HMGB-1 Levels May be Markers of Haematological Dysfunction after Severe Trauma

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Abstract

Introduction: HMGB-1 is a nuclear protein that acts as an alarmin to tissue repair in sepsis and is one of multiple mediators in the systemic inflammatory response (SIRS). Its role in clinical models of severe trauma is less well studied.

Objectives: The aim of this study was to study the release pattern of HMGB-1 in the first 72 hours after severe trauma and the association of HMGB-1 levels with tissue damage, shock, coagulation disorders and thrombocytopenia.

Materials and Methods: A prospective cohort study enrolling all adult trauma patients with injury severity score (ISS)>15 admitted to a Trauma Room. Analytical variables assessed were: creatine kinase (CK), myoglobin (MIO) lactate, coagulation times and platelets at admission; HMGB-1 levels were measured at admission 24, 48 and 72h.

Results: Ninety-nine patients were enrolled with median ISS of 29, age 31 (18-60) years and 83% were male. Shock was found in 17%, hyperlactacidemia in 46%, coagulopathy in 26%, and thrombocytopenia in 19%. Outcomes were ICU admission-66%, MODS-34%, and Death-28%. The HMGB-1 highest level was found at admission. The study showed correlations between HMGB-1 and shock at admission (p<0.047), coagulopathy at 24h (p<0.01), and thrombocytopenia at 48h (p<0.026). Coagulopathy was associated with death and thrombocytopenia with ICU admission and death. HMGB-1 did not show correlation with ISS, CK or MIO or with any of the outcomes.

Conclusions: In this group of patientsHMGB-1 levels at admission, at 24 h and at 48h after severe trauma were respectively associated with the existence of shock, coagulopathy and thrombocytopenia.

Keywords: HMGB-1; Polytrauma; Shock; Coagulopathy; Thrombocytopenia; Haematological dysfunction

Introduction

HMGB-1 is a structural protein bound to nuclear DNA, participating in the transcription process and stabilizing nucleosomes. Its release into the circulation occurs passively from necrotic cells and actively from mononuclear cells [1]. Published studies suggest that HMGB-1 acts as an alarmin in the stimulation and modulation of several mediators of systemic inflammatory response syndrome (SIRS), following microbial sepsis in human [2] and in experimental models [3]. In aseptic SIRS models, such as severe trauma, HMGB-1 acts in the extracellular milieu signaling important cell necrosis through the TLR4 and RAGE receptors [4]. Through this function, it promotes the recruitment of mononuclear cells (allowing the removal of necrotic cells) and initiates the process of cell repair, having a chemotactic effect on smooth muscle cells, vascular stem cells and endothelial cell precursors [5] and inducing repair of skeletal muscle cells [6]. Besides acting on tissue repair processes, it also acts as a pro-inflammatory cytokine in pathological processes such as ischemia, burns, sepsis, inflammatory diseases, malignancies and trauma [4]. Its pro-inflammatory action leads to stimulation of cytokine production (IL-6, IL-1, TNF, IL-1 alpha and IL-8) from monocytes, neutrophils, dendritic cells, T cells and endothelial cells [7] and to increased expression of ICAM-1 and VCAM-1 on the surface of endothelial cells, enhancing adhesion of inflammatory cells [8]. HMGB-1 has been associated with development of coagulopathy and tissue hypoperfusion and with worst outcome in experimental models of trauma-related SIRS and multiple organ dysfunction syndrome (MODS) [9-11,13].

The aim of this study was to study the release pattern of HMGB-1 in the first 72 hours after severe trauma and the association of HMGB-1 levels with tissue damage, shock, coagulation disorders and thrombocytopenia.

Materials and Methods

A prospective cohort study was carried out during 12 months at a Level 1 Trauma Centre, in the North of Portugal. All consecutive adults admitted to the Trauma Room with severe polytrauma satisfying...
inclusion criteria were enrolled. Inclusion criteria were polytrauma, injury severity score (ISS)>15, and age >18 and <65 years. Exclusion criteria were death in the Trauma Room, accident-admission period longer than 360 minutes, noncompliance with the emergency department protocol for severe trauma patients, and transference to a level 2 trauma centre. Ethical approval for this work was obtained through local authority. This was a substudy of a larger study recently published (Disease Markers, February 2015, Hindawi Publishing Corporation) [14]. All patients were assessed and treated according to a specific emergency department protocol for severe trauma patients, based in international recommendations. Demographic, clinical and injury mechanism, severity scores and analytical parameters were obtained at admission and HMGB-1 was measured at admission and at 24, 48 and 72 hours, by the same researcher, using the Elisa method, following technical recommendations of SHINO-TEST CORPORATION®. SIRS and MODS criteria used were those proposed by the American College of Chest Physicians and the Society of Critical Care Medicine 1992 Consensus Conference [15]. Shock (SIRS associated with hypotension refractory to fluids and requiring vasopressor support), hyperlactacidemia (serum lactate >4 mmol/L), coagulopathy (increase by 1.5 of either activated partial thromboplastin time (APTT) or prothrombin time (PT), thrombocytopenia (platelets <150,000) at admission, were recorded. Outcomes considered were ICU admission, MODS and death.

Statistical analysis was carried out in SPSS v.20.0. Categorical variables were described as absolute and relative frequencies and continued variables by the median and as percentiles, minimums and maximums. To test the hypotheses about categorical variables independence, Chi-square test or Fisher's exact test was applied. To test continuous variables with no normal distribution, Mann-Whitney test was used. Relationships between variables were assessed with Spearman's correlation coefficient. Logistic regression was used to study the relationship between HMGB-1 and outcomes. The level of significance was <0.05.

Results

The 99 patients meeting the inclusion criteria had a median age of 31(18-60) years, median ISS of 29 (17-52) and 83% were male. Injury mechanisms were: traffic accident-81%, work accident-6% and other-13%. Shock, hyperlactacidemia, coagulopathy and thrombocytopenia occurred in 17, 46, 26 and 19%, respectively. Sixty-six percent of the patients were admitted to the ICU, 34% developed MODS and 28% died.

The highest levels of HMGB-1 occurred at admission (median level 10,3ng/mL), decreasing to about one third at 24h and maintaining stable levels from 48 to 72h (Table 1).

| med (P05-P95) | Admission | 24h | 48h | 72h |
|---------------|-----------|-----|-----|-----|
| HMGB-1 (ng/mL) | 10,3 (1,0-52,6) | 3,00 (1,0-19,6) | 4,25 (1,0-22,0) | 3,46 (1,0-17,5) |

Table 1: HMGB-1 levels on admission and at 24,48 and 72 hours.

No correlation was found between HMGB-1 levels and ISS score, CK, MIO or lactate at admission (Mann-Whitney test). HMGB-1 levels were associated with shock at admission (table 2). HMGB-1 levels at 24 hours were associated with coagulopathy and at 48 hours with thrombocytopenia (Table 2). Coagulopathy was associated with death and thrombocytopenia with ICU admission and death (Table 3). No association was found between HMGB-1 levels and the outcomes considered.

Table 2: Association between HMGB-1 (ng/mL) and shock, thrombocytopenia and coagulopathy (Mann-Whitney test).

$$|\text{Coagulopathy}|$$

| Coagulopathy | HMGB-1 (24h) | | | |
|---------------|---------------|---------------|---------------|---------------|
| no | med 1,73 | yes 4,40 | | |
| P05-P95 | 0,17-14,5 | 0,33-21,8 | | |
| p | 0,01 | | | |

$$|\text{Thrombocytopenia}|$$

| Thrombocytopenia | HMGB-1 (48h) | | | |
|-----------------|---------------|---------------|---------------|---------------|
| no | med 1,55 | yes 4,8 | | |
| P05-P95 | 0,3-15 | 0,5-22 | | |
| p | 0,026 | | | |

$$|\text{Shock}|$$

| Shock | HMGB-1 (admission) | | | |
|-------|---------------------|---------------|---------------|---------------|
| no | med 8,40 | yes 15,9 | | |
| P05-P95 | 0,52-44,4 | 1,12-77,9 | | |
| p | 0,047 | | | |

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Discussion

In this study, increased levels of HMGB-1 were found at an early phase after severe trauma, with higher values at admission. In spite of being considered an alarmin after cell necrosis, HMGB-1 level was not correlated with ISS and tissue damage markers, as CK and MIO. However, a good correlation was found between HMGB-1 levels during the first 48 hours and shock, thrombocytopenia and coagulopathy.

In human trauma, highest levels of HMGB-1 have been described to occur at an early stage [16,17]. In our group of severe trauma patients, the high HMGB-1 level was also found at admission (first 6 hours after trauma). The value (10.3ng/mL) is clearly lower than levels published by other authors in the first 2-6 hours [18], but similar to those found by Cohen et al [19] and Giannoudis et al. [16]. In our study, as in Peltz et al. [18] and contrary to Cohen et al. [19], HMGB-1 did not correlate with injury severity or with tissue injury markers, such as MIO or CK, and therefore cannot be used as an alarmin signaling important cell necrosis [4]. Literature is inconsistent regarding the association between HMGB-1 levels and trauma and hypoperfusion parameters as shock and arterial base deficit (BD). Cohen et al. showed that it HMGB-I was correlated with BD [19], but Peltz et al. didn't find correlation with BD or with shock [18]. We have found an association between HMGB-1 level at admission and the existence of shock, but not with hyperlactacidemia (lactate=>4mm/mL), an analytic parameter of hypoperfusion.

Haematological dysfunction, either coagulopathy or thrombocytopenia, are related with poor outcomes in trauma [20], and our study confirms these data, as coagulopathy was associated with death and thrombocytopenia with ICU admission and death. In our population of patients, HMGB-1 levels at 24 hours were associated with coagulopathy, defined as an increase by 1.5 of APTT or PT, and HMGB-1 at 48 hours was associated with thrombocytopenia. Our data, such as those of Cohen et al. [19] seem to confirm data from experimental models of trauma-related SIRS and MODS, linking HMGB-1 to haematological dysfunction induced by systemic inflammation. However, we have found no correlation between HMGB-1 levels at 0, 24, 48 and 72 hours and any of the outcome variables studied, namely ICU admission, MODS development and death, probably because the outcome in trauma is clearly multifactorial and both dependant on the initial lesions and syndrome and on hospital acquired second-hit lesions.

Conclusions

This study shows that HMGB-1 is released soon after severe trauma, reaching its highest level at emergency department admission. HMGB-1 levels in the first 48 hours were associated with shock, thrombocytopenia and coagulopathy. Further studies are needed to prove HMGB-1 as a marker of haematological dysfunction and systemic inflammation after severe trauma, in face of these results, tight monitoring and control of coagulation parameters to avoid persistent bleeding is advisable in patients with augmented HMGB-1 level.

Table 3: Association between coagulopathy and thrombocytopenia and outcomes (Chi-square test - Pearson).

| p   | 0.019 | ns   |

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