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Proinflammatory biomarkers' level and functional genetic polymorphisms in periprosthetic joint infection

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ABSTRACT

Objective: The aims of this study were 1) to identify the level of inflammatory biomarkers interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-17, C-reactive protein (CRP), granulocyte colony-stimulating factor (GCSF), ferritin, and tumor necrosis factor (TNF)-α in serum and synovial fluid samples of patients who underwent revision arthroplasty surgery; 2) to establish the relationship between serum and synovial fluid levels; 3) to determine if any of the 11 genetic polymorphisms of TNFα, IL-1, IL-6, IL-8, IL-17, and GCSF on the encoding genes was associated with periprosthetic joint infection (PJI).
Methods: Synovial fluid and serum was collected from 88 patients who underwent revision arthroplasty surgery. The Musculoskeletal Infection Society definition was used to classify these patients into 2 groups: 36 PJIs and 52 aseptic failures. Synovial fluid and serum samples were tested for 9 biomarkers using a micro enzyme-linked immunosorbent assay. Genetic polymorphisms were evaluated with polymerase chain reaction and restriction endonuclease analysis.
Results: Synovial fluid-derived IL-1α, IL-1β, IL-8, IL-17, CRP, GCSF, TNFα, and serum-derived IL-6, IL-17, ferritin, CRP were found suitable to classify PJI and aseptic failure. In addition, IL-17 and CRP levels demonstrated a positive correlation between synovial fluid and serum. TNFα-238, IL6-174, GCSF3R, and IL1 RN-VNTR genetic polymorphisms occurred more frequently in individuals with septic failure.
Conclusion: Significant differences between the two groups were observed in the functional polymorphisms of the genes encoding the cytokines investigated. These differences could be interpreted as indicating that there is an association between PJI and genetic polymorphisms.
Level of evidence: Level III, diagnostic study.

Introduction

Total joint replacement, especially of the hip or knee, is one of the most successful surgical interventions worldwide.[1] Though the reported complication rate is 1%–3%, among all lower extremity (hip–knee) arthroplasties, periprosthetic joint infection (PJI) is the most common reason for revision of total knee surgeries and the third most common reason for total hip revision arthroplasties.[2] There have been studies in recent years that suggested that the real reason for aseptic arthroplasty failure was inflammation caused by bacteria and their synthesized products. These studies relied on evidence from implants previously revised due to aseptic arthroplasty failure.[3]

The use of synovial fluid and blood serum biomarkers, which include inflammatory proteins, such as cytokines, to diagnose PJI has been confirmed in several studies.[1,4,5] Jacovides et al[6] suggested that in the future, it may be possible for a dipstick test, the fast, easy, and successful method used in the diagnosis of pregnancy and

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urinary tract infections, to be performed to diagnose PJI based on their study of the levels of 46 inflammatory biomarkers in 74 samples of synovial fluid.

Functional polymorphisms defined in cytokine genes, such as interleukin (IL)-1, IL-6, IL-8, IL-17 and granulocyte colony-stimulating factor (GCSF), influence the onset, severity, and duration of inflammation by affecting both basal and stimulated cytokine levels.[7–14] The role of these polymorphisms in the onset and clinical outcome of various autoimmune, infectious, inflammatory, and malignant diseases has been extensively studied.[7–14] Results regarding the value of these inflammatory markers in the diagnosis of PJI have been contradictory. The hypothesis of the present study was that the polymorphisms affecting transcription levels may contribute to cytokine levels as well as to inflammation. To our knowledge, there is no other study in the literature investigating the relationship between functional polymorphisms defined in the genes encoding the investigated cytokines or their receptors, all of which have been identified as markers for PJI in various studies, and the occurrence of PJI.

Based on previous investigations, the aims of this study were 1) to identify the level of inflammatory biomarkers IL-1α, IL-1β, IL-6, IL-8, IL-17, C-reactive protein (CRP), GCSF, ferritin, and tumor necrosis factor (TNF)-α in serum and synovial fluid samples of patients who underwent revision arthroplasty surgery (as a result of either septic or aseptic reasons), 2) to establish the relationship between serum and synovial fluid levels of these markers and PJI in order to identify them as biomarkers of PJI, and 3) to determine if any of the 11 functional polymorphisms of TNFα, IL-1, IL-6, IL-8, IL-17, and GCSF on the encoding genes was associated with PJI among patients undergoing surgery for infection or aseptic loosening.

Patients and methods

The study was approved by our Institutional Ethics Review Board. All of the participating patients provided signed informed consent prior to being enrolled in the study, and the study was conducted in accordance with the Declaration of Helsinki.

This prospective, single-center, controlled study was conducted with a total of 88 patients who underwent revision arthroplasty surgery for either septic or aseptic reasons. Patients with malignancy or antibiotic treatment within 2 months before revision surgery were excluded. The study group included 27 men and 61 women with a mean age of 68 years (range: 44–83 years). Fifty-nine of the samples were knee samples, while 29 were hip samples. Patients undergoing revision arthroplastic surgery had preoperative laboratory tests, which included measurement of sedimentation (ESR), CRP, and synovial fluid white blood cell (WBC) count with a differential cell count. Patients who met the study criteria were classified as septic or aseptic on the basis of the Musculoskeletal Infection Society (MSIS) definition of PJI.[15] Thirty-six of the 88 patients had surgery for presumed infection, including irrigation and debridement or first stage of a two-stage exchange; 52 patients had surgery for presumed aseptic loosening or a mechanical complication of a hip or knee arthroplasty. Intraoperative deep tissue samples were collected for conventional microbiological culture.

All 88 patients with total joint arthroplasty requiring a reoperation due to failure underwent surgery and were followed prospectively between November 2010 and May 2014. Synovial fluid and peripheral blood samples were collected from the patients. Peripheral whole blood samples were collected 30 min before the revision total joint arthroplasty, prior to the administration of preoperative antibiotic prophylaxis in the operating room. Synovial fluid samples were collected intraoperatively before joint capsulotomy. All samples were centrifuged in the hospital clinical laboratory; the separated serum from the peripheral blood sample and supernatant of the synovial fluid were transferred to 2 mL sterile cryotubes and stored at −80 °C until studied. Blood samples from all the patients enrolled in the study were also drawn in tubes containing ethylenediaminetetraacetic acid in order to extract total DNA for analysis of the functional polymorphisms.

Levels of biomarkers in the serum and joint aspirates were determined using an enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis, MN, USA). All assays were carried out according to the manufacturer's instructions. Total DNA was extracted from the blood samples using a total DNA extraction kit (Qiagen, N.V., Hilden, Germany) per the manufacturer's instructions. Polymerase chain reaction-restriction endonuclease analyses were performed to determine the functional polymorphisms defined in the genes encoding the analyzed cytokines according to previously published methods.[7–14,16–19]

Biomarker expression levels in the serum and synovial fluid were compared in patients with and without PJI. The Kruskal–Wallis test was used to assess biomarkers of serum and synovial fluid expression. Pairwise comparisons were performed using the Mann–Whitney U test.

Within-patient comparisons of serum and synovial fluid biomarkers levels were performed using the Wilcoxon test for paired data. Spearman's test was used to evaluate correlations. Receiver operating characteristic curve analysis was used to determine serum and synovial fluid-derived biomarker levels for the diagnosis of PJI. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of each assay were calculated.

The allele and genotype frequencies of genes in the patients were compared to controls using a chi-square test. The linkage disequilibrium between 2 single nucleotide polymorphisms (SNPs) was examined using the SNPAnalyzer 2.0 (Istech Corp., Goyang, South Korea). The Hardy–Weinberg equilibrium was tested using a goodness-of-fit chi-square test with 1 degree of freedom to compare the observed genotype frequencies between the patients with the expected genotype frequencies. Comparisons between groups were made with two test (nominal data) or Student's t-test (interval data). Values of $p < 0.05$ were considered significant.

Results

The final cohort contained synovial fluid and serum samples from 88 patients with a minimum of 2 years (mean: 43 months, range: 28–71 months) of clinical and laboratory follow-up. The patients were classified as infected ($n = 36$; 40.9%) or uninfected ($n = 52$; 59.1%). Mean age was 68.7 years, and did not differ significantly between the 2 groups. There were more female patients in the overall cohort (female: 69.3%, male: 30.7%) and more knees than hips were revised (knees: 67%, hips: 33%) but these variables did not differ significantly between the 2 groups ($p > 0.05$). Of the 88 patients, 8 had systemic inflammatory disease (2 patients with PJI and 6 patients with aseptic loosening), and there was no significant difference between the study groups ($p > 0.05$).

No bacterial pathogen was cultured in 9 patients in the septic group, while an organism was isolated preoperatively or intraoperatively in 27 patients of that group (Table 1). No purulence was observed intraoperatively in any patient with aseptic loosening, and no organisms were isolated in cultures.

Significant local increases in the biomarkers IL-1α ($p < 0.002$), IL-1β ($p < 0.001$), IL-8 ($p = 0.002$), IL-17 ($p < 0.001$), CRP ($p < 0.001$), GCSF ($p < 0.001$), and TNF-α ($p < 0.001$) were observed in the synovial fluid of patients with PJI compared with those with aseptic loosening. Only IL-6 ($p < 0.001$), IL-17 ($p < 0.001$), ferritin ($p < 0.001$), and CRP ($p = 0.001$) serum levels were significantly

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