International Journal of Research in Medical Sciences
Mitila T et al. Int J Res Med Sci. 2023 Apr;11(4):1186-1191
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20230859

Original Research Article

Utility of nucleated RBCs in critical care patients in a tertiary care centre

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Received: 06 February 2023
Revised: 07 March 2023
Accepted: 14 March 2023

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ABSTRACT

Background: In healthy adults and older children, NRBCs are normally found only in the blood-building bone marrow where they mature. Their appearance in peripheral blood points to extramedullary erythropoiesis or disruption of the blood-bone marrow barrier. Aim of current study was to evaluate and compare the prognostic significance of NRBCs in the peripheral blood of intensive care patients (ICU) and non-intensive care patients (non-ICI) and to assess the morbidity and mortality risk associated with NRBCs among ICU and non-ICU patients.
Methods: Relevant clinical details and investigations were collected from the Haematology nominal Register of the Department of Pathology, Saveetha Medical College. Blood samples were routinely drawn in the morning. The presence of NRBCs in the peripheral blood was detected with the help of an automated analyser (Sysmex XN 1000) and confirmed by a peripheral smear.
Results: Among the NRBC-positive study population, mortality rate was 28% and was associated with ICU admission status, and death was predominantly due to cardiovascular causes. The highest NRBC value during the period of admission was significantly associated with deceased patients and ICU patients (p values of <0.001 and 0.002 respectively). The Pearson correlation of NRBC shows a significant positive correlation with serum creatinine and a negative correlation with platelets.
Conclusions: The presence of NRBCs in the peripheral blood of critically ill adults is a significant prognostic marker of morbidity and mortality, laying down the emphasis on daily screening of peripheral smears for NRBCs.

Keywords: Nucleated RBCs, Mortality, ICU, Peripheral smear, Normoblastemia

INTRODUCTION

Nucleated red blood cells (NRBCs), are immature precursors of red blood cells, normally seen in the peripheral blood circulation of new-borns and disappear during the first few weeks of life. During haematopoiesis, nuclear chromatin of proerythroblast gradually condense and forms orthochromatic erythroblast, also known as erythroblasts or obsolete normoblasts.[1] These immature cells RBC's do not enter the peripheral circulation normally. Fenestrations in the bone marrow work as a physical filter, prevent the release of large NRBCs into the circulation. Even if some NRBCs escape this barrier, enter into circulation, are rapidly cleared from the peripheral circulation by the spleen. Hence the presence of NRBC in the peripheral blood circulation in adults is indicative of a disorder in the blood-producing mechanism, thus reflecting a marked increase in erythropoietic activity or

failure of the blood filtration mechanism. An increased demand for red blood cells produces an outpouring of all cellular elements into the peripheral blood because there is a certain amount of nonspecific stimulation that will increase the white blood cells with a concomitant increase in primitive white blood cells. The rise in immature red blood cells, reticulocytes, and, in the worst case, an increase in nucleated red blood cells, which is the focus of concern here, will result from the specific stimulation. Stimuli sufficient to cause NRBCs to cross the barrier between the marrow and the peripheral circulation are pernicious anaemia, marrow displacement, haemorrhage, haemolysis, which may be intrinsic or extrinsic, and anoxia from other causes.[2] At the time of birth, 3 to 10 NRBCs per 100 WBCs are normally seen. Premature mature birth and foetal hypoxia act as cause for NRBC count to increase in circulation.[3] The presence of NRBCs in the peripheral smear of the critically ill is associated with increased in-hospital mortality.[4] In automated haematology analyzer, a high NRBC count raises the WBC count. The samples should be manually inspected once the majority of analysers generate suspicion flags for aberrant cell identification. Sadly, analysers might not be able to detect NRBCs at low quantities. The WBC count should be updated and recorded as "occasional NRBCs seen" if there is even one NRBC per 100 WBCs. As a result, clinicians are made aware of the relevance of unexplained normoblastemia (Figure 1).[5]

Objectives

Objectives of current study were to analyse age distribution of the study population, to evaluate the morbidity and mortality risk associated with NRBC among ICU and Non-ICU patients and to emphasize the significance of evaluation of the peripheral blood smears for NRBCs.

METHODS

This study was conducted on a total of 100 patients with peripheral blood positive for NRBCs. After obtaining ethical committee clearance, relevant clinical details and investigation data were collected from the Haematology nominal register of the Department of Pathology, Saveetha Medical College. It's a longitudinal study, conducted from August 2021 to December 2021. Blood samples were routinely drawn in the morning. 2ml of blood was collected in a vacutainer containing Ethylene diamine tetra acetic acid (EDTA) by venipuncture. The presence of NRBCs in the peripheral blood was detected with the help of an automated analyser (Sysmex XN 1000).

Inclusion and exclusion criteria

Patients with NRBCs in their peripheral blood who were admitted in the Intensive Care Unit (ICU) and wards (non-ICU) and age above 18 years were included. NRBC-negative patients, outpatients, and patients below 18 years of age were excluded.

Statistical analysis

Data was entered in Microsoft office Excel sheet and Statistical analysis was done with SPSS version 23.0 software. Categorical variables were expressed as frequencies and percentages. Continuous variables are represented in mean, median, mode, and standard deviation. Nominal categorical data between the groups were compared using the Chi -square test. For statistical tests, a $p<0.05$ was taken to indicate a significant difference. Furthermore, Pearson correlation was used. The correlation between the highest NRBC value and lowest platelet, highest total leukocyte count, lowest prothrombin time, highest creatinine, and highest RDW during the period of admission was seen.

RESULTS

A total case 100 patients were taken for the study, out of which 50 were male and 50 were female. The distribution was 41 patients admitted to ICU and 59 patients were admitted to wards (non-ICU) (Figure 1).

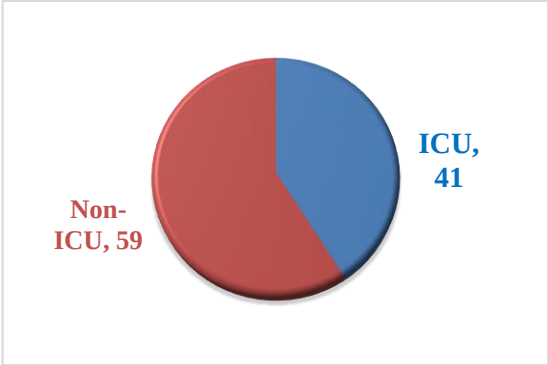

Figure 1: The distribution of patients ICU and Non-ICU.

The age groups with maximum and the least number of cases was 58-68 years and above 78 years respectively. The mean age of the patients of the study population was 48.9 years (Figure 2).

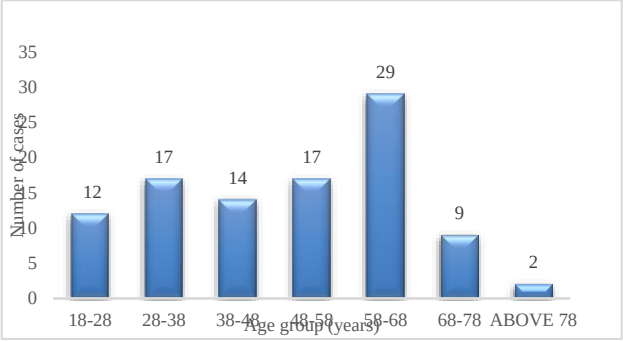

Figure 2: Age group distribution.

The highest number of cases (30 cases) was seen in the haemoglobin range 8-10g/dl among the study population

considering the lowest value during the period of admission (Figure 3).

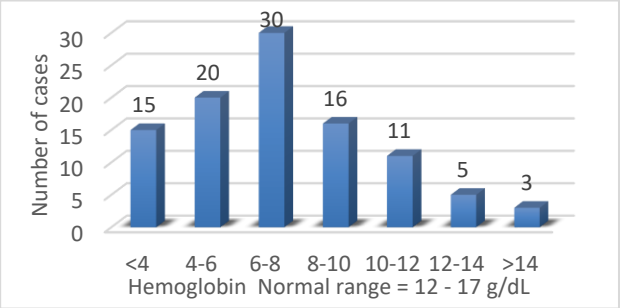

Figure 3: The distribution of lowest haemoglobin levels. Hemoglobin Normal range = 12 - 17 g/dl.

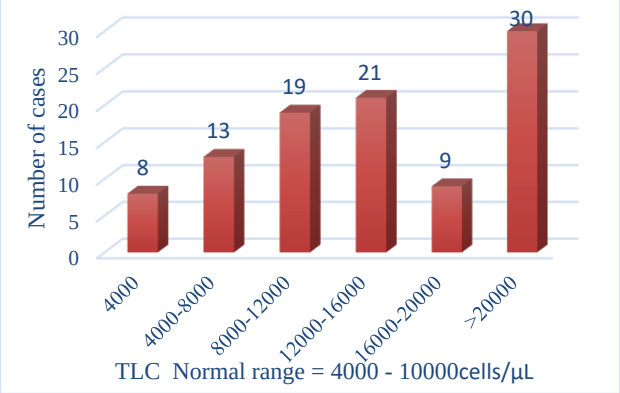

Figure 4: The distribution of total leukocyte count. Normal range = 4000 - 10000cells/µl.

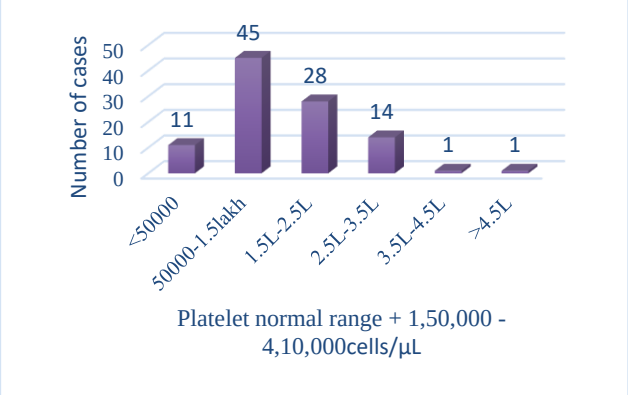

Figure 5: The distribution of platelet count, normal range: 1,50,000- 4,10,000 cells/µl.

Taking the highest total leukocyte value during the period of admission into consideration, most cases had a total leukocyte count of more than 20000 cells/µl. Only 8 cases had a TLC of less than 4000 cells/µl (Figure 4). The maximum number of patients (45 patients) had lowest platelet count during the period of admission in the range 50000-1.5 lakh. The least common platelet range was 3.5-4.5 lakhs and 4.5-5.5 lakhs per µl. The number of patients with platelet counts of less than 5000 was 11 (Figure 5).

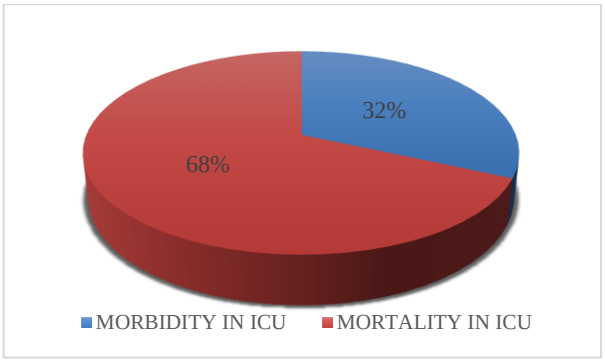

Figure 6: The distribution of mortality and morbidity in ICU.

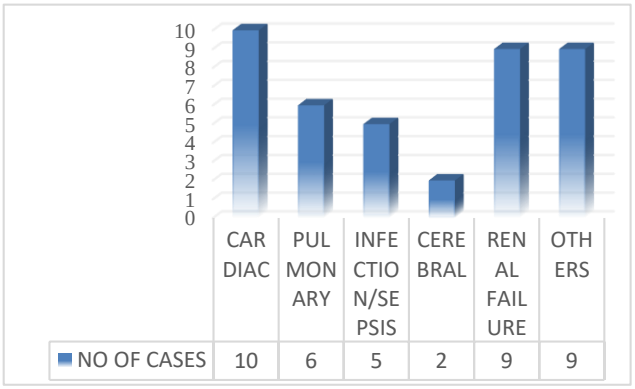

Figure 7: Distribution of cause of death in deceased ICU patients.

The percent of morbidity and mortality of patients admitted to ICU are 32% and 68% respectively (Figure 6). The various causes of death of patients admitted to ICU, encountered in our study population with the respective number of patients are cardiac causes (10 patients), pulmonary causes (6 patients), infections/sepsis (5 patients), cerebral causes (2 patients), renal failure (9 patients), other causes (9 patients) (Figure 7).

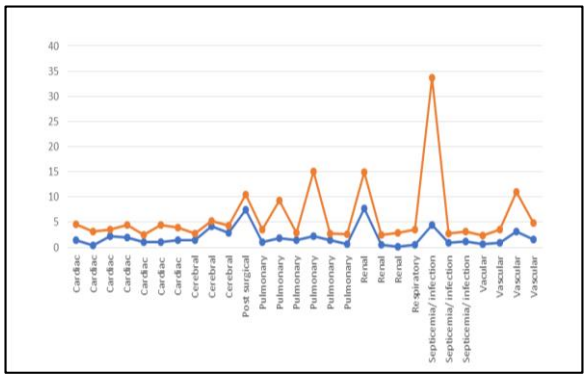

Figure 8: The increase in NRBC from the day of admission to the day of discharge in deceased ICU patients.

NRBC level increased from the day of admission (blue line graph) to the day of discharge (orange line graph)

compared with the systemic disease in deceased ICU patients.

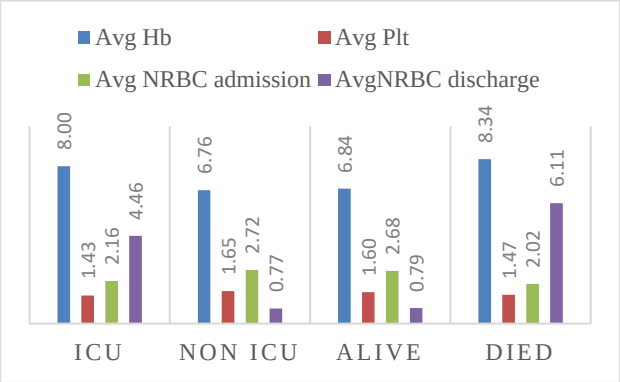

Figure 9: Comparison between average values of the platelet, haemoglobin, NRBCs at the time of admission and at time of discharge/death.

The proportion of increase in NRBCs was higher when death due to sepsis, infection, or renal failure than other causes (Figure 8).

Table 1: Correlation of highest NRBC values of with the status of the patients.

Variable	Status	N	Mean	SD	P value
NRBC	Deceased	28	6.093	6.4947	<0.001
	Alive	72	2.679	2.0694	
	ICU	41	4.944	5.6881	0.007
	Non-ICU	59	2.725	2.1361	

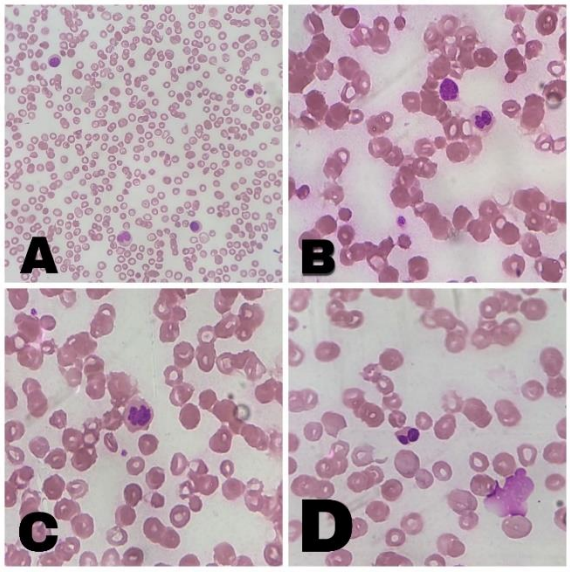

Figure 10: Leishman-stained blood peripheral smear A) A low-power field (10X) showing 3 NRBCs. B-D) High power field (40X) showing dyserythropoietic NRBCs.

The average values of the platelet, haemoglobin, NRBCs at the time of admission and at time of discharge/death in ICU patients was compared to the corresponding values in non-ICU patients, and between deceased and discharged patients. The NRBC level was increased at time of discharge/death than at the time of admission in ICU and deceased patients, whereas there was a reduction in the NRBC level at the time of discharge when compared to the levels at the time of admission in non-ICU and alive patients. The average platelet values are slightly lower in ICU and deceased patients compared to the platelet values of discharged and non-ICU patients. However, the reverse was true on comparing the average haemoglobin levels (Figure 9). The association of highest NRBC value during the period of admission with the status of ICU patients at discharge (if deceased or alive) was significant ($p<0.001$). In addition, there was significance on association of the highest NRBC values with the status of non-ICU patients at discharge (if deceased or alive) ($p=0.02$) (Table 1). In both ICU and Non-ICU patients, the correlation of the highest NRBC value with mortality is significant, especially in ICU patients. Hence, it is important to monitor the progress of the course of the patient and use the presence of NRBCs as an indicator to monitor the responsiveness of a patient to the treatment. Furthermore, there was significant positive correlation of the creatinine level and platelet count on association with highest NRBC value, (p values of 0.004 and 0.013 respectively) (Table 2). No such correlation was seen on association of NRBCs with other parameters.

DISCUSSION

Immature RBCs known as nucleated RBCs (NRBCs) are typically absent from peripheral blood beyond the neonatal period. Adults who have them in their peripheral blood have bone marrow injury or stress, which may be a symptom of a significant underlying condition.[6] Although the precise mechanism by which NRBCs are expelled from the bone marrow is unknown, research has shown that increased hematopoietic stress brought on by inflammation, hypoxia, or both can result in the release of immature red cells. Patients with circulating NRBCs have been found to have higher levels of inflammatory cytokines interleukin 6 and 3 and erythropoietin in their plasma as well as lower arterial oxygen partial pressure. Erythropoiesis is influenced by a number of substances in addition to erythropoietin (EPO), including glucocorticoids, interleukins, tumour necrosis factor, and granulocyte-macrophage colony-stimulating factor. Hypoxia promotes the generation of EPO, which is mostly produced by the kidney. Within 1.5 hours, acute hypoxia can result in an erythropoietin response. By promoting the production of inflammatory mediators, sepsis causes an inflammatory response.[7] In our study, the average age of patients is 49 years with equal male and female populations. This contrasted with the study by Shubha et al, in which the age group 21-30 years had the maximum number of cases, showing a mild male predominance in their study population, as was the case in Stachon et al study (55% male study population).

Table 2: Correlation of highest NRBC values with the various parameters.

Variables		Creatinine	ALT	INR	PT	RDW	Platelet	TLC
NRBC	Pearson correlation	0.288	-0.14	-0.57	-0.57	0.72	-0.248	-0.069
	Significance (P Value)	0.004	0.89	0.581	0.575	0.479	0.013	0.496

The percentage of mortality was 28%, and among the deceased patients, the maximum number was seen between the ages 60 and 70 years and 64% were male patients. Among these deceased patients, 42% of patients had the lowest total leucocyte count during their period of admission in the range 11000-20000 cells/µl and 64 % of patients had thrombocytopenia (platelet count <1.5 lakh/µl) and 85% were anaemic (haemoglobin <10 g/dl). Of the 41 ICU patients, the mortality rate was found to be 68%. The average total leukocyte count in ICU patients was found to be much higher (15136/µl) than that seen in non-ICU patients (9807/µl). There were no significant differences in platelets and haemoglobin levels in ICU and non-ICU patients.

On association of the highest NRBC value during the period of admission with the status of patient on discharge (recovered/deceased), high statistical significance was noted (p value <0.0001). In addition, the p value on association of highest NRBC value during the period of admission with the critical status (ICU and non-ICU) was 0.002 (statistically significant). 89% of recovered patients' NRBC value was <4/100 WBC, but in the deceased patients, 43% of the NRBC value was >4/100 WBC. In both ICU & Non-ICU patients, the p-value is significant <0.05 implying that with the increase in the NRBC, there increase in the risk of mortality.[8] The Pearson correlation of NRBC was done with creatinine and platelet values, and the p-value was 0.4 for creatinine which is a positive correlation, and -4 for platelet count, which is a negative correlation. This indicates that with an increase in NRBC value, there is a decrease in platelet count and an increase in creatinine levels. In the Stachon et al study, the detection of NRBCs is significantly associated with an increased mortality rate.[9]

In Narci et al study, NRBC value was higher in patients who died than in control. In addition, leukocyte, PLCR, RDW and CRP values were found to be statistically higher in the patients who died. There was no difference in Hb value between the two groups. In the multivariate logistic regression analysis, the NRBC was associated with all-cause mortality in the ED. Most of the patients were admitted for cardiac diseases which include coronary artery disease, cardiac arrest, and aortic stenosis, followed closely by renal causes in which chronic renal failure contributed to most of the cases. This was in accordance with the studies by Stachon et al and Narci et al, where 38% of cases were caused by cardiovascular diseases. The range of difference between the number of NRBCs from the time of admission to the time of discharge/death was observed to be higher in septicaemia.[10,11]

Mostly patients died because of cardiac causes followed by respiratory, renal, and infectious causes.[12,13] Therefore, it may be assumed that systemic inflammation and hypoxia contributed to the elevated NRBC level in our study groups' deceased patients. Since normoblastemia is a response to hypoxia in both anaemia and cardiopulmonary ailments, the majority of our patients passed away from cardiovascular and pulmonary causes.[14,15]

Limitations

The limitations of this study is that it is a single centre and for a short period of time.

CONCLUSION

The increase in the levels of NRBCs in peripheral blood may be an early predictor of death in patients admitted to the intensive care unit. This marker is routinely measured by analysis of haematological parameters and can therefore be used for further evaluation at no additional cost. Detection of NRBCs is an independent risk of poor outcome, where the mortality increases with the increasing NRBC concentration, so monitoring the levels of NRBC by automated method or routine screening of peripheral smears aid in the early detection of high-risk patients. This study should raise the suspicion among clinicians that the presence of NRBC in peripheral blood may be a predictor of all-cause death in patients admitted to the ICU. These patients should be under close follow-up.

ACKNOWLEDGEMENTS

Authors are thankful to Saveetha medical college and hospital, Chennai, for their continued support in the procurement of the data. Authors also extend heartfelt gratitude to, all the faculties in the department of pathology, to Dr Chitra Srinivasan. Authors thank all patients who participated in this study. Thanks to all health care workers in our hospital for their efforts in caring for these patients.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. May JE, Marques MB, Reddy VVB, Gangaraju R. Three neglected numbers in the CBC: The RDW, MPV, and NRBC count. Cleve Clin J Med. 2019;86(3): 167-72.

2. Schwartz SO, Stansbury F. Significance of nucleated red blood cells in peripheral blood; analysis of 1,496 cases. J Am Med Assoc. 1954;154(16):1339-40.
3. Krafte-Jacobs B, Bock GH. Circulating erythropoietin and interleukin-6 concentrations increase in critically ill children with sepsis and septic shock. Crit Care Med. 1996;24(9):1455-9.
4. Narcı H, Oktay MM, Ayrık C, Çimen MBY. Nucleated red blood cells as predictor of all-cause mortality in emergency department. Am J Emerg Med. 2021;46: 335-8.
5. Shubha H, Tg DV. A study of hundred adults cases presenting with normoblastemia. Int J Clin Diagn Pathol. 2019;2(2):08-13.
6. Kawthalkar SM. Essentials of Haematology. JP Medical Ltd; 2012:561.
7. Danise P, Maconi M, Barrella F, Palma AD, Avino D, Rovetti A, et al. Evaluation of nucleated red blood cells in the peripheral blood of hematological diseases. Clin Chem Lab Med. 2012;50(2):357-60.
8. Stachon A, Segbers E, Holland-Letz T, Kempf R, Hering S, Krieg M. Nucleated red blood cells in the blood of medical intensive care patients indicate increased mortality risk: a prospective cohort study. Crit Care Lond Engl. 2007;11(3):62.
9. Stachon A, Sondermann N, Imohl M, Krieg M. Nucleated red blood cells indicate high risk of in-hospital mortality. J Lab Clin Med. 2002;140(6):407-12.
10. Frey B, Duke T, Horton SB. Nucleated red blood cells after cardiopulmonary bypass in infants and children: is there a relationship to the systemic inflammatory response syndrome? Perfusion. 1999. May;14(3):173-80.
11. Stachon A, Kempf R, Holland-Letz T, Friese J, Becker A, Krieg M. Daily monitoring of nucleated red blood cells in the blood of surgical intensive care patients. Clin Chim Acta Int J Clin Chem. 2006;366(2):329-35.
12. Menk M, Giebelhäuser L, Vorderwülbecke G, Gassner M, Graw JA, Weiss B, et al. Nucleated red blood cells as predictors of mortality in patients with acute respiratory distress syndrome (ARDS): an observational study. Ann Intensive Care. 2018;8(1):42.
13. Desai S, Jones SL, Turner KL, Hall J, Moore LJ. Nucleated red blood cells are associated with a higher mortality rate in patients with surgical sepsis. Surg Infect. 2012;13(6):360-5.
14. Purtle SW, Horkan CM, Moromizato T, Gibbons FK, Christopher KB. Nucleated red blood cells, critical illness survivors and postdischarge outcomes: a cohort study. Crit Care. 2017;21(1):154.
15. Gullupınar B, Saglam C, Yavru EV, Aksoy EE, Koran S, Evrin T, et al. New hematological parameters as early diagnosis and prognostic markers in critically patients. Disaster Emerg Med J. 2022;7(2):100-6.

Cite this article as: Mitila T, Vinothkumar B, Balaji S, Yogalakshmi E. Utility of nucleated RBCs in critical care patients. Int J Res Med Sci 2023;11:1186-91.