REDUCED RESPONSIVENESS OF BLOOD LEUKOCYTES TO LIPOPOLYSACCHARIDE DOES NOT PREDICT NOSOCOMIAL INFECTIONS IN CRITICALLY ILL PATIENTS

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INTRODUCTION

Nosocomial infection is a common complication in critically ill patients and strongly associated with prolonged stay in the intensive care unit (ICU) and increased morbidity and mortality (1). Critical illness results in a disturbed homeostasis leading to features of both hyperinflammation and immune suppression (2). Although originally immune suppression was considered to follow the initial proinflammatory phase (3,4), a recent study conducted in trauma and burn patients revealed evidence for both activation and impairment of immune pathways within hours of the injury using whole-blood leukocyte transcriptional profiling (5). A hallmark functional feature of immune suppression accompanying critical illness is the reduced capacity of whole blood to produce proinflammatory cytokines on stimulation with bacterial agonists ex vivo (3,4,6).

Immune suppression is considered an important risk factor for secondary infection in ICU patients, and immunostimulatory therapy is a newly proposed treatment strategy in this population (4). A clinical challenge herein is to identify patients who may benefit from such therapies, that is, those at high risk for nosocomial infections. Although multiple ways of immune monitoring have been developed (6), whole-blood stimulation tests provide the potential advantage that they can be done in a rapid and reproducible way in a routine setting (7). In this study, we aimed to determine whether the extent of reduced whole-blood leukocyte responsiveness to lipopolysaccharide (LPS) relates to the subsequent development of ICU-acquired infections in critically ill patients.
cytokine levels in samples incubated without LPS from those measured in samples obtained after incubation with LPS.

An ICU-acquired infection was defined by the systemic therapeutic administration of antibiotics for a suspected new infection of more than 48 h after ICU admission. The presence of infection (either on admission or ICU acquired) was established for every affected organ or site using Center for Disease criteria (11) and International Sepsis Forum consensus definitions (12) as described in detail elsewhere (8). Dedicated research physicians categorized the plausibility of infection based on a post hoc review of all available clinical, radiological, and microbiological evidence (8); patients treated for a suspected infection but with a post hoc infection likelihood of none were not considered infectious.

**Statistical analysis**

All results are presented as numbers (percentages) for categorical variables, median and interquartile ranges (IQRs) for nonparametric quantitative variables, and mean ± SD for parametric quantitative variables. Continuous nonparametric data were analyzed using a Mann-Whitney U test or a Kruskal-Wallis test; categorical data were analyzed using a χ² or Fisher exact test. Continuous parametric data were analyzed using a Student t test or analysis of variance when appropriate. A value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Patients and ICU-acquired infections**

Seventy-three critically ill patients (Table 1) and 18 healthy age (median, 63 years [IQR, 52 – 71 years])- and sex-matched (39% male) controls were included. Admission diagnoses are provided in Table 2. In total, 47 patients (64%) had a sepsis admission diagnosis, whereas 26 patients (36%) were admitted for a noninfectious condition. Ten patients developed an

| Table 1. Patient characteristics at baseline | All | ICU-AI | No ICU-AI | P |
|--------------------------------------------|-----|--------|----------|---|
| **Age, years**                             |     |        |          |   |
| Median (IQR)                               |     |        |          |   |
|                                         | n = 73 | n = 10 | n = 63 | 0.59 |
| Sex, n (%)                                 |     |        |          |   |
| Male                                       | 43 (58) | 4 (40) | 39 (62) | 0.42 |
| BMI                                        |     |        |          |   |
| Median (IQR)                               |     |        |          |   |
|                                         | 25 (23 – 29) | 25 (24 – 30) | 25 (23 – 29) | 0.86 |
| Race, n (%)                                |     |        |          |   |
| White                                      | 60 (82) | 8 (80) | 52 (83) | 0.33 |
| Comorbidity, n (%)                         |     |        |          |   |
| None                                       | 20 (27) | 2 (20) | 18 (29) | 0.72 |
| Cancer (nonhematologic)                    | 6 (8) | 1 (10) | 5 (8) | 1.00 |
| COPD                                       | 9 (12) | —      | 9 (14) | 0.34 |
| Diabetes mellitus                          | 12 (16) | 2 (20) | 10 (16) | 1.00 |
| Hypertension                               | 23 (32) | 4 (40) | 19 (30) | 0.70 |
| Immune deficiency                          | 6 (8) | 1 (10) | 5 (8) | 1.00 |
| History of myocardial infarction           | 7 (10) | 1 (10) | 6 (10) | 1.00 |
| Acute morbidity on admission               |     |        |          |   |
| Sepsis, n (%)                              | 47 (64) | 8 (80) | 39 (62) | 0.31 |
| APACHE IV score, mean (SD)                 | 78 (27) | 86 (35) | 77 (26) | 0.44 |
| SOFA score, median (IQR)                   | 7 (5 – 8) | 7 (5 – 8) | 7 (5 – 8) | 1.00 |
| Organ failure at admissions, n (%)         |     |        |          |   |
| None                                       | 4 (5) | —      | 4 (6) | 0.63 |
| Cardiovascular failure                     | 56 (77) | 6 (60) | 50 (79) | 0.08 |
| Respiratory failure                        | 35 (48) | 5 (50) | 30 (48) | 1.00 |
| Renal failure                              | 12 (16) | 3 (30) | 9 (14) | 0.34 |
| Coagulation failure                        | 1 (1) | —      | 1 (2) | 1.00 |
| Shock                                      | 18 (25) | 3 (30) | 15 (24) | 0.06 |
| Mechanical ventilation                     | 57 (78) | 8 (80) | 49 (78) | 1.00 |

**Laboratory parameters first 24 h**

| Parameter                  | All | ICU-AI | No ICU-AI | P |
|----------------------------|-----|--------|----------|---|
| CRP, U/L                   | 103 (13 – 183) | 183 (147 – 200) | 81 (10 – 152) | 0.24 |
| Lactate max, mm/L          | 2.4 (1.6 – 6.1) | 3.4 (2.0 – 7.4) | 2.4 (1.6 – 6.0) | 0.63 |
| Platelet count, *10⁹/L     | 157 (121 – 211) | 147 (108 – 174) | 158 (129 – 212) | 0.37 |
| WBC max, *10⁹/L            | 13 (10 – 18) | 13 (12 – 16) | 13 (10 – 18) | 0.60 |
| Neutrophils, *10⁹/L        | 9.61 (7.2 – 12.3) | 8.88 (7.48 – 11.63) | 10.28 (7.12 – 12.37) | 0.94 |
| Lymphocytes, *10⁹/L        | 0.92 (0.67 – 1.45) | 1.44 (0.72 – 1.58) | 0.89 (0.68 – 1.33) | 0.54 |
| Monocytes, *10⁹/L          | 0.59 (0.40 – 0.93) | 0.64 (0.57 – 0.94) | 0.58 (0.38 – 0.93) | 0.70 |

APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ICU-AI, intensive care unit-acquired infection; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell count.

*All laboratory parameters are given in medians (IQR).
Overall ICU mortality was 15% (30% in cases and 13% in ICU controls [IQR, 2–6 days] vs. 3 days in ICU controls [IQR, 2–6 days]). Cases had a longer length of ICU stay (median, 22 days [IQR, 16–24 days]; P = 0.35). The incidence of secondary infection in our ICU cohort was 13.7%. Previous studies have reported incidence rates varying between 9% and 37%, largely dependent on the population studied and the definitions used (1). Our study was conducted in a mixed surgical-medical ICU in an academic hospital. We used strict definitions and post hoc classification by dedicated research physicians to diagnose ICU-acquired infections (8).

In theory, functional tests, such as ex vivo stimulation of whole blood, represent the best method to establish the function of the innate immune system because they directly measure the capacity of relevant cells to react to a microbial challenge (6). Whole-blood stimulation is an easy-to-perform test that could be implemented in routine practice (7). In the present study, we intentionally used a short incubation period (3 h) considering that, if deemed clinically relevant, the test should yield results relatively quickly. The cytokines measured on a 3-h LPS stimulation of whole blood likely are mainly produced by monocytes. In accordance, monocytes demonstrated a reduced capacity to release proinflammatory cytokines in response to LPS in a variety of clinical settings, including sepsis and noninfectious systemic inflammatory conditions (3,6). In our cohort, whole-blood cytokine production corrected for absolute monocyte counts (collected in 81% of the patients included) also did not discriminate between patients who did and those who did not develop an ICU-acquired infection (data not shown).

**DISCUSSION**

In accordance with previous investigations (3,4,6), we report a strongly impaired release of TNF-α, IL-1β, and IL-6 in LPS-stimulated whole blood obtained from critically ill patients when compared with healthy controls. However, the extent of the reduction in cytokine release capacity did not relate to the subsequent development of ICU-acquired infections. These results argue against the use of whole-blood stimulation as a functional test of innate immunity applied early after ICU admission to predict nosocomial infection.

The whole-blood cytokine production capacity did not differ between those who did and those who did not develop an ICU-acquired infection (data not shown).

**Whole-blood stimulations**

Whole-blood leukocytes of critically ill patients, harvested on the first morning after ICU admission, released significantly less TNF-α, IL-1β, and IL-6 on stimulation with LPS than blood leukocytes from healthy controls (Fig. 1). Whole blood from patients with a sepsis admission diagnosis released less TNF-α when compared with whole blood from patients with a noninfectious admission diagnosis (P = 0.002), whereas IL-1β and IL-6 release did not differ between these groups (Fig. 2). The whole-blood cytokine production capacity did not differ between patients who subsequently developed an ICU-acquired infection and ICU controls (Fig. 1). Similarly, when analyzed separately, whole-blood cytokine production capacity in patients with a sepsis admission diagnosis did not differ between those who did and those who did not develop an ICU-acquired infection (data not shown).

| Table 2. Admission diagnosis |
|-----------------------------|
| n  | %   |
|-----------------------------|
| Sepsis admission diagnosis  | 47  | 64  |
| Community-acquired pneumonia| 25  |     |
| Abdominal sepsis            | 5   |     |
| Urinary tract infection     | 5   |     |
| Hospital-acquired pneumonia| 4   |     |
| Skin infection              | 3   |     |
| Brain abscess               | 1   |     |
| Mediastinitis               | 1   |     |
| Primary meningitis          | 1   |     |
| Pharyngitis                 | 1   |     |
| Sinusitis                   | 1   |     |
| Noninfectious admission diagnosis | 26  | 36  |
| Carcinoma                   | 6   |     |
| Subdural hematoma/intracranial hemorrhage | 5 |     |
| Cerebrovascular accident/stroke | 3 |     |
| Cardiogenic shock           | 2   |     |
| Exacerbation chronic obstructive pulmonary disease | 2 |     |
| Anaphylaxis                 | 1   |     |
| Asthma                      | 1   |     |
| Coma/change in level of consciousness | 1 |     |
| Gastrointestinal ischemia   | 1   |     |
| Cardiomyopathy              | 1   |     |
| Gastrointestinal bleeding   | 1   |     |
| Pulmonary hemorrhage        | 1   |     |
| Dissected thoracic aortic aneurysm | 1 |     |

ICU-acquired infection (cases) 10 days (median) (IQR, 8–13 days) after ICU admission, whereas 63 patients did not develop an infection during ICU stay (ICU controls). Intensive care unit–acquired infections were composed of catheter-related bloodstream infections (n = 3), pneumonia (n = 3), abdominal infections (n = 2), secondary meningitis, eye infection, wound infection, and skin infection (all n = 1). Two patients developed two ICU-acquired infections at different times during their ICU admission. Baseline characteristics, including comorbidities, a sepsis admission diagnosis, severity of illness (Acute Physiology and Chronic Health Evaluation IV and Sequential Organ Failure Assessment scores, number of organ failures and shock), and white blood cell counts, were not different between cases and ICU controls (Table 1). Cases had a longer length of ICU stay (median, 22 days [IQR, 16–24 days] vs. 3 days in ICU controls [IQR, 2–6 days]; P < 0.0001). Overall ICU mortality was 15% (30% in cases and 13% in controls, P = 0.35).

The results obtained with LPS-induced whole-blood stimulation were not able to predict the subsequent development of ICU-acquired infections. One might argue that our sample size was too small. However, although median TNF-α levels were slightly lower in patients who developed a secondary infection, based on the variation in TNF-α concentrations in the 73 patients included in the present study, we calculated that a study encompassing more than 800 patients would be required to show a statistically significant difference. Hence, such a test will unlikely be of clinical value in daily practice. We observed a relatively uniformly depressed blood leukocyte production with a noninfectious admission diagnosis (P = 0.002), whereas IL-1β and IL-6 release did not differ between these groups (Fig. 2). The whole-blood cytokine production capacity did not differ between patients who subsequently developed an ICU-acquired infection and ICU controls (Fig. 1). Similarly, when analyzed separately, whole-blood cytokine production capacity in patients with a sepsis admission diagnosis did not differ between those who did and those who did not develop an ICU-acquired infection (data not shown).
responsiveness in critically ill patients, suggesting that other factors such as the presence of absence of lines, surgical interventions, and the way care is delivered to the individual patient influence the risk for the development of nosocomial infections to a greater extent than the capacity of innate immune cells to respond to bacterial agonists such as LPS.

Previous studies investigated the value of surrogate markers of suppression of the adaptive immune system to predict secondary infections in ICU patients, especially in those admitted with sepsis. Specifically, reduced expression of human leukocyte antigen-DR and increased expression of programmed cell death (PD)-1, PD-ligand 1, and PD-ligand 2 on blood monocytes, determined by flow cytometry 3 to 5 days after ICU admission, correlated with an enhanced incidence of secondary infections in patients with septic shock (13,14). Measurements were not done earlier after ICU admission. In both earlier investigations, the incidence of nosocomial infections was much higher (24.2% and 29.7%, respectively) (13,14) than observed here (13.7%), suggesting that the populations studied and/or the definitions used for ICU-acquired infection differed. We performed whole-blood stimulation on the first morning after ICU admission, at 9:00 AM, seeking to evaluate a potential early test and avoiding potential circadian variation. Further research is needed to establish whether whole-blood stimulation conducted at later time points after ICU admission can assist in identifying patients at risk for nosocomial infections. Indeed, in a study encompassing 70 critically ill children, among whom 30 with sepsis, a reduced \textit{ex vivo} LPS-induced TNF-\(\alpha\) response in whole blood on day 7 after admission was associated with development of nosocomial infection and death (15).

**CONCLUSIONS**

The extent of reduced LPS responsiveness of whole-blood leukocytes in critically ill patients determined on the first
morning after ICU admission does not relate to the subsequent development of ICU-acquired infections.

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