

OPEN

Point-of-Care Analysis of Neutrophil Phenotypes: A First Step Toward Immuno-Based Precision Medicine in the Trauma ICU

Roy Spijkerman, MD^{1,2}; Lillian Hesselink, MD, PhD^{1,2}; Suzanne Bongers, MD^{1,2}; Karlijn J.P. van Wessem, MD, PhD¹; Nienke Vrisekoop, PhD^{2,3}; Falco Hietbrink, MD, PhD¹; Leo Koenderman, PhD^{2,3}; Luke P.H. Leenen, MD, PhD, FACS¹

Objectives: The amount of tissue damage and the amplitude of the immune response after trauma are related to the development of infectious complications later on. Changes in the neutrophil compartment can be used as read out of the amplitude of the immune response after trauma. The study aim was to test whether 24/7 point-of-care analysis of neutrophil marker expression by automated flow cytometry can be achieved after trauma.

Design: A prospective cohort study was performed. Polytrauma patients who developed infectious complications were compared with polytrauma patients who did not develop infectious complications.

Setting: The study was performed in a level 1 trauma center.

Patients: All trauma patients presented in the trauma bay were included.

Interventions: An extra blood tube was drawn from all patients. Thereafter, a member of the trauma team placed the blood tube in the fully automated flow cytometer, which was located in the corner of the trauma room. Next, a modified and tailored protocol for this study was automatically performed.

Main Results: The trauma team was able to successfully start the point-of-care automated flow cytometry analysis in 156 of 164 patients, resulting in a 95% success rate. Polytrauma patients

who developed infectious complications had a significantly higher %CD16^{dim}/CD62L^{bright} neutrophils compared with polytrauma patients who did not develop infectious complications ($p = 0.002$). Area under the curve value for %CD16^{dim}/CD62L^{bright} neutrophils is 0.90 (0.83–0.97).

Conclusions: This study showed the feasibility of the implementation of a fully automated point-of-care flow cytometry system for the characterization of the cellular innate immune response in trauma patients. This study supports the concept that the assessment of CD16^{dim}/CD62L^{bright} neutrophils can be used for early detection of patients at risk for infectious complications. Furthermore, this can be used as first step toward immuno-based precision medicine of polytrauma patients at the ICU.

Key Words: critical care; point-of-care systems; infection; innate immunity; wounds and injuries

The inflammatory response directly after trauma is related to the development of infectious complications during hospital admission (1–4). Injury causes tissue damage, and it is assumed that the resulting production of cytokines and damage-associated molecular patterns are the fuel in this inflammatory process. However, these mediators are numerous, diverse, and many cannot be timely and adequately identified nor quantified. Neutrophils, as part of the final common pathway in inflammation, are responsive to these mediators. These cells integrate the signals into a specific cellular response (5, 6). This cellular response can then be used as a “simple” biomarker of complex systemic inflammation (4). Changes in neutrophil phenotype can be seen within minutes after trauma, and these changes are dynamic over time (3). Differential expression of neutrophil markers is thought to be a putative measurement of the amplitude of the tissue damage and the following immunologic response after

¹Department of Trauma Surgery, University Medical Center Utrecht, Utrecht, The Netherlands.

²Center for Translational Immunology (CTI), University Medical Center Utrecht, Utrecht, The Netherlands.

³Department of Respiratory Medicine, University Medical Center Utrecht, Utrecht, The Netherlands.

Copyright © 2020 The Authors. Published by Wolters Kluwer Health, Inc. on behalf of the Society of Critical Care Medicine. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Crit Care Expl 2020; 2:e0158

DOI: 10.1097/CCE.000000000000158

trauma (5–9). Furthermore, the literature suggests that neutrophil marker expression immediately after trauma has predictive value for infectious complications later on (10, 11). Early detection of patients at risk for infectious complications allows for immunobased treatment decisions after trauma such as timing and intensity of surgery (4, 12).

Until recently, analysis of neutrophil marker expression by flow cytometry was time consuming, labor intensive, and subject to many manual steps that cause data variability. These technical difficulties hamper the clinical application of neutrophil analyses early after trauma.

However, with the introduction of an easy to use, fully automated flow cytometer, it is now possible to obtain flow cytometry results point-of-care within 20 minutes (13, 14). This method enables the phenotyping of neutrophils and other immune cells that can be performed quickly as a point-of-care test by any health-care worker. Standardization and shortening of ex-vivo processing steps provide highly reproducible data (13). Furthermore, such an approach minimizes alterations of neutrophil marker expression due to ex vivo manipulation. Therefore, this method might give better and new insights into the cell's true biological features.

The study aim was to test whether 24/7 point-of-care analysis of neutrophil marker expression by automated flow cytometry can be achieved after trauma. Second, the hypothesis was tested whether shifts in neutrophil phenotype were associated with the injury severity score and infectious complications later on.

MATERIALS AND METHODS

Study Design

All trauma patients presented in the trauma bay of the emergency department of the University Medical Center Utrecht were prospectively screened for inclusion from November 26, 2018, to February 12, 2019. Exclusion criteria were as follows: 1) age less than 18 years, 2) transfer from another hospital, and 3) no diagnostic blood sampling needed. If a patient was eligible for inclusion, blood was drawn and analyzed. Informed consent was obtained in the period thereafter, details are provided in the **Supplemental Methods** (Supplemental Digital Content 1, <http://links.lww.com/CCX/A224>). The medical ethical committee of the University Medical Center Utrecht approved this study under study protocol no. 17/899/D. All procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments. The study was approved and registered online by the Central Committee on Research Involving Human Subjects in The Netherlands under protocol no. NL64100.041.17.

Study Procedure

If blood was drawn from the patient for standard-of-care diagnostic workup during resuscitation, one extra 4-mL Vacutainer sodium heparin blood tube (Becton Dickinson, Oakville, ON) was drawn specifically for this study. Thereafter, a member of the trauma team placed the blood tube in the automated AQUIOS CL “Load & Go” flow cytometer (Beckman Coulter, Miami, FL) that was located in the corner of the trauma room. Data generated by the machine were stored on the computer with a study identification code, generated by the machine.

Flow Cytometry Analysis by the Automated AQUIOS CL “Load & Go” Flow Cytometer

The AQUIOS CL combines robotic automated sample preparation with automated analysis of cells using flow cytometry (13). The details of these procedures are provided in the Supplemental Methods (Supplemental Digital Content 1, <http://links.lww.com/CCX/A224>).

For this research purpose, two customized antibody mixes were made, and both panels were tested in the presence and absence of the bacterial/mitochondrial derived stimulus formyl-methionyl-leucyl-phenylalanine (fMLF) (Sigma Aldrich, St. Louis, MO) with an end concentration of 10^{-6} M. Details of antibody mixes are provided in the Supplemental Methods (Supplemental Digital Content 1, <http://links.lww.com/CCX/A224>).

Analysis of Flow Cytometry Data

Multidimensional FLOW cytometric Orthogonal Orientation for Diagnosis (FLOOD) analysis was used to do explorative data analysis (15). For definitive analysis, the.lmd data files were exported from the AQUIOS CL and imported into FlowJo analysis software (Tree Star Inc., Ashland, OR). Neutrophils were gated based on forward scatter and side scatter. Neutrophils in the polymorphonuclear leukocytes gate were identified based on CD16 positivity, thereby excluding eosinophils. Then, neutrophil markers were analyzed in the absence (resting) and presence (activated) of fMLF.

Neutrophil phenotypes were identified by the expression of CD16 and CD62L as described in detail before (16). The markers CD16 and CD62L were present in panel 1 and panel 2 allowing for bench marking between both panels. **Online supplement figure 1** (Supplemental Digital Content 2, <http://links.lww.com/CCX/A225>) shows the gating strategy to identify neutrophil phenotypes. The average percentage of neutrophil phenotypes as determined by both panels was used. Gating strategy was checked for every individual patient by two independent researchers.

Clinical Data

Baseline characteristics and clinical outcomes of the included patients were recorded. Data were collected by the treating clinician and anonymously analyzed. Details of reported infectious complications are provided in the Supplemental Methods (Supplemental Digital Content 1, <http://links.lww.com/CCX/A224>).

Statistical Analysis

Data were analyzed with IBM SPSS version 23 (IBM Corporation, North Castle, NY) and GraphPad Prism Version 7 (GraphPad Software Inc., San Diego, CA). Statistical significance was defined as a *p* value of less than 0.05. Data are presented as median with interquartile range. Clinical outcomes and demographics were compared between polytrauma patients developing infectious complications and those who did not. To test whether the %CD16^{dim}/CD62L^{bright} neutrophils could be univariate predictors for infectious complications in trauma patients, receiver operating characteristic curves were calculated.

RESULTS

Study Overview

In the period from November 26, 2018, to February 2, 2019, a total of 233 patients were presented in the trauma bay of our emergency

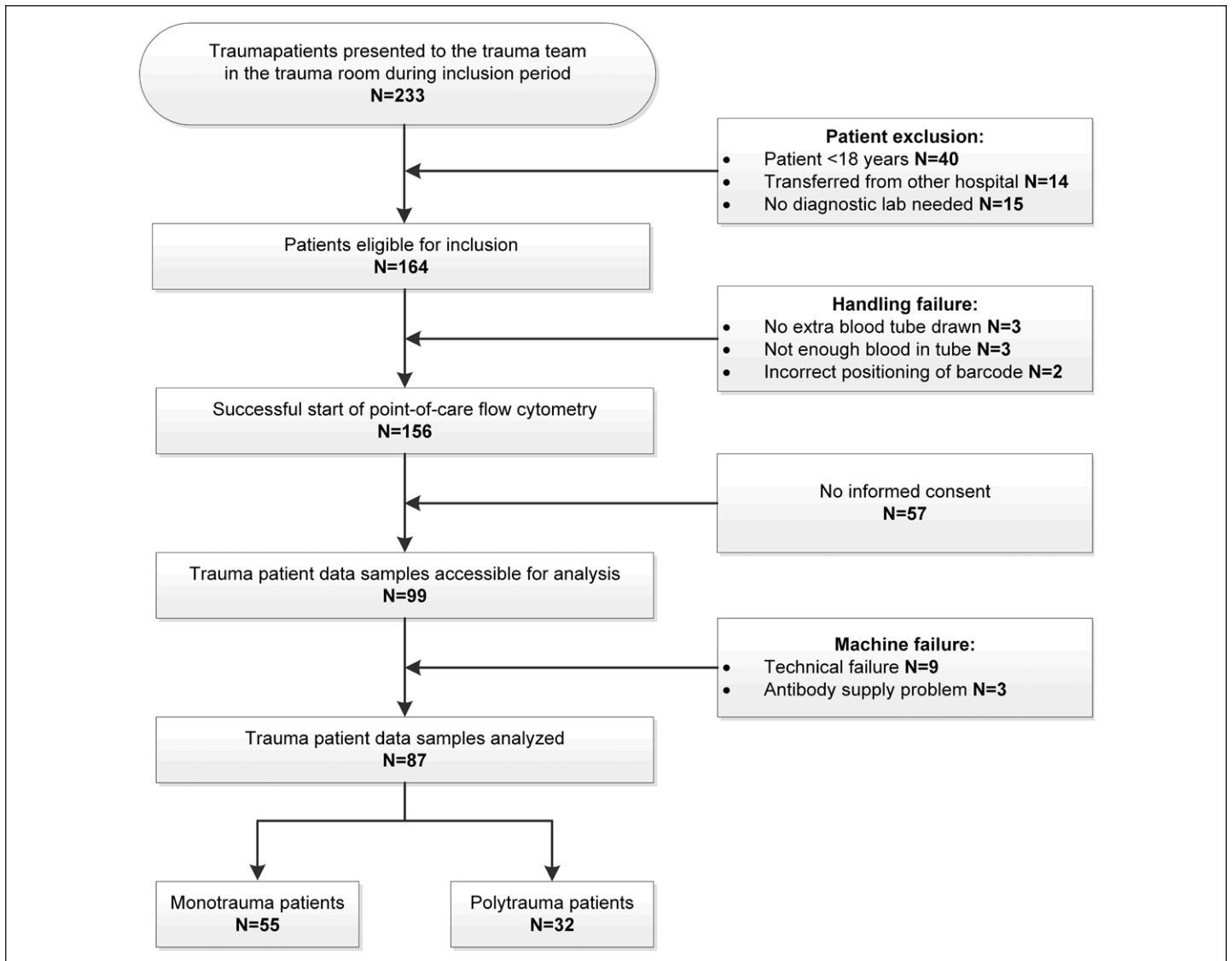


Figure 1. Flowchart of patient inclusion.

department that led to the activation of the whole trauma team. The following patients were excluded from our study (**Fig. 1**): patients less than 18 years ($n = 40$), patients who did not need standard diagnostic blood drawing ($n = 15$), and patients who were transferred from another hospital ($n = 14$). This resulted in a total of 164 patients who were eligible for inclusion. In three patients, the trauma team did not succeed to obtain an extra tube of blood; the blood tube was incorrectly placed in the machine in two cases, and too little blood was drawn in the tubes in three cases. Thus, the trauma team was able to successfully start the point-of-care flow cytometry analysis in 156 of 164 patients (95%). A total of 57 of 156 patients (37%) did not give informed consent to use their clinical and/or flow cytometry data. This finally resulted in a total of 87 patients who were included for further analysis of neutrophil markers.

Baseline Characteristics

Of these 87 patients, 32 patients sustained severe injuries (Injury Severity Score [ISS] ≥ 16), and 55 patients had isolated injuries

(ISS < 16). During hospital admission, a third of the polytrauma patients (11/32; 34%) developed infectious complications. Baseline characteristics are shown in **Table 1**. Only three of 55 monotrauma patients (5%) developed infectious complications. Polytrauma patients who developed infectious complications had a lower Glasgow Coma Scale (10 [3–14] vs 14 [11–15]; $p = 0.009$) and a lower systolic blood pressure (113 [90–130] vs 140 [119–155] mm Hg, $p = 0.015$) upon trauma bay presentation. This was associated with a significantly higher ISS (29 [22–34] vs 19 [17–23]; $p = 0.002$) and new ISS (41 [27–48] vs 22 [22–27]; $p = 0.004$). These patients also had a significantly longer ICU stay (9 [7–21] vs 1 d [0–3 d]; $p < 0.001$) and total hospital stay (33 [18–47] vs 7 d [3–11 d]; $p < 0.001$).

Baseline Neutrophil Activation

Counterintuitively and in contrast with the current literature (3, 11), no signs of direct neutrophil activation were seen in either monotrauma patients or polytrauma patients, as measured by the panel of antibodies directed against activation markers. The baseline

TABLE 1. Comparison of Baseline Characteristics of Polytrauma Patients (Injury Severity Score ≥ 16) Who Developed Complications Compared With Polytrauma Patients Who Did Not Develop Complications

Baseline characteristics	ISS ≥ 16 , No Infectious Complications, <i>n</i> = 21	ISS ≥ 16 , Infectious Complications, <i>n</i> = 11	<i>p</i>
Age at trauma, yr, median (interquartile range)	61 (32–74)	55 (26–67)	0.65
Male, <i>n</i> (%)	13 (62)	7 (63)	0.93
ASA score, median (interquartile range)	1 (1–3)	3 (1–3)	0.11
Mechanism of injury, <i>n</i> (%)			
Car occupant	3 (14)	4 (36)	
Motor cyclist	1 (5)	1 (9)	
Pedal cyclist	5 (24)	0 (0)	0.32
Pedestrian	1 (5)	2 (18)	
Fall	9 (42)	4 (36)	
Other	2 (10)	0 (0)	
Patients with Abbreviated Injury Scale score > 2 , <i>n</i> (%)			
Head	15 (71)	7 (63)	0.66
Face	5 (24)	2 (18)	0.39
Thorax	11 (52)	8 (72)	0.11
Abdomen	2 (10)	2 (18)	0.31
Extremities	12 (57)	4 (36)	0.70
External	1 (5)	1 (9)	0.31
ISS, median (interquartile range)	19 (17–23)	29 (22–34)	0.002
New Injury Severity Score, median (interquartile range)	22 (22–27)	41 (27–48)	0.003
Glasgow Coma Scale, median (interquartile range)	14 (11–15)	10 (3–14)	0.009
Systolic blood pressure, mm Hg, median (interquartile range)	140 (119–155)	113 (90–130)	0.014
Respiratory rate, beats/min, median (interquartile range)	18 (15–28)	21 (20–28)	0.55
Serum hemoglobin, mmol/L, median (interquartile range)	8.9 (8.5–9.3)	8.4 (7.4–9.5)	0.35
pH, median (interquartile range)	7.38 (7.26–7.40)	7.26 (7.18–7.34)	0.06
Lactate, mmol/L, median (interquartile range)	1.90 (1.50–3.05)	2.60 (2.40–3.50)	0.12
Base excess, mEq/L, median (interquartile range)	1.5 (–5.5 to –2.0)	–4.0 (–6.5 to –2.5)	0.10
Total leukocytes, <i>n</i> $\times 10^6$ /mL, median (interquartile range)	10.2 (8.4–14.1)	14.5 (8.7–18.2)	0.09
Lymphocytes, <i>n</i> $\times 10^6$ /mL, median (interquartile range)	1.4 (0.8–2.1)	1.6 (1.2–2.4)	0.57
Monocytes, <i>n</i> $\times 10^6$ /mL, median (interquartile range)	0.4 (0.3–0.6)	0.5 (0.2–0.8)	0.92
Granulocytes, <i>n</i> $\times 10^6$ /mL, median (interquartile range)	7.4 (6.1–11.3)	11.6 (6.4–14.4)	0.05
Neutrophils, <i>n</i> $\times 10^6$ /mL, median (interquartile range)	7.1 (5.9–10.7)	11.4 (6.3–14.1)	0.042
Eosinophils, <i>n</i> $\times 10^6$ /mL, median (interquartile range)	0.09 (0.05–0.15)	0.09 (0.04–0.18)	0.55
Hospital admission, <i>n</i> (%)	21 (100)	11 (100)	NA
Infectious complications, <i>n</i> (%)	0 (0)	11 (100)	NA
Length of stay, d, median (interquartile range)	7 (3–11)	33 (18–47)	< 0.001
ICU stay, d, median (interquartile range)	1 (0–)	9 (7–21)	< 0.001
Mortality, <i>n</i> (%)	3 (14)	4 (36)	0.16

ISS = Injury Severity Score, NA = not applicable.

expression of these neutrophil activation markers of all included patients is shown in **Figure 2A–F**. The activation markers CD35, CD11c, CD11b, CBRM1/5, CD10, and CD66b showed no significant differences between polytrauma patients who developed infectious complications during hospital admission and polytrauma patients who did not.

Neutrophil Responsiveness

Neutrophil responsiveness that is determined by the expression of neutrophil activation markers in the presence of the bacterial stimulus fMLF is shown in **Figure 2G–L**. Significantly lower responsiveness of neutrophils for the activation-specific epitope of CD11b (CBRM1/5) was found in the infection group compared with the no infection group ($p = 0.034$). No significant difference in responsiveness was found for the other neutrophil markers CD35, CD11c, CD11b, CD10, and CD66b although trends toward less neutrophil responsiveness were visible for some markers.

Neutrophil Phenotypes

After trauma, two neutrophil phenotypes were found in the peripheral blood that is characteristic for systemic acute inflammation: CD16^{dim}/CD62L^{bright} neutrophils and CD16^{bright}/CD62L^{dim} neutrophils (16). Representative examples of differences in the percentage of these neutrophil phenotypes under different conditions (healthy controls, monotrauma patients, polytrauma patients and very severely injured polytrauma patients) are shown in **Figure 3**. Monotrauma patients show up to 6% CD16^{dim}/CD62L^{bright} neutrophils, and polytrauma patients show up to 22% CD16^{dim}/CD62L^{bright} neutrophils. In very severe polytrauma patients, mature neutrophils seem to have partially disappeared from the circulation, and many progenitor cells are left. This phenotype is in this cohort associated with a 100% mortality rate.

Next, it was evaluated whether an association was present between the presence of neutrophil phenotypes and infectious complications. The percentage CD16^{dim}/CD62L^{bright} and CD16^{bright}/CD62L^{dim} neutrophils in mono- and polytrauma patients was plotted in **Figure 4**. Monotrauma patients were characterized by the presence of 0.5% (0.3–1.5%) CD16^{dim}/CD62L^{bright} neutrophils and 1.2% (0.6–3.2%) CD16^{bright}/CD62L^{dim} cells. Polytrauma patients who developed infectious complications had

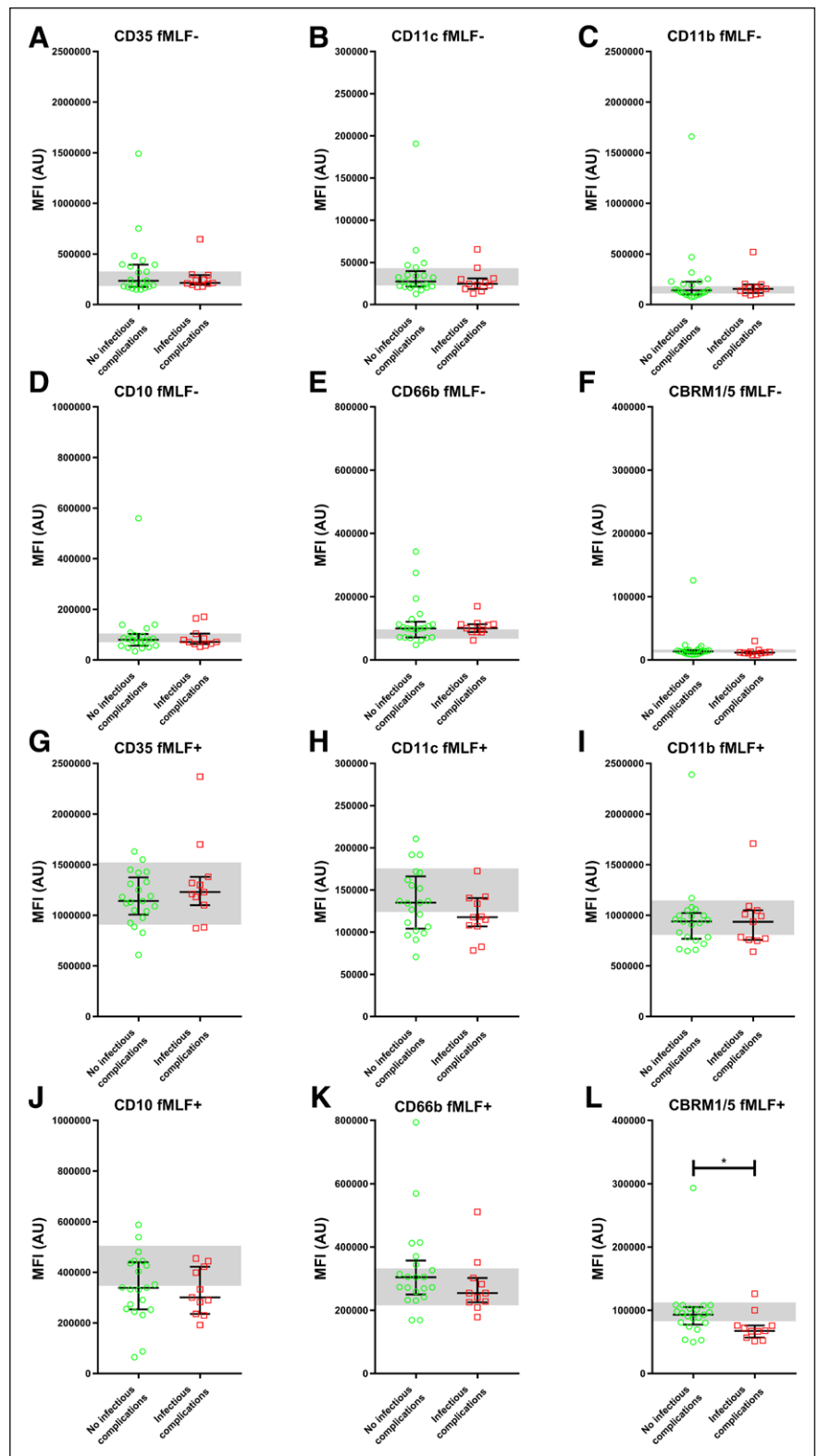


Figure 2. Expression of neutrophil activation markers of all included polytrauma patients (Injury Severity Score [ISS] ≥ 16). Neutrophil markers CD35 (**A**), CD11c (**B**), CD11b (**C**), CD10 (**D**), CD66b (**E**), and active CD11b (CBRM1/5) (**F**) were measured without the bacterial stimulus fMLF. The effect of activation (N-formyl-methionyl-leucyl-phenylalanine [fMLF] +) on the expression of neutrophil activation markers CD35 (**G**), CD11c (**H**), CD11b (**I**), CD10 (**J**), CD66b (**K**), and active CD11b (CBRM1/5) (**L**) is shown. Polytrauma patients without infections ($n = 21$) (●) were compared with polytrauma patients with infections ($n = 11$) (■) with the use of a Mann-Whitney U test. Data are presented as scatter plot with median and interquartile range. Reference values (gray area) show interquartile range of mono-trauma patients (ISS < 16) ($n = 55$). AU = arbitrary units, MFI = median fluorescent intensity.

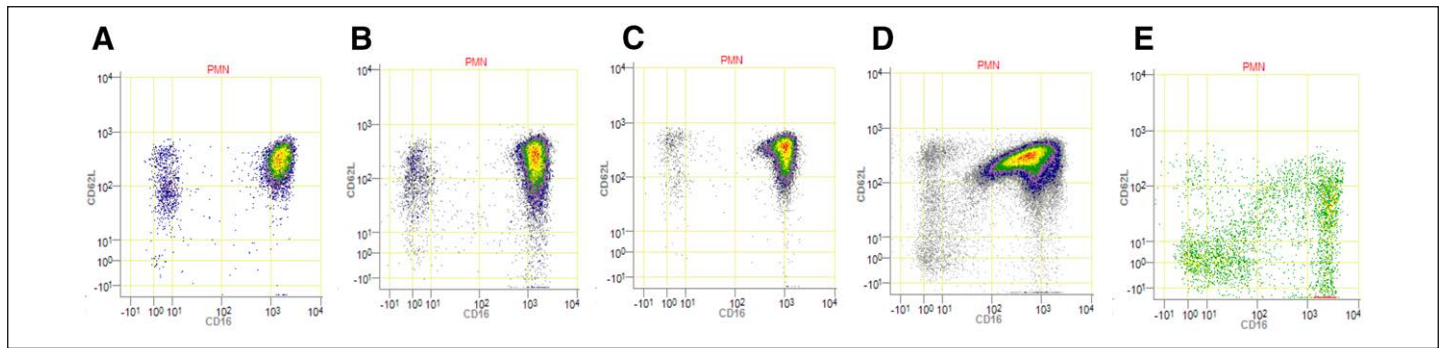


Figure 3. Representative examples of differences in neutrophil phenotypes in patients with different injury severity. Healthy controls (A) and trauma patients without any injuries (B) show very little CD16^{dim}/CD62L^{bright} neutrophils. Monotrauma patients (Injury Severity Score [ISS] < 16) show up to 6% CD16^{dim}/CD62L^{bright} neutrophils (C) and polytrauma patients (ISS ≥ 16) show up to 22% CD16^{dim}/CD62L^{bright} neutrophils (D). In very severe polytrauma patients, mature neutrophils seem to have partially disappeared from the circulation and many progenitor cells are left. This phenotype is associated with a 100% mortality rate (E). PMN = polymorphonuclear leukocytes.

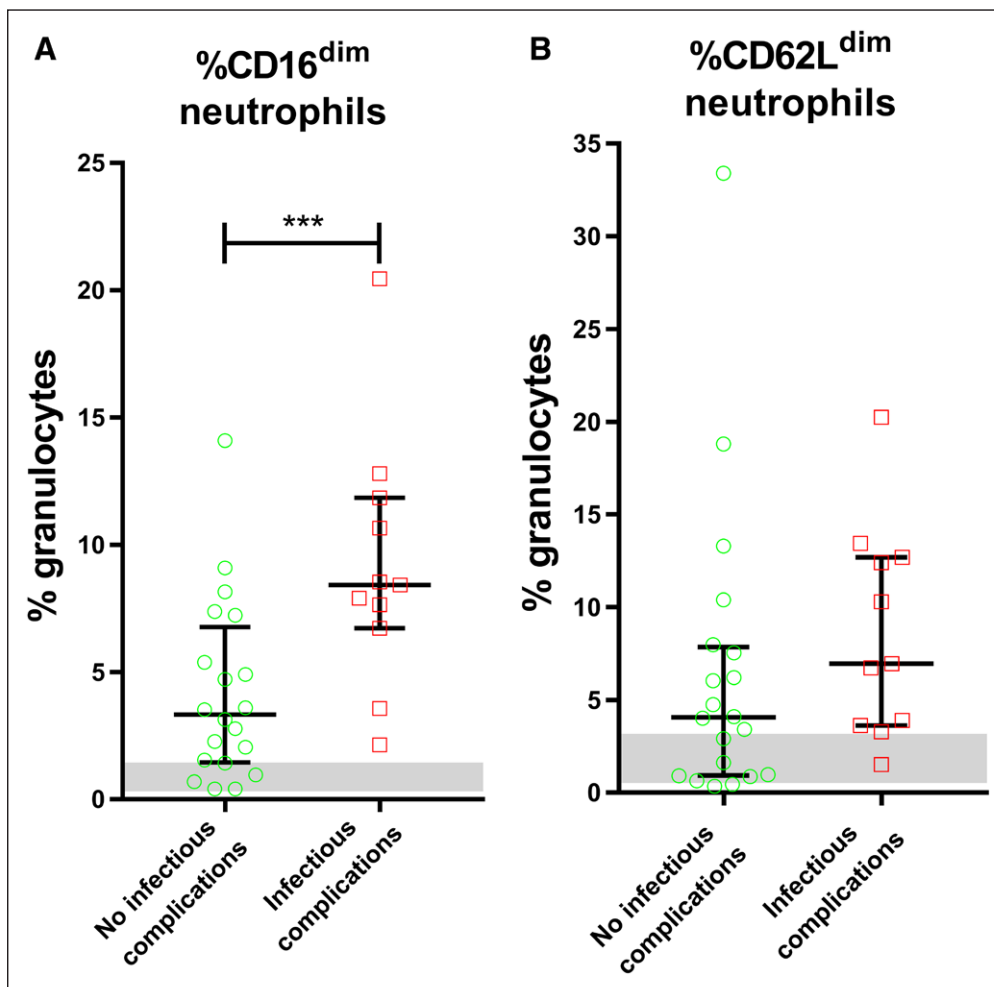


Figure 4. Percentage of CD16^{dim}/CD62L^{bright} neutrophils (A) and CD16^{bright}/CD62L^{dim} neutrophils (B) in polytrauma patients without infections ($n = 21$) (●) and with infections ($n = 11$) (■). Data are presented as a scatter plot with median and interquartile range and analyzed with the use of a Mann-Whitney U test. Reference values (gray area) show interquartile range of monotrauma patients (Injury Severity Score [ISS] < 16) ($n = 55$). *** $p < 0.005$.

a significantly higher percentage CD16^{dim}/CD62L^{bright} neutrophils than polytrauma patients without infections (8.4% [6.7–11.8%] vs 3.1% [1.3–6.8%]; $p = 0.002$) despite no significant difference in percentage CD16^{bright}/CD62L^{dim} neutrophils (6.9% [3.6–12.7%] vs 3.6% [1.0–9.4%]; $p = 0.090$). Area under the curve value with

is essential for quick and reliable measurements of neutrophil markers (13) and 2) The machine needs to be 24/7 available to start immune analysis in trauma patients. The AQUIOS CL is made for 24/7 measurements provided that reagents are checked and quality control is implemented.

95% clearance interval for %CD16^{dim}/CD62L^{bright} neutrophils is 0.90 (0.83–0.97) (online supplement fig. 2, Supplemental Digital Content 2, <http://links.lww.com/CCX/A225>).

DISCUSSION

Feasibility of Flow Analysis of Innate Immune Cells by a Point-of-Care Automated Flow Cytometer in the Trauma Bay

This study demonstrates the feasibility of a fully automated flow cytometer used point-of-care in the emergency bay to analyze the state of the innate immune response in trauma patients presented without the help of experienced laboratory personnel. The trauma team on-duty was able to include 156 of 164 patients eligible for inclusion during the 2.5-month inclusion period resulting in a success rate of 95%.

With the introduction of the fully automated flow cytometer in the trauma bay, several steps had to be taken to ensure an optimal workflow: 1) Correct placement of the automated flow cytometer is essential. The machine needs to be placed in a point-of-care setting or in any place where the time from blood drawing until analysis will be maximum 30 minutes because minimal time delay

Handling Failure

All the aforementioned steps were taken care of before the start of this study, which resulted in a high success percentage of 95%. However, there were several learning points for further implementation. In three cases, too little blood was drawn because blood was not visible in the tube due to incorrect placement of the barcode. In two additional cases, the blood tube with the barcode was placed incorrectly in the tray precluding the adequate reading of the barcode and the start of the machine. These issues were resolved by using multiple barcode stickers.

Informed Consent Procedure

For this study, written informed consent was required to collect clinical data and flow cytometry results of the included trauma patients. Informed consent procedures in emergency medicine are a challenge for testing fast procedures, particularly when proxy consent is needed that is often the case with severely injured patients (17). Therefore, our institutional ethical review board approved delayed consent. Despite the fact that delayed informed consent could be obtained, still 37% of the patients did not give written informed consent to participate in this study. The most important reason for this was that a significant part of the patients was sent home after the initial medical examination or soon after admission. The low response rate after hospital discharge is a known problem in the trauma population (18–20). The informed consent procedure caused a selection bias in this study, as it led to the exclusion of less severely injured trauma patients who were rapidly discharged from the hospital. To minimize selection bias, this analysis was only performed with the cell markers obtained from the polytrauma patients. In this subgroup, a total of 87% did give informed consent.

Biomarkers Associated With Injury Severity and Infectious Complications

Up to now, no clear biomarker has been identified that adequately correlates with the severity of tissue damage and the following immune response directly after trauma. The ISS and new ISS are widely used scoring methods for trauma patients who show a correlation with clinical outcomes but are obtained after final evaluation (21, 22). Hence, it is not a useful tool for the initial evaluation of the clinical (immune) status of trauma patients. Base excess at admission has a clear correlation with injury severity and mortality after trauma, as it is indicative of inadequate perfusion and tissue hypoxia (23–25). However, there are a number of comorbidities that can change acid-base deficit as well, for example, diabetic ketoacidosis, substance intoxication, or a prevalent condition as kidney insufficiency (25).

Several studies have tried to find early markers with predictive value for posttraumatic infectious complications. Biomarkers that seemed useful included the expression of human leukocyte antigen-DR isotype on circulating monocytes and procalcitonin levels in the peripheral blood (26–28). The best predictive value for septic complications was found for procalcitonin at day 1 post trauma, with only a reasonable sensitivity and specificity of up to 70% in a multivariate model (26).

Literature shows a huge cytokine storm 4–12 hours after trauma (3). Pro- and antiinflammatory cytokines are released in this timeframe (3). However, measuring only proinflammatory cytokines

as interleukin-8, C5a, and leukotrienes such as Leukotriene B4 would not show the real status of the innate immune system as the antagonizing antiinflammatory markers will affect overall status of the system. Measuring neutrophil receptor expression, as an integrator of all pro- and antiinflammatory cytokines, is a better indicator of the status of the innate immune response. Recently, neutrophil marker analysis showed a better prognostic value in the prediction of septic shock, with a sensitivity of up to 90% (3, 8, 11). Therefore in this study, baseline neutrophil activation, neutrophil responsiveness to fMLF, and analysis of neutrophil phenotypes were used to find potentially a better clinical applicable biomarker that would be correlated with injury severity and the resulting immune response.

Baseline Neutrophil Activation

With the introduction of an automated flow cytometer in a point-of-care setting, a more accurate measurement method of neutrophil activation markers was applied (13). Surprisingly, applying this improved method, no significant differences were found between the expression of activation markers on neutrophils of mono-trauma patients and polytrauma patients. In marked contrast, several studies in the literature showed a significant up-regulation of neutrophil activation markers after trauma (e.g. CD11b) (3, 11, 29, 30). However, marked variances in marker expression were demonstrated, and no clear correlation was found with tissue damage nor infectious complications. Some studies found a higher expression of CD11b, whereas some studies found a lower expression of CD11b in polytrauma patients compared with monotrauma patients (2, 3). A putative explanation for this discrepancy is that the increase in neutrophil activation markers shown in previous studies was at least in part a result of ex vivo manipulation (13).

Neutrophil Responsiveness

Automated flow cytometry analysis of neutrophil responsiveness to the bacterial stimulus fMLF was also performed in every trauma patient. Several studies showed that neutrophil responsiveness provides useful information (2, 8, 11–13, 31). In line with the literature, we found lower neutrophil responsiveness in polytrauma patients with infectious complications compared with polytrauma patients without infectious complications. However, this was only significantly found for the fMLF-induced expression of the neoepitope of CD11b (CBRM1/5) that recognizes only an active configuration of this integrin (32).

Neutrophil Phenotypes

Literature showed a significant increase in CD16^{bright}/CD62L^{dim} neutrophils in trauma patient samples collected within 1 hour after trauma, as well as 4–12 and 48–72 hours post injury (3). Up to now, no detailed kinetics on the appearance of CD16^{dim}/CD62L^{bright} neutrophils after trauma has been published. The CD16^{dim}/CD62L^{bright} neutrophils usually characterized by a banded shaped nucleus are most likely released from the bone marrow (16, 33). It is tempting to speculate that the amount of tissue damage leading to the liberation of damage-associated molecular patterns and production of immune mediators correlated with the release of new neutrophils from the bone marrow. This hypothesis is corroborated

with the correlation that was found between CD16^{dim}/CD62L^{bright} neutrophils and the ISS. However, there is only a limited correlation between the ISS and the amount of tissue damage (34). Therefore, it seems plausible that the bone marrow response leading to mobilization of CD16^{dim}/CD62L^{bright} neutrophils might be a better measure for the cumulative amount of tissue damage.

We found a significant relation between CD16^{dim}/CD62L^{bright} neutrophils and infectious complications in polytrauma patients. This can be used for the prediction of infectious complications in trauma patients, as supported by the high predictive values found in this study (online supplement fig. 2, Supplemental Digital Content 2, <http://links.lww.com/CCX/A225>). Furthermore, the %CD16^{dim}/CD62L^{bright} neutrophils was a significant covariate, when tested in a binary logistic regression model. However, the number of patients was too low to analyze the predictive value of CD16^{dim}/CD62L^{bright} neutrophils in a multivariate analysis that includes other factors as well.

CONCLUSIONS

This study showed the feasibility of the implementation of a fully automated point-of-care flow cytometry system for the characterization of the cellular innate immune response in trauma patients. This study supports the concept that assessment of CD16^{dim}/CD62L^{bright} neutrophils can be used for early detection of patients at risk for infectious complications. Furthermore, this can be used as a first step toward immuno-based precision medicine of polytrauma patients at the ICU.

ACKNOWLEDGMENTS

We want to thank all trauma surgery staff members, surgery residents, emergency department physicians, emergency department nurses, and other trauma bay members for the participation in this study. We also want to acknowledge Ruben Hoepelman for his contribution to data collection. For technical support, we want to thank Paul van Hoof, Roelof-Jan van der Lei, Geert Weijers, Andreas Boehmler, and Markus Kaymer from the Beckman Coulter team.

Prof. Koenderman and Leenen contributed equally to the study. Prof. Spijkerman, Ms. Hesselink, Mr. Hietbrink, and Prof. Koenderman and Leenen made substantial contributions to the conception of the work. Prof. Spijkerman, Ms. Hesselink, and Ms. Bongers worked on the data acquisition. Prof. Spijkerman and Ms. Hesselink performed the data analysis and made a substantial contribution to the drafting of the work. All authors worked on the interpretation of data, made a substantial contribution to revising the work critically for important intellectual content, approved the final version to be published, agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Supplemental digital content is available for this article. Direct URL citations appear in the HTML and PDF versions of this article on the journal's website (<http://journals.lww.com/ccejournal>).

The AQUIOS CL "load & go" flow cytometer is provided by the company Beckman Coulter Life Sciences, Miami, FL. Supported, in part, by a grant of Dutch Research Council in the framework of the "Startimpulse" Dutch National Research Agenda.

The authors have disclosed that they do not have any potential conflicts of interest.

For information regarding this article, E-mail: l.p.h.leenen@umcutrecht.nl

REFERENCES

- Cabrera CP, Manson J, Shepherd JM, et al: Signatures of inflammation and impending multiple organ dysfunction in the hyperacute phase of trauma: A prospective cohort study. *Plos Med* 2017; 14:e1002352
- Hietbrink F, Oudijk EJ, Braams R, et al: Aberrant regulation of polymorphonuclear phagocyte responsiveness in multitrauma patients. *Shock* 2006; 26:558–564
- Hazeldine J, Naumann DN, Toman E, et al: Prehospital immune responses and development of multiple organ dysfunction syndrome following traumatic injury: A prospective cohort study. *Plos Med* 2017; 14:e1002338
- Hesselink L, Spijkerman R, van Wessem KJP, et al: Neutrophil heterogeneity and its role in infectious complications after severe trauma. *World J Emerg Surg* 2019; 14:24
- Nuytinck HK, Offermans XJ, Kubat K, et al: Whole-body inflammation in trauma patients. An autopsy study. *Arch Surg* 1988; 123:1519–1524
- Botha AJ, Moore FA, Moore EE, et al: Postinjury neutrophil priming and activation: An early vulnerable window. *Surgery* 1995; 118:358–364
- Botha AJ, Moore FA, Moore EE, et al: Early neutrophil sequestration after injury: A pathogenic mechanism for multiple organ failure. *J Trauma* 1995; 39:411–417
- Hietbrink F, Koenderman L, Althuisen M, et al: Modulation of the innate immune response after trauma visualised by a change in functional PMN phenotype. *Injury* 2009; 40:851–855
- Pasquale MD, Cipolle MD, Monaco J, et al: Early inflammatory response correlates with the severity of injury. *Crit Care Med* 1996; 24:1238–1242
- Liefveld PHC, Pillay J, Vrisekoop N, et al: Differential antibacterial control by neutrophil subsets. *Blood Adv* 2018; 2:1344–1355
- Groeneveld KM, Koenderman L, Warren BL, et al: Early decreased neutrophil responsiveness is related to late onset sepsis in multitrauma patients: An international cohort study. *PLoS One* 2017; 12:e0180145
- Pillay J, Hietbrink F, Koenderman L, et al: The systemic inflammatory response induced by trauma is reflected by multiple phenotypes of blood neutrophils. *Injury* 2007; 38:1365–1372
- Spijkerman R, Hesselink L, Hellebrekers P, et al: Automated flow cytometry enables high performance point-of-care analysis of leukocyte phenotypes. *J Immunol Methods* 2019; 474:112646.
- Pallister I, Bhatia R, Katpalli G, et al: Alteration of polymorphonuclear neutrophil surface receptor expression and migratory activity after isolation: Comparison of whole blood and isolated PMN preparations from normal and postfracture trauma patients. *J Trauma* 2006; 60:844–850
- Jansen JJ, Hilvering B, van den Doel A, et al: FLOOD: FLOW cytometric orthogonal orientation for diagnosis. *Chemom Intell Lab Syst* 2016; 151:126–135
- Pillay J, Kamp VM, van Hoffen E, et al: A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J Clin Invest* 2012; 122:327–336
- Moskop JC: Informed consent and refusal of treatment: Challenges for emergency physicians. *Emerg Med Clin North Am* 2006; 24:605–618
- Rios-Diaz AJ, Herrera-Escobar JP, Lilley EJ, et al: Routine inclusion of long-term functional and patient-reported outcomes into trauma registries: The FORTE project. *J Trauma Acute Care Surg* 2017; 83:97–104
- Rainer TH, Yeung HH, Gabbe BJ, et al: A comparison of functional outcome in patients sustaining major trauma: A multicentre, prospective, international study. *PLoS One* 2014; 9:e103396
- Dutton RP, Stansbury LG, Hemlock B, et al: Impediments to obtaining informed consent for clinical research in trauma patients. *J Trauma* 2008; 64:1106–1112
- Chawda MN, Hildebrand F, Pape HC, et al: Predicting outcome after multiple trauma: Which scoring system? *Injury* 2004; 35:347–358
- Baker SP, O'Neill B, Haddon W, et al: The injury severity score: A method for describing patients with multiple injuries and evaluating emergency care. *J Trauma* 1974; 14:187–196
- Kroezen F, Bijlsma TS, Liem MS, et al: Base deficit-based predictive modeling of outcome in trauma patients admitted to intensive care units in Dutch trauma centers. *J Trauma* 2007; 63:908–913
- Husain FA, Martin MJ, Mullenix PS, et al: Serum lactate and base deficit as predictors of mortality and morbidity. *Am J Surg* 2003; 185:485–491

25. Raux M, Le Manach Y, Gauss T, et al; TRAUMABASE Group: Comparison of the prognostic significance of initial blood lactate and base deficit in trauma patients. *Anesthesiology* 2017; 126:522–533
26. Ciriello V, Gudipati S, Stavrou PZ, et al: Biomarkers predicting sepsis in polytrauma patients: Current evidence. *Injury* 2013; 44:1680–1692
27. Mathur P, Misra M, Rajkumari N, et al: Procalcitonin as a predictor of sepsis and outcome in severe trauma patients: A prospective study. *J Lab Physicians* 2013; 5:100–108
28. Cheron A, Floccard B, Allaouchiche B, et al: Lack of recovery in monocyte human leukocyte antigen-DR expression is independently associated with the development of sepsis after major trauma. *Crit Care* 2010; 14:R208
29. Hietbrink F, Koenderman L, Althuisen M, et al: Kinetics of the innate immune response after trauma: Implications for the development of late onset sepsis. *Shock* 2013; 40:21–27
30. Maekawa K, Futami S, Nishida M, et al: Effects of trauma and sepsis on soluble L-selectin and cell surface expression of L-selectin and CD11b. *J Trauma* 1998; 44:460–468
31. Pillay J, Ramakers BP, Kamp VM, et al: Functional heterogeneity and differential priming of circulating neutrophils in human experimental endotoxemia. *J Leukoc Biol* 2010; 88:211–220
32. Diamond MS, Springer TA: A subpopulation of Mac-1 (CD11b/CD18) molecules mediates neutrophil adhesion to ICAM-1 and fibrinogen. *J Cell Biol* 1993; 120:545–556
33. Tak T, Tesselaar K, Pillay J, et al: What's your age again? Determination of human neutrophil half-lives revisited. *J Leukoc Biol* 2013; 94:595–601
34. Frantz TL, Steenburg SD, Gaski GE, et al: Tissue damage volume predicts organ dysfunction and inflammation after injury. *J Surg Res* 2016; 202:188–195