Original Article

Mortality at 180-days is affected by serum haptoglobin levels in septic patients with high magnitude serum high mobility group box-1 levels

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Aim: High mobility group box-1 (HMGB1) is a lethal mediator of sepsis that binds to haptoglobin (Hp) and is associated with its prognosis. We investigated the effect of the combination of HMGB1 and Hp on sepsis prognosis.

Methods: This single-center, retrospective study registered 78 patients with sepsis according to Sepsis-3 criteria on day 1 of diagnosis from July 2016 to November 2018. We divided the patients into four groups according to the serum concentration of 6.2 ng/mL HMGB1 and the median value of Hp. The 180-day mortality rates and cytokine concentrations of the low and high HMGB1 groups were compared.

Results: There was no difference in the 180-day mortality rate between the low Hp group and the high Hp group in the low HMGB1 group ($P = 0.691$). In the high HMGB1 group, a statistically significant difference was found between the low Hp group and the high Hp group ($P = 0.002$). In the high HMGB1 group, high Hp was associated with a better prognosis in univariate analysis (odds ratio, 0.131; 95% confidence interval [CI], 0.027–0.629; $P = 0.011$), and multivariate analysis (adjusted odds ratio, 0.086; 95% CI, 0.013–0.582; $P = 0.009$). In addition, in the high HMGB1 group, interleukin-8 levels were significantly higher in the low Hp group than in the high Hp group ($P = 0.004$).

Conclusion: Patients with sepsis-induced high serum HMGB1 levels and low serum Hp levels could have a poor long-term prognosis.

Key words: Cytokine, haptoglobin, HMGB1 protein, prognosis, sepsis

INTRODUCTION

SEPSIS IS A fatal disease caused by infection and is a leading cause of health problems worldwide.[1] Sepsis has been redefined as "a life-threatening organ dysfunction caused by an abnormal host response to infection."[2] Although humoral mediators have been reported to be associated with multiple organ failure and high mortality,[1] the pathophysiology and molecular mechanisms of sepsis are complex and remain unclear.

High mobility group box-1 (HMGB1) is a nuclear protein measuring approximately 30 kDa. It is a late and lethal mediator in sepsis, and treatment with an antibody against HMGB1 can attenuate its lethality in mice.[3] Since that study, HMGB1 has been identified as a key mediator and a therapeutic target of sepsis with its levels related to exacerbation of inflammation.[4] In clinical settings, a high mortality rate was found in severe septic patients with a serum HMGB1 level exceeding 6.2 ng/mL.[5]

Haptoglobin (Hp) is an acute-phase protein that is regulated by the induction of interleukin (IL)-6 in human hepatocytes.[6–8] It has been reported that Hp suppresses IL-10 production in human mononuclear cells exposed to lipopolysaccharide.[4] In vivo, Hp induces cytoprotective responses through heme oxygenase-1 activity.[9] As a control mechanism of HMGB1, complexes of HMGB1 and Hp elicit the production of anti-inflammatory substances such as IL-10 through CD163 in macrophages.[3]

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In light of the aforementioned findings, we investigated the combined effects of HMGB1 and Hp on prognosis and production of cytokines such as IL-10 in septic patients.

METHODS

Study design and setting

THIS WAS A single-center, retrospective study. Patients who were diagnosed with sepsis based on the Sepsis-3 criteria and admitted to the intensive care unit (ICU) at the hospital of Shiga University of Medical Science between July 2016 and November 2018 were enrolled. The study protocol was approved by the Institutional Review Board of Shiga University of Medical Sciences (approval number R2015-22) and conforms to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). Written informed consent was obtained from each patient or a family member. Patients younger than 16 years were excluded from the study.

Outcome

The primary outcome was the 180-day mortality, based on the report that the difference in mortality due to high HMGB1 levels is more pronounced in the late stage of septic patients.[1] The secondary outcome was the level of cytokines (IL-6, IL-8, and IL10) in the blood because it has been reported that the formation of Hp and HMGB1 complexes cause changes in cytokine production.

Target cases and grouping

In this study, we divided the patients into two groups based on HMGB1 levels of 6.2 ng/mL because a previous study reported that the mortality rate was higher in septic patients with HMGB1 levels of 6.2 ng/mL or higher.[5] To investigate the prognostic impact of Hp in the high and low HMGB1 groups, each HMGB1 group was divided into high and low Hp groups for analysis. We divided the patients into two groups at the median value of Hp because a previous study reported that patients with a lower than median value of Hp (113.2 [50.8–289.0] mg/dL) tended toward poor prognosis,[7] and the median value of Hp in our study was close to the value of the previous report.

Data collection

The patients' medical records were retrospectively searched to collect their demographic and clinical data. Body mass index was calculated from the data at the time of admission to the ICU. The Charlson comorbidity index was calculated using clinical records. The severity of the illness was evaluated using the Sequential Organ Failure Assessment (SOFA) score, which was determined using the most aberrant clinical and laboratory results from variables during the first 24 h after ICU admission.

Measurement of serum HMGB1 and Hp levels

Blood was collected from patients within 24 h of admission to the ICU. Serum was centrifuged at 1,470 ***g*** for 10 min and stored at −80°C until the measurement date. Serum HMGB1 levels were measured by the HMGB1 ELISA Kit II (Shino-Test) and serum Hp levels were measured by the Quantikine Human Haptoglobin ELISA Kit (R&D Systems) according to the manufacturer's instructions.

Measurement of serum cytokines

Blood serum samples obtained from the patients within 24 h of admission to the ICU were kept at −80°C until the measurement day. Serum cytokines (IL-6, IL-8, and IL-10) levels were measured using an automated chemiluminescence assay (Immulite; Siemens Healthcare Diagnostics; Siemens AG, Tokyo, Japan), according to the manufacturer's instructions.

Statistical analysis

Continuous variables are presented as median and interquartile range (IQR, 25th–75th percentiles) and were tested using the *t*-test or Mann–Whitney *U*-test, and categorical variables were tested using χ^2-test. The Kaplan–Meier analysis and log–rank test were used to evaluate the effect of HMGB1 and Hp on 180-day survival. Univariate and multivariate Cox hazard regression analyses were undertaken to examine the factors influencing 180-day survival, and the SOFA score was selected as a confounder of 180-day survival. Statistical significance was set at $P < 0.025$ as the test was carried out in the high HMGB1 group and low HMGB1 group. SPSS software (SPSS Statistics 25; IBM, Chicago, IL, USA) was used for the analysis.

RESULTS

Clinical characteristics

A TOTAL OF 78 septic patients (23 women and 55 men) with a median age of 73 (IQR, 67–80) years were enrolled in the study. The median SOFA score was 9 (IQR, 6–12), and the 28-day mortality rate was 25.6% (20/

78). The incidence of abdominal infection and pulmonary infection was 41.0% (32/78) and 28.2% (22/78), respectively. Of the 78 septic patients, 36 patients had HMGB1 levels that exceeded 6.2 ng/mL. Baseline characteristics and levels of HMGB1, Hp, and cytokines of the 78 patients are shown in Table 1.

We compared the SOFA scores and cytokine and lactate levels between the low Hp and high Hp groups in both the low HMGB1 and high HMGB1 groups, and found that IL-8 and lactate levels were significantly different between the low Hp and high Hp groups (P = 0.01 and 0.024, respectively) in the high HMGB1 group. The SOFA scores and IL-6 and IL-10 levels did not significantly differ between the low Hp and high Hp groups in either the low HMGB1 or high HMGB1 groups (Table 1). These findings indicate that the severity of sepsis was similar between the low HP and high Hp groups, based on SOFA scores and IL-6 levels. Additionally, there was no significant difference in HMGB1 levels between the low and high Hp groups in either the low HMGB1 or high HMGB1 groups.

180-Day mortality in low and high HMGB1 groups

For the primary outcome, 180-day mortality, there was no difference between the low Hp and high HP group in the low HMGB1 group (Fig. 1A). However, in the high HMGB1 group, a statistically significant difference was found between the low Hp and high Hp groups (P = 0.002; Fig. 1B).

For 180-day mortality, in the low HMGB1 group, there was no association between Hp ≥ 109 mg/dL and prognosis in the univariate and multivariate analyses. However, in the high HMGB1 group, high Hp was associated with a better prognosis in univariate analysis (odds ratio, 0.131; 95% confidence interval [CI], 0.027–0.629; P = 0.011). Multivariate analysis was carried out using the SOFA score as a confounding factor, and high Hp was shown to improve prognosis (adjusted odds ratio, 0.086; 95% CI, 0.013–0.582; P = 0.009) (Table 2).

Inflammatory and anti-inflammatory cytokines were measured using blood collected at the time of ICU admission. In the low HMGB1 group, there was no difference in IL-6, IL-8, or IL-10 levels between the low Hp and high Hp groups. However, in the high HMGB1 group, IL-8 levels were significantly higher in the low Hp group compared to the high Hp group (Fig. 2).

DISCUSSION

THIS STUDY INVESTIGATED the combined effects of HMGB1 and Hp in patients with sepsis. Our results showed that a systemic reaction to HMGB1 and Hp induced by sepsis could affect the production of cytokines; following admission to the ICU, IL-8 levels were significantly lower in patients with high Hp levels than in those with low Hp levels who had restrictively high HMGB1 levels. These findings suggest that Hp has an anti-inflammatory effect on HMGB1. Indeed, patients with high serum Hp levels showed a good long-term prognosis even if high serum HMGB1 levels were induced by sepsis.

Haptoglobin itself might not have an effect on long-term prognosis. In severe sepsis, in-hospital mortality has been reported to be significantly affected by low serum Hp levels[7] and the median serum Hp level was 113.2 mg/dL (50.8–289 mg/dL) for all cases, 75 mg/dL (40.4–242.1 mg/dL), and 123.4 mg/dL (56.9–303.7 mg/dL) in the low and high Hp groups, respectively. However, in our study, there was no decrease in mortality due to Hp in the low HMGB1 group. Therefore, Hp might be reducing the effect of HMGB1.

Extracellularly secreted HMGB1 has been shown to function as a damage-associated molecular pattern[10] and act on monocytes, macrophages, and vascular endothelial cells to promote the production of cytokines such as IL-6, IL-8, tumor necrosis factor-α, and IL-1β to induce inflammation.[10–12] In contrast, Hp has been shown to have no effect on the production of IL-6 and IL-8 in sepsis models,[4] but Yang *et al*.[3] reported that Hp dose-dependently inhibits the production of IL-6 and IL-8 in macrophages stimulated by HMGB1 in vitro. In the present study, IL-8 levels were significantly lower in the high Hp group than in the low Hp group within the high HMGB1 group, indicating that Hp could inhibit the stimulation to produce IL-8 mediated by HMGB1 in patients with sepsis.

In a sepsis model, IL-10 production was induced.[12–14] The HMGB1–Hp complex produces IL-10 through CD163 in macrophages;[3] in contrast, Hp alone has been reported to inhibit the production of IL-10 in vitro and in vivo.[4] High mobility group box-1 undergoes redox reactions and transforms into three subtypes. Fully reduced HMGB1 has a chemotactic effect. Under moderately oxidizing conditions, HMGB1 transforms into the disulfide subtype that induces cytokine production, and under heavily oxidizing conditions, into the sulfonyl subtype.[15] Haptoglobin is known to bind to the fully reduced and disulfide forms of HMGB1.[15] In this study, IL-10 levels were not statistically different in the two groups. Taken together, the production of IL-10 would depend on the amount of both the disulfide-subtype HMGB1 and Hp. Therefore, it is considered to be necessary to measure the disulfide subtype of HMGB1 in clinical settings.

Table 1. Baseline characteristics of 78 patients with sepsis and overall values for the four study groups

	Overall values	HMGB1 < 6.2 ng/mL			HMGB1 ≥ 6.2 ng/mL		
		Hp < 109 mg/dL	Hp ≥ 109 mg/dL	*P*-value	Hp < 109 mg/dL	Hp ≥ 109 mg/dL	*P*-value
N	78	26	16		13	23	
Age, years	73 (67–80)	76 (67–82)	77 (70–81)	0.940	72 (67–82.5)	71 (66–76)	0.409
Gender, F/M (%)	23/55 (41.8)	8/18 (44.4)	4/12 (33.3)	0.740	4/9 (44.4)	7/17 (41.2)	0.984
BMI, kg/m^2	21.4 (18.7–24.3)	20.8 (18.4–24)	21.3 (16.1–25.5)	0.940	21.5 (20.5–24.2)	21.5 (15.1–24.3)	0.802
Charlson comorbidity index	2 (1–4)	2 (1–4)	3 (1–4)	0.751	3 (2–4)	2 (1–3)	0.229
SOFA score	9 (6–12)	9 (7–12)	6 (4–11)	0.819	11 (8.5–15.5)	10 (4.3–14.5)	0.276
HMGB1, ng/mL	5.2 (2.8–12.1)	2.9 (1.8–4.3)	2.8 (1.9–4.1)	0.751	17.6 (10.1–26.1)	11.8 (8.81–20.0)	0.488
Hp, mg/dL	109.1 (45.4–173.4)	48.8 (38.3–81.1)	146.8 (119.9–188.2)	< 0.001*	44.7 (4.65–75.5)	215.97(148.7–280.3)	0.001*
IL-6, pg/mL	619 (149–6,835)	1,059 (104.5–7 452.3)	345 (44.3–3,717.5)	0.365	2,126 (783–15,020)	221 (190–2,404)	0.107
IL-8, pg/mL	354 (55.38–2,559.4)	255.5 (40.1–2,995.3)	275 (33.6–1702.3)	0.623	1,969 (553–5,004)	225 (72.4–740.5)	0.001*
IL-10, pg/mL	33.9 (14.83–133)	40.4 (22.73–125.5)	21.9 (8.74–81.1)	0.218	134 (27.5–639)	18.6 (11.6–71.1)	0.123
Lactate	20 (13–38.8)	21 (15–41.5)	16 (10.3–28.8)	0.710	44 (22.0–101.0)	20 (11.0–49.0)	0.024*
Septic shock, *n* (%)	37 (47.4)	12 (46.1)	6 (37.5)	0.750	9 (69.2)	10 (43.5)	0.177
Infection sites, *n*							
Abdominal	32	10	8	0.585	7	7	0.477
Pulmonary	22	8	5		2	7	
Soft tissue	8	3	0		1	4	
Others	16	5	3		3	5	

Note: Data are given as median (quartile 1–quartile 3), unless otherwise indicated.
Abbreviations: BMI, body mass index; F, female; HMGB1, high mobility group box-1; Hp, haptoglobin; IL-, interleukin; M, male; SOFA, Sequential Organ Failure Assessment.
*$P < 0.05$.

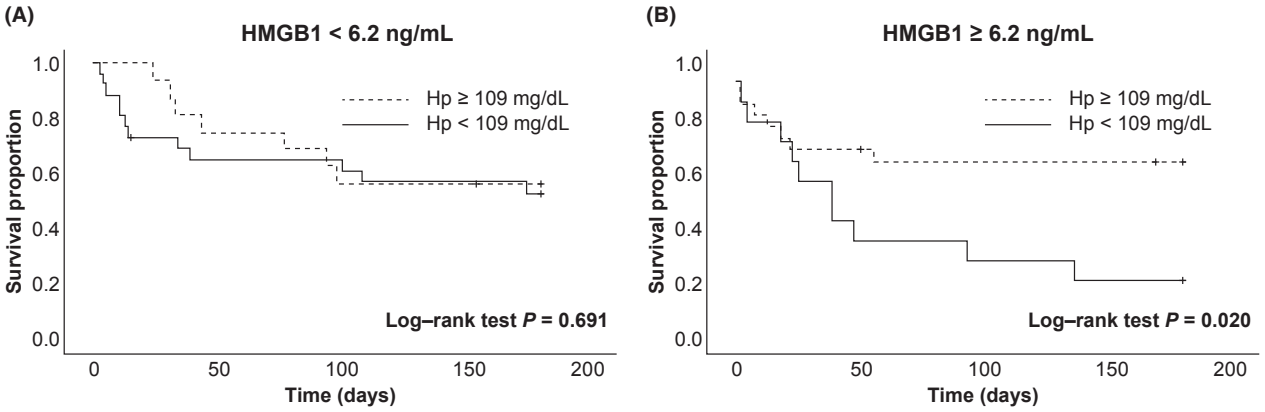

Fig. 1. Kaplan–Meier survival curves of 78 septic patients at 180 days after admission to the intensive care unit. A, There was no significant difference in 180-day mortality between low haptoglobin (Hp) and high Hp in the group with low levels of high mobility group box-1 (HMGB1) ($P = 0.691$). B, In the high HMGB1 group, 180-day mortality rates were significantly different between the low Hp and high Hp groups, as determined using the log–rank test ($P = 0.020$).

Table 2. Summary of univariate and multivariate Cox hazard regression analyses for 180-day mortality in patients with sepsis

		Univariate analysis			Multivariate analysis		
		OR	95% CI	*P*-value	aOR	95% CI	*P* value
Low HMGB1 group	High Hp group	0.907	0.259–3.177	0.879	1.115	0.291–4.264	0.874
	SOFA score	1.224	1.014–1.479	0.036	1.227	1.014–1.484	0.035
High HMGB1 group	High Hp group	0.131	0.027–0.629	0.011*	0.086	0.013–0.582	0.012*
	SOFA score	1.291	1.070–1.557	0.008*	1.35	1.076–1.694	0.009*

Note: High high mobility group box-1 (HMGB1) group, ≥6.2 mg/mL serum HMGB1; Low HMGB1 group, <6.2 mg/mL serum HMGB1; High haptoglobin (Hp) group, ≥109 mg/dL serum Hp.
Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio; SOFA, Sequential Organ Failure Assessment.
*$P < 0.025$.

Gibot *et al*.[5] reported that persistently elevated HMGB1 concentrations within 72 h after admission to the ICU were related to the prognosis of sepsis, and that HMGB1 is a valid prognostic factor after 30 days in patients with septic acute respiratory distress syndrome.[16] Shibata *et al*.[17] also suggested HMGB1 as a long-term prognostic factor (90 and 180 days). In the previous decade, standardization of diagnosis and treatment in patients with sepsis has been popular in a clinical setting, therefore, humoral mediators such as cytokines could be regulated by intervention for sepsis in the early phase. In the present study, the 180-day mortality rates were significantly higher in patients with low Hp than those with high Hp under high HMGB1 levels. These findings suggest that nonbinding of HMGB1 to Hp could act as a late phase humoral mediator.

Limitations

This study has some limitations. First, the number of cases was small; thus, we could not undertake a sufficient multivariate analysis. Multivariate analysis was carried out using only the SOFA score as a confounding factor, which is strongly associated with the prognosis of sepsis. Therefore, we were unable to examine other confounding factors thoroughly. Second, the cut-off value of HMGB1 concentration was 6.2 ng/mL, because Gibot *et al*. reported that the median blood HMGB1 concentration in patients with sepsis in the nonsurvivor group was 6.2 ng/mL,[5] however, the results would be different if another cut-off value was used. Finally, the direct relationship between the HMGB1–Hp complex and mortality was not investigated because the levels of HMGB1 alone and the complex were not measured separately.

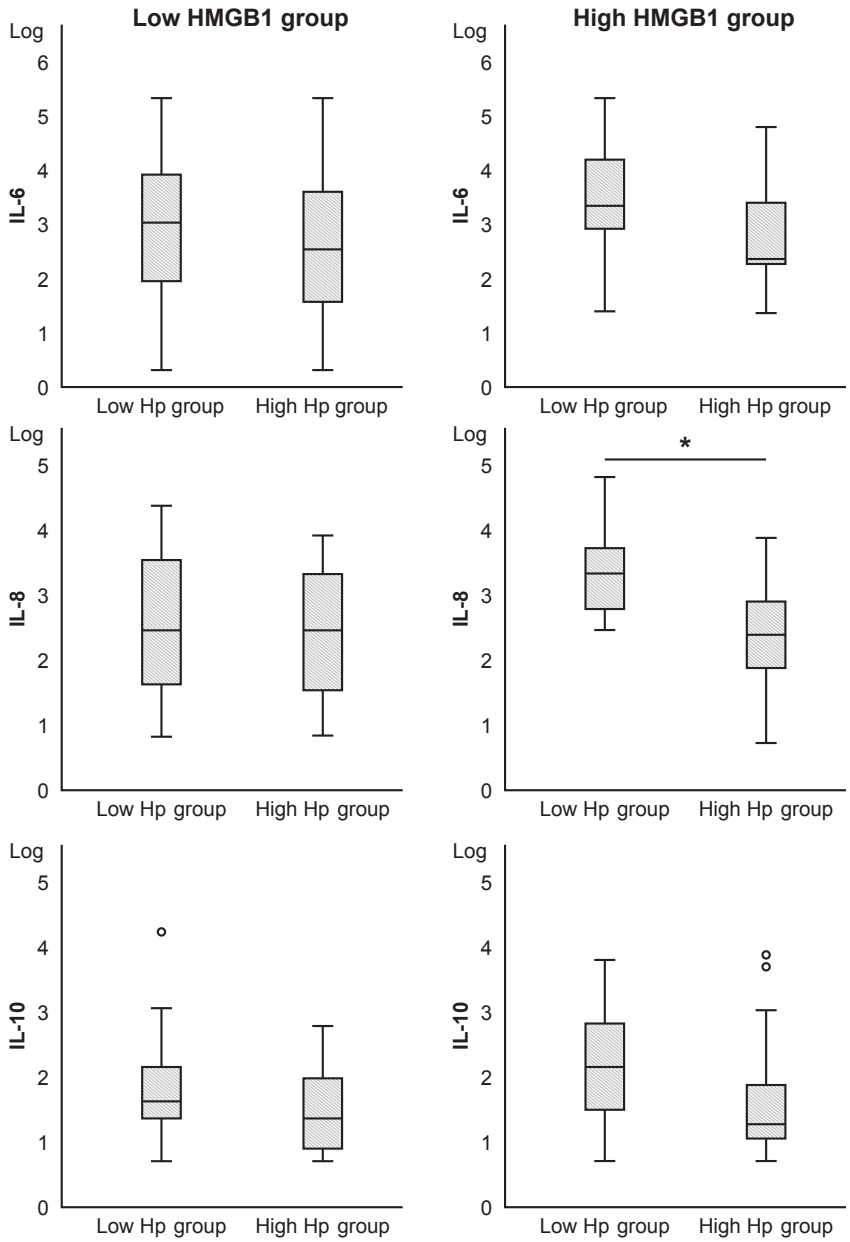

Fig. 2. Cytokines were compared in 78 septic patients according to haptoglobin (Hp) and high mobility group box-1 (HMGB1) levels. Low/high Hp groups of low/high HMGB1 are represented in box-and-whisker diagrams. Serum cytokine levels were not significantly different in low HMGB1 groups. In high HMGB1 groups, interleukin (IL)-6 and IL-10 were not significantly different, but IL-8 was lower in the high Hp group ($P = 0.004$).

CONCLUSION

PATIENTS WITH SEPSIS who have Hp levels less than 109 mg/dL have a poor long-term prognosis, even if their HMGB1 levels are higher than 6.2 ng/mL. In clinical settings, simultaneous measurement of Hp and HMGB1 could be valuable to predict the long-term prognosis of sepsis and could affect the decision-making of the initial treatment for sepsis.

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DISCLOSURE

APPROVAL OF RESEARCH protocol with approval no. and committee name: The study protocol was approved by the Institutional Review Board of Shiga University of Medical Sciences (approval no. R2015-22) and conforms to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013).

Informed consent: Written informed consent was obtained from each patient or a family member. Patients younger than 16 years were excluded from the study.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

Conflict of interest: None.

REFERENCES

1 Rudd KE, Johnson SC, Agesa KM *et al*. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet 2020; 395: 200–11.
2 Singer M, Deutschman CS, Seymour CW *et al*. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016; 315: 801–10.
3 Yang H, Wang H, Levine YA *et al*. Identification of CD163 as an antiinflammatory receptor for HMGB1-haptoglobin complexes. JCI Insight 2016; 1: e85375.
4 Arredouani MS, Kasran A, Vanoirbeek JA, Berger FG, Baumann H, Ceuppens JL. Haptoglobin dampens endotoxin-induced inflammatory effects both *in vitro* and *in vivo*. Immunology 2005; 114: 263–71.
5 Gibot S, Massin F, Cravoisy A *et al*. High-mobility group box 1 protein plasma concentrations during septic shock. Intensive Care Med. 2007; 33: 1347–53.
6 Ramji DP, Vitelli A, Tronche F, Cortese R, Ciliberto G. The two C/EBP isoforms, IL-6DBP/NF-IL6 and C/EBP delta/NF-IL6 beta, are induced by IL-6 to promote acute phase gene transcription via different mechanisms. Nucleic Acids Res. 1993; 21: 289–94.
7 Janz DR, Bastarache JA, Sills G *et al*. Association between haptoglobin, hemopexin and mortality in adults with sepsis. Crit. Care 2013; 17: R272.
8 Li SC, Lee CC, Hsu CM, Huang HB, Su YC. IL-6 induces haptoglobin expression through activating STAT3 in human head and neck cancer. J. Oral Pathol. Med. 2020; 49: 49–54.
9 Belcher JD, Chen C, Nguyen J *et al*. Haptoglobin and hemopexin inhibit vaso-occlusion and inflammation in murine sickle cell disease: role of heme oxygenase-1 induction. PLoS One 2018; 13: e0196455.
10 Bae JS. Role of high mobility group box 1 in inflammatory disease: focus on sepsis. Arch. Pharm. Res. 2012; 35: 1511–23.
11 Yang H, Wang H, Czura CJ, Tracey KJ. The cytokine activity of HMGB1. J. Leukoc. Biol. 2005; 78: 1–8.
12 Andersson U, Wang H, Palmblad K *et al*. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J. Exp. Med. 2000; 192: 565–70.
13 Park J, Arcaroli J, Yum HK. Activation of gene expression in human neutrophils by high mobility group box 1 protein. Am. J. Physiol. Cell Physiol. 2003; 284: C870–9.
14 Williams L, Bradley L, Smith A, Foxwell B. Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. J. Immunol. 2004; 172: 567–76.
15 Antoine DJ, Harris HE, Andersson U, Tracey KJ, Bianchi ME. A systematic nomenclature for the redox states of high mobility group box (HMGB) proteins. Mol. Med. 2014; 20: 135–7.
16 Kikuchi S, Masahiro K, Shigehiro S. Assessment of high mobility group box 1 values in the early stage after the onset of ALI/ARDS and outcome. J. Iwate Med. Assoc. 2009; 61: 283–93.
17 Shibata S, Gaku T, Yasushi S. Pathology and clinical findings of acute respiratory distress syndrome. J. Jap. Soc. Clin. Anesthesia 2013; 33: 926–31.