Utility of CD64 on Neutrophils in Orthopedic Infection

Akihiro Fujita,* Shinji Tanishima,* Yoshihiro Kato,* Ryota Teshima* and Hideki Nagashima*

**Division of Orthopedic Surgery, Department of Sensory and Motor Organs, School of Medicine, Tottori University Faculty of Medicine, Yonago 683-8504, Japan*

ABSTRACT

Background Musculoskeletal infections are often seen in the daily practice of orthopedics. Several markers [white blood cell (WBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT)] have been used for diagnosing these infections. However, these markers may be elevated due to surgery or trauma, and may not be infection-related. These markers also show drug-dependent dynamics during infection that differ from its usual dynamics. Such situations make diagnosis of infections difficult, and Cluster of Differentiation 64 (CD64) has been brought to attention. This study aimed to clarify the utility of CD64 on neutrophils by comparing it with conventional infection markers (CRP, PCT) in musculoskeletal infection.

Methods Forty-four patients who were suspected of having musculoskeletal infection between May 2010 and November 2013 in our hospital were enrolled in this study. Patients were divided into subgroups according to their culture results, antibiotics administration, measurement timing, and if they were immunocompromised. The measurements of the infection markers were compared between each group. In addition, the positive rates of each infection marker were compared between groups.

Results There was no difference in the infection marker measurements between several groups. There was no statistically significant difference between groups for the positive rates of CD64, CRP, and PCT.

Conclusion We evaluated the utility of CD64 on neutrophils in musculoskeletal infection. CD64 showed the utility that was equivalent to conventional infection markers in diagnoses of various musculoskeletal infections.

Key words Cluster of Differentiation 64; C-reactive protein; musculoskeletal infections; procalcitonin

Musculoskeletal infections are often seen in the daily practice of orthopedics. Several markers [white blood cell (WBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT)] have been used to diagnose these infections, but elevated levels can be due to reasons other than infection, for instance surgery or trauma. These markers can also exhibit altered drug-dependent dynamics during infection. Such situations make the diagnosis of infections difficult, and for this reason attention has recently been paid to the cluster of differentiation 64 (CD64).

CD64 is an integral membrane glycoprotein known as an Fc receptor, with a molecular weight of 72 kDa. It binds monomeric IgG-type antibodies with high affinity and is more commonly known as Fc-gamma receptor 1 (FcγRI). It is constitutively expressed on macrophages, monocytes, and eosinophils, but expressed at only low levels on normal neutrophils. The expression of CD64 on neutrophils is upregulated as a physiological response to microbial wall components, complement split products, and several cytokines such as interferon-c (IFN-c), interleukin-8 (IL-8), IL-12, and granulocyte-colony stimulating factor (G-CSF).[1–3] Therefore, CD64 may be a marker that is specific for infection, and its efficacy in the diagnosis of both systemic and local infections has been reported.[4–6] For the diagnosis of musculoskeletal infections seen in orthopedics practice, conventional markers such as CRP or PCT may not be effective. In particular, in patients with rheumatoid arthritis (RA) it is difficult to differentiate between disease activity and infection as the cause of the inflammatory reaction. CD64 was shown to be useful in this regard, as it is not influenced by the disease activity of RA but is increased by a variety of bacterial, acid-fast bacilli, viral, and fungal infections.[4, 7, 8] In the early phase after joint replacement, CD64 helped to differentiate whether elevations in other infection markers were due to infection or surgical stress.[9–11] Furthermore, CD64 was shown to have decreased sensitivity for old or local infections.[5, 12] However, few studies have evaluated the efficacy of CD64 in the diagnosis of usual infections that did not occur in the context of RA or after joint replacement.[12] This study aimed to compare neutrophil CD64 expression with

Corresponding author: Akihiro Fujita
fujita-desu@tottori-u.ac.jp
Received 2018 September 20
Accepted 2018 December 5
Abbreviations: CD64, cluster of differentiation 64; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FcγRI, Fc-gamma receptor 1; G-CSF, granulocyte-colony stimulating factor; IL-8, interleukin-8; IFN-c, interferon-c; MSSA, methicillin-sensitive Staphylococcus aureus; PCT, procalcitonin; RA, rheumatoid arthritis; WBC, white blood cell

conventional infection markers (CRP and PCT) in terms of diagnosing musculoskeletal infection in a general population of patients.

MATERIALS AND METHODS

Study design

This investigation was a single-facility observational study and was approved by the ethics committee of the Faculty of Medicine at Tottori University (No.1803). Forty-four patients in our hospital who were suspected of having musculoskeletal infections between May 2010 and November 2013 were enrolled in this study.

Following the approach of Matsui et al., an infection was considered to be present if one of the following conditions was met: 1) the presence of a pathogen was demonstrated by microbiologic culture or PCR; 2) infection was diagnosed by radiological or other imaging findings by at least two physicians; and 3) the patient had obvious symptoms of infection or obvious clinical effects of treatment with antimicrobial, antiviral, or antifungal agents.[4] As followed it, we diagnosed infections by positive blood or local cultures. Patients with negative cultures were diagnosed after consideration of clinical findings (recurrent symptoms, no improvement without antibiotics, and infectious inflammation on pathological examination). All patients in the blood culture–positive group also had same bacteria detected in local cultures, and we therefore concluded that there was no contamination in these cases.

Blood samples (2 mL per patient) were collected for routine blood tests and CD64 evaluation using EDTA-2K blood collection tubes, and measurements were carried out within 2 hours. CD64 expression on neutrophils was measured by the following methods according to past literature.[4] QuantiBrite CD64PE/CD45PerCP (Beckton Dickinson, San Jose, CA) was added to 20 μL to 50 μL of whole blood and incubated for 60 minutes in the dark at 20 °C. After erythrocyte lysis with Versalysis (Beckman Coulter, Brea, CA) for 12 minutes, the samples were incubated for an additional 60 minutes to allow for equilibration and to reduce nonspecific background staining. The expression of CD64 was examined with a Gallios flow cytometer (Beckman Coulter) calibrated using QuantiBrite PE beads (Beckton Dickinson). These beads were conjugated with four different levels of phycoerythrin (PE), which made it possible to create a standard curve for determining the mean number of PE molecules present on a cell. The mean number of CD64 molecules expressed on the cell surface was calculated using the PE fluorescence quantification kit with QuantiBrite PE beads. Three different cell populations, namely lymphocytes, monocytes, and granulocytes, were identified and gated by their CD45/side-scatter profile.

The data are expressed as median and interquartile ranges (IQR). The cut-off values were set at 2000 molecules/cell for CD64, 1.0 mg/dL for CRP, and 0.05 ng/mL for PCT.[13–15]

We assessed the diagnostic utility of CD64 by retrospectively analyzing patients with musculoskeletal infections and evaluating whether CD64 was able to identify an infection more accurately than conventional infection markers even if the infection had characteristics that increased its likelihood of being masked.

We classified patients by culture results and also divided them into subgroups based on the following factors that contribute to masking of an infection: antibiotic administration, late CD64 measurement, and presence of an immunodeficient state. First, patients were classified based on whether or not they received antibiotics before CD64 measurement. Second, they were classified based on the timing of CD64 measurement, either within 7 days of infection onset or afterward. This classification was based on a report describing that CD64 expression increased within 24 hours after infection onset and returned to baseline after 7 days.[16] Finally, patients were classified based on whether they were immunocompromised; immunodeficient patients included diabetes, malignant tumor, hepatic failure, renal failure and those receiving immunosuppressive drugs such as steroids. The positive rates and levels of each infection marker in each subgroup were compared and evaluated.

Statistical analysis

Marker measurements were compared using the Mann-Whitney *U* test. For culture results, we performed a multiplex test using the Steel-Dwass test. The positive rate of each infection marker was compared between groups by the Fisher's exact test. A value of $P < 0.05$ was considered statistically significant. SPSS Statistics Version 24.0 was used for statistical analysis.

RESULTS

Demographic data

Forty-four patients (26 males and 18 females) were enrolled in the study. The average age was 62.9 (20–85) years. There were 10 cases of surgical-site infection, eight of arthritis or osteomyelitis, six of myositis or fasciitis, six of perispondylitis, four of cellulitis, four of tendinitis or tenosynovitis, three of diabetic gangrene, two of skin ulcer infection, and one of bursitis (Table 1).

Cultures

Among the 44 patients, nine had positive blood cultures,

Table 1. Clinical and biological data of patients and characteristics of infections

Number of patients	44
Age	62.9 (20–85)
Gender	
M : F	26 : 18
Diagnosis of infection	
SSI : surgical-site infection	10
Arthritis or osteomyelitis	8
Myositis or fascitis	6
Peri-spondylitis	6
Cellulitis	4
Tendinitis or tenosynovitis	4
Diabetic gangrene	3
Infection of skin ulcer	2
Brusitis	1
CD64 (molecules/cell)	6723 (701–38640)
CRP (mg/dL)	13.4 (0.1–41.7)
PCT (ng/mL)	3.1 (0–50.3)

CD64, cluster of differentiation 64; CRP, C-reactive protein; F, female; M, male; PCT, procalcitonin.

21 had positive local cultures, and 14 were negative for both. Among patients with positive blood cultures, methicillin-sensitive *Staphylococcus aureus* (MSSA) was detected in five cases, *Streptococcus pyogenes* in two cases, and *Streptococcus intermedius* and *Streptococcus agalactia* in one case each. Among patients with positive local cultures, MSSA was detected in eight cases, methicillin-resistant *Staphylococcus* aureus in five cases, *Streptococcus pyogenes* in two cases, *Pseudomonas aeruginosa* in two cases, and *Streptococcus agalactia, Staphylococcus logrunner*, and *Enterobacter cloacae* in one case each (Table 2).

There were no significant differences in age or gender between the three culture groups. The median (IQR) neutrophil CD64 expression was 12,820 molecules/cell (5,002–18,710) in the patients with positive blood cultures, 2,717 molecules/cell (1,341–10,987) in those with positive local cultures, and 2,553 molecules/cell (2,199–3,411) in those with negative cultures. CD64 expression was significantly higher in patients with positive blood cultures than in those with positive local cultures. There was no significant difference between patients with positive local cultures and those who were culture negative (Fig. 1). There were also no significant differences in the positive rates of CD64, CRP, or PCT between the three groups (Table 3).

Antibiotics

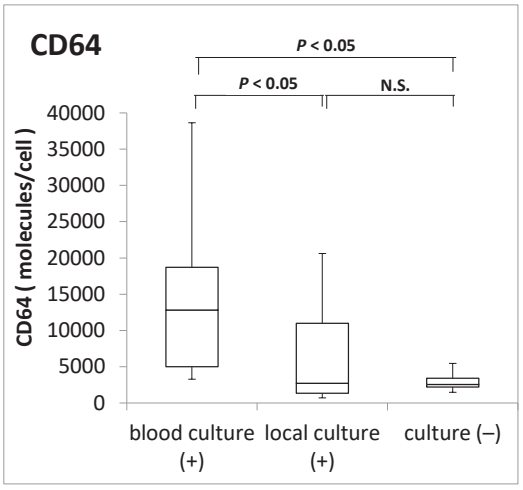
Antibiotics were administered to 22 of the 44 patients before marker measurements (Table 4). The median neutrophil CD64 expression was 5,400 molecules/cell (1,463–10,352) in the antibiotic group and 7,765 molecules/cell (2,340–11,684) in the no-antibiotic group. CD64 expression was higher in the former group, but the difference was not statistically significant. Antibiotics were administered to 15 patients in the positive local culture group and to seven in the culture-negative group,

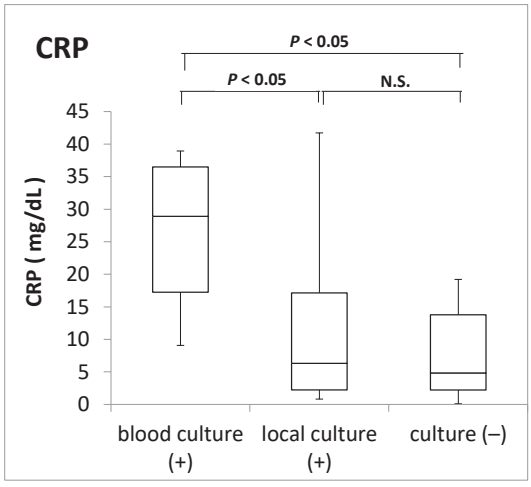
Table 2. Result of culture and laboratory data of patients

	Blood culture (+)	Local culture (+)	Culture (–)	*P** Value
n	9	21	14	
CD64 (molecules/cell)	12820 (5002–18710)	2717 (1341–10987.5)	2553 (2199–3411)	B:L < 0.05 L:C 0.761 B:C < 0.05
CRP (mg/dL)	28.9 (17.24–36.49)	6.31 (2.23–17.13)	4.83 (2.21–13.77)	B:L < 0.05 L:C 0.544 B:C < 0.05
PCT (ng/mL)	0.5 (0.43–6.75)	0.08 (0.03–1.38)	0.06 (0.03–0.44)	B:L < 0.05 L:C 0.542 B:C < 0.05
Pathogens (*n*)	MSSA (5)	MSSA (8)	(–)	
	Streptococcus pyogenes (2)	MRSA (5)		
	Streptococcus intermedius (1)	*Streptococcus pyogenes* (2)		
	Streptococcus agalactiae (1)	*Pseudomonas aeruginosa* (2)		
		Klebsiella pneumoniae (1)		
		Streptococcus agalactiae (1)		
		Staphylococcus lugdunen (1)		
		Enterobacter cloacae (1)		

*Steel-Dwass test

B, Blood culture (+); C, Culture (–); CD64, cluster of differentiation 64; CRP, C-reactive protein; L, Local culture (+); MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PCT, procalcitonin.





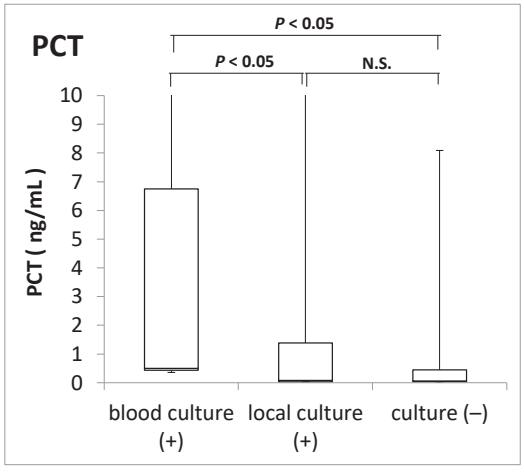


Fig. 1. Neutrophil CD64 expression, CRP, and PCT levels in groups with positive blood culture, positive local culture, and negative culture. The box shows the 25–75th percentiles, whereas the whiskers indicate the 10th and 90th percentiles. N.S. means not significant by the Steel-Dwass test. CD64, cluster of differentiation 64; CRP, C-reactive protein; PCT, procalcitonin.

but to none in the positive blood culture group. The antibiotic and no-antibiotic groups showed no significant differences in CD64, CRP, or PCT levels (Fig. 2), and the positive rate of each infection marker did not differ between the two groups (Table 5).

Timing of CD64 measurement

CD64 was measured within 7 days after infection onset in 15 patients, and later than 7 days after onset in 29 patients (Table 6). Levels of each infection marker were significantly higher when measurements were performed within 7 days (Fig. 3). The positive rate of CD64 was lower within 7 days than afterward, but there was no difference in CRP or PCT (Table 7).

Immunodeficiency

Twenty-three of 44 patients were immunodeficient (Table 8). CD64 levels were not significantly different between immunodeficient and non-immunodeficient patients (Fig. 4), and there were no significant differences between these two groups in the positive rate of any of the infection markers (Table 9).

Table 3. Positive rate of each group classified by culture results

	Blood culture (+) $n = 9$	Local culture (+) $n = 21$	Culture (–) $n = 14$	P* Value
CD64				
(+)	9	13	12	0.056
(–)	0	8	2	
CRP				
(+)	9	21	12	0.134
(–)	0	0	2	
PCT				
(+)	9	14	11	0.163
(–)	0	7	3	

*Fisher's exact test
CD64, cluster of differentiation 64; CRP, C-reactive protein; PCT, procalcitonin.

DISCUSSION

There have been many reports on the efficacy of CD64 as a diagnostic marker for infection in patients with RA and those in the early postoperative period.[4, 8–11, 15, 19, 20] However, the efficacy of CD64 as a diagnostic marker for common musculoskeletal infections has not yet been examined. Therefore, we measured CD64 expression in patients with various musculoskeletal infections in a number of different clinical settings, and compared its diagnostic utility with that of conventional infection markers.

Although CD64 has been shown to have good sen-

Table 4. Clinical details and laboratory data of patients with and without antibiotics administration

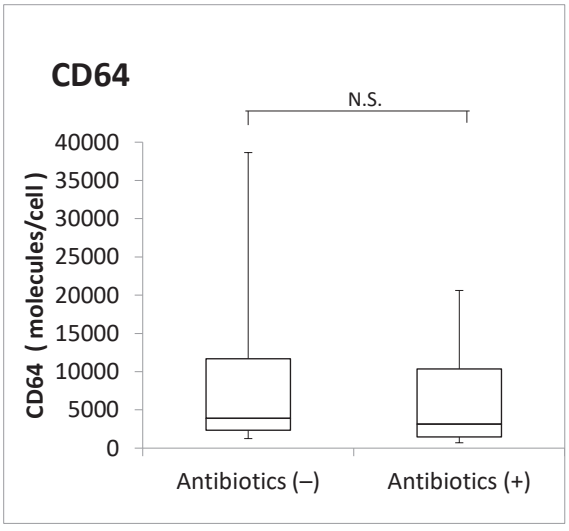
	Antibiotics (+)	Antibiotics (–)	*P** Value
n	22	22	
Age	61.0 (30–85)	64.7 (20–85)	
Gender (*n*)	M (12) F (10)	M (14) F (8)	
Culture (*n*)	Blood culture (+) (0) Local culture (+) (15) Culture (–) (7)	Blood culture (+) (9) Local culture (+) (6) Culture (–) (7)	
CD64 (molecules/cell)	3141 (1463–10352)	3896 (2340–11684)	0.301
CRP (mg/dL)	8.82 (3.13–18.55)	9.35 (2.75–27.45)	0.981
PCT (ng/mL)	0.17 (0.04–1.8)	0.2 (0.04–0.53)	0.473

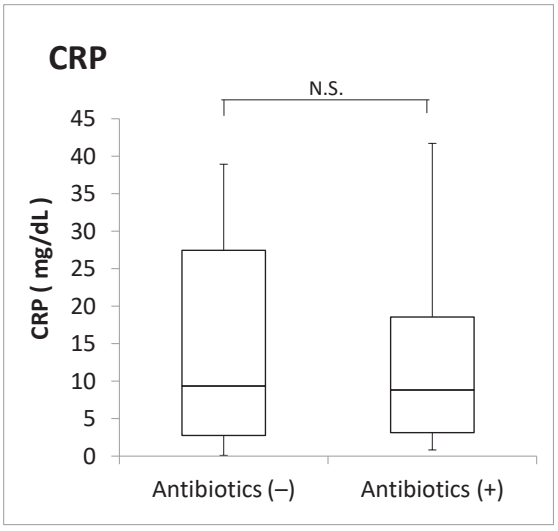
*Mann-Whitney *U* test
CD64, cluster of differentiation 64; CRP, C-reactive protein; F, female; M, male; PCT, procalcitonin.

Table 5. Positive rate of each group classified with or without antibioticsadministration

	Antibiotics (+) *n* = 23	Antibiotics (–) *n* = 21	*P** Value
CD64			
(+)	9	13	0.066
(–)	0	8	
CRP			
(+)	9	21	1.000
(–)	0	0	
PCT			
(+)	9	14	0.071
(–)	0	7	

*Fisher's exact test
CD64, cluster of differentiation 64; CRP, C-reactive protein; PCT, procalcitonin.





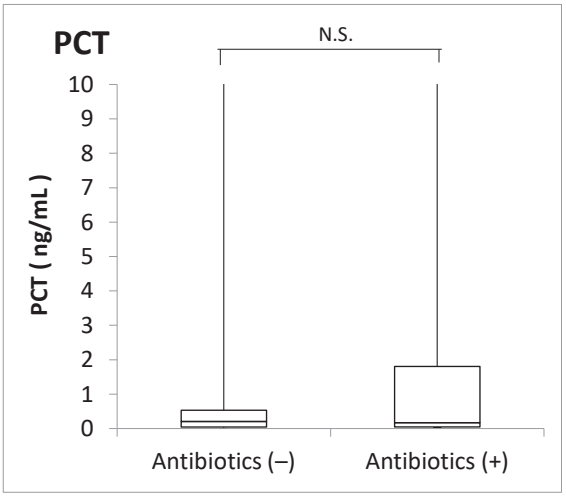


Fig. 2. Neutrophil CD64 expression, CRP, and PCT levels in groups with and without antibiotic administration. The box shows the 25–75th percentiles, whereas the whiskers indicate the 10th and 90th percentiles. N.S. means not significant by the Mann-Whitney *U* test. CD64, cluster of differentiation 64; CRP, C-reactive protein; PCT, procalcitonin.

Table 6. Clinical details and laboratory data of patients measured at under or over 7th day after onset

n	≤ 7th day 15	7th day < 29	*P** Value
Age	65.5 (31–80)	61.6 (20–85)	
Gender (*n*)	M (9) F (6)	M (17) F (12)	
Culture (*n*)	Blood culture (+) (0) Local culture (+) (15) Culture (–) (7)	Blood culture (+) (9) Local culture (+) (6) Culture (–) (7)	
CD64 (molecules/cell)	10427 (3277–17052)	2589 (1470–4626)	< 0.05
CRP (mg/dL)	18.34 (6.31–34.98)	5.64 (2.56–14.4)	< 0.05
PCT (ng/mL)	0.5 (0.3–3.5)	0.06 (0.03–0.52)	< 0.05

*Mann-Whitney *U* test
CD64, cluster of differentiation 64; CRP, C-reactive protein; F, female; M, male; PCT, procalcitonin.

Table 7. Positive rate of each group measured at under or over 7th day after onset

	≤ 7th day *n* = 15	> 7th day *n* = 29	*P** Value
CD64			
(+)	15	19	< 0.05
(–)	0	10	
CRP			
(+)	15	27	0.540
(–)	0	2	
PCT			
(+)	13	21	0.071
(–)	2	8	

*Fisher's exact test
CD64, cluster of differentiation 64; CRP, C-reactive protein; PCT, procalcitonin.

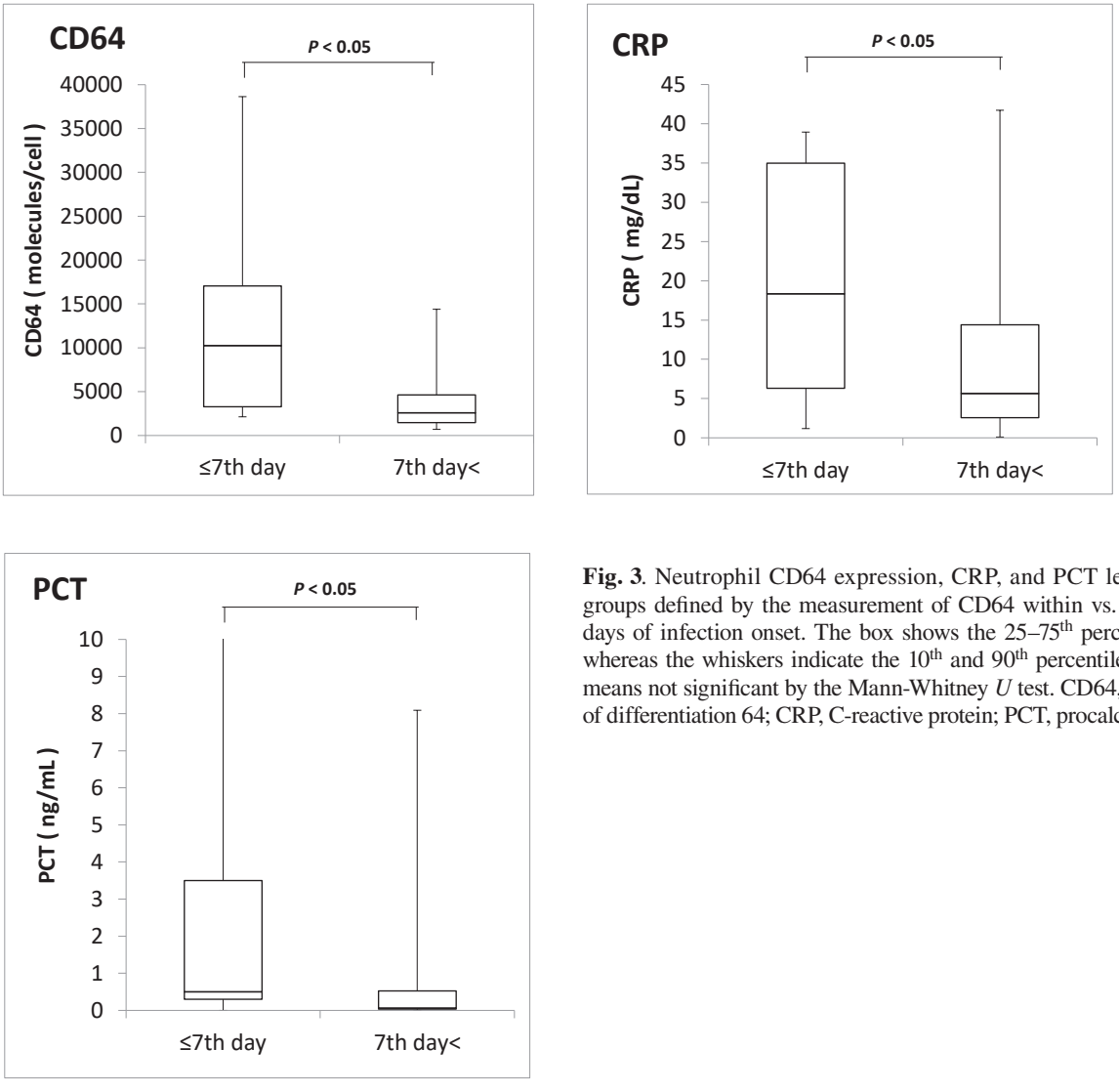


Fig. 3. Neutrophil CD64 expression, CRP, and PCT levels in groups defined by the measurement of CD64 within vs. after 7 days of infection onset. The box shows the 25–75th percentiles, whereas the whiskers indicate the 10th and 90th percentiles. N.S. means not significant by the Mann-Whitney *U* test. CD64, cluster of differentiation 64; CRP, C-reactive protein; PCT, procalcitonin.

Table 8. Clinical details and laboratory data of patients with and without immunodeficiency

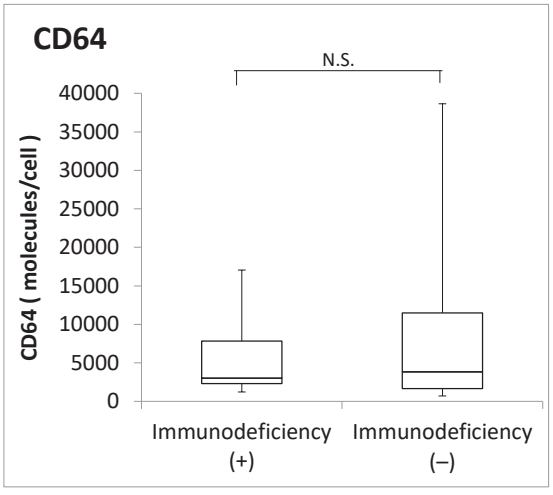
n	Immunodeficiency (+) 23	Immunodeficiency (–) 21	*P** Value
Age	69.0 (45–85)	56.2 (20–85)	
Gender (*n*)	M (8) F (15)	M (12) F (9)	
Culture (*n*)	Blood culture (+) (5) Local culture (+) (8) Culture (–) (10)	Blood culture (+) (4) Local culture (+) (13) Culture (–) (4)	
CD64 (molecules/cell)	3009 (2308–7826)	3828 (1658–11482)	0.991
CRP (mg/dL)	7.67 (3.33–12.25)	15.93 (2.36–28.04)	0.177
PCT (ng/mL)	0.11 (0.06–0.63)	0.42 (0.04–2.53)	0.437

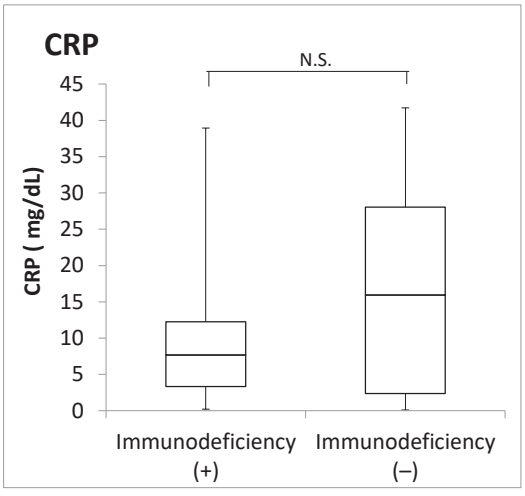
*Mann-Whitney *U* test
CD64, cluster of differentiation 64; CRP, C-reactive protein; F, female; M, male; PCT, procalcitonin.

Table 9. Positive rate of each group classified with or without immunodeficiency

	Immunodeficiency (+) *n* = 23	Immunodeficiency (–) *n* = 21	*P** Value
CD64			
(+)	20	14	0.155
(–)	3	7	
CRP			
(+)	22	20	1.000
(–)	1	1	
PCT			
(+)	18	16	1.000
(–)	5	5	

*Fisher's exact test
CD64, cluster of differentiation 64; CRP, C-reactive protein; PCT, procalcitonin.





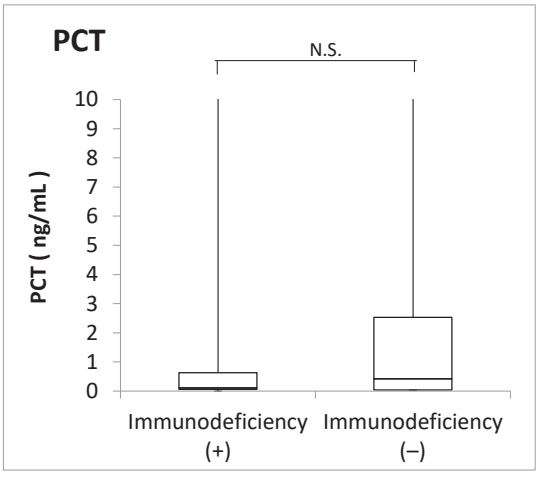


Fig. 4. Neutrophil CD64 expression, CRP, and PCT levels in groups with and without immunodeficiency. The box shows the 25–75th percentiles, whereas the whiskers indicate the 10th and 90th percentiles. N.S. means not significant by the Mann-Whitney *U* test. CD64, cluster of differentiation 64; CRP, C-reactive protein; PCT, procalcitonin.

sitivity for the diagnosis of systemic infection, there are conflicting results regarding its diagnostic utility in local infection.[11, 16, 17] When the CD64-positive cut-off level was set at 2000 molecules/cell,[4] the sensitivity of CD64 in diagnosing systemic infection was 92.7% and the specificity was 96.5%. For local infection, on the other hand, the sensitivity was 66.0% and the specificity was 95.7%.[18] In our study, CD64 expression was significantly lower in patients with a positive local culture or negative cultures than in those with a positive blood culture, findings that support those of the report.[18] These results were similar to those of CRP and PCT, two conventional infection markers, and all three markers showed similar positive rates regardless of culture results. Based on these results, CD64 seemed to have similar utility as conventional infection markers regardless of whether infection was systemic or local.

Antibiotic administration before CD64 measurement was shown to decrease the sensitivity and specificity of CD64 as an infection marker.[15] In our study, the positive rate of each infection marker was similar between the antibiotic and no-antibiotic groups. However, no patients in the positive blood culture group received antibiotics before CD64 measurement. The ratios of patients who received antibiotics were different among groups, and this might have influenced our results.

Regarding the timing of CD64 assessment, patients in whom CD64 was measured later than 7 days after infection onset showed significantly lower CD64 values than patients in whom CD64 was measured within 7 days. Cid et al. demonstrated that CD64 increased within 24 hours after infection onset, began to decrease 48 hours later, and reached baseline levels within 7 days.[16] Therefore, the diagnostic sensitivity of CD64 is decreased in chronic infection.[13] Conventional infection markers show a similar temporal relation to infection onset. In our study, the positive rate of CD64 was lower within 7 days than afterward, but there was no difference in CRP or PCT.

CD64 levels were unaffected by immunodeficiency, and there were no significant differences in the positive rates of any of the three markers. Gros et al. indicated that the diagnostic sensitivity of CD64 was decreased in patients in the intensive care unit with poor overall clinical status.[18] Therefore, it was expected that CD64 levels would be decreased in immunodeficient patients, but this was not observed in our study. However, our results may have been influenced by the fact that there were different percentages of immunodeficient patients in the three infection status groups: 79% (five patients) in the positive blood culture group, 56% (seven patients) in the positive local culture group, and 56% (11 patients) in the culture-negative group.

We showed that the utility of CD64 was equivalent to conventional infection markers in patients with a variety of conditions. However, there are several challenges to routinely using CD64 in clinical settings. First, measurement of CD64 expression on neutrophils requires flow cytometry and takes approximately 2 hours. Matsui et al. reported that measurements remained stable for at least 24 hours after blood samples were obtained.[4] While this issue was not statistically evaluated in this study, measurement values tended to decrease several hours after sampling, and it may therefore be necessary to measure CD64 levels immediately. Second, the cost of this measurement is not currently covered by health insurance in Japan. Third, measurement of CD64 is more complicated and time-consuming than the evaluation of conventional markers, and there are many issues that must be resolved before CD64 assessment can replace standard tests.

There were several limitations to this study. The small sample size contributed to significant variation between groups, particularly regarding antibiotic administration and timing of CD64 measurement, and this might have reduced the reliability of the results. In addition, the reason for the diagnosis of infection in culture-negative patients differed in each case, and a clear criterion should have been established instead.

In conclusion, we evaluated the utility and efficacy of neutrophil CD64 expression in diagnosing musculoskeletal infection. Several previous studies reported that CD64 was useful in diagnosing infection in patients with RA and those in the early post-surgical phase. Nevertheless, to the best of our knowledge, this is the first study to evaluate the utility of CD64 in musculoskeletal infection. It revealed that CD64 had equivalent diagnostic utility as conventional infection markers in various musculoskeletal infections.

The authors declare no conflict of interest.

REFERENCES

1 van der Meer W, Pickkers P, Scott CS, van der Hoeven JG, Gunnewiek JK. Hematological indices, inflammatory markers and neutrophil CD64 expression: comparative trends during experimental human endotoxemia. J Endotoxin Res. 2007;13:94-100. doi: 10.1177/0968051907079101. PMID: 17621550.

2 Repp R, Valerius T, Sendler A, Gramatzki M, Iro H, Kalden JR, et al. Neutrophils express the high affinity receptor for IgG (Fc gamma RI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. Blood. 1991;78:885-9. PMID: 1714327.

3 Valerius T, Repp R, de Wit TP, Berthold S, Platzer E, Kalden JR, et al. Involvement of the high-affinity receptor for IgG

(Fc gamma RI; CD64) in enhanced tumor cell cytotoxicity of neutrophils during granulocyte colony-stimulating factor therapy. Blood. 1993;82:931-9. PMID: 7687898.

4 Matsui T, Ohsumi K, Ozawa N, Shimada K, Sumitomo S, Shimane K, et al. CD64 on neutrophils is a sensitive and specific marker for detection of infection in patients with rheumatoid arthritis. J Rheumatol. 2006;33:2416-24. PMID: 17080517.

5 Gilbert DN. Use of plasma procalcitonin levels as an adjunct to clinical microbiology. J Clin Microbiol. 2010;48: 2325-9. doi: 10.1128/JCM.00655-10. PMID: 20421436; PMCID: PMC2897488.

6 Kaya B, Sana B, Eris C, Karabulut K, Bat O, Kutanis R. The diagnostic value of D-dimer, procalcitonin and CRP in acute appendicitis. Int J Med Sci. 2012;9:909-15. doi: 10.7150/ijms.4733. PMID: 23236260; PMCID: PMC3520016.

7 Oludag T, Gode F, Caglayan E, Saatli B, Okyay RE, Altunyurt S. Value of maternal procalcitonin levels for predicting subclinical intra-amniotic infection in preterm premature rupture of membranes. J Obstet Gynaecol Res. 2014;40:954-60. doi: 10.1111/jog.12273. PMID: 24320627.

8 Nishino J, Tanaka S, Kadono Y, Matsui T, Komiya A, Nishimura K, et al. The usefulness of neutrophil CD64 expression in the diagnosis of local infection in patients with rheumatoid arthritis in daily practice. J Orthop Sci. 2010;15:547-52. doi: 10.1007/s00776-010-1498-5. PMID: 20721724.

9 Katoh N, Nishino J, Nishimura K, Kawabata C, Hotta Y, Matsui T, et al. Normal sequential changes in neutrophil CD64 expression after total joint arthroplasty. J Orthop Sci. 2013;18:949-54. doi: 10.1007/s00776-013-0451-9. PMID: 23943224; PMCID: PMC3838574.

10 Perry J, Reed MR, Refaie R, Sprowson AP, Rankin KS, Refaie R. The assessment of neutrophil CD64 count as an early warning marker of joint replacement infection. Arch Orthop Trauma Surg. 2013;133:1351-8. doi: 10.1007/s00402-013-1816-4. PMID: 23860675.

11 Daryapeyma A, Pedersen G, Laxdal E, Corbascio M, Johannessen HB, Aune S, et al. Neutrophil CD64 as a marker for postoperative infection: a pilot study. Eur J Vasc Endovasc Surg. 2009;38:100-3. doi: 10.1016/j.ejvs.2009.03.012. PMID: 19359198.

12 Tanaka S, Nishino J, Matsui T, Komiya A, Nishimura K, Tohma S. Neutrophil CD64 expression in the diagnosis of local musculoskeletal infection and the impact of antibiotics. J Bone Joint Surg Br. 2009;91:1237-42. doi: 10.1302/0301-620x.91b9.22051. PMID: 19721054.

13 Li S, Huang X, Chen Z, Zhong H, Peng Q, Deng Y, et al. Neutrophil CD64 expression as a biomarker in the early diagnosis of bacterial infection: a meta-analysis. Int J Infect Dis. 2013;17:e12-23. doi: 10.1016/j.ijid.2012.07.017. PMID: 22940278.

14 Davis BH, Olsen SH, Ahmad E, Bigelow NC. Neutrophil CD64 is an improved indicator of infection or sepsis in emergency department patients. Arch Pathol Lab Med. 2006;130:654-61. doi: 10.1043/1543-2165(2006)130[654:nci-aii]2.0.co;2. PMID: 16683883.

15 Allen E, Bakke AC, Purtzer MZ, Deodhar A. Neutrophil CD64 expression: distinguishing acute inflammatory autoimmune disease from systemic infections. Ann Rheum Dis. 2002;61:522-5. Epub 2002/05/15. PMID: 12006325; PMCID: PMC1754114.

16 Cid J, Aguinaco R, Sanchez R, Garcia-Pardo G, Llorente A. Neutrophil CD64 expression as marker of bacterial infection: a systematic review and meta-analysis. J Infect. 2010;60:313-9. doi: 10.1016/j.jinf.2010.02.013. PMID: 20206205.

17 Oppegaard O, Skodvin B, Halse AK, Langeland N. CD64 as a potential biomarker in septic arthritis. BMC Infect Dis. 2013;13:278. doi: 10.1186/1471-2334-13-278. PMID: 23783182; PMCID: PMC3689602.

18 Gros A, Roussel M, Sauvadet E, Gacouin A, Marque S, Chimot L, et al. The sensitivity of neutrophil CD64 expression as a biomarker of bacterial infection is low in critically ill patients. Intensive Care Med. 2012;38:445-52. doi: 10.1007/s00134-012-2483-6. PMID: 22310872.

19 Matsui T, Komiya A, Shimada K, Nakayama H, Tohma S. Neutrophil CD64 as a marker of infection in patients treated with tocilizumab. Modern Rheumatology. 2009;19:696-7. doi: 10.3109/s10165-009-0223-8.

20 Komiya A, Matsui T, Horie K, Fukuda H, Nogi S, Iwata K, et al. Neutrophil CD64 for monitoring the activity of nontuberculous mycobacteria infection in patients with rheumatoid arthritis. Modern Rheumatology. 2014;24:770-4. doi: 10.3109/14397595.2013.871108.