Original article

Early inflammatory response in polytraumatized patients: Cytokines and heat shock proteins. A pilot study

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ABSTRACT

Introduction: In the initial phases after polytrauma there is an hyperinflammatory state that sometimes leads to multiple organ dysfunction syndrome (MODS) and death, and that appears to be responsible for posttraumatic immunosuppression; among the trigger endogenous stimuli, heat shock proteins (HSPs) have been proposed. The objectives of this study were to analyze if some inflammatory mediators can be considered prognostic biomarkers of outcome, and the possible role of HSPA1A in posttraumatic immunosuppression.

Hypothesis: Cytokines and HSPs could be early prognostic biomarkers of inflammatory and immune response in polytrauma patients.

Materials and methods: A prospective observational descriptive pilot study was carried out, evaluating the early inflammatory and stress response of 18 polytraumatized patients (ISS > 16) on hospital admission, at 12 hours, 24 hours, and 48 hours posttrauma. Variable means were compared using non-parametric tests; qualitative and quantitative variables were analyzed using a Spearman's correlation test.

Results: Seven patients met criteria for MODS. Statistically significant changes were recorded in leukocyte count, C-reactive-protein (CRP), IL-6, TNF-α, and IL-1ß concentrations. HSPA1A levels were significantly higher immediately after the accident followed by gradual lowering. Anti-Hsp70 antibodies showed a significant reduction in all the studied time-points. MODS did not influence either plasma levels of leukocytes, fibrinogen, RCP or anti-Hsp70, but patients with MODS had higher plasma levels of IL-6 and TNF-α and a slower decrease of HSPA1A concentrations.

Discussion: The higher serum concentrations of TNF-α and IL-6 found in patients with MODS, suggests a possible role as potential early predictive markers for systemic inflammatory response and clinical complications. The higher levels of HSPA1A in patients with MODS, allows proposing HSPA1A as a useful prognostic trauma biomarker early after severe injury and to consider a "damage control surgery". The significant reduction in the levels of anti-Hsp70 antibodies could reflect a part of posttraumatic immunosuppression and hydrocortisone treatment might be suggested.

Level of evidence: Level III: case-control study.

1. Introduction

In the initial phases after injury, local tissue damage induces a local and systemic inflammatory response (SIRS), characterized by the production and release of a variety of "dangerous molecules" (danger-associated-molecular-patterns [DAMPs]) which lead to an early inflammatory and immune response [1]. Depending on the severity of the aggression and the immune status of the patients, the posttraumatic response sometimes leads to multiple organ failure (multiple organ dysfunction syndrome [MODS]) and death [2].

Elevated serum levels of cytokines TNF-α, IL-1ß or IL-8 have been observed in patients with systemic inflammation, and interleukin 6 (IL-6) has been correlated with the Injury Severity Score (ISS), incidence of multiple organ failure (MODS), sepsis and survival prognosis [3]. IL-6 also has anti-inflammatory properties, both due to induction of release of prostaglandin E2 and by promoting release of IL-1ß and TNF-α receptor antagonists [4]. This, together with the production of specific anti-inflammatory cytokines such as

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IL-10 or IL-4, causes the initial proinflammatory immune response to be followed by a compensatory anti-inflammatory response syndrome (CARS). This hyperinflammatory state appears to be responsible for posttraumatic immunosuppression; also called *sterile inflammation* due to the absence of bacterial infection at the time of the traumatic event, has led to it being suggest that this posttraumatic immunosuppression must be triggered by endogenous stimuli, among which heat shock proteins (HSPs) have been proposed [5].

Within the superfamily of the HSPs, the most widely studied family of proteins related to the biology of inflammation is HSPA1A (Hsp70) [6]. Extracellular HSPA1A has powerful immune properties participating in the processing and presentation of exogenous antigens [7]. HSPA1A also has anti-inflammatory properties through inhibition of the expression of proinflammatory cytokines and proinflammatory transcription factors such as nuclear factor κß [8]. Although there are multiple studies implicating immunity to Hsp70 in the promotion of chronic inflammatory conditions such as diabetes and cardiovascular disease, many others show that this autoimmune response attenuates inflammatory diseases [9].

The present study has a dual objective; on one hand to analyze whether the mediators of early inflammatory response in severely injured patients can be considered prognostic biomarkers of outcome, and on the other to study the possible role of HSPA1A in posttraumatic immunosuppression. Our hypothesis was that cytokines and HSPs could be early prognostic biomarkers of inflammatory and immune response in polytraumatized patients.

2. Patients and methods

2.1. Patients

Eighteen patients who were victims of multiple trauma of mechanical origin (ISS > 16) were included in a prospective observational pilot study with analytical components. Eighteen healthy volunteers grouped by similar age and sex comprised the control group. Patients with ISS < 16, malignant tumors, or chronic lung, liver or kidney disease were excluded. The ISS is defined as the sum of the squares of the single highest injury score in each of the three most severely injury body regions [10]. Additionally, the Abbreviated Injury Scale (AIS) [11], an anatomic classification that values from 1 to 6 the degree of injury of body systems (head and neck, chest, abdomen, extremities abd/or pelvic girdle, and general), and New Injury Severity Score (NISS) [12] based on the sum of the squares of the three most severe injuries regardless of the body region, were calculated. A manifest MODS was considered when the score was > 12 points on two consecutive days according to the Marshall scale based on the degree (from 0 to 4) of dysfunction of six organ systems [13]. The study was approved by the Clinical Research Ethics Committee of our institution. All patients or their direct relatives signed consent prior to inclusion in the study.

2.2. Blood samples and serum determinations

Samples of venous blood were taken for routine tests and quantification of fibrinogen, C-reactive-protein (CRP), cytokines (TNF-α, IL-6, IL-1ß), HSPA1A and anti-Hsp70 antibodies. Data collection was performed on hospital admission (T0), at 12 hours (T1), 24 hours (T2), and 48 hours posttrauma (T3). A single blood sample was drawn from control subjects after a 12 hour fast. Blood was centrifuged at 3500 rpm, 15 minutes at 4 °C, and serum samples were frozen at −80 °C until assayed.

CRP and cytokines were quantified using commercial ELISA kits according to the manufacturer's instructions (DRG Instruments GMBH, Marburg, Germany; Diaclone Research, France).

HSPA1A were quantified in diluted serum 1:5 using the Hsp70 ELISA kit (EKS-715, Assay-Designs-Stressgen, AnnArbor, MI, USA). The working range (linearity) for HSPA1A resulted in 0.34–6.25 ng/mL, and sensitivity was 0.30 ng/mL. The inter-assay and intra-assay precisions were < 10%.

Titers of anti-Hsp70 antibodies in serum samples (diluted 1:1000) were measured using the EKS-750 ELISA Kits, Assay-Designs-Stressgen. The working range resulted 31.25–1000 μg/mL and sensitivity was 6.79 μg/mL. The inter-assay and intra-assay precision were < 10%.

2.3. Statistical analysis

To examine the distribution of data a Kolmorogov-Smirnoff test was applied. As data were normally distributed variable means were compared using non-parametric tests, Mann-Whitney's U test for two independent samples, and Kruskal-Wallis test for three independent samples and an ANOVA with Bonferroni correction for normally distributed data. All probability values are derived from 2-tailed analyses and the statistical significance level selected was $P < 0.05$. SPSS 18.0 software for Windows was used throughout.

3. Results

3.1. Demographic characteristics

Eighteen polytraumatized patients (10 men and 8 women) with a median age of 43.50 years (range 18–77), and 18 control subjects (11 men and 7 women) with a median age of 30 years (range 18–53) were included in the study. There were no statistically significant demographic differences between both groups. Only one of the patients died 48 hours after admission due to irreversible brain injuries.

3.2. Pattern and severity of injury

All patients met the defining criteria for polytrauma. All had an AIS greater than two in at least two ISS body regions and an ISS > 16. The traumatic mechanisms were: road traffic accident ($n = 8$, 44.4%), pedestrians struck by motor vehicles ($n = 6$, 33.3%) and accidental falls ($n = 4$, 22.2%). Overall, ISS was 26.83 ± 9.43, NISS was 33.72 ± 10.55, and AIS was 14.61 ± 4.60. Seven patients met criteria for MODS detailed data are shown in Table 1.

3.3. Serum and plasma parameters

Leukocyte count was significantly higher on arrival to the emergency department compared to the control group, and remained so in the subsequent hours until it was similar to 48 hours from admission (Mann-Whitney test, $P < 0.05$). There was a high consumption of fibrinogen in the first hours after polytrauma, which gradually recovered until concentrations significantly higher than in the control group, at all the times analyzed. This progression over time in

Table 1
Demographic data of included patients.

Variable	MODS	No MODS	Total
Patients (n)	7	11	18
Age (years)	49.57 ± 18.22	37.8 ± 14.03	42.00 ± 16.48
Gender (m/w)	3/4	7/4	10/8
AIS	18.86 ± 3.02	11.91 ± 3.14	14.61 ± 4.60
ISS	36.29 ± 7.67	20.82 ± 3.69	26.83 ± 9.43
NISS	42.14 ± 0.37	28.36 ± 6.62	33.72 ± 10.56

MODS: multiple organ dysfunction syndrome; m: man; w: woman; AIS: Abbreviated Injury Scale; ISS: Injury Severity Score; NISS: New Injury Severity Score. Data are expressed as mean ± SD.