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ORIGINAL ARTICLE

The terminal complement pathway is activated in septic but not in aseptic shoulder revision arthroplasties

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Background: The early diagnosis of suspected periprosthetic low-grade infections in shoulder arthroplasties is important for the outcome of the revision surgical procedures. The aim of this study was to investigate new biomarkers of infection in revision shoulder arthroplasties, taking into account the implant design, patient age, and comorbidities.

Methods: The study included 33 patients with shoulder arthroplasties undergoing revision surgical procedures. Microbiological diagnostic testing was performed in all cases. C-reactive protein serum levels and white blood cell counts were evaluated, and the periprosthetic tissue was stained immunohistologically for the terminal complement pathway components (C3, C5, and C9) and for CD68 and α-defensin.

Results: Microbiological diagnostic testing detected a periprosthetic infection in 10 reverse shoulder arthroplasties and in 4 anatomic shoulder arthroplasties, while the remaining 19 shoulder arthroplasties were classified as aseptic. We observed more *Staphylococcus epidermidis* infections in reverse shoulder arthroplasties and more *Staphylococcus aureus* infections in anatomic shoulder arthroplasties. The revision rate correlated with pre-existing comorbidities and number of previous surgical procedures. The C-reactive protein values and the incidence of specific periprosthetic radiolucent lines were significantly increased in septic revision cases. We found increased staining for all tested complement factors (C3, C5, and C9) but not for α-defensin and CD68 in septic tissue. The most interesting finding was that C9 separated septic from aseptic tissue with a predictive specificity of 100% and a sensitivity of 88.89%.

Conclusion: We observed a strong correlation between C9 expressions in septic revision tissue. We propose that the terminal complement pathway, especially C9 deposition, may be a potential biomarker to identify septic complications using tissue biopsy specimens.

Ethical approval for this study was given by the Institutional Review Board of the Otto-von-Guericke University Medical School, Magdeburg (IRB No. 150/12).

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The number of total shoulder arthroplasties is rapidly growing, with a 7-fold increase predicted during the next 15 years.[9] Today, various different types of shoulder prostheses for different medical and individual anatomic conditions exist. However, the design of these prostheses can be distinguished by 2 basic biomechanical principles: anatomic and reverse shoulder implants.

Because of the increasing number of failed primary shoulder arthroplasties, the number of septic and aseptic revision surgical procedures is a rising challenge in shoulder surgery. The rate of infection in shoulder endoprostheses is approximately 1%, comparable with the infection rate of other joints.[27] The most frequently detected pathogens in total shoulder joint arthroplasty are *Cutibacterium* (formerly *Propionibacterium*) *acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.[31] The cause of infection can be a hematogenic infection (eg, pneumonia, urinary tract infection, or dental sinuses) or an intraoperative or perioperative infection, which can appear as an early or late infection. Clinically, an early periprosthetic joint infection (PJI) is accompanied by the onset of pain, loss of function, and other signs of inflammation such as fever, wound-healing disorders, or the development of local erythema. Furthermore, the presence of a systemic reaction is indicated by increased systemic inflammation parameters such as the white blood cell (WBC) count and C-reactive protein (CRP) level.[28] A late infection, however, is often more difficult to diagnose because of the lack of systemic inflammation owing to the formation of a biofilm. Sometimes, radiologically detectable periprosthetic radiolucent lines (RLLs) indicate the failure of secure fixation of the implant in the case of low-grade septic complications. Microbiological analysis of the synovial fluid, however, detects low-grade infection cases only at very late stages, when the biofilm is already producing planktonic bacteria. A second surgical intervention using a spacer implant, consisting of antibiotics and bone cement, is inevitable in most cases. Because low-grade infections are difficult to diagnose at early stages and a late diagnosis makes appropriate treatment impossible without explantation of the prosthesis, there is a need for biomarkers to diagnose the infection at an early time point. To develop biomarkers for the early diagnosis of a low-grade infection, the understanding of different pathways activated during the infection is of utmost importance.

The detection of α-defensin has been proposed to be a marker for PJI.[4,13,46] The α-defensin protein is a 2- to 6-kDa antimicrobial peptide, which is predominantly activated by gram-negative and -positive bacteria. It is secreted by neutrophils and macrophages and is able to bind pathogens in the synovial fluid and impede cell wall synthesis.[13,46] However, there have been reports of false-positive test results in the case of adverse tissue reactions.[11]

Another important component of the immune response to bacterial infection is the complement system.[41] The main purpose of the complement system is the destruction of foreign or dead cells, activation of immune defense cells, and opsonization of pathogens.[23] Therefore, the activation of the complement system is predominantly active in the early infection phase.[17,20] The system recognizes foreign structures activating 3 different pathways, which converge to the common component C3; the terminal common pathway is initiated with C5 being cleaved into C5a and C5b. C5b starts the formation of the membrane attack complex by recruiting C6, C7, C8, and C9. The membrane attack complex is the cytolytic end product of the terminal complement cascade resulting in osmotic lysis and thereby cell death.[10]

The presence of macrophages in tissue biopsy specimens has been proposed to be an indicator for septic complications, as they are part of the nonspecific immune response by removing pathogens via phagocytosis and also part of the adaptive immune response by recruiting other immune cells. Immunostaining for CD68 shows the presence of monocytes and macrophages, as a first hint of the inflammatory tissue response.[29,38]

The hypothesis of this study was that the terminal complement pathway in combination with α-defensin would provide better evidence of discrimination between aseptic loosening and PJI in total shoulder arthroplasties. To test this hypothesis, we investigated aseptic and septic tissues of shoulder endoprosthesis revision surgical procedures regarding the design of the shoulder implant, patient characteristics, bacterial diagnostic testing, and proposed biomarkers.

Materials and methods

Patients

In this retrospective basic research study, 33 consecutive shoulder revision surgical procedures performed for aseptic and septic revision reasons between February 2011 and April 2016 were included. The demographic data of all patients (age at surgery, implantation time, radiologically detected RLLs, number of previous surgical procedures, and comorbidities) were recorded (Table I). Before surgery, serum levels of CRP (in milligrams per liter) and WBC count (in Gpt per liter) were determined. Infections were identified according to Musculoskeletal Infection Society criteria and Infectious Diseases Society of America criteria.[26,28]