Nucleated red blood cells are predictive of in-hospital mortality for pediatric patients

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Abbreviations: cardiopulmonary resuscitation (CPR), complete blood count (CBC), extracorporeal membrane oxygenation (ECMO), INR (international nationalized ratio), intensive care unit (ICU), length of stay (LOS), NRBCs (nucleated red blood cells), PED (pediatric emergency department), pediatric intensive care unit (PICU), electronic medical record (EMR)

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Abstract

Objectives: We sought to establish whether nucleated red blood cells (NRBCs) are predictive of disposition, morbidity, and mortality for pediatric patients presenting to the emergency department (ED).

Methods: A single center retrospective cohort study examining all ED encounters from patients<19 years old between January 2016 and March 2020, during which a CBC was obtained. Univariate analysis and multivariable logistic regression were used to test presence of NRBCs as independent predictor of patient-related outcomes.

Results: The prevalence of NRBCs was 8.9% (4,195/46,991 patient encounters). Patient with NRBCs were younger (median age 4.58 vs 8.23 years; P<0.001). Those with NRBCs had higher rates of in-hospital mortality (30/2,465 [1.22%] vs 65/21,741 [0.30%]; P<0.001), sepsis (19% vs 12%; P<0.001), shock (7% vs 4%; P<0.001), and CPR (0.62% vs 0.09%; P<0.001). They were more likely to be admitted (59% vs 51%; P<0.001), have longer median hospital length of stay (LOS) (1.3 [IQR: 0.22, 4.14] vs. 0.8 days [0.23, 2.64]; P<0.001), and median intensive care unit (ICU) LOS (3.9 [IQR: 1.87, 8.72] vs 2.6 days [IQR: 1.27, 5.83]; P<0.001). Multivariable regression revealed presence of NRBCs as an independent predictor for in-hospital mortality (adjusted odds ratio [aOR] 2.21 95% CI 1.38-3.53; p<0.001), ICU admission (aOR 1.30 95% CI 1.11, 1.51), CPR (aOR 3.83 95% CI 2.33-6.30; p<0.001), and 30-day return to the ED (aOR 1.15 95% CI 1.15-1.26; P<0.001).

Conclusions: Presence of NRBCs is an independent predictor for in-hospital mortality, ICU admission, CPR, and readmission within 30 days for children presenting to the ED.

Introduction

Nucleated red blood cells (NRBCs) are early erythrocyte precursors usually absent in the peripheral blood of healthy children and adults.[1] Disease processes involving extreme increases in erythropoiesis or failure of the normal bone marrow filtration mechanisms can lead to an influx of NRBCs into the circulation. Hematopoietic stress occurs in a multitude of acute care clinical scenarios due to triggered inflammatory responses, hematologic stress, or severe hypoxia[1]. Approximately 10 to 30% of adults in the critical care setting have NRBCs in the peripheral blood[2–5]. NRBCs are a useful biomarker of disease severity and poor clinical outcomes in critically ill adults, as they are independently associated with in-hospital mortality, poor prognosis, post-discharge mortality, and readmission, and a complementary marker to acute scoring systems for risk prediction[3,6–9].

Limited data exist on the clinical utility of NRBCs as a biomarker for clinical outcomes in children[4,9]. An association has been described between the length of cardiopulmonary bypass and presence of NRBCs, and presence of NRBCs has been described in a small case series of critically ill children following cardiopulmonary arrest.[9,10] An observational prospective study of 670 patients in the pediatric intensive care unit (PICU) found in-hospital mortality was significantly higher among children with circulating NRBCs, however presence of NRBCs was not confirmed to be an independent predictor of composite outcome (death and/or ventilation and/or renal replacement therapy, and/or inotropic support) at multivariate analysis [4]. Unfortunately, in this study the number of children>28 days of life was limited. A recent neonatal intensive care unit (NICU) study showed infants with NRBCs had a higher risk of mortality and those with higher NRBC count had shorter survival (cit?). However, studies on

older children are currently missing. Ascertaining whether NRBCs may predict short-term patients' outcomes in the emergency department (ED) could add value to existing knowledge and practice by improving systems for triaging, delegating resources, and deciding discharge with no additional cost by repurposing available information. In this study, we sought to establish whether NRBCs identified in the pediatric ED may predict disposition, morbidity, and in-hospital mortality.

Material and Methods:

Study Design and Patient Selection

We performed a retrospective cohort study including all encounters of patients who presented to a single tertiary pediatric ED (Boston Children's Hospital, BCH, Boston, Massachusetts, U.S.) between January 1, 2016, and March 31, 2020, from whom a CBC was obtained within 5 hours of arrival. The study end date was specifically selected to avoid any confounding from the emerging COVID-19 pandemic. The BCH ED has approximately 60,000 encounters per year from children and older patients with acute and chronic pediatric conditions. The study was approved by the local Institutional Review Board with a waiver of informed consent.

Data Collection and Categorization

Demographic, clinical characteristics and outcomes of patients included were extracted from the electronic medical record (EMR) using a structured query language (SQL, DBeaver UI Tool, IBM Netezza Data Warehouse, IBM Inc) from time-stamped electronic health medical records. We collected the following variables: age, race, gender, laboratory values (first absolute NRBC

count, International Normalized Ratio [INR], creatinine, aspartate transaminase [AST], alanine transaminase [ALT] within <5 hours from ED admission), emergency severity index score (ESI), admission category (hospital floor vs ICU), presence of sepsis or shock, use of oral or IV steroids within 72 hours of arrival, use of erythropoiesis enhancing medication (darbepoetin alfa, erythropoietin, corticotropin IV, methylprednisolone IV, prednisone IV, hydrocortisone IV), use and type of vasoactive support (norepinephrine, dopamine, epinephrine, milrinone, and vasopressin), administration of blood products (platelets, packed red blood cells, cryoprecipitate, and fresh frozen plasma), need for dialysis, mechanical ventilation, non-invasive positive pressure ventilation, or supplemental oxygen, need for cardiopulmonary resuscitation (CPR), extracorporeal membrane oxygenation (ECMO), hospital and ICU length of stay (LOS), and in-hospital mortality. The ESI is a five-level ED triage algorithm validated in children that provides clinically relevant stratification of patients into five groups from 1 (most urgent) to 5 (least urgent) on the basis of acuity and resource needs[11,12] and was used as the primary measure of severity of illness[11–13]. Patients with sepsis and shock were determined by administrative *International Classification of Diseases -9* (ICD-9, before October 1, 2015) and *International Classification of Diseases*-10 (ICD-10, after October 1, 2015)[14–17]. Patients were classified to have renal dysfunction if they had a creatinine > upper limit for age based on BCH Laboratory cut-offs, and hepatic dysfunction if more than two or more of the following were present: AST >100 units/L, conjugated bilirubin >1.2umol/L, ALT > 500 units/L and or International Normalized Ratio (INR) >1.5 in absence of warfarin anticoagulation.

Laboratory Methods

NRBC counts were performed at the BCH laboratory, following a standardized protocol. NRBCs were measured using a Sysmex XN-series multi-parameter automated hematology analyzer (Sysmex Corporations, Kobe, Japan). Blood samples were mixed with the Lysercell WNR reagent and stains. Nucleated erythrocytes were counted by the WNR channel based on the intensity of forward scattered light intensity and depth of fluorescent staining. NRBCs were expressed as an absolute number of thousand cells per microliter (Kcells/μL) and not per 100 white blood cells because of the risk of confounding effects related to the leucocyte count. NRBC positivity (NRBC+) was defined as an absolute count >0.

Outcomes

The primary outcome of this study was in-hospital mortality. Secondary outcomes included hospital admission, ICU admission, need for CPR, and return to the ED within 30 days. Additionally, mortality was evaluated 1 year after presentation and through March of 2021, i.e. the time of data acquisition[18].

Statistical Analysis

Descriptive statistics include frequencies and percentages for categorical variables; and median and inter-quartile range (IQR, 25th-75th percentile) for continuous variables. Demographic, clinical characteristics, and outcomes were compared between patient encounters with NRBCs and those without NRBCs. The ED encounter was the unit of analysis; multiple encounters from the same patient were accounted for in all analyses using robust standard errors that cluster by patient. Categorical variables were compared using a Rao-Scott chi-squared test (clustering by patient)[19] and continuous variables were compared using the Mann-Whitney-Wilcoxon test (clustering by patient).

A Kaplan-Meier survival analysis was performed to visualize survival using single medical record numbers. The starting time for a an NRBC+ patient was the encounter at which NRBC > 0 even if a prior encounter existed without NRBC detection; to find the appropriate controls for these NRBC+ patients, we used the pool of patients without NRBC detection at any encounter during our study period. We then matched two NRBC- patients to an NRBC+ patient by encounter number (of the NRBC+ patient), age, ESI, and whether the patient was on glucocorticoids. Balance of the main confounders was then confirmed by comparing these factors between the two matched cohort using XXX tests. A Kaplan-Meier survival curve (Figure 1A) for NRBC negative patients vs NRBC positive patients were compared using a logrank test, clustering by matched set[20,21]. In order to evaluate the effect of NRBC number on mortality, we also created a Kaplan-Meier survival curve for NRBC+ patients broken up into 4 groups: 20 cells/μL, 21-100 cells/μL, 101-1000 cells/μL and > 1000 cells/μL (the minimal number of NRBCs reported was 20 cells/μL) as shown in figure 1B.

To test for the adjusted association between NRBCs and in-hospital mortality, we used all encounters in which patients were admitted to the hospital. With multiple hospital admissions from the same patients, we can consider this analysis a conditional analysis in which patients must survive the previous admission to be 'at-risk' for in-hospital mortality at the next hospital admission. For each admission, a logistic regression was used to model the probability on in-hospital mortality as a function of NRBC status (present, not present) at the current admission, as well admission number during our study period of the current admission (coded as 1,2,3,4, or ≥5), and potential confounders: recent glucocorticoid therapy within 72 hours of ED presentation[22], age (in years, as well as testing for a quadratic age effect) and ESI of 1-2[11,13] (Reference ESI of 3-4). To assess if the effect of being NRBC positive increases the risk of in-

hospital mortality as the number of admissions increases, we tested for the significance of an interaction between NRBC and admission number. Furthermore, to account for the multiple admissions from the same patient, we used a robust standard error[23,24]. Lastly, we present results of a logistic regression model for in-patient mortality based on the first admission from each patient. Similar analyses were performed for testing the association between NRBCs and secondary outcomes (hospital admission, ICU admission, need for CPR, and return to ED in 30 days). A P value less than 0.05 was considered statistically significant. All analyses were performed using R version 4.0.3 (R Foundation, Vienna, Austria) and SAS 9.4.

Results:

Cohort Description

A total of 46,991 ED encounters (51% Caucasian, 49% female, median age of 7.9 years [IQR 2.7, 14.1 years]) from 31,756 unique pediatric patients formed the analytic cohort (Table 1). Most patients (n=25,347, 79.8%) had a single encounter. Subjects who presented more than once to the ED were distributed as follows: 3,656 (11.5%) twice, 1,147 (3.6%) three times, 529 (1.7%) four times with the remaining 1,077 (3.4%) five or more times. Neonates (≤28 days old) comprised 1.9% (88/46,991) of the encounters. Almost half of patient encounters (21,525, 46%) had an ESI triage score of 1 or 2 out of 5, indicative of high acuity patients. Fifty-two percent of the encounters resulted in admission, of whom 3.399 (7.2%) were admitted to the ICU. The median LOS was 0.82 days (IQR: 0.23, 2.77 days).

Comparison of NRBC+ and NRBC- encounters

Out of the 46,991 ED encounters, 4,195 (8.9%) were NRBC+ (NRBC count >0) with the remaining having undetectable NRBCs (NRBC-). The absolute number of NRBCs measured had a highly skewed distribution, ranging from 20 to 33,420 cells/μL, with a median of 30 cells/μL (IQR 20, 90). NRBC+ patients were younger than those without NRBCs (median age 4.58 [IQR 1.24, 12.29] vs 8.23 [IQR 2.96, 14.24] years; p <0.001). Patients with NRBC+ encounters more frequently had a lower ESI, received more than one inotrope, required CPR, received supplemental oxygen or positive pressure support, required intubation, received blood products, had sepsis or shock, received glucocorticoids, needed dialysis or had hepatic dysfunction (each P<0.001, Table 1). NRBC+ encounters more often led to admission, longer hospital LOS, more frequent admission to the ICU, and had more frequently an ICU stay >48 hours, and were more likely to return to the ED within 30 days (each p<0.001) (Table 1).

Association between NRBC and Outcomes

To study the association between NRBC and in-hospital mortality, we restricted the analysis to the 24,270 patient encounters that resulted in admission, 10% (2,465 patients) of which were NRBC+. Overall, a total of 95 patients suffered in-hospital mortality (95/24,270; 0.39%). Children with NRBCs at the time of admission (29/2,465; 1.18%) were at higher risk of in-hospital mortality than children without (65/21,740; 0.3%), with an unadjusted OR 3.98 (95% CI 2.57-6.18, P<0.001; Table 1). After adjusting for potential confounders, NRBCs remained associated with in-hospital death (aOR 2.21, 95% CI 1.38-3.53; Table 2). Although there was no significant association between the overall number of admission and being NRBC+ (P=0.640), having 4 or , ≥5 admission was significantly associated with in-hospital mortality (aOR 2.57, 95% CI 1.20-5.49; and 2.97, 95% CI 1.76-5.03, respectively). A sub-analysis of each child's first

encounter revealed a similar effect of NRBC positivity on in-patient mortality (aOR 2.28, 95% CI 1.04-4.98, P=0.038; Supplemental Table 1), giving a similar adjusted odds ratio. Using an adjusted logistic regression analysis accounting for multiple patient encounters, NRBC+ patients were more likely to return to the ED within 30 days (aOR 1.15, 95% CI 1.05-1.26), to be admitted to the ICU (aOR 1.30, 95% CI 1.11,1.51) and receive CPR (aOR 3.83, 95% CI 2.33-6.30). Notably, using this approach, NRBC+ patients were no more likely to be admitted to the hospital compared to those without NRBCs (aOR 0.91, 95% CI 0.83,1.01).

Association between absolute number of NRBCs and survival

Of the 31,757 individual pediatric patients who presented to the ED during the study period, 8.4% (2,661) had detectable NRBCs during at least one study encounter. Analysis was restricted to the first NRBC+ encounter for each patient and their two matched NRBC- encounters (matched by encounter number during the study period, age, ESI, and glucocorticoid use). In the matched analysis, there were 7,948 patients (2,661 NRBC+ patients and 5,287 NRBC- patients), and there were 232 deaths in the matched (104 in the NRBC- group and 128 in the NRBC+ group). Supplemental Table 2 gives the patient characteristics of the matched sample and shows that that the NRBC+ and NRBC- patients are well-balanced on the important possible confounders. A Kaplan Meier estimate (Figure 1A) of survival comparing these groups gave a significant logrank test, accounting for clustering to matching (P=0.024), showing that NRBC are associated with in-hospital mortality. Particularly, there were no significant differences in mortality at 30 days (1.47% for NRBC+ vs 1.98 % for NRBC-; P=0.282); borderline significance at 90 days (3.53% for NRBC+ vs 2.37 % for NRBC-; P=0.053); and significance at 1 year (9.7% for NRBC+ vs 7.3 % for NRBC-; P=0.405). The most significant difference (P=0.001) between

groups was at 2.5 years in which the mortality probability for NRBC+ patients was 20.6% (95% CI: 16.6-24.4 %) and 11.9% NRBC- patients (95% CI: 9.3%-14.4 %). In order to assess if there was an effect of NRBC number on mortality, NRBC+ patients were subdivided into 4 groups based on increasing NRBC+ (1,368 with 20 cells/μL, 970 with 21-100 cells/μL, 278 with 101-1000 cells/μL, > 1000 cells/μL). The Kaplan-Meier curve (Figure 1B) comparing just the three groups (for ease of presentation of the KM plot, we left out the NRBC- patients) showed no differences between the groups (logrank P=0.5254). From the Kaplan Meier curve in Figure 1B, the 1 year mortality estimates in the three NRBC+ groups were (9.2% for 20 cells/μL, 10.8% for 21-100 cells/μL; and 9.9% for 01-1000 cells/μL; P=0.803).

Discussion:

Among 31,757 children evaluated at a tertiary care ED, the presence of circulating NRBCs was found to be associated with in-hospital mortality, need for CPR, need for ICU admission, and return to the ED within 30 days. Detection of NRBCs was independently associated with these clinically important outcomes, over and above the current standard ESI ED triage scoring system.

Previous studies have demonstrated the association of NRBCs with adverse outcomes in adults and more recently in neonates managed in critical care units, however no such association has been previously demonstrated for the general pediatric population in the ED [3,6,8]. Additionally, the limited studies in the pediatric population outside the neonatal ICU have been unable to detect an association [3,6,8], likely due to being underpowered. Our study design intentionally encompasses a large study population of over 45,000 ED encounters from all

comers, who underwent laboratory studies including a CBC at the discretion of the treating clinician, and includes repeat encounters, making results generalizable to standard pediatric ED practice. We demonstrate that pediatric patients with NRBCs had a 2.2 times higher risk of in-hospital mortality. Additionally, we found the presence of NRBCs was associated with longer lengths of hospital and ICU stay, and administration of intensive care therapies. Similar to another study in children, we found that NRBC+ patients in our cohort were at high risk for receiving CPR compared with those without NRBCs[10].

As opposed to other potential biomarkers, key advantages of using NRBC count are that it is typically included in a relatively inexpensive and routinely ordered screening test, a CBC with differential. Screening for NRBCs at the time of ED presentation, when a CBC is obtained, may serve as an additional clinical "clue", alerting the provider that the child may have a heightened risk of poor outcomes. Given the widespread availability of CBC, NRBC detection is a pragmatic variable to alert any busy healthcare provider to a subgroup of patients who may need admission, additional considerations, and closer follow-up. Presence of NRBC was not associated with in-hospital admission itself, but was significantly associated with higher criticality as need for ICU therapies and ICU admission, as well as in-hospital mortality. Additionally, and not surprisingly, NRBC+ patients were more likely to return to the ED within 30 days. This suggests that NRBCs may have a role in assisting the ED clinician in considering disposition for borderline cases, as well as arranging the appropriate level of follow-up for patients at high risk for adverse outcomes.

In regard to NRBC levels, prior studies in neonatal and adult patients have shown that even a modest rise in NRBCs was associated with increased risk of morbidity and mortality and that

those with higher peak NRBC counts appeared to be at increased risk compared to those with lower non-zero counts[25,26]. In this study we found that increasing NRBC counts were associated with higher mortality rates at 30 and 90 days when compared to patients with no NRBCs except for the subgroup of NRBC+ patients with >1000 cells/μL, likely because this subgroup was very small. Mortality risk for patients with NRBCs increased over time, being highest at 2.5 years, the longest time analyzed in the current study. While this association may not be applicable to the ED setting, it demonstrates that detectable circulating NRBC counts may serve to identify a population of pediatric patients who might benefit from additional care. While not addressed in this study, it is possible that for patients admitted or with prolonged follow-up, the trajectory of NRBC counts may prove to be most clinically useful. This is supported by some studies that have found that higher NRBCs counts were associated with shorter survival in addition to increased overall mortality[2,25,27] whereas the disappearance of circulating NRBC was associated with recovery and higher survival compared with those with persistent NRBCs[2]. Perhaps, at this point, the presence rather than a specific number of NRBCs should be considered sufficient to elicit increased vigilance. Future studies will be needed to clarify this point and to investigate the trajectory of NRBC counts with adverse outcome and potential recovery.

Our study has several limitations. First, we did not account for all the possible confounding variables thought to potentially influence NRBC counts such as genetic syndromes, congenital infections, markers of systemic inflammation, hematologic malignancy, extramedullary hematopoiesis or chronic hypoxemia[1,28]. While these may be contributing to observed differences in outcomes, we wanted our study to be generalizable to the population evaluated in the ED. Second, we limited NRBC measurements to the first measurement on presentation. We intentionally restricted our database inquiry to NRBCs within the timeframe of

the ED encounter to make the study applicable to the data available to the ED clinician. CBCs are rarely repeated in the same ED encounter and therefore the ED physician must make clinical decisions from a singular data point. Third, reliance on ICD codes to determine comorbidities, sepsis, and shock will likely underestimate the true incidence[29]. Additionally, we chose not to control for packed red blood cell (PRBC) transfusions prior to NRBC measurement, which can alter the NRBC measurement. We minimized the likelihood that a patient would have received a PRBC transfusion immediately before the NRBC measurement by using the first available blood sample following ED presentation. Finally, while we obtained detailed mortality data from the medical record, there is a possibility that an event occurred at an outside facility that was not reported to our institution and therefore deaths may be undercounted. Despite these limitation, we believe this study may help physician understanding the significance of NRBCs in pediatric patients, and its potential value as a predictor of severity in pediatric patients in the ED setting.

Conclusion: The presence of circulating NRBCs is an independent risk factor for need for higher level of care and adverse clinically relevant outcomes including in-hospital and long-term mortality in pediatric patients presenting to the pediatric ED. Our data support the clinical utility of NRBCs as an adjunctive biomarker to prognose children in a busy ED environment. Future studies should look at the trajectory of NRBC counts to assess the relationship to time to and recovery from adverse outcomes.

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Figure 1A: KM analysis for first visit (NRBC-) or first visit with NRBCs. NRBC+ patients had higher mortality at 3 months, 6 months, and 1 year after presentation.

Figure 1B: Percent mortality for first visit (NRBC-) or first visit with NRBCs calculated at 30- and 90-days post ED presentation or at any point during the study period. NRBC+ patients were grouped based on number of NRBCs detected. No deaths occurred during the study period for the patients in the >1000 NRBC group (N=44).