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# Associations between Single Nucleotide Polymorphisms of High Mobility Group Box 1 Protein and Clinical Outcomes in Korean Sepsis Patients

Kwangha Lee[1], Youjin Chang[2], Kyuyoung Song[3], Yun Young Park[4], Jin Won Huh[4], Sang-Bum Hong[4], Chae-Man Lim[4], and Younsuck Koh[4]

[1]Department of Internal Medicine, Pusan National University School of Medicine, Busan;
[2]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, College of Medicine, Chungbuk National University, Cheongju;
Departments of [3]Biochemistry and Molecular Biology and [4]Pulmonary and Critical Care Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

**Purpose:** High mobility group box 1 (HMGB1) plays a central role in the pathogenesis of sepsis and multiple organ dysfunction syndromes. We investigated the associations of a single nucleotide polymorphism (SNP; rs1045411) in *HMGB1* with various clinical parameters, severity, and prognosis in patients with sepsis, severe sepsis, or septic shock.
**Materials and Methods:** We enrolled 212 adult patients followed for 28 days. All patients were genotyped for rs1045411, and the serum levels of HMGB1 and several cytokines were measured.
**Results:** The proportions of patients according to genotype were GG (71.2%), GA (26.4%), and AA (2.4%). Among patients with chronic lung disease comorbidity, patients with a variant A allele had higher positive blood culture rates and higher levels of various cytokines [interleukin (IL)-1β, IL-6, IL-10, IL-17, and tumor necrosis factor-α] than those with the GG genotype. In the analysis of those with diabetes as a comorbidity, patients with a variant A allele had higher blood culture and Gram-negative culture rates than those with GG genotypes; these patients also had a higher levels of IL-17. In the analysis of those with sepsis caused by a respiratory tract infection, patients with a variant A allele had higher levels of IL-10 and IL-17 (all $p<0.05$). This polymorphism had no significant impact on patient survival.
**Conclusion:** The variant A allele of rs1045411 appears to be associated with a more severe inflammatory response than the GG genotype under specific conditions.

**Key Words:** High mobility group box 1, single nucleotide polymorphisms, rs1045411, sepsis, the variant A allele

## INTRODUCTION

Sepsis is a devastating clinical condition characterized by systemic inflammation occurring during a severe infection. Severe sepsis and septic shock are leading causes of morbidity and mortality in the intensive care unit (ICU).[1,2] The reason that some patients die while others survive similar insults is partially understood, although some of this patient outcome variability may be caused by genetic variation. Several reports have confirmed that susceptibility and outcomes from infectious disease are inheritable.[3-5] Indeed, numerous studies have demonstrated that innate immune responses to pathogens exhibit interindividual variability strongly influenced by genetic factors, which may affect disease susceptibility and severity.[6-12] In the pathophysiology of sepsis, the innate immune system is activated prior to the acquired immune system: cells of the innate immune system, such as monocytes, macrophages, and neu-

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**Corresponding author:** Dr. Younsuck Koh, Department of Pulmonary and Critical Care Medicine, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea.
Tel: 82-2-3010-3134, Fax: 82-2-3010-6968, E-mail: yskoh@amc.seoul.kr

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trophils, represent the front line of the host response to infection, invasion, and injury.[13]

High mobility group box 1 (HMGB1) is a highly conserved, ubiquitously expressed protein, originally discovered as a nonhistonal nuclear DNA binding protein.[14-16] It is located on chromosome 13 and present in the nuclei and cytoplasm of nearly all cell types.[17] In response to infection and injury, HMGB1 is secreted by activated innate immune cells and/or passively released by necrotic or damaged cell.[18] Some studies have demonstrated that HMGB1 is a late mediator of sepsis in amplifying the inflammatory response and that serum/plasma HMGB1 concentrations are elevated in patients with sepsis.[19,20] Accumulating evidence supports a central pathogenic role for HMGB1 in the pathogenesis of sepsis and multiple organ dysfunction syndromes.[21-24]

The past decade has witnessed important advances in the understanding of genetic polymorphisms in sepsis; numerous studies have identified that these sepsis-related genetic polymorphisms are associated with severity and/or outcomes.[11,12] Although, several studies reported the clinical relevance of *HMGB1* genetic variation,[25-27] there are limited data on the relationship between single nucleotide polymorphisms (SNPs) of *HMGB1* and clinical outcomes in patients with sepsis. Moreover, the characteristics of these polymorphisms differ according to ethnicity, although few data have been reported in the Korean population. Therefore, we hypothesized that SNPs of *HMGB1* could influence clinical outcomes in Korean patients with sepsis.

In this study, we genotyped a SNP of known genetic variants within *HMGB1* in patients diagnosed with sepsis (including severe sepsis and septic shock), and analyzed its relationship with various clinical parameters, including disease severity and prognosis. We also investigated the relationship between this *HMGB1* polymorphism and serum concentrations of HMGB1 and various cytokines.

## MATERIALS AND METHODS

### Study subjects

Inclusion criteria were adult patients diagnosed with sepsis, including severe sepsis and septic shock. There was no exclusion criterion. In total, 212 patients were enrolled from March 1, 2011 to October 31, 2012. All patients were >20 years of age [median 67.5 (range 29–95) years, M:F=149:63] and had been admitted to the ICU of a Asan Medical Center (Seoul, Korea). Sepsis, severe sepsis, and septic shock were defined using American College of Chest Physicians/Society of Critical Care Medicine.[28,29] All patients were managed according to therapeutic recommendations based on early goal-directed therapy and lung-protective ventilator strategy.[29,30] Survivors were defined as patients who had survived for 28 days after ICU admission. The study objectives and procedures were fully disclosed, and a case report form for this study was completed. All data were collected from the medical records and laboratory and radiographic findings in all patients. This study was approved by the Institutional Review Board (IRB) of the Asan Medical Center (2012-0878). Informed consent was confirmed by the IRB, and written informed consent was obtained from all study participants or their surrogates.

### Data collection

The following data were gathered from the medical records of patients: age, gender, the primary cause of sepsis on initial admission, underlying comorbidities, duration of mechanical ventilation, and lengths of stay in the ICU and hospital. All patients were categorized as sepsis, severe sepsis, and septic shock on ICU admission. Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores were calculated on the sampling day for this study.[31,32] We also identified the causative pathogen for sepsis in patients with positive blood culture, and classified them accordingly. In addition, we recorded laboratory data (complete blood count, lactate, C-reactive protein, procalcitonin) on sampling day, and surveyed for the presence of neutropenia (absolute neutrophil count <1500/mm$^3$).

### SNPs genotyping

Blood samples were drawn within 24 hrs after ICU admission. Genomic DNA was isolated from 5 mL of ethylenediaminetetraacetic acid (EDTA)-anticoagulated venous blood by the standard method using proteinase K and phenol/chloroform extraction. SNP data for the HMGB1 gene [chromosome 13, position 29930000-29939000 (9 kb total)] was obtained from the HapMap data (version 2, release 21) for 45 unrelated Han Chinese individuals from Beijing, China (CHB) and 44 unrelated Japanese individuals from Tokyo, Japan (JPT) samples. From the database, a total of three SNPs with a minor allele frequency >0.05 (rs1045411, rs3742305, rs2249825) were identified in *HMGB1* (excluding 5'- and 3'-flanking regions) and selected for genotyping; all are common SNPs with a minor allele frequency >0.05. Among the three SNPs, rs1045411 located in the 3'-untranslated region (Fig. 1) was chosen for further analyses, because this SNP seemed to show the most significant difference in allele frequencies between patients and normal healthy persons ($\chi^2$ test, $p<0.05$) with the lowest $p$ value and highest odds ratio, compared to the other two SNPs (data not shown). Genotypes for rs1045411 in all patients were determined by Sanger sequencing. The target region of HMGB1 was amplified using forward primer 5'-TGGAAGTGGGAGGCAATTTA-3' (HMGB1_1045411_F) and reverse primer 5'-TGCTGTGCAAA GGTTGAGAG-3' (HMGB1_1045411_R). Amplification conditions were one cycle of 95°C for 7 min, 30 cycle of 95°C for 30 s, 56°C for 30 s, 72°C for 1 min, plus one cycle of 72°C for 5 min. Amplification conditions were one cycle of 95°C for 7 min, 30 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 1


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min, plus one final cycle of 72°C for 5 min. Amplified products of 303 bp were confirmed by agarose gel electrophoresis and purified by treating with Exo-SAP (10:1 U ratio mixture of exonuclease I and shrimp alkaline phosphatase, USB corp., Cleveland, OH, USA) at 37°C for 15 min, 80°C for 15 min, and a 4°C hold. Sequencing was carried out using an ABI 3730XL sequencer by Cosmogenetech, Seoul, Korea. Briefly, sequencing amplifications were performed using the BigDye terminator (ver. 3.1) cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). PCR products of 30–90 ng were used as templates and the cycling reaction consisted of one cycle of 95°C for 90 s, 25 cycles of 95°C for 30 s, 50°C for 5 s, and 60°C for 4 min, followed by a 4°C hold with HMGB1_ 1045411_F and HMGB1_1045411_R. After purifying the cycle sequencing reaction products with magnetic beads (MagneSil GREEN, Promega, Madison, WI, USA), the sequencer instrument was run according to the manufacturer's protocol.

### Serum HMGB1 and cytokines measurement

Blood samples for cytokine measurement were immediately centrifuged, and serum was frozen -80°C until the assay could be performed. We measured serum HMGB1 levels and several inflammatory [interleukin (IL)-1β, IL-6, IL-17, and tumor necrosis factor (TNF)-α] and anti-inflammatory (IL-10) cytokines from 190 patients, because we did not have sufficient quantities of samples; we could not measure serum levels of HMGB1 or other cytokines in 22 patients. The serum HMGB1 level was measured using a sandwich enzyme-linked immunosorbent assay (HMGB1 ELISA; IBL International, Hamburg, Germany). Intra-assay and inter-assay coefficients of variation (CV) were 5.5–13.7% and 7.6–13.7%, respectively. Also, functional sensitivity was 0.2 ng/mL (lowest HMGB1 concentration with ≤20%). The serum levels of cytokines were determined by Luminex® Performance Assay multiplex kits (R&D Systems, Minneapolis, MN, USA). Analyses were performed in accordance with manufacturer's protocol.

### Statistical analysis

The genotype frequencies of three SNPs were tested for Hardy-Weinberg equilibrium using Haploview (v4.2) (http://www.broadinstitute.org/haploview). No significant deviation from Hardy-Weinberg equilibrium was observed. Continuous variables are expressed as median with range. Student's t-test or the Mann-Whitney U-test, depending on the normality of distribution, were used to compare continuous variables between two groups, and the Kruskal-Wallis test was used for comparisons among three groups. Also, the $\chi^2$ and Fisher's exact tests (for small numbers) were used to compare categorical variables. To evaluate whether these SNPs could influence clinical outcomes, univariate and multivariate Cox regression analyses were performed including all clinical data from medical records, with adjustment for age, gender. Survival curves were obtained with using the Kaplan-Meier method with the log-rank test. All statistical analyses were performed using the Statistical Package for the Social Sciences (ver. 19.0; IBM, Armonk, NY, USA). A two-tailed $p$ value<0.05 was considered to indicate significant difference.

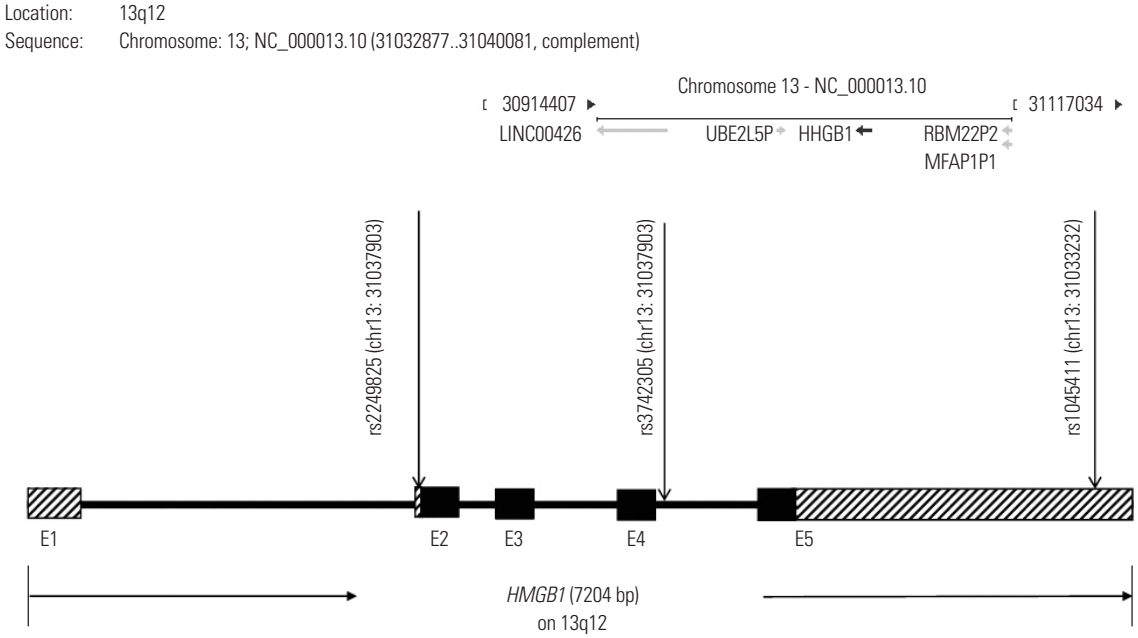


**Fig. 1.** Genetic map of the three single nucleotide polymorphism (SNP) of the *HMGB1*. The three long vertical arrows indicate the locations of the three SNPs. E1 to E5 show the locations of the exons. The black boxes are the protein-coding regions, and boxes including oblique lines are untranslated regions (UTR). rs2249825, rs3742305, and rs1045411 are located in the 5'-UTR, intron 4, and the 3'-UTR, respectively.

## RESULTS

### Baseline characteristics of the study subjects

The clinical characteristics and comparisons of survivors and non-survivors are presented in Table 1. The median durations of stay in the ICU and hospital were six (range 1–104) and 20 (1–830) days, respectively. The ICU and hospital mortality rates were 29.2% and 36.3%, respectively. In total, 150 patients (70.8%) were diagnosed with septic shock; 143 (67.5%) received ventilator care. Also, 86 (40.6%) and 6 (2.8%) patients had renal failure and neutropenia on admission, respectively. The most common primary site of infection was respiratory tract (57.5%), and 72 patients (34.0%) had positive blood cultures (Table 1). The underlying diseases present in all the patients are shown in Table 2. Oncologic and chronic heart diseases were the most common.

### Analyses according to rs1045411 genotypes

When we performed rs1045411 genotyping, the proportion of patients groups by genotype was GG (71.2%), GA (26.4%), and AA (2.4%), respectively. There was no significant difference in other clinical characteristics and outcomes among these three groups (data not shown). When patients were categorized into those with GG (n=151) versus those with GA+AA (n=61), the proportions of A alleles among patients with septic shock, severe sepsis, and sepsis were 31.3%, 23.7%, and 20.8%, respec-

**Table 1.** Baseline Characteristics of Patients at Admission to the Intensive Care Unit

| Characteristics (n) | Total | Survivors (n=155) | Non-survivors (n=57) | *p* value |
|---|---|---|---|---|
| Age, yrs | 68 (29–95) | 66 (29–95) | 71 (29–91) | 0.218 |
| Male | 149 (70.3) | 115 (74.2) | 34 (59.6) | 0.044 |
| APACHE II score | 23 (6–40) | 22 (6–38) | 28 (15–40) | <0.001 |
| SOFA score | 10 (3–18) | 10 (3–18) | 12 (4–18) | <0.001 |
| Primary site of infection | | | | |
| Respiratory tract | 122 (57.5) | 84 (54.2) | 38 (66.7) | 0.118 |
| Hepatobiliary tracts | 34 (18.0) | 29 (18.7) | 5 (8.8) | 0.093 |
| Urinary tract | 18 (8.5) | 15 (9.7) | 3 (5.3) | 0.410 |
| Gastrointestinal tracts | 14 (6.6) | 10 (6.5) | 4 (7.0) | >0.999 |
| Cutaneous/soft tissue | 7 (3.3) | 5 (3.2) | 2 (3.5) | >0.999 |
| Central nervous system | 3 (1.4) | 2 (1.3) | 1 (1.8) | >0.999 |
| Others | 10 (4.7) | 8 (5.2) | 2 (3.5) | >0.999 |
| Unknown | 5 (2.4) | 3 (1.9) | 2 (3.5) | 0.613 |
| Positive blood culture | 72 (34.0) | 58 (37.4) | 14 (24.6) | 0.102 |
| Gram-positive* | 22 (10.4) | 20 (12.9) | 2 (3.5) | 0.072 |
| Gram-negative† | 49 (23.1) | 39 (25.2) | 10 (17.5) | 0.275 |
| Polymicrobial | 11 (5.2) | 8 (5.2) | 3 (5.3) | >0.999 |
| Fungi‡ | 3 (1.4) | 1 (0.6) | 2 (3.5) | 0.177 |
| White blood cell ($10^3$/$mm^3$) (211) | 12600 (600–86700) | 12300 (600–86700) | 12700 (3300–47400) | 0.344 |
| C-reactive protein (mg/dL) (139) | 16.1 (0.1–67.4) | 17.6 (0.1–67.4) | 14.1 (0.9–46.7) | 0.693 |
| Procalcitonin (ng/mL) (57) | 5.1 (0.1–200.0) | 5.1 (0.1–200.0) | 7.0 (0.1–87.6) | 0.661 |
| Lactate (mmol/L) (210) | 1.8 (0.3–15.0) | 1.6 (0.3–13.8) | 2.4 (0.4–15.0) | 0.001 |
| IL-1β (pg/mL) (190) | 6.5 (1.5–219.0) | 6.3 (1.5–219.0) | 6.7 (1.8–133.0) | 0.890 |
| IL-6 (pg/mL) (190) | 140.4 (3.5–16164.5) | 134.0 (3.5–16164.5) | 202.8 (15.6–11562.5) | 0.086 |
| IL-10 (pg/mL) (190) | 33.4 (3.5–22427.5) | 31.7 (3.5–1492.3) | 40.5 (4.2–22427.5) | 0.029 |
| IL-17 (pg/mL) (190) | 6.4 (1.8–317.1) | 6.8 (317.1) | 5.3 (1.9–91.2) | 0.077 |
| HMGB1 (ng/mL) (190) | 16.1 (0.1–310.3) | 15.4 (0.1–84.4) | 18.0 (0.1–310.3) | 0.687 |
| TNF-α (pg/mL) (190) | 17.2 (3.9–249.4) | 17.3 (4.5–249.4) | 17.0 (3.9–193.5) | 0.953 |

APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; IL, interleukin; HMGB1, high mobility group box 1; TNF, tumor necrosis factor.

Data are presented as means (range) (continuous variables) or number (%) (categorical values). Student's t-test or the Mann-Whitney U-test, depending on normality of the distribution, was used to compare continuous variables between two groups. Also, the $\chi^2$ and Fisher's exact tests (for small numbers) were used to compare categorical variables.

*Gram-positive bacteria included *Streptococcus pneumonia* (7), *Staphylococcus aureus* (5), *Streptococcus anginosus* (3), *Streptococcus constellatus* (2), *Streptococcus mitis* (1), *Streptococcus dysgalactiae* (1), *Streptococcus agalactiae* (1), *Streptococcus intermedius* (1), *Enterococcus gallinarum* (1), *Enterococcus casseliflavus* (1), and *Peptostreptococcus spp.* (1), †Gram-negative bacteria included *E.coli* (24), *Klebsiella pneumonia* (12), *Pseudomonas aeruginosa* (8), *Klebsiella oxytoca* (5), *Aeromonas hydrophilia* (2), *Bacteroides fragilis* (2), *Citrobacter koseri* (1), *Citrobacter freundii* (1), *Burholderia cepacia* (1), *Acinetobacter baumanii* (1), *Providencia rittgeri* (1), and *Enterobacter aerogens* (1), ‡Fungi included *Candida grabrata* (1) and *Candida albicans* (1).


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tively. However, there were no significant differences in this allele according to sepsis severity (data not shown).

As underlying comorbidities or primary site of infection could influence clinical outcomes, we also evaluated whether the categories of SNPs (GG and GA+AA) had different implications according to specific comorbid conditions or primary site of infection. In patients with chronic lung diseases or diabetes, patients with the variant A allele had higher positive blood culture rates than those with GG genotype. This allele was also associated with a higher Gram-positive blood culture rate in male patients and Gram-negative culture rate in female and non-survivors. Patients with chronic renal disease had a higher incidence of shock. In cytokine analysis of patients with chronic lung diseases, patients with the variant A allele had significantly higher levels of IL-1β, IL-6, IL-10, IL-17, and TNF-α than those with GG genotype. In analysis of those with diabetes, patients with the variant A allele had higher IL-17 levels. In patients with sepsis caused by respiratory tract infection, patients with this allele had higher levels of IL-10 and IL-17 than those with GG genotype (Table 3).

**Table 2.** Underlying Diseases of Total Patients

| Underlying diseases | n (%) |
|---|---|
| Oncologic disease | 60 (28.3) |
| Chronic heart diseases (ischemic heart disease, arrhythmia, valvular heart disease) | 60 (28.3) |
| Chronic lung diseases (COPD, ILD, destroyed lung caused by various origins) | 57 (26.9) |
| Endocrine disease | 54 (25.5) |
| Chronic liver disease | 31 (14.6) |
| Chronic kidney disease | 23 (10.8) |
| Cerebrovascular disease | 21 (9.9) |
| Musculoskeletal disease | 21 (9.9) |
| Neuromuscular disease | 19 (9.0) |
| Hematologic disease | 14 (6.6) |
| Biliary disease | 13 (6.1) |
| Rheumatologic disease | 8 (3.8) |
| Alimentary disease | 6 (2.8) |
| Others* | 6 (2.8) |

COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease.
Data are presented as number (%).
*Others included chronic alcoholics (2), mood disorder (1), panic disorder (1), arteriosclerosis obliterans (1), and aortic thrombus (1).

### Influence of rs1045411 genotypes on patient survival

A univariate Cox proportional hazards model was performed to evaluate the influence of the variant A allele of rs1045411 on survival in all patients, as well as some subgroups that showed significant findings. However, the A allele was not significantly associated with survival (Table 4), and thus, we could not clarify a significant Kaplan-Meier survival curve for this allele (data not shown).

**Table 3.** Significant Differences between Patients with GG Genotype and Those with GA+AA Genotype in Subgroups Analyses

| Subgroups | Genotype | | *p* value |
|---|---|---|---|
| | GG | GA+AA | |
| Positive Gram-positive culture in male | 5/108 (4.6) | 7/41 (17.1) | 0.020 |
| Positive Gram-negative culture in female | 6/43 (14.0) | 8/20 (40.0) | 0.047 |
| Positive Gram-negative culture in nonsurvivors | 4/41 (9.8) | 6/16 (37.5) | 0.022 |
| Patients with chronic lung diseases | | | |
| Positive blood culture | 4/42 (9.5) | 5/15 (33.3) | 0.044 |
| IL-1β (pg/mL) (n=52) | 5.4 (2.2–74.4) | 21.2 (2.9–131.3) | 0.039 |
| IL-6 (pg/mL) (n=52) | 84.1 (7.5–4236.9) | 406.5 (3.5–11562.5) | 0.026 |
| IL-10 (pg/mL) (n=52) | 25.6 (3.9–391.6) | 88.9 (16.5–2398.5) | 0.003 |
| IL-17 (pg/mL) (n=52) | 3.4 (1.8–150.9) | 12.0 (1.8–111.8) | 0.041 |
| TNF-α (pg/mL) (n=52) | 8.6 (4.5–121.4) | 22.8 (7.2–124.2) | 0.007 |
| Patients with diabetes | | | |
| Positive blood culture | 9/35 (25.7) | 10/17 (58.8) | 0.032 |
| Positive Gram-negative culture | 5/35 (14.3) | 8/17 (47.1) | 0.017 |
| IL-17 (pg/mL) (n=44) | 7.9 (2.0–158.5) | 22.4 (5.0–274.5) | 0.014 |
| Patients with chronic renal disease | | | |
| Development of shock | 8/14 (57.1) | 9/9 (100) | 0.048 |
| Patients with sepsis caused by respiratory tract infection | | | |
| IL-10 (pg/mL) (n=108) | 23.9 (3.9–322.1) | 33.5 (7.9–2398.5) | 0.033 |
| IL-17 (pg/mL) (n=108) | 3.6 (1.8–120.1) | 7.0 (1.8–177.5) | 0.048 |

TNF, tumor necrosis factor; IL, interleukin.
Data are presented as median (range) (continuous variables) or applicable patients/total patients (%) (categorical values). Statistical significance was determined using the Mann-Whitney U-test when comparing continuous values, or the chi-square test or Fisher's exact test when comparing categorical values (for small numbers).

## DISCUSSION

Although HMGB1 has been investigated extensively as an extracellular protein with cytokine-like activity that plays a pivotal role as a late mediator of sepsis,[19,20,22-24,33] few studies have suggested a role for *HMGB1* genetics in critically ill patients.[25,26] To our knowledge, this is the first reported study to investigate the clinical relevance of rs1045411, one of the known *HMGB1* SNPs, in patients diagnosed with sepsis, severe sepsis, and septic shock in Korean populations. Because of the lack of reported data regarding any association between this SNP and various cytokines, our study also assessed this relationship. In view of our results, the rs1045411 genotype likely plays a distinct role in the pathophysiology of sepsis in the Korean population.

In this study, there was no significant difference in the clinical characteristics examined according to rs1045411 genotype in all patients; however, the variant A allele of the rs1045411 polymorphism was associated with higher positive blood culture rates and elevated cytokine levels, particularly in patients with chronic lung diseases or diabetes as comorbidities (Table 4). These findings suggest that patients with the variant A allele might experience more severe inflammatory responses under specific conditions. Patients without these comorbidities, however, showed no significant difference in blood culture rates or cytokine levels between the GG genotype and the GA+AA genotype. Although we could not determine the mechanism underlying the association of this polymorphism with various causes of chronic lung diseases or diabetic control, our rs1045411 results might be more useful for predicting sepsis severity in patients with these comorbidities. It is also important to evaluate the impact of this polymorphism on prognosis through further analysis of a larger number of sepsis patients with these conditions.

In studying human *HMGB1*, Kornblit, et al.[25] identified six SNPs (rs1412125, rs41369348, rs2249825, rs1060348, rs3472305, rs41376448), and reported significant associations of rs1060348 and rs41369348 with early and late mortality in patients who were diagnosed with systemic inflammatory response syndrome in Denmark.[26] Zeng, et al.[27] studied the clinical relevance of *HMGB1* in patients with major trauma in South China. Among the three SNPs (rs1412125, rs2249825, rs1045411) selected using the HapMap database, they reported that rs2249825 and the haplotype TCG might be used as a relevant risk factor for the development of sepsis and multiorgan dysfunction syndrome in patients with major trauma. Compared with these reports, our study enrolled patients with sepsis (including severe sepsis and septic shock) and evaluated the clinical utility of genotype using subgroup analysis and cytokine level measurements. In addition, severity would have differed among the three studies because additional mechanical ventilation, higher SOFA and APACHE II scores, and longer ICU stays were included in our report.

This study had several limitations. First, we expected that there would be significant differences in clinical outcomes (e.g., ICU and hospital length of stay, duration mechanical ventilation, organ dysfunctions, and causative organisms) according to rs1045411 polymorphism; however, we found no such difference, possibly due to the small sample size. Second, this study was performed in only one hospital and the sample size was small; thus, it may not be representative of the general population of Korea. However, all patients were managed according to the "Surviving Sepsis Campaign Guideline for Management of Severe Sepsis and Septic Shock,"[29] and standardized procedures in this hospital were performed as a rule to reduce any confounding factors related to the outcome of sepsis. Third, we could not measure serum HMGB1 levels using Western blot analysis, which shows higher values than those using ELISA. Fourth, the number of total enrolled patients was not based on our preliminary sample size analysis.

In conclusion, we investigated the clinical relevance of rs1045411 genotypes, one of the known *HMGB1* SNPs, in patients diagnosed with sepsis, severe sepsis, or septic shock. In this study, patients with the variant A allele had higher positive blood culture rates and higher cytokine levels than did those with the GG genotype under some specific conditions. Although this polymorphism had no significant impact on survival, patients with the variant A allele might experience an aggravated inflammatory response to these conditions. Also, there may be interactions between our studied haplotype and other genes involved in immune responses. Large-scale studies are required to determine the clinical significance of this polymorphism.

**Table 4.** Influence of the Variant A Allele of rs1045411 on Patient Mortality Using Univariate Cox Proportional Hazards Models

| | The variant A allele of rs1045411 | | |
|---|---|---|---|
| | HR | 95% CI | *p* value |
| Total patients | 0.903 | 0.505–1.614 | 0.731 |
| Subgroups | | | |
| Septic shock | 0.798 | 0.406–1.566 | 0.511 |
| Patients with positive blood culture | 1.431 | 0.493–4.151 | 0.509 |
| Primary site of infection–respiratory tract | 1.163 | 0.576–2.348 | 0.673 |
| Comorbidity–chronic lung disease | 2.117 | 0.820–5.466 | 0.121 |
| Comorbidity–diabetes | 0.632 | 0.201–1.989 | 0.433 |

HR, hazard ratio; CI, confidence interval.


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## REFERENCES

1. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003;348:1546-54.
2. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29:1303-10.
3. Burgner D, Levin M. Genetic susceptibility to infectious diseases. Pediatr Infect Dis J 2003;22:1-6.
4. Bellamy R, Hill AV. Genetic susceptibility to mycobacteria and other infectious pathogens in humans. Curr Opin Immunol 1998;10:483-7.
5. Choi EH, Zimmerman PA, Foster CB, Zhu S, Kumaraswami V, Nutman TB, et al. Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with Wuchereria bancrofti in South India. Genes Immun 2001;2:248-53.
6. Holmes CL, Russell JA, Walley KR. Genetic polymorphisms in sepsis and septic shock: role in prognosis and potential for therapy. Chest 2003;124:1103-15.
7. De Maio A, Torres MB, Reeves RH. Genetic determinants influencing the response to injury, inflammation, and sepsis. Shock 2005;23:11-7.
8. Wurfel MM, Park WY, Radella F, Ruzinski J, Sandstrom A, Strout J, et al. Identification of high and low responders to lipopolysaccharide in normal subjects: an unbiased approach to identify modulators of innate immunity. J Immunol 2005;175:2570-8.
9. de Craen AJ, Posthuma D, Remarque EJ, van den Biggelaar AH, Westendorp RG, Boomsma DI. Heritability estimates of innate immunity: an extended twin study. Genes Immun 2005;6:167-70.
10. Aziz RK, Kansal R, Abdeltawab NF, Rowe SL, Su Y, Carrigan D, et al. Susceptibility to severe Streptococcal sepsis: use of a large set of isogenic mouse lines to study genetic and environmental factors. Genes Immun 2007;8:404-15.
11. Sutherland AM, Walley KR. Bench-to-bedside review: association of genetic variation with sepsis. Crit Care 2009;13:210.
12. Namath A, Patterson AJ. Genetic polymorphisms in sepsis. Crit Care Clin 2009;25:835-56.
13. Cinel I, Opal SM. Molecular biology of inflammation and sepsis: a primer. Crit Care Med 2009;37:291-304.
14. Wang H, Zhu S, Zhou R, Li W, Sama AE. Therapeutic potential of HMGB1-targeting agents in sepsis. Expert Rev Mol Med 2008;10:e32.
15. Bustin M, Lehn DA, Landsman D. Structural features of the HMG chromosomal proteins and their genes. Biochim Biophys Acta 1990;1049:231-43.
16. Müller S, Ronfani L, Bianchi ME. Regulated expression and subcellular localization of HMGB1, a chromatin protein with a cytokine function. J Intern Med 2004;255:332-43.
17. Javaherian K, Liu JF, Wang JC. Nonhistone proteins HMG1 and HMG2 change the DNA helical structure. Science 1978;199:1345-6.
18. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418:191-5.
19. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999;285:248-51.
20. Ueno H, Matsuda T, Hashimoto S, Amaya F, Kitamura Y, Tanaka M, et al. Contributions of high mobility group box protein in experimental and clinical acute lung injury. Am J Respir Crit Care Med 2004;170:1310-6.
21. Angus DC, Yang L, Kong L, Kellum JA, Delude RL, Tracey KJ, et al. Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. Crit Care Med 2007;35:1061-7.
22. Gibot S, Massin F, Cravoisy A, Barraud D, Nace L, Levy B, et al. High-mobility group box 1 protein plasma concentrations during septic shock. Intensive Care Med 2007;33:1347-53.
23. Sundén-Cullberg J, Norrby-Teglund A, Rouhiainen A, Rauvala H, Herman G, Tracey KJ, et al. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Crit Care Med 2005;33:564-73.
24. Wang H, Yang H, Czura CJ, Sama AE, Tracey KJ. HMGB1 as a late mediator of lethal systemic inflammation. Am J Respir Crit Care Med 2001;164:1768-73.
25. Kornblit B, Munthe-Fog L, Petersen SL, Madsen HO, Vindeløv L, Garred P. The genetic variation of the human HMGB1 gene. Tissue Antigens 2007;70:151-6.
26. Kornblit B, Munthe-Fog L, Madsen HO, Strøm J, Vindeløv L, Garred P. Association of HMGB1 polymorphisms with outcome in patients with systemic inflammatory response syndrome. Crit Care 2008;12:R83.
27. Zeng L, Zhang AQ, Gu W, Chen KH, Jiang DP, Zhang LY, et al. Clinical relevance of single nucleotide polymorphisms of the high mobility group box 1 protein gene in patients with major trauma in southwest China. Surgery 2012;151:427-36.
28. Bone RC, Sibbald WJ, Sprung CL. The ACCP-SCCM consensus conference on sepsis and organ failure. Chest 1992;101:1481-3.
29. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Intensive Care Med 2008;34:17-60.
30. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. N Engl J Med 2000;342:1301-8.
31. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985;13:818-29.
32. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996;22:707-10.
33. van Zoelen MA, Laterre PF, van Veen SQ, van Till JW, Wittebole X, Bresser P, et al. Systemic and local high mobility group box 1 concentrations during severe infection. Crit Care Med 2007;35:2799-804.