Alberto Francés Borrego · Fernando Marco Martínez ·
Juan Luis Cebrian Parra · David Serfaty Grañeda ·
Rodrigo García Crespo · Luis López-Durán Stern

Diagnosis of infection in hip and knee revision surgery: intraoperative frozen section analysis

Received: 7 November 2005 / Revised: 2 December 2005 / Accepted: 5 December 2005 / Published online: 18 March 2006
© Springer-Verlag 2006

Abstract In this study we validated intraoperatively the analysis of polymorphonuclear leucocyte frozen sections for diagnosis of infection in hip and knee revisions. Between 1996 and 2002 we examined sections and cultured periprosthetic tissues in prosthetic revision in 170 cases, including 146 cases (83 hips and 63 knees). We assessed sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), Youden index, positive likelihood ratio (PLR) and negative likelihood ratio (NLR). We compared intraoperative findings and paraffin-embedded samples. The results in the knee group were SE=66.7%, SP=89.7% [95% confidence interval (CI)], PPV=81% (95% CI), NPV=81.4% (95% CI), Youden index=0.56, PLR=6.5 (95% CI) and NLR=0.4 (95% CI). In the paraffin-embedded samples the results were SE=91%, SP=87% (95% CI), PPV=81% (95% CI), NPV=94% (95% CI), PLR=7 (95% CI) and NLR=8.7 (95% CI). We found a significant difference. The results in the hip group were SE=50%, SP=100% (95% CI), PPV=100% (95% CI), NPV=94.9% (95% CI), Youden index=0.5 and PLR=0.5 (95% CI). In hip and knee prosthetic revision surgery the presence of polymorphonuclear cells correlates with infection, but their absence does not exclude it. It is a quick and inexpensive test that should be included in the diagnostic protocol in revision surgery. Level of evidence: diagnostic Study (investigating a diagnostic test), level I. See instructions to authors for a complete description of levels of evidence.

Résumé Nous avons analysé des coupes de leucocytes polymorphonucléaires, prélevés per-opératoirement pour le diagnostic des infections de hanche et de genou lors des révisions prothétiques. Entre 1996 et 2002, nous avons examiné les coupes et les cultures des tissus périprothétiques prélevés lors des révisions de prothèses (170 cas), 146 cas ont été inclus dans cette étude (83 hanches et 63 genoux). Nous avons évalué la sensibilité (SE), la spécificité (SP), la valeur prédictive positive (PPV), la valeur prédictive négative (NPV), l'index de Youden, le ratio positif de probabilité (PLR) et le ratio négatif de probabilité (NLR). Nous avons comparé les prélèvements per-opératoires et les échantillons inclus dans la paraffine. Résultats : Dans le groupe des genoux : SE=66,7%; SP=89,7% (CI 95%); PPV=81% (CI 95%); NPV=81,4% (CI 95%). L'index de Youden=0,56; PLR=6,5 (CI 95%); NLR=0,4 (CI 95%). Paraffine: SE=91%; SP=87% (CI 95%); PPV=81% (CI 95%); NPV=94% (CI 95%); PLR=7 (CI 95%); NLR=8,7 (CI 95%). Nous avons trouvé une différence significative. Dans le groupe de la hanche SE=50%; SP=100% (CI 95%); PPV=100% (CI 95%); NPV=94,9% (CI 95%); l'index de Youden=0,5; PLR=0,5 (CI 95%). Conclusions: Dans la chirurgie de révision des prothèses de hanches et des prothèses de genoux, la présence de cellules polymorphonucléaires est bien corrélée avec l'infection, mais, son absence n'exclue pas celle-ci. Il s'agit là d'un test rapide, peu invasif qui peut être pratiqué systématiquement dans le protocole de ce diagnostic dans la chirurgie de révision articulaire.

A. Francés Borrego · F. M. Martínez · J. L. Cebrian Parra ·
D. S. Grañeda · R. G. Crespo · L. López-Durán Stern
Servicio de Cirugía Ortopédica y Traumatología,
Hospital Clínico San Carlos de Madrid,
Madrid, Spain

A. Francés Borrego (✉)
C/ Valle Inclán 8, portal 5, piso 3ºA,
Boadilla del Monte,
28660 Madrid, Spain
e-mail: albertofrances@hotmail.com

Introduction

Diagnosis of infection in prosthetic revision surgery is of paramount importance. Revision of implants, debridement, antibiotics and one- or two-stage prosthesis implantation should be done [19]. Other alternatives include acute debridement, antibiotic suppression and resection arthroplasty. The treatment of aseptic loosening allows implantation of a new prosthesis during the same surgery. The

relatively high rate of infection (5–10%) in revisions [7] compels us to make a differential diagnosis of infection before implantation of a new prosthesis.

For preoperative diagnosis, we use the history (pain, drainage), laboratory analysis [erythrocyte sedimentation rate (ESR), protein C, blood count], radiology, nuclear medicine and synovial fluid analysis. Nevertheless these tests show low confidence levels [14, 16, 23].

For intraoperative diagnosis, we rely on the appearance of periprosthetic tissues, Gram sampling and articular fluid analysis. They also show low confidence levels [7].

It has been shown that histological examination of periprosthetic soft tissue has high sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) [3, 11, 17, 25]. Inflammatory foci with polymorphonuclear leucocytes in periprosthetic interfaces correlate with infection, and although this has been questioned [10], it is generally accepted. We assessed the validity of intraoperative frozen sections.

Materials and methods

We made a cross-sectional study where the results of 146 cases in 146 patients (83 hips and 63 knees) were collected prospectively, representing the entire hip and knee revision surgery cases between 1996 and 2002 with complete data. We have excluded 24 cases with incomplete data. All patients received intraoperative antibiotic doses. Three intraoperative soft tissue samples were taken at the beginning of the procedure with a scalpel blade, choosing highly suspicious or inflammatory areas. Half of the samples were sent for culture to the Microbiology Department (general microbiology, fungus and mycobacterium cultures) in a sealed container in the operative field to avoid contamination. They were cultured on blood agar, chocolate agar and CNA-MacConkey at 37°C for 5 days, for fungus, on Sabouraud medium at 35°C for 7 days and for mycobacterium on Lowenstein medium for no more than 8 weeks. It was considered that the culture was negative if no growth was seen in 10 days for general bacteria, 4–5 weeks for fungus and 8 weeks for mycobacterium. It was positive if any colony growth appeared during the mean period.

The other half of each sample was sent to the Pathology Department for examination. The most suspicious areas were processed. The sample was frozen in liquid nitrogen, cut and dyed with haematoxylin-eosin. Once under the microscope, five low-power fields were selected (the most inflammatory). These areas were examined at high power ($\times 40$), considering the sample negative when less than ten polymorphonuclear cells per high-power field were found in any of the selected areas (Fig. 1a) and positive when more than ten polymorphonuclear cells were found (Fig. 1b). In the knee group the rest of the samples were examined in paraffin and dyed with haematoxylin-eosin in the postoperative period.

Fig. 1 **a** <10 polymorphonuclear cells per high-power field (microscopic view, frozen section, haematoxylin-eosin). **b** >10 polymorphonuclear cells per high-power field (microscopic view, frozen section, haematoxylin-eosin)

Statistical methods

The results of intraoperative frozen sections and intraoperative cultured samples were correlated (considering culture as the gold standard) to calculate the SE (relation between true positives and the addition of false negatives and true positives), the SP (relation between true negatives and the addition of false positives and true negatives), the PPV (relation between true positives and the addition of true positives and false positives), NPV (relation between true negatives and the addition of true negatives and false negatives), Youden index ($SE + SP - 1$), positive likelihood ratio ($PLR = SE / (1 - SP)$) and negative likelihood ratio ($NLR = (1 - SE) / SP$). This type of study was performed to assess the validity of a diagnostic test (intraoperative frozen sections). We also correlated the results of frozen sections and paraffin sections in the same samples in the knee group. We calculated the chi-square test, looking for statistical differences ($p < 0.05$). We calculated the confidence interval for all of the parameters (95% CI).

Results

We divided the results into two different groups: the knee and the hip revision group. In the knee group, we

had 63 cases of knee prosthesis revision (62 total knee and one unicompartmental knee replacement) with a mean age of 73 years (63–89); the male/female ratio was 19/54. In 24 cases, the culture was positive and in 39 it was negative. The infection rate was 38.1% (37.2–39%). All of the results are shown in Table 1. The statistical results were: for the SE 66.7% (95% CI: 64.5–68.8%), for the SP 89.7% (95% CI: 88.4–91.1%), for the validity index 81% (95% CI: 80.1–81.8%), for the PPV 80% (95% CI: 77.4–82.6%), for the NPV 81.4% (95% CI: 80.2–82.6%), for the Youden index 0.56, for the PLR 6.5 (95% CI) and for the NLR 0.37 (95% CI). In the knee group we correlated the results of the frozen sections (intraoperative) and the paraffin group (postoperative) to assess the accuracy of the frozen section analysis to determine the amount of polymorphonuclear cells in the samples. We found statistically significant differences between the two groups ($p < 0.05$). There was an increase in the SE of 25%, diminishing the SP in 3%, increasing the PPV in 1% and diminishing the NPV in 13%.

In the hip group, we had 83 cases of hip prosthesis revision with a mean age of 74 years (61–86); the male/female ratio was 29/54. In eight cases, the culture was positive and in 75 it was negative. The infection rate was 9.6% (9–10.3%). All of the results are shown in Table 2. The statistical results were: for the SE 50% (95% CI: 43–56.5%), for the SP 100% (95% CI: 87.5–100%), for the validity index 95.2% (95% CI: 94.6–95.8%), for the PPV 100% (95% CI: 87.5–100%), for the NPV 94.9% (95% CI: 94.3–95.6%), for the Youden index 0.56, for the PLR 6.5 (95% CI) and for the NLR 0.37 (95% CI).

Discussion

Diagnosis of infection in hip and knee arthroplasty is not accurate. This fact makes it difficult to decide the correct protocol in revision arthroplasty, since it is mandatory to know for sure if the loosening we are revising is septic or aseptic [7]. It is essential to find a valid diagnostic test. Preoperative tests (protein C, ESR, radionuclide images, blood cell count, X-ray, physical examination) are not conclusive [1, 7, 20]. Some intraoperative tests have the same restrictions (Gram, tissue appearance and synovial fluid analysis) [7, 23]. We need a reliable test to detect prosthesis infection. Many authors give excellent validity to the intraoperative frozen section examination [3, 11, 24, 25]; nevertheless, we have found some controversy [7, 8,

Table 1 Knee results. *TP* true positive, *FP* false positive, *FN* false negative, *TN* true negative

Cultures			
PMN	Positive	Negative	Total
Positive	TP=16	FP=4	20
Negative	FN=8	TN=35	43
Total	24	39	63

Table 2 Hip results. *TP* true positive, *FP* false positive, *FN* false negative, *TN* true negative

Cultures			
PMN	Positive	Negative	Total
Positive	TP=4	FP=0	4
Negative	FN=4	TN=75	79
Total	8	75	83

[10]. We reviewed our series of patients to reach reliable conclusions in our daily practice.

We validated a previously developed diagnostic test in a group of consecutive patients (with universally applied “gold” standard), so the type of study is level I (level of evidence). Both tests were done by different, independent investigators who knew nothing about the study. These were also different from the investigators who designed the study and treated all the patients of the group. The statistical analysis was done by an independent person.

There were 146 cases. We included all patients who underwent hip or knee arthroplasty revision surgery during the period studied, so we do not have selection bias. The intraoperative sample examination (pathologists), the culture (microbiologists), the statistical analysis (epidemiologists) and the surgeons were independent of one another.

In the first part of the work, we assessed the validity of the frozen section examination comparing intraoperative results with postoperative final culture results. We need to know for certain that the prosthesis will be implanted in a non-infected field. If the test we use shows infection (positive test), the infection should exist (positive culture), so the test would have no false positives. That means that we have a test with high specificity. That will enable us to use the protocol of infection arthroplasty in all of the infected patients. This is a good point, however, if a non-infected patient is treated as an infected patient (increasing false positives diminishes the specificity) this is a bad situation. The key during revision surgery is that the non-infected patient should have a negative test and false negative cases should not exist, indicating that the sensitivity is very high. It would be catastrophic to treat an infected patient as a non-infected one because the test used was negative (false negative, because the postoperative culture will reveal the infection). Although we need a specific test, the goal is to have a very sensitive test. In the knee group the specificity is acceptable (>75%), but the sensitivity is not good enough. The PPV and the NPV are acceptable and similar to the results found in other series (Table 3). This lack of correlation among SP, SE and predictive values results from of the relatively low prevalence of infection in this group. In the hip group the specificity is very good (>75%), but the sensitivity is not good enough. The PPV and the PNV are very good and similar to the results found in other series (Table 3).

These not very enthusiastic results are similar to others [2, 8–10, 22] and differ from better results in other publications [3, 11–13, 15, 17, 24, 25]. There is con-

Table 3 Published results (most significant published data). *SE* sensitivity, *SP* specificity, *PPV* positive predictive value, *NPV* negative predictive value

Author	SE	SP	PPV	NPV	Number
Fehring and McAlister [10]	18%	89%			107
Feldman et al. [11]	100%	96%			33
Athanasou et al. [3]	90%	96%	88%	98%	106
Lonner et al. [17]	84%	99%	89%	98%	175
Pace et al. [24]	93%	95%			18
Abdul-Karim et al. [2]	43%	97%			64
Pandey et al. [25]	100%	97%	92%	100%	602
Della Valle et al. [9]	25%	98%	50%	95%	62
Banit et al. [8]	67%	93%	67%	93%	121
Musso et al. [22]	50%	95%	60%	92%	45

troversty over the validity of the frozen section examination to diagnose infection in revision surgery.

Our results of SE, SP, PPV and NPV allow us to validate or not to validate the frozen section test in our hospital and with our patients. Our relatively low prevalence of infection influences the results (9.6% in the hip group and 38.1% in the knee group). To eliminate this influence, we calculated the likelihood that is not prevalence dependent.

In the knee group the PLR was 6.5, which is a good result (good results are considered when the PLR is over 5). The NLR was 0.37 (good results are considered when the NLR is below 0.1). In the hip group, the PLR was infinite (because the specificity was 1) and the NLR was 0.5.

We can conclude that intraoperative examination of polymorphonuclear cells in frozen section samples in hip and knee arthroplasty revision surgery is highly reliable, and when the test is positive the arthroplasty is infected. However, when it is negative, we cannot rely on the test. So our goals have not been achieved, leaving us in doubt when the test is negative. For the decision, we should keep in mind the results obtained in the other preoperative and postoperative tests.

We found differences between the hip and knee groups. The sensitivity is higher with statistical significance in the knee group. In contrast, the specificity is statistically higher in the hip group. As others have reported [6], we do not know the reason for that.

To assess the quality of frozen section tests, in the knee group we compared the results of the intraoperative test with the histological examination in paraffin postoperatively. We found an increase in the sensitivity from 66.7% to 91%. The specificity decreased by 3%. The PPV increased 1 point and the NPV increased 13 points. The PLR was 7 and the NLR was 0.11 ($p < 0.05$). Better results, nevertheless, it is not possible to perform the paraffin test intraoperatively and although valid is not useful for intraoperative diagnosis. The quality of frozen sections is lower than the paraffin samples. Only a few publications found identical results when comparing frozen sections and paraffin [11]. In our study we found increasing SE and NPV ($p < 0.05$), which indicates that paraffin is more

accurate, as have others [25]. We found higher infection rates than others, with general positive tests below 10% [7].

Differences appeared among authors. The culture of intraoperative samples, the gold standard, has false positives secondary to sample contamination and false negatives in patients treated with an antibiotic preoperatively [18]. Our cases received intravenous antibiotics at the beginning of the surgery according to our antibiotic prophylaxis protocol. We do not know if this fact has increased the rate of false negatives (diminishing the rate of positive cultures).

In the first publications of the 1970s, authors described a qualitative histology difference between the inflammatory response in aseptic loosening and the response observed in the aseptic loosening [4, 5]. In retrospective reports, offering quantitative data [21], intervals ranged from five [11, 17] to ten [7, 8] per high power field. This carries a statistical consequence: the fewer the number of cells needed for diagnosis, the greater the sensitivity will be. So the goals for the test would be achieved. This may be the cause of the low sensitivity of the test in this review.

The place where samples are taken exerts an influence; they should be obtained from highly suspicious areas and divided into two fragments, one for culture and the other for histology. The distribution of polymorphonuclear cells is heterogeneous. So the first examination of the sample in a low-power field is the key to finding the inflammatory areas for diagnosis [25]. No evidence has been found of a correlation between other groups of inflammatory cells and infection [25]. Most authors did not obtain significant differences of validity between groups, with the exception of Banit et al. [8].

The conclusion reached is that the analysis of polymorphonuclear cells in frozen section samples of periprosthetic soft tissues examined with the criteria established in this work is a reliable test for the diagnosis of septic loosening when the test is positive. If the test is negative, the final decision of the surgeon will rest not only on frozen section analysis, but also on other preoperative and intraoperative tests. It is a quick test and we recommend it for the protocol of diagnosis in prosthetic revision surgery bearing in mind its limitations.

For future studies, we are planning to decrease the required number of polymorphonuclear cells necessary for the diagnosis of infection in order to increase the sensitivity.

References

1. Aalto K, Osterman K, Peltola H, Rasanen J (1984) Changes in erythrocyte sedimentation rate and C-reactive protein after total hip arthroplasty. *Clin Orthop* 184:118–120
2. Abdul-Karim FW, McGinnis MG, Kraay M, Emancipator SN, Gold V (1998) Frozen section biopsy assessment for the presence of polymorphonuclear leukocytes in patients undergoing revision of arthroplasties. *Mod Pathol* 11:427–431
3. Athanasou NA, Pandey R, De Steiger R, Crook D, Smith PM (1995) Diagnosis of infection by frozen section during revision arthroplasty. *J Bone Joint Surg Br* 77:28–33
4. Bullough PG (1973) Tissue reaction to wear debris generated from total hip replacements. In the hip. *Proceedings of the First Open Scientific Meeting of The Hip Society*. Mosby, St. Louis, MO, pp 80–91
5. Charosky CB, Bullough PG, Wilson PD Jr (1973) Total hip replacement failures. A histological evaluation. *J Bone Joint Surg Am* 55:49–58
6. Cherney DL, Amstutz HC (1983) Total hip replacement in the previously septic hip. *J Bone Joint Surg Am* 65:1256–1265
7. Della Valle CJ, Zuckerman JD, Di Cesare PE (2004) Periprosthetic sepsis. *Clin Orthop* 420:26–31
8. Banit DM, Kaufer H, M Hartford JM (2002) Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop* 401:230–238
9. Della Valle CJ, Bogner E, Desai P, Lonner JH et al (1999) 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* 81:684–689
10. Fehring TK, McAlister JA (1994) Frozen histologic section as a guide to sepsis in revision joint arthroplasty. *Clin Orthop* 304:229–232
11. Feldman DS, Lonner JH, Desai P, Zuckerman JD (1995) The role of intraoperative frozen sections in revision total joint arthroplasty. *J Bone Joint Surg Am* 77:1807–1813
12. Grobbelaar CJ (1975) *Proceedings: Hip replacement: frozen section as an additional method of detecting latent infection*. *J Bone Joint Surg Br* 57(2):259
13. Hugues PW, Salvati EA, Wilson PD, Blumenfeld EL (1979) Treatment of subacute sepsis of the hip by antibiotics and joint replacement: criteria for diagnosis with evaluation of twenty-six cases. *Clin Orthop* 141:143–157
14. Johnson JA, Christie MJ, Sandler MP (1988) Detection of occult infection following total joint arthroplasty using sequential technetium bone scintigraphy and indium-111 WBC imaging. *J Nucl Med* 29:1347–1353
15. Kataoka M, Torisu T, Tsumura H, Yoshida S, Takashita M (2002) An assessment of histopathological criteria for infection in joint arthroplasty in rheumatoid synovium. *Clin Rheumatol* 21:159–163
16. Levitsky KA, Hozack WJ, Balderston RA et al (1991) Evaluation of a painful prosthetic joint: relative value of bone scan, sedimentation rate and joint aspiration. *J Arthroplasty* 6:237–244
17. Lonner JH, Desai P, Dicesare PE, Steiner G, Zuckerman JD (1996) The reliability of analysis of intraoperative frozen sections for identifying active infection during revision hip or knee arthroplasty. *J Bone Joint Surg Am* 78:1553–1558
18. Maale G (2004) Aseptic versus septic failures in total hip arthroplasties- difficulty in detection of bacterial biofilm. *J Bone Joint Surg Br* 86:7
19. McDonald DJ, Fitzgerald RH Jr, Ilstrup DM (1989) Two-stage reconstruction of a total hip arthroplasty because of infection. *J Bone Joint Surg Am* 71:828–834
20. Merkel KD, Brown ML, Dewangee MK, Fitzgerald RH Jr (1985) Comparison of indium-labeled-imaging with sequential technetium-galium scanning in the diagnosis of low-grade musculoskeletal sepsis. A prospective study. *J Bone Joint Surg Am* 67:465–476
21. Mirra JM, Amstutz HC, Matos M, Gold R (1976) The pathology of the joint tissues and its clinical relevance in prosthesis failure. *Clin Orthop* 117:221–240
22. Musso AD, Mohanty K, Spencer-Jones R (2003) Role of frozen section histology in diagnosis of infection during revision arthroplasty. *Postgrad Med J* 79:590–593
23. O'Neill DA, Harris WH (1984) Failed total hip replacement: assessment by plain radiographs, arthrograms, and aspiration of hip joint. *J Bone Joint Surg Am* 66:540–546
24. Pace TB, Jeray KJ, Latham JT Jr (1997) Synovial tissue examination by frozen section as an indicator of infection in hip and knee arthroplasty in community hospitals. *J Arthroplasty* 12:64–69
25. Pandey R, Drakoulakis E, Athanasou NA (1999) An assessment of the histological criteria used to diagnose infection in hip revision arthroplasty tissues. *J Clin Pathol* 52:118–123