Changes in neutrophil CD64 expression after elective orthopedic surgery

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Introduction
Post-operative musculoskeletal infection has been one of the most devastating complications in orthopedic surgeries. Although its early and accurate diagnosis is required, it often fails because there have been no adequate procedures for detecting such infection at an early stage after surgery. Findings of the clinical local symptoms or elevations of levels in laboratory markers, for example, may overlap with physiological responses to the surgical stress. A microbiological culture could consume time and often indicate false negative results.

CD64, one of the Fc receptors for IgG, is constitutively expressed on macrophages, monocytes and eosinophils, and is rapidly upregulated on neutrophils by stimulation of bacterium, virus or mycobacterium [1, 2]. Based on these findings, the measurement of neutrophil CD64 expression was considered to be a strong candidate for use in the early diagnosis of post-operative local infection.

The purpose of the current study, therefore, was to clarify the 2-week sequential changes of neutrophil CD64 expression after elective orthopedic surgery, and to evaluate its usefulness as a specific marker for post-operative musculoskeletal infection.

Materials and Methods

Patients. Included in this study were patients who gave their written informed consent and who underwent elective orthopedic surgeries between January and June of 2008 at Teikyo University Hospital in Tokyo. The study was approved by the local ethics committee. Among 68 cases enrolled, 19 were excluded from further analysis because of comorbidities such as microscopic polyangitis or chronic pneumonitis.

Finally, 49 patients (41 women and 8 men; median age [IQR]: 65 [54-74]) were enrolled (19 TKAs, 24 THAs and 6 pelvic rotational acetabular osteotomies).

Measurement of CD64 expression. The peripheral blood samples used for CD64 measurement were obtained at one day before and at days 1, 3, 5, 7, and 14 after the surgery. Levels of neutrophil CD64 expression per cell were quantitative-measured [1]. Briefly, after staining of all of the blood with QuantiBRITE CD64PE/CD45PerCP (Beckton-Dickinson, San Jose, CA), granulocyte population was gated by the CD45/side-scatter profile. The CD64 levels were analyzed using a FACScan flow cytometer (BD) calibrated with QuantiBRITE PE beads (BD). Levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white blood cell count (WBC), as other inflammatory markers, were simultaneously measured in the corresponding sample.

Statistical analysis. The measurement variables were presented as the median and interquartile range [IQR]. Comparison among the continuous variables was carried out using Mann-Whitney U test. Correlations among the variables were evaluated by calculated Spearman’s rank correlation coefficient. Statistical significance was set at p < 0.05.

Results

Clinical Results. No cases complicated with post-operative local infection were observed for the full investigated 2 weeks following the surgeries, nor for the ensuing period to the end of December 2008.

Sequential changes in neutrophil CD64 expression. The levels of neutrophil CD64 expression elevated at day 1 after surgery, peaked at day 3, and thereafter decreased: 725.0 [550.5 - 1118.0] molecules/cell at a day before surgery, 1186.0 [874.5 - 1716.5] at day 1, 1492.0 [1091.5 - 2485.5] at day 3, 1040.0 [680.5 - 1488.5] at day 5, 906.0 [595.0 - 1335.0] at day 7, and 818.0 [673.5 - 1272.5] at day 14 after surgery. Statistical analysis confirmed that significant differences existed between the pre-operative levels and the levels at days 1, 3 and 5, while no significant differences existed between the pre-operative levels and those at days 7 or 14 (Figure).

Of the total 49 cases, 40 showed their peaks at day 3 after surgery, whereas the remaining 9 cases showed different peak points: three cases at day 1, four at day 5, and two at day 7 or 14 after surgery. However, there were no differences among the 40 and the 9 cases in their baseline nor peri-operative profiles.

Sequential changes in the other parameter levels. The levels of CRP and ESR significantly elevated at day 1 after surgery (p < 0.01, p < 0.01, respectively), peaked at day 3 (p < 0.01, p < 0.01, respectively), and thereafter tended to decrease in a manner similar to that of CD64 (p < 0.01, p < 0.01, respectively). Their levels at day 14, however, still remained significantly higher compared to their baseline levels (p < 0.01, p < 0.01, respectively).

The level of WBC elevated immediately after surgery (p < 0.01), peaked at day 3 (p < 0.01), and alleviated at day 5. Thereafter, however, it elevated again (p < 0.05).

Analysis of the highest CD64 level. The median value of the highest CD64 level in the observed postoperative 2 weeks (peak CD64 level) was 1650.0 molecules/cell [1135.0 – 2487.5]. Eighteen patients showed peak levels over 2000 molecules/cell, the level which has been reported to be a cut-off value for distinguishing infection.

The peak CD64 level positively correlated with the preoperative CD64 level and negatively correlated with the preoperative WBC level. The results of the multiple regression analysis, however, showed that the sole parameter of preoperative CD64 level significantly explained the peak CD64 level. In a comparison between the preoperative and the peak postoperative CD64 levels, a peak of 2.16 [1.62 – 2.71] times the preoperative level was reached.

Discussion

The results obtained from the present study demonstrated that the sequential changes in CD64 expression are more distinguishable than those in CRP, ESR or WBC. Of note, is that the elevation ratio of CD64 peaked at about 2 times the preoperative level in all cases, even exceeding the red-flag 2000 molecules/cell level without infection.

In conclusion, the neutrophil CD64 expression level can increases to about double the preoperative levels in early post-operative days without cause for concern about infection, so long as it is followed by a rapid return to nearly basal levels by day 7. The measurement of CD64 is, therefore, thought to be more useful for early diagnosis of postoperative local infection than that of other conventionally measured inflammatory markers.

Figure: Sequential changes in neutrophil CD64 expression level

The neutrophil CD64 expression levels elevated at day 1 after surgery, peaked at day 3, and thereafter decreased. The elevation ratio reached about 2 times the preoperative level. (**: p < 0.01)

References