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# Influence of gender on systemic IL-6 levels, complication rates and outcome after major trauma

Katharina Mörs, Olivia Braun, Nils Wagner, Birgit Auner, Maika Voth, Philipp Störmann, Sebastian Wutzler, Ingo Marzi, Borna Relja*

*Department of Trauma, Hand and Reconstructive Surgery, University Hospital Frankfurt, Goethe University, 60590 Frankfurt, Germany*

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ABSTRACT

*Background:* While female gender was associated with lower rates of systemic inflammatory response syndrome (SIRS), sepsis and single and/or multiple organ failure (MOF), contradictory data suggest no correlation between gender and complication rates and/or outcome in trauma patients (TP). Here, we analyzed the gender influence on systemic interleukin (IL)-6 levels and outcome in TP.

*Patients/Methods:* 343 TP with injury severity scores (ISS) $\geq$16 were included upon admittance to the emergency department (ED) and grouped to male (n = 257) *vs.* female (n = 86). Injury severity, vital signs, physiological parameters, length of intensive care unit (ICU) and in-hospital stay, outcome parameters including SIRS, sepsis, respiratory complications, single- and/or MOF and in-hospital mortality were analyzed. Systemic IL-6 levels during the first 10 post-injury days were determined daily.

*Results:* Age (45.0 ± 1.0 *vs.* 48.2 ± 2.1) and ISS (27.1 ± 0.8 *vs.* 24.7 ± 1.2) were comparable between both groups. Abbreviated Injury Scale (AIS) $\geq$3 of chest and abdominal body regions were significantly higher in male TP (chest:51.02% *vs.* 36.05%, abdomen:19.84% *vs.* 10.47%, $p < 0.05$). IL-6 was significantly increased in male TP on post-injury days 1 and 2 (d1:363.9 ± 72.58 *vs.* 163.7 ± 25.98; d2:194.3 ± 31.38 *vs.* 114.3 ± 17.81 pg/ml, $p < 0.05$). Multivariate analysis excluded an association of increased chest or abdominal injury occurrence with IL-6 levels. Female *vs.* male TP had significantly lower SIRS and sepsis occurrence (SIRS:40.70% *vs.* 53.31%, sepsis:6.98% *vs.* 19.46%, $p < 0.05$). There were no gender-based differences regarding ICU or in-hospital stay, single and/or MOF and respiratory complications.

*Conclusions:* Taken together, higher systemic IL-6 levels after trauma are associated with enhanced susceptibility for SIRS and sepsis in male patients.



## 1. Introduction

Traumatic injury is one of the leading causes of morbidity and mortality, with men being affected more frequently than women (Alberdi et al., 2014; Bardenheuer et al., 2000; Sakran et al., 2012). Next to fatal initial injuries resulting in early mortality after trauma, secondary infectious complications often leading to single or multiple organ failure (MOF) constitute predominant causes of death in the later clinical post-injury course of trauma patients (Wafaisade et al., 2011). Thus, recovery and outcome after major trauma are strongly influenced by the occurrence of post-injury complications.

It is well accepted that gender influences both, manifestation and recovery of several acute and chronic diseases in humans (Pinn, 2003). Regarding major trauma patients numerous studies have suggested gender-based differences in the recovery and outcome (Napolitano et al., 2001; Yang et al., 2014). Clinical studies have shown improved outcome after circulatory and septic shock as well as a lower overall mortality after blunt trauma in females (Trentzsch et al., 2014; George et al., 2003a). Trentzsch et al. (2015) and Mostafa et al. (2002) reported less MOF occurrence, shorter stay at the intensive care unit (ICU) and decreased mortality rates after severe trauma in females. Frink et al. (2007) found significantly lower occurrence of multiple organ dysfunction syndrome (MODS) and sepsis in female trauma patients (TP). Moreover, female TP were less susceptible to systemic inflammatory response syndrome (SIRS) and had less infectious complications such as pneumonia or sepsis after major trauma in other clinical studies (Napolitano et al., 2001; Haider et al., 2009; Schoeneberg et al., 2015; Gannon et al., 2004; Mica et al., 2013). Taken together, these studies indicate gender-specific results. In contrast to this data, Heffernan et al. observed increased incidence of acute respiratory distress syndrom (ARDS) in female patients after trauma (Heffernan et al., 2011),

* Corresponding author.
*E-mail address:* info@bornarelja.com (B. Relja).

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while others have reported no association between gender and the frequency of infectious complications or mortality rates after severe injury (Angele et al., 2006; Zellweger et al., 1997; Sheth et al., 2011; Wichmann et al., 1997).

Rationales for the observed gender differences are delivered mainly by experimental *in vivo* models showing that female sex hormones, notably estrogen, were associated with beneficial effects on immune status and organ integrity in models of hemorrhage and sepsis (Angele et al., 2006; Zellweger et al., 1997; Knöferl et al., 2002). Nevertheless, observations regarding the immune status and female sex hormones in humans are sparse, yet. In response to trauma initially developed SIRS, which is characterized by increased circulating levels of the pro-inflammatory cytokine interleukin (IL)-6, has been described (Wutzler et al., 2013; Biberthaler and van Griensven, 2014; Lenz et al., 2007). As demonstrated by Gebhard et al. (2000) IL-6 acts as an acute phase protein and denotes a reliable marker of the injury severity in trauma patients or severity of sepsis in critically ill patients (Damas et al., 1992; Stensballe et al., 2009; Okeny et al., 2015). To our knowledge, there is only sparse data referring possible differences in systemic IL-6 levels between female and male trauma patients or their association with the clinical course regarding post-injury complications or outcome.

This study aimed at elaborating the role of gender on the inflammatory status of TP reflected by systemic IL-6 levels. We also examined the influence of gender on the occurrence of SIRS and clinical complications, single and/or MOF, pneumonia, ARDS and sepsis after major trauma in our study population.

## 2. Patients and methods

### 2.1. Ethics

The study was performed in the University Hospital Frankfurt of the Goethe-University with institutional ethics committee approval in accordance with the Declaration of Helsinki and following STROBE-guidelines (von Elm et al., 2008). All included subjects signed the written informed consent forms themselves or informed consent was obtained from the nominated legally authorized representative consented on the behalf of participants as approved by the ethical committee.

### 2.2. Patients

343 patients admitted to our emergency department (ED) with a history of acute blunt or penetrating trauma from 2008 until 2012 were included in this study. Inclusion criteria consisted of an injury severity score (ISS) $\geq$16 and an age between 18 and 80 years. Patients who died in the ED or within 24 h of hospital admission as well as patients with known pre-existing immunological disorders, immunosuppressive and anti-coagulant medication, burns, concomitant acute myocardial infarction or thromboembolic events were excluded.

### 2.3. Study setting

All trauma patients were treated in the ED according to the Advanced Trauma Life Support (ATLS) standards and the up-to-date guidelines for polytrauma management (Marzi and Rose, 2012; Bouillon et al., 2013). Injury severity from trauma was calculated using the Injury Severity Score (ISS) (Baker et al., 1974) based on the Abbreviated Injury Scale (AIS) (Palmer et al., 2015).

The demographic data (age and gender), injury severity parameters (ISS, AIS head, chest, abdomen, extremities), systolic blood pressure (SBP), prehospital shock index (heart rate (HR)/(SBP)), amount of packed red blood cells (PRBC) and fresh frozen plasma (FFP) administered within the initial 24 h or in total, lactate, hemoglobin, length of the stay at the ICU or in hospital stay and in-hospital mortality were recorded. A defined laboratory profile of coagulation parameters including platelets, fibrinogen and international normalized ratio (INR) were measured from samples obtained at the ED. Serum level of IL-6 was measured beginning at the admission to the ED until day 10 daily after trauma.

SIRS and sepsis were assessed using the 2005 criteria outlined by the International Sepsis Forum (Calandra and Cohen, 2005). SIRS was defined by fulfilling two or more of the following criteria: heart rate >90 beats per minute; respiratory rate >20 breaths per minute or arterial carbon dioxide tension ($PaCO_2$) <32 mmHg; body temperature >38 °C or <36 °C; and white blood cell count >12.000 cells/mm$^3$ or <4.000 cells/mm$^3$, or with >10% immature (band) forms as previously described (Relja et al., 2013). Sepsis was diagnosed by fullfilling SIRS criteria and having a proven infection. ARDS was defined as previously described (Bernard et al., 1994; Ferguson et al., 2012). Pneumonia was defined by clinical, radiologic and bacteriologic findings including new pulmonary infiltrates on chest X-ray as well as one of the following criteria: positive blood culture, bronchial alveolar lavage and/or sputum culture (Bauer et al., 2005). Organ failure was defined using the SOFA-score (Vincent et al., 1996).

### 2.4. Blood processing and analysis

Blood samples were obtained as early as possible after admission of the patient to the ED as well as on day 1 until day 10 daily after trauma for routine diagnostics and laboratory investigations. The blood sample was obtained in pre-chilled ethylenediaminetetraacetic acid (EDTA) tubes (BD vacutainer, Becton Dickinson Diagnostics, Aalst, Belgium) and kept on ice. Blood was centrifuged at 2000*g* for 15 min at 4 °C. Subsequently the supernatant was stored at −80 °C until IL-6 and IL-10 analysis. Interleukin-6 and IL-10 concentrations were measured by IL-6 or IL_10 Eli-pair ELISA-Assays (Diaclone, Hoelzel Diagnostica, Cologne, Germany) according to the manufacturer‘s instructions. Blood counts were obtained by standard clinical methods using the Sysmex XE-2100 automated blood cell counter (Sysmex Europe GmbH, Norderstedt, Germany). Fibrinogen concentration was determined using the Clauss method and both, fibrinogen and the INR were assayed by an automated coagulation analyzer (STA-R Evolution, Roche AG, Grenzach, Germany).

### 2.5. Statistics

The Kolmogoroff-Smirnoff-Lillieford’s test showed that the plasma concentrations of IL-6 did not have a Gaussian distribution. Continuous variables were compared between female *vs.* male group using the Mann-Whitney *U* test and the Bonferroni adjustment of the p-value in order to deal post-hoc. Categorical variables were analyzed by the two-sided Fisher‘s exact test. The multivariate analysis was performed by one-way MANOVA and LSD *post-hoc* test. Data are shown as the mean ± standard error of the mean (sem) unless otherwise stated. P-value <0.05 was considered statistically significant. GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA) and Statistical Package for Social Sciences 16.0 (SPSS, Chicago, Illinois, USA) were used to perform the statistical analysis.

## 3. Results

A total of 343 patients, therefrom 86 female and 257 male met the inclusion criteria while showing non of the exclusion criteria, and were therefore included in this study. The mean age with 45.0 ± 1.1 in male was statistically comparable to 48.2 ± 2.1 years of age in female trauma patients (Table 1). All patients were substantially injured with mean ISS scores of 27.1 ± 0.8 and 24.7 ± 1.2 in