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Original Article PATHOLOGY

Should we rely on frozen section during the reimplantation stage of revision knee arthroplasty?

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ABSTRACT

OBJECTIVE: To compare Frozen Section (FS) results during the reimplantation stage of revision knee arthroplasty, in patients without clinical signs of infection but with preoperative inconclusive serum inflammatory markers.

METHODS: Sections were revisited the day after surgery. Intraoperative FS (iFS) was accepted as positive when the presence of >5 polymorphonuclear neutrophils (PMNLs) in 5 separate high-power fields was determined according to the consensus criteria of the International Consensus on Musculoskeletal Infection. The clinical outcomes, cultures and diagnostic values of iFS and review FS (rFS) were analyzed.

RESULTS: No complications developed after reimplantation in 66 (84.6%) of the 78 evaluated patients. Complications developed in 12 patients, six of whom were treated with re-explantation, four with arthrodesis and two with above-the-knee amputation. Both iFS and rFS yielded insignificant sensitivity and specificity (25% and 45.5%, 25% and 45%, respectively). There was no statistically significant difference between definitive culture and iFS and rFS.

CONCLUSION: iFS evaluation is insufficient to exclude recovery from periprosthetic joint infection (PJI). Diagnosis of recurrence of infection in patients with indefinite serum inflammatory markers between the explantation and reimplantation interval remains challenging due to massive fibrosis that makes proper tissue sampling difficult. The attending physician should closely monitor clinical findings.

Keywords: C-reactive protein; erythrocyte sedimentation rate; frozen; reimplantation.

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Both the diagnosis and treatment of periprosthetic joint infection (PJI) are major challenges in orthopaedic surgery. Currently, the most widely accepted treatment for PJI is two-stage replacement arthroplasty, in which careful surgical debridement using a cement spacer loaded with antibiotics (AB) is followed by long-term (at least 6 weeks) (iv) AB therapy to eliminate the infection. The Musculoskeletal Infection Society (MSIS) criteria recommend serum, synovial fluid and histological specimens for diagnosis [1]. Although clinical suspicion with various intra-articular or serum infection markers or soft tissue samples can be used to rule out previous PJI, there is no established method that accurately discounts infection [2].

Recent technological advances allow the use of synovial C-reactive protein, α-defensin, IL-6, TNF-α, or procalcitonin to help the treating surgeon decide the effect of the first stage and dominate the last stage. However, the traditional methods to detect possible ongoing PJI such as aspiration, tissue culture (frozen section - FS) and serum infection markers are still used worldwide due to the simplicity and technical availability of these evaluations.

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The most commonly used method is serum markers. In the absence of clinical signs, sustained normalization of both erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) is recommended for reimplantation. Although normalization has not been scientifically confirmed in the literature, these markers are commonly used to monitor response to treatment in the outpatient setting [3]. MSIS suggests the conventional thresholds of 30 mm/hour and 10 mg/L for ESR and CRP, respectively. Nevertheless, surgeons are frequently confronted with borderline cases in which serum ESR and CRP results are equivocal and this complicates the decision of whether or not to initiate the second phase [1]. Similarly, in an excellent meta-analysis by Huerfano et al. [4], it was reported that with an ESR of <30 mm/h and a CRP of <10 mg/L, the likelihood of eradication of infection is quite high, but it was concluded that additional testing is needed in cases exceeding these values. However, this statement may differ in acute and chronic PPJI. Although Alijanipour et al. [5] claimed that the optimal threshold for CRP in knees should be 23.5 mg/L, quite different from the rest of the literature, they noted that this value may be different in acute and late chronic situations.

As a rapid and inexpensive method, fresh frozen soft tissue analysis (FS) is often used intraoperatively with high specificity but inconsistent sensitivity (SE) ranging from 18% to 100%. It is mostly used to rule in an infection rather than rule it out. Outcomes depend on both the surgeon and the pathologist and there is a high rate of false-negative results [6–8]. Previous studies have also reported low SE at the time of reimplantation [9].

The aim of this study was to evaluate the effectiveness of FS. Evaluations were made to determine whether iFS (intraoperative frozen section) had diagnostic value in ruling out infection. A secondary aim of the study was to analyze if iFS or reviewed FS (rFS) of the first postoperative day had different sensitivity, specificity, positive and negative predictive values.

MATERIAL AND METHODS

Patient Selection

This retrospective study evaluated the results of revision total knee arthroplasty (R-TKA) due to PJI. Yildirim Beyazit University Faculty of Medicine Ethics Committee reviewed and approved the study design (date 22.03.2017, decision no: 26379996/61). All consecutive patients (n=94) with a PJI diagnosis between March 2009 and January 2014, operated on by the same surgeon (KK) using the Scorpio TS Total Knee Revision System (Stryker®) were included. Patients with negative serum inflammatory markers (n=9) on at least two consecutive visits, those with incomplete data (n=3), and patients with a history of inflammatory arthritis (n=4) were excluded from the study (Fig. 1). The medical records of 78 patients were retrospectively reviewed. Approval was obtained from the hospital IRB, and the study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. All patients gave written and verbal consent to be included in the study.

The 78 patients included in the study comprised 18 males and 60 females with a mean age of 67.2 years (range, 49–80 years). All had a cement spacer impregnated with AB in the joint. The mean time window between the first (explantation and debridement) and second stage (revision) was 13.9 weeks (range 12–17 weeks). The demographic data of the patients are presented in Table 1. During this period, all patients received parenteral AB sensitive to isolated microorganisms from culture me-

Highlight key points

- Intraoperative frozen section evaluation is insufficient to exclude recovery from periprosthetic joint infection.
- The negative predicting values of all kinds of frozen sections have an acceptable value, but sensitivity, specificity and positive predictive values are significantly lower.
- The diagnosis of recurring infection in patients with indefinite serum inflammatory markers in the period between explantation and reimplantation remains challenging.

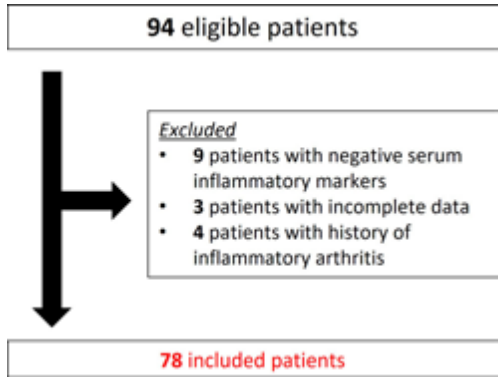

Figure 1. Diagram depicting inclusion and exclusion process of the study patients.

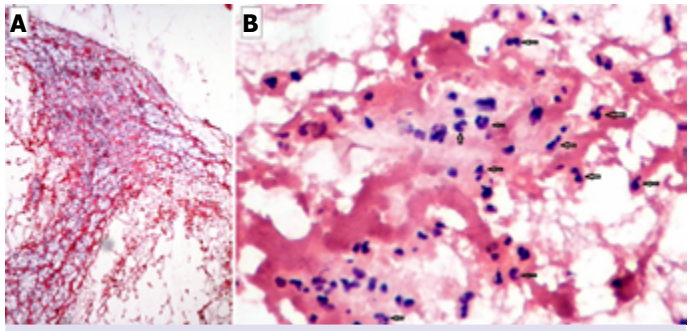

FIGURE 2. Intraoperative frozen section examination (iFS) shows intense chronic inflation within the tissue (x40) **(A)**. At high magnification (x400) 10 polymorphonuclear leukocytes (arrow) were detected **(B)**.

dia obtained from tissue samples taken during the first stage. These microorganisms were methicillin-resistant and coagulase-negative Staph. aureus in 31 cases, methicillin-sensitive and coagulase-negative Staph. aureus in 17 cases, *E. coli* in 5 cases, mixed microorganisms in 10 cases, Pseud. aeruginosa in 3 cases, methicillin-sensitive Staph. aureus in 6 cases and Candida albicans in 6 cases. The duration of AB therapy was at least 6 weeks, and after the end of antibiotic treatment, the patients were followed up at least twice at 3-week intervals to determine acute phase reactants in serum ESR and CRP and peripheral blood cells.

Surgical Procedure and Histological Evaluation

All revision surgeries were performed according to a standard procedure. Reimplantation was planned if the patient had no signs of infection, including pain or discharge from the surgical site, after two consecutive visits and without AB. Careful debridement was performed, and two tissue samples were obtained from both the lateral and medial suprapatellar pouches. These two samples were divided into two equal parts to be sent for fresh FS and microbial analysis. After staining with hematoxylin and eosin, the most inflamed area was traced under low magnification. Subsequently, each of these areas was examined for the number of polymorphonuclear neutrophils (PMNLs) under high magnification (hpf-x400 magnification), excluding both intravascular and peri-synovial PMNLs. All four sections were examined, and the highest number was noted. The Mirra et al. [10] criteria were used to diagnose infection, and the presence of >5 PMNLs per hpf was defined as positive (Fig. 2). Later, the FS from each sample, along with paired samples from the same tissues, were processed into permanent kerosene sections after fixation with formalin, and all were reexamined the following day (rFS) (Fig. 3). All iFS and rFS sections were reviewed separately by two experienced pathologists. Tissue samples from both sections were also cultured for at least 14 days.

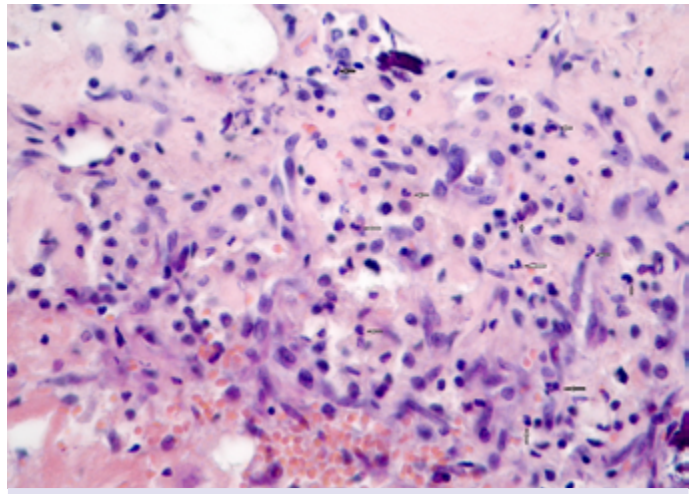

FIGURE 3. Examination of a section of the same tissue (fFS) the following day again revealed (rFS) numerous (>5) polymorphonuclear leukocytes (arrow) in the stroma at high magnification (x400).

TABLE 1. Demographic data of the study group

	Total (n=78)
Gender (%)	
Male	23.1
Female	76.9
Age, Mean±SD	67.2±8.1
Time in-between surgeries, Mean±SD	13.9±3.4
Isolated microorganisms (%)	
Staph. aureus (MR)	39.7
Staph. aureus (MS)	21.8
E. coli	6.4
Preud. aureginosa	3.8
Candida albicans	7.7
Mixed microorganisms	20.6
Preop CRP, Mean±SD	7.8±1.7
Preop ESR, Mean±SD	36.4±10.9
Preop WBC, Mean±SD	7.1±2.2

SD: Standard deviation; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; WBC: White blood cell.

Statistical Analysis

Data analyses were performed using SPSS version 11.5 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as mean±standard deviation (SD) values, and categorical variables as number (n) and percentage (%). An Intra-class Correlation Coefficient (ICC) was calculated to analyze inter-observer reliability. The SE, specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were calculated for both iFS and rFS using culture results as the gold standard. The Chi-square test was used to compare culture results between iFS and rFS. A p value of <0.05 was considered statistically significant.

RESULTS

The mean preoperative ESR, CRP levels and WBC count were 36.38±10.98, 7.75±1.67 and 7.14±2.22, respectively (Table 1).

The overall results of the iFS and rFS were similar. While there were 39 positive or negative common sections, when the permanent sections were examined, 12 negative sections were seen in a subgroup. This discrepancy was evident among permanent sections where 66 positive sections decreased to 54 (12 patients). Both iFS and rFS yielded insignificant SE and SP (50% and 50%, 55.56% and 62.5%, respectively). There was no statistically significant difference between the culture results of iFS and rFS (p=0.103 and p=0.073 respectively). Of these, 18 (69.2%) patients still had positive and 8 (30.8%) had negative culture results for the same microorganisms. Both iFS and rFSs had acceptable SE and SP (94.44% and 37.5%, 100% and 100%, respectively) and although there was no statistically significant balance between culture and iFS (Chi Square test; p=0.72), the match between rFS and culture result was statistically significant (Chi Square test; p<0.0001). Positive and negative predictive values for iFS and rFS were 69.23% and 30.76%, respectively (Table 2).

Of the 78 patients included in this cohort, 66 (84.6%) presented with no complications at the final follow-up examination. Complications were experienced after the second stage in 12 patients. Of these, six patients had undergone the exchange arthroplasty procedure (implant removal, irrigation & debridement, spacer application and iv AB therapy) twice and at the third stage the final revision prosthesis was implanted. Further follow-up revealed no additional complications. Four patients with negative culture results underwent R-TKA at the second stage, but both clinical and serum infection markers revealed recurrence of the infection and thus an exchange arthroplasty procedure was performed again. At the fourth stage these patients underwent R-TKA, but clinical cure could not be obtained and finally knee arthrodesis with intramedullary nailing was performed. Above-the-knee amputation as the fifth surgical procedure was only performed on two patients who had isolated Candida preoperatively after one unsatisfactory exchange arthroplasty and two R-TKA procedures.

Table 2. Data for comparison of Sensitivity, Specificity, and Positive and Negative Predictive values for original FS, permanent FS, Review of original FS and review of permanent FS with definitive culture result

	Culture		
	Positive	Negative	Total
FS			
+	3	36	39
-	9	30	39
Total	12	66	78
PFS			
+	9	57	66
-	3	9	12
Total	12	66	78
R-FS			
+	3	36	39
-	9	30	39
Total	12	66	78
R-PFS			
+	6	48	54
-	6	18	24
Total	12	66	78

FS: Frozen section; P: Permanent; R: Review.

DISCUSSION

This study showed that the result of an intraoperative frozen section evaluation could not definitively diagnose an ongoing infection. Among the methods evaluated to indicate the infection status, rFS was the most valuable test. Furthermore, an acceptable satisfaction rate (84.6%) was detected in the second phase of replacement arthroplasty among patients without negative serum infection markers.

It is of utmost importance to perform careful irrigation and debridement with removal of the prosthesis during the first stage of PJI. In this way, the joint usually

recovers in the long term with the help of AB and the recovery of infection can be confirmed with negative serum infection markers. However, in some patients, it is quite difficult to decide whether the infection is cured or not based on serum markers alone.

A growing body of literature has focused on additional methods for diagnosing deep PJI. Most of these studies have shown promising results with the use of procalcitonin, TNF-α, synovial CRP, α-defensin, human β-defensin-2 and -3, leukocyte esterase or cathelicidin LL-37 biomarkers [11–13]. Bottner et al. [11] compared the diagnostic value of WBC, CRP, IL-6, procalcitonin and TNF-α and found that a combination of C-reactive protein and interleukin-6 measurement provided excellent screening tests for infection of a deep implant. They suggested that specific markers such as procalcitonin and pre-operative aspiration of the joint could be useful in identifying patients with true positive C-reactive protein and/or interleukin-6 levels.

The exclusive inclusion of patients without negative infection markers in the current study raises the question of whether and to what extent the findings are transferable to patients with at least 6 weeks of negative serum levels of both ESR and CRP. To rule out infection, the cut-off values for ESR, CRP and WBC were accepted as 30 mm/h, 10 mg/L and 10.0x109/L, respectively [1]. Although the current study is underpowered to make a definite statement, with the exception of fungal infection, positive serum CRP and ESR levels are not a contraindication for the second stage of exchange arthroplasty.

Although recent evidence supports the role of histological examination as a crucial parameter in PJI [9, 14], previous studies have accepted FS as a complementary tool for decision making because of potential diagnostic error [15, 16]. It has been shown that the most valuable diagnostic tissue is the pseudomembrane of the bone implant, but it is not possible to obtain it from a joint with spacer [17]. Moreover, the threshold for PMNL counts with an acceptable range of SE and SP is controversial, and the literature indicates a range of 1 to 5 PMNs in 5 to 10 hpf [8, 17, 18].

To confirm low-grade PJI, Thotz et al. evaluated the iFS of periprosthetic membranes according to the consensus of Morawietz et al. and recommended iFS as a diagnostic tool for decision making in replacement procedures [19, 20]. Although a high correlation was found between iFS and rFS, and they had similar cases with inconsistent preoperative findings, the current authors disagree with that conclusion. In the current study, slightly lower values were obtained for SE, SP, PPV, and NPV for iFS. Therefore, these results suggest that intraoperative tissue samples should be used for culture media and reviewed sections can be used to validate and document the diagnosis. This study also differs in terms of iFS accuracy, as Nunez et al. and Buttaro et al. found relatively high SE, SP, PPV, and NPV for FS [6, 14]. Buttaro et al. [6] suggested the use of synovial CRP instead of FS when there is persistent PJI with dry joints, especially in joints with spacers.

There were some limitations to this study, primarily the relatively small number of cases. Second, this study cannot answer the question of how to increase the diagnostic accuracy of iFS instead of rFS, which will be reviewed in the follow-up period and may ultimately affect the subsequent outcome. Third, there were no aspiration or other laboratory tests such as interleukins, procalcitonin, or synovial cytokines but only ESR and CRP were monitored. The accuracy of CRP is well known, but the SE and SP of ESR is low and not accepted as a monitoring tool for response to treatment after deep infection [11]. Currently, the most valuable serological test for patients still using AB at the time of aspiration is a synovial cytokine. Although Xie et al. [12] showed that α-defensin is more sensitive and specific than current diagnostic tools, the significance and cost-effectiveness of the test are still unknown. To date, the statement of Parvizi et al. [17] has not changed. All these new methods require experienced personnel, special equipment, a long time for analysis and the procedures incur higher costs. Finally, two pathologists analyzed the histological sections to determine possible analytical bias. However, interobserver agreement data have been shown to be similar when two to five pathologists are involved in a study [19].

Conclusion

The NPV of all kinds of FS analyses have an acceptable value, but SE, SP and PPVs are significantly lower. Intraoperative FS evaluation is insufficient to exclude recovery from PJI. The diagnosis of recurrence of infection in patients with indefinite serum inflammatory markers during the period between explantation and reimplantation remains challenging due to massive fibrosis that makes proper tissue sampling difficult. The attending physician should closely monitor clinical findings. The question simply remains whether positive CRP and ESR values are dependent on microorganisms with low virulence.

Ethics Committee Approval: The Yildirim Beyazit University Clinical Research Ethics Committee granted approval for this study (date: 22.03.2017, number: 26379996/61).

Authorship Contributions: Concept – AK, KY, NS; Design – AK, KY, NS; Supervision – AK, KY, NS; Fundings – AK, KY, NS; Materials – AK, KY, NS; Data collection and/or processing – AK, KY, NS; Analysis and/or interpretation – AK, KY, NS; Literature review – AK, KY, NS; Writing – AK, KY, NS; Critical review – AK, KY, NS.

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