.30±1.41	12.10±0.78	1.200	0.390ns
	SEM	2.10	1.14	1.60	1.07	1.00	0.45		
MCHC (g/dl)	Mean±SD	4.17±0.04	3.88±0.16	4.32±0.24	4.08±0.07	4.37±0.05	4.02±0.17	3.770	0.075ns
	SEM	0.03	0.09	0.14	0.04	0.04	0.10		
MCH (pg)	Mean±SD	18.21±1.01	17.31±0.84	18.53±0.08	17.61±0.43	18.11±0.25	17.67±0.56	1.677	0.085ns
	SEM	0.72	0.48	0.04	0.25	0.18	0.32		
MCV (fl)	Mean±SD	43.66±1.96	44.59±0.87	42.98±2.31	43.12±1.21	41.48±0.14	43.97±0.48	1.382	0.024*
	SEM	1.39	0.50	1.34	0.70	0.99	0.27		

PCV: Packed cell volume; RBC: Red blood cell; Hb: Hemoglobin; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean cell hemoglobin concentration; WBC: White blood cell; LYM: Lymphocyte; PLT: Platelets; NEUT: Neutrophil; MID: include –Eosinophil, monocyte and basophil.

**Table 4:** The comparison of some normal hematological parameters according to a time frame critical for utilization of supplement per male.

Haematological indices		Treatment						F value	Pr>F
		T1	T2	T3	T4	T5	T6		
RBC (10 <sup>12</sup> /L)	Mean±SD	5.13±1.00	6.00±0.14	5.71±0.81	5.91±0.42	6.20±0.62	5.79±0.53	0.965	0.148ns
	SEM	0.58	0.08	0.47	0.24	0.36	0.30		
PCV (%)	Mean±SD	22.43±5.70	26.30±2.18	24.83±4.56	26.03±3.10	28.13±4.01	26.20±3.39	0.683	0.440ns
	SEM	3.29	1.26	2.63	1.79	2.31	1.96		
PLT (10 <sup>9</sup> /L)	Mean±SD	766.97±615.50	900.00±574.09	832.00±619.72	877.33±373.45	512.67±542.90	533.00±327.93	0.326	0.544ns
	SEM	355.36	331.45	357.80	215.61	313.44	189.33		
WBC (10 <sup>9</sup> /L)	Mean±SD	10.00±2.19	10.20±0.95	11.57±2.40	8.53±1.54	8.93±3.10	7.80±1.73	1.245	0.655ns
	SEM	1.27	0.55	1.39	0.89	1.79	1.00		
Hb (g/dl)	Mean±SD	9.73±0.75	10.87±0.42	9.97±1.07	10.57±1.03	10.90±0.66	10.10±1.25	0.880	0.623ns
	SEM	0.43	0.24	0.62	0.59	0.38	0.72		
LYMP (%)	Mean±SD	62.07±4.11	58.23±8.67	53.41±7.25	51.93±15.88	57.20±4.54	52.80±3.06	0.647	0.069ns
	SEM	2.38	5.01	4.19	9.17	2.62	1.77		
NEUT (%)	Mean±SD	26.47±2.51	29.93±6.66	33.93±5.64	37.23±13.77	30.97±2.73	33.50±2.61	0.872	0.048*
	SEM	1.45	3.84	3.25	7.95	1.58	1.50		
MID (%)	Mean±SD	11.47±1.63	11.83±2.85	12.57±2.45	10.83±2.40	11.83±3.30	13.70±0.50	0.524	0.291ns
	SEM	0.94	1.65	1.42	1.39	1.91	0.29		
MCHC (g/dl)	Mean±SD	4.51±1.03	4.15±0.35	4.05±0.33	4.07±0.27	3.91±0.36	3.86±0.11	0.640	0.026ns
	SEM	0.60	0.20	0.19	0.16	0.21	0.06		
MCH (pg)	Mean±SD	19.35±3.01	18.12±0.65	17.51±0.62	17.88±0.92	17.63±1.07	17.40±0.58	0.764	0.075ns
	SEM	1.74	0.38	0.36	0.53	0.62	0.34		
MCV (fl)	Mean±SD	43.34±3.02	43.80±2.62	43.31±2.00	43.97±2.21	45.25±3.05	45.13±1.85	0.353	0.750ns
	SEM	1.75	1.52	1.16	1.28	1.76	1.07		

PCV: Packed cell volume; RBC: Red blood cell; Hb: Hemoglobin; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean cell hemoglobin concentration; WBC: White blood cell; LYMP: Lymphocyte; PLT: Platelets; NEUT: Neutrophil; MID: include –Eosinophil, monocyte and basophil.

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