

## Study of High Mobility Group Box 1 Values in Septic Disseminated Intravascular Coagulation Treated by Polymyxin-B Immobilized Fiber-Direct Hemoperfusion

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### Abstract

**Background:** High mobility group box 1 (HMGB1) is a pro-inflammatory cytokine that acts as a final -phase endotoxin mediator. HMGB1 also increases the expression of tissue factors in human peripheral blood monocytes, and induces DIC. We investigated HMGB1 values when polymyxin-B immobilized fiber-direct hemoperfusion (PMX-DHP) was performed for the treatment of septic DIC patients with endotoxemia.

**Methods:** Serum high mobility group box 1 (HMGB1) levels were examined in 16 patients with septic disseminated intravascular coagulation (DIC) undergoing polymyxin-B-immobilized fiber-direct hemoperfusion (PMX-DHP), whose serum endotoxin levels were  $\geq 1.1$  pg/mL and who exhibited shock symptoms.

**Results:** Average acute physiology and chronic health evaluation (APACHE II) score was 32.2, average sequential organ failure assessment (SOFA) score 12.4, and average DIC score 5.5. Following PMX-DHP, the serum endotoxin level decreased below the limit of detection in all the patients. The serum HMGB1 level significantly decreased and the DIC score improved on days 1 and 2 after PMX-DHP ( $P < 0.05$ ). There was a significant correlation between HMGB1 values and DIC score ( $P < 0.05$ ).

**Conclusions:** PMX-DHP was effective therapy for septic DIC and HMGB1 was a useful index for this clinical condition.

**Keywords:** Disseminated intravascular coagulation; Hemoperfusion; Endotoxic shock

**Abbreviations** HMGB1: High Mobility Group Box 1; MODS: Multiple Organ Dysfunction; PMX-DHP: Polymyxin-B Immobilized Fiber-Direct Hemoperfusion; APACHE: Acute Physiology and Chronic Health Evaluation; ARF: Acute Respiratory Failure; DIC: Disseminated Intravascular Coagulation; ELISA: Enzyme-linked Immunosorbent Assay; FDP: Fibrinogen Degradation Products; PRP: Platelet-Rich Plasma; SIRS: Systemic Inflammatory Response Syndrome; SOFA: Sequential Organ Failure Assessment

### Background

High mobility group box 1 (HMGB1) is a pro-inflammatory cytokine that acts as a final-phase endotoxin mediator, and reportedly has a very important function in inflammatory diseases. More specifically, it reportedly acts as a lethal factor in endotoxic shock [1] and studies have been conducted regarding many diseases and HMGB1 [2,3]. Locally, HMGB1 exhibits hemostasis-promoting and tissue-repair activity. However, when HMGB1 is present in excess during endotoxemia, it can act as a lethal factor in the individual [4]. HMGB1 increases the expression of tissue factors in human peripheral blood monocytes, and induces DIC [5] HMGB1 is deeply involved in

the pathogenesis of DIC and MODS, blood HMGB1 value is high in DIC patients, correlation is found between DIC score and HMGB1 value [4]. In addition, patients with MODS complications have higher HMGB1 values than noncompliant patients.

HMGB1 accelerates the development of DIC and causes irreversible and lethal MODS [6]. We investigated HMGB1 values when polymyxin-B immobilized fiber-direct hemoperfusion (PMX-DHP) was performed for the treatment of septic DIC patients with endotoxemia.

### Methods

Before performing this study, informed consent was obtained from the patient or family, and approval was obtained from the ethics committee of Iwate Medical University.

The subjects were 16 patients with endotoxin values of  $\geq 7.4$  EU/L who underwent PMX-DHP for septic shock between 2005 and 2008. Sepsis was diagnosed according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee (ACCP/SCCM Consensus Conference Committee) [7]. Japanese Association for Acute Medicine criteria were used to diagnose DIC [8]. The acute physiology and chronic health evaluation (APACHE II) [9] and sequential organ failure assessment

(SOFA) [10] scores were used as indicators of the severity. More than one physician with qualifications of an infection control doctor and emergency physician and the clinical research coordinator calculated the scores in conference.

Antibiotics (Biapenem 0.6 g/day: 8 cases, panipenem/Betamipronand 1 g/day: 6cases, Flomoxef 2 g/day: 2 cases) and Y-globulin preparation (5 g/day for 3 days, total 15 g) were used to treat sepsis. Gabexate mesilate (2 g/day) (Ono Pharmaceutical Co., Ltd., Osaka) was used to treat DIC in all the patients, and an antithrombin-III (AT-III) preparation (1500E/day for 3days, total 4500E) was used as appropriate.

Blood samples to measure endotoxin and HMGB1 were collected into an endotoxin-free heparinised syringe in the early phase after diagnosis and in the post-therapeutic phase with PMX-DHP. The specimen was centrifuged immediately for 40 seconds at 3000 revolutions per minute (rpm), and platelet-rich plasma (PRP) was obtained. Endotoxin was measured immediately after PRP collection. The remaining PRP was stored at  $-80^{\circ}\text{C}$ , and the HMGB1 in all the specimens was measured in a batch at a later date. Endotoxin values were measured using a high-sensitivity endotoxin-specific turbidimetry method (Endotoxin-Single Waco; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and Toxinometer<sup>®</sup> ET-500 (Wako Pure Chemical Industries, Ltd.). A cutoff value of 7.4 EU/L was adopted for endotoxemia.

PMX-DHP (Toray Medical Co., Ltd., Chiba, Japan) was enforced to remove endotoxin from patient blood, and nafamostat mesilate (Torii Pharmaceutical Co., Ltd., Tokyo, Japan) was used as an anticoagulant. Other blood examinations related to the diagnosis of DIC were conducted as needed. PMX-DHP was performed in all the patients with an endotoxin value of  $\geq 1.1$  pg/mL and who were also in shock. The HMGB1 was measured by an enzyme-linked immunosorbent assay (ELISA; Shino-Test Corporation, Tokyo, Japan). The measurement limit was 1 ng/mL.

Values were expressed as mean  $\pm$  standard deviation. The data were tested for significant differences by the unpaired t-test, and Pearson's formula was used for correlations.  $P < 0.05$  was considered significant according to both of the tests.

## Results

The mean age of the 11 male and five female patients was  $71.9 \pm 9.5$ . DIC score was  $5.6 \pm 3.1$ , APACHE II score  $32.2 \pm 5.5$ , and SOFA score  $12.4 \pm 4.0$ . PMX-DHP was performed once in six patients and twice in 10. Another blood purification method besides PMX-DHP was performed in combination in 10 patients. A significant correlation was found between the HMGB1 values and the DIC scores at initial examination as shown in Figure 1A. There were no significant correlations between the HMGB1 values and the other shock scores. The endotoxin values of all the patients decreased to  $<7.4$  EU/L just after PMX-DHP. The acute-phase DIC score decreased significantly from  $5.6 \pm 3.1$  on hospital day 0 to  $3.9 \pm 1.9$  on hospital day 1 and  $2.9 \pm 1.9$  on hospital day 2 as shown in Figure 1B. HMGB1 values decreased significantly from  $31.3 \pm 19.2$  to  $16.6 \pm 7.2$  and  $7.9 \pm 2.9$  ng/mL, respectively as shown in Figure 1C. The total number of the criteria for systemic inflammatory response syndrome (SIRS) [7] that were met decreased significantly from  $3.3 \pm 0.9$  to  $2.0 \pm 0.5$  and  $1.8 \pm 0.4$ , respectively as shown in Figure 1D. The blood platelet count ( $6.8 \pm 2.4 \times 10^4/\text{mm}^3$  on hospital day 0) decreased slightly to  $6.5 \pm 2.0 \times 10^4/\text{mm}^3$  on hospital day 1, and then increased, although not

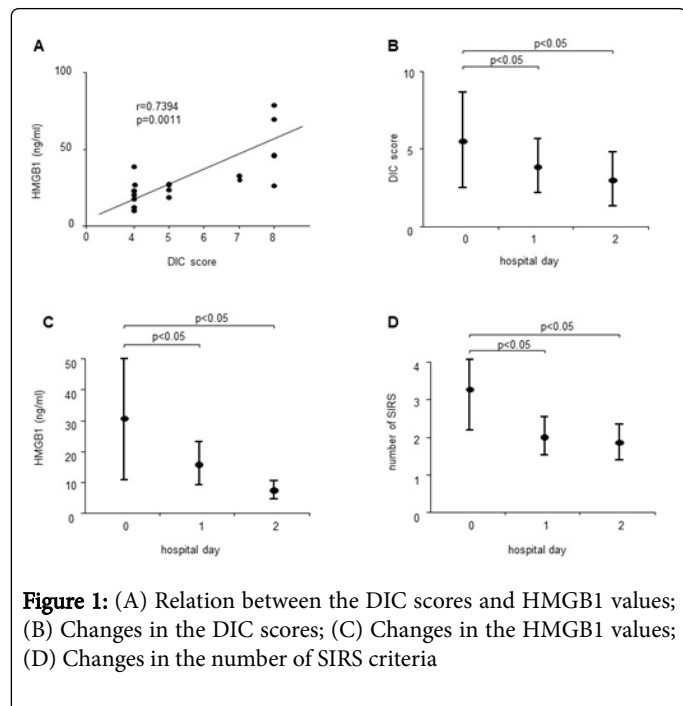
significantly, to  $8.2 \pm 2.6 \times 10^4/\text{mm}^3$  on hospital day 2 as shown in Figure 2A. The prothrombin time-international normalized ratio (PT-INR) decreased significantly from  $1.31 \pm 0.18$  to  $1.22 \pm 0.16$  and  $1.13 \pm 0.15$ , respectively as shown in Figure 2B. Fibrin and fibrinogen degradation products (FDP) decreased significantly from  $21.8 \pm 8.1$  to  $16.1 \pm 4.9$  and  $13.6 \pm 4.2$   $\mu\text{g}/\text{mL}$ , respectively as shown in Figure 2C. Antithrombin III (AT-III) activity increased significantly from  $62.6 \pm 14.1\%$  to  $84.1 \pm 9.6\%$  and  $96.8 \pm 8.5\%$ , respectively as shown in Figure 2D. Overall, the 30-, 60-, 90-, and 180- day mortality rates were 0%, 6.3%, 12.5%, and 12.5%, respectively. The cause of death in cases 3 and 12 was pre-existing heart failure. The difference between the HMGB1 values in the groups that survived and died was not significant.

## Discussion

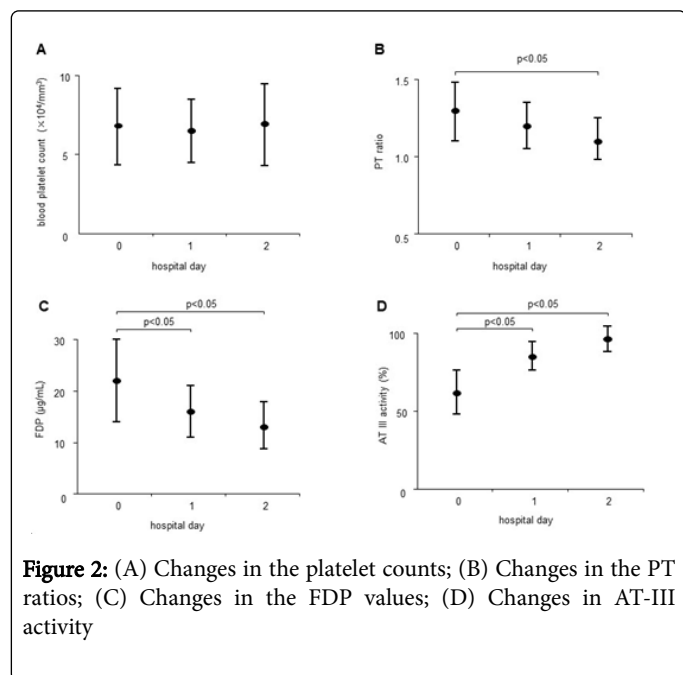
When inflammatory cytokines are produced in excess in endotoxemia, a variety of diseases are induced, including SIRS, multiple organ failure, liver injury, lung injury, and DIC. Several previous reports exist in regard to cytokine involvement in the onset of DIC [11,12]. Cytokines have many actions, but in their roles in the onset of DIC, by acting on a variety of cells, exerting an activating or an inhibitory action on the direct clotting and fibrinolysis system, and activating or damaging blood cells or vascular endothelial cells, they appear to have mechanisms of action that cause organ dysfunction and secondarily lead to DIC.

In contrast to other inflammatory cytokines, HMGB1 has been described as a late-phase mediator of the inflammatory response that is produced by activated macrophages, dendritic cells, and necrotic cells 24 to 48 h after experiencing an insult [1]. However, in our previous septic acute respiratory failure (ARF) patients, HMGB1 in the early phase of septic ARF development acted as a factor that determined the outcome in the final phase. For this reason as well, HMGB1 may always have an important role in sepsis regardless of the phase. HMGB1 activates macrophages and increases tissue repair, but when it is produced in excess throughout the body, it causes organ dysfunction and shock. Not only have early-phase humoral factors, such as cytokines, been reported as the causes of DIC and organ dysfunction, but also the involvement of HMGB1 has been reported as a humoral factor that governs the outcome in the final phase [5]. In sepsis, a pathologic state is thought to exist in which vascular endothelial dysfunction and inhibition of fibrinolysis induce the onset of circulatory disorders, and, consequently, cause cell necrosis, and that excessive HMGB1 present in the cells is released throughout the body and causes multiple organ failure. In our study, HMGB1 did not increase significantly in the patients with sepsis who did not have DIC (unpublished data), and the HMGB1 values increased greatly at the onset of septic DIC as shown in Figure 1C. These findings seemed to indicate the role of HMGB1 in the onset of DIC. All the patients had shock as a complication, and all had positive endotoxin values as well; however, when PMX-DHP was performed in these patients, their endotoxin values rapidly decreased to within the normal range, and their HMGB1 values decreased almost simultaneously as shown in Figure 1C. These changes were associated with improvements in the number of SIRS criteria, platelet count, PT-INR, and FDP values, all of which are factors that constitute the diagnostic criteria for acute-phase DIC as shown in Figures 1D and 2A-2C. Consequently, all the patients were able to recover from DIC as shown in Figure 1B. In addition, the AT-III activity values also increased significantly as shown in Figure 2D. Thus, PMX-DHP suppressed the inflammatory response, and, consequently decreased the HMGB1 values, suggesting that it also may

be a useful tool to treat septic DIC. In conclusion, we suggested that PMX-DHP might have a therapeutic effect on septic DIC and HMGB1 might be a useful index to evaluate its clinical state.



**Figure 1:** (A) Relation between the DIC scores and HMGB1 values; (B) Changes in the DIC scores; (C) Changes in the HMGB1 values; (D) Changes in the number of SIRS criteria



**Figure 2:** (A) Changes in the platelet counts; (B) Changes in the PT ratios; (C) Changes in the FDP values; (D) Changes in AT-III activity

## Conclusions

In this study, we examined serum HMGB1 levels in 16 patients with septic DIC undergoing polymyxin-B-immobilized fiber-direct hemoperfusion (PMX-DHP), whose serum endotoxin levels were  $\geq 1.1$  pg/mL and who exhibited shock symptoms. In our study, HMGB1 did not increase significantly in the sepsis patients who did not have DIC, but the HMGB1 values increased greatly at the onset of septic DIC.

These findings seemed to indicate the role of HMGB1 in the onset of DIC. All patients had shock as a complication, and all had positive endotoxin values as well, but when PMX-DHP was performed in these patients, their endotoxin values rapidly decreased to within the normal range, and their HMGB1 values decreased almost simultaneously. The findings suggest that PMX-DHP was effective therapy for septic DIC and HMGB1 was a useful index for this clinical condition.

## Ethics Approval and Consent to Participate

Before performing this study, informed consent was obtained from the patient or family, and approval was obtained from the ethics committee of Iwate Medical University. (Approval number H14-3)

## Consent for Publication

Written informed consent was obtained from the patients for publication of this article. A copy of the written consent is available for review by the editor of this journal.

## Availability of Data and Materials

The data generated and analyzed in this study are included in this published article and its additional files. The original datasets used for this study are not publicly available due to the existing regulation, and only can be shared upon the approval of the directors of the corresponding hospitals.

## Competing Interests

The authors declare that they have no competing interests.

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## Authors Contributions

GT managed the case and redaction and correction of the manuscript. SE assisted with redaction, correction, and reconstruction of the manuscript. YI assisted with clinical management of the case and correction of the manuscript. All authors read and approved the final manuscript.

## Acknowledgements

Not applicable

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