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Degenerative changes of the interface membrane as a possible reason for prosthesis loosening

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Summary. Objective: The aim of the present study was to perform a comparative evaluation of septic and aseptic interface membranes, assessing histological features, inflammatory infiltrate, and expression of inflammatory cytokines. Methods: Septic and aseptic interface membranes from 102 patients were examined by histology, histochemistry, and immunohistochemistry (tissue arrays). The cell subpopulations were characterized by quantification of CD3, CD4, CD8, CD20, and CD163 positive cells. Additionally, a semiquantitative evaluation of inflammatory cytokines (TNFα, TGF-$ß_1$, IL-1, IL-6, CRP, MMP-1, MMP-6) was performed to complete the analysis of inflammatory infiltrates. Results: The histological analysis revealed three different types of aseptic interface membranes: wear particle, degenerative, and mixed type. The expression of inflammatory molecules did not differ between septic and wear particle interface membranes. Significantly lower expression of cytokines, MMPs and CRP was observed, however, in degenerative interface membranes compared to other types. No expression of TNFα was observed in the degenerative interface membranes. Over 88% of patients with degenerative interface membranes had had a clinical record of osteoarthritis. Conclusion: Aseptic interface membranes were represented by wear particle, degenerative and mixed type. The expression of inflammatory factors in wear particle type is similar to this in septic membranes and can contribute to the bone destruction and prosthesis loosening. These factors seem not to play a major role in the degenerative membranes.

Key words: Interface membrane, Degeneration, Aseptic

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Introduction

The increasing number of arthroplasty (about 1.5 million implantations per year worldwide) and expanding indications for this surgical intervention have consequently led to a growing number of revisions (about 10% of patients) (Morawitz et al., 2003, 2006a,b). The results of revision surgery are not as good as the primary arthroplasty due to reasons such as increased polyethylene wear, aseptic loosening, misalignment of components, instability, extensive mechanical problems, infection, or stiffness (Riaz and Umar, 2006; Vince et al., 2006). Revision arthroplasty of the knee is a technically and economically demanding procedure and its successful performance requires careful preoperative planning, adherence to the principles of revision knee arthroplasty, availability of diverse implant options and an adequate bone graft (Parker et al., 2003). Because of the high complexity of this procedure, a complete understanding of the mechanisms involved in prosthesis loosening is essential for better surgical outcomes.

The interface membrane is a fibrous pseudo-capsule around prosthesis. According to available data this membrane plays a crucial role in the loosening of the prosthesis. There are several different pathological mechanisms which lead to the development of the interface membrane. Infection-associated inflammatory reaction, foreign body reaction (Sun et al., 2006) as well as combined infection- and wear particle-induced reaction have been discussed in the literature as possible causes for prosthesis loosening (Yokohama et al., 1995; Imai et al., 1998; Ren et al., 2006). Moravitz et al. (2006a,b) suggest a classification of the interface membrane into wear particle induced type, infectious type, and combined type with aspects of both. However, the classification of interface membranes into the septic and aseptic type is more appropriate for treatment and prognosis. The object of our interest in the present study was the aseptic type of the interface membrane. The key

role of the wear particle reaction accompanied by a release of inflammatory cytokines, such as tumor necrosis factor (TNFα), interleukins IL-6, IL-1, prostaglandin E (PGE) in this type of interface membrane was previously described (Li et al., 2000; Otto et al., 2006). Moreover, an increased release of free radicals in aseptic interface membrane has also been shown (Kinov et al., 2006).

Careful morphological analysis of the interface membranes in our patients revealed a high number of interface membranes with degenerative changes. This aspect has not been studied previously. We suspect an essential role of the degenerative processes in aseptic prosthesis loosening. To address this hypothesis, the present study aimed at analyzing the cell composition and expression of inflammatory factors in aseptic interface membranes with degenerative changes and wear particle reaction, and compare these findings to those of septic interface membranes.

Material and methods

Tissue samples

Material retrieved at prosthesis revision from 102 subsequent patients was collected between 2004 and 2006 from tissue bank of the Department for Orthopedic Pathology at the Institute of Pathology, University of Heidelberg, Germany.

The interface membranes were resected in total at revision surgery due to prosthesis loosening. The whole tissue was divided into smaller parts fitting into 2x3cm paraffin blocks and 5-8 blocks were prepared for each patient. All samples were fixed in formalin and embedded in paraffin according to routine procedure performed for tissue preparation.

Clinical data

The following clinical data were analyzed using a clinical questionnaire: age and sex of patients, affected joints, clinical diagnosis, the time between the primary arthroplasty and the revision surgery, clinical results of microbiological analysis (standard microbiological culturing). Based on histomorphological criteria, four types of periprosthetic membrane were defined: wear particle induced type (detection of foreign body particles; macrophages and multinucleated giant cells occupy at least 20% of the area; type I); infectious type (granulation tissue with neutrophilic granulocytes, plasma cells and few, if any, wear particles; type II); combined type (aspects of type I and type II occur simultaneously; type III); and indeterminate type (neither criteria for type I nor type II are fulfilled; type IV) (Morawietz et al. 2006a,b).

In the present study 102 unselected cases with prosthesis loosening were analyzed. Interface membranes were collected at revision arthroplasty. The affected joints were the knee in 57.8% (59 patients), the hip joint in 42.8% (42 patients), and ankle in 0.98% (1 patient).

In 82 patients the first prosthesis was replaced. Second revision was performed in 17 patients and third revision was made in three patients.

Females (58) were affected more often than males (44).

The mean age of the patients was 71 years, ranged from 59 to 82 years. The mean age of patients with first revision was 70 years, ranged from 60 to 82 years. The mean age of patients with re-revision was 68 years, ranged from 59 to 79 years. The mean age of patients with third revision was 72 years, ranged from 68 to 76 years.

At revision surgery, all patients showed radiographic osteolysis around the implants.

The time between primary arthroplasty and revision surgery was 6 years on average for patients with the first revision (ranged from 1 year to 14 years). The mean period between revision and re-revision surgery was 5 years (ranged from 8 months to 9 years).

Cement fixation was used in 33% of all cases and cement-less fixation was used in other cases. Cement fixation was used in 28% in Low grade infect, 37% in Distinct infect, 43% in Wear particle type, 27% in Degenerative type, and 29% in Mixed type.

Histology and histochemistry

All samples were primary investigated by conventional histology. Additional histochemical staining for presenting collagen and elastic fibrils were performed using respectivelly Masson & Goldner and Elastica van Gieson stainings. Histological diagnosis applied to inflammatory infiltration, foreign body reaction, fibrosis and degenerative changes of prosthetic membrane, such as hyalinosis, myxoid degeneration or chondroid metaplasia.

Tissue microarrays

Sections prepared from each paraffin block were stained with hematoxylin and eosin (HE). Representative regions of the interface membranes were selected acoording to the classification by Moravietz et al. (2006). Tissue cylinders (diameter 1.5 mm) were obtained from regions including inflammatory infiltrations, macrophages with wear particles, and giant cells, as well as degenative changes (myxoid degeneration, hyalinosis and chondroid metaplasia). The cylinders were arrayed into a recipient paraffin block using a tissue chip microarrayer (Beecher Instruments, Micro-Array-Technology). Subsequent 3 μm sections were cut from the recipient block and mounted on sialinised glass slides to support adhesion of the tissue samples. All the immunohistochemistry assessments were made on tissue arrays.

Immunohistochemistry

All samples were investigated by immunohistochemistry. The immunohistochemical markers were used to differentiate cell populations involved in inflammatory infiltrates (CD 68 for macrophages, CD3, CD4/CD8 for T-lymphocytes, CD20 for B lymphocytes,). Additionally, cell mediatrors involved in proliferative, degenerative processes and in inflammation: C-reactive protein (CRP), transforming growth factor ß$_1$ (TGF-ß$_1$), tumor necrosis factor α (TNFα), interleukin 1 (IL-1), matrix metalloproteinase (MMP) 1, and MMP-13 were also assessed. The antibodies used in the study and detection system are summarized in table 1.

For screening of CD68, CD20, CD3, CD8, and CD4 in the various samples immunostaining was processed automatically using an automated platform-Dako Autostainer.

Quantitative and semi-quantitative evaluation

Quantitative evaluation was performed independently by two pathologists. In 15 cases the results were the same. If the results differed the mean of he two results was used for analysis. The expression of investigated proteins was quantified using a semiquantitative scoring system from 0-12. The score is the product of the semi-quantitative score of the immunostaining (0=negative, 1= poor intensity, and 3=severe intensity) and the semiquantitative score for the percentage of the positive stained cells (0= negative, 1≤ 10%, 2= 10-50%, 3= 51-80%, and 4≤ 80%).

Statistical analysis

Data are presented as mean values ± SD or frequency. Differences between groups were analyzed by one-way ANOVA followed by Duncan's test, Student's t-test or log-linear analysis of frequency as appropriate. P value less than 0.05 was accepted as being statistically significant. All statistical analyses were performed using Statistica 6.0 software (StatSoft, Inc. Tulsa, USA).

Results

Histological findings

Conventional histological analysis revealed a wide spectrum of pathological processes. The infection of the periprosthatic membrane was confirmed by a bacterial culture. The most common bacterium found was *Staphylococcus epidermidis* (13 cases) and other bacteria present were *Enterococcus faecali* (3 cases), *Prop. Acnes* (5 cases), and *Baccilus megaterium* (2 cases). Morphological features of inflammatory reaction due to infection were found in 26.4% of cases (27/102 patients). In these cases, variably dense inflammatory

Table 1. Antibodies used for immunohistochemistry and detection system.

Marker	Antigen retrieval method	Primary antibody	Secondary antibody
TGF-ß1	none	Affinity purified Rabbit polyclonal (Santa Cruz Biotechnologies, Germany) 4 μg/ml in 2% normal goat serum (Dako, Germany)	SS Link goat anti-rabbit (BioGenex, USA)
CRP	20 min 0.1% trypsine in 0.1% CaCl at 37°C	Affinity purified Sheep polyclonal (Biotrend, Germany) 0.4 μg/ml in universal AB diluent (DCS, Germany)	Rabbit anti-sheep (Jackson ImmunoResearch, UK) 0.4 μg/ml in universal AB diluent (DCS, Germany)
IL-1	20 min boiling in 10 mM citrate buffer, pH 6.0	Affinity purified Rabbit polyclonal (Santa Cruz Biotechnologies, Germany) 2 μg/ml in 2% normal goat serum (Dako, Germany)	SS Link goat anti-rabbit (BioGenex, USA)
IL-6	20 min Target Unmasking Fluid (Invitrogen, Germany) at 37°C	Affinity purified Sheep polyclonal (Biotrend, Germany) 1 μg/ml in universal AB diluent (DCS, Germany).	Rabbit anti-sheep (Jackson ImmunoResearch, UK) 0.2 μg/ml in universal AB diluent (DCS, Germany)
MMP-1	20 min boiling in 10 mM citrate buffer, pH 6.0	Affinity purified Mouse monoclonal, (R&D System, Germany) 4 μg/ml in universal AB diluent (DCS, Germany)	SS Multilink (BioGenex, USA)
MMP-13	20 min boiling in 10 mM citrate buffer, pH 6.0	Affinity purified Mouse monoclonal, (R&D System, Germany) 4 μg/ml in universal AB diluent (DCS, Germany)	SS Multilink (BioGenex, USA)
TNF-α	20 min boiling in 10 mM citrate buffer, pH 6.0	Affinity purified Rabbit polyclonal (Genzyme, Germany) 4 μg/ml in 2% normal goat serum (Dako, Germany)	SS Link goat anti-rabbit (BioGenex, USA)
CD20	none	Affinity purified Mouse monoclonal 2 μg/ml in universal AB diluent (DCS, Germany)	Vectastasin Universal Quick Kit (Linaris, Germany)
CD8	none	Affinity purified Mouse monoclonal 20 μg/ml in universal AB diluent (DCS, Germany)	Vectastasin Universal Quick Kit (Linaris, Germany)
CD68	none	Affinity purified Mouse monoclonal 0.4 μg/ml in universal AB diluent (DCS, Germany)	Vectastasin Universal Quick Kit (Linaris, Germany)
CD4	none	Affinity purified Mouse monoclonal 5 μg/ml in universal AB diluent (DCS, Germany)	Vectastasin Universal Quick Kit (Linaris, Germany)
CD3	none	Affinity purified Mouse monoclonal 2 μg/ml in universal AB diluent (DCS, Germany)	Vectastasin Universal Quick Kit (Linaris, Germany)

infiltrate was found, composed of mononuclear lymphocytes and granulocytes were found. These infiltrates distinguish two forms of infect induced reaction: low grade infect and distinct infection. The morphological features of the low grade infection are characterized by lymphocytic infiltrations making up to 60% of the cell populations (CD8, CD4 and CD20 positive cells proven by immunohostochemistry) with several granulocytes. No fibrin exudations or necrotic changes could be found. The morphological signs of low grade infection were established in 88.8% of patients with inflammatory type of interface membranes (24/27) and in 23.5 % of all investigated patients (24/102).

The morphological features of a distinct infection include lymphocytic infiltrations with a high numbers of granulocytes accompanying by fibrin exudations and necrotic changes of the interface membrane (Fig.1f). Small bone inclusions within the prosthetic membrane point to early bone destruction. Only in three cases were histological findings of a distinct infection detected (11.1% of the patients with inflammaroty type of the interface membrane, 2.9% of all cases).

Wear particle reaction was found in most of the investigated cases (95 of 102 patients, 87.5%). However, it showed a different intensity with a wide spectrum of foreign body reaction, from spare accumulations of macrophages (Fig. 1e), to severe dense macrophage and giant cell infiltration of the prosthetic membrane. Low grade wear particle reaction (up to 20% of macrophages (CD68 positive cells) was found in 33.6 % of cases (32/95), a moderate wear particle reaction (21-50% of macrophages) was detected in 36.8% of cases (35/95), and severe wear particle reactions were found in 18.9% of cases (18/95).

Degenerative changes of the interface membrane were shown as myxoid degeneration, hyalinosis and focal chondroid metaplasia (Fig. 1a-d). A number of the tissue breaks within the degenerative areas have been seen. The degenerative changes were detected in 28.4% of cases (29/102.). In 13.6% of the investigated cases (14/102) degenerative processes were low distinguished and found as an accompanying finding in interface membrane of wear particle type. In 14.7% of cases (15 of 102 patients) degenerative processes were dominant.

Based on the conventional histological findings we classified following groups: interface membrane with infection-induced inflammatory reaction; interface membrane with predominant wear particle reaction; interface membrane with predominant degenerative changes and mixed type. According to this grouping we performed immunohistochemical analysis of an expression pattern of pro-inflammation factors.

Immunohistochemical analysis

Immunohistochemical analysis revealed essential differences between the defined types of the interface membranes regarding both cell composition and the expression of the inflammatory factors.

Interface membranes with an infection-induced inflammatory reaction showed significant higher number of lymphocytes ($p<0.001$) compared with other groups. The cytotoxic (CD8 positive) lymphocytes were highly represented in inflammatory infiltrations (up to 35% of cells involved in inflammation). B-lymphocytes were diffusely distributed within prosthetic membrane (2-18% of the investigated cell populations).

The expression of all investigated inflammatory factors (CRP, TNFα, TGF-$ß_1$, MMP-1, MMP-13, IL-1, IL-6) were also statistically significant higher in this group of interface membranes in comparison with degenerative type (Table 2).

Interface membranes with predominant wear particle reaction showed a predomination of macrophages (CD68 positive cells). These cells express the metallo-proteinases (MMP-1 and MMP-13), IL-1 and IL-6 and CRP (Figs. 2f, 3a,c). We did not find any significant differences between septic and wear particle interface membranes regarding expression level of all investigated inflammation factors (Table 2).

In contrast to this, the interface membrane with predominant degenerative changes showed low expression levels of CRP, MMP-1, MMP-13 and IL-1, IL-6 (Figs. 2a-e, 3b). No expression of TNFα was detected in this type of interface membrane (Fig. 3d).

An expression level of TGF-$ß_1$ was similar to other

Table 2. Data of comparative semiquantitative analysis of investigated pro-inflammation factors in different types of interface membranes (score 0-12).

Marker	Septic interface membranes		Aseptic interface membranes			ANOVA
	Low grade infect	Distinct infect	Wear particle type	Degenerative type	Mixed type	
TNFα	1.09±0.8	1.49±1.01	1.6±0.89	$0.00^{1,2,3}$	1.02±0.44	p<0.05
CRP	3.2±0.76	3.6±1.67	4.4±1.58	$0.87±0.4^{1,2,3}$	2.33±1.87	p<0.05
IL-6	3.2±1.78	3.9±1.44	2.8±1.09	$1.41±1.01^{1,2,3}$	2.02±1.83	p<0.05
TGF-ß1	3.11±1.03	3.6±1.09	6.8±1.09	2.0±1.41	4.34±1.58	NS
IL-1	3.9±0.96	5.6±1.78	3.2±1.66	$0.98±0.6^{1,2,3}$	3.88±2.40	p<0.05
MMP-1	2.4±0.89	3.07±1.09	3.2±1.78	$0.89±0.4^{1,2,3}$	1.09±0.8	p<0.05
MMP-13	2.68±1.21	3.10±1.67	3.8±1.66	$0.88±0.60^{1,2,3}$	2.4±1.53	p<0.05

1: p<0.05 vs. Low grade infection, 2: p<0.05 vs. Distinct infection, 3: p<0.05 vs. Wear particle type, 4: p<0.05 vs. Degenerative type.

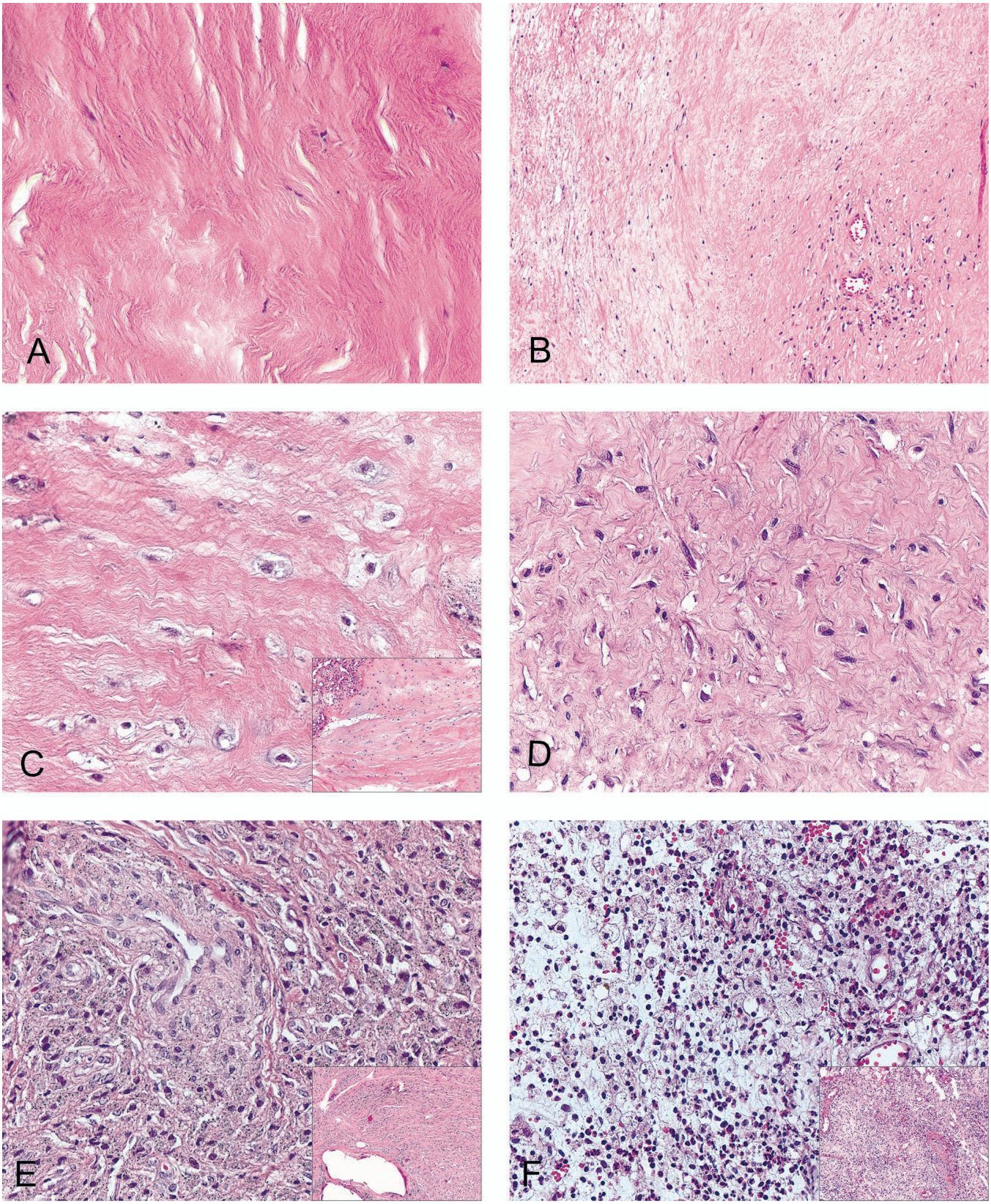

Fig. 1. A-D. Representative sections of the interface membranes with degenerative changes showing a hyalinosis (**A,B**) and chondroid metaplasia (**C,D**). A low number of fibroblasts and metaplastic chondrocyted are rarely distributed within degenerative areas. H&E staining. A, x 15.6; B, x 6.4; C, x 31.2 (insert x 15.6). **E.** Periprosthetic interface membrane of wear particle type with a high number of macrophages including wear particle. H&E staining. x 15.6 (insert x 31.2). **F.** Peroprosthetic interface membrane of infectious type showing diffuse lymphocytic inflammatory infiltrations. H&E staining. x 15.6 (insert x 31.2).

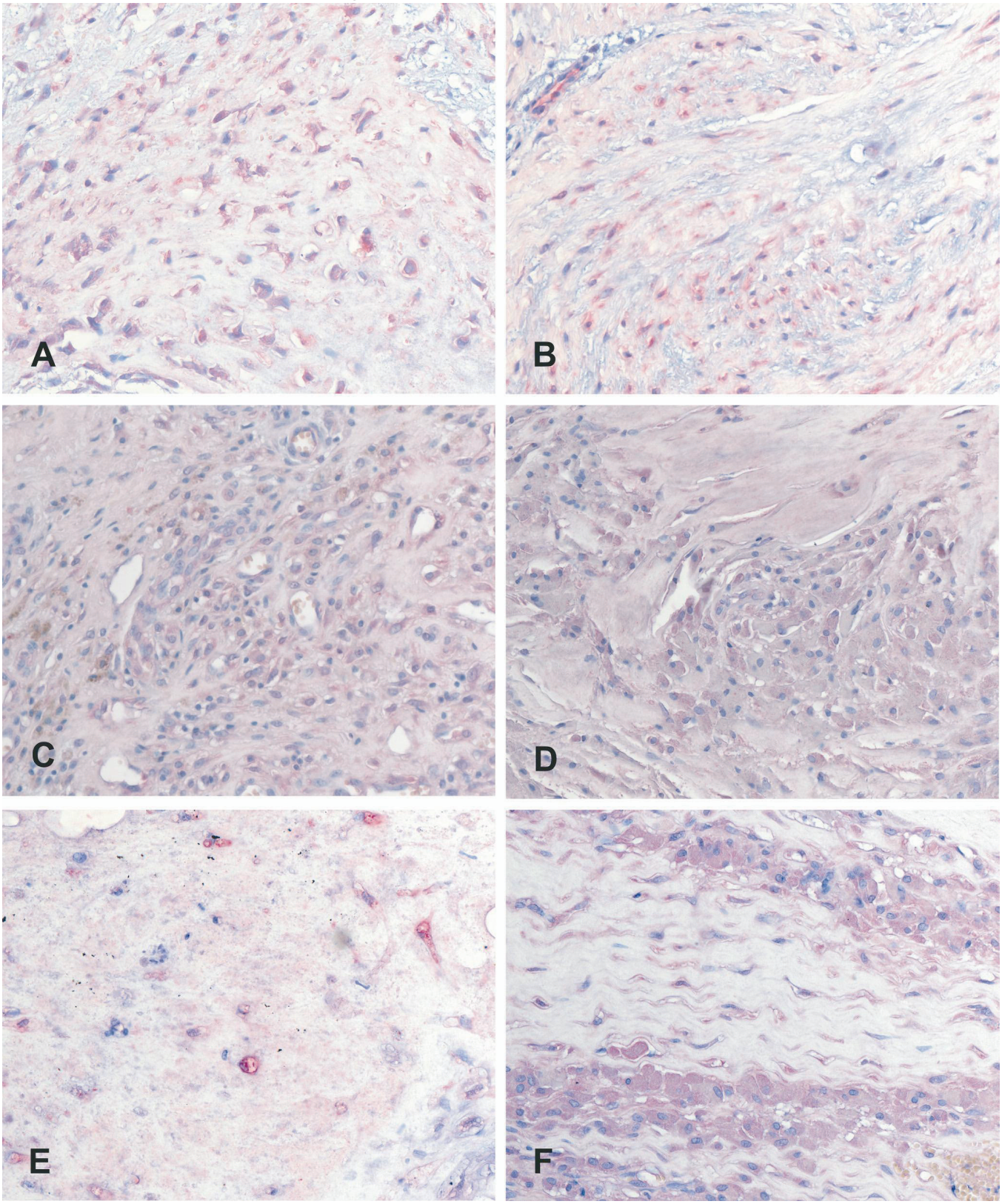

Fig. 2. A. Degenerative area with a hyalinosis and chondroid metaplasia. Metaplastic chondrocytes with a moderate cytoplasmic expression of TGFß. x 31.2. **B.** Cytoplasmic expression of IL-6 in fibroblasts of degenerative interface membranes. x 31.2. **C.** Interface membrane with low grade infection with expression of IL-6 in cytoplasm of macrophages. x 31.2. **D.** Wear particle type of the interface membrane with an low expression of IL-6 in cytoplasm of foreign body macrophages. x 31.2. **E.** Periprosthetic interface membrane with degenerative changes with a low to moderate cytoplasmic expression of MMP13 in some fibroblasts and metaplastic chondrocytes. x 31.2. **F.** Periprosthesic interface membrane of wear particle type with a diffuse moderate expression of MMP13 in cytoplasm of macrophages. x 31.2

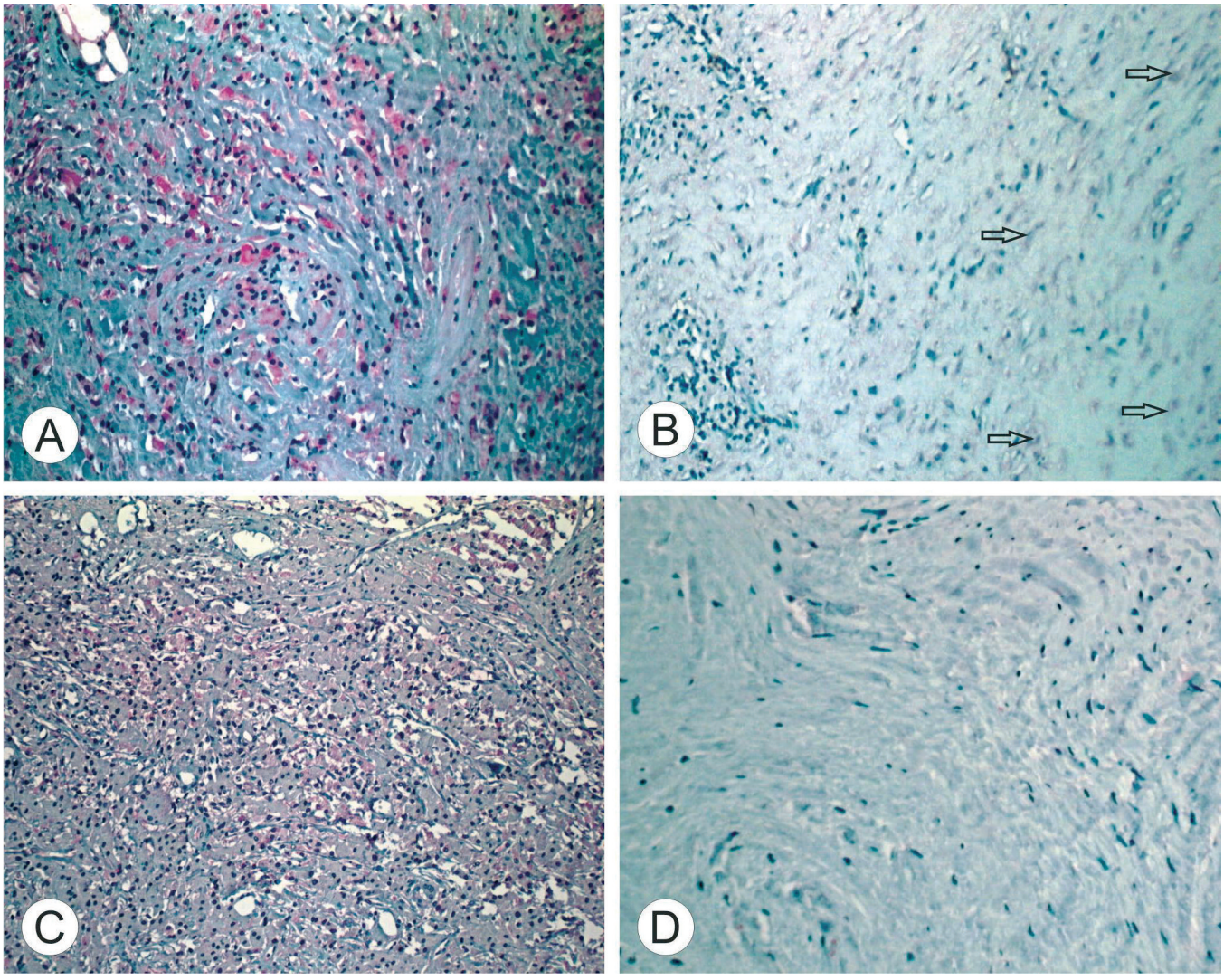

Fig. 3. A. High level of CRP expression in cytoplasm of macrophages in wear particle type of interface membrane. x 31.2. **B.** Very low expression of CRP in cytoplasm of metaplastic chondrocytes within degenerative interface membrane. x 33.8. **C.** Diffuse moderate cytoplasmic expression of TNF_ in macrophages of the wear particle type of interface membrane. x 15,6. **D.** No expression of TNFα in degenerative area of interface membrane. x 33,8

types of interface membranes (Table 2).

Correlation of clinical data and histological findings

A comparative analysis of the clinical data and histological findings revealed no significant correlation between age, sex of the patients, implant survival or localization of endoprosthesis and histological type of interface membranes. The mean age of the patients with inflammatory type of interface membrane was 71±7 years. The mean age of the patients with significant wear particle reaction was 68±10 years. The mean age of patients with degenerative changes of the interface membrane was 72±6 years. The mean age of patients with a combined wear particle and degenerative reaction was 73±8 years.

Moreover, in 88.8 % of patients with degenerative interface membranes (24 of 27 patients) a severe destruction of the joint by osteoarthritis was the indication for arthroplasty.

Discussion

The present study aimed at analyzing the morphological characteristics of aseptic interface membranes compared to septic ones. The results of this study revealed essential differences between investigated groups regarding the cell composition of inflammatory

processes and the expression level of the inflammatory mediators.

In 26.4% of the investigated patients infectious-inflammatory type of the interface membrane was observed. A low grade infection was found in a predominant (over 88%) number of these patients. In agreement with previous publications we found a high expression level of the inflammatory factors in this type of the interface membrane. Even low grade infection induced increased abundance of the inflammatory factors IL-1, IL-6, MMPs and TNF-α.

Wear particle reaction was found in most cases. In spite of cement-free arthroplasty and modern arthroplasty technology, a phagocytic reaction seems to play an important role in the interface membranes. In this group of patients we found a similar pattern of the investigated cytokines compared to the group with septic interface membranes. The differences in cell populations involved in these two types of interface membrane must be taken into account. The septic interface membranes were rich in lymphocytes and neutrophils, while in aseptic ones macrophages were predominant. It is possible that further investigations might reveal differences in other molecules between these cell populations. The expression of MMPs was maximal in the wear particle type of interface membranes. In view of the destructive effect of metalloproteinases (Takagi et al., 1994; Takei et al., 1999, 2000) we suspected an essential impact of MMPs in prosthesis loosening in low grade infection, as well as in the wear particle type interface membranes. An interesting finding of our study is a similar expression level of all investigated inflammatory factors in septic and in aseptic interface membranes. From this we conclude that in spite of different stimuli leading to inflammation the pathological mechanisms of the progression of inflammation seem to be similar in both, septic and aseptic interface membranes of wear particle type.

Another interesting finding in our study was an evidence of degenerative processes in interface membranes. Nearly one third of patients revealed degenerative changes of interface membranes. In a group of 15 patients (14.7%) the degenerative processes were a superficial histological feature. Neither significant wear particle reaction nor morphological signs of infection-induced inflammatory processes were found in this group. Degenerative changes were represented by myxoid degeneration of the connective tissue or hyalinosis with focal chondroid metaplasia. These processes lower the stability of the connective tissue and are able to induce rupture of collagen bundles. This phenomenon is well known as a reason of tendon ruptures (Sharma et al., 2006; Riley, 2005). Based on our results, we conclude that degenerative processes can lead to the destruction of the connective tissue of the periprosthetic membrane. Our hypothesis is supported by the results of the immunohistochemical analysis. The investigated inflammatory factors were negative (TNF) or weaky expressed (CRP, MMPs, IL-1, IL-6, TGFß) in this group. We found few inflammatory cells (macrophages and lymphocytes) in this kind of interface membrane. Fibroblasts and metaplastic chondrocytes were observed as a predominant cell population. The pro-inflammatory mechanisms seem not to play a key role in this kind of interface membrane. This aspect separates the degenerative interface membranes from each other type of interface membrane. More than 14% of the patients in our study developed prosthesis loosening without any signs of inflammation. From a pathogenic view, the conventional therapeutic tactic based on anti-inflammation treatment (Syggelos et al., 2006) cannot be successful in this group of patients.

Conclusion:

Our study revealed a significant part of patients with distinct degenerative changes of interface membranes, which were characterised by myxoid degeneration, hyalinosis, and chondroid metaplasia. In contrast to wear particle type, the immunohistochemical analysis did not show any relevant expression of pro-inflammatory cytokines in this type of aseptic interface membrane. Over 80% of patients with degenerative changes of the interface membranes had a previous clinical record of osteoarthritis.

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