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Morphological study of synovial changes in two-stage reconstructions of the infected hip and knee arthroplasties

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ABSTRACT

Objectives: To study the morphological changes of the regenerating synovium in two-stage revision arthroplasty, which is the gold standard for treatment of periprosthetic joint infection.

Design: The authors analysed a series of synovial biopsies to examine morphological changes in healing periprosthetic tissues damaged by previous surgery and infection.

Methods: Synovial tissues from 19 patients (10 knees and 9 hips) who underwent a two-stage exchange surgery for periprosthetic infection were reviewed and correlated with clinical and laboratory findings.

Setting: Retrospective morphological study.

Participants: Archival tissues from 19 two-stage revision arthroplasties in adult patients.

Results: Healing synovial tissue obtained at the reimplantation surgery showed characteristic layering: superficial fibrin exudate, immature richly vascularised granulation tissue and deeper maturing granulation tissue and fibrosis. Although increased neutrophil counts were found in the majority of cases, 2 of 19 cases showed dense infiltrates indicative of persistent infection, which correlated with positive microbiology in one case. One of the cases failed due to acetabular loosening and two cases failed due to late superinfection. One case showed a dense infiltration of eosinophils suggestive of a hypersensitivity reaction, which was subsequently proven by cutaneous tests. Foci of extramedullary haematopoiesis were detected in two cases.

Conclusions: We observed characteristic morphological changes in the healing synovial tissue during reimplantation surgery for periprosthetic infection in serologically and microbiologically sterile tissues. Substantial increased counts of synovial neutrophils (>200 cells/10 high-power fields) seem to be indicative of persistent infection of the joint; therefore, prolonged antibiotic therapy should be considered in positive cases.

ARTICLE SUMMARY

Article focus

- Does the regenerating synovium in two-stage revision arthroplasty display characteristic morphological changes?
- Can histopathological analysis improve interdisciplinary diagnosis of persistent infection in two-stage revision arthroplasty?

Key messages

- Our study demonstrated characteristic morphological pattern of the regenerating synovium in a clinical setting of surgically and antibiotic treated periprosthetic infection.
- Substantially increased counts of synovial neutrophils can be linked with persistent periprosthetic infection.
- Non-neoplastic synovial extramedullary haematopoiesis was observed within a regenerating synovial tissue.

Strength and limitations of this study

- This pilot morphological study defined characteristic features of the regenerating synovium in two-stage revision arthroplasty.
- The major limitation in this study is the limited number of cases.

INTRODUCTION

Periprosthetic joint infection remains one of the most challenging complications of arthroplasty and is associated with immense physiological, psychological and financial costs.[1] Even though many tests are used to help identify possible infection in patients with symptoms of a failed arthroplasty, in many cases, diagnosis remains difficult. The current definitions of periprosthetic infection, by the American Academy of Orthopaedic Surgeons[2] and the Musculoskeletal Infection Society,[3 4] recommend several laboratory tests, including histopathological evaluation of the periprosthetic tissues (erythrocyte sedimentation rate (ESR) and C reactive protein (CRP)). Although recent recommendations consider a cell count greater than five neutrophils in five high-power fields (HPFs) characteristic of infection in cases that also fulfil other clinical

and/or laboratory criteria, in the past, the number of neutrophils required for histopathological diagnosis of periprosthetic infection has varied from 1 to 23 neutrophils in 10 HPFs.[5–15]

Two-stage exchange arthroplasty has become the preferred method of treatment for periprosthetic joint infection in North America[16–20] and parts of Europe. The procedure entails surgical removal of all infected tissue, the prosthesis and all foreign material, and insertion of either a static or dynamic antibiotic-impregnated spacer during the first stage, a so-called resection arthroplasty. The patient is then given a course of antibiotic treatment, usually for 6–12 weeks, to treat underlying osteomyelitis followed by reimplantation of new prostheses whenever appropriate.[19 21–23] The function of the spacer is to release the antibiotic into the infected bed of the prosthesis, minimise soft tissue contractures, retain soft tissue tension and so maintain reasonable functionality until a new prosthesis can be implanted.[24 25] Although two-stage exchange arthroplasty controls infection in the majority of cases, failures still occur. Although surgeons managing periprosthetic joint infections usually use serum markers, in particular the ESR and CRP, to guide reimplantation,[21 22] the potential usefulness of histopathological analysis of the healing synovial membrane following debridement has not yet been established.

In the current study, we first wished to characterise morphological changes of the healing synovial membrane following implantation of the dynamic antibiotic-impregnated spacer and, second, to investigate morphological changes predictive of resolution of the joint infection. We hypothesised that synovial membranes obtained during the final implantation surgery from healing synovial tissue would show characteristic morphological patterns common to all cases, and that substantial high neutrophile counts observed within the tissues might be considered unspecific for persistent active bacterial infection.

MATERIAL AND METHODS

We searched the databases of the Institute of Pathology, University Medical Center Hamburg-Eppendorf, for cases with a diagnosis of periprosthetic infection between September 2008 and September 2011. We specifically focused on cases in which biopsies were removed during both surgeries, at the first revision surgery, and again during final implantation of the new prosthesis. The study was performed according to the Declaration of Helsinki.

Patients

We studied 19 patients (9 women (median age: 80 years old, range 62–91) and 10 men (median age: 72 years old, range 33–84), p=0.086) who had undergone two-stage revision of an infected hip (9 cases) or knee (10 cases) prosthesis and whose spacers contained antibiotic-loaded cement (see supplementary table S1). The primary diagnosis which led to the initial implantation surgery was primary osteoarthritis in 13 cases, rheumatoid arthritis in 4 cases and both femoral neck fracture and high-grade osteosarcoma in 1 case. In addition, five patients had diabetes mellitus.

Periprosthetic infection was diagnosed using clinical, radiological and laboratory tests. Histopathological and microbiological analyses were performed in all cases for both the revision and reimplantation surgeries. Follow-up data were collected during control visits. All subjects gave informed consent to participate in the study.

Clinical and microbiological investigation

A synovial fluid aspiration was obtained preoperatively, and 4–6 tissue probes were obtained during the first revision surgery for each case for microbiological analysis. Similarly, 4–6 tissue probes from the reimplantation surgery were sent to the laboratory. Second-stage infection was diagnosed based on second-stage cultures, as either superinfection (new organism) or persistence of the original infection (previously isolated organism).

Tissue processing

The tissues analysed were synovial probes from the joint capsule obtained from both surgeries, fixed in buffered formalin immediately after excision and sent to the laboratory. Bone tissue or intraosseous fibrous membranes were not analysed.

STAINING METHODS

Archival paraffin blocks were cut in the vertical planes and stained simultaneously with H&E and chloracetate esterase stains.

Histopathological criteria

We considered periprosthetic infection in cases where five or more neutrophils were found in 10 HPF (magnification ×400). Cells located within the superficial fibrin exudate, or intravascularly, were not considered. Similarly, cell counts of eosinophils and lymphocytes per 1 HPF were recorded. The periprosthetic fibrous membranes were classified according to so-called consensus classification schema.[26] The same criteria were used for the evaluation of both biopsies (obtained during the revision and reimplantation surgeries) in each case.

Immunohistochemistry

Fresh cut sections from selected cases were stained with CD61 (platelet glycoprotein IIIa, clone Y2/51, Dako M0753, Glostrup, Denmark, dilution 1 : 50), myeloperoxidase (Dako, Glostrup, Denmark, A0398, dilution 1 : 50), glycophorin C (Dako, M0820, dilution 1 : 500) and Ki-67 (Dako, M7240, Glostrup, Denmark, dilution 1 : 400) antibodies.

Statistical evaluation

Descriptive statistics were performed to describe the median and range. As the patient's age and number of

blood transfusions deviated from a normal distribution, a non-parametric analytical method was used (Mann-Whitney U test). All statistical analyses were performed using SPSS V.18.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

First revision surgery

Synovial membranes were found to contain neutrophils in all specimens (median cell count: 170 cells/10 HPF, range 6–1750). Cells entrapped in superficial fibrin and adherent to endothelium or small veins were not encountered. Eosinophils were present in 13/19 biopsies (median cell count: 1 cell/HPF, range 0–8) and lymphocyte infiltration was apparent in 18/19 specimens (median cell count: 12 cells/HPF, range 0–107).

All patients had increased CRP values (median CRP: 5.90, range 0.70–20.20).

Perioperative blood loss at the time of revision surgery was treated with allogeneic blood transfusion in 17/19 operations (median number of intraoperative blood transfusions in men: 2, range 0–6; median number of intraoperative blood transfusion in women: 4, range 2–10; p=0.018).

Final or reimplantation surgery

The final surgery was performed after a median 43 days postimplantation of a cement-loaded dynamic spacer (range 34–141 days).

Microscopically, the synovial specimens (figure 1A) showed a characteristic layering structure, with superficial proliferating spindle-shaped and stellate fibroblasts (figure 1B), along with blood capillaries lined with fibrin exudate and fresh blood. Neutrophils (figure 1C) were present in 17/19 specimens (median cell count: 24 cells/10 HPF, range 0–420). Deeper tissues displayed more mature vessel walls; vascular density decreased with growing distance from the synovial surface. In the majority of cases, the neutrophil cell count was relatively low (<100/10 HPF). In 2/17 cases with a positive finding of neutrophils, the infiltrates were quite cellular (>200/10 HPF) and formed focal microabscesses, we therefore suspected an infection. Indeed, the microbiology was positive in one (knee joint; a 66-year-old female patient) of the two cases showing numerous neutrophils. Eosinophils (figure 1D) were present in 10/19 biopsies (median cell count: 1 cell/HPF, range 0–620). Their cell counts were low (<6/HPF) in most cases but surprisingly high in one case (620/HPF); therefore, a hypersensitivity reaction was suspected. The patient was subsequently tested with standard epicutaneous patch tests and showed a positive result for cobalt chloride. Perivascular and diffuse lymphocyte infiltration was apparent in all cases (median cell count: 29 cells/HPF, range 1–93). Soft tissue necrosis was not observed. Surprisingly, we observed focal accumulations of blasts (figure 1E) with hyperchromatic nuclei and a clear or pink ring of cytoplasm in the healing synovial membranes of 2/19 patients. Although we suspected extramedullary haematopoiesis, a unilineage proliferation of erythroid precursor cells was proven immunohistochemically, consistent with extramedullary erythropoiesis. Megakaryocytes and immature myeloid cells were not found. Most specimens contained macrophages with ingested cement particles (figure 1F) and foreign body-type cement granulomas (figure 1G) were found in few cases. All patients had increased CRP values (median CRP: 1.10, range 0.30–8.40).

Follow-up data after the two-stage revision arthroplasty

We investigated the clinical outcome of all patients for a median time of 28 months (range 7–40) postoperatively. We recorded two late periprosthetic infections in knee joints of two patients (a 74-year-old man and a 33-year-old man) at 21 and 25 months after the two-stage revision surgery, respectively. The latter patient, with osteosarcoma of the distal femur, experienced severe periprosthetic infection complicated with sepsis and his lower extremity had to be amputated. The last recorded postoperative complication was an aseptic loosening of the acetabular component in an 86-year-old female patient, which was managed with revision surgery 22 months after the two-stage revision surgery (the results are summarised in supplementary table S1).

DISCUSSION

Although two-stage exchange arthroplasty remains the preferred surgical treatment for periprosthetic joint infection, little is known about the synovial changes around the implanted antibiotic-loaded cement spacer. Because the final reimplantation surgery is usually performed 6–12 weeks after removal of the infected prosthesis, similar morphological changes can be expected within the healing synovium in all cases. Although most two-stage revisions of infected joints with prostheses are successful, some cases fail due to persistent infection. Nonetheless, recent studies were unable to identify variables that could guide the surgeon in identifying acceptable circumstances in which to perform the second-stage operation,[20 27] and the appropriate conditions under which to reimplant remain unclear. In the current study we analysed synovial tissues from 19 patients who underwent two-stage revision arthroplasty in order to characterise morphological features of the healing synovium, and to investigate the variables potentially associated with persistent infection of the joint at the time of the reimplantation surgery.

Synovial membrane pattern at the time of reimplantation surgery

We observed characteristic morphological changes in healing synovium, with superficial fibrin exudation, loose fibrosis and proliferation of blood vessels, consistent with maturing granulation tissue and deeper fibrosis.

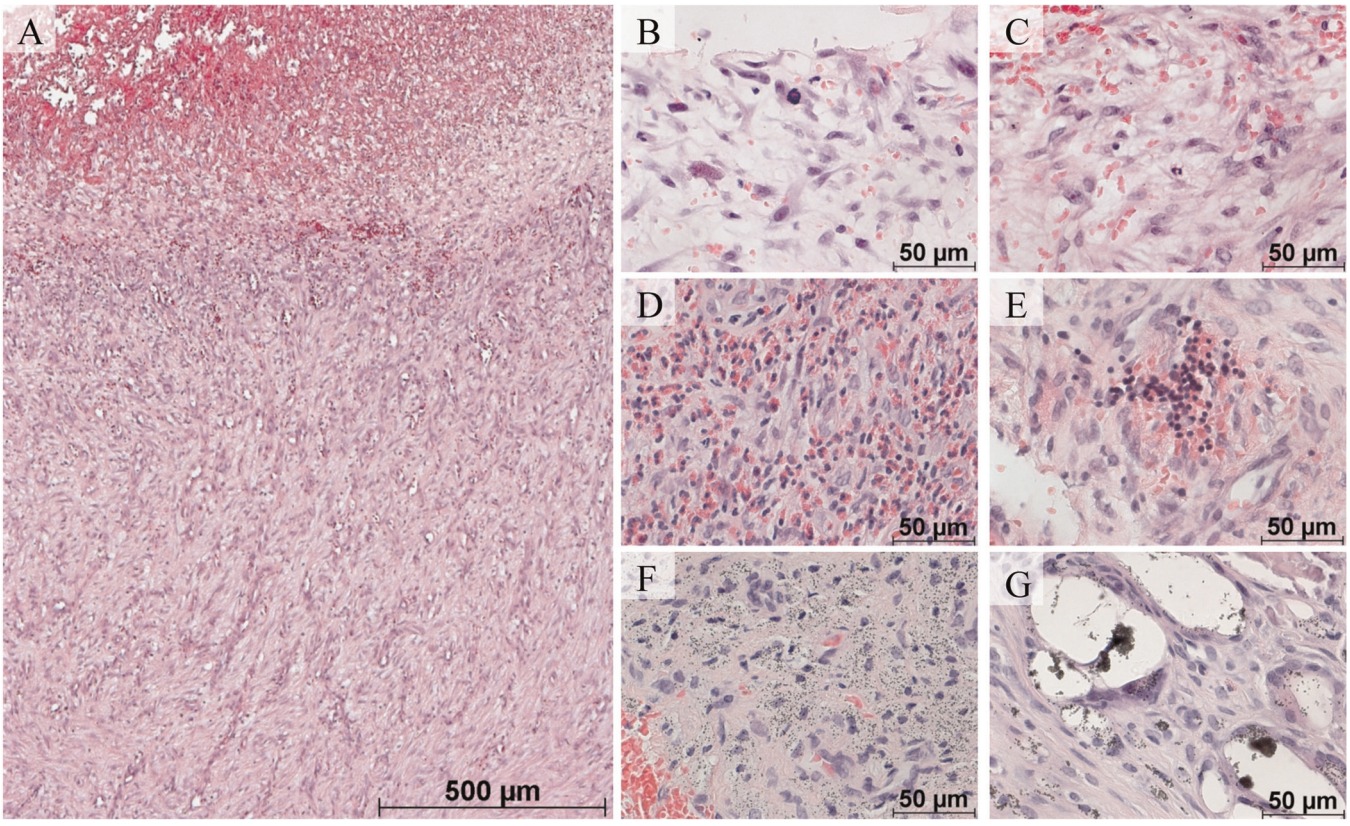

Figure 1 Synovial biopsy from the reimplantation surgery in two-stage revision arthroplasty. (A) The healing synovial membrane showed a characteristic layering structure, with superficial bleeding and fibrin exudation next to proliferating granulation tissue in the middle portions of the synovial membrane, and more mature granulation tissue at the base of the regenerating synovium. (B) Proliferating granulation tissue, with spindle-shaped and stellate fibroblasts and blood capillaries, was seen in the superficial layer of the synovial membrane. (C) Neutrophils were found in the majority of specimens; however, their cell counts varied substantially. (D) We observed dense infiltration of eosinophils in one case. (E) Foci of extramedullary erythropoiesis were present in regenerating synovium from two patients. (F) Tissue macrophages with ingested cement particles and (G) cement granulomas were found in some specimens.

Soft tissue necrosis or necrobiosis were not observed. Infiltration of neutrophils has traditionally been considered the most important histopathological sign of active periprosthetic infection; however, it was not clear as to whether this criterion was also applicable to the analysis of the persistent infection in the second reimplantation surgery in two-stage revision arthroplasty. Our data demonstrated the presence of low cell counts of neutrophils in the majority of specimens analysed, but in a few cases, the cell counts were considerably higher (>200 cells/10 HPF); this finding correlated with the microbiology cultivation results.

Eosinophils within the healing synovial membrane

The role of low numbers of eosinophils in the healing synovium remained unclear; however, our single case showing dense eosinophil infiltration was suggestive of a hypersensitivity reaction of the immediate type (type I according to the Coombs and Gell classification scheme[28]). Although the patient underwent epicutaneous tests several weeks following the two-stage surgery and tested positive for cobalt chromium, further studies would be necessary to explore the role of the eosinophils in the synovial membranes.

Lymphocyte infiltration within the healing synovial membrane

In the past, lymphocytic infiltration within periprosthetic synovium has been considered suggestive of a hypersensitivity reaction (delayed type; type IV according to the Coombs and Gell classification scheme[28]), especially in cases of failed metal-on-metal arthroplasties.[29–35] Other microscopic lesions that have been observed in cases with suggested metal hypersensitivity are intraosseous lymphocyte infiltrations,[29 32] necrotising granulomas[36–38] with macrophages, proliferative synovitis,[32 39] and changes at the bone-cement interface,[40] particularly hyperosteoidosis of the interface bone trabeculae.[29 41 42] Because lymphocytes can be seen in virtually all periprosthetic synovial biopsies, and also other bearing couples,[43–46] we used a conservative cut-off value of more than 300 lymphocytes/HPF in order for lymphocyte infiltrates to be considered suggestive of a hypersensitivity reaction in our previous studies.[29 32 44] As

specimens in the current study did not fulfil this criterion, a delayed hypersensitivity reaction was not indicated in any of the cases. Also, it seems unlikely that a time period of 6 weeks (from revision to reimplantation surgery) would be sufficient for the development of a delayed hypersensitivity reaction. Even though there is growing evidence for delayed hypersensitivity reactions in the failure of arthroplasties, little is currently known about the effectiveness/potency of sensitisation of the synovial membrane and its risk assessment.

Extramedullary erythropoiesis within the healing synovial membrane

The finding of focal extramedullary haematopoiesis in regenerating synovium of adult patients obtained during reimplantation surgery was one of the most surprising outcomes of the current study. In general, there are three circumstances which underlie abnormal extramedullary proliferation of normal haematopoietic elements:[47] (1) filtration, where immature cells are trapped by the spleen or other sites and proliferate; (2) inadequate marrow space to produce appropriate numbers of marrow elements or damage to the bone marrow microenvironment leading to increased numbers of circulating haematopoietic stem cells;[48] (3) abnormal cytokine or other circulating haematopoietic growth factors causing stem cells to differentiate into haematopoietic cells, or other local effects simulating the marrow microenvironment.[49] Even though tumefactive extramedullary haematopoiesis has been described, although exceedingly rarely, in the synovium of patients suffering from haematopoietic disorders,[50–52] microscopic extramedullary haematopoiesis in haematologically healthy adults has not been reported previously.

In our study, the biopsies were taken from healing synovial membranes following debridement of the infected tissues without direct contact with bone marrow; extramedullary haematopoiesis was apparent in the richly vascularised maturing granulation tissue in proximity to the synovial surface. On the basis of both conventional histology and immunohistochemistry, unilineage proliferation of erythroid cells was demonstrated. Although the patients with extramedullary erythropoiesis were a 60-year-old man and a 91-year-old woman, whose intraoperative blood losses were treated via blood transfusion (6×500 ml in both patients), the erythropoietic proliferation can possibly in general be explained by their large intraoperative blood losses. It must be mentioned that these patients did not suffer from any haematological disease; therefore, the finding of extramedullary haematopoiesis seems best explained by local changes in the richly vascularised regenerating synovial tissues simulating the marrow microenvironment. Indeed, extramedullary haematopoiesis has been reported in vascular lesions such as hemangioma[53] or pyogenic granuloma,[54] in healing but not early acute stages of myocardial infarcts[55] and in chronic subdural haematoma.[56–58]

CONCLUSION

To summarise, in the present study, we characterised morphological changes in healing synovial tissue during reimplantation surgery for periprosthetic infection in microbiologically sterile tissues. Substantially increased counts of synovial neutrophils, and the formation of microabscesses, seem to be indicative of persistent infection of the joint; therefore, prolonged antibiotic therapy should be considered in positive cases. Foci of extramedullary erythropoiesis were also detected in patients with higher intraoperative blood losses, which were treated with blood transfusions.

Contributors JZ and WR: conception and design of the study. AG, ON, LT and LG: data acquisition. AG, ON, LT, LG, WR and JZ: data analysis. JZ: drafting and revision of the manuscript. AG, ON, LT, LG and WR: revision and approval of the manuscript.

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