The Potential Role of Nucleated Red Blood Cell (NRBC) Count as a Marker of Illness Severity in Critically Ill Neonates

Abstract

Background

The aim of this study was to assess the diagnostic and prognostic utility of nucleated red blood cells (nRBCs) in critically ill neonates, to help early diagnosis, since they have high risk of death.

Patients and methods

This cohort study involved 102 neonates with critical illness who were admitted to the Neonatal Intensive Care Unit on their 1st day of life. Data regarding patient demographics, perinatal risk factors, clinical characteristics, morbidities, and outcomes were collected. Laboratory investigations were performed, including a complete blood picture as well as a peripheral blood film in which nRBCs were counted.

Results

Non-survivors had a considerably greater nRBC count than survivors, *P* <0.001. Elevated nRBCs were found to be an independent predictor of newborn mortality, *P*=0.042. Elevated nRBCs were substantially linked to higher mortality, and the requirement for mechanical ventilation. The diagnostic performance test demonstrated a sensitivity of 85.7% and 81.2%, and a specificity of 85.2% and 72.9%, with area under the curve value of 0.906 and 0.799, *P* <0.001 and *P* <0.001, respectively.

Conclusion

According to our results, the nRBC count can help clinicians make prompt decisions by acting as a useful early prognostic indicator for death and the need for mechanical ventilation in severely ill newborns.

Key-words:

nRBCs, nucleated red blood cells, critically ill neonates, neonatal mortality

Key messages:

The nRBC count serves as a reliable marker for assessing illness severity and independently predicting mortality in critically ill neonates. It can enhance outcomes by informing treatment strategies and supporting early risk assessment for this vulnerable group.

Introduction

Neonates, a highly vulnerable population, require prompt diagnosis and treatment of severe illnesses to improve outcomes **[1].** Critically ill neonates include those with perinatal asphyxia/fetal distress, suspected or proven infections, and respiratory distress syndrome (RDS) in preterm neonates **[2].**

Early detection of critical illness can be aided by biomarkers like leukocyte and neutrophil counts, C-reactive protein (CRP), procalcitonin, blood gases, lactate, and troponin high sensitivity **[1, 3].** Moreover, scoring systems such as the Clinical Risk Index for Babies (CRIB II), Neonatal Multiple Organ Dysfunction (NEOMOD) score, Score for Neonatal Acute Physiology (SNAP), and SNAP-Perinatal Extension (SNAPPE-II) assess illness severity in neonates **[4–7].**

Despite these tools, additional metrics are needed to enhance neonatal care by identifying and evaluating disease severity early **[1].** Nucleated red blood cells (nRBCs), undetectable in healthy adults but naturally present at birth, have emerged as a potential diagnostic and prognostic indicator for critically ill neonates **[1, 8–9].** Increased nRBC counts, influenced by stresses like perinatal difficulties and fetal acidemia, correlate with higher mortality rates **[1, 8–10].**

Earlier research shows that nRBCs are a strong predictor of mortality in critically ill individuals, including adults and children, with values exceeding 200

nRBC/microliter linked to higher death rates during or after ICU admission **[11–15].** While numerous studies have explored nRBC relevance in identifying critically ill newborns, their diagnostic and prognostic significance remains unclear. This study aims to investigate the role of nRBC count as a predictor of mortality and illness severity in critically ill neonates.

Subjects and Methods

Study design:

All critically ill newborns admitted to the Neonatal Intensive Care Unit (NICU) on their first day of life between December 2022 and November 2023 were included in this cohort study. The study excluded neonates who were not deemed severely ill or who presented after the first day of life.

Critical illness was diagnosed according to the fulfillment of any of the following criteria **[2, 16].**

- **Preterm or full-term neonates with congenital anomalies** such as congenital heart disease or congenital gastrointestinal defects requiring surgical intervention.
- **Preterm or full-term neonates with critical medical illnesses** such as sepsis, RDS, perinatal hypoxia, and multiorgan failure.

- **Neonates with high disease severity scores** (Score for Neonatal Acute Physiology-Perinatal Extension (SNAPPE-II) >30), particularly premature ones (birthweight <1500 g or gestational age <32 weeks). Given that in extremely preterm newborns, neonatal disease severity scores are a higher predictor of time-dependent death and short-term morbidities than birth weight and gestational age.

Data were collected on patient demographics, perinatal risk factors, clinical characteristics, and morbidities. The SNAPPE II and NEOMOD scoring systems were computed for each neonate at the time of NICU admission (for preterm neonates with RDS or neonates with perinatal asphyxia/fetal distress) and at the onset of the illness (for neonates with suspected or confirmed sepsis) **[17].** The outcomes assessed included hospital admission duration and mortality, which was defined as death prior to NICU discharge.

Venous blood samples were obtained under sterile conditions for laboratory testing. On the first day of life, blood was drawn for a complete blood count (CBC), peripheral blood film examination, CRP, blood gas analysis, and blood culture (if neonatal sepsis was suspected). For blood cultures, samples were collected in specialized pediatric culture bottles. On the second day of life, additional venous samples were collected to assess biochemical markers, including liver function

tests (serum albumin, aspartate aminotransferase [AST], and alanine aminotransferase [ALT]) and kidney function tests (serum creatinine and blood urea). All samples were promptly processed and analyzed to ensure accurate and reliable results.

A complete blood count was performed using a Sysmex XN-550 hematology analyzer (Sysmex, Kobe, Japan) for the automated count following the ICSH guidelines for blood cell analyzers **[18].** The immature neutrophil count and the manual differential leukocytic count were assessed in peripheral blood smears made with Giemsa stain. The immature to total neutrophil count ratio (IT ratio) was determined. The nRBCs were counted from the peripheral smear; the count was stated as the nRBC count/100 white blood cells (WBCs) **[19].**

Ethical approval:

The ethical guidelines outlined in the 1975 Declaration of Helsinki and its subsequent revisions were followed when conducting this investigation **[20].** Before neonates were enrolled in this study, their parents' or guardians' informed consent was sought, and this was done with permission from the local Research Ethics Committee.

Statistical methods:

Statistical Package for Social Sciences (SPSS) version 28 was used for data management and statistical analysis (IBM, Armonk, New York, United States). The Shapiro-Wilk test and direct data visualization techniques were used to evaluate the normality of quantitative data. Means and standard deviations, or medians and ranges, were used to summarize quantitative data based on normality. Numbers and percentages were used to summarize categorical data. The independent t-test and the Mann-Whitney U test were used to evaluate quantitative data based on mortality for normally and non-normally distributed quantitative variables, respectively. Fisher's exact test, or the Chi-square test, was used to compare categorical data.

To predict mortality and the need for mechanical ventilation (MV), nRBCs underwent receiver operating characteristic (ROC) curve analysis. The diagnostic indices, optimal cutoff point, and area under the curve with 95% confidence intervals were computed. MV use and mortality were predicted using multivariate logistic regression analysis. A 95% confidence interval for the odds ratio was computed. There were two sides to every statistical test. *P* values were deemed significant if they were less than 0.05.

Results

This study involved 102 neonates with critical illness, 58 (56.9%) neonates were preterm, and 44 (43.1%) neonates were full-term. They were divided into two subgroups according to their survival: survivors (79.4%) and non-survivors (20.6%). Regarding perinatal risk factors, survivors and non-survivors were no significantly different. The sociodemographic data, clinical characteristics, morbidities and outcomes of the study groups are defined in **table 1,** and their laboratory test results are mentioned in **table 2.**

On comparing the survivor group to non-survivors, gestational age and birth weight were significantly lower (P <0.001 and <0.001, respectively), and prematurity was more prevalent (P <0.001) among non-survivors. Meanwhile, no significant difference was found between the two groups regarding sex. Normal vaginal delivery was considerably more common among non-survivors, constituting the mode of delivery in one-third of them, compared to 8.6% of survivors (P=0.003). APGAR score was significantly lower in non-survivors than survivors at 1 minute and 5 minutes (P <0.001, and 0.003, respectively), SNAPPE II score and NEOMOD score were meaningfully elevated in the non-survivor group (P <0.001, and <0.001, respectively) as shown in **figure 1. (table 1).**

On investigating the morbidities, RDS was more prevalent among non-survivors (*P* <0.001), and intraventricular hemorrhage (IVH) was reported in one-third of the non-survivors in comparison with 6.2% of the survivors (*P* <0.001), while congenital pneumonia was more common among survivors (*P*=0.036). Need for cardiac support, and MV were significantly more reported in the non-survivors (*P* <0.001, and <0.001, respectively), while continuous positive airway pressure (CPAP) use was more frequent among survivors (*P*=0.02). Regarding the outcomes, the length of NICU stay was significantly longer among survivors (*P* <0.001) **(table 1).**

Regarding laboratory parameters. nRBCs were considerably greater in the non-survivor group [24 (range 8 - 72) nRBCs/100 WBCs], compared to the survivor group [8 (range 1 - 48) nRBCs/100 WBCs, *P* <0.001] **(Figure 2)**. Also, there were significant differences with respect to platelet counts, blood gas parameters, serum creatinine, and serum bilirubin between both groups **(table 2).** In comparing preterm and full-term neonates, preterm neonates had significantly lower survival **(table 3).**

According to our analysis, there was a negative correlation between nRBCs and gestational age (*P*=<0.001), birth weight (*P*=<0.001), blood PH (*P*=<0.001), and length of hospital admission (*P*=0.009), while there was a positive correlation between nRBCs and SNAPPE II score and NEOMOD score.

Univariate logistic regression analysis was performed to assess the predictive ability of different morbidities for neonatal mortality, RDS, necrotizing enterocolitis (NEC), and IVH. The results showed that these morbidities were significantly associated with higher odds of neonatal mortality **(Table 4).**

On the assessment of the diagnostic performance of nRBCs to predict mortality in both full-term and preterm groups, and in the preterm group only, an AUC of 0.906, $P < 0.001$ at cutoff point of >17 nRBCs/100 WBCs, and AUC of 0.8895, $P < 0.001$ at cutoff point of >18 nRBCs/100 WBCs, were found respectively, with reasonable sensitivities and specificities. Additionally, nRBCs demonstrated significant diagnostic performance for predicting the need for mechanical ventilation, with an AUC of 0.799 ($P < 0.001$) at a cutoff point of >11 nRBCs/100 WBCs **(Table 5, Figures 3–5).**

Moreover, multivariate logistic regression was made to determine the independent predictors of neonatal mortality and mechanical ventilation use. It revealed that lower birth weight and higher nRBCs were the only independent predictors of death, as one unit increase in nRBCs was significantly associated with an 8% increased risk of mortality (OR = 1.083, 95% CI = 1.003 – 1.17, $P = 0.042$), and one unit decrease in birth weight was considerably correlated to an 17% higher risk of mortality (OR=0.173, 95% CI = 0.031 - 0.973, $P = 0.046$) **(table 6).**

Discussion

In the current investigation, we aimed to assess the clinical significance of nRBCs as a marker of illness severity in critically ill newborns. According to the literature, normal newborns typically exhibit 3-10 nRBCs per/WBCs in their peripheral blood at birth, a tiny number that may last until the fifth day of life **[21].** Physiological stressors, including inflammation, massive bleeding, acidemia, hypoxic-ischemic encephalopathy, and meconium aspiration, have been found to be specifically linked to elevations in nRBC count in previous analyses. This is due to the fact that these conditions increase erythropoietic pressure and hinder the spleen's capacity to remove these cells from the circulation **[1, 8].**

In the present work, the median nRBCs count was threefold higher in non-survivors than in survivors, P <0.001. Additionally, multivariate analysis identified elevated nRBCs as an independent predictor of neonatal mortality (OR 1.083, P=0.042). On performing ROC curve analysis, elevated nRBCs were significantly associated with increased mortality with an AUC of 0.906. At a cutoff value >17.0, it had a sensitivity of 85.7% and a specificity of 85.2%, P <0.001. Also, we found that a high nRBC count at a cutoff value >11.0 could predict the need for MV (AUC 0.799, with 81.2% sensitivity and 72.9% specificity).

Previous research has reported similar outcomes. The Morton et al. study found a significant relationship between the number of nRBCs and the elevated risk of

newborn mortality in critically ill neonates admitted to the NICU for seven days **[8].** Moreover, Zakerihamidi et al. found that nRBC counts in non-survivors were observed to be considerably greater than those in live newborns who were discharged **[22],** and increased nRBC counts on days 2–5 after birth was found to be linked to high mortality **[10].** A recent investigation by Schmidt et al. further highlighted that neonates with detectable peripheral blood nRBCs required prolonged mechanical ventilation compared to nRBC-negative patients **[23].** Collectively, these findings suggest that, for critically ill neonates, nRBC counts might be regarded as an early diagnostic and prognostic marker of illness severity **[9].**

On the other hand, this measure did not exhibit good discriminative power for predicting mortality in the overall study population, despite the median value of nRBCs occasionally being higher for non-survivors in the Sokou et al. study. The small number of cases and the considerable variability of nRBCs are probably the causes of this. However, the ROC curve analysis demonstrated that nRBCs were discriminatively effective as both a diagnostic and prognostic marker (AUC 0.565, $P < 0.001$) **[1].**

Furthermore, nRBCs could serve as an indicator of the severity of various diseases in neonates. The nRBC count has been shown by Boskabadi et al. to be a predictive marker for neonatal asphyxia, which could suggest high infant mortality,

particularly when combined with the hypoxic-ischemic encephalopathy grade **[24].** Additionally, it could help recognize perinatal hypoxia early, facilitating timely intervention and potentially improving neonatal outcomes **[2, 25].** With an AUC of 0.921, Sokou et al. showed that nRBCs may predict newborn hypoxia, particularly in preterm neonates. Using a cut-off value of ≥11.2%, nRBCs showed good diagnostic performance, with a sensitivity of 80% and a specificity of 88.7%, respectively. **[1].**

Neonatal sepsis may be diagnosed more easily if peripheral blood contains excessive nRBCs. Furthermore, nRBCs have been associated with increased disease severity, as evidenced by longer hospital stays, greater transfusion rates, and a higher likelihood of a poor prognosis and neonatal mortality in septic neonates **[26, 27].** According to Sokou's research, nRBCs with a cutoff value of ≥1% demonstrated considerable predictive significance for death among neonates with sepsis, particularly preterm, with 81.60% sensitivity and 78.10% specificity **[1].**

According to Boskabadi et al. and Bin-Nun et al., there is a correlation between increased nRBC and a higher risk of unfavorable newborn outcomes as well as significant neonatal disorders like infections, retinopathy of prematurity, white matter injury, necrotizing enterocolitis, open arterial duct, and IVH **[3, 28].**

The current work established a substantial inverse relationship between nRBC count and both gestational age and birth weight. In addition, preterm neonates had

twice the median nRBC counts than full-term neonates, P <0.001. These findings align with those of Valina et al., who found significant variations in the number of nRBCs between the term and preterm infant groups, particularly on the first day of life **[29].** Also, according to reports, preterm neonates have a larger number of nRBCs upon delivery. Erythropoiesis rises in premature babies, allowing for the observation of a substantial number of nRBCs for a longer time after birth **[2].**

Moreover, in the current study, nRBC count was positively correlated with acidosis and higher SNAPPE II and NEOMOD scores, both of which were significantly elevated among non-survivors. This finding supports the role of nRBC count as an indicator of illness severity, comparable to established neonatal severity scoring systems, as previously reported by **[1].** Moreover, we found that nRBCs were inversely correlated with the duration of NICU stay, reflecting a shorter time to mortality, which agrees with a previous study **[8].**

According to our analysis, the mortality rate among critically ill neonates was 20.6%. Similarly, in a study by Seoud et al. that involved 826 neonates admitted to the NICU, the mortality rate was found to be 29.1% **[30].** In the Sokou et al. study conducted on 467 neonates with critical illness, 9.6% did not survive **[1].** In contrast, Morton et al., who carried out a study on all neonates admitted to the NICU, found that 4.2% of them died in-hospital before NICU discharge **[8].** The

difference in mortality rates among studies could be attributed to the variable diagnoses and different illness severities among the neonates involved.

We found that a higher mortality rate was associated with vaginal delivery and a lower 1- and 5-minute APGAR scores. This agrees with Boskabadi et al. study **[9].** RDS, intraventricular hemorrhage, need for MV and cardiac support were more prevalent among non survivors. Moreover, RDS, NEC, and IVH were discovered as predictors of neonatal mortality by univariate logistic regression. In comparison, pneumonia and CPAP use were more frequent among survivors. In other studies, RDS was a substantial predictor of death by univariate analysis, OR 5.61 (2.45–12.85), P <0.0001, and the use of oxygen therapy (P <0.001) and mechanical ventilation (P=0.036) had a statistically important correlation with the infant survival rate **[1, 9].**

We also found that lower platelet counts, higher acidosis, and elevated serum creatinine and bilirubin levels were associated with higher mortality rates. Similar results were found by Sokou et al., who reported that platelet count was considerably lower, while serum creatinine and bilirubin were significantly higher among non-survivors. No significant difference was found regarding base deficit **[1].**

Herein, we also compared the outcomes of preterm and full-term newborns. It was evident that preterm neonates were responsible for most of the deaths, and they had

higher SNAPPE and NEOMOD scores as well as a higher need for cardiac and respiratory support. Previous literature has shown that the morbidity of newborns is significantly influenced by preterm labor. Preterm labor and prematurity were cited by the World Bank in 2015 as the primary causes of 85% of infant deaths worldwide **[31].** Premature birth is therefore thought to be the cause of one million deaths every year **[32].**

We determined that low birth weight is an independent predictor of newborn mortality, as indicated by multivariate analysis. Being small for gestational age has been shown to significantly increase the mortality rates of both early-term and late-preterm newborns **[33].** Low birth weight and premature birth are thought to be the best indicators of neonatal mortality **[29].**

The comparatively small sample size is one of the study's limitations. We recommend conducting further multicenter studies to support our results. Additionally, we measured the nRBC count only once on the first day, and we recommend further research with serial measurement of the nRBC count to assess the impact of critical illness on the count and the rate of disappearance of nRBCs from peripheral blood. The absence of long-term follow-up is another issue. We only assessed outcomes during the neonatal period; it would be valuable to include long-term follow-up to evaluate the impact of elevated nRBCs on neonatal outcomes, including neurodevelopmental progress, cognitive function, and growth.

Incorporating additional markers of inflammation, infection, or organ dysfunction could provide a more comprehensive picture of illness severity and prognosis.

Conclusion

This prospective study performed on critically ill neonates found that nRBC count represented a good prognostic indicator of illness severity and an independent predictor of death in neonates with critical illness. This could improve their result by guiding the treatment of this crucial demographic and aiding in early risk classification.

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Tables

Table 1. A comparison between survivors to non-survivors concerning sociodemographic data, clinical characteristics, morbidities, and outcomes of the study groups.

Clinical features	**Total (N = 102)** N (%)	**Non-survivors (N = 21)** N (%)	**Survivors (N = 81)** N (%)	**p-value**
Gestational age (weeks), mean ± SD	35 ±3	31 ±3	36 ±2	**<0.001***
Birth weight (kg), median (range)	2.49 (0.49 - 4)	1.22 (0.49 - 4)	2.6 (0.72 - 3.88)	**<0.001***
Sex				
Males	64 (62.7)	16 (76.2)	48 (59.3)	0.153
Females	38 (37.3)	5 (23.8)	33 (40.7)	
Maturity				
Pre-term	58 (56.9)	20 (95.2)	38 (46.9)	**<0.001***
Full-term	44 (43.1)	1 (4.8)	43 (53.1)	
Mode of delivery				
NVD	14 (13.7)	7 (33.3)	7 (8.6)	**0.003***
CS	88 (86.3)	14 (66.7)	74 (91.4)	
APGAR, median (range)				
APGAR at 1 min.	8 (2 – 9)	5 (2 – 8)	8 (3 – 9)	**<0.001***
APGAR at 5 min.	9 (4 – 10)	7 (4 – 9)	9 (5 – 10)	**0.003***
SNAPPE II score, median (range)	5 (0 - 84)	62 (31 - 84)	5 (0 - 67)	**<0.001***
NEOMOD score, median (range)	4 (0 - 10)	8 (5 - 10)	3 (0 - 9)	**<0.001***
Morbidity				
RDS	46 (45.1)	18 (85.7)	28 (34.6)	**<0.001***

Pneumonia	22 (21.6)	1 (4.8)	21 (25.9)	**0.036***
Neonatal sepsis	29 (28.4)	6 (28.6)	23 (28.4)	0.987
Pulmonary hypertension	11 (10.8)	1 (4.8)	10 (12.3)	0.318
CHD	3 (2.9)	0 (0)	3 (3.7)	1.0
HIE	2 (2)	1 (4.8)	1 (1.2)	0.371
Congenital renal anomaly	1 (1)	0 (0)	1 (1.2)	1.0
TOF	1 (1)	0 (0)	1 (1.2)	1.0
NEC	5 (4.9)	3 (14.3)	2 (2.5)	0.058
IVH	12 (11.8)	7 (33.3)	5 (6.2)	**<0.001***
BPD	3 (2.9)	1 (4.8)	2 (2.5)	0.503
AKI	1 (1)	1 (4.8)	0 (0)	0.206
ROP	1 (1)	0 (0)	1 (1.2)	1.0
Cardiac support use	26 (25.5)	12 (57.1)	14 (17.3)	**<0.001***
MV use	32 (31.4)	16 (76.2)	16 (19.8)	**<0.001***
CPAP use	70 (68.6)	10 (47.6)	60 (74.1)	**0.02***
Outcome				
Length of NICU stay (days), median (range)	11 (1 - 32)	5 (1 - 25)	12 (10 - 32)	**<0.001***

NVD: Normal vaginal deviation; CS: Cesarean section; RDS: Respiratory distress syndrome; CHD: Congenital heart disease; HIE: Hypoxic ischemic encephalopathy; TOF: Tetralogy of Fallot; NEC: Necrotizing enterocolitis; IVH: Intraventricular hemorrhage; BPD: Bronchopulmonary dysplasia; AKI: Acute kidney injury; ROP: Retinopathy of prematurity; CPAP: Continuous positive airway pressure; MV: Mechanical ventilation; NICU: Neonatal intensive care unit.

Table 2. Laboratory test results of the study group, comparing survivors to non-survivors

Laboratory test results	Total (N = 102)	Non-survivors (N = 21)	Survivors (N = 81)	P value
nRBCs (%)	10 (1 - 72)	24 (8 - 72)	8 (1 - 48)	**<0.001***
Hb (g/dL)	15.15 ±2.92	14.6 ±3.2	15.29 ±2.84	0.338
HCT (%)	45.2 ±9.9	44 ±8.9	45.5 ±10.1	0.539
Platelets $x10^3$/uL	217 (63 - 788)	182 (63 - 788)	246 (63 - 527)	**0.012***
Corrected TLC $x10^3$/uL	11.63 (2.5 - 51)	11.56 (2.5 - 51.0)	11.7 (2.53 – 51.0)	0.571
Neutrophils $x10^3$/uL	5.43 (0.7 - 40.4)	2.9 (0.7 - 40.4)	5.48 (0.7 - 17.53)	0.413
IT ratio	0.2 (0 - 2.9)	0.22 (0 - 0.9)	0.185 (0 - 2.9)	0.236
Lowest PH	7.235 ±0.15	7.031 ±0.138	7.288 ±0.1	**<0.001***
PCO_2 (mmHg)	43.15 (13.3 - 119.8)	50 (14 - 119.8)	40 (13.3 - 68)	**0.002***
HCO_3 (mEq/L)	18.5 ±4.8	14.6 ±5.9	19.5 ±3.9	**0.001***
Base deficit	-8 (-28.1 - 6)	-17 (-28.1 - 0.1)	-7.3 (-17.6 - 6)	**<0.001***
Positive blood culture	28 (27.5)	6 (28.6)	22 (27.2)	0.897
ALT (U/L)	15.3 (8 - 144)	15 (8 - 144)	16.5 (11 - 54)	0.188
AST (U/L)	41 (12.5 - 167)	38 (20 - 167)	42.5 (12.5 - 122)	0.224
Urea (mg/dl)	30 (1 - 137)	36 (14 - 137)	29.5 (1 - 96)	0.221
Serum creat. (mg/dl)	0.7 (0.3 - 2.2)	0.8 (0.5 - 2.2)	0.6 (0.3 - 1.5)	**<0.001***
Total bili. (mg/dL)	6.7 (0.5 - 17)	3.8 (1.4 - 11)	7.1 (0.5 - 17)	**0.027***
Direct bili. (mg/dL)	0.5 (0.2 - 1.3)	0.5 (0.2 - 1.2)	0.5 (0.2 - 1.3)	0.704
CRP (mg/dL)	30 (11 - 124)	49 (15 - 96)	26 (11 - 124)	0.118

Values are expressed as mean ±SD, or median (range); nRBCs: Nucleated red blood cells; Hb: Hemoglobin; HCT: Hematocrit; TLC: Total leucocytic count; IT ratio: Immature to total neutrophil ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; creat.: Creatinine; Bili.: Bilirubin; CRP: C-reactive protein.

Table 3. NRBCs count, clinical characteristics, and different outcomes of preterm and full-term neonates

Parameter	**Preterm (n = 58)**	**Full-term (n=44)**	**P-value**
nRBCs (%), median (range)	16 (1 - 72)	8 (1 - 22)	**<0.001***
SNAPPE II score, median (range)	23 (5 - 84)	5 (0 - 31)	**<0.001***
NEOMOD score, median (range)	5 (1 - 10)	3 (0 - 8)	**<0.001***
CPAP use, N (%)	40 (69.0)	30 (68.2)	0.933
MV use, N (%)	26 (44.8)	6 (13.6)	**<0.001***
Cardiac support, N (%)	21 (36.2)	5 (11.4)	**0.004***
Length of NICU stay (days), median (range)	10 (1 - 30)	13 (10 - 32)	**0.003***
Mortality, N (%)	20 (34.5)	1 (2.3)	**<0.001***

nRBCs: Nucleated red blood cells; CPAP: Continuous positive airway pressure; MV: Mechanical ventilation; NICU: Neonatal intensive care unit.

Table 4. Univariate logistic regression analysis for different morbidities as mortality predictors

Morbidity	OR (95% CI)	P-value
RDS	11.357 (3.079 - 41.89)	**<0.001***
Pneumonia	0.143 (0.018 - 1.131)	0.065
Neonatal sepsis	1.009 (0.348 - 2.92)	0.987
Pulmonary hypertension	0.355 (0.043 - 2.942)	0.337
NEC	6.583 (1.024 - 42.331)	**0.047***
IVH	7.6 (2.11 - 27.373)	**0.002***

*Significant P-value; RDS: Respiratory distress syndrome; NEC: Necrotizing enterocolitis; IVH: Intraventricular hemorrhage.

Table 5. Diagnostic performance of nRBCs for the prediction of neonatal mortality and mechanical ventilation use

nRBCs prediction	AUC	p value	95% CI		Cut off point	Sensitivity %	Specificity %	PPV	NPV
			Min	Max					
Mortality	0.906	**<0.001**	0.841	0.972	>17	85.7	85.2	60	95.8
Mortality in preterm	0.895	**<0.001**	0.762	0.956	>18	75	79	65.2	85.7
MV use	0.799	**<0.001**	0.700	0.899	>11	81.2	72.9	57.8	89.5

nRBCs: Nucleated red blood cells; AUC: Area under the curve; 95%CI: 95% confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; MV: Mechanical ventilation.

Table 6: Multivariate logistic regression analysis to predict neonatal mortality and mechanical ventilation use

Parameter	Mortality		Mechanical ventilation	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Gestational age (weeks)	0.849 (0.609 - 1.182)	0.331	0.864 (0.654 - 1.142)	0.305
Sex	0.586 (0.105 - 3.262)	0.542	0.336 (0.103 - 1.097)	0.071
Birth weight (kg)	0.173 (0.031 - 0.973)	**0.046**	0.575 (0.178 - 1.861)	0.356
Mode of delivery	0.5 (0.074 - 3.396)	0.478	0.702 (0.156 - 3.173)	0.646
nRBCs	1.083 (1.003 - 1.17)	**0.042**	1.067 (0.999 - 1.139)	0.053

nRBCs: Nucleated red blood cells; 95%CI: 95% confidence interval.

Figure Legends

Figure number	Figure title
Figure 1.	Total SNAPPE II and NEOMOD scores in the study group, comparing survivors to non-survivors
Figure 2.	nRBCs in the study group, comparing survivors to non-survivors
Figure 3.	ROC analysis of nRBCs to predict neonatal mortality in both preterm and full-term
Figure 4.	ROC analysis of nRBCs to predict neonatal mortality in preterm neonates
Figure 5.	ROC analysis of nRBCs to predict use of mechanical ventilation