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Interface membrane is the best sample for histological study to diagnose prosthetic joint infection

Guillem Bori[1], Ernesto Muñoz-Mahamud[1], Sebastián Garcia[1], Carme Mallofre[2], Xavier Gallart[1], Jordi Bosch[3], Ester Garcia[1], Josep Riba[1], Josep Mensa[4] and Alex Soriano[4]

[1]*Department of Orthopaedic and Traumatology, Hospital Clínic, University of Barcelona, Barcelona, Spain;* [2]*Department of Pathology, Hospital Clínic, University of Barcelona, Barcelona, Spain;* [3]*Department of Microbiology, Hospital Clínic, University of Barcelona, Barcelona, Spain and* [4]*Department of Infectious Diseases, Hospital Clínic, University of Barcelona, Barcelona, Spain*

The objective of our study was to study which is the most accurate specimen for histological diagnosis of prosthetic joint infections (pseudocapsule or interface membrane). This is a prospective study including hip revision arthroplasties performed from January 2007 to June 2009. Specimens from pseudocapsule and from interface membrane were obtained from each patient. The histology was considered positive for infection when $\geq$5 neutrophils per high-power field ($\times$40) were found. Definitive diagnosis of infection was considered when $\geq$2 cultures were positive for the same microorganism. According to the definition of infection, patients were classified in two groups: (A) patients with aseptic loosening in whom cultures obtained during surgery were negative and (B) patients with prosthetic joint infection. A total of 69 revisions were included in the study; 57 were classified in group A and 12 in group B. In group B, the percentage of positive interface membrane histology was significantly higher than the percentage of positive pseudocapsule histology (83 *vs* 42%, $P = 0.04$, Fisher's exact test). The results suggest that periprosthetic interface membrane is the best specimen for the histological diagnosis of prosthetic joint infection.
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Infection after total joint arthroplasty is a challenging problem.[1] Microorganisms colonizing the implant surface are associated with prosthesis loosening. Cultures and polymorphonuclear leukocytes count from periprosthetic tissue are the gold standard for the diagnosis of prosthetic joint infections.[2] The specificity and sensitivity of histology has never been 100%[3–21] and this may be for different reasons.[5,12,22,23] False-positive results of histology could be because culture and histological specimens are taken from different areas.[5,12,22] False-negative results could be attributed to: (1) low virulent microorganisms like *Staphylococcus epidermidis*[6,7,22,24,25] or *Propionibacterium* spp[26,27] that do not stimulate neutrophile infiltration, (2) bacteriological contamination of the specimen obtained for culture[12] or (3) the cut-off point (number of neutrophils per field) to establish the diagnosis of infection.[2,4,5,10,13,28–30] Another possibility for inconsistencies in the histological results could be the type of specimen submitted to the laboratory. The majority of investigators obtain specimens from pseudocapsule, synovial surface, interface membrane and any tissue area suspected of infection.[6–8,12,15,16,22] The objective of our study was to study which is the most accurate specimen for histological diagnosis of prosthetic joint infection.

Materials and methods

Prospective study including hip revision arthroplasties performed in our hospital from January 2007 to June 2009.

Correspondence: Dr G Bori, PhD, Department of Orthopedic and Traumatology, Hospital Clínic. University of Barcelona, C/Villarroel 170 Escalera 12 Piso 4, 08036 Barcelona (Catalunya), Spain.
E-mail: gbori@clinic.ub.es
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Histological Protocol

Specimens from pseudocapsule and interface membrane were obtained for each patient. The pseudocapsule specimens studied were obtained from the part in contact with the neck of the prosthesis. The surface of the pseudocapsule that faces the joint cavity was identified and the sections were taken perpendicular to it. These surfaces were histologically identified in each specimen examined. Interface membranes from the femoral stem and from the acetabular cup were taken. The specimens for paraffin histology sections were formalin-fixed and paraffin-embedded before hematoxylin-eosin staining. The pathologists of our hospital used the Mirras' criteria (adapted by Feldman).[2,13] The histology was considered positive for infection when ≥ 5 neutrophils per high-power field ($\times 40$) in at least five separate microscopic fields were found. The study was performed in paraffin sections and not in frozen sections avoiding histological technical bias.

Microbiology

The specimens for the microbiological study were always taken before the administration of antibiotic prophylaxis. At the time of implant removal, at least five periprosthetic specimens from different sites were submitted to the laboratory for culture. Liquid samples were aspirated from the operative site using a sterile syringe and immediately inoculated into blood culture flasks (Bactec 9400, Becton Dickinson Diagnostic Instruments, Sparks, MD, USA) and incubated for 5 days. Positive flasks were subcultured in aerobic and anaerobic agar media. Swab cultures were obtained by rubbing a sterile swab (Deltalab, invasive sterile eurotube collection swab with Stuart transport medium, Rubí, Catalonia, Spain) over the tissue area, bone or fluid suspected of infection. Solid tissue samples from pseudocapsule, periprosthetic membranes or tissue suspected to be infected were immediately placed into a separate sterile universal bottle. Solid tissue and swabs were cultured in aerobic and anaerobic agar media and in thioglicolate broth enriched with vitamin K and hemin and incubated for 10 days. Positive cultures were sent for identification and sensitivity testing.

Patient Classification

Preoperative diagnosis of aseptic loosening was made when the patient had pain, erythrocyte sedimentation rate <30 mm/h and a serum concentration of C-reactive protein <1.3 mg per 100 ml, radiological signs of loosening, Technetium^{99m} methylene diphosphonate scintigraphy and the Tecnetium^{99m} hexamethylpropylene-amineoxine-labelled leukocytes scintigraphy were negative for infection. In these patients revision was performed using one stage exchange.

Preoperative diagnosis of septic loosening was made when the patient had pain in the hip and/or fistula, erythrocyte sedimentation rate >30 mm/h and serum concentration of C-reactive protein >1.3 mg per 100 ml, radiological signs of loosening, Technetium^{99m} methylene diphosphonate scintigraphy and the Tecnetium^{99m} hexamethylpropylene-amineoxine-labelled leukocytes scintigraphy were positive for infection and when a culture of synovial fluid obtained by joint aspiration was positive. In these patients, revision was performed using a two stage exchange.

Definitive diagnosis of infection was considered when ≥ 2 intraoperative cultures were positive for the same microorganism or when there was pus surrounding the prosthesis.[31] Patients with ≤ 1 intraoperative positive culture were classified as non-infected.

Patients included in the study were classified in two groups:

Group A: patients submitted to hip revision arthroplasty because of a preoperative diagnosis of an aseptic loosening in whom the definitive diagnosis was as non-infected.

Group B: patients submitted to hip revision arthroplasty because of a preoperative diagnosis of a septic loosening in whom the definitive diagnosis was confirmed as infection.

Patients who underwent hip revision arthroplasty because of periprosthetic fracture[4,12,15] and patients with a preoperative diagnosis of aseptic loosening and a definitive diagnosis of infection were excluded for the study.[6]

Statistical Analysis

The specificity (true negatives/false positives + true negatives), sensitivity (true positives/false negatives + true positives), positive predictive value (true positives/true positives + false positives) and negative predictive value (true negatives/true negatives + false negatives) of Mirras' criteria were evaluated. For comparison of proportions, a Fisher's exact test was applied and the differences were considered significant when $P<0.05$.

Results

During the study period, a total of 69 revisions hip arthroplasties were included; 57 were classified in group A and 12 in group B. The mean age was 65 years (range 45–85 years), 34 were women and 35 were men. The results of interface membrane, pseudocapsule histology and microbiology from group A and B are shown in Table 1.

The sensitivity, specificity, positive and negative predictive value of interface membrane histology were 83, 98, 91 and 96%, respectively (Table 2) and

Table 1 Patients with preoperative diagnosis of aseptic loosening and definitive diagnosis of non-infected prosthesis (group A) and patients with preoperative diagnosis of septic loosening and definitive diagnosis of infected prosthesis (group B)

Interface membrane histology[a]	*Pseudocapsule histology*[a]	*Culture*	*NP*	*Microorganism*[b]	*Li*	*So*	*Sw*
Group A							
Negative	Negative	–	55	—	—	—	—
Negative	Positive	–	1	—	—	—	—
Positive	Negative	–	1	—	—	—	—
Group B							
Positive	Positive	+	1	*Staphylococcus aureus*	1/2	1/2	1/2
Positive	Positive	+	1	*Pseudomonas aeruginosa*	2/2	0/2	0/2
Positive	Positive	+	1	*Klebsiella pneumoniae*	2/2	2/2	2/2
Positive	Positive	+	1	*Pseudomonas aeruginosa*/CNS	2/2	2/2	2/2
Positive	Positive	+	1	CNS	2/2	2/2	2/2
Positive	Negative	+	1	CNS	2/2	1/2	0/2
Positive	Negative	+	1	CNS	1/2	3/3	0/2
Positive	Negative	+	1	*Escherichia coli*	1/1	1/2	2/2
Positive	Negative	+	1	CNS	1/2	2/4	1/3
Positive	Negative	+	1	CNS	2/2	0/2	0/2
Negative	Negative	+	1	CNS	1/2	1/2	0/2
Negative	Negative	+	1	*Staphylococcus aureus*	2/2	2/2	0/2

Abbreviations: CNS, coagulase-negative staphylococci; NP, number of patients; Li, liquid samples; So, solid samples; Sw, swab samples.
[a]The result was considered positive for infection if there were more than five PMN per high-power field (×40) in at least five separate microscopic fields.
[b]Number of positive samples/number of samples taken for culture.

Table 2 Interface membrane histology and culture results in the 69 patients

Interface membrane histology[a]	*Definitive diagnosis (culture)*[b]		*Total*
	Positive	*Negative*	
Positive	10	1	11
Negative	2	56	58
Total	12	57	

[a]The result was considered positive for infection if there were more than 5 PMN per high-power field (×40) in at least five separate microscopic fields in paraffin histology sections.
[b]Definitive diagnosis was considered positive for infection when ≥2 intraoperative cultures were positive for the same microorganism or the presence of pus around the prosthesis.

Table 3 Pseudocapsule histology and culture results in the 69 patients

Pseudocapsule histology[a]	*Definitive diagnosis (culture)*[b]		*Total*
	Positive	*Negative*	
Positive	5	1	6
Negative	7	56	63
Total	12	57	

[a]The result was considered positive for infection if there were more than 5 PMN per high-power field (×40) in at least five separate microscopic fields in paraffin histology sections.
[b]Definitive diagnosis was considered positive for infection when ≥2 intraoperative cultures were positive for the same microorganism or the presence of pus around the prosthesis.

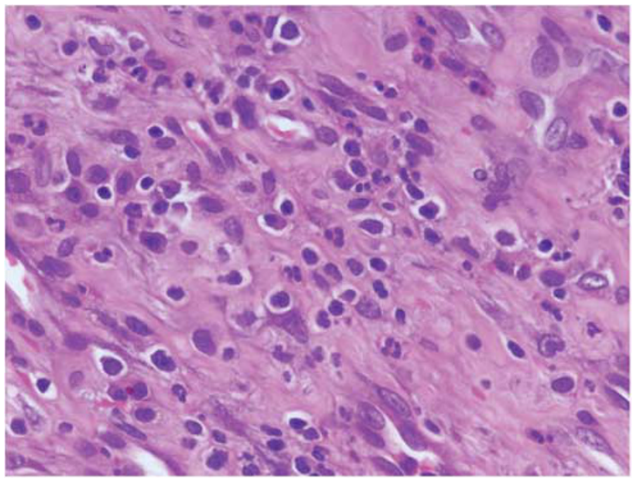

Figure 1 This photomicrograph is a paraffin section from periprosthetic interface membrane using hematoxylin-eosin in the first-stage arthroplasty. There are more than five neutrophils out of the vessels per high-power field (×40). It is a positive criteria for infection, using Feldman's criteria.

of pseudocapsule histology were 42, 98, 83 and 83%, respectively (Table 3). In group B, the percentage of positive interface membrane histology (Figure 1) was significantly higher than the percentage of positive pseudocapsule histology (83 *vs* 42%, $P=0.04$, Fisher's exact test).

The types of specimens used for the histological study in previous articles that evaluate the usefulness of histology in prosthetic loosening are summarized in Table 4.

Table 4 Summary of the main articles and type of specimens used for the histological study

Article	*Specimen*
Mirra *et al*[13,14]	Synovial and capsular tissues
Fehring *et al*[9]	Joint pseudocapsule, interface membrane and any area that appeared suspicious for possible infection[a]
Feldman *et al*[2]	Joint pseudocapsule and interface membrane
Athanasou *et al*[4]	Joint pseudocapsule and interface membrane
Lonner *et al*[12]	Joint pseudocapsule, interface membrane and any area that appeared suspicious for possible infection
Pace *et al*[17]	Joint pseudocapsule and interface membrane
Abdul-Karim *et al*[3]	Interface membrane (aseptic suspicion). Interface membrane, synovial tissue and unusually discolored tissue (septic suspicion)
Spangehl *et al*[19]	Synovial surface
Pandey *et al*[28,35]	Joint pseudocapsule and interface membrane
Pons *et al*[18]	Synovial surface
Della Valle *et al*[8]	Joint pseudocapsule, granulation tissue and any area that appeared suspicious for possible infection
Banit *et al*[5]	Joint pseudocapsule and any area that appeared suspicious for possible infection
Musso *et al*[15]	Joint pseudocapsule, interface membrane and any area that appeared suspicious for possible infection
Ko *et al*[11]	Joint pseudocapsule, interface membrane and any area that appeared suspicious for possible infection[a]
Wong *et al*[20]	Synovial surface, joint pseudocapsule and interface membrane
Francés *et al*[10]	Periprosthetic soft tissue
Bori *et al*[6,7,22]	Joint pseudocapsule, interface membrane and any area that appeared suspicious for possible infection
Morawietz *et al*[29,30]	Interface membrane
Nuñez *et al*[16]	Joint pseudocapsule, interface membrane and any area that appeared suspicious for possible infection
Nilsdotter *et al*[36]	Synovial surface and interface membrane
Della Valle *et al*[34]	Synovial surface
Kanner *et al*[21]	Periprosthetic soft tissue
Müller *et al*[37,38]	Interface membrane
Schinsky *et al*[33]	Synovial surface
Tohtz *et al*[32]	Interface membrane

[a]Include synovial proliferation, unusually pigmented tissue and areas of bone erosion.

Discussion

Histology has been considered one of the gold standards for the diagnosis of prosthetic joint infection,[2,4,18,29] however, a low sensitivity has been observed by several authors.[3,6–9,11,15,21] These inconsistencies could be attributed to the type of patients included in each study,[6–8] the microbiological[19,29,32] or histological criteria applied for the diagnosis of infection[2,4,5,28–30] or the different specimens (interface membrane or pseudocapsule) submitted for the analysis.[4] Reviewing the literature (Table 4), there is a lot of variability in the specimen submitted for histological evaluation. Some investigators did not obtain interface membrane[5,18,33,34] and others did not define the type of specimen analyzed.[10,13,21] It is generally accepted that there is no important differences between tissue specimens.[28,29,35] To our knowledge, this is the first study comparing the results of the histology in two different specimens (interface membrane and pseudocapsule). The interface membrane had a higher sensitivity and predictive values than pseudocapsule. In fact, the proportion of infected patients with positive interface membrane was significantly higher than those with positive pseudocapsule (83 *vs* 42%, $P=0.04$). Using only pseudocapsule, 7 out of 12 infected patients would not have been correctly diagnosed. Previously, Athanasou *et al*[4] suggested that more florid inflammation was generally found in femoral interface membrane than in joint pseudocapsule, however, this information was not quantified. A possible reasons for our results could be the presence of fibrosis (Figure 2) in pseudocapsule that makes difficult the neutrophil infiltration[7] and the fact that the major bacterial biofilm is found between implant and bone.

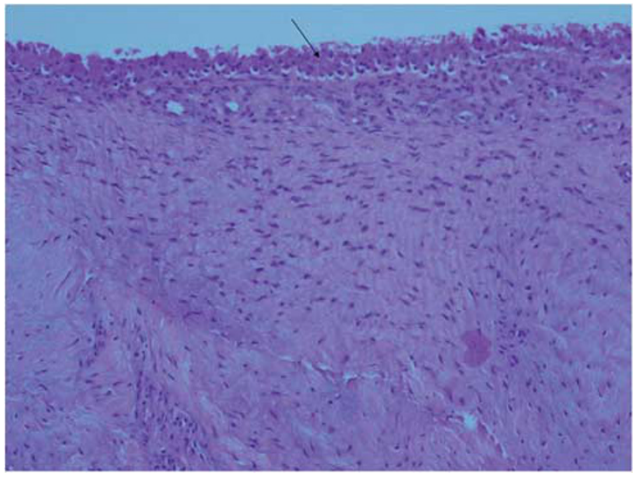

Figure 2 This photomicrograph is a paraffin section from pseudocapsule without neutrophil infiltration from the same patient showed in Figure 1. Below the synovial surface (head of black arrow), a dense fibrous tissue with mature collagen fibers and ordered fibroblasts is shown.

Recently, there are a group of investigators[29,30,32,37,38] that have used only membranes (not

pseudocapsule) and have proposed a histopathological consensus classification for a standardized evaluation of the periprosthetic tissues. Our results support the selection of the interface membrane as a reference tissue for histological classification.

Although frozen section gives surgeons intraoperative information about the diagnosis, our study was performed on paraffin sections to avoid histological technical bias. It has been described that frozen sections have inferior quality than paraffin ones.[10,19,32] For instance, Tohtz *et al*[32] described a 19% of discrepancies (in 14 out of 64 cases) between frozen section and paraffin sections.[32]

The main drawback of our study was the low number of infected patients included. However, this study had enough statistical power to reveal differences between interface membrane and pseudocapsule specimens. Patients who underwent hip revision arthroplasty because of periprosthetic fracture were not included since it is a cause of false-positive histology results.[4,12,15] Patients with a preoperative diagnosis of aseptic loosening and a definitive diagnosis of infection were also not included since in our experience,[6] the sensitivity of the histology (using Mirras' criteria) in this group of patients is low.

In conclusion, our results suggest that the best specimen of periprosthetic soft tissue for histological diagnosis of the infection in a total hip revision arthroplasty is the periprosthetic interface membrane.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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