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RESEARCH ARTICLE

Comparison between the prognostic value of the white blood cell differential count and morphological parameters of neutrophils and lymphocytes in severely injured patients for 7-day in-hospital mortality

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Abstract

Context: Current laboratory parameters provide limited information about the prognosis of severely injured patients; therefore, novel laboratory parameters are needed.

Objective: We investigated the prognostic value of morphological parameters of neutrophils and lymphocytes in severely injured patients ($n = 477$).

Materials and methods: We compared the morphological parameters of neutrophils and lymphocytes, and white blood cell (WBC) differential count between survivors and nonsurvivors within 7 days after admission. Multiple logistic regression analysis was conducted to identify independent prognostic factors for 7-day in-hospital mortality.

Results: Neutrophil cell size was significantly different between survivors and nonsurvivors ($p = 0.04$), whereas WBC count and differential were not significantly different. Multiple logistic regression showed that neutrophil cell size was a significant predictor of poor outcome.

Conclusions: Neutrophil cell size at admission is a prognostic factor for 7-day in-hospital mortality in severely injured trauma patients, whereas conventional WBC count and differential have no prognostic value.

Keywords: Trauma, neutrophil morphology, hematology, prognosis

Introduction

Early identification of trauma patients with poor outcome remains a clinical challenge. The mortality rate of severely injured trauma patients can be up to 17% (Probst et al. 2009), thus this specific group of patients will mostly benefit from early prognostic biomarkers that can be used in routine clinical practice.

Among laboratory parameters, white blood cell (WBC) count and differential have been well studied in trauma patients. A marked increase of WBC count and its subpopulations, especially neutrophil granulocytes, have been noted after trauma. Several investigations have studied the prognostic value of the WBC count at admission or serial WBC counts (Rainer et al. 1999; Chang et al. 2003; Lam et al. 2011). However, these results often lack prognostic value.

In response to tissue damage due to trauma, neutrophil granulocytes become activated and this may lead to a dysfunctional immune response. The overwhelming immune response is considered to be a major risk factor in the development of the systemic inflammatory response syndrome (SIRS) and subsequent posttraumatic organ failure (Pillay et al. 2007). Rapid and reliable recognition of activated neutrophils could therefore serve as valuable prognostic biomarker to identify trauma patients with poor risk.

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In addition to WBC count and differential, recent advances in fully automated hematology analyzers enable the rapid study of detailed morphological parameters related to WBCs. These parameters are used by hematology analyzers to generate the WBC differential count and may allow analysis of morphological characteristics of large numbers of WBCs in one run. The potential of these novel parameters has been shown in diagnosis and outcome prediction of various clinical conditions (Leckie et al. 2000; Leckie et al. 2004; Chaves et al. 2005; Chaves et al. 2006; Silva et al. 2006; Bagdasaryan et al. 2007; Velthove et al. 2009; Campuzano-Zuluaga et al. 2010; Furundarena et al. 2010; Lee et al. 2010; Mardi et al. 2010; Charafeddine et al. 2011; Inaba et al. 2011; Celik et al. 2012; Zhu et al. 2012). The goal of this study was to assess whether WBC morphological parameters could be used as potential predictors of 7-day in-hospital mortality after severe trauma. Furthermore, WBC count and WBC differential count were compared as outcome predictor in these severely injured trauma patients.

Methods

Study population

From a prospectively collected trauma register, we evaluated all severely injured trauma patients aged 18 years or older, who required clinical admission to the University Medical Center Utrecht (UMC Utrecht) from 1 January 2005 to 31 December 2007. Severe trauma was defined as injury severity score (ISS) > 15 (Baker et al. 1974; Copes et al. 1988). The following clinical data were obtained: demographic information, trauma mechanism, ISS, total hospital stay, intensive care unit (ICU) stay and 7-day in-hospital mortality rate. Missing data were retrieved from the hospital's main frame if possible. The collection of clinical data was performed by a medical coding specialist. The study population described in this paper is a subset of the population that has been described in one of our previous papers (Lam et al. 2011). An institutional review board approval for this retrospective database study was not required.

WBC differential count and morphological data

As part of the trauma admitting protocol, blood was drawn from all patients within 1 h after arrival at the emergency department. The results of laboratory tests were obtained from the Utrecht Patient Oriented Database (UPOD). The technical details of UPOD are described elsewhere (ten Berg et al. 2007). In short, UPOD is an infrastructure of relational databases that allows (semi)automated transfer, processing and storage of data including administrative information, medical and surgical procedures, medication orders and laboratory test results for all clinically admitted patients and patients attending the outpatient clinic of the UMC Utrecht since 2004. The process and storage of data are in accordance with privacy and ethics regulations.

Routine hematological analysis was performed by using the Cell-Dyn Sapphire hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA). This analyzer is equipped with an integrated 488-nm blue diode laser and uses spectrophotometry, electrical impedance, laser light scattering (multiangle polarized scatter separation (MAPSS)), and three-color fluorescent technologies (three detectors at 90°: FL-1 (530 ± 30 nm), FL-2 (580 ± 30 nm) and FL-3 (630 ± 30 nm)) to measure morphological parameters of WBC for WBC classification and enumeration. The morphological parameters entail the following five optical scatter signals: cell size (0° scatter, axial light loss (ALL)), cell complexity and granularity (7° scatter, intermediate angle scatter (IAS)), nuclear lobularity (90° scatter, polarized side scatter (PSS)), depolarization (90° depolarized side scatter (DSS)) and viability (red fluorescence (FL-3), 630 ± 30 nm) (Müller et al. 2006; Kang et al. 2008; Groeneveld et al. 2012). The morphological parameters were reported as mean arbitrary unit.

The results of WBC count included are as follows: total WBC count, absolute neutrophil count (ANC), neutrophil band count, immature neutrophil (IG) count, lymphocyte count, monocyte count, eosinophil count and basophil count. All parameters are stored within the UPOD database. The reliability and validity of the laboratory results are monitored through routine quality control.

Statistical analysis

For comparison between groups, Pearson's χ^2 test and Mann–Whitney *U* test were used as appropriate. Comparisons between survivors and nonsurvivors within 7 days after admission for WBC count, WBC differential count and morphological parameters of neutrophils and lymphocytes were made. The IG count was dichotomized as presence and absence. The neutrophil band count was also dichotomized to normal ($<0.6 \times 10^9$ cells/L) and abnormal ($\geq 0.6 \times 10^9$ cells/L) according to the reference interval used in our institution. Significant variables from the univariate analysis were selected for further analysis.

Multiple logistic regression analysis was performed to identify prognostic factors for the 7-day in-hospital mortality while controlling for potential confounders (age, gender and dichotomized ISS). All results were expressed as median and its interquartile range (IQR), actual value or percentage. Statistical significance was defined as two-sided p value < 0.05. Statistical analyses were carried out using SPSS version 15 (SPSS Inc, Chicago, IL, USA).

Results

A total of 477 severely injured trauma patients were identified in the 3-year study period (Table 1). There were 319 male and 158 female patients with a median age of 49 years (IQR: 31–66). Blunt trauma was the most common trauma mechanism (98%). The overall 7-day in-hospital mortality rate was 16.1% (n = 77). Complete data were available for all variables.

Table 1. Patient's demographics.

	Study population (n = 477)	Survivors (n = 400)	Nonsurvivors (n = 77)	p value[a]
Age	49 (31–66)	48 (29–63)	59 (39–75)	0.002
Gender (M/F)	319/158	132/268	26/51	0.90
ISS	25 (20–33)	25 (19–29)	32 (25–42)	<0.001
ICU (days)	1 (0–9)	1 (0–12)	1 (0–2)	0.12
LOS (days)	12 (5-33)	16 (8–38)	2 (1–3)	<0.001

The results are expressed as median and IQR or actual numbers.
ICU, intensive care unit; IQR, interquartile range; ISS, injury severity score; LOS, length of hospital stay.
[a]Comparison between survivor and nonsurvivors.

As shown in Table 1, nonsurvivors were significantly older and sustained more severe injury compared with survivors. There were no significant differences between admission WBC count and differential in survivors and nonsurvivors. However, among the WBC morphological parameters, neutrophil cell size was significantly different between groups (p = 0.04; Table 2). A significant increase in neutrophil cell size was seen in nonsurvivors.

Given that the 7-day in-hospital mortality rates between severe (ISS = 16–24) and critically injured (ISS > 24) trauma patients were substantially different (5.7 versus 23.2%; $p < 0.001$), we sought to examine whether WBC morphological parameters are useful in this specific subgroup. Indeed, in the group of critically trauma patients (n = 284), comparison between survivor and nonsurvivors also showed significant increase in neutrophil cell size (p = 0.03; Table 3). No significant differences were found in WBC count and differential.

When adjusting for age, gender and injury severity, multiple logistic regression analysis showed that neutrophil cell size (as continuous variable) was independently associated with 7-day in-hospital mortality for the study population (p = 0.04; Table 4). The significant association of neutrophil cell size with 7-day in-hospital mortality was also present in critically injured trauma patients (p = 0.025; Table 5).

Discussion

There was a significant difference in neutrophil cell size at admission between survivors and nonsurvivors of severe trauma. In addition, neutrophil cell size was significantly associated with 7-day in-hospital mortality, independent of age, gender and injury severity. On the other hand, admission WBC count and differential did not show any prognostic value. To our knowledge, this is the first study that investigates the prognostic value of morphological parameters of WBC, as provided by modern hematology analyzers, in severely injured trauma patients.

The relation between elevated WBC count and increasing ISS, as a measurement of sustained anatomical injuries, has been reported previously by many authors (Chang et al. 2003). In addition, an increasing ISS was generally associated with higher mortality (Baker et al. 1974). This observation has initiated numerous attempts to explore the utility of WBC count to predict the overall outcome in trauma patients. Previously, Malone et al. tried to determine the predictive ability of each components needed to calculate the SIRS score (Malone et al. 2001). Among the four SIRS components (fever or hypothermia, tachycardia, tachypnea, abnormal WBC count defined as ANC $>12 \times 10^9$ cells/L), only hypothermia was associated with mortality in 9539 trauma patients included for analysis. In our previous study of a heterogeneous group of 1673 adult trauma patients, we demonstrated that the admission WBC count and differential were not independently associated with short-term mortality (Lam et al. 2011). The results were consistent with numerous studies (Malone et al. 2001; Chang et al. 2003; Keller et al. 2004; Swenson et al. 2007), showing that admission WBC count and WBC differential count did not have any prognostic value for in-hospital mortality in trauma patients. Using a subset of trauma population with severe injury, the prognostic value of the WBC count and WBC differential count remained limited in this study. Other studies have investigated the WBC count in more defined trauma population, that is, head injury (Keskil et al. 1994; Rovlias & Kotsou, 2001), pediatric trauma patients (Keller et al. 2004), surgical patients with blood stream infection (Swenson et al. 2007), also these studies have been unsuccessful to demonstrate clinically significant results.

A significant increase in neutrophil cell size was observed in nonsurvivors after severe trauma. As shown previously, IGs are released into the peripheral circulation as a result of inflammatory reaction induced by trauma (Köller et al. 2001). This release of "younger" neutrophils with distinct morphological appearance, that is, large cell size can be detected by hematology analyzer. Although, in the current study, abnormal neutrophil band count and IG count were detected in survivors and nonsurvivors, the differences were not significant between both groups. Furthermore, a larger proportion of patients in the survivor group had abnormal neutrophil band count and IG count, which is counterintuitive with the observed increased neutrophil cell size in nonsurvivors. Although, there was a significant correlation between neutrophil cell size and neutrophil band count (Spearman's correlation = 0.188; $p < 0.001$) or IG count (Spearman's correlation = 0.130; p = 0.005), the correlations are considered weak. This suggests that the increased neutrophil cell size is partially explained by the presence of younger neutrophils. Furthermore, the results suggest that not the absolute number of circulating neutrophils, but the morphological changes may be of prognostic significance in trauma patients. However, the underlying mechanism of increased neutrophil cell size and its prognostic significance need to be elucidated.

A number of studies have investigated morphological parameters in different clinical conditions (Leckie et al. 2000; Leckie et al. 2004; Chaves et al. 2005; Chaves et al. 2006; Silva et al. 2006; Bagdasaryan et al. 2007; Velthove et al. 2009; Campuzano-Zuluaga et al. 2010; Furundarena

Table 2. Comparisons of WBC count and morphological parameters between survivors and nonsurvivors within 7 days after admission.

	Survivors (n = 400)	Nonsurvivors (n = 77)	p value
WBC[a]	12.67 (9.57–16.44)	12.53 (10.21–16.42)	0.62
ANC[a]	9.44 (6.47–13.26)	9.30 (6.64–12.79)	0.54
Segmented neutrophil count[a]	9.06 (6.26–12.74)	8.80 (6.33–11.73)	0.46
Band neutrophil count ≥0.6 × 10^9 cells/L	63 (15.8%)	10 (13%)	0.54
Presence of IG[a]	43 (10.8%)	4 (5.2%)	0.13
Lymphocyte count[a]	2.01 (1.35–3.04)	2.20 (1.28–3.37)	0.79
Monocyte count[a]	0.70 (0.54–0.97)	0.66 (0.45–0.95)	0.16
Basophil count[a]	0.03 (0.02–0.06)	0.03 (0.02–0.06)	0.68
Eosinophil count[a]	0.11 (0.06–0.20)	0.09 (0.04–0.19)	0.59
Neutrophil morphological parameters			
Cell size[b]	147.88 (138.34–155.91)	150.93 (142.02–161.16)	0.04
Cell complexity[b]	131.91 (127.53–136.39)	131.90 (126.40–135.19)	0.33
Lobularity[b]	129.39 (120.82–136.46)	129.91 (118.78–137.79)	0.81
Depolarization[b]	29.32 (27.00–31.82)	30.23 (26.74–32.91)	0.27
Lymphocyte morphological parameters			
Cell size[b]	97.35 (93.85–100.55)	96.85 (92.36–100.99)	0.49
Cell complexity[b]	76.79 (74.11–79.13)	75.97 (73.99–79.61)	0.80

The results are expressed as median and IQR, actual numbers or percentage.
ANC, absolute neutrophil count; IQR, interquartile range; WBC, white blood cell.
[a]Value × 10^9 cells/L.
[b]Mean arbitrary unit.

Table 3. Subgroup analysis of critically injured patients (ISS > 24): comparisons of WBC count and morphological parameters between survivors and nonsurvivors 7 days after admission.

	Survivors (n = 218)	Nonsurvivors (n = 66)	p value
WBC[a]	12.74 (9.74–17.50)	12.54 (8.82–15.61)	0.21
ANC[a]	9.71 (6.73–13.55)	9.03 (6.16–12.03)	0.15
Segmented neutrophil count[a]	9.22 (6.45–13.19)	8.64 (6.05–11.55)	0.16
Band neutrophil count ≥0.6 × 10^9/L	40 (18.3%)	6 (9.1%)	0.07
Presence of IG[a]	23 (10.6%)	3 (4.5%)	0.14
Lymphocyte count[a]	2.10 (1.39–3.11)	2.28 (1.28–3.56)	0.86
Monocyte count[a]	0.69 (0.51–0.95)	0.63 (0.45–0.90)	0.19
Basophil count[a]	0.03 (0.02–0.06)	0.03 (0.02–0.06)	0.77
Eosinophil count[a]	0.12 (0.06–0.22)	0.09 (0.04–0.19)	0.30
Neutrophil morphological parameters			
Cell size[b]	147.77 (137.32–156.93)	152.59 (143.46–162.26)	0.03
Cell complexity[b]	131.91 (127.69–136.67)	132.10 (126.98–135.36)	0.48
Lobularity[b]	130.13 (121.97–136.26)	130.70 (119.70–138.19)	0.78
Depolarization[b]	29.45 (27.29–31.89)	30.78 (26.74-33.19)	0.35
Lymphocyte morphological parameters			
Cell size[b]	97.25 (93.74–100.20)	96.46 (90.60–101.57)	0.51
Cell complexity[b]	77.06 (74.45–79.25)	76.06 (73.40–79.61)	0.46

The results are expressed as median and IQR, actual numbers or percentage.
ANC, absolute neutrophil count; IQR, interquartile range; WBC, white blood cell.
[a]Value × 10^9 cells/L.
[b]Mean arbitrary unit.

et al. 2010; Lee et al. 2010; Mardi et al. 2010; Charafeddine et al. 2011; Inaba et al. 2011; Celik et al. 2012; Zhu et al. 2012). The results indicate great potential for diagnosing several inflammatory (Leckie et al. 2000;Velthove et al. 2009), infectious (Chaves et al. 2005; Chaves et al. 2006; Bagdasaryan et al. 2007; Campuzano-Zuluaga et al. 2010; Lee et al. 2010; Mardi et al. 2010; Charafeddine et al. 2011; Celik et al. 2012; Zhu et al. 2012), cardiac (Leckie et al. 2004), myelodysplastic (Furundarena et al. 2010; Inaba et al. 2011) and lymphoproliferative diseases (Silva et al. 2006). Chaves et al. demonstrated that mean neutrophil volume (MNV) and variability of neutrophil volume were sensitive indicators for bacteremia compared with WBC count and neutrophil percentage. Of note, neutrophils were larger in bacterial infection in comparison with controls (Chaves et al. 2005; Chaves et al. 2006). In the case–control study of Mardi et al., MNV showed comparable sensitivity and specificity as CRP for discriminating sepsis from nonsystemic infections. Furthermore, this study also demonstrated that larger neutrophils were associated with more severe infection or

Table 4. Neutrophil cell size is independently associated with in-hospital mortality after severe trauma.

	Adjusted OR[a] (95% CI)	SE	*Wald*	*p* value
WBC	0.990 (0.943–1.039)	0.025	0.178	0.67
ANC	0.983 (0.932–1.038)	0.027	0.373	0.54
Neutrophil cell size	1.020 (1.001–1.040)	0.010	4.116	0.04

Odd ratios were expressed per log unit increase in variable.
ANC, absolute neutrophil count; CI, confidence interval; OR, Odd ratio; SE: standard error; WBC, white blood cell.
[a]Adjusted for age, gender and dichotomized ISS (severe injury: ISS = 16–24 and very severe injury: ISS > 24).

Table 5. Neutrophil cell size is independently associated with in-hospital mortality in critically injured patients (ISS > 24).

	Adjusted OR[a] (95% CI)	SE	*Wald*	*p* value
WBC	0.962 (0.913–1.013)	0.026	2.203	0.14
ANC	0.952 (0.898–1.010)	0.030	2.698	0.10
Neutrophil cell size	1.024 (1.003–1.045)	0.010	5.050	0.025

Odd ratios were expressed per log unit increase in variable.
ANC, absolute neutrophil count; CI, confidence interval; OR, Odd ratio; SE: standard error; WBC, white blood cell.
[a]Adjusted for age and gender.

sepsis (Mardi et al. 2010). Although most of the morphological parameters in previous studies were generated by a different hematology analyzer than the one used in this study, these data indicate that morphological parameters of WBC are useful attributes for diagnosing or predicting several diseases. However, more studies are needed to further investigate the potential clinical application of these novel parameters.

Several limitations need to be acknowledged. Although this is a retrospective analysis of a prospective filled trauma register, the complete clinical and laboratory data were used for the analysis. Only patients with severe trauma were included. We selected this specific group of patients, because of the poor prognosis at initial presentation, the high mortality rate after admission and the difficulty to identify patients at risk. Therefore, this group of patients will most likely benefit from novel prognostic biomarkers. Finally, there are several types of automated hematology analyzers available that have the capability to provide additional morphological parameters of WBCs. Some analyzers use different technology for WBC characterization; therefore, further comparison of results should taken this difference in account.

In conclusion, increased neutrophil cell size at admission is a prognostic factor for 7-day in-hospital mortality in adult patients with severe trauma. This novel biomarker is readily available in most clinics and may aid to identify severe trauma patients at risk, but further investigation is needed to confirm the found results.

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Declaration of interest

The authors report no conflict of interest.

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