

## Frozen sections of samples taken intraoperatively for diagnosis of infection in revision hip surgery

Leandro V Nuñez<sup>1</sup>, Martín A Buttaró<sup>1</sup>, Ana Morandi<sup>2</sup>, Rodolfo Pusso<sup>1</sup> and Francisco Piccaluga<sup>1</sup>

<sup>1</sup>Institute of Orthopedics “Carlos E. Ottolenghi”, <sup>2</sup>Pathology Department, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina  
Correspondence LN: leandro.nunez@hospitalitaliano.org.ar  
Submitted 06-05-21. Accepted 06-07-31

**Background** The diagnosis of a suspected infected prosthesis is often difficult, but is important for the choice of treatment. Even at surgery, it is not easy to assess whether the prosthesis is infected or not—even though this may be important for the choice of surgical procedure.

**Patients and methods** We assessed the sensitivity, specificity, predictive value, and reliability of the results of the analysis of frozen sections from samples of tissues taken during revision hip surgery of 136 probably infected prostheses. Samples of tissues were taken to be analyzed immediately from frozen sections, to be processed on a routine basis later, and to be referred for bacteriological cultures. A finding of 5 or more polymorphonuclear leukocytes per field at a magnification of 400× was considered positive for infection.

**Results** The analysis of frozen sections for infection was in agreement with the results of routine histopathology in 134 of 136 cases. Comparison with the results of culture showed a sensitivity of 85%, a specificity of 87%, a PPV of 79%, an NPV of 91%, and a Kendall’s tau correlation coefficient of 0.72.

**Interpretation** We believe that the method we have tested is of value in revision surgery when infection cannot be ruled out.

The differential diagnosis between aseptic loosening and infected prosthesis is often difficult, but is very important since the treatments are different. The diagnosis of infection is based on anamnesis, clinical findings, radiographs, scintigraphy, labora-

tory studies and needle biopsy if necessary. However, the sensitivity and specificity of the different methods has been questioned (Mirra et al. 1976, Wukich et al. 1987, Barrack et al. 1993, Banit et al. 2002). For most authors, the results of culture from specimens taken intraoperatively are considered to be the “gold standard” for detection of a prosthetic infection (Feldman et al. 1995, Lonner et al. 1996). Histopathological analysis of frozen sections from samples taken during surgery may be of diagnostic value (Carlson 1978, Athanasou et al. 1995, Lonner et al. 1996, Pace et al. 1997). However, routine use of this method in revision hip surgery has been questioned (Fehring and McAlister 1994).

We determined the sensitivity, specificity, predictive value, and accuracy of histopathological analysis of such frozen sections by comparing the results to the corresponding results from routinely processed samples and bacteriological culture in 136 patients with suspicion of an infected THA.

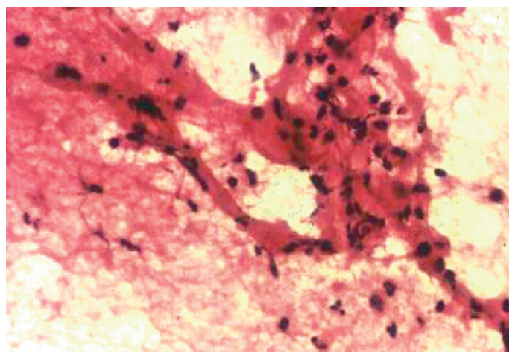
### Patients and methods

Between January 1998 and December 2003, 291 revisions of total hip arthroplasties were performed in our unit. 145 hips were discarded either because there was no suspicion of infection (105/145) or because they were obviously infected according to clinical, radiological and intraoperative signs (40/145), so frozen sections were not taken during surgery. Thus, 136 patients (87 females) were studied, with a mean age of 64 (28–90) years.

Samples of tissues to be analyzed were taken during surgery from the pseudocapsule, the cement-bone interface of the femur and acetabulum, and any other tissues involved according to the surgeon's judgement. All samples were referred for frozen sections and to be processed on a routine basis. Samples for cultures were taken from the same areas and were packed into a sterile surgical container to avoid contamination. No patient received antibiotic therapy before the samples were obtained, and all the surgical procedures were supervised by one of the authors (FP). All samples were processed and analyzed by the pathology and bacteriology units of our hospital.

Histological analysis was performed on all material. Smear tissue staining was hematoxylin-phloxine. Serial sections (4 mm thick) were processed in a freezing chamber and stained with hematoxylin-phloxine and toluidine blue. The material for standard processing was fixed in 10% formol and paraffin and embedded in an automatic tissue processor. 6 microsections stained with hematoxylin-eosin and Mason's-trichrome were observed with an optical microscope under high magnification (total magnification 400×) and polarized-light lens (Axiostar).

All samples were analyzed by two pathologists who counted the number of cells in 10 fields. Neutrophils, lymphocytes, plasma cells, macrophages, multinuclear giant cells, acrylic material, and polystyrene particles were included. For the specific purposes of this study, only neutrophils were considered. The limit to considering a sample to be infected was 5 or more polymorphonuclear leukocytes (Mirra et al. 1982) (Figure). Bacteriological analyses were performed in at least 5 samples from the same sites as those that were taken for histopathological examination. The samples for aerobic and anaerobic cultures were plated on chocolate agar, blood agar and different enriched culture media. They were incubated for 5 days and subcultured daily for aerobic and anaerobic bacteria. Cultures were considered negative when there was no growth of bacteria in 14 days, no growth of fungus in 4 weeks, and no growth of mycobacteria in 8 weeks. Cultures were considered to be positive if there was growth of at least one colony.



Frozen section from sample taken intraoperatively showing more than 5 polymorphonuclear leukocytes per high-power field (hematoxylin-phloxine and toluidine blue. 400×).

## Results

The average follow-up period was 34 (3–70) months. Of the 136 patients, 70 were revised in one stage due to there being no signs of infection and negative histological analysis. In the remaining 66 cases an extraction procedure was carried out. In these cases, frozen sections from 53 patients were positive for infection, so these patients were considered to be infected. In 49 patients, a two-stage procedure was decided, and in 4 patients, a Girdlestone's procedure was done. In the remaining 13 cases, although the histological analyses were negative for infection, a two-stage procedure was decided on either because of the appearance of the tissues or the technical impossibility of properly reconstructing the bone structure in one stage (due to the magnitude of the bone defect or the clinical condition of the patient). In this last group the cultures were positive in 4 cases, confirming the surgeon's suspicion, and negative in 9. Six of these patients were reimplanted within 3 months, and in the remaining 3, a definitive Girdlestone's procedure was done.

Of the total of 136 cases studied, the result from the frozen section samples coincided with that from routine sections in 134 cases (the exceptions being 1 false positive and 1 false negative) (Table 1). From the 49 positive cultures obtained, 46 were accepted as being genuine infections and 3 were considered to be contaminations, because only one colony had grown and the histopathological examination was negative. The most frequent pathogens found were coagulase-negative *Staphylococcus*

Table 1. Comparison of results from analysis of frozen sections and deferred histopathological analysis

Frozen sections	Deferred		Total
	Positive	Negative	
Positive	52	1	53
Negative	1	82	83
Total	53	83	136

Table 2. Microorganisms found in cultures

Bacteria	No.
Coagulase-negative <i>Staphylococcus</i>	12
Methicillin-resistant <i>Staphylococcus aureus</i>	9
<i>Enterococcus</i> sp.	5
<i>Enterococcus faecalis</i>	5
Methicillin-sensitive <i>Staphylococcus aureus</i>	4
<i>Escherichia coli</i>	4
<i>Proteus mirabilis</i>	3
<i>Pseudomona aeruginosa</i>	2
<i>Enterobacter</i>	2
β-hemolytic group B <i>Streptococcus</i>	1
<i>Streptococcus viridans</i>	1
<i>Enterobacter cloacae</i>	1

*aureus* in 12 cases and methicillin-resistant *Staphylococcus aureus* in 9 cases (Table 2).

The intraoperative frozen sections were considered negative in 76 cases. In 68 of the patients, a one-stage revision was performed. In the remaining 8 cases, either the tissues had a dubious appearance or there was an important bone defect that could not be reconstructed. In 7 of these patients an antibiotic-loaded cement spacer was set pending the results of culture, and a new implant was inserted within 6 months. The other patient was treated using a Girdlestone procedure due to a history of infection. 42 (31%) of the 136 cases were considered to be true positives and thus to be infected, so a two-stage procedure was performed.

In 11 patients (8%), the analysis of the frozen sections was positive but the cultures were negative. In these patients the prostheses were explanted and antibiotic therapy was given 6–8 weeks before re-implant. One of these patients was treated as an infection, based on the frozen section, but the postoperative histological study was negative.

Table 3. Comparison of results from analysis of frozen sections and culture

Frozen sections	Cultures		Total
	Positive	Negative	
Positive	42	11	53
Negative	7	76	83
Total	49	87	136

7 patients (5%) had false-negative frozen sections. In 3 of these cases, we considered the positive cultures to be sampling contaminants (*Staphylococcus epidermidis*) because the clinical and intraoperative diagnosis was negative and the frozen section did not contain polymorphonuclear leukocytes. These patients underwent a one-stage exchange. Another patient with a negative intraoperative frozen section had a positive definitive histology, but the surgeon's impression intraoperatively and previous clinical findings led to a two-stage procedure. Despite the negative results from intraoperative frozen sections, the remaining 3 patients had delayed re-implantation and postoperative antibiotics because of intraoperative findings and previous suspicion of infection.

Compared to the definitive bacteriological analysis, the sensitivity of the analysis of frozen sections in detecting an infection was 85% (95% CI: 73–93) and the specificity was 87% (CI: 79–93). The NPV was 91%, and PPV 79% with a Kendall's correlation tau of 0.72 ( $p < 0.001$ ) (Table 3).

## Discussion

In our patients, analysis of frozen sections of samples taken intraoperatively helped to differentiate between mechanical and septic failure of a total hip arthroplasty in almost all the suspected cases. In comparing analysis of frozen sections with definitive histopathological analysis, we found agreement in 134/136 cases.

The possibility of differentiating an aseptic loosening from an infected prosthesis is rather difficult when clinical findings are poor or even absent. Laboratory tests and radiographic and scintigraphic

findings are not specific. Needle biopsy allows aspiration of the periprosthetic fluid (Merkel et al. 1985, Rand and Fitzgerald 1989, Rand and Brown 1990). However, the results that have been published show considerable variation; the sensitivity has ranged from 50% to 93% (Barrack et al. 1993, Tigges et al. 1993, Fehring and Cohen 1996) and the specificity has been between 82% and 97% (Phillips et al. 1983, Roberts et al. 1992, Fehring and Cohen 1996). This procedure is useful when positive, to determine the antibiotic to be added to the spacer.

In the absence of a universally accepted diagnostic method, the histological analysis of frozen sections of samples taken during surgery has been proposed to aid the choice between a one-stage and a two-stage surgical procedure (Charosky et al. 1973, Mirra et al. 1976, Rand and Fitzgerald 1989, Feldman et al. 1995). In contrast to needle biopsy, the samples can be taken under visual control. The method is not a new one; the first report was by Charosky et al. in 1973. Mirra et al. (1976) quantified the infection according to the number of polymorphonuclear leukocytes per high-power field (more than 5) in 15 patients, and found that the results of analysis of frozen sections and culture were the same in 21 of 22 patients (Mirra et al. 1982).

Using a similar criterion, and after analyzing 33 hips and knee revision prostheses, Feldman et al. (1995) found the same results from frozen sections and cultures in 9 patients. In 24 patients with negative cultures, 23 had negative frozen sections. Their study supports the conclusion that examination of frozen sections of samples taken intraoperatively is a reliable method for detection of the presence of active infection during a THA revision. The conclusions of these reports were questioned by Fehring and McAlister (1994), who reported a sensitivity of 18% and a specificity of 89% in 97 patients. In 106 knee and hip arthroplasties, Athanasou et al. (1995) found a sensitivity of 90% and a specificity of 96%, when comparing the results of analysis of frozen sections with the results of culture. In a prospective study to determine the reliability of analysis of frozen sections and culture from samples taken intraoperatively in 175 revisions of hip and knee arthroplasty, Lonner et al. (1996) obtained a sensitivity of 84% and a

specificity of 96%, and considered this method to be useful in the detection of infection during the surgical procedure. In a prospective study of 121 arthroplasty revisions comparing intraoperative frozen sections and cultures, Banit et al. (2002) obtained a sensitivity of 67% and a specificity of 84% when considering more than 5 polymorphonuclear leukocytes per field to be the positive cut-off point. When only hips were analyzed, a sensitivity of 45% and a specificity of 92% was obtained. In another study published by Abdul-Karim et al. (1998), the reliability of analysis of intraoperative frozen sections was compared to that of culture from 64 patients, giving a sensitivity of 43% and a specificity of 97%. Della Valle et al. (1999) reported a sensitivity of 25% and a specificity of 98%, concluding that analysis of frozen sections of samples taken intraoperatively is an important method to rule out the presence of infection, but not for detecting it. The study was performed during the second stage of re-implantation, which might explain the low sensitivity (due to alterations of scar tissue by prior infection). Re-implantations were not included in our survey.

We found a sensitivity of 85% for detection of infection by analysis of frozen sections of samples taken during the operation. The specificity of the method in determining the absence of infection was 87%. In comparison with the results of other methods, we can infer that analysis of intraoperative frozen sections is a relatively sensitive method (Fehring and McAlister 1994, Abdul-Karim et al. 1998, Banit et al. 2002), although other authors found lower sensitivity percentages (Athanasou et al. 1995, Feldman et al. 1995, and Lonner et al. 1996). It is difficult to find a suitable explanation for the discrepancy between our own results and those of Fehring and McAlister (1994). When analyzing low-sensitivity studies comparing results from intraoperative frozen sections with those from definitive bacteriology, some standards and errors must be taken into account. One error could be made by the surgeon by not taking samples from the areas of greatest suspicion. Another error might be made by the pathologist, when handling the samples. There is a chance that the area chosen is not infected. A third error might be related to bacteria that require special culture media and longer incubation periods.

Our results agree with those of other authors, and reinforce the belief that the presence of more than 5 polymorphonuclear leukocytes per high-power field in frozen sections of samples taken intraoperatively confirms the existence of an infection. Based on our results, if there is no clinical suspicion of infection prior to operation and intraoperative frozen sections do not reveal 5 or more polymorphonuclear leukocytes per high-power field, re-implantation may be carried out at that moment, with a 91% chance of absence of infection. In suspected cases without positive preoperative diagnostic results but in the presence of more than 5 polymorphonuclear leukocytes in the frozen sections, the surgeon must proceed with caution and it would be convenient in such a case to defer re-implantation until a later stage.

#### *Contributions of authors*

LVN: Collected all data, performed statistical analysis and wrote the paper. MAB: Collaborated in writing of the paper. AM: head of Pathology Department, analysis of frozen sections. RP: previous head of the Center of Hip Surgery, translation. FP: head of the Center of Hip Surgery, surgeon.

No competing interests declared.

- Abdul-Karim F W, McGinnis M G, Kraay M, Emancipator S N, Goldberg V. Frozen section biopsy assesment for the presence of polymorphonuclear leukocytes in patients undergoing revision of arthroplasties. *Mod Pathol* 1998; 11: 427-31
- Athanasou N A, Pandey R, DeSteiger R, Crook D, McLardy Smith P. Diagnosis of infection by frozen section during revision arthroplasty. *J Bone Joint Surg (Br)* 1995; 77: 28-33.
- Banit D M, Kaufer H, Hartford J M. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop* 2002; (401): 230-8.
- Barrack R L, Harris W H. The value of aspiration of the hip before total hip arthroplasty. *J Bone Joint Surg (Am)* 1993; 75: 66-76.
- Carlson A S. Erythrocyte sedimentation rate in infected and non infected total hip arthroplasties. *Acta Orthop Scand*. 1978; 49: 287-90.
- Charosky C B, Bullough P G, Wilson Jr P D. Total hip replacement failures: A histological evaluation. *J Bone Joint Surg (Am)* 1973; 55: 49-58.

- Della Valle C J, Bogner B A, Desai P, Lonner J H, Adler E, Zuckerman J D, Di Cesare P E. Analysis of frozen sections of intraoperative specimens obtained at the time of reoperation after hip or knee resection arthroplasty for the treatment of infection. *J Bone Joint Surg (Am)* 1999; 81: 684-9.
- Fehring T K, Cohen B. Aspiration as a guide to sepsis in revision total hip arthroplasty. *J Arthroplasty* 1996; 11: 543-7.
- Fehring T K, McAlister Jr J A. Frozen histologic section as a guide to sepsis in revision joint arthroplasty. *Clin Orthop* 1994; (304): 229-37.
- Feldman D S, Lonner J H, Desai P, Zuckerman J D. The role of intraoperative frozen sections in revision total joint arthroplasty. *J Bone Joint Surg (Am)* 1995; 77: 1807-13.
- Lonner J H, Desai P, DiCesare P E, Steiner G, Zuckerman J D. The reliability of analysis of intraoperative frozen sections for identifying active infection during revision hip and knee arthroplasty. *J Bone Joint Surg (Am)* 1996; 78: 1553-8.
- Merkel K D, Brown M L, Dewangee M K, Fitzgerald Jr R H. Comparison of indium-labeled leukocyte imaging with sequential technetium-galium scanning in the diagnosis of low grade musculoskeletal sepsis: A prospective study. *J Bone Joint Surg (Am)* 1985; 67: 465-76.
- Mirra J M, Amstutz H C, Matos M, Gold R. The pathology of joint tissue and its clinical relevance in prosthesis failure. *Clin Orthop* 1976; (117): 221-40.
- Mirra J M, Marder R A, Amstutz H C. Pathology of failed total joint arthroplasty. *Clin Orthop* 1982; (170): 175-83.
- Pace T B, Jeray K J, Latham J T. Synovial tissue examination by frozen section as an indicator of infection in hip and knee arthroplasty in community hospitals. *J Arthroplasty* 1997; 12: 64-9.
- Phillips W C, Kattapuram S V. Efficacy of preoperative hip aspiration performed in the radiology department. *Clin Orthop* 1983; (179): 141-6.
- Rand J A, Fitzgerald Jr R H. Diagnosis and management of the infected total knee arthroplasty. *Orthop Clin North Am* 1989; 20: 201-10.
- Rand J A, Brown M L. The value of indium-111 leukocyte scanning in the evaluation of painful or infected total knee arthroplasties. *Clin Orthop* 1990; (259): 179-82.
- Roberts P, Walters A J, McMinn D J W. Diagnosing infection in hip replacements. The use of fine-needle aspiration and radiometric culture. *J Bone Joint Surg (Br)* 1992; 72: 265-9.
- Tigges S, Stiles R G, Meli R J, Roberson J R. Hip aspiration: A cost-effective and accurate method of evaluating the potentially infected hip prosthesis. *Radiology* 1993; 189: 485-8.
- Wukich K, Abreu H, Callaghan J, Van Nostrand D, Savory G, Egli F, Garcia E, Berrey H. Diagnosis of infection by preoperative scintigraphy with indium labeled white blood cell. *J Bone Joint Surg (Am)* 1987; 69: 1353-60.