[Original]

Alpha-Defensin-1 in Synovial Fluid is Useful for Diagnosis of Joint Infection

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Abstract : The distinction between bacterial infectious and noninfectious arthritis is typically challenging in the early stages; however, it is critical for treatment decision making. Here, we investigated the diagnostic relevance of alpha- and beta-defensin levels in serum and synovial fluid as biomarkers of joint infection in patients presenting with fever and arthritis. The study included 12 patients who presented with fever ($\geq 37^{\circ}\text{C}$) and arthritis (pain in the knee or hip joint). The diagnostic criteria for periprosthetic joint infection proposed by the Musculoskeletal Infection Society were used to detect joint infection and categorize the patients into infection and non-infection groups. Alpha-defensin-1 and beta-defensin-3 levels in serum and synovial fluid were measured using enzyme-linked immunosorbent assay. No significant between-group difference was observed with respect to serum alpha-defensin-1 levels; however, synovial fluid alpha-defensin-1 levels were significantly higher in the infection group ($33.6 \pm 26.2 \text{ ng/ml}$) than in the non-infection group ($0.9 \pm 0.4 \text{ ng/ml}$). No significant between-group differences were observed with respect to serum or synovial fluid beta-defensin-3 levels. Furthermore, synovial fluid alpha-defensin-1 levels were increased in patients without prosthesis in the infection group. In conclusion, in patients with fever and arthritis, synovial fluid alpha-defensin-1 levels were significantly higher in patients with infectious arthritis than in those with noninfectious arthritis. Therefore, synovial fluid alpha-defensin-1 levels is a useful diagnostic marker for joint infection.

Keywords : diagnosis of joint infection, alpha-defensin, beta-defensin.

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Introduction

Joint infection is considered in the differential diagnosis of patients presenting with fever and arthritis (pain in joints). Early diagnosis of joint infection is typically challenging and relies heavily on the clinical course and symptoms. A definitive diagnosis necessitates culture of synovial fluid or blood, which are time consuming.

Recent studies have identified procalcitonin as the serological marker of septic disease [1], however, the definitive significance of these markers during local infection or the transition from local infection to bacteremia or sepsis is unclear [2, 3]. Therefore, the identification of biological markers in blood or synovial fluid to detect joint infection in musculoskeletal diseases is clinically relevant and may facilitate appropriate treatment for

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joint infection.

Recent studies have identified several highly sensitive and specific markers of periprosthetic joint infection (PJI) in aspirated joint fluid; these include, human alpha-defensin, neutrophil elastase 2, bactericidal/permeability-increasing protein, neutrophil gelatinase-associated lipocalin, lactoferrin [4], human beta-defensin [5, 6], leukocyte esterase, and interleukin 6 and 8 [7, 8]. In particular, the utility of alpha-defensin as a marker of PJI has been corroborated by several systematic reviews and meta-analyses [7, 9, 10]. In addition, a rapid lateral flow device (Synovasure, Zimmer Biomet, Indiana, USA) that measures alpha-defensin levels is commercially available in Japan since 2016; its usefulness has been reported in several studies [11–14]. Defensin is one of the antimicrobial peptides produced by neutrophils in response to infection [15, 16]. Alpha- and beta-defensins have been identified in humans and are believed to function by disrupting the plasma membrane of pathogenic bacteria [15, 16].

Thus, the detection of alpha- and beta-defensins in synovial fluid has been shown to be helpful in the diagnosis of PJI; however, the clinical relevance of these markers with regard to joint infection in the absence of prosthesis is yet unclear. Moreover, no studies have investigated the clinical relevance of serum alpha- and beta-defensin levels. Therefore, this study aimed to investigate whether alpha- and beta-defensin levels in serum and synovial fluid are useful diagnostic markers of joint infection in patients with fever and arthritis.

Subjects and Methods

Patients who presented at the Department of Orthopedic Surgery at our hospital between October 2015 and October 2016 with fever (body temperature $\geq 37^{\circ}\text{C}$) and arthritis in the knee or hip joints were eligible for inclusion. Of these, patients who consented to participate in this study were included. The patients who had not had joint fluid aspiration and children under 15 years old were excluded.

We retrospectively examined the body temperature at presentation, number of days from onset to visit, results of hematological investigations (total and differential white blood cell [WBC] count, C-reactive protein [CRP] level, erythrocyte sedimentation rate

[ESR]), synovial fluid microscopy, and bacteriology with blood and synovial fluid culture. The day of onset was defined as the day of onset of symptoms such as pain and fever or, in the case of prosthetic surgery, the day of first surgery. Blood and synovial fluid samples for enzyme-linked immunosorbent assay (ELISA) were collected after obtaining written informed consent from the patients. Blood was stored overnight at 4°C , followed by centrifugation at 1,000 rpm for 15 minutes to collect serum; synovial fluid was similarly centrifuged, and only sediment-free portions were collected and stored at -80°C until measurement. Serum and synovial fluid alpha-defensin levels were measured using Human defensin, Alpha 1 ELISA kit (MyBioSource, California, USA); beta-defensin levels were measured using Human Beta-Defensin 3 ELISA kit (Alpha Diagnostic International, Texas, USA).

According to the definition of PJI proposed by the Musculoskeletal Infection Society (MSIS) [17], a definite PJI was confirmed when: (1) there is a sinus tract communicating with the prosthesis; or (2) a pathogen is isolated by culture from 2 or more separate tissue or fluid samples obtained from the affected prosthetic joint; or (3) when 4 of the following 6 criteria exist; (a) elevated serum ESR and CRP, (b) elevated synovial WBC count, (c) elevated synovial polymorphonuclear percentage, (d) presence of purulence in the affected joint, (e) isolation of a microorganism in one culture of periprosthetic tissue or fluid, or (f) greater than 5 neutrophils per high-power field in 5 high-power fields observed from histologic analysis of periprosthetic tissue at $\times 400$ magnification. The cutoff values with regard to acute infection (<6 weeks after onset) were as follows: serum CRP > 10 mg/dl; serum ESR > 30 mm/hr; synovial fluid WBC count $> 3,000/\mu\text{l}$; and synovial fluid neutrophils % $> 65\%$.

All results are presented as mean $\pm$ standard deviation (SD). Statistical analysis was performed using SPSS 22 (IBM, NY). Differences between the infection and non-infection groups were assessed using the Mann-Whitney's *U*-test, with *P* values < 0.05 considered to be statistically significant.

This study was reviewed by the Ethics Committee of the University of Occupational and Environmental Health Japan, and its implementation content was approved by the same committee (No. H26-207).

Results

Based on the definition of PJI proposed by MSIS, 6 patients each were included in the infection and non-infection groups. The infection group included three patients with postoperative prosthetic joint infection and one patient each with post-intra-articular-injection infection, post-osteosynthesis infection, and idiopathic infection. The non-infection group included four patients with acute attacks of pseudogout and two patients with idiopathic inflammation (Table 1). The

bacteria was detected in the infection group by the blood bacterial culture analysis: methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, and *Staphylococcus lugdunensis* were isolated in one patient each; no pathogenic bacteria were isolated in 2 patients.

The results of laboratory investigations are shown in Table 2 and 3. No significant difference was observed between the infection and non-infection groups with respect to body temperature and any of the hemato-

Table 1. Clinical characteristics of individual patients in the infection and non-infection groups

	No.	Sex	Age	Joint	Pathology	Days from onset	Pathogen
infection	1	F	76	Knee	post TKA	910	unknown
	2	F	59	Knee	idiopathic	3	MSSA
	3	M	20	Hip	post ORIF	4	MRSA
	4	F	72	Hip	post THA	3	<i>Staphylococcus epidermidis</i>
	5	F	77	Knee	post injection	80	<i>Staphylococcus lugdunensis</i>
	6	F	57	Knee	post TKA	270	unknown
non infection	7	F	81	Knee	pseudogout	1	none
	8	F	43	Knee	pseudogout	3	
	9	F	77	Knee	idiopathic	37	
	10	F	79	Knee	pseudogout	9	
	11	F	63	Knee	pseudogout	3	
	12	M	75	Hip	idiopathic	9	

TKA: total knee arthroplasty, ORIF: open reduction and internal fixation, THA: total hip arthroplasty, MSSA: methicillin-sensitive *Staphylococcus aureus*, MRSA: methicillin-resistant *Staphylococcus aureus*

Table 2. Detailed laboratory results of each case

	No.	WBC (/μl)	Neutro (%)	CRP (mg/dl)	ESR (mm/h)	serum alpha- defensin-1 (ng/ml)	fluid alpha- defensin-1 (ng/ml)	serum beta- defensin-3 (pg/ml)	fluid beta- defensin-1 (pg/ml)
infection	1	13,900	51.8	2.75	54	0	55.1	149.2	159.9
	2	9,000	83.3	10.55	82	0.11	51.2	206.6	172.7
	3	15,800	70.0	13.29	–	1.23	14.6	202.2	207.5
	4	6,700	88.5	8.48	44	2.14	70.5	191.0	234.7
	5	6,200	73.0	5.22	104	1.76	6.49	199.9	141.9
	6	10,000	64.6	2.43	32	0	3.78	201.0	475.5
non infection	7	13,800	88.3	2.67	–	2.78	1.14	343.2	226.2
	8	5,100	–	0.17	8	0.02	1.18	177.3	221.9
	9	8,300	75.9	20.11	73	1.32	1.46	106.9	144.4
	10	15,000	86.1	14.84	126	–	0.78	–	81.7
	11	12,400	78.1	16.57	58	0.95	0.74	103.0	146.8
	12	6,200	79.6	7.93	118	0.02	0.21	282.3	134.6

WBC: white blood cell, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate

Table 3. Results of laboratory investigations in the infection and non-infection groups

	infection (n = 6)	non-infection (n = 6)	P-value
BT (°C)	37.8 ± 0.8	37.8 ± 0.6	0.936
serum WBC (/μl)	10267 ± 3529	10133 ± 3795	0.688
serum Neutro (%)	71.9 ± 12.0	81.6 ± 4.8	0.201
CRP (mg/dl)	7.1 ± 4.0	10.4 ± 7.3	0.522
ESR (mm/h)	63.2 ± 26.3	76.6 ± 42.9	0.465
serum alpha-defensin-1 (ng/ml)	0.87 ± 0.89	1.02 ± 1.02	0.714
fluid alpha-defensin-1 (ng/ml)	33.6 ± 26.2	0.9 ± 0.4	0.004
serum beta-defensin-3 (pg/ml)	191 ± 20	203 ± 96	0.715
fluid beta-defensin-3 (pg/ml)	231 ± 113	159 ± 51	0.200

Only synovial fluid alpha-defensin-1 levels were significantly higher in the infection group than in the non-infection group. BT: body temperature, WBC: white blood cell, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate

logical parameters. Results of ELISA with regard to serum alpha-defensin-1 levels were not significantly different between the two groups; however, synovial fluid alpha-defensin-1 levels were significantly higher in the infection group (33.6 ± 26.2 ng/ml) than in the non-infection group (0.9 ± 0.4 ng/ml). Furthermore, synovial fluid alpha-defensin-1 levels were increased in patients without prosthesis in the infection group. No significant between-group differences were observed with respect to serum and synovial fluid beta-defensin-3 levels.

Synovial fluid alpha-defensin levels in the infection group did not show any association with the number of days from onset.

Discussion

In this study, synovial fluid alpha-defensin-1 levels were significantly higher in the infection group than in the non-infection group, which is consistent with previous reports [3, 11–14, 18, 19]. In addition, serum alpha-defensin-1 levels and serum and synovial fluid beta-defensin-3 levels were measured; however, the between-group differences were not significant. No

significant between-group difference was observed with respect to serum WBC count, CRP level, or ESR.

We found high synovial fluid alpha-defensin-1 levels in the infection group; these included PJI and non-PJI cases, such as patients with post-intra-articular-injection, post-osteosynthesis, and idiopathic infections. Several previous studies have demonstrated increased defensin levels in patients with PJI [2–4, 6, 8, 10–14, 18, 19]. Because defensin is an antimicrobial peptide produced by neutrophils in response to infection [15], it is produced regardless of the presence of implants. Therefore, our results suggest that the synovial fluid alpha-defensin-1 level is a useful diagnostic marker for infection in patients without prosthesis.

In addition, synovial fluid alpha-defensin levels in the infection group were increased both in patients with acute and chronic infection. Despite the small sample size, this suggests that synovial fluid alpha-defensin levels increase in the presence of bacteria in the joint regardless of the number of days from onset.

No significant between-group difference was observed with respect to serum alpha-defensin-1 levels, despite the significant between-group difference with respect to synovial fluid alpha-defensin-1 levels. This may be because of the lack of systemic effect of alpha-defensin-1, which is produced by neutrophils at the site of infection. Antimicrobial peptides function as local antimicrobial agents and were shown to exhibit negligible systemic effects [20]. Our data suggests that the alpha-defensin-1 level in synovial fluid, but not in serum, is a useful marker of joint infection.

In a previous study, immunostaining of periprosthetic tissues and cancellous bone with regard to beta-defensin-3 in patients with PJI was shown to be significantly greater than that in patients with aseptic loosening of prosthesis [5]; in the study, tissue sections were studied under a microscope to examine the association between beta-defensin-3 expression and infectious loosening of the joint prosthesis. The periprosthetic tissue sections were subjected to immunofluorescence staining; however, no serum or synovial fluid samples were used. Although immunofluorescence staining of tissue specimen may enable more accurate diagnosis of infection, the procedure is time consuming. Therefore, in our study, we used serum and synovial fluid samples to facilitate early diagnosis;

however, no differences in beta-defensin-3 levels were observed in serum and synovial fluid samples in our study.

Some limitations of this study must be considered. The first limitation pertains to the accuracy of the diagnostic criteria for joint infection. In the absence of more appropriate diagnostic criteria, we used the diagnostic criteria for PJI to detect joint infection without prostheses. In a previous report, only two-thirds of PJI cases fulfilled the diagnostic criteria proposed by MSIS [21]. The second limitation is the small sample size. Future studies with larger sample size are required to confirm our findings and to determine the cutoff level of alpha-defensin-1 in synovial fluid for the diagnosis of joint infection.

Conclusion

Among patients with fever and arthritis, those with joint infection showed significantly higher synovial fluid alpha-defensin-1 levels than those with noninfectious arthritis. In contrast, no significant between-group difference was observed with respect to serum alpha-defensin-1 levels or serum or synovial fluid beta-defensin-3 levels. Synovial fluid alpha-defensin-1 levels were increased regardless of the presence or absence of prosthesis; therefore, synovial fluid alpha-defensin-1 levels may be a useful diagnostic marker for joint infection.

Conflicts of Interest

We declare that we have no conflicts of interest with regard to this paper.

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化膿性関節炎の診断における関節液中 α ディフェンシン1の有用性

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要 旨： 日常診療において、細菌感染による化膿性関節炎と偽痛風発作などの非化膿性関節炎の鑑別は手術加療を要するか否かを判定する上で非常に重要であるにもかかわらず、早期段階では困難であることが多い。本研究では、発熱を有する関節炎患者において、血清および関節液中の α -defensinおよび β -defensinが感染の診断に有用であるか否かを検討することを目的とした。対象は、37°C以上の発熱を有し、膝関節もしくは股関節に疼痛を伴った関節炎を発症した患者12例である。化膿性関節炎の診断の確定は、MSIS (Musculoskeletal Infection Society) によるPJI (Periprosthetic Joint Infection) 定義を参照とし、感染群と非感染群に分けた。ELISA法で血清および関節液中の α -defensin-1および β -defensin-3を測定した。血清中 α -defensin-1は両群で有意差はなかった一方で、関節液中 α -defensin-1 (ng / ml) は感染群で 33.6 ± 26.2 , 非感染群で 0.9 ± 0.4 と有意に感染群で高値を示した。 β -defensin-3は血清、関節液ともに有意差は認めなかった。感染群において、人工関節のない症例においても関節液中 α -defensin-1は高値であった。発熱を有する関節炎患者において、化膿性関節炎では関節液中 α -defensin-1は非化膿性関節炎と比較して有意に高値であった。関節液中 α -defensin-1は化膿性関節炎の診断において有用なマーカーである。

キーワード： 化膿性関節炎診断, α ディフェンシン, β ディフェンシン.