The Presence of Nucleated Red Blood Cells as an Indicator for Increased Mortality and Morbidity in Burn Patients

Phillip M Jenkins, DO,* Fadi Al Daoud, MD,* Leo Mercer, MD,* Donald Scholten, MD,* Kristoffer Wong, DO,* Vinu Perinjelil, MD,* Karl Majeske, PhD,† James Cranford, PhD,* Ghaith Elian, BS,‡ Tina Nigam, BS,‡ Chase A. Carto, BS,‡ and Gul R. Sachwani-Daswani, DO*,*

Nucleated red blood cells (NRBCs) have been studied in critically ill and injured patients as a predictor of increased in-hospital mortality and poor clinical outcomes. While prior studies have demonstrated the prognostic power of NRBCs in the critical patient, there has been a paucity of literature available describing their value as a prognostic indicator in the severely burned patient. This retrospective observational study was conducted from 2012 to 2017. Inclusion criteria for this study included all burn patients with total body surface area > 10% who were aged ≥ 15 years. Demographic and clinical data were collected from the electronic medical record system. Data analysis consisted of descriptive and comparative analysis using SPSS. Two hundred and nineteen patients (17.5%) met inclusion criteria with 51 (23.3%) patients positive for NRBCs. The presence of NRBCs had an increased mortality rate with an odds ratio of 6.0 (P = .001; 2.5, 14.5); was more likely to appear in older patients (P < .001); and was associated with increased hospital length of stay (P < .001), injury severity scores (P < .001), and complications. The presence of NRBCs even at the low concentrations reported in our study showed a 6-fold increase in the rate of mortality. With the current improvements in burn care leading to higher survival rates, the need to improve upon the numerous models that have been developed to predict mortality in severe burn patients is clear given the significantly increased risk of death that the presence of NRBCs portends.

METHODS

In this retrospective observational study (2012–2017), we evaluated the prognostic value that the presence of NRBCs had on in-hospital mortality in burn patients compared with those who remained NRBC negative throughout their hospital stay. The study was conducted at our American College of Surgeons (ACS) verified Level 1 Adult and Level 2 Pediatric Trauma Center. All patients included were admitted to our burn unit.

Data Collection

Following Institutional Review Board approval, we collected data for patients sustaining ≥ 10% TBSA from our trauma
registry and patient electronic medical record (EMR) system. Inclusion criteria for this study included all burns patients with TBSA > 10% and patients aged ≥ 15 years. Data collected from the EMR included age, gender, race, TBSA, degree of burn, injury severity score (ISS), hospital length of stay (LOS), complications and comorbidity, mechanism of injury, and management. Siemens A2120I blood analyzer (BA) was used to detect the presence of NRBCs in the peripheral blood of patients. A manual verification via peripheral blood smear was conducted to confirm the presence of NRBCs flagged by the automated BA and was reported as the number of NRBCs/100 white blood cells (WBCs). NRBCs were measured from the day of admission until resolution or discharge. We also evaluated complete blood count and lactate values.

**Statistical Analysis**

Statistical analyses were conducted using SPSS (Version 27.0; IBM Corporation, Armonk, New York). For quantitative univariables, a two-sample t-test was used for comparing groups of patients on continuous variables. Fisher’s Exact test was used for comparing two groups of patients binary variables with the Chi-Squared test used for three or more groups of patients. Statistical tests were deemed significant for P-values less than .05.

**RESULTS**

A total of 1249 patients were admitted for burns from 2012 to 2017. Of those, 219 patients (17.5%) met inclusion criteria with TBSA > 10% and age ≥ 15 years, and the presence of NRBCs was detected in 51 patients (23.3%) (Figure 1). Patient demographics and clinical characteristics are presented in Table 1. The majority of patients were white (83.1%), male (79.5%), and presented with thermal injuries (90.0%). The mean ISS was 11.1 (±9.9) with an average hospital LOS of 17.3 (±22.6) days. For comorbidities, only atrial fibrillation and congestive heart failure were found to have statistical significance (P-value: .012 and .011, respectively). The distribution of %TBSA was as follows: 10% to 19% (n = 123; 56.2%), 20% to 30% (n = 43; 19.6%), and >30% (n = 53; 24.2%). The majority of patients sustained second- and third-degree burns (n = 205; 95%). One hundred and fifteen (52.5%) required surgical debridement and skin grafting. The remaining patients were treated with local debridement and local wound care.

Bivariate analyses based on the presence of NRBCs are reported in Tables 2 and 3. Patients with positive NRBCs were older (52.9 ± 16 vs 43.7 ± 17.9; P = .001), had a higher ISS (18.9 ± 12 vs 8.7 ± 11.2; P < .001), and also had a longer hospital stay (39.8 ± 39.6 vs 10.1 ± 9.2; P < .001). Thermal injuries were the most common mechanism of injury noted for both groups (NRBC+ n = 50 and NRBC- n = 147). There was a larger number of NRBC+ patients with a TBSA >30% when compared with NRBC- patients, respectively (n = 32, 64.7%; n = 20, 11.9%). In both groups, there was an equivalent proportion of patients sustaining second- and third-degree burns. Patients with circulating NRBCs had a higher mortality rate when compared with patients who did not have NRBCs (n = 14/51, 27.5% vs n = 10/168, 6%; P < .001). Patients who developed acute respiratory distress syndrome (ARDS; n = 10, 19.6% vs n = 3, 1.8%; P < .001), burn-induced anemia (mean hemoglobin value 10.1 mg/dL ± 1.6 vs 13.2 mg/dL ± 2; P < .001), pneumonia (n = 10, 19.6% vs n = 3, 1.8%; P < .001), and UTI (n = 9, 17.7% vs n = 2; 1.2%; P < .001) were noted to be to have a higher incidence of circulating NRBCs.

For the NRBC-positive patients, we compared the NRBC profile and other laboratory values between survivors and deceased patients as presented in Table 4. The mean NRBC count for deceased patients was statistically higher than those who survived (6 ± 8.5 vs 1.4 ± 0.6; P = .002). Patients who died had an earlier appearance of NRBC when compared with patients who survived. While not statistically significant, the days to appearance of NRBCs were on average 5.2 (±3.4) days for deceased patients vs 9.1 (±7.9) days for survivors (P-value: .080) with days to resolution at 15.8 (±26.8) days for deceased patients vs 23.0 (±17.0) days for survivors (P-value: .255). Patients who had NRBCs that survived had a lower NRBC count (1.4 ± 0.6), and the first NRBCs were detected later in their admission and resolved by day 23. NRBC count is reported as the mean concentration over the hospital course. No statistical significance was found between the groups when considering WBC, hemoglobin, and hematocrit lab values; however, lactate with an average of 4.5 (±4.7) for deceased patients vs 1.8 (±0.5) for survivors and platelets at 171.0 (±70.6) vs 334.6 (±115.5) were significant (P-values: .001 and <.001, respectively).

**DISCUSSION**

In this single-center retrospective study, we investigated the value of monitoring NRBCs as a marker for monitoring critically ill burned patients. Our study demonstrated that the presence of circulating NRBCs in severely burned patients is associated with a higher mortality rate. This is consistent with previously published studies stating that circulating NRBCs can serve as a prognostic indicator for increased mortality in critically burned patients.2,11,12 Despite low concentrations of

---

Figure 1. Study outline describing the total number of burn admits during the study period and those meeting inclusion criteria grouped by the presence of nucleated red blood cells (NRBCs).
NRBCs, our study revealed that the presence of NRBCs in the peripheral blood is associated with a 6-fold increased risk of death.

While the mechanism of NRBC release into the peripheral blood is not clearly understood, studies looking at the presence of NRBCs in critically ill patients suggest that hypoxia and inflammation are the key driving factors.\(^1\)\(^-\)\(^6\), \(^9\), \(^10\), \(^13\) Previously, it was reported that 90% of NRBC-positive burn patients died of sepsis compared with 54% of NRBC-negative patients indicating a strong association with systemic inflammation.\(^6\)

Also, severe hypoxemia (and inflammation) has been reported as a leading cause for the presence of NRBCs, and, even for patients with overt signs of severe disease, shock, ARDS, or severe trauma, NRBCs may be the only strong signal for disease severity.\(^10\), \(^14\) While not statistically different (P = .08), the earlier appearance of NRBCs was linked to higher mortality and higher NRBC counts. Interestingly, we found that the following clinical characteristics (age, severity of injury, degree of burn, and TBSA), comorbidities (atrial fibrillation, chronic heart failure, and hypertension), and complications (ARDS, cardiopulmonary resuscitation performed during admission, clostridium difficile colitis, deep vein thrombus/pulmonary embolism, burn-induced anemia, pneumonia, sepsis, and urinary tract infections [UTIs]) leading to increased oxygen demand and subsequent hypoxemia had a higher rate of NRBC occurrence and the amount of NRBCs present. The anemia of critical illness especially when related to burn injuries is extremely complex. Some data suggest that erythropoiesis in the bone marrow is dampened after a burn injury, leading to a decrease in the overall erythrocyte production.\(^15\), \(^16\) Given the disruption in the native mechanism of red blood cell proliferation, NRBCs (erythroblasts) begin to appear in the circulating blood. Consistent with the literature, our study demonstrates that patients with a significant reduction in hemoglobin due to the burn injury were more likely to have circulating NRBCs present when compared with patients without signs of anemia (10.1 ± 1.6 vs 13.2 ± 2, P < .001). Also, patients developing ARDS after sustaining burn injury had a higher incidence of circulating NRBCs (ARDS/NRBC+: 10 [19.6%] vs ARDS/NRBC−: 3 [1.8%], P < .001). The overall incidence of ARDS patients with circulating NRBCs was 19.6%. However, from the 14 NRBC-positive patients who died, 71% developed ARDS.

As previously mentioned, severe burn injuries are highly inflammatory disease states and depending on the presence of inhalation injuries potentially include significant hypoxia. This leads to a prolonged metabolically heightened state with increased adrenergic activity, inflammatory stress, metabolic derangement, and loss of lean body mass that may be present post-injury up to and beyond 2 years. This hypermetabolic state complicates delivering the necessary energy and nutritional requirements leading to cachexia and an increase in whole body oxygen consumption compounding any hypoxic injuries that are present.\(^8\) The leading cause of death in burn patients is multiorgan failure and burn sepsis.\(^7\), \(^8\), \(^17\), \(^18\) As such, NRBCs should be considered as a surrogate marker for complications following moderate to severe burns that increase the inflammatory and hypoxic burden placed on the patient. A high index of suspicion for the development of sepsis, ARDS, acute kidney injury, pneumonia, UTI, and post-burn anemia should be investigated early during the hospital course.

### Table 1. Patient demographics and clinical characteristics

|                | 2012–2017 |
|----------------|-----------|
| Adult burn admits | 942       |
| Study sample     | 219 (17.5%) |
| Age             | mean (±SD) \(45.6 (±17.9)\) |
| Gender          | M 174 (79.5%) |
|                | F 45 (20.5%) |
| Race/ethnicity  | W 182 (83.1%) |
|                | AA 23 (10.5%) |
|                | Other 14 (6.4%) |
| Comorbidities   | AFib 3 (1.4%) |
|                | CHF 5 (2.3%) |
|                | COPD 23 (10.5%) |
|                | DM 21 (9.6%) |
|                | HTN 44 (20.1%) |
|                | Obesity 8 (3.7%) |
|                | Smoking 96 (43.8%) |
|                | Other 47 (21.5%) |
| ISS             | Mean (±SD) \(11.1 (±9.9)\) |
| Hospital LOS    | Mean (±SD) \(17.3 (±22.6)\) |
| MOI            | Thermal 197 (90.0%) |
|                | Chemical 21 (9.6%) |
|                | Electrical 1 (0.5%) |
| TBSA           | 10–19% 123 (56.2%) |
|                | 20–30% 43 (19.6%) |
|                | ≥30% 53 (24.2%) |
| Degree of burn  | 2/3 208 (95.0%) |
|                | 1/2/3 11 (5.0%) |
| Management      | LD + WC 96 (43.8%) |
|                | SD + Grafting 115 (52.5%) |
|                | None 8 (3.7%) |

Abbreviations: AA, African American; AFib, atrial fibrillation; CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HTN, hypertension; ISS, injury severity score; LOS, length of stay; LD, local debridement; MOI, mechanism of injury; W, white; WC, wound care.

### Table 2. Bivariate analyses evaluating the impact of nucleated red blood cells (NRBCs) in burn patients

|                | NRBC+ | NRBC− | P-value |
|----------------|-------|-------|---------|
| Study sample   | 51 (23.3%) | 168 (76.7%) |         |
| Age            | mean (±SD) \(52.9 (±16.0)\) | \(43.7 (±17.9)\) | .001 |
| ISS            | mean (±SD) \(18.9 (±12.1)\) | \(8.7 (±11.2)\) | <.001 |
| Hospital LOS   | mean (±SD) \(39.8 (±39.6)\) | \(10.1 (±9.2)\) | <.001 |
| Mortality      | Thermal 14 (27.5%) | 10 (6.0%) | <.001 |
|                | Chemical 1 (2.0%) | 20 (11.9%) |       |
|                | Electrical 0 | 1 (0.6%) |       |
| TBSA           | 10–19.9% 9 (17.7%) | 114 (67.9%) | <.001* |
|                | 20–29.9% 9 (17.7%) | 34 (20.2%) |       |
|                | ≥30% 32 (64.7%) | 20 (11.9%) |       |
| Degree of burns| 2/3 48 (94.1%) | 160 (95.2%) | .753* |
|                | 1/2/3 3 (5.9%) | 4 (4.8%) |       |

Abbreviations: ISS, injury severity score; LOS, length of stay; MOI, mechanism of injury.

*Chi-square analysis.
Complications

| ACS   | 1 (2.0%) | 2 (1.2%) | .551 |
|-------|----------|----------|------|
| AKI   | 1 (2.0%) | 0        | .233 |
| ARDS  | 10 (19.6%) | 3 (1.8%) | <.001 |
| CPR performed | 3 (5.9%) | 1 (0.6%) | .01 |
| C-diff | 2 (3.9%) | 0        | .01 |
| DVT   | 3 (5.9%) | 2 (1.2%) | .049 |
| Burn-induced anemia* | 10.1 (±1.6) | 13.2 (±2.0) | <.001 |

Abbreviations: HTC, hematocrit; WBC, white blood cell.

**Table 3. Mortality and morbidity in burn patients with nucleated red blood cells (NRBCs)**

| NRBC+ | NRBC− | P-value |
|-------|-------|---------|
| Mortality | 14 (27.5%) | 10 (6.0%) | <.001 |
| Complications | | | |
| ACS | 1 (2.0%) | 2 (1.2%) | .551 |
| AKI | 1 (2.0%) | 0 | .233 |
| ARDS | 10 (19.6%) | 3 (1.8%) | <.001 |
| CPR performed | 3 (5.9%) | 1 (0.6%) | .01 |
| C-diff | 2 (3.9%) | 0 | .01 |
| DVT | 3 (5.9%) | 2 (1.2%) | .049 |
| Burn-induced anemia* | 10.1 (±1.6) | 13.2 (±2.0) | <.001 |

Abbreviations: AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; C-diff, clostridium difficile colitis; CPR, cardiopulmonary resuscitation; DVT, deep vein thrombus; UTI, urinary tract infections.

*Reported as mean hemoglobin (mg/dL).

**Table 4. Nucleated red blood cells (NRBC) profile and other lab profiles for NRBC-positive survivors vs deceased**

| Deceased | Survivor | P-value |
|----------|----------|---------|
| NRBC positive | | |
| NRBC count Mean (±SD) | 6.0 (±8.5) | 1.4 (±0.6) | .002 |
| Days to appearance Mean (±SD) | 5.2 (±3.4) | 9.1 (±7.9) | .08 |
| Days to resolution Mean (±SD) | 15.8 (±26.8) | 23.0 (±17.0) | .255 |
| Other lab profiles | | |
| WBC | 11.8 (±5.8) | 11.4 (±5.5) | .75 |
| Hemoglobin | 10.6 (±2.0) | 10.0 (±1.4) | .27 |
| HTC | 32.7 (±6.0) | 31.0 (±4.2) | .246 |
| Lactate | 4.5 (±4.7) | 1.8 (±0.5) | .001 |
| Platelets | 171.0 (±70.6) | 334.6 (±115.5) | <.001 |

Abbreviations: HTC, hematocrit; WBC, white blood cell.

The clinical relevance of NRBCs as an indicator for in-hospital mortality is well established, but it is unclear how this information can improve poorer outcomes. NRBCs have been shown to be independent of established risk models such as acute physiology and chronic health evaluation (APACHE II) and simplified acute physiology score (SAPS II) meaning that making adjustments to these scores for the level of NRBCs led to improved prediction of outcome. It should be noted that the presence of NRBCs is typically delayed by several days after admission, but previous studies showed that with increasing APACHE II and SAPS II scores, there was an increase in the concentration of NRBCs present. While the early predictive ability of these scoring systems does not directly benefit from screening for the presence of NRBCs that typically appear ≥ 5 days post-injury, the presence of NRBCs combined with these scoring systems should prompt focused investigation into burn-related complications and/or intervention before those complications become clinically apparent. As stated in the literature, NRBCs may be the only indication that a complication is present. Future prospective studies to determine if accounting for NRBCs in the revised Baux score, Ryan, Smith, McGwin, Abbreviated Burn Severity Index (ABSI), Belgian Outcome of Burn Injury (BOBI), and the Fatality by Longevity, APACHE II, Measured Extent of burn, and Sex score (FLAMES) may improve the predictability of outcomes, inform interventions, and more accurately predict the point of futility in treatment.

**CONCLUSIONS**

Even though our study is limited by sample size and being that it is a single-center retrospective study, our study demonstrates that circulating NRBCs can be used as a potential marker for critical illness in burn patients. NRBCs can be detected as early as 5 days after admission. Early detection and identification of NRBCs in the circulating blood of patients should trigger burn clinicians to aggressively workup and treat conditions such as anemia, sepsis, and respiratory conditions (ARDS and pneumonia). Future prospective studies at our institution will seek to incorporate NRBCs into the various tools available in predicting the outcomes for severely burned patients.

**REFERENCES**

1. Kuert S, Holland-Letz T, Friese J, Stachon A. Association of nucleated red blood cells in blood and arterial oxygen partial tension. Clin Chem Lab Med 2011;49:257–63.
2. Stachon A, Becker A, Kempf R, Holland-Letz T, Friese J, Krieg M. Re-evaluation of established risk scores by measurement of nucleated red blood cells in blood of surgical intensive care patients. J Trauma 2008;65:666–73.
3. 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 2007;11:R62.
4. Stachon A, Bolulu O, Holland-Letz T, Krieg M. Association between nucleated red blood cells in blood and the levels of cytokines, interleukin 3, interleukin 6, and interleukin 12p70. Shock 2005;24:34–9.
5. Andes WR. Normoblastemia after thermal injury. Am J Surg 1976;131:725–6.
6. Lehnhardt M, Katzy Y, Langer S, et al. Prognostic significance of erythroblasts in burns. Plast Reconstr Surg 2005;115:120–7.
7. Tejiram S, Romanowski KS, Palmieri TL. Initial management of severe burn injury. Curr Opin Crit Care 2019;25:647–52.
8. Porter C, Tompkins RG, Finnerty CC, Sidossis LS, Suman OE, Herndon DN. The metabolic stress response to burn trauma: current understanding and therapies. Lancet 2016;388:1417–26.
9. Monteiro Júnior JG, Torres Dde O, da Silva MC, et al. Nucleated red blood cells as predictors of all-cause mortality in cardiac intensive care unit patients: a prospective cohort study. PLoS One 2015;10:e0144259.
10. Menk M, Giebelhäuser L, Vorderwülbecke G, 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;8:42.
11. Stachon A, Holland-Letz T, Kempf R, Becker A, Fries J, Krieg M. Poor prognosis indicated by nucleated red blood cells in peripheral blood is not associated with organ failure of the liver or kidney. Clin Chem Lab Med 2006;44:955–61.
12. Stachon A, Sondermann N, Imoh M, Krieg M. Nucleated red blood cells indicate high risk of in-hospital mortality. J Lab Clin Med 2002;140:497–12.
13. May JE, Marques MB, Reddy VVB, Gangaraju R. Three neglected numbers in the CBC: the RDW, MPV, and NRBC count. Cleve J Med 2019;86:167–72.
14. Kho AN, Hui S, Kesterson JG, McDonald CJ. Which observations from the complete blood cell count predict mortality for hospitalized patients? J Hosp Med 2007;2(1):5–12.
15. Johnson NB, Poslusny JA, He LK, et al. Perturbed MafB/GATA1 axis after burn trauma bares the potential mechanism for immune suppression and anemia of critical illness. J Leukoc Biol 2016;100:725–36.
16. Williams KN, Srilagyi A, Conrad P, et al. Peripheral blood mononuclear cell-derived erythroid progenitors and erythroblasts are decreased in burn patients. J Burn Care Res 2013;34:133–41.
17. Zuo KJ, Medina A, Tredget EE. Important developments in burn care. Plast Reconstr Surg 2017;139:120e–38e.
18. ISBI Practice Guidelines Committee; Steering Subcommittee; Advisory Subcommittee. ISBI practice guidelines for burn care. Burns 2016;42:953–1021.
19. Stachon A, Kempf R, Holland-Letz T, Fries J, Becker A, Krieg M. Daily monitoring of nucleated red blood cells in the blood of surgical intensive care patients. Clin Chim Acta 2006;366:329–35.
20. Shah R, Reddy S, Horst HM, Stassinopoulos J, Jordan J, Rubinfeld I. Getting back to zero with nucleated red blood cells: following trends is not necessarily a bad thing. Am J Surg 2012;203:343–46.
21. 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 (Larchmt) 2012;13:360–5.
22. Halgas B, Bay C, Foster K. A comparison of injury scoring systems in predicting burn mortality. Ann Burns Fire Disasters 2018;31:89–93.