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Original article

The role of intraoperative frozen section in arthroplasty revision surgery: our experience

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Summary. *Background and aim of the work:* Due to the increasing the number of hip and knee replacement in the future will be increasing the number of cases of prosthetic revision. Our aim is to test the validity of extemporaneous exam for differentiation between septic and aseptic loosening of prosthetic. *Methods:* 159 patients underwent surgery for the prosthesis revision from 2008 to 2014 An intraoperative histological examination was performed during all the surgeries and multiple samples were taken for the conclusive histological examination and culture. *Results:* Sensitivity of the intraoperative histological examination resulted 38,3% (IC 0,26; 0,51); specificity 82,5% (IC 0,73; 0,90), where positive predictive value was 57,5% (IC 0,41; 0,73) and negative predictive value 68,4% (IC 0,59; 0,76). *Conclusions:* In the absence of a universally accepted method to diagnose infection in patients with mobilization of the prosthesis, intraoperative histological examination is, in spite of everything, a method easy to perform and reproduce, it shows high specificity and sensitivity in the presence of highly virulent pathogens. (www.actabiomedica.it)

Key words: periprosthetic infection, hip replacement, knee replacement, histological examination, GRAM, infection, prosthesis revision

Introduction

Hip and Knee replacement is nowadays the most successful operation in orthopaedic surgery, especially as far as the improvement of pain and the restoration of functional integrity in cases of degenerative arthropathy are concerned. Nonetheless, within 10 years from the replacement of the joint, 5 to 12% of implantations become painful, dysfunctional or display symptoms of chronic inflammation (2), reflecting a mobilization of the prosthesis. Basically, two clinical pictures may occur: an "aseptic" mobilization and a "septic" mobilization (3), which entail different clinical-surgical approaches. In the former case, surgery consists in removing the old prosthesis and replacing it with a new one; in the latter, a more complex and difficult path lies ahead of the patient, involving prolonged antibacterial therapies and the replacement of the prosthesis in two-time surgery. This lets us understand how the diagnosis of infection becomes an extremely important element in order to outline a correct operative strategy for prosthesis revision surgeries.(1)

Clinical manifestations and different tests - as for instance C reactive protein levels (17), erythrocyte sedimentation rate (17), radiological techniques, biochemical analyses, culture and synovial fluid leukocyte count (4, 5, 16) – can be useful in preoperative diagnosis. In several cases, in particular during chronic or late infections, a correct definition of the ongoing pathological process could turn out to be difficult. All these methods, although useful and necessary for an appropriate definition of the overall preoperative clinical picture, can result limited owing to factors like their low level of accuracy and specificity, which can lead to

an ambiguous outcome. This has resulted in searching for new possibilities in diagnosis, aiming at a higher definition of prosthesis infections.

Only two intraoperative tests - Gram staining and intraoperative histological examination - provide immediate information about the etiology of the mobilization of the prosthesis (4). According to the related literature, the first method seems to have a controversial role (6), especially because of its low sensitivity and consequently poor reliability (5).

Intraoperative histological examination is more precise in defining whether the mobilization of the prosthesis is septic or aseptic. The number of polymorphonuclear leukocytes in a 40X magnification (High-Power Field magnification - HPF) is a crucial parameter in histopathological diagnosis . (7)

Several studies have assessed the effectiveness of this method in intraoperative diagnosis, using different numbers of polymorphonuclear leukocytes as criterion of infection, more often 10 per HPF (1, 13, 14), but also lowering the limit to 5 or less polymorphonuclear leukocytes (10-12), and the outcome shows acceptable sensitivity and specificity levels (1, 8-14).

The prevailing opinion among the Authors is that intraoperative histological examination is useful to diagnose the infection, thanks to its high specificity, but that it has nonetheless a low sensitivity, so that resorting to clinical and instrumental evaluations as well (1, 8, 10, 11, 14) is necessary in order to establish the correct surgical approach.

This study aims at examining the hypothesis that the presence of at least 5 polymorphonuclear neutrophils per field (40X) in the samples sent for intraoperative examination is a discriminating factor between septic and aseptic failure of a prosthesis. The study also aims at correlating the number of polymorphonuclear leukocytes with the results of the definitive histological examination and culture, the indexes of inflammation and the preoperative nuclear medicine investigations.

Materials and methods

From December 2008 to March 2014 159 patients underwent surgery for the revision of hip, knee and shoulder prosthesis; precisely, 38 patients underwent a total hip revision, 62 total knee revision, 7 unicompartimental knee revision and 5 shoulder revision. 55,9% of patients were female and 44,1% male, the average age was 74,7 years (range 50-92).

The diagnosis of periprosthesic infection was established by clinical presentation (pain, fever, presence of fistulae), hematochemical findings, level of inflammation (ESR > 10 mm/h and PCR > 5 mg/mL) and X-ray investigations (standard Rx, bone scintigraphy).

An intraoperative histological examination was performed during all the surgeries and multiple samples were taken for the conclusive histological examination and culture.

Patients suffering from rheumatologic diseases, as for instance rheumatoid arthritis, and patients which hadn't undergone all the intraoperative examinations (intraoperative, histological, definitive and culture) were excluded from the study in order to prevent false positives in the intraoperative histological examination.

Intraoperative histological examinations were carried out and multiple blood samples were taken during each single surgery, for the definitive histological diagnosis and culture.

For every patent the following parameters were recorded:

- ESR and preoperative CRP
- Total-body scintigraphy
- Intraoperative culture
- Type of revision surgery
- Type and n. of days of antibacterial treatment received.

All patients received antibacterial prophylaxis with 12 mg/kg teicoplanin or 2 gr cefazolin 30' before the surgical incision.

All surgeries were performed by the same surgeon.

In order to guarantee the most accurate diagnosis, the following criterion was established for taking samples for the intraoperative histological examination: the pathologist had to analyze a fragment from the pseudocapsule, taken considering that the most suitable areas where those apparently infected at macroscopic level, while the tissues had to be taken close to the prosthesis-bone interface. After freezing and standard staining with haematoxylin and eosin, the tissues were analyzed as described by Feldman (12), aiming at minimizing errors in sampling:

1. Preferentially analyzing the granulated tissue
2. Analyzing at least 3 samples of periprosthetic tissue
3. Considering the 5 samples with the highest number of polymorphonuclear cells
4. Performing cell count per high magnification (40X)
5. The polymorphonuclear leukocytes were considered in the count only where the cytoplasmic membrane was perfectly recognizable (Fig. 1).

All samples taken for the intraoperative histological examination were analyzed by the same anatomical pathologist.

Three tissue samples were removed from the same periprosthetic regions from every patient. One sample was sent for intraoperative histological examination, while the remaining two served as a control for definitive histological examination and culture.

Intraoperative histological examination was performed according to Feldman's classification (12), being thus considered positive when 5 or more polymorphonuclear leukocytes were found per HPF (40 X), negative when the amount of those cells was lower (< 5) (Fig. 2).

Both synovial fluid and intraoperative tissue samples were sent to the microbiology laboratory for culture. As for the diagnosis of infection, in case of highly virulent pathogens like *Staphylococcus aureus*, the result was considered positive when at least one of the intraoperative samples resulted positive. In the event of low-virulence pathogens, like *coagulase-negative*

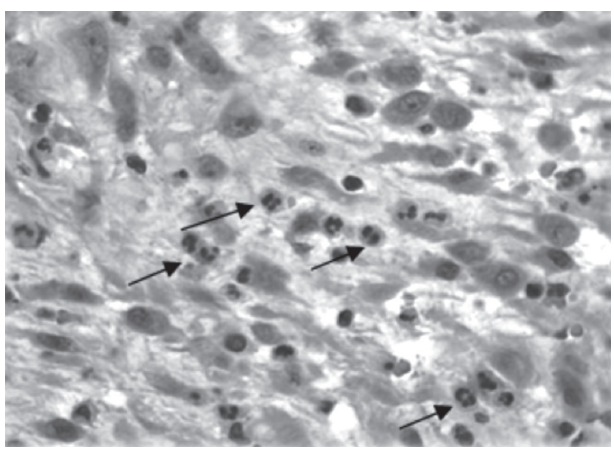

Figure 1. PMN cells (black arrows) in frozen section

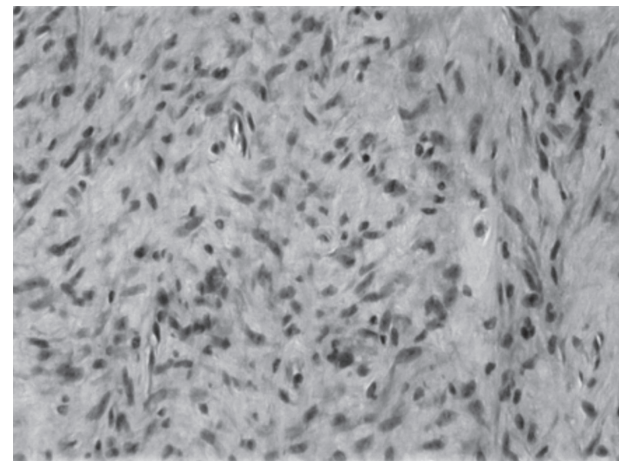

Figure 2. Frozen section examination positive for infection: > 5 PMN

Staphylococcus or *Propinebacterium*, the test result was considered positive when growth was recorded in at least three samples, in accordance to Morawietz' criteria (15).

The tissue samples collected for the definitive histological examination have been immediately fixed in 4% formaline, consequently 5 μm-thin slices were prepared and stained with haematoxylin and eosin. The samples thus prepared have then been analyzed with optical microscope and polarized light microscope, applying Krenn and Morawietz' classification of periprosthetic tissues (18).

The data obtained were used to calculate sensitivity, specificity, confidence interval, positive and negative predictive values, and to verify the validity of intraoperative histological examination as a diagnostic test for periprosthetic infection in comparison to the definitive histological examination and culture.

Results

This study took into consideration 159 patients, because 10 subjects were excluded owing to incompleteness of the data collected.

ESR and preoperative CRP of each patient were observed. ESR avarage value was 28,04 mm/h (range 2-120), CRP was on avarage 21,24 mg/L (range 0,5-120).

Scintigraphy was performed on 67 patients (three-phase scintigraphy: 33 patients; scintigraphy with marked leukocytes: 27 patients; scintigraphy with nanocolloids: 4 patients; antigranulocyte antibody scintigraphy: 3 patients). The reports pointed out positive results in 13 patients owing to the presence of sepsis, in 16 patients the accumulation of tracking was compatible with a mobilization of the implant, 23 patients exhibited a localized accumulation that could be due to non specific inflammations (as for instance medullary expansion/compression, periprosthetic osteolysis), while the exam turned out negative for 15 patients.

Culture resulted positive in 60 patients, 31,3% of which had polymicrobial flora (1 patient was positive for Aspergillus niger, 1 for Aspergillus flavus, 10 for Staphylococcus aureus, 4 for MRSA, 4 for Staphylococcus haemolyticus, 18 for Staphylococcus epidermidis, 1 of which methicillin resistant, 1 for Listeria monocytogenes, 1 for Staphylococcus agalactiae, 3 for methicillin resistant Staphylococcus haemolyticus, 4 for Staphylococcus capitis, 3 for Pseudomonas aeruginosa, 2 for non specified coagulase negative Staphylococcus, 2 for methicillin resistant Staphylococcus saprophyticus, 4 for Streptococcus warnerii, 3 for Stahphylococcus Lugdunensis, 1 for E.Coli, 6 for Staphylococcus Hominis, 1 for Coryneacterium, 1 for Bacillus Cereus, 4 for Propionibacterium Acnes, 1 for Streptococcus Bovis, 1 for Bacillus spp, 1 Streptococcus Gordonii).

All patients underwent intraoperative and definitive histological examinations.

The results of intraoperative histological examination were then compared with those of definitive culture, which acted as a reference for the diagnosis of infection.

The following results were obtained:

- 80 patients negative to intraoperative histological examination and culture;
- 17 patients positive to intraoperative histological examination and negative to culture;
- 37 patients negative to intraoperative histological examination and positive to culture;
- 23 patients negative to intraoperative histological examination and culture;

Sensitivity of the intraoperative histological examination resulted 38,3% (IC 0,26; 0,51); specificity 82,5% (IC 0,73; 0,90), where positive predictive value was 57,5% (IC 0,41; 0,73) and negative predictive value 68,4% (IC 0,59; 0,76).

Discussion

An infection following a prosthesis surgery is a complication that can occur in almost 1% of cases, depending on the clinical records (19-21).

Reaching the diagnosis of infection is often difficult, especially in patients who exhibit pathognomonic signs like fever, flush, tumefaction or fistulae.

Routine haematochemical tests, as leukocyte count and ESR, demonstrate low specificity and sensitivity (22, 23), while other markers, like CRP and IL-6 levels, are apparently more accurate, even though their diagnostic usefulness in the event of a prosthetic infection has not been made completely clear yet (23). Chemical and physichal analysis and culture of the synovial fluid, intra-operative Gram staining and the surgeon's evaluation during the operation are further elements that can help discriminating an aseptic prosthetic mobilization from a periprosthetic infection, nevertheless these methods show not irrelevant limits (24-26).

Intraoperative histological examination, first proposed by Charosky et al. in 1973 (27), is often applied to establish the presence or absence of infections during prosthesis revision surgeries (28, 29). In the literature up to date, only few studies have analyzed the effectiveness of this method in defining a septic or aseptic mobilization (11, 25).

Della Valle et al. (30) applied a cut-off of at least 10 neutrophils per field in high magnification, and obtained a sensitivity of 25% and a specificity of 98%, results that reflect those of Bori et al. (28,5% sensitivity and 98% specificity) (11), who had applied the same criterion proposed by Feldman (at least 5 neutrophils per field). Anyway, Bori et al. (11) resorted to Athanasou's method to increase test sensitivity, which means that the sample is considered positive to infections when the average number of neutrophils found per HPF is one, after analyzing 10 fields (36). When this criterion was applied, sensitivity increased to over 70%, while specificity shrinked to 64,2% (31).

In 2006 Moravietz et al. presented a new classification for samples subjected to intraoperative histological examination, with the aim of sorting the different types of periprosthetic tissue into four classes. This classification is based on the number of neutrophil granulocites (at least 2 PMN in 10 HPF) and other histological criteria like the presence of lymphocytes, plasma cells, multinucleate cells and components from the coating (35).

In their study, Tohtz et al. (32) applied this classification, obtaining a specificity of 100%, but a considerable percentage (19%) of results that could not be univocally defined and fell into none of Moravietz' four categories. This problem also depends on the lower quality of *frozen sections* in comparison to the samples fixed in formaline for the intraoperative histological examination, the laboratory technicians' expertise and the anatomical pathologist's skills. The classification systems created by Feldman et al. (12) or Athanasou et al. (36) do not show interpretation-related problems, but exhibit a low sensitivity level owing to the high amount of false negatives.

The outcomes demonstrated in the most recent studies highlight a strong similarity between the results of intraoperative examination and the gold standard tests carried out to confirm the presence of infection (culture or definitive histological examination) (32-34) and point out high specificity levels (100% in Tohtz et al., 97% Ko et al., 95% Kanner et al.), to the detritment of lower sensitivity levels (86,6% in Tohtz et al., 67% in Ko et al., 29% in Kanner et al.) (9, 25, 29).

In this study, intraoperative histological examination obtained a sensitivity of 42%, a specificity of 81%, a positive predictive value of 50% and a negative predictive value of 76%, values in line with the results achieved by the other Authors, even though the specificity level is lower.

When applying Feldman's criteria, the different sensitivity observed in the intraoperative investigation could be caused by the low inflammatory response produced by low-virulence pathogens, like *coagulase negative staphylococci*. Feldman's criteria have been first described by Mirra in 1976 (41) in patients suffering from infections caused by *Pseudomonas*, *Escherichia coli*, *Proteus* and *Staphylococcus aureus* (highly virulent bacteria). As a consequence, finding less than 5 neutrophils per field in a high magnification does not exclude the presence of a low virulence pathogen (*Propinebacterium Acnes*, *Corynebacterium* or *coagulase negative Staphylococci*) (11). This has been confirmed in 22 cases of our set in which intraoperative histological examination turned out negative (less than 5 neutrophils per field), while culture resulted positive (*S. haemolyticus, S. lugdunensis, S. epidermidis, S warneri, S. capitis, S. hominis*). Under these circumstances it is the macroscopic appearance of the tissues, the patient's medical history and the results of laboratory and instrumental examinations that, during the surgery, make the surgeon opt for a one- or two-time revision surgery.

Tunney et al. analyzed the results of intraoperative histological examination in a set of 18 patients with sure diagnosis of infection with low-virulence pathogens, and in 8 cases found no neutrophil per field in high magnification, reaching therefore the conclusion that the infection by these microorganisms cannot be excluded even in case of absence of neutrophils (38). Bori et al. drew the same conclusions in their work, in which they compared intraoperative histological examination with the result of definitive culture. All intraoperative examinations resulting in more than 5 neutrophil granulocytes corresponded to positive cultures, except for 2 out of the 13 intraoperative examinations positive for coagulase negative Staphylococcus (39).

A high number of false positives could also be determined by factors like the administration of antibacterial in the period before surgery, which could alter the count of granulocytes, or mistakes in taking the samples. In some cases, the tissues taken for culture or histological examination may not have been removed from the same areas, or delays in the procedure could imply a higher number of polymorphonuclears owing to the extravasation of these cells from blood vessels following surgical treatments (32). The presence of active inflammatory arthritis or fractures could also lead to an increase in neutrophils, without infections being present (40).

Conclusions

In conclusion, in the absence of a universally accepted method to diagnose infection in patients with mobilization of the prosthesis, intraoperative histo-

logical examination is, in spite of everything, a method easy to perform and reproduce, it shows high specificity and sensitivity in the presence of highly virulent pathogens (12) and is therefore a valid tool to support the surgeon in deciding which therapeutic approach to adopt. A few limits remain nonetheless, especially in the event of infections caused by low-virulence pathogens, among which coagulase negative Staphylococci, that rank among the most common agents related to periprosthetic infections (41).

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