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ORIGINAL ARTICLE – OPEN ACCESS

High Mobility Group Box – 1 in Patients with Bacterial Septic Arthritis of the Knee: A Controlled Prospective Study

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ABSTRACT

Objective: High mobility group Box–1 (HMGB-1) is related to inflammation and many kinds of arthritic diseases. Septic arthritis is acute infectious arthritis with potentially devastating outcomes and needs to be diagnosed and treated in an emergent manner. It is not always easy to distinguish septic arthritis from other forms of acute arthritis. In this study, we aimed to find out if serum or synovial HMGB1 can be used for diagnosis and differentiation of septic arthritis.

Materials and Methods: Consecutive patients who were admitted to the emergency department with suspected knee septic arthritis were included in this study. Patients were divided into two groups as septic and non-septic arthritis regarding Newman's criteria. All patients underwent a laboratory analysis of serum and synovial fluid for white blood cell count, c-reactive protein, sedimentation, cultures, and HMGB1.

Results: There were 23 patients with acute bacterial septic arthritis and 21 with acute non-bacterial arthritis. No difference was observed regarding age and sex. In the septic group, serum WBC, body temperature, sedimentation, CRP and synovial WBC were significantly higher. However, no difference was observed between groups regarding serum and synovial HMGB1 levels.

Conclusion: Although HMGB1 may predict articular damage in any form of arthritis, serum or synovial HMGB1 of patients with bacterial septic arthritis are similar to patients with non-bacterial arthritis.

Keywords: HMGB1, septic arthritis, CRP, orthopedic emergency

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INTRODUCTION

Septic arthritis of the knee is an orthopedic emergency. Early differentiation between septic and non-septic arthritis and the possible decision for urgent treatment is challenging and also crucial to avoid potentially life-threatening and disabling results (1). Although the disease is uncommon, delayed, or inadequate treatment causes irreversible joint destruction. All the native joints may be affected by septic arthritis, but the knee is the most common (1). The synovium of the knee is a vascular tissue that lacks a protective basement membrane making it vulnerable to bacteremic seeding (2). The most common findings of septic arthritis are fever, warmth, redness, and swelling of the joint and elevated laboratory markers as white blood cell count (WBC), erythrocyte sedimentation rate (ESR), CRP and synovial fluid (SF) assessment. However, these findings may be insufficient to distinguish septic arthritis from other forms of acute arthritis and the absence of these findings does not exclude the diagnosis of septic arthritis (3). Several laboratory markers as serum procalcitonin, leukocyte esterase were tested before for accurate differentiation of septic arthritis from other forms of acute arthritis.

High mobility group box 1 (HMGB1) is a nuclear DNA binding protein that facilitates DNA transcription, recombination, replication, and repair (4). When HMGB1 reaches the extracellular compartment by passive release from necrotic/apoptotic cells or active secretion from monocytes, macrophages, and dendritic cells, it acts as a potent proinflammatory mediator and may lead to an augmented inflammatory reaction (5). Extracellular HMGB1 was reported to play a crucial role in the pathogenesis of arthritis (6) and further studies demonstrated that HMGB1 triggers joint inflammation resulting in chondrocyte death leading arthritis (7, 8). Intraarticular injection of HMGB1 resulted in synovitis and arthritis in an animal study, and the authors described HMGB1 as a trigger of joint arthritis (7).

Septic arthritis may cause severe chondrocyte and synoviocyte death and may lead to significant synovial HMGB1 elevation, which may have potential use in diagnosis or differentiation of septic arthritis from other causes of acute arthritis. A controlled prospective study was conducted to find out the synovial and serum levels of HMGB1 in patients with bacterial septic arthritis of the knee.

MATERIALS and METHODS

This study was undertaken in the Department of Orthopaedics and Clinical Biochemistry in the institute and approved

by the IRB of the authors' affiliated institution. A prospective controlled study was conducted, and all patients signed the consent form.

Patients

The consecutive patients who were admitted to the emergency department with suspected acute septic arthritis of knee from June 2016 to June 2017 were included in this prospective study. Patients' baseline characteristics (age, sex, history of previous diseases, and operations) were recorded. Diagnostic laboratory workup was performed as WBC, CRP, ESR, and arthrocentesis (SF culture and SF-WBC) for all patients who were admitted to the emergency department with suspected septic arthritis of the knee. All patients underwent weight-bearing knee x-rays to find out the level of osteoarthritis.

Patients who met the Newman criteria (9) were diagnosed as septic arthritis and treated using urgent arthroscopic joint drainage and antibiotics (Table 1). These patients constituted group I. Patients that did not meet the criteria were diagnosed as non-septic arthritis and observed with rest and oral non-steroid medication and these patients enrolled group II (control group).

Patients were excluded if they had a major knee operation before (knee replacement), antibiotics taken within one week, end-stage diseases as cancer, and a diagnosed chronic rheumatoid disease. All patients' knee x rays were examined for osteoarthritis and graded with the Kellegren-Lawrence grading system. Patients who were graded as KL-3, 4 (moderate and severe osteoarthritis) were not included in this study.

Laboratory Analysis

SF and blood samples were centrifuged and saved at -80° for future HMGB1 analysis. HMGB1 in serum and SF were tested with a commercial ELISA (enzyme-linked immunosorbent assay) kit (Human High mobility group protein B1, HMGB-1 ELISA Kit Shanghai YL Biotech Co). ELISA was conducted in the biochemistry laboratory of the hospital with ETI-Max 3000 Diasorin S.p.A Saluggia (VC) – Italy.

Statistics

Statistical analysis was performed using SPSS ver. 20.0 for Windows (IBM SPSS Inc., NY USA). We used the Shapiro-Wilk test to assess the normal distribution of the data. Continuous variables were expressed as the mean and standard derivations. Categorical variables, such as gender, were summarized as frequencies and percentages. The groups were compared using the Mann-Whitney U test for non-normally distributed variables. Categorical variables were compared using the Chi-Square test. The diagnostic power of HMGB1, WBC, CRP, ESR, and SF-WBC were tested with the area under the corresponding receiver operating characteristic analysis. Spearmen rank correlation coefficient was employed to determine the correlation between HMGB1 levels and other variables. Any p-values less than 0.05 were considered significant.

Table 1. Newman criteria for septic arthritis

1. Isolated pathogen from the joint
2. Isolated pathogen from elsewhere
3. None pathogen isolated but
    a. Radiological or histological infection evidence
    b. Turbid joint aspiration

RESULTS

There were 44 patients in this study, and 23 of them were diagnosed with acute bacterial septic arthritis (Group I). The remaining 21 patients were classified as non-septic arthritis and these patients constituted group II. The mean age of all patients was 59.9±18 (24–90) and 61.9±18.5 in group I, while 57.9±17.8 in group II (p=0.445). Eight of 23 patients (%35) in group I and seven of 21 patients (%33) in group II were females and no significant differences were observed between groups regarding patients' sex (p=0.919) (Table 2).

Patients in group I had higher mean body temperature (mean±SD; Celsius; 38,1±0.9 vs 37.3±0.8, p=0.008), serum WBC (mean±SD; cell/mm^3; 12030±4128 vs 8167±2445, p=0.001), ESR (mean±SD; mm/h; 57.7±29.6 vs 29.3±26, p=0.002), CRP (mean±SD; mg/L; 134.6±115.2 vs 22.2±31.3, p=0.001) and SF-WBC (mean±SD; cell/mm^3; 92909±117100 vs 7438±7264, p=0.001) in comparison with patients in group II (Table 2).

Table 2. Demographics and laboratory findings of patients' thorough groups

	Group I n=23 (Acute bacterial septic arthritis)	Group II n=21 (Acute non-septic arthritis)	p
Age (years, mean±SD)	61.9±18.5	57.9±17.8	0.445
Sex (female/male)	8/15	7/14	0.919
Body temperature (celcius mean±SD)	38.1±0.9	37.3±0.8	**0.008**
Serum WBC (cells/mm^3 mean±SD)	12090±4128	8167±2445	**0.001**
Sedimentation (mm/h mean±SD	57.7±29.6	29.3±26	**0.002**
CRP (mg/L mean±SD)	134.6±115.2	22.2±31.3	**0.001**
Synovial fluid WBC (cells/mm^3 mean±SD)	92909±117100	7438±7264	**0.001**
Serum HMGB1	1.41±0.8	1.95±1.5	0.503
Synovial HMGB1	1.46±0.6	1.72±0.4	0.055

SD: Standard deviation; WBC: White blood cell; CRP: C-reactive protein; HMGB1: High mobility group box 1

Table 3. Isolated pathogens in patients with acute bacterial septic arthritis

Pathogen	n	%
Staphylococcus aureus	6	26
Group *B streptococcus*	3	13
Burkholderia cepaica	2	8.6
Klebsiella pneumonia	1	4.4
Neisseria gonorrhea	1	4.4
Pseudomonas auruginosa	1	4.4
Pathogen not isolated	9	39.2

In 14 of 23 patients (61%) in group I, the pathogen was isolated in SF culture and there was no positive SF culture in the control group. The most common isolated pathogen was Staphylococcus aureus in six patients (43%, n=6) followed by group B streptococcus (21%, n=3), Burkholderia cepaica (14%, n=2), Klebsiella pneumonia (7%, n=1), Neisseria gonorrhea (7%, n=1) and Pseudomonas aeuruginosa (7%, n=1) (Table 3).

Both serum and synovial HMGB1 studies demonstrated no significant differences between groups (Serum HMGB1; mean±SD; 1.41±0.8 ng/ml vs 1.95±1.5 ng/ml (p=0.503) and synovial HMGB1; mean±SD; 1.46±0.69 ng/ml vs 1.72±0.4 ng/ml (p=0,055) in group I and II respectively) (Table 2). Therewithal there were no correlation with patients' serum or synovial HMGB1 and the patient's age, body temperature, WBC, ESR, CRP and SF-WBC (Table 4). The area under the ROC curve was assessed to evaluate the diagnostic performance of the body temperature, WBC, CRP, ESR, SF-WBC, serum and synovial HMGB1. SF-WBC and CRP had the highest area under the curve in differentiation of the acute bacterial septic arthritis as 0.924 (0.842–1 95% CI) and 0.901 (0.795–1 95% CI) respectively. The area under the curve was 0.821 (0.673–0.969 95% CI) for ESR, 0.643 (0.456–0.829 95% CI) for WBC and 0.810 (0.673–0.948 95% CI) for body temperature. The lowest areas under the curve were 0.203 (0.055–0.350 95% CI) for synovial HMGB1 and 0.368 (0.182–0.553 95 CI%) for serum HMGB1 (Table 5 and Fig. 1).

DISCUSSION

Acute bacterial septic arthritis is diagnosed by detecting the pathogen organism in SF, but synovial cultures are usually negative, and it is not useful in emergency diagnosis. The diagnosis of acute

Table 5. The area under the curve and the confidence intervals

Tests	Area under curve	Confidence interval	p
Synovial HMGB1	0.203	0.055–0.350	0.055
Serum HMGB1	0.368	0.182–0.553	0.503
CRP	0.901	0.795–1.000	**0.001**
Sedimentation	0.821	0.673–0.969	**0.002**
White blood cell	0.643	0.456–0.829	**0.001**
Synovial white blood cells	0.924	0.842–1.000	**0.001**
Body temperature	0.810	0.673–0.948	**0.001**

HMGB1: High mobility group box 1; CRP: C-reactive protein

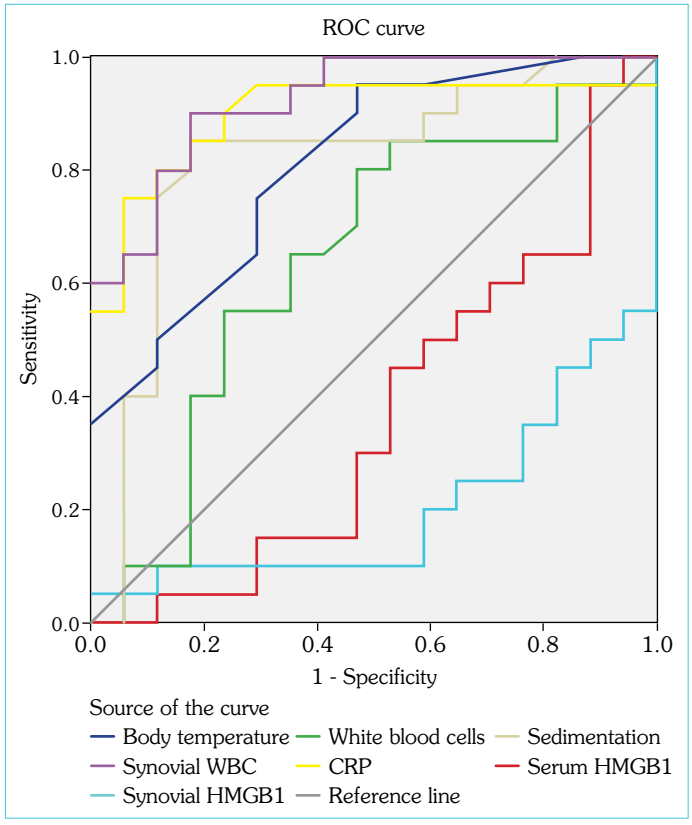

Figure 1. The ROC curve for the tests

Table 4. Correlation Coefficient of Serum and Synovial HMGB1 and other findings

	Age	Body temperature	WBC	ESR	CRP	SF-WBC
Serum HMGB1						
Rho	-0.153	-0.123	-0.018	-0.125	-0.072	0.014
p	0.321	0.425	0.907	0.437	0.656	0.932
Synovial HMGB1						
Rho	-0.16	-0.097	0.145	-0.101	-0.273	-0.245
p	0.3	0.53	0.347	0.529	0.084	0.128

HMGB1: High mobility group box 1; WBC: White blood cell; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; SF: Synovial fluid

bacterial septic arthritis must be ruled out rapidly because of the potentially devastating complications of delayed treatment. Many laboratory findings as CRP, ESR, WBC, and body temperature are helpful for diagnosis but the absence of these acute-phase responses would not exclude the diagnosis (3, 10). HMGB1 is a dual functioning protein, it binds DNA inside the cell and outside the cell, it activates the innate system and mediates a wide range of physiological and pathological responses (11). Necrotic and activated cells as chondrocytes and macrophages release HMGB1 into the extracellular cartilage matrix (12) and HMGB1 is blamed to be a triggering agent in joint inflammation by activating macrophages (8). HMGB1 concentrations in SF were assumed as the pro-inflammatory mediator in arthritic joints (6) and higher levels of HMGB1 were correlated with destructive arthritis in long term follow-up (13). Considering all these given studies, we evaluated the HMGB1 in septic arthritis -which is one of the most destructive arthritis- and also tested its role in distinction acute bacterial septic arthritis from non-septic arthritis.

The most surprising finding of our study was that patients with septic arthritis had similar serum and synovial HMGB1 compared with patients with aseptic arthritis. Furthermore, in both groups, the HMGB1 was similar to the levels of healthy adults that were reported before (14). HMGB1, either in serum or SF cannot be used as a marker for diagnosis of acute bacterial septic arthritis as this study demonstrated. Although HMGB1 was directly relevant with acute disorders as acute liver failure (15), acute lung injury (16) and sepsis (17), and even more, it is reported to be elevated within hours of trauma (18), it did not elevate in patients with such a severe and devastating disease. The blood and synovial samples were collected in the emergency department and inflammatory responses might be in the initial phases as the HMGB1 is passively released during cell necrosis and it activates an augmented inflammation. Collection of the samples in the early inflammation phase might be responsible for the 'normal' HMGB1 levels; however, Gaïni et al. (19) also collected the serum and plasma samples at the time of admission in patients with community-acquired infections. HMGB1 levels were significantly higher in patients compared to the healthy controls in their study although there was no difference between the infected and the non-infected patients. Furthermore, levels of HMGB1 correlated only very weakly to other pro-inflammatory markers in their cohort of infection/sepsis.

Contrary to previous reports regarding rheumatoid arthritis, our study did not demonstrate a significant HMBG1 increase in patients with acute septic or non-septic arthritis (7, 19). Thus, we can conclude that elevated HMGB1 is not expected in patients with acute arthritis and elevated HMGB1 in a patient with septic arthritis may indicate a delayed diagnosis or an underlying chronic rheumatoid disease and may predict a poor prognosis because of severe chondrocyte loss, but this must be addressed in future studies.

There is a serious need for a reliable biomarker in the differentiation of septic and aseptic arthritis since the treatment plan changes dramatically and the prognosis is different. Joint destruction is almost inevitable in bacterial arthritis. Thus, emergent drainage and antibiotic treatment are necessary before the cellular damage of the joint cartilage (20). Most of the aseptic arthritis is self-limiting and needs only observation and supportive therapy. A novel and accurate biomarker would help the management of patients with septic and aseptic arthritis.

When the infection is not quickly cleared by the host, the potent activation of the immune response with the associated high levels of cytokines and reactive oxygen species leads to joint destruction. High cytokine concentrations increase the release of host matrix metalloproteinases and other collagen-degrading enzymes (21). Serum C reactive protein was found to be the only significant variable in comparison to culture-negative, acute atraumatic joint effusion with septic arthritis. Serum C-reactive protein may worsen tissue damage in certain cases due to the activation of the complement system or passively released from necrotic cells from any tissue (22). Serum CRP and synovial WBC markedly differentiate septic arthritis from non-septic arthritis in this study, similar to previous reports. On the other hand, HMGB1 has been proven to be associated with divergent clinical conditions, such as sepsis, rheumatoid arthritis, and atherosclerosis (23). Although the role of the HMGB1 in arthritis was found while investigating the pathogenesis of infection, its role in septic arthritis still has not been described yet. In this study, we evaluated the serum and synovial HMGB1 of patients with septic and aseptic arthritis and no difference was observed.

Patients with septic arthritis were elder than the patients with aseptic arthritis in the current study but this is probably not a fact since aging did not found to affect serum HMGB1 levels in healthy subjects (24). The samples were collected before the administration of drugs, but as a weak side of our study, we did not assess the relation between HMGB1 levels and the drugs that are used by the patients for their chronic conditions. Several drugs are related to the HMGB1 levels. Statins were proven to lower HMGB1 levels in patients having atherosclerosis treatment (25). Other drugs may also influence serum HMGB1 levels, such as corticosteroids and metformin (26). Several diseases were related to HMGB1 as Osteoarthritis (OA) and Rheumatoid arthritis (RA). HMGB-1 was shown in the pathogenesis of cartilage destruction in OA (8). The average age of both groups was high and although osteoarthritis is common in this age, we have not established a correlation with these diseases. Hence, pathogenesis of the cartilage degeneration and the role of HMGB-1 on it must be studied further.

The low number of patients was the major limitation of this study. Notwithstanding, this study was prospective, and the datum was clear and solid enough to conclude that HMGB1 was similar in patients with septic arthritis and non-septic arthritis of the knee at least in the early phases of the disease in contrast to OA and RA.

CONCLUSION

Although serum or synovial HMGB1 is elevated in several inflammatory arthritis and may indicate the articular damage, it is in the normal range in patients with acute septic and non-septic arthritis of the knee.

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Peer-review: Externally peer-reviewed.

Author Contributions: Concept – AÖ; Design – AÖ, YGB; Supervision – ÖE, AY; Resource – EÇ, AY; Materials – ME, EÇ; Data Collection and/or Processing – ME, EÇ; Analysis and/or Interpretation – HAA; Literature Search – HAA; Writing – AÖ, HAA; Critical Reviews – ÖE.

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