Journal of Infection (2017) 74, 163—171

BIA
British Infection Association

www.elsevierhealth.com/journals/jinf

Survival after multiple traumas is associated with improved outcomes from gram-negative sepsis: Clinical and experimental evidence

Eleftherios Mandragos [a], Aikaterini Pistiki [a], Iraklis Tsangaris [b], Christina Routsi [c], Michael Paraschos [d], Dionyssia-Irene Droggiti [a], Olga Savvidou [e], Dimitrios Mastrokalos [e], Panayiotis J. Papagelopoulos [e], Mihai G. Netea [f], Evangelos J. Giamarellos-Bourboulis [a,*]

[a] *4th Department of Internal Medicine, National and Kapodistrian University of Athens, Medical School, Greece*
[b] *2nd Department of Critical Care Medicine, National and Kapodistrian University of Athens, Medical School, Greece*
[c] *1st Department of Critical Care Medicine, National and Kapodistrian University of Athens, Medical School, Greece*
[d] *Intensive Care Unit, "Korgialeneion-Benakeion" General Hospital, Athens, Greece*
[e] *1st Department of Orthopaedics, National and Kapodistrian University of Athens, Medical School, Greece*
[f] *Department of Internal Medicine, Radboud University Nijmegen, The Netherlands*

Accepted 28 October 2016
Available online 5 November 2016

KEYWORDS
Multiple trauma;
Sepsis;
Cytokines;
Interleukin-10;
Survival

Summary *Objectives:* We investigated the susceptibility to Gram-negative sepsis after multiple traumas (MT).
Methods: From a prospective cohort of 5076 Greek patients with sepsis, 16 with Gram-negative bacteremia after MT were compared with 204 patients well-matched for severity, comorbidities and appropriateness of antimicrobials; circulating mononuclear cells were isolated and stimulated for the release of interleukin (IL)-10. Male C57Bl6J mice were subject to MT (right pneumothorax and right femur fracture) followed after 72 h by the intravenous challenge with *Pseudomonas aeruginosa*. Survival was recorded and splenocytes were isolated for cytokine stimulation.

* Corresponding author. 4th Department of Internal Medicine, ATTIKON University Hospital, 1 Rimini Street, 12462 Athens, Greece. Fax: +30 210 53 26 446.
E-mail address: egiamarel@med.uoa.gr (E.J. Giamarellos-Bourboulis).

http://dx.doi.org/10.1016/j.jinf.2016.10.007

Results: 28-day mortality after MT was 18.8% compared to 48.0% of comparators (48.0%) (odds ratio 0.25, p: 0.035). This was confirmed after logistic regression analysis taking into consideration comorbidities and age. Stimulation of IL-10 was enhanced from MT patients. Survival of mice challenged by *P. aeruginosa* 72 h after MT was prolonged compared to mice challenged by *P. aeruginosa* without prior MT. Cytokine production was decreased 24 h after MT and restored 96 h thereafter. Production of IL-10 was particularly pronounced from splenocytes of mice challenged by *P. aeruginosa* after MT.
Conclusions: Survival after MT is accompanied by favorable immune responses allowing survival benefit from Gram-negative sepsis. This is associated with increased IL-10 release.

Introduction

Sepsis develops when well-conserved structures of microorganisms (also known as pathogen-associated molecular patterns, PAMPs) bind to transmembrane or intracellular receptors of the innate immune system and particularly to blood monocytes and tissue macrophages. This binding leads to the release of pro-inflammatory and anti-inflammatory cytokines which induce clinical sepsis.[1] The most widely studied PAMP is endotoxin (lipopolysaccharide, LPS) of the cell wall of Gram-negative bacteria which binds to the transmembrane Toll-like receptor (TLR-4) on monocytes and macrophages. Exposure to bacterial LPS causes monocytes and macrophages to become tolerant showing decreased cytokine production upon LPS re-exposure.[2]

Multiple traumas are a major cause of death of patients hospitalized in Intensive Care Units (ICU).[3] Patients usually present with systemic inflammatory response of non-bacterial etiology at their initial admission to the ICU. Patients surviving this post-traumatic response often develop sepsis as a consequence of bacterial superinfection. The initial post-traumatic systemic inflammatory response is caused by the stimulation of leukocytes to release inflammatory mediators during binding of endogenous ligands released after injury to the TLRs. Examples of such components are uric acid, mitochondrial DNA and non-histone nuclear proteins known as "alarmins" or danger-associated molecular patterns (DAMPs).[4,5]

In analogy to the phenomenon of tolerance to bacterial LPS, it would be expected that the innate immune cells of the trauma patients manifest tolerance to alarmins and consequently would release less cytokines after re-exposure to bacterial PAMPs. If this hypothesis holds true, patients bearing multiple traumas when they develop severe sepsis may have a relatively better prognosis compared to patients without prior multiple traumas as the predisposing factor for severe sepsis. On the other hand, long-term depression of immune responses after multiple traumas could also be associated with increased susceptibility to opportunistic infections. This study attempts to investigate the impact of multiple traumas on the outcome of sepsis using a two-step approach. At the first step, the impact of previous multiple traumas on sepsis outcome is explored after analysis of a large scale database. At the second step an animal model is studied to analyze the mechanism of modulation of the innate immune responses by preceding multiple traumas.

Patients and methods

Clinical study

Since 2007, the clinical course of patients with sepsis syndrome has been recorded by a prospective registry conducted by the Hellenic Sepsis Study Group; 43 Departments of Internal Medicine, 19 ICUs and eight Department of Surgery participate in this registry after approval by the Ethics Committees of the hospitals. Written informed consent was provided by the patients or their first-degree relatives if patients were unable to provide the consent. Inclusion criteria were: a) age $\geq$18 years old; b) one of the following infections: acute pyelonephritis, community acquired pneumonia, acute intra-abdominal infection, primary Gram-negative bacteremia, ventilator-associated pneumonia; and c) uncomplicated sepsis, severe sepsis or septic shock. Exclusion criteria were: a) HIV-1 infection; b) neutropenia defined as less than 1000 neutrophils/mm^3; and c) chronic use of corticosteroids defined as more than 1 mg/kg/day of prednisone equivalent for more than 15 days. Inclusion criteria (b) and (c) were defined according to international definitions.[6,7]

Of all patients entered in the database, those who developed ICU-acquired sepsis due to Gram-negative bacteremia during their ICU hospitalization for multiple injuries were selected. These patients were compared to a group of patients without multiple injuries selected to be well-matched for severity, Charlson's Comorbidity Index (CCI) and appropriateness of the administered antimicrobials. The CCI was calculated for each patient as defined elsewhere.[8] Antimicrobial treatment was considered appropriate when the isolated pathogen was susceptible to at least one of the empirically prescribed antimicrobials according to the antibiogram. Meanwhile, 10 ml of heparinized venous blood was obtained after venipuncture of the forearm under aseptic conditions and within 24 h of the onset of sepsis from patients with and without multiple traumas hospitalized in the ICU of Korgialeneion-Benakeion hospital of Athens. Peripheral mononuclear blood cells (PBMCs) were isolated after centrifugation of the blood over Ficoll-Hypaque (Biochrom). After three successive washings with ice-cold PBS (phosphate buffered saline, pH: 7.2) the number of PBMCs was counted in a