Title

The injured monocyte: The link to chronic critical illness and mortality following injury.

Journal

The Journal of Trauma and Acute Care Surgery, 96(2)

Authors

Cuschieri, Joseph
Kornblith, Lucy
Pati, Shibani
et al.

Publication Date

2024-02-01

DOI

10.1097/TA.0000000000004173

Peer reviewed

HHS Public Access

Author manuscript

J Trauma Acute Care Surg. Author manuscript; available in PMC 2025 February 01.

Published in final edited form as:

J Trauma Acute Care Surg. 2024 February 01; 96(2): 195–202. doi:10.1097/TA.0000000000004173.

The Injured Monocyte: The Link to Chronic Critical Illness and Mortality Following Injury

Joseph Cuschieri, MD,
Department of Surgery, University of California San Francisco

Lucy Kornblith, MD,
Department of Surgery, University of California San Francisco

Shibani Pati, PhD,
Department of Laboratory Medicine, University of California San Francisco

Adrian Piliponsky, PhD
Department of Pediatrics, University of Washington

Abstract

Background: This study aimed to understand the altered innate immune response in severely injured patients leading to chronic critical illness (CCI). Specifically, it focused on characterizing the monocyte populations and their correlation with CCI development and long-term complications.

Methods: Over a 3-year period, we monitored patients with severe injuries for up to 1-year post-injury. CCI was defined as an ICU stay exceeding 14 days with persistent organ failure. Blood samples were collected on days 1 and 5 for monocyte phenotypic expression analysis using cytometry by time flight. The monocyte subpopulations studied were classical (CL), intermediate (INT), and non-classical (NC), along with cell surface receptor expression and activation.

Results: Out of 80 enrolled patients, 26 (32.5%) developed CCI. Patients with CCI had more severe injuries (injury severity score 32.4+5.2 vs. 29.6+4.1, p=0.01) and received a higher number of red blood cells (8.9+4.1 vs. 4.7+3.8 units, p<0.01) compared to those without CCI. In patients with CCI, the NC monocytes were significantly reduced by over 2-fold early, and significantly increased later, compared to those without CCI. Moreover, significant changes in intracellular cytokine expression and cell receptors were observed within each monocyte subpopulation in patients with CCI, indicating an increased pro-inflammatory phenotype but decreased phagocytic capacity and antigen presentation. The development of CCI and the presence of this unique monocyte phenotype were associated with a significantly increased risk of infection, discharge to a long-term care facility, and 1-year mortality of 27%.

Corresponding Author: Joseph Cuschieri MD, Zuckerberg San Francisco General Hospital and Trauma Center, Department of Surgery, 3A, 1001 Potrero Avenue, San Francisco, CA 94143, USA, Office: 628-206-4631, Fax: 628-206-5484, Joseph.Cuschieri@UCSF.edu.

Author Contribution: J.C and A.P. were responsible for study design, J.C. was responsible for patient recruitment, J.C. and A.P. were responsible for data collection and sample preparation, J.C. and A.P. were responsible for data analysis, J.C., K.L. S.P. and A.P. were responsible for data interpretation, J.C. was responsible for writing, K.L., S.P. and A.P. were responsible for critical reivsions.

Presentation Disclosure: This work to be presented as part of the 82 Annual AAST Annual Meeting on September 20–23, 2023 in Anaheim, California

Conclusions: Development of CCI following severe injury is associated with significant long-term morbidity and unacceptably high mortality. The altered NC phenotype with reduced phagocytic capacity and antigen presentation in patients developing CCI after severe injury is appears partially responsible. Early identification of this unique phenotype may help predict and treat patients at risk for CCI, leading to improved outcomes.

Level of Evidence:

Level III, Prognostic/Epidemiological

Social media summary:

Sustained dysregulation and alteration in monocyte phenotypes were associated with chronic critical illness, nosocomial infection and late death following injury. #ChronicCriticalIllness#InnateImuneResponse#UCSF

Keywords

Immunity; Chronic Critical Illness; Injury; Shock

INTRODUCTION

For the past four decades, traumatic injury has been the leading cause of death among individuals under 45 years of age in high-income countries.(1) However, encouragingly, advancements in pre-hospital care and trauma systems have improved in-hospital survival rates after injury and hemorrhage.(2, 3) Rapid bleeding control and enhanced blood product resuscitation have reduced hemorrhage-associated mortality.(4) These advances have led to fewer early trauma related deaths, but increased intensive care unit (ICU) stays.(5) This prolonged ICU care often correlates with persistent organ dysfunction, a condition known as chronic critical illness (CCI).(6, 7) Despite varied definitions of CCI, it's recognized as a distinct clinical entity, spurring research on its prediction, diagnosis and management.(8–10)

Traumatic injury triggers pro-inflammatory systemic and anti-inflammatory response, linked to CCI.(6, 11) This immune dysregulation is similar to that seen in conditions like sepsis and Coronavirus 2019 (COVID-19).(7, 12–14) Subsequent infections in CCI patients contribute to late deaths weeks after injury, suggesting that a unifying mechanism of immunologic dysfunction.(15–18) Several studies have indicated that among the immunologic changes, early alterations in innate immunity, specifically within monocytes, may play a central role in the development of persistent organ dysfunction after injury, possibly even within the first few hours, providing an opportunity to identify and mitigate early immune dysfunction and potentially reduce the risk of CCI.(6, 9, 19) However, a critical gap in our understanding of the distinct role that circulating monocytes play in the development of CCI exists.

Monocytes represent a heterogeneous cell population in both phenotype and function that compromise 5–10% of the circulating peripheral blood leukocytes. Monocytes play a critical role in tissue homeostasis and the inflammatory cascade by regulating inflammation through regulated pro- and anti-inflammatory cytokine production. Recent data has demonstrated that monocytes consist of phenotypically and functional distinct subpopulations with

distinct function.(20) These subpopulations are based on differential expression of cluster of differentiation (CD)14 Lipopolysaccharide (LPS) receptor and CD16 low-affinity Fcγ receptor. These subsets are designated as classical (CD14+CD16−), intermediate (CD14+CD16+), and non-classical (CD14-CD16+).(21) Although the last two subsets play critical distinct roles in innate immune responses, they normally only account for 10% of the overall circulating monocyte population in healthy individuals.

The equilibrium among the various monocyte subpopulations relies on complex mechanisms that are dependent on individual responses to immune demands.(22) Regulated CD16+ expansion, in reaction to infectious and inflammatory stimuli, enhances differentiation of classical monocytes to intermediate and non-classical subpopulations. This aids in responding to tissue injury and bacterial infection by enhancing phagocytosis and antigen presentation. Although subpopulation differentiation is critical to inflammatory responses, dysregulated alterations in these subpopulations are known to lead to tissue injury and chronic inflammatory states, akin to CCI.

Therefore, the aim of this study is to characterize the monocyte phenotype following severe hemorrhagic trauma, and determine the relationship of the various subset populations on the development of CCI. We hypothesize that patients who develop CCI will exhibit unique alterations in the expression of monocyte subpopulations early following severe injury, and these differences will be associated with unique monocyte phenotypes that are characterized by variations in cytokine production, antigen uptake, antigen presentation, and migratory ability.

MATERIALS AND METHODS

Study Design

We conducted a prospective, observational cohort study over a 3-year period (September 2018 to August 2021) at a single Level 1 trauma center. The institutional review board granted approval prior to study initiation. The STROBE checklist was used to assure the highest scientific methodological transparency and scientific standards were meet (Supplemental Data). Subjects were initially enrolled under a 96-hour waiver of informed consent. Inclusion criteria included patients 18 years and older, confirmation of severe blunt traumatic injury with hemorrhagic shock (systolic blood pressure less than 90 mmHg or base deficit ≥ 6 meq/L within 60 minutes of arrival). Patients expected to survive 48 hours or less and those with severe traumatic brain injury (Glasgow coma scale less than 8 and abnormal head computed tomography [CT]) were excluded. These inclusion criteria were consistent with the “Trauma Glue Grant” and were utilized to select for patients likely to survive their initial injuries but at a significant risk for multiple organ failure (MOF), as previously described.(5, 23) All consecutive patients meeting these criteria in which consent was obtained within 96 hours were enrolled to minimize bias.

Demographic, clinical, and physiologic data were obtained and studied for the first 28 days after injury, or until ICU discharge. To determine one year mortality following injury, we both attempted to contact the patient and queried the State’s Death Registry. To determine the initial immunologic response to severe blunt trauma and hemorrhagic shock,

blood samples were obtained on hospital day 1 (day after presentation) and day 5. Five milliliters of blood were drawn into ethylenediaminetetraacetic acid (EDTA) treated tubes (BD Biosciences, USA) at both time points.

Sample size was based on the study by Mira and colleagues on patients that developed CCI following severe blunt trauma.(24) In that study, 19% of surviving patients developed CCI and were thus at highest risk for sustained inflammatory dysfunction. For the current study, a similar frequency was used despite limited phenotypic human data available to determine an accurate biologic estimate. Based on the 28-day rate of CCI development, we estimated that approximately 75 patients were required to detect a difference for a power of 0.9, alpha 0.05, and doubling the standard deviation.

Definitions and Outcomes

The primary outcome variable was the incidence of CCI. Secondary outcomes included nosocomial infections, discharge disposition, and mortality. Currently there is no consensus definition for CCI so we elected to define CCI based upon the "Trauma Glue Grant" experience that severely injured patients in the ICU (≥ 14d) with a dysregulated genomic response to injury develop persistent organ dysfunction, and adverse outcomes.(5) As a result, we defined CCI as prolonged ICU admission (≥ 14d) with evidence of ongoing organ dysfunction or early death. We defined persistent organ dysfunction using the Modified Marshall's Multiple Organ Dysfunction Score criteria requiring either ≥ 2 in the renal (serum creatinine ≥ 1.9 mg/dl [without dialysis]) or pulmonary (Pao2/Fio2 ≤ 300) categories, or ≥ 1 in the cardiac category (systolic blood pressure < 90 mm Hg, or use of vasopressors). We specifically excluded the neurologic component because of potential concurrent traumatic brain injury. "Time-to-recovery" was defined as number of days after study entry to resolution of organ dysfunction, without subsequent recurrence.(5, 24) Patients with an ICU stay less than 14 days without persistent organ dysfunction were classified as not suffering from CCI.

Cytometry by time of flight (CyTOF) determination of monocyte phenotypes and activation

Whole blood samples (n = 80) were collected from patients on days 1 and 5 following injury using BD Vacutainer® tubes with EDTA as the anticoagulant. The samples were gently inverted several times to ensure proper mixing of the whole blood and anticoagulant. Following blood collection, RBC lysis was performed using the Ammonium-Chloride-Potassium (ACK) Lysing Buffer (Gibco, Thermo Fisher Scientific, USA). Briefly, an aliquot of whole blood was incubated with the ACK Lysing Buffer for 10 minutes at room temperature to lyse the RBCs and facilitate white blood cell analysis. The lysed blood samples were centrifuged at 400 × g for 5 minutes at 4°C. The supernatant was discarded, and the cell pellet was washed twice with phosphate-buffered saline (PBS, pH 7.4) to remove any residual lysing buffer or cellular debris. The washed cell pellet was resuspended in a fixation buffer containing 1.6% paraformaldehyde (Electron Microscopy Sciences, USA) and 0.05% w/v saponin (Sigma-Aldrich, USA) in PBS. The cells were then incubated for 15 minutes at room temperature to ensure proper fixation. Fixed cell samples were stored in 1.5 mL microcentrifuge tubes at 4°C in the dark until analysis. Samples were transferred

to cryovials and stored in liquid nitrogen or a −80°C freezer to maintain sample integrity until analysis.

Cells Surface Antibody Staining:

Cells were incubated with a 22-panel of isotypeconjugated surface antibodies towards multiple cell types, those specifically targeting monocytes were: 160Gd-CD14, 209Bi-CD16, 174Yb-HLA-DR, 161Dy-CD80, 156Gd-CD86, 145Nd-CD163, 152Sm-CD36 and 142Nd-CX_3CR1 from Standard Biotools-Fluidigm, USA, and 158Gd-CCR2 from Biolegend, USA. In addition, cells were also labeled with 162Dy-CD66, 10Nd-CD63, 169Tm-CD203c, 165Ho-CD19, 141Pr-CD3 and 176Yb-CCR3 (Standard Biotoools-Fludigm,) as isotype controls to label neutrophils, basophils, B cells, T cells and dendritic cells to optimize monocyte gating and serve as negative controls for non-specific binding. Antibody concentrations were optimized according to the manufacturer's recommendations. Incubation was carried out at 4°C for 30 minutes. After incubation, cells were washed with PBS to remove unbound antibodies.

Intracellular Antibody Staining for Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-α:

For intracellular staining of IL-6 and TNF-α, cells were fixed and permeabilized using dilute Iradium-Interchelator solution (Fluidigm, USA). Cells were then incubated with a isotopeeconjugated antibody 154Sm-IL-6 and 175Lu-TNF-α at 4°C for 30 minutes. Following incubation, cells were washed to remove any excess antibody.

CyTOF Analysis:

Stained cells were resuspended in CyTOF acquisition buffer. Helios CyTOF system running CyTOF Software (Fluidigm, USA) at a rate of 300–500 events per second. Prior to data acquisition, the CyTOF instrument was calibrated and normalized using calibration beads. FCS files are subsequently generated using CyTOF Software for subsequent analysis.

Statistical Analysis

Categorical variables are presented as frequency and percentage and compared using the Pearson chi-square test or Fisher exact test. We use log rank tests to compare Kaplan-Meier product limit estimates of organ dysfunction recovery between CCI and no CCI. Baseline demographic data and clinical outcome scores are expressed as mean ± SD. All biomarker values were treated as normally distributed continuous variables and expressed as mean ± SD. For statistical analyses, Student-t test was used for continuous variables, and $\chi 2$ test was used for categorical predictor variables. The nonparametric Mann-Whitney U test was used for continuous variables that were not normally distributed.

CyTOF files were analyzed using CyTOF software from Fluidigm, USA. Gating strategies were employed to identify monocytes, initially CD45 positive cells followed by characteristic scatter properties to exclude non-cellular debris and non-leukocyte cells. Monocyte gating was based on surface marker expression, of CD14 and CD16 while excluding cells that expressed CD66, CD63, CD19 and CCR3 (Supplemental Data). Only events falling within these thresholds were considered pure monocytes, The use of these specific isotype controls improved the monocyte gating and ensured that the identified cell

populations were not the result of non-specific antibody binding. Marker expression values and absolute cell counts for surface expression CD14, CD16, HLA-DR, CD80, CD86, CD36, CD163, CX_3CR1 and CCR2 were calculated for each monocyte subset and analyzed on the clinical development of CCI. Similar, marker expression of intracellular TNF-α and IL-6 was calculated for each monocyte subset, and further characterized and analyzed on the clinical development of CCI.

Additionally, the non-classical/classical monocyte phenotype ratio was determined for both patients that did and did not develop CCI at both time points. Serial comparisons between groups were made using repeated-measures analysis of variance (ANOVA) with post hoc Bonferroni/Dunn testing. All analyses were two-tailed, and $P < 0.05$ was considered statistically significant. Additionally, the non-classical/classical monocyte phenotype ratio correlation with subsequent mortality was determined by developing a receiver operating curve (ROC) and the optimal ratio for development of CCI was determined using the Youden index.

For multivariable logistic regression analysis, we a priori selected explanatory variables including age, sex, injury severity score, shock as defined as either initial lactate (> 4) and/or initial base deficit (BD) (≤ 6), red blood transfusion greater than 6 units in the first 12 hours, and the non-classical/classical monocyte phenotype ratio. We report adjusted odds ratios with 95% CIs. Area under the receiver operating curve values and Hosmer-Lemeshow goodness-of-fit test were used to assess model discrimination and fit.

RESULTS

A total of eighty patients were enrolled (Supplemental Data). Among them, 26 (32.5%) developed CCI. Patients that developed CCI did not significantly differ in age, sex, or body mass index (BMI) from those that did not (Table 1). However, CCI patients had more sever injuries (ISS 32.4±5.2 vs. 29.6±4.1, p=0.11), presented in shock more often (88% vs. 72%, p=0.038), and received more blood products (P'RBC transfusion 8.9±3.8 units vs. 4.7±3.8 units, p<0.001).

CCI patients had fewer ventilator-free days, and longer ICU and hospital stays (Table 1). Their time to recover from organ dysfunction was significantly longer (26.9±5.7 vs. 7.9±6.7, p<0.001), and they experience more . infectious complications (88.5% vs. 35.2%, p<0.001). Discharge disposition also differed significantly, with more CCI having a "bad" discharge disposition (69.2% vs. 29.6%, p=0.002). One-year mortality was found to be only 4% in patients that did not develop CCI, compared to an alarming 27% for those that did.

We then evaluated the monocyte subpopulations and phenotypes associated with CCI. Total monocyte counts didn't differ between patients that went on to develop CCI compared to those that did not on either day 1 ($1.14±0.23 \times 10^3$ cells/μl vs. $1.06±.017 \times 10^3$ cells/μl, p=0.084) or day 5 ($0.96±0.21 \times 10^3$ cells/μl vs. $0.89±0.12 \times 10^3$ cells/μl, p=0.616). No significant difference in the distribution of classical or intermediate subpopulations was found in patients that developed CCI. However, the distribution of the non-classical subpopulation in patients with CCI demonstrated significant variations

(Figure 1). Immediately following injury there was a reduction in the overall non-classical subpopulation in patients that developed CCI in comparison to patients that did not (2.44±0.99% vs. 4.73±1.95%, p<0.0001). This was followed by a significant increase by day 5 in patients that developed CCI in comparison to patients that did not (8.95±2.99% vs. 4.91±1.58%, p<0.0001). To standardize this difference amongst individual patients, the non-classical/classical monocyte subpopulation ratio was determined. A decrease in the non-classical/classical monocyte subpopulation ratio was found at day 1 (0.062±0.030 without CCI vs. 0.032±0.014 with CCI, p<0.0001), but an increase in day 5 (0.064±0.022 without CCI vs. 0.122±0.048 with CCI, p<0.0001).

To assess the correlation of the non-classical subpopulation early following injury and CCI development, a ROC curve was established, The day 1 non-classical/classical subpopulation monocyte ratio was found to be inversely correlated with the development of CCI, with an AUC of 0.79 (Figure 2). Using the Youden index (sensitivity + specificity-1) a non-classical/classical monocyte ratio of 0.035 or less was determined to be the ratio of the non-classical subset that is most highly associated with the development of CCI.

The overall monocytic phenotypes were further explored by evaluating the surface expression of several key receptors (Figure 3). In patients that developed CCI, there was a rapid increase in the expression of the scavenger receptors CD36 and CD163. Although a moderate increase in CD36 occurred early in non-classical subpopulation, it was not until day 5 when a significant increase in expression was noted in all three subpopulations. Significant increase in CD163 occurred in classical and non-classical subpopulations at day 1, but was not sustained. Similar, patients that developed CCI had significant increases in the migratory receptors CCR2 and CX_3CR1. These changes occurred early and were sustained through day 5, with CCR2 being increased in classical monocytes and CX_3CR1 being increased in non-classical monocytes.

The surface expression of antigen presenting receptors, HLA-DR, CD80 and 86, were attenuated early and remained diminished through day 5 in patients that developed CCI in comparison to those that did not in all subpopulations. Importantly, the attenuation of HLA-DR, CD80 and CD86 were most pronounced in intermediate and non-classical monocytes.

These receptor changes were associated with a rapid increase pro-inflammatory cytokine expression of both IL-6 and TNF-α within all mononuclear subpopulations after injury, but were significantly increased in patients that developed CCI. The increase in IL-6 was not present on day 5, but sustained increases in TNF-α was present in all subpopulations that went on to develop CCI.

Multivariable logistic regression was performed to assess the effect of various admission factors, including the potential effect of non-classical monocyte expression, on development of CCI. A significant association with the presence of severe injury and massive blood product resuscitation defined as 6 units of packed red blood cells administered in the first 12 hours following injury were found to independently associated with the development of CCI (Table 2). Biologically, a non-classical/classical monocyte ratio of 0.035 or less was independently associated with CCI development (OR 3.46 [1.68–6.84], p=0.009) despite

the significant degree of injury. These relationships remained independently associated with 1-year mortality in the overall patient population, with the additional independent variable of age (Table 2).

DISCUSSION

The changing epidemiology of trauma-related morbidity and mortality demands an investigation into late adverse outcomes. Currently, it is becoming increasingly apparent that surviving patients with severe injuries face significant morbidity and mortality weeks later, raising questions about lasting biological changes and the emergence of CCI as a potential underlying disease process.(1, 5, 25–27)

This prospective observational study demonstrates that multiple organ failure occurs frequently in patients receiving blood product resuscitation, similar to previous studies.(28) Importantly, this study expands on previous work, demonstrating that the development of multiple organ failure can persist in the form of CCI. (24) Of those surviving their initial injury, we demonstrate approximately one-third of patients develop CCI that is associated with increased resource utilization and infectious complications. Additionally, a significant number of patients are discharged to skilled nursing facilities or long-term acute care facilities, consistent with previous studies in trauma and sepsis.(24, 29)

Unfortunately, despite substantial resources applied, the one-year mortality rate for injured patients developing CCI is excessively high, similar to previous data in trauma and sepsis.(9, 24, 29, 30) As a result, it is essential that the biology resulting in CCI be unraveled to improve the outcome in following severe traumatic injury. The current study specifically aims to evaluate a part but important component of the inflammatory response following injury, the peripheral blood monocyte. Monocytes have been implicated as key contributors to organ failure development and CCI following injury.(8, 9, 19) Furthermore, monocytes have been demonstrated to have unique phenotypic alterations in several different inflammatory diseases such as severe COVID-19 infections and sepsis, especially in those that develop long-term complications.(8, 19, 31–33)

In the current study, we explore the dynamic alterations in monocyte subset frequencies following severe traumatic injury associated with hemorrhagic shock. Given the dysregulated innate inflammatory nature of the initial response associated with organ failure and nosocomial infection, we focused our study on the phenotypically and functionally distinct monocyte subsets in circulation, which play variable roles: classical, intermediate, and non-classical.

This study provides strong evidence of a significant link between changes in the mononuclear phenotype and CCI. Notably, alterations in the early expression of non-classical monocytes are both immediate and sustained in CCI patients. Following injury, there is a reduction in non-classical monocyte expression, independent of shock, injury severity, and blood transfusions. This decrease may result from either reduced monopoiesis, or increased interstitial migration in response to tissue injury.(34) Since non-classical monocytes play a crucial role in regulating inflammation and maintaining endothelial

integrity, their reduction, coupled with increased expression of inflammatory markers like IL-6 and TNF-α, could contribute to early tissue and organ injury.(21) This might occur due to inefficient clearance of cellular debris at injury sites caused by decreased monopoiesis, or an uncontrolled pro-inflammatory state due to overzealous migration by non-classical monocytes. Similar reduction in non-classical monocytes have been observed in other pro-inflammatory diseases, like sepsis and COVID-19.(31–33)

Interestingly, each monocytic subpopulation demonstrates unique alterations in membrane receptors expression early that are most pronounced within non-classical monocytes. The alteration in cell membrane receptor expression and intracellular cytokine production are consistent with a pro-inflammatory endotype. Among the receptor changes, increased expression of scavenger receptors CD36 and CD163 occurs. CD36, a lipid scavenger, promotes sterile inflammation within monocytes, and thus could directly lead to tissue injury and organ failure.(35) Moreover, increased CD36 expression persists beyond the initial injury, particularly in non-classical monocytes, possibly leading to long-term arterial and organ injury. This sustained change could result in increased thrombotic complications and atherosclerotic disease progression, akin to long COVID-19.(36–40) The early elevation in CD163, on the other hand, may be a compensatory and important scavenger receptor for free hemoglobin limiting tissue injury. However, significant elevations have been demonstrated in patients with severe sepsis going on to develop organ failure.(41)

Alterations in receptor expression were not limited to scavenger receptors, early and sustained changes in elevation both CCR2 in classical monocytes and CX_3CR1 in non-classical monocytes were found. Both CCR2 and CX_3CR1 are chemokine receptors and involved in cell migration. CCR2, normally expressed on classical monocytes, is involved in the initial inflammatory response and tissue repair. CX_3CR1 is mainly expressed in non-classical monocytes and involved in tissue repair and attenuating inflammation. However, enhanced expression of both CCR2 and CX_3CR1 was found in patients developed CCI. This would result in dysregulated pro-inflammatory recruitment and subsequent tissue injury similar to other disease processes including atherosclerosis, inflammatory disorders, neurodegenerative disease and most recently COVID-19.(42)

Surprisingly, the initial downregulation of non-classical monocytes is not sustained, and by day 5, a significant increase in this monocytic phenotype is observed in patients who develop CCI. This would be consistent with the development of a chronic inflammatory state, like that seen in patients with chronic inflammatory conditions, Thus, it is plausible that the increased non-classical monocytes sustained after injury would result in continued organ injury and late death. Interesting, a similar alteration in non-classical monocytes has been recently observed in patients with severe COVID-19 infections.(43) No significant population changes were found in classical or intermediate monocytes.

At this later time, a significant decrease in surface receptors for phagocytosis and antigen presentation was observed in each subpopulation, particularly CD80, CD86 and HLA-DR. CD80 and CD86 are critical glycoproteins involved in antigen presentation and T-cell activation, heightening the risk of nosocomial infections when they are diminished. Similar alterations in CD80 and CD86 expression, have been linked to long-COVID-19

infectious complications.(44, 45) These glycoproteins, along with HLADR, are upregulated by interferon (INF)-γ, which is downregulated after severe injury.(6) These modifications in receptor expression, especially HLA-DR, increase the risk of infectious insults. The reduced expression of HLA-DR is most evident in non-classical monocyte subpopulation but also present in intermediate subset., Similar reductions in HLA-DR expression have been observed in other immune conditions, including post-surgical states and severe COVID-19 infections, associated with reduced antigen presentation and an increased risk of nosocomial infections.(33, 46, 47) This overall late increase in the non-classical subpopulation combined with decreased HLA-DR expression and sustained TNF-α production, creates a dysregulated pro-inflammatory state that hinders the clearance of subsequent infections. This unique biological discovery aligns with clinical course of CCI.

Several key limitations in this study to clarify how to interpret the finding optimally. This prospective observational study collected blood samples at early time points, limiting a comprehensive assessment of the extent and duration of the phenotypic changes. Consequently, extending these to later clinical conditions, such as nosocomial infections, CCI, and late death, can only suggest an association without causation. Profiling of monocyte subsets relied on cell membrane receptors, CD45, CD14 and CD16, followed by scatter properties, potentially reducing purity. Nonetheless, the use of specific isotype controls (CD66, CD63, CD19 and CCR3) to the gating strategy enhanced the likelihood of identifying pure monocyte populations. Given the relatively small sample size, generalizability may be limited to important patient demographics, such as age and sex, despite no statistical difference among the patients studied. Lastly, it's worth noting that we focused only on the response of circulating monocytes. Compartmentalization of the inflammatory response is a well-known phenomenon and could restrict the generalizability to the immune findings, despite previous data highlighting the importance of monocytes. (9, 48)

Over the past 50 years, trauma care has witnessed continuous improvement in pre-hospital, operative, and post-operative care. However, patients who survive their initial trauma are at risk of long-term physiologic changes and disability that is unfortunately associated with a significant risk of late mortality. As our ability to keep patients alive through their acute resuscitation improves, understanding the biology associated with CCI and long-term complications is increasingly imperative. This study provides a framework for the significant alterations within monocytes, in particular non-classical monocytes, as a key contributor to the development of CCI. Future investigations will provide improved understanding of the complex immunological state following severe injury, helping to determine novel targets for therapeutic intervention and attenuation of CCI, leading to an improvement in overall long-term outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Conflict of Interest Statement:

JTACS Disclosure forms for all authors are provided as supplemental documents. I, Dr. Joseph Cuschieri, attest on behalf of all authors, that we had full access to the data of the study, conducted all data analyses independently from any funding entity, and take complete responsibility for the integrity and accuracy of the data reported in the manuscript.

Funding:

This work was funded in part by the NIH, R01HL141094 (PI: Piliponsky)

References

1. Hedegaard H, Warner M. Evaluating the cause-of-death information needed for estimating the burden of injury mortality: United States, 2019. Natl Vital Stat Rep. 2021;70(13):1–23.
2. Sasser SM, Varghese M, Joshipura M, Kellermann A. Preventing death and disability through the timely provision of prehospital trauma care. Bull World Health Organ. 2006;84(7):507. [PubMed: 16878215]
3. MacKenzie EJ, Rivara FP, Jurkovich GJ, Nathens AB, Frey KP, Egleston BL, et al. A national evaluation of the effect of trauma-center care on mortality. N Engl J Med. 2006;354(4):366–78. [PubMed: 16436768]
4. Holcomb JB, Tilley BC, Baraniuk S, Fox EE, Wade CE, Podbielski JM, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA. 2015;313(5):471–82. [PubMed: 25647203]
5. Cuschieri J, Johnson JL, Sperry J, West MA, Moore EE, Minei JP, et al. Benchmarking outcomes in the critically injured trauma patient and the effect of implementing standard operating procedures. Ann Surg. 2012;255(5):993–9. [PubMed: 22470077]
6. Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, et al. A genomic storm in critically injured humans. J Exp Med. 2011;208(13):2581–90. [PubMed: 22110166]
7. Mira JC, Brakenridge SC, Moldawer LL, Moore FA. Persistent Inflammation, Immunosuppression and Catabolism Syndrome. Crit Care Clin. 2017;33(2):245–58. [PubMed: 28284293]
8. Brakenridge SC, Wang Z, Cox M, Raymond S, Hawkins R, Darden D, et al. Distinct immunologic endotypes are associated with clinical trajectory after severe blunt trauma and hemorrhagic shock. J Trauma Acute Care Surg. 2021;90(2):257–67. [PubMed: 33214489]
9. Miranda D, Maine R, Cook M, Brakenridge S, Moldawer L, Arbabi S, et al. Chronic critical illness after hypothermia in trauma patients. Trauma Surg Acute Care Open. 2021;6(1):e000747. [PubMed: 34423134]
10. Raymond SL, Hawkins RB, Wang Z, Mira JC, Stortz JA, Han F, et al. Prospective Validation of a Transcriptomic Metric in Severe Trauma. Ann Surg. 2020;271(5):802–10. [PubMed: 30688688]
11. Ward NS, Casserly B, Ayala A. The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. Clin Chest Med. 2008;29(4):617–25, viii. [PubMed: 18954697]
12. Philip B, Mukherjee P, Khare Y, Ramesh P, Zaidi S, Sabry H, et al. COVID-19 and its long-term impact on the cardiovascular system. Expert Rev Cardiovasc Ther. 2023;21(3):211–8. [PubMed: 36856339]
13. Maity S, Santra A, Hebbani AV, Pulakuntla S, Chatterjee A, Badri KR, et al. Targeting cytokine storm as the potential anti-viral therapy: Implications in regulating SARS-CoV-2 pathogenicity. Gene. 2023;881:147612. [PubMed: 37423400]
14. Parotto M, Gyongyosi M, Howe K, Myatra SN, Ranzani O, Shankar-Hari M, et al. Postacute sequelae of COVID-19: understanding and addressing the burden of multisystem manifestations. Lancet Respir Med. 2023.
15. Hakkarainen TW, Arbabi S, Willis MM, Davidson GH, Flum DR. Outcomes of Patients Discharged to Skilled Nursing Facilities After Acute Care Hospitalizations. Ann Surg. 2016;263(2):280–5. [PubMed: 26445466]

16. McIntyre LK, Arbabi S, Robinson EF, Maier RV. Analysis of Risk Factors for Patient Readmission 30 Days Following Discharge From General Surgery. JAMA Surg. 2016;151(9):855–61. [PubMed: 27303913]
17. Nehra D, Nixon ZA, Lengenfelder C, Bulger EM, Cuschieri J, Maier RV, et al. Acute Rehabilitation after Trauma: Does it Really Matter? J Am Coll Surg. 2016;223(6):755–63. [PubMed: 28193321]
18. Thornblade LW, Arbabi S, Flum DR, Qiu Q, Fawcett VJ, Davidson GH. Facility-Level Factors and Outcomes After Skilled Nursing Facility Admission for Trauma and Surgical Patients. J Am Med Dir Assoc. 2018;19(1):70–6 e1. [PubMed: 29042263]
19. Chen T, Delano MJ, Chen K, Sperry JL, Namas RA, Lamparello AJ, et al. A road map from single-cell transcriptome to patient classification for the immune response to trauma. JCI Insight. 2021;6(2).
20. Ziegler-Heitbrock L, Hofer TP. Toward a refined definition of monocyte subsets. Front Immunol. 2013;4:23. [PubMed: 23382732]
21. Kapellos TS, Bonaguro L, Gemund I, Reusch N, Saglam A, Hinkley ER, et al. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front Immunol. 2019;10:2035. [PubMed: 31543877]
22. Ozanska A, Szymczak D, Rybka J. Pattern of human monocyte subpopulations in health and disease. Scand J Immunol. 2020;92(1):e12883. [PubMed: 32243617]
23. Cuenca AG, Maier RV, Cuschieri J, Moore EE, Moldawer LL, Tompkins RG, et al. The Glue Grant experience: characterizing the post injury genomic response. Eur J Trauma Emerg Surg. 2011;37(6):549–58. [PubMed: 26815465]
24. Mira JC, Cuschieri J, Ozrazgat-Baslanti T, Wang Z, Ghita GL, Loftus TJ, et al. The Epidemiology of Chronic Critical Illness After Severe Traumatic Injury at Two Level-One Trauma Centers. Crit Care Med. 2017;45(12):1989–96. [PubMed: 28837430]
25. Claridge JA, Leukhardt WH, Golob JF, McCoy AM, Malangoni MA. Moving beyond traditional measurement of mortality after injury: evaluation of risks for late death. J Am Coll Surg. 2010;210(5):788–94, 94–6. [PubMed: 20421051]
26. Davidson GH, Hamlat CA, Rivara FP, Koepsell TD, Jurkovich GJ, Arbabi S. Long-term survival of adult trauma patients. JAMA. 2011;305(10):1001–7. [PubMed: 21386078]
27. Fawcett VJ, Flynn-O'Brien KT, Shorter Z, Davidson GH, Bulger E, Rivara FP, et al. Risk factors for unplanned readmissions in older adult trauma patients in Washington State: a competing risk analysis. J Am Coll Surg. 2015;220(3):330–8. [PubMed: 25542280]
28. Brakenridge SC, Phelan HA, Henley SS, Golden RM, Kashner TM, Eastman AE, et al. Early blood product and crystalloid volume resuscitation: risk association with multiple organ dysfunction after severe blunt traumatic injury. J Trauma. 2011;71(2):299–305. [PubMed: 21825930]
29. Carmichael ED, Apple CG, Kannan KB, Gardener A, Anton S, Efron PA, et al. Chronic Critical Illness in Patients With Sepsis is Associated With Persistent Anemia, Inflammation, and Impaired Functional Outcomes. Am Surg. 2023;89(6):2563–71. [PubMed: 35593749]
30. Stern K, Qiu Q, Weykamp M, O'Keefe G, Brakenridge SC. Defining Posttraumatic Sepsis for Population-Level Research. JAMA Netw Open. 2023;6(1):e2251445. [PubMed: 36652245]
31. Balch JA, Chen UI, Liesenfeld O, Starostik P, Loftus TJ, Efron PA, et al. Defining critical illness using immunological endotypes in patients with and without of sepsis: A cohort study. Res Sq. 2023.
32. Ferreira da Mota NV, Brunialti MKC, Santos SS, Machado FR, Assuncao M, Azevedo LCP, et al. Immunophenotyping of Monocytes During Human Sepsis Shows Impairment in Antigen Presentation: A Shift Toward Nonclassical Differentiation and Upregulation of FCgammaRi-Receptor. Shock. 2018;50(3):293–300. [PubMed: 29668526]
33. Tincati C, Cannizzo ES, Giacomelli M, Badolato R, d'Arminio Monforte A, Marchetti G. Heightened Circulating Interferon-Inducible Chemokines, and Activated Pro-Cytolytic Th1-Cell Phenotype Features Covid-19 Aggravation in the Second Week of Illness. Front Immunol. 2020;11:580987. [PubMed: 33193384]

34. Patel AA, Zhang Y, Fullerton JN, Boelen L, Rongvaux A, Maini AA, et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. J Exp Med. 2017;214(7):1913–23. [PubMed: 28606987]
35. Chen Y, Zhang J, Cui W, Silverstein RL. CD36, a signaling receptor and fatty acid transporter that regulates immune cell metabolism and fate. J Exp Med. 2022;219(6).
36. Haslbauer JD, Zinner C, Stalder AK, Schneeberger J, Menter T, Bassetti S, et al. Vascular Damage, Thromboinflammation, Plasmablast Activation, T-Cell Dysregulation and Pathological Histiocytic Response in Pulmonary Draining Lymph Nodes of COVID-19. Front Immunol. 2021;12:763098. [PubMed: 34966385]
37. Xu Y, Ma Q, Ren J, Chen L, Guo W, Feng K, et al. Using Machine Learning Methods in Identifying Genes Associated with COVID-19 in Cardiomyocytes and Cardiac Vascular Endothelial Cells. Life (Basel). 2023;13(4).
38. Yang CA, Huang YL, Chiang BL. Innate immune response analysis in COVID-19 and kawasaki disease reveals MIS-C predictors. J Formos Med Assoc. 2022;121(3):623–32. [PubMed: 34193364]
39. Zhao L, Varghese Z, Moorhead JF, Chen Y, Ruan XZ. CD36 and lipid metabolism in the evolution of atherosclerosis. Br Med Bull. 2018;126(1):101–12. [PubMed: 29534172]
40. Wypasek E, Padjas A, Szymanska M, Plens K, Siedlar M, Undas A. Non-classical and intermediate monocytes in patients following venous thromboembolism: Links with inflammation. Adv Clin Exp Med. 2019;28(1):51–8. [PubMed: 30088349]
41. Feng L, Zhou X, Su LX, Feng D, Jia YH, Xie LX. Clinical significance of soluble hemoglobin scavenger receptor CD163 (sCD163) in sepsis, a prospective study. PLoS One. 2012;7(7):e38400. [PubMed: 22911680]
42. Ranjbar M, Rahimi A, Baghernejadan Z, Ghorbani A, Khorramdelazad H. Role of CCL2/CCR2 axis in the pathogenesis of COVID-19 and possible Treatments: All options on the Table. Int Immunopharmacol. 2022;113(Pt A):109325. [PubMed: 36252475]
43. Utrero-Rico A, Gonzalez-Cuadrado C, Chivite-Lacaba M, Cabrera-Marante O, Laguna-Goya R, Almendro-Vazquez P, et al. Alterations in Circulating Monocytes Predict COVID-19 Severity and Include Chromatin Modifications Still Detectable Six Months after Recovery. Biomedicines. 2021;9(9).
44. Nabilah MH, Wardhani P, Aryati A, Hernaningsih Y, Semedi BP. The Comparison of Monocyte Human Leukocyte Antigen-D-Related (mHLA-DR) Expression Levels Between Corona Virus Disease 2019 (COVID-19) Patients and Healthy Subjects. Acta Med Indones. 2023;55(1):52–60. [PubMed: 36999265]
45. Raghuwanshi B, Singh A, Singh A. Recurrent Viral Infections Post COVID 19. Mymensingh Med J. 2023;32(2):587–9. [PubMed: 37002775]
46. Rajamanickam A, Kumar NP, Pandiarajan AN, Selvaraj N, Munisankar S, Renji RM, et al. Dynamic alterations in monocyte numbers, subset frequencies and activation markers in acute and convalescent COVID-19 individuals. Sci Rep. 2021;11(1):20254. [PubMed: 34642411]
47. Sebastian A, Sanju S, Jain P, Priya VV, Varma PK, Mony U. Non-classical monocytes and its potential in diagnosing sepsis post cardiac surgery. Int Immunopharmacol. 2021;99:108037. [PubMed: 34426113]
48. Marcos-Morales A, Barea-Mendoza JA, Garcia-Fuentes C, Cueto-Felgueroso C, Lopez-Jimenez A, Martin-Loeches I, et al. Elevated monocyte distribution width in trauma: An early cellular biomarker of organ dysfunction. Injury. 2022;53(3):959–65. [PubMed: 34893306]

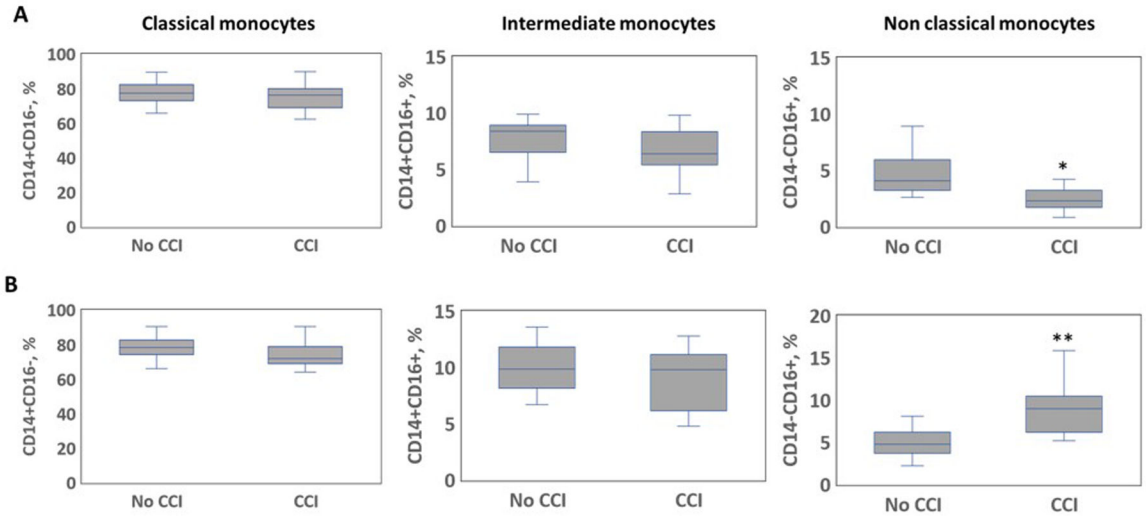

Figure 1: Monocyte subset distribution on day 1 (A) and day 5 (B) following severe hemorrhagic trauma.

The distribution data for patient that did not develop CCI (n=54) and patients that develop CCI (p=16) were evaluated by CyTOF for monocyte phenotype distribution of classical (CL), intermediate (INT) and non-classical (NC). With only significant difference within NC monocytes at day 1 (*p=0.005) and day 5 (**p=0.019)

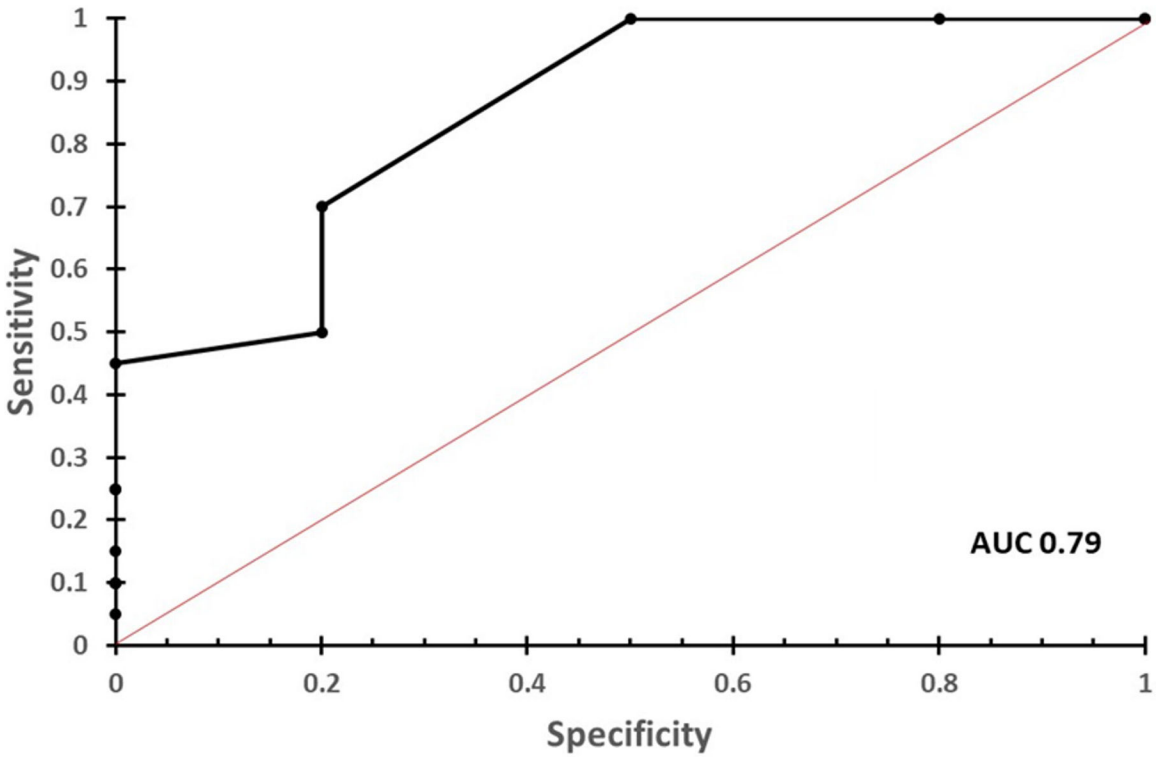

Figure 2: Receiver operative curve of non-classical/classical monocyte ratio correlation with the development of CCI.

ROC analysis was performed for the inverse ratio of non-classical/classical monocyte and development of CCI. The Area Under the Curve (AUC) was determined to be 0.82, with a $p<0.05$.

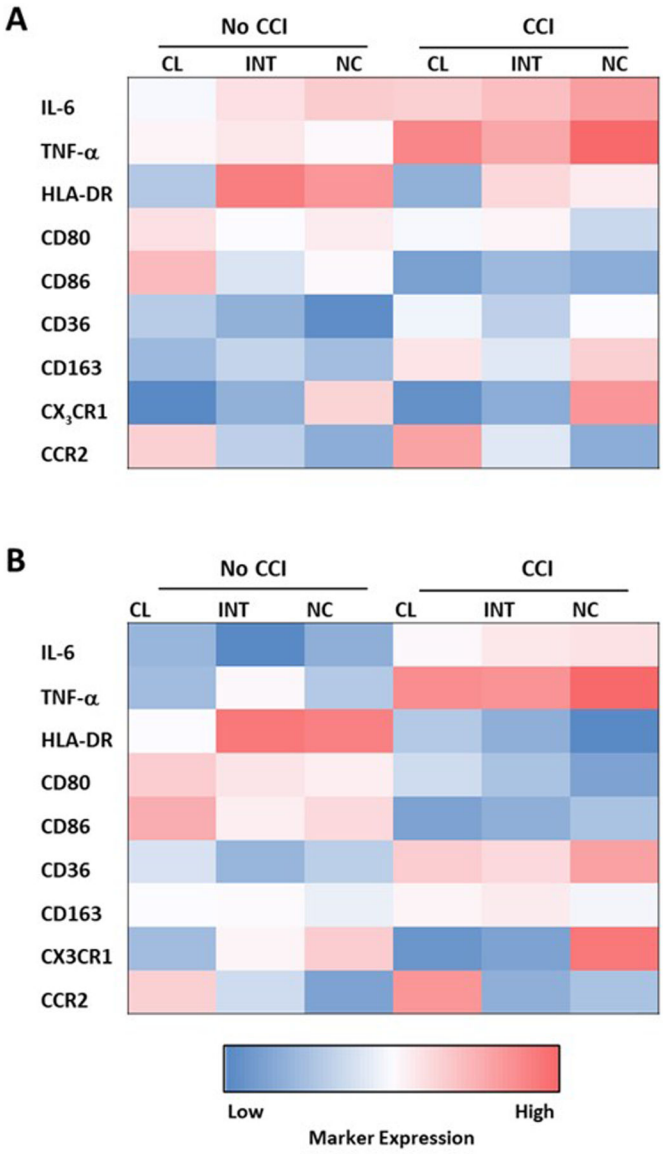

Figure 3:

CyTOF heatmap demonstrating expression changes of intracellular cytokines and cell surface expression in Classical (CL), Intermediate (INT) and Non-Classical (NC) monocytes on day 1 (A) and day 5 (B) following severe hemorrhagic trauma.

Table 1.

Patient demographics and outcome with and without CCI

Variable	No CCI (N-54)	CCI (N-26)	P-value
Age, years – (mean, SD)	42 ± 16	44 ±15	0.594
Male sex, n(%)	41 (76)	21 (81)	0.627
Body mass index (BMI) – (mean, SD)	26.4 + 4.6	27.2 + 5.2	0.487
Shock*, n(%)	36 (72)	23 (88)	0.038
Base Def >6, n(%)	34 (63)	20 (77)	
Lactate >4 mmol/L, n(%)	32 (59)	19 (73)	
Hypotension (systolic blood pressure (SBP) <90), n(%)	33 (61)	20 (77)	0.161
Injury severity score – (mean, SD)	29.6 ± 4.1	32.4 ± 5.2	0.011
Red Blood Cell Transfusion, units – (mean, SD)	4.7 ± 3.8	8.9 ± 3.8	<0.001
Plasma (FFP) Transfusion, units – (mean, SD)	4.2 ± 4.3	7.2 ± 5.1	0.007
Platelets (PLT) Transfusion, units – (mean, SD)	1.2 ± 1.4	2.2 ± 1.9	0.010
Mechanical Ventilation, n(%)	47(87.0)	25(96.2)	0.380
Ventilator-Free Days (28 d), (mean, SD)	23.8 + 4.6	8.9 + 10.6	<0.001
Time to recovery, days – (mean, SD)	7.9 + 6.7	26.9 + 5.7	<0.001
In-Hospital Mortality – no (%)	0(0)	2 (7.7)	0.194
ICU Length of Stay, days – (mean, SD)	8.1 + 7.4	27.8 + 11.6	<0.001
Hospital Length of Stay, days – (mean, SD)	19.4 + 10.8	37.2 + 12.3	<0.001
Nosocomial infections, n(%)	19(35.2)	23(88.5)	<0.001
Discharge Disposition			0.002
“Good” disposition, n(%)	38(70.4)	8(30.8)	
“Bad” disposition, n(%)	16(29.6)	18(69.2)	
1 Year Mortality – no (%)	2(3.8)	7(26.9)	0.007

* Defined as initial base deficit >6 and/or initial lactate >4

Table 2.

Multivariable analysis of impact of non-classical monocytes on CCI and 1 year mortality.

Outcome	Variable	Odds Ratio	95% CI	p-value
CCI	Injury Severity Score	1.05	1.01 – 1.23	0.015
	Red blood transfusion > 6 units/12 hrs	1.72	1.16 – 3.42	0.011
	Non classical/classical ratio < 0.035	3.46	1.68 – 6.84	0.009
1 year mortality	Age	1.04	1.01 – 1.36	0.026
	Injury Severity Score	1.06	1.01 −1.29	0.021
	Red blood transfusions > 6 units/12 hrs	1.49	1.12 – 3.58	0.019
	Non classical/classical ratio < 0.035	2.76	1.43 – 7.21	0.013