Neutrophil CD64 expression in the diagnosis of local musculoskeletal infection and the impact of antibiotics

We examined the usefulness of neutrophil CD64 expression in detecting local musculoskeletal infection and the impact of antibiotics on its expression. Of 141 patients suspected of musculoskeletal infection, 46 were confirmed by microbiological culture to be infected and 95 had infection excluded. The median CD64 count of patients with localised infection was 2230 molecules per cell (interquartile range (IQR) 918 to 4592) and that of the patients without infection was 937 molecules per cell (IQR 648 to 1309) (p < 0.001). The level of CD64 correlated with the CRP level in patients with infection, but not in those without infection (r = 0.59, p < 0.01). Receiver operator characteristic curve analysis revealed that CD64 was a good predictor of local infection. When the patients were subdivided into two groups based on the administration of antibiotics at the time of CD64 sampling, the sensitivity for detecting infection was better in those who had not received antibiotics.

These results suggest that measurement of CD64 expression is a useful marker for local musculoskeletal infection.

Infection in musculoskeletal surgery results in morbidity and mortality, but also imposes an enormous socio-economic burden on society.1,2 An early and accurate diagnosis of local infection remains a major challenge for clinicians to improve the prognosis and to reduce cost. However, clinical symptoms such as pain, swelling, redness and a local increase in temperature are not sufficiently specific to confirm infection. General inflammatory markers such as the level of CRP, the ESR and the white blood cell (WBC) count are diagnostically useful,3 but again lack specificity. Although a positive microbiological culture is widely regarded as the absolute gold standard, the specificity of microbiological culture has been reported to be 40% to 65% in some cases.4,5 Sometimes, the culture results can be misleading, with false- negative results. The rate of false-negative results for microbiological culture is known to be high in cases of infection with anaerobic organisms or fungi.4,6

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positive microbiological culture and careful observation of the clinical course. Patients with a spontaneous improvement of symptoms without antibiotics were considered to have had no infection. Of 156 patients examined, 15 had systemic infection and were thus excluded from further analysis. Of the remaining 141, there were 70 women and 71 men with a median age of 65 years (interquartile range (IQR) 47 to 74.3). Of these, 43 had rheumatoid arthritis, 12 osteoarthritis, 28 traumatic injuries, 11 crystal-induced arthritis, three gout and 44 other diseases.

Quantitative measurement of CD64 expression. The blood samples used for CD64 measurement were not collected specifically for our study. These were unused portions of samples obtained for routine blood tests. CD64 expression on neutrophils was measured as previously reported. In short, 20 μl of QuantiBrite CD64PE/CD45PerCP (Becton-Dickinson, San Jose, California) were added to 50 μl of whole blood and incubated for 60 minutes in the dark, at room temperature. After the erythrocytes had been lysed with 2 ml of 1× FACS lysis solution (Becton-Dickinson), the samples were incubated for an additional 60 minutes to allow equilibration and to reduce non-specific background staining. The expression of CD64 was examined using a FACScan flow cytometer (Becton-Dickinson) calibrated with QuantiBrite phycoerythrin beads (Becton-Dickinson). These contained four different beads with known numbers of phycoerythrin molecules which made it possible to create a standard curve for determining the mean number of phycoerythrin molecules present on a cell. The mean number of CD64 molecules expressed on the cell surface was calculated using the phycoerythrin fluorescence quantification kit with QuantiBrite phycoerythrin beads. There were three different cell populations comprising lymphocytes, monocytes and granulocytes, which were identified and gated by their CD45/side-scatter profile.

Statistical analysis. The data were presented as the median and IQR. Comparison between the groups was carried out using the non-parametric Mann-Whitney U test. Correlations were evaluated by Spearman’s rank correlation coefficient. Receiver-operator characteristic curve analysis and the area under the curve with the 95% confidence interval (CI) were used to compare the discrimination capacity of CD64, the CRP, the ESR and the WBC count in diagnosing local infection, and to establish an optimal cut-off value for CD64 expression. Applying a previously published cut-off value, the usefulness of CD64 to discriminate local infection was evaluated by comparison with the clinical results using the chi-squared or Fisher’s exact test depending on the number of cases. In addition, the negative predictive value and positive predictive value were calculated. The SigmaStat statistical program (SPSS Inc., Chicago, Illinois) was used for statistical analysis. A p-value < 0.05 was used to define statistical significance.

Results
Neutrophil CD64 expression, CRP, ESR and WBC count. Of the 141 patients studied, infection was confirmed in 46 patients (Table I) and included late infection in total knee replacement in two, cellulitis in ten, infected bursitis in two, infected spondylitis in two, septic arthritis in five, osteomyelitis in eleven, surgical-site infection in nine, an infected skin ulcer in two, folliculitis in two and infected tenosynovitis in one. There were significantly more men identified with infection (p = 0.028) possibly because of the number of men with post-traumatic osteomyelitis in the group. Comparison of the median ages between the group with confirmed infection and without confirmed infection exhibited no statistical difference (p = 0.17, Table I). Comparison of the median neutrophil CD64 expression between the groups was statistically significantly different (Mann-Whitney U test, p < 0.001; Table I, Fig. 1). When the cut-off value of the CD64 count was set at 2000 molecules/cell in accordance with previous studies, the negative and positive predictive values for infection were 83.8% and 93.3%, respectively. The sensitivity of detecting infection was 60.9% and the specificity was 97.9%.

The difference in the median CRP, ESR and WBC counts in the two groups were also compared (Table I). There was a statistically significant difference in the CRP (p = 0.001) and WBC count (p = 0.007), but not in the ESR (p = 0.108).

In an attempt to examine the relationship between CD64 and the severity of infection, we analysed the correlation of CD64 with the CRP in the presence and absence of proven infection. Neutrophil CD64 expression was significantly correlated with the CRP in the group with confirmed infection (r = 0.590, p < 0.01), but not in the group in which infection was not proven, suggesting that CD64 is correlated with the severity of infection (Fig. 2).
Surgical treatment was required in 35 patients in the infected group and CD64 measurement was performed before operation in these cases. There was no statistically significant difference in the median levels of CD64 between patients with proven infection who required surgical treatment (2373, IQR 1106 to 6345) and those in whom surgery was not required (1837, IQR 1606 to 3337) (p = 0.183).

Impact of antibiotics. We then subdivided the infected and infection-free groups into those who had been treated with antibiotics (antibiotics (+) group) and those who had not.

| Table II. Impact of antibiotics on median (IQR) CD64, CRP, ESR and the white blood cell (WBC) count in patients with and without proven infection |
|-----------------|-----------------|----------------|----------------|-----------------|-----------------|----------------|
|                 | Antibiotic (+)  | Antibiotic (-) | p-value*       | Antibiotic (+)  | Antibiotic (-) | p-value*       |
| Number of patients | Infection (-) | Infection (+)  | Infection (-) | Infection (+)  | Infection (-) | Infection (+)  |
| 41              | 35              | 54             | 11             | 0.086           |
| Female:male ratio | 19:22           | 15:20          | 0.64           | 33:21           | 3:8            | 0.086           |
| Age in yrs (range) | 65 (40.5 to 79) | 64 (47 to 75.5) | 0.52           | 68 (56.5 to 78) | 64.0 (50.75 to 74.25) | 0.397 |
| CD64 in molecules/cell (range) | 965 (651 to 1365) | 2124 (918 to 4204) | < 0.001 | 884 (626 to 1265) | 2680 (848 to 11635) | 0.001 |
| CRP in mg/dl (range) | 2.2 (0.50 to 6.10) | 5.43 (2.11 to 12.01) | 0.007 | 2.25 (0.45 to 5.98) | 5.52 (1.23 to 24.44) | 0.112 |
| ESR in mm/hour (range) | 38 (38.75 to 75.5) | 67 (67.0 to 101.5) | 0.06 | 32 (9.5 to 64.5) | 26 (12.0 to 34.5) | 0.373 |
| WBC in cells/mm³ (range) | 7270 (7270 to 9608) | 8500 (8500 to 12250) | 0.19 | 6600 (5483 to 9473) | 8850 (6575 to 14572) | 0.022 |

* Mann-Whitney U test

Box plots showing a) the CD64, b) the CRP, c) the ESR and d) the white blood cell (WBC) count for patients with and without proven local musculoskeletal infection (infection (+) and infection (-), respectively). The lines inside the box represent the median, the boxes show the 25th and 75th percentiles and the bars indicate the 10th and 90th percentiles.
Patients included in the antibiotic (+) group had received antibiotics within five days of the blood samples being taken. In contrast, the antibiotic (-) group had never received antibiotics or had stopped them more than five days before the blood sampling. In the antibiotic (+) group, 35 patients were diagnosed with infection and 41 without and in the antibiotic (-) group 11 patients were diagnosed with infection and 54 without (Table II).

In the antibiotic (+) group, the median neutrophil CD64 expression in the patients without infection and in those with infection was significantly different (Mann-Whitney U test, p < 0.001) (Table II). Comparison of the medians of the CRP, ESR and WBC counts in patients without infection and in those with infection showed a significant difference in the CRP (p = 0.007) but no statistically significant differences were found in the ESR or WBC count (p = 0.6 and p = 0.19 respectively) (Table II).

In the antibiotic (-) group, the median result of the neutrophil CD64 expression for patients with and without infection was statistically significantly different (Mann-Whitney U test, p = 0.001) (Table II). However, the matching analysis for the median CRP and ESR measurements did not reveal any statistical difference (p = 0.112 and p = 0.373, respectively) (Table II).

The negative predictive value of CD64 in the antibiotic (+) group (70.4%) was significantly lower than in the antibiotic (-) group (96.5%) (p < 0.001), while the positive predictive value did not show any statistical significance (90.9% vs 100%; p = 0.383). The sensitivity of the CD64 count for detecting local infection was only 55.6% in the antibiotic (+) group and in the antibiotic (-) group was superior at 80% (p < 0.001). The specificity was 95.0% and 100%, respectively.

**Receiver operator characteristic curve analysis.** Receiver-operator characteristic curves were generated to compare the predictive value of the laboratory data in identifying the presence of infection (Fig. 3). The areas under the curves with their 95% CIs for CD64, the CRP, ESR and the WBC count were 0.791 (95% CI 0.674 to 0.909), 0.641 (95% CI 0.521 to 0.761), 0.610 (95% CI 0.488 to 0.732) and 0.603 (95% CI 0.480 to 0.727), respectively. In the antibiotic (+) group, these values were 0.795 (95% CI 0.657 to 0.932), 0.682 (95% CI 0.528 to 0.836), 0.644 (95% CI 0.483 to 0.805) and 0.570 (95% CI 0.403 to 0.737), and in the antibiotic (-) group, 0.801 (95% CI 0.548 to 1.035), 0.639 (95% CI 0.432 to 0.846), 0.491 (95% CI 0.310 to 0.671) and 0.761 (95% CI 0.595 to 0.927), respectively. The area under the curve of the CD64 count was the highest in both groups.

**Discussion**

The diagnosis of musculoskeletal infection can be difficult since haematological investigations are not absolutely reliable and a negative bacterial culture does not necessarily exclude infection.4,5 In addition, the CRP and ESR can increase in the presence of an inflammatory process even in the absence of infection.4,5 It is increased in patients with systemic infection, but not in those with a flare-up of rheumatoid arthritis or other inflammatory disorders when infection is absent.10 We have shown that CD64 expression can be helpful in the diagnosis of local musculoskeletal infection. It was significantly higher in patients with local infection than in those without, and the
levels of CD64 were significantly correlated with the CRP in patients with proven infection ($r = 0.59$, $p < 0.01$), but not in those in whom infection had been excluded. The area under the curve of CD64 was higher than that of the CRP, ESR and WBC count, confirming that CD64 expression is a good predictor of local infection.

At a cut-off value of 2000 molecules/cell, the sensitivity and specificity of CD64 for the diagnosis of infection were 66.0% and 95.7%, respectively. These values were lower than those obtained in a previous study on systemic infections, in which the sensitivity was 92.7% and the specificity 96.5%. We suspect that this was at least partly because some patients had received antibiotics before its measurement reduces its negative predictive value, and antibiotics reduced the number of bacteria in the inflammatory foci, which led to the reduction of neutrophil CD64 expression. Therefore it is recommended that CD64 expression should be measured before starting treatment with antibiotics. However, CD64 yielded a larger area under the curve than that of the CRP, ESR and WBC count even in the antibiotic (+) group, further confirming the superiority of CD64 for the diagnosis of local infection. Additionally, it is possible that a smaller number of pathogens is present in local infections than that in systemic infections, which may result in a weaker neutrophil response.

The limitations of our study were the small number of patients and the lack of a control group since this was an observational study performed in the course of daily clinical practice. Future prospective studies with a large number of patients will be required to confirm that measurement of neutrophil CD64 expression is a better predictor of local infection than other markers. Recent studies by Bottner et al have demonstrated the role of IL-6, procalcitonin and TNF-$\alpha$ in the diagnosis of deep infection in implants. They found that these markers were very specific, but had a low sensitivity. Future studies are required to establish their value and neutrophil CD64 expression.

Our study suggests that the level of neutrophil CD64 expression is helpful in confirming the presence of musculoskeletal infection. Although the administration of antibiotics before its measurement reduces its negative predictive value, it still appears to be a better predictor of local infection than the CRP, ESR and/or the WBC count.

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References

