Role of serum high-motility group box-1 (HMGB1) concentration as a prognostic factor in canine acute pancreatitis

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Abstract

Background High-mobility group box-1 (HMGB1) is an intranuclear molecule that is released extracellularly in cytotoxic conditions. In acute pancreatitis, extracellular HMGB1 acts as stimulating factor in the mechanism associated with pancreatic injury. The serum level of HMGB1 may be associated with the severity and prognosis of pancreatitis.

Methods To evaluate the prognostic property of serum HMGB1 levels at the time of diagnosis of pancreatitis, case control study using serum samples collected over 10 months from canine patients in Seoul National University Veterinary Medical Teaching Hospital (SNU VMTH). The HMGB1 levels in serum were measured by ELSIA and they were analyzed with patient's death, hospitalization cost and hospitalization period.

Results Serum levels of HMGB1 in patients with acute pancreatitis (n=19) were higher than those in normal individuals (n=10). Mean (± standard deviation) values were 76.00 (±46.99) and 31.65 (±18.41) ng/mL, respectively (p=0.004). The levels of HMGB1 were significantly higher in non-survivors than in survivors with acute pancreatitis (p = 0.019). Clinical severity of acute pancreatitis was categorized into three stages—mild, moderate, and severe—based on the disease activity index (DAI). Higher HMGB1 levels in serum were related to mortality in patients with moderate DAI (p = 0.049). Furthermore, higher levels of serum HMGB1 among hospitalized patients were positively associated with higher costs and longer duration of hospital stays. Conclusions The evaluation of serum HMGB1 levels at the time of diagnosis was identified as a potential prognostic factor to assess the outcome of acute pancreatitis in canines.

Background

High-mobility group box-1 (HMGB1) is a 30-kDa non-histone chromosomal protein with several functions depending on its location (extra- or intracellular). As a nuclear molecule, it typically regulates gene transcription, repair, and recombination processes, and determines the structure and stability of nucleosides. HMGB1 may be detected extracellularly in two specific conditions [1]. The first is an active process in stimulated macrophages, which is particularly gradual and involves post-translational modification and its secretion. The second is passive cell leakage caused by necrosis or apoptosis-associated cellular damage. Since both conditions are not particularly common, HMGB1 is considered to be a damage-associated molecular pattern [2]. Therefore, the intracellular substances exit the cell and occupy the extracellular space in a cell-destructive event, act as a danger signal causing the infiltration of several inflammatory cells. Intensive reactions can eventually disrupt organ function and cause tumor growth and metastasis, which may promote septicemia, resulting in fatal consequences.

Acute pancreatitis (AP) elicits various responses to the same stimuli with different progression and outcomes. Mild AP is a localized process presenting uncomplicated full recovery, but severe AP (SAP) with necrosis, although localized, can cause systemic inflammatory response syndrome (SIRS) resulting in poorer responses. Extracellular HMGB1 is considered to be a novel pro-inflammatory cytokine in

humans [3], and several studies have significantly contributed to elucidating its pathophysiologic role in SAP [4, 5]. HMGB1 is initially produced by pancreatic and peritoneal macrophages in SAP in response to inflammation and is then partially released into the blood, thereby damaging remote organs. The damaged organs subsequently release HMGB1, resulting in a series of cascading effects [6]. Gang Li [7] reported that HMGB1 acts as a stimulating factor using the TLR4-mediated NF-κB signaling pathway to cause pancreatic injury in mice. Human SAP patients [8] and SAP-induced rat models [9] demonstrated significantly increased serum and pancreatic HMGB1 levels. Furthermore, a study recently confirmed the same in dogs with SAP [10].

The clinical symptoms of pancreatitis are an important part of the diagnosis, but the severity of the clinical symptoms is often inconsistent with the prediction of the patient's prognosis. A study demonstrated its prognostic aspect in SIRS-associated conditions as compared to canine C-reactive protein (CRP) [11]. Considering the lack of specific treatment modalities for AP, canine pancreas-specific lipase (cPL) tendency and CRP levels are referred to as treatment indicators during symptomatic treatment. Furthermore, canine AP, hyponatremia, and azotemia [12] demonstrating low platelet counts, cPL tendency, and CRP levels [13] appear to be associated with an increased mortality risk. However, a number of conflicting study results have also been reported that limit their use in veterinary clinical applications. HMGB1 is not specific to pancreatitis, but is more closely associated with SIRS conditions associated with SAP. Therefore, serum HMGB1 could be useful with the prediction of the patient's prognosis in patients with ambiguous clinical symptoms.

This study aimed to clarify if HMGB1 could function as a prognostic factor for AP, and if patients who had previously visited university hospitals for the treatment of AP were confirmed to have elevated values. The correlation with the survival rate, length of stay, and cost of hospitalization was investigated, and the future of HMGB1 as a pancreatitis prognostic factor was discussed.

Results

Study population

The study included 29 dogs of several breeds, of which 10 healthy dogs as controls were grouped into normal group. Table 1 showed age, weight, sex and neuter status of normal group. They included 4 spayed females and 6 males, of which 5 were neutered. The median age of the dogs 7 years (range, 2–11 years). The clinical data of the patients with AP (n = 19), who met the inclusion criteria during the study recruitment period, are summarized in Table 2 and were classified as the AP group. They included 8 females, of which 5 were spayed, and 11 neutered males, and the median age of the dogs was 11 years (range, 4–17 years). They had various diseases at the time of their visit, such as chronic kidney disease, chronic valvular heart disease, enteritis, etc. (Table 2).

Table 1

Summary of data of normal dogs included in this study as controls, presenting age, breed, sex, and body condition score and serum HMGB1 level

Num.	Breed	Age (year)	Sex	BCS	Serum HMGB1 level
1	Cavalier King Charles spaniel	3	MC	5	26.26 ng/ml
2	Mixed	11	FS	5	20.606 ng/ml
3	Pekingese	10	FS	5	31.879 ng/ml
4	Golden retriever	8	MC	6	55.2388 ng/ml
5	Poodles	9	IM	5	66.72528 ng/ml
6	Chihuahua	8	FS	5	4.365079 ng/ml
7	Japanese Spitz	2	MC	5	37.93651 ng/ml
8	Mixed	3	FS	5	17.77778 ng/ml
9	Greyhound	5	MC	5	20.47619 ng/ml
10	Labrador retriever	11	MC	4	35.2381 ng/ml
MC: male castrated; FS: female spayed; IM: intact male;					

Table 2

Summary of clinical data of acute pancreatitis canine patients included in this study, presenting age, breed, sex, body condition score, and concurrent diseases at the time of AP diagnosis.

Num.	Breed	Age (year)	Sex	BCS	Concurrent disease
1	Poodles	14	FS	4	Enteritis
2	Maltese	11	IF	5	CVHD, PDA
3	Maltese	12	MC	5	Anemia, CVHD
4	Mixed	14	MC	6	B cell lymphoma, chronic bronchitis
5	Mixed	14	MC	3	CKD, CVHD, hyporeninemic hypoaldosteronism, idiopathic vestibular disease
6	Maltese	13	IF	3	HCC, pulmonary metastasis suspected, MGT, enteritis, tracheal collapse
7	Coton de Tulear	4	FS	4	2nd AV block, hematuria
8	Dachshund	8	MC	5	Bone marrow dysplasia
9	Yorkshire terrier	8	MC	6	Renal calculi
10	Maltese	12	IF	6	CVHD, enteritis, renal calculi
11	Chihuahua	4	MC	6	-
12	Maltese	14	FS	5	CKD, CVHD, hyperadrenocorticism
13	Maltese	10	MC	5	Acute liver failure, cholecystitis
14	Schnauzers	15	MC	5	Chronic hepatitis, CKD, CVHD, enteritis
15	Poodles	14	FS	5	EHBO, GB rupture
16	Maltese	17	FS	4	CKD, CVHD, proteinuria
17	Yorkshire terrier	12	MC	5	-
18	Pekingese	14	MC	6	CKD, CVHD, Tracheal collapse, renal caculi, cystic calculi,
19	Poodles	8	MC	5	Duodenitis, idiopathic epilepsy

MC: male castrated; IF: intact female; FS: female spayed; CKD: chronic kidney disease; CVHD: chronic valvular heart disease; PDA: patent ductus arteriosus; HCC: hepatic cellular carcinoma; MGT: mammary gland tumor; AV: atrioventricular; EHBO: extrahepatic biliary obstruction; GB: gall bladder

Serum HMGB1 levels in dogs with acute pancreatitis and comparison with the normal group

The test results are shown in Fig. 1. The serum concentration of HMGB1 in the normal group ranged from 4.365–66.725 ng/mL (mean = 31.65 ng/mL, standard deviation [SD] = 18.41 ng/mL). In the AP group, serum HMGB1 levels ranged from 22.572-200 ng/mL (mean = 76 ng/mL, SD = 46.99 ng/mL). The values of the two groups showed a difference, and reached statistical significance (p = 0.004).

Serum HMGB1 levels in dogs with acute pancreatitis and comparison with survivors

Canine patients with AP who died after the onset of disease, and patients who survived the disease were divided into the survivor and non-survivor groups, respectively, to compare the differences in HMGB1 levels (Fig. 2). Serum HMGB1 levels in the non-survivor group (mean = 121 ng/mL, SD = 46.9 ng/mL) were significantly higher (p = 0.019) than that of the survival group (mean = 59.91 ng/mL, SD = 36.34 ng/mL).

Serum HMGB1 levels in dogs with acute pancreatitis and correlation with disease activity index

Table 3 summarizes the data regarding the HMGB1 level, survival, disease activity index (DAI), hospitalization duration, and hospitalization cost to confirm the data of all 19 patients of the AP group. The sum of DAI of each patient was classified as mild (0–2), moderate (3–5), and severe (6–8). To evaluate the tendency of other observations according to the HMGB1 concentration, the survival of the patients was calculated based on the DAI (Table 4). All patients with mild and severe DAI demonstrated consistent results, contrary to those with moderate DAI, among which 25% of the animals were non-survivors. In Fig. 3, analyses of the HMGB1 concentrations in patients with moderate DAI reached statistical significance (p = 0.049). This suggested that HMGB1 concentration could be used as a basis for assessing the patient's prognosis in patients with moderate DAI.

Table 3
Summary of serum HMGB1 concentration, survival rate, DAI and hospitalization information for each AP patient.

Num.	Serum HMGB1 level	Survival	DAI	Hospitalization		
				Admitted	Length	Total cost
1	32.278 ng/ml	Live	2 (mild)	Yes	2 days	1,941,244 ₩
2	96.479 ng/ml	Dead	8 (severe)	Yes	3 days	2,686,091 ₩
3	33.82 ng/ml	Live	4 (moderate)	No	-	-
4	22.574 ng/ml	Live	2 (mild)	Yes	4 days	1,633,460 ₩
5	24.293 ng/ml	Live	5 (moderate)	Yes	3 days	2,786,786 ₩
6	27.377 ng/ml	Live	3 (moderate)	Yes	3 days	1,115,340 ₩
7	133.869 ng/ml	Live	3 (moderate)	Yes	10 days	4,445,200 ₩
8	37.25 ng/ml	Live	2 (mild)	No	-	-
9	32.801 ng/ml	Live	2 (mild)	No	-	-
10	77.454 ng/ml	Dead	6 (severe)	Yes	8 days	3,053,780 ₩
11	116.954 ng/ml	Dead	4 (moderate)	Yes	6 days	2,135,469 ₩
12	114.348 ng/ml	Dead	5 (moderate)	Yes	5 days	7,893,136 ₩
13	66.562 ng/ml	Live	3 (moderate)	Yes	11 days	4,602,044 ₩
14	200 ng/ml	Dead	4 (moderate)	Yes	4 days	2,807,110 ₩
15	99.341 ng/ml	Live	4 (moderate)	Yes	7 days	3,639,040 ₩
16	76.277 ng/ml	Live	4 (moderate)	No	-	-
17	81.253 ng/ml	Live	2 (mild)	No	-	-
18	114.269 ng/ml	Live	4 (moderate)	Yes	5 days	2,053,960 ₩
19	56.768 ng/ml	Live	4 (moderate)	Yes	12 days	8,472,030 ₩

Table 4
Comparison of survived and dead patients according to DAI in AP group.

	Mild	Moderate	Severe	Total
Live	5	9	0	14
Dead	0	3	2	5
Total	5	12	2	19
Mortality	0%	25%	100%	-

Relationship among serum HMGB1 levels, length of hospitalization and cost of hospitalization in dogs with acute pancreatitis

Based on Table 3, the correlation between the serum HMGB1 concentration and the hospitalization duration and cost of admission was investigated (Fig. 4). The correlation coefficient between serum HMGB1 level and hospitalization cost was 0.154, 0.141 between serum HMGB1 level and hospitalization duration, and 0.632 between hospitalization cost and duration, respectively. The cost and period of hospitalization of AP patients showed a moderate positive relationship. In addition, serum HMGB1 levels were weakly positively correlated with each total cost and period of hospitalization. In Fig. 5, the four parameters have been represented in one graph. Each dot represents acute pancreatitis patients that were hospitalized in SNU VMTH. The size of the dots reflected the cost, and they were marked in different colors depending on their survival.

Regression analysis (Supplementary Fig. 1) was performed to evaluate the casual relationship between serum HMGB1 concentration and patient's hospitalization cost, or period of the hospital stay. The regression curve between the serum HMGB1 concentration and the patient's hospitalization cost with log values was best suited for following the S equation ($p = 0.07$). The S equation was also observed between the serum HMGB1 concentration and the hospitalization period ($p = 0.067$).

Discussion

In this study, the association between serum HMGB1 levels and mortality in canine patients with AP was investigated. The diagnosis of pancreatitis in dogs requires a combination of investigating the clinical symptoms, serum chemistry, and abdominal imaging. However, these diagnostic tools cannot predict if the patient will suffer severely due to pancreatitis, or the risk of the disease when the patient is being diagnosed. Significant differences were observed in the serum levels of HMGB1 at the time of diagnosis of pancreatitis between the normal group and the AP group. Also, statistical significance was shown between the survivors and the non-survivors. These results suggest that high serum HMGB1 levels may be associated with a poor prognosis in patients with AP.

The terms mild, moderate, and severe are used to describe the severity of pancreatitis. They could classify the severity of pancreatitis intuitively and used to determine the survival and prognosis of the patient. In general, the criterion for severe AP is neutrophilic inflammation or necrosis in pancreatic tissues confirmed by histologic examination. However, clinically assessing the severity of the disease through biopsies in every case is difficult. Therefore, patients with multiple organ failures or those needing intensive treatment are considered as severe acute pancreatitis (SAP). When multiple organ failure occurs, pancreatitis is already in the severe state and therapeutic intervention could be delayed, thus, the value of prognostic evaluation is low. Accordingly, there is a need for a method to measure the disease activity in patients with AP, clinically. There are several criteria used in humans and dogs, and they have been summarized in a previous paper [14], but establishment of such criteria based only on the clinical symptoms is needed. In this study, the screening criteria for DAI was established in canine patients and used to analyze the population. The mild DAI group recovered from AP and returned to normal condition, the severe DAI group showed irreversible results, while those with moderate DAI had a 75% survival rate. There was a statistically significant difference in their serum HMGB1 levels at the time of diagnosis. Therefore, it may be helpful to measure serum HMGB1 levels in estimating mortality of AP group whose clinical prognosis is ambiguous.

In this study, the AP group received the necessary symptomatic treatment as needed. The relationship among HMGB1 level, hospitalization cost, and hospitalization duration was evaluated using multiple scatter-plot and regression analysis. The multiple scatter-plot visualized the relationship between the three variables and correlation coefficient values showed that the HMGB1 level, hospitalization cost, and hospitalization period had a positive correlation with each other. Also, the regression analysis was applied to outline a causal view of the increased monetary and temporal effort required to treat patients with AP at high serum HMGB1 levels when serum HMGB1 levels were high (Supplementary Fig. 1). When the S equation was applied, the R squared values were found to be 0.25 and 0.249. These are values for real patients and are considered to be meaningful.

This study included 10 normal dogs and 19 AP patients. Patients with an average age of 11 years and with several concurrent diseases were recruited. About the exclusion criteria and normal group inclusion, some dogs showed serum HMGB1 levels significantly lower than normal dogs, despite a high DAI of AP and their poor prognosis. Their commonality was taking oral steroids against immune-mediated thrombocytopenia (IMT) disease. There has been no clear study that has reported that oral prednisolone administration lowers blood HMGB1 levels in dogs, but E. Mysler [15] showed that prednisolone suppresses HMGB1-induced inflammatory responses in cell-based screening. In this matter, there is a possibility of a correlation between the mechanism of action of HMGB1 and that of prednisolone in vivo.

In AP patients, the severity is typically associated with sepsis or SIRS, which is classified as SAP. When the AP group were screened for SIRS criteria [16], 4 patients (No. 2, 7, 11, 19) met at least two out of four, and one patient (No. 11) showed severe weakness, bradycardia and hypothermia, and was strongly suspected as SIRS. It died despite being hospitalized for 6 days. In particular, papers on AP patients and HMGB1 concentrations published to date have demonstrated an upward trend in SAP patients with SIRS

[17]. This result suggests that HMGB1 levels could be used as a prognostic factor in non-SIRS or early SIRS in AP patients, which needs further investigations.

In addition, HMGB1 is also elevated in immune mediated inflammatory diseases including inflammatory bowel disease, rheumatoid arthritis. Although not attached to this paper, the number of patients with immune-mediated diseases other than pancreatitis has been found to have excessively high values. Therefore, HMGB1 is not suitable as a tool for diagnosing pancreatitis, but is rather applicable in the prognostic evaluation in patients who have already been diagnosed with AP.

One study [8] confirmed that HMGB1, a late mediator of inflammation, reached its peak within 48–72 hours in SAP human patients. Patients in this study had their serum collected within 48 hours when diagnosed with AP. However, it may be different from the onset of pancreatitis because the time taken by the caretaker to notice the symptoms of the dog and visit the hospital may vary. In this study, although the trend of HMGB1 level was not confirmed, the clinical symptoms of 2 patients collected serially at 24-hour intervals improved, but there was little change in HMGB1 concentration. However, for CRP [18] or cPL [19], which confirms the presence of an acute inflammatory response, their concentrations could rapidly increase and decrease within 24 hours. They reflect the immediate inflammatory situation, and the tendency to increase or decrease is more important. On the other hand, HMGB1 levels tend to climb slowly [11], indicating that the error due to time of visit does not have a big influence.

Some limitations were encountered in the study. First, most AP cases in the SNU VMTH were already managed for the various underlying diseases. If study participants had only AP, the correlation between serum HMGB1 concentration and prognosis would be more intuitive. Second, the study confirmed the possibility of serum HMGB1 concentration as a prognostic factor of pancreatitis despite the small sample size. The next step should be to conduct a systematic assessment of increasing the evaluation period and the number of cases.

In this study, the serum HMGB1 levels in canine patients diagnosed with AP were significantly higher than normal dogs without physical abnormalities. And statistical significance between patients that died from the disease and those that recovered successfully was also identified. Furthermore, there was a tendency of longer hospital stay and higher cost in AP patients with higher HMGB1 levels. Serum levels at the time of diagnosis could be used to predict the prognosis of patients with AP, especially in patients with moderate DAI.

Materials And Methods

Case selection

This study included canine patients referred to the SNU VMTH, between October 2018 and July 2019, and included normal dogs as controls, and dogs diagnosed with AP as cases. This study was approved by Institutional Animal Care and Use Committee of SNU (SNU-191108-1). Written or verbal informed consent for collecting blood was obtained from the owners and was recorded in the medical chart.

Inclusion criteria

Dogs enrolled in this study were screened for abnormalities during the general health examination (overall physical examination, complete blood count, serum biochemistry, survey thoracic and abdominal radiographs, and abdominal ultrasonography). AP diagnosis was based on the following criteria: 1) presence of one or more of the clinical signs of anorexia/hyporexia, diarrhea, lethargy, vomiting for < 7 days; 2) concurrent presence of increased serum cPL level (> 200 ng/L, VCheck cPL®, Bionote, Seoul, South Korea) or abnormal SNAP cPL test result (Idexx Laboratories, Milan, Italy); and 3) ultrasonographic evidence of pancreatitis (ProSound Alpha 7, Hitachi, Japan and UGEO H60 Samsung Medison, South Korea). Ultrasonographic findings included enlarged and hypoechoic pancreas, cavitary lesions such as abscess or pseudocyst, dilated pancreatic duct, swollen hypomotile duodenum, biliary dilatation, and peritoneal fluid [20]. Normal dogs that showed no clinical signs and did not show any abnormalities in general examination were recruited into the control group.

Exclusion criteria

Among patients with AP, dogs had undergone treatment with prednisolone for their concurrent diseases were excluded to avoid the effect of external steroids on the results. Additionally, clinical and experimental studies suggested that HMGB1 could majorly influence the pathogenesis of autoimmune diseases [1]. Patients with concurrent diseases such as immune-mediated hemolytic anemia, IMT, protein-losing enteropathy, and systemic lupus erythematosus were excluded to eliminate the possibility of synergism with the autoimmune diseases.

Development of the disease activity index on acute pancreatitis

DAI was designed to clinically quantify AP severity. The scoring system was based on the careful monitoring of patients. To capture the immediate disease activity during diagnosis in non-invasive way, clinical symptoms were grouped into 4 categories: anorexia/hyporexia, diarrhea, lethargy, and vomiting. Symptoms was scored on a scale of 0–2 as follows: 1) Appetite: 0 - no abnormalities, 1 - no enteral food intake for < 3 days or decreased appetite < 50%, 2 - no enteral food intake or decreased appetite > 50% for > 3 days; 2) Defecation: 0 - no abnormalities, 1 - Bristol type [21] 5–6, 2 - Bristol type 6–7, or presence of hematochezia or melena; 3) Vitality: 0 - Karnofsky's performance score [22] grade 0, 1 - Karnofsky's performance score grade 1, 2 - Karnofsky's performance score grade 2–3; 4) Vomiting: 0 - no abnormalities, 1 - vomiting or regurgitation < 3 days, 2 - vomiting or regurgitation > 3 days or > 5 times in a day. The scores were summed and the total score was evaluated as the DAI.

Serum collection

Blood was collected by venipuncture method and placed in heparin tubes within 48 hours of diagnosis. After the samples were centrifuged, the collected plasma was stored at -80 ℃ until analysis.

Assay for high-mobility group box chromosomal protein-1 concentrations

The serum HMGB1 concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Canine High Mobility Group Box-1 Protein ELISA kit; MyBioSource, Inc., San Diego, USA) and following the manufacturer's instructions, and each sample was tested twice for precision.

Medical records

The following data of all patients was reviewed from the medical records on the electronic charting program (E-friends, pnV, Seoul, Korea): age, breed, sex, clinical symptoms, body condition score [23], results of clinicopathologic evaluation (hematologic and serum biochemical analyses and urinalysis) and radiologic results, costs for hospital stays, length of hospital stay, and survival. The cost of hospitalization was calculated as the total cost of all medications, supplies, and injections used during hospitalization.

Treatment for acute pancreatitis

Each AP patient was treated according to their specific conditions. General treatment principles involved replacing fluid loss, maintaining hydrostatic pressure, controlling nausea, and providing pain relief [24]. Furthermore, the patients were managed under hospitalization for cases that were not permitted to consume fluids orally and had severe dehydration, or high inflammation (white blood cells > 15000 /ul, CRP > 35 ng/L) [25]. The patients were discharged when their appetites were restored, vitality had improved, and cPL levels were normalized. The period from the time of admission until discharge was regarded as the length of hospital stay.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software), R software and SPSS. Shapiro-Wilk test was performed for normality. Student's t-test was performed if the data passed equal variance test. If equal variance tests failed, Welch's t-test was used. Differences between groups were assessed for statistical significance by using unpaired t-test. The p-value was calculated by one-tailed testing and considered as a statistically significant difference if $p < 0.05$. After multicollinearity was screened, the correlation analysis and regression analysis were performed between level of serum HMGB1 concentration, patient's hospitalization period, and hospitalization cost.

Declarations

Competing interests
The authors declare that they have no competing interests
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Authors' contributions

JHL, WJS, and HYY conceived and designed this study, JHA, HKC, SMP, and QL collected and analyzed data, and JHL and WJS wrote manuscript. All authors have read and approved the final manuscript.

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Ethics approval and consent to participate

This study protocol and design were approved by the Seoul National University Institutional Animal Care and Use Committee (IACUC) and ethical approval has been granted (SNU-191108-1).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study will be available from the corresponding author on reasonable request.

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Figures

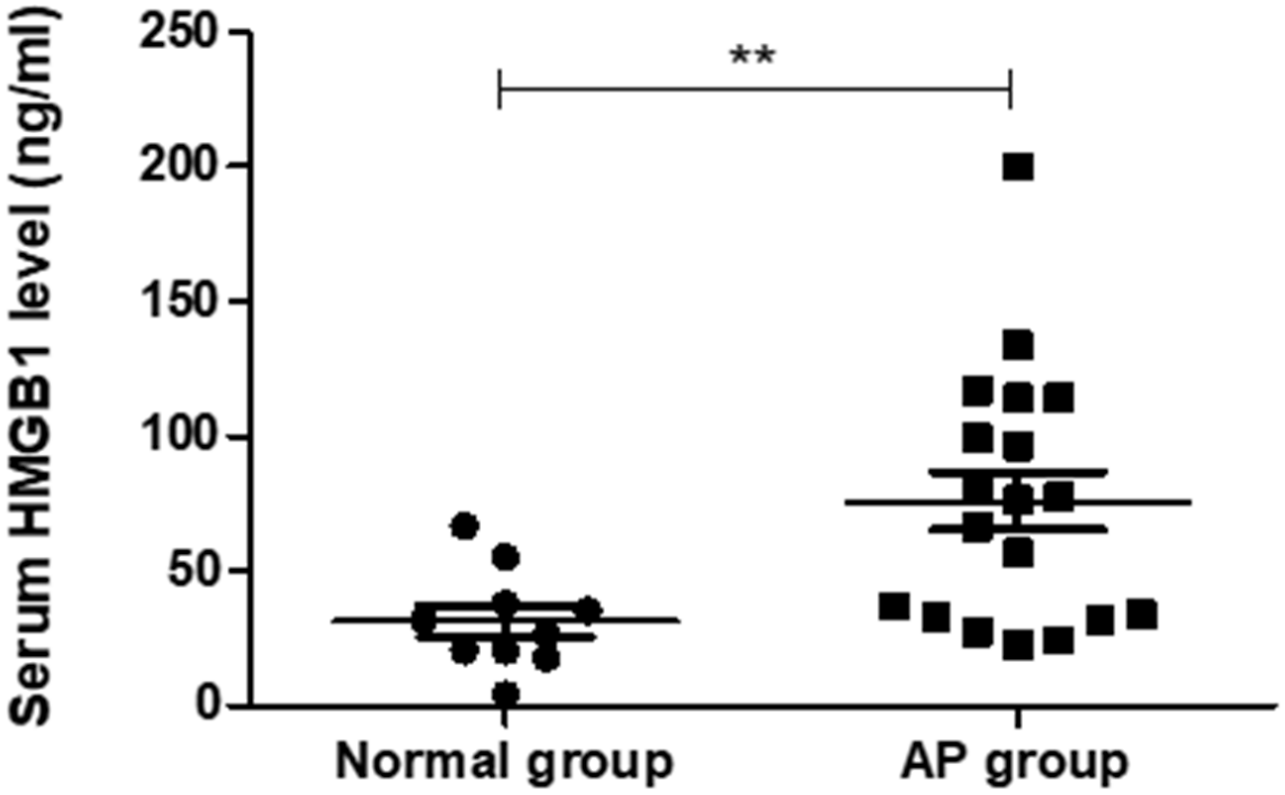

Figure 1

Serum high mobility group box-1 protein (HMGB1) levels in normal group and acute pancreatitis (AP) group

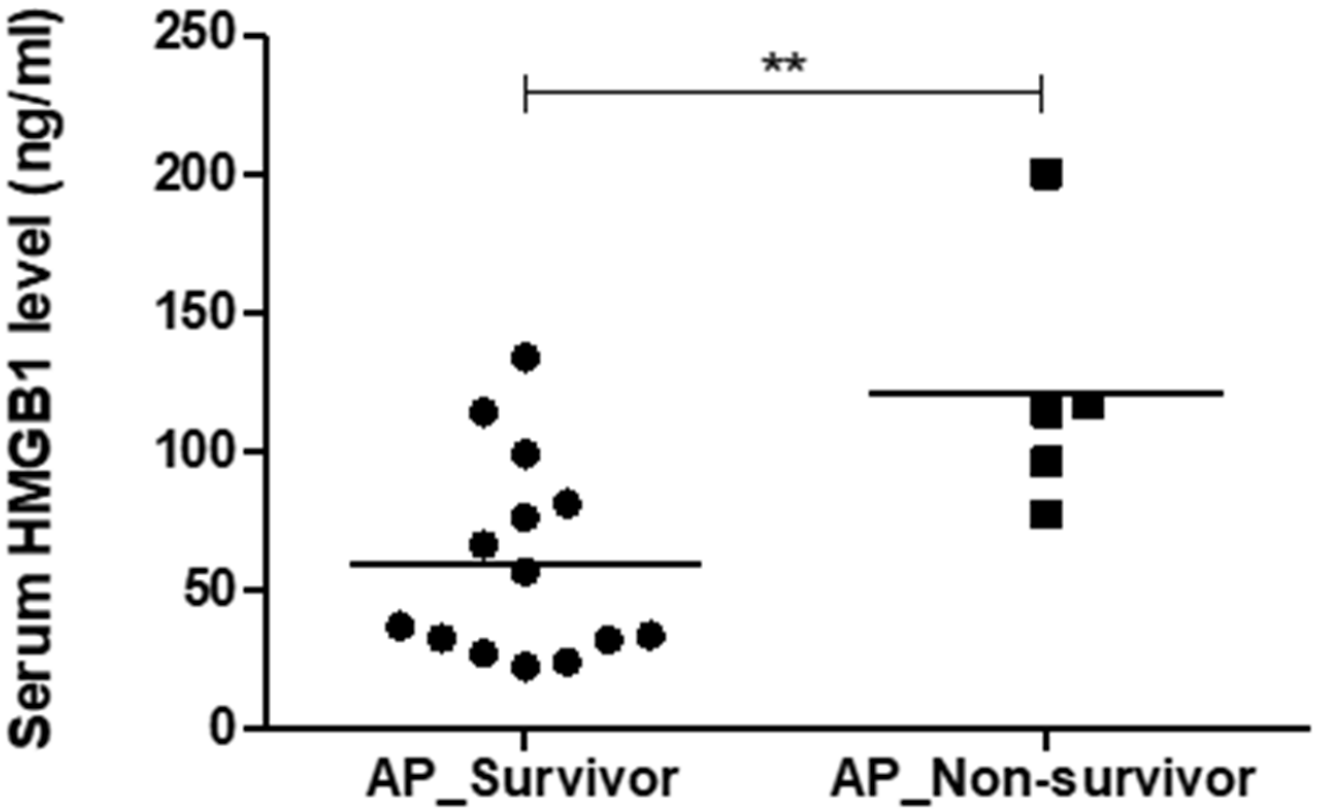

Figure 2

Comparison of serum high mobility group box-1 protein (HMGB1) concentrations between the survivor group and non-survivor group (** $p < 0.01$)

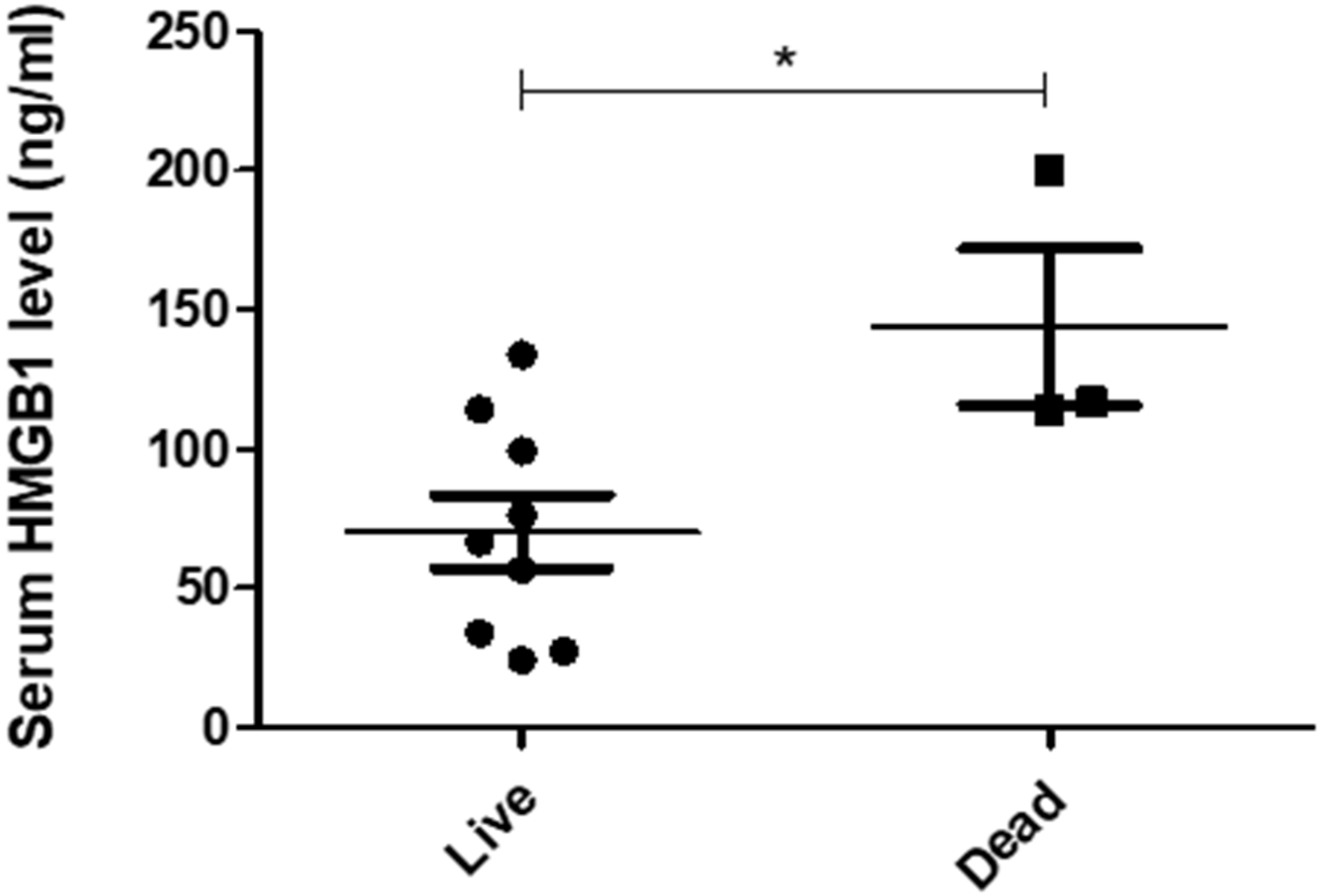

Figure 3

Comparison of serum high mobility group box-1 protein (HMGB1) and mortality of acute pancreatitis (AP) patients with moderate disease activity index (DAI) (* $p < 0.05$)

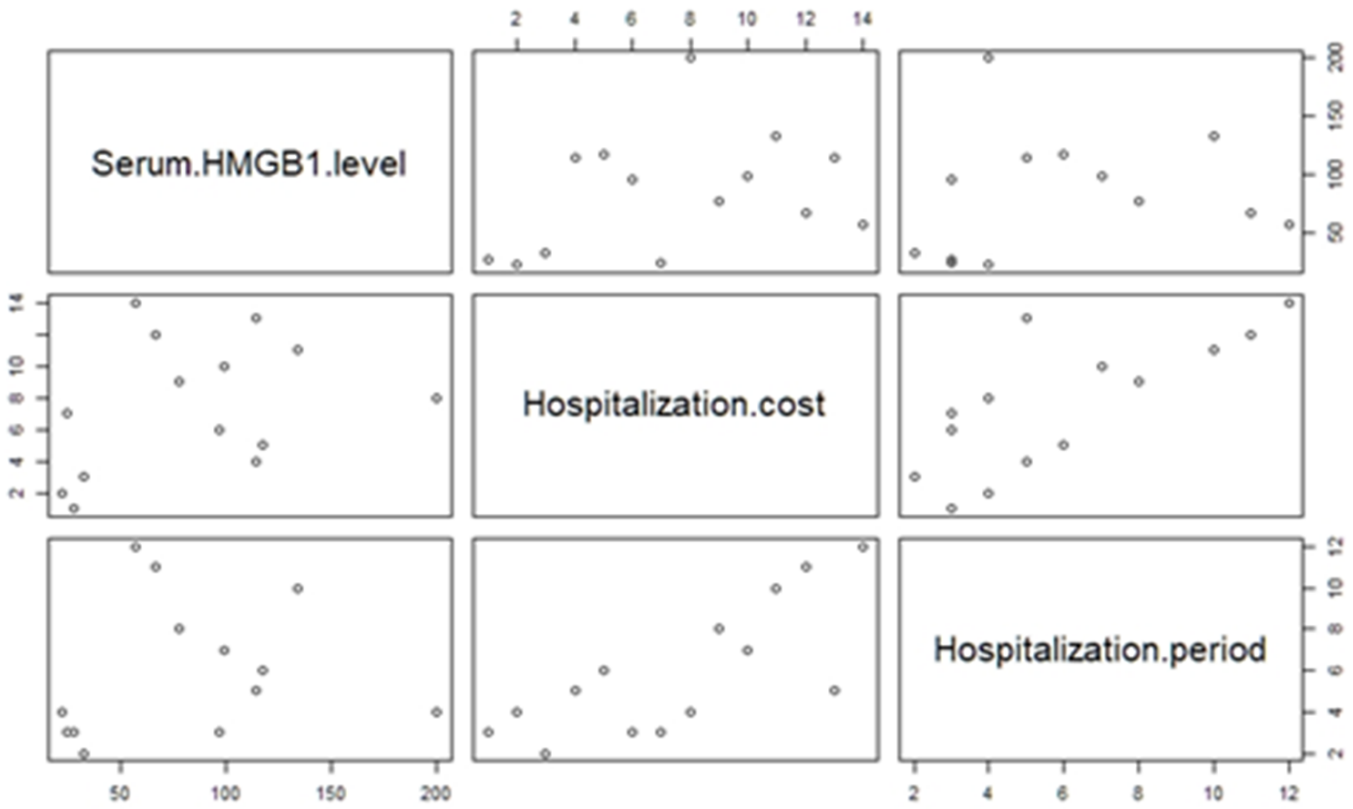

Figure 4

Multiple scatter-plot of serum high mobility group box-1 protein (HMGB1) level and hospitalization cost and period

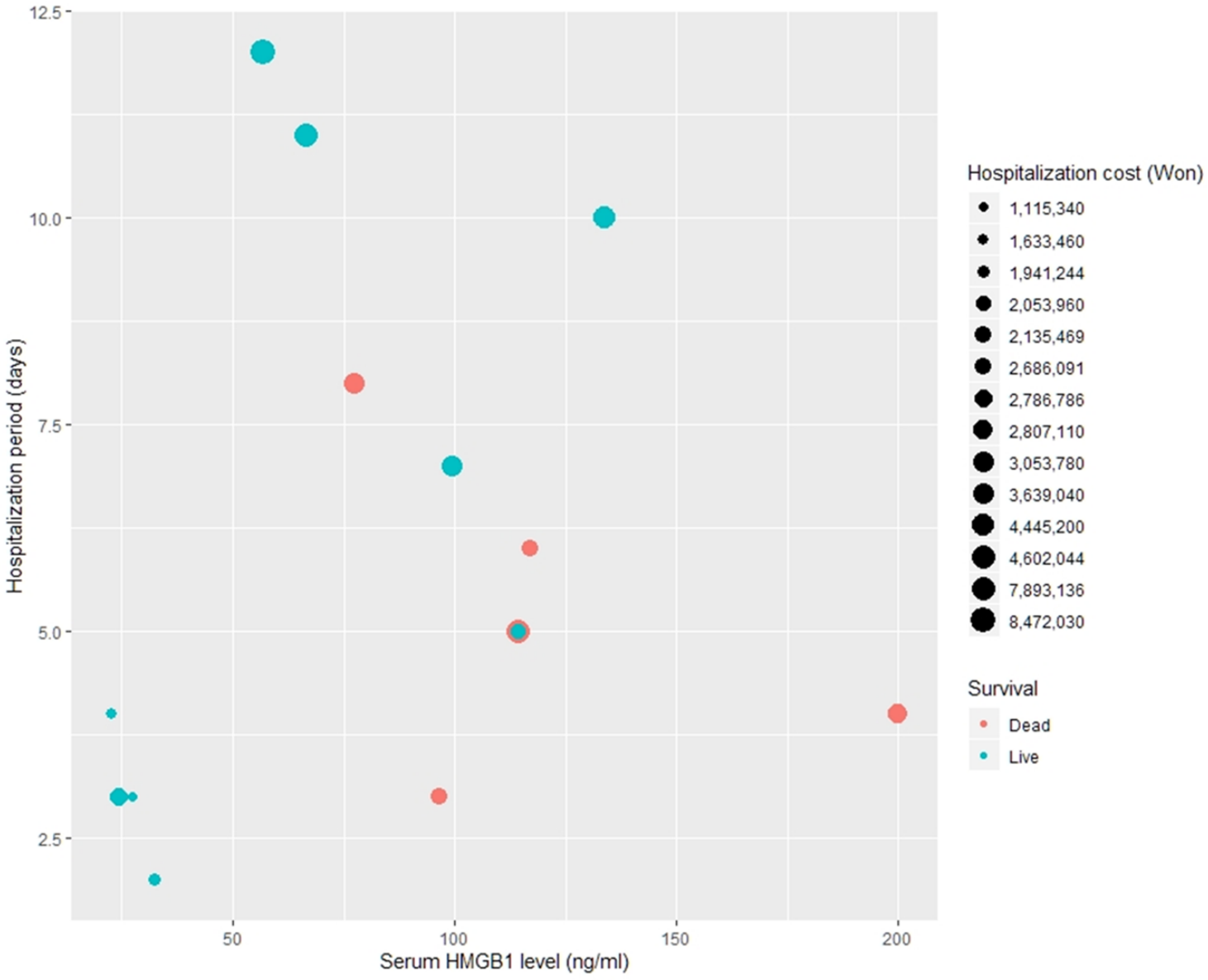

Figure 5

Comparison of serum high mobility group box-1 protein (HMGB1) level and hospitalization period, hospitalization cost in patients with acute pancreatitis (AP). Each patient was marked with a different colour to indicate survival (orange for death, blue for alive).