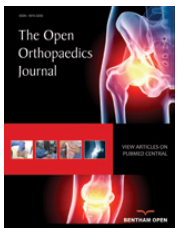

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RESEARCH ARTICLE

Intraoperative Cultures in Reimplantation of a Two-Stage Protocol: Only 1 *vs.* At Least 2 Positive Microbiological Results

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Abstract:

Background:

The main reason for using a two-stage exchange in Prosthetic Joint Infection (PJI) is that bacteria are completely eradicated in reimplantation surgery. However, reports of a positive culture in the second surgery are growing. The number of positive intraoperative cultures and their influence on final results is not well-established.

Objectives:

To compare epidemiological characteristics, infection recurrence and clinical evolution of patients with only one *vs.* at least two positive cultures based on our series of cases with positive cultures in reimplantation surgery.

Material and Methods:

Retrospective study of 55 patients was conducted prospectively. They were diagnosed with chronic PJI, treated with a two-stage protocol and at least three intraoperative cultures were obtained in the second stage. These cultures were negative in 28 patients. Fourteen patients showed two or more cultures with the same microorganism and they were denominated patients with positive cultures. Thirteen patients showed only one positive culture, and they were considered contaminated. Both groups of patients (positive cultures and contaminated ones) received the second cycle of oral antibiotics for 6 months. Functional results were evaluated with the Harris Hip Score (hips) or Knee Society Clinical Rating Score (KSCRS) (knees).

Results:

There were no significant differences between patients with positive or contaminated cultures for age (p=0.420) and sex (p=0.385). The knee was involved in 13/14 positive and in only 6/13 contaminated patients (p=0.013). *Staphylococcus epidermidis* was the predominant isolate, but there were differences between positive (methicillin-resistant in 7/14 patients) and contaminated cultures (methicillin-sensitive in 6/13). There were no differences in the prevalence of polymicrobial cultures (p=0.785) or coincidence with cultures from the first stage (p=0.257). Three infection recurrences have appeared in patients with positive cultures (3/13, 21%) and none in patients with contaminated cultures. There are no differences in HSS or KSCRS when comparing final functional results between groups (p=0.411).

Conclusion:

The prevalence of positive cultures in reimplantation surgery is higher than expected (25%), and more frequent in women and in knee arthroplasties. The most frequently involved microorganism is *Staphylococcus epidermidis*, but antibiotic sensitivity varies between patients with positive cultures (methicillin-resistant) and those with contaminated cultures (methicillin-sensitive). There were no infection recurrences in patients with contaminated cultures, but those with positive cultures present a risk of over 20%.

Keywords: Prosthetic joint infection, Two-stage exchange arthroplasty, Reimplantation culture, Clinical evolution, *Staphylococcus epidermidis*, predominant isolate.

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1. INTRODUCTION

Two-stage exchange is the most used treatment for chronic Prosthetic Joint Infection (PJI). The main advantage of a two-stage protocol is that infection is supposedly cured when reimplantation is done. Infection eradication can be evaluated by different diagnostic methods: serological markers [1 - 6], scintigraphy [4], preoperative aspiration [5, 7], or intraoperative histology of frozen samples [8]. Despite these tests, some

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patients present positive intraoperative cultures in their second surgery for reimplantation.

Few authors have studied the prevalence of positive cultures in the second surgery, or the epidemiology and final clinical result in these patients [9 - 13]. Moreover, antibiotic-loaded cement spacer is a foreign body and may increase the risk of infection persistence [9, 11 - 13].

The aims of this study are: 1) To analyze epidemiological characteristics of patients with positive intraoperative cultures in reimplantation surgery; and 2) To compare infective, functional and radiographic results in patients with positive intraoperative cultures *vs.* those with a single contaminated culture.

2. MATERIALS AND METHODS

2.1. Design

Retrospective study of a case series followed prospectively in the outpatient clinic.

2.2. Inclusion Criteria

[1] Patients diagnosed with PJI according to the Musculoskeletal Infection Society criteria [14].
[2] Late/chronic PJI (more than 3 months after the index surgery) [15].
[3] Treatment with a two-stage protocol.
[4] At least 3 intraoperative cultures obtained during the second surgery (reimplantation).

2.3. Exclusion Criteria

Patient non-compliance with antibiotic treatment; second stage not performed (patient rejection, worsening of medical condition, and/or unacceptable anesthetic or surgical risks); inadequate follow-up.

2.4. Ethics

All patients included gave written and oral informed consent for the inclusion of their clinical data (not personal ones) for the study. Confidentiality for personal data has been maintained during the entire study. This study was approved by the Local Ethics Committee with the number 3261 on December 14th, 2017.

2.5. First Surgery

All patients received a first stage surgery with removal of all implants and cement, aggressive debridement and irrigation with 12 liters of saline supplemented with 120 ml of iodine (Betadine, Viatris Manufacturing, Mundipharma AG, CH). No spacer was used in any hip, so patients remained in a temporary Girdlestone resection-arthroplasty. Knees were implanted with a static hand-made spacer manufactured with Copal cement (polymethylmetacrylate with gentamicin 1g and clindamycin 1 g, Biomet Merck GmbH, Ried b Kerzers, CH).

2.6. Antibiotic Treatment

After the first stage (removal surgery) patients were treated with a combination of two oral antibiotics for six months [2]. Antibiotics were selected taking into account the sensitivity in antibiograms, as well as their activity inside biofilm and against intracellular bacteria. Those used were: linezolid, rifampin, ciprofloxacin, levofloxacin, fosfomicin, doxycycline and amoxicillin-clavulanic acid. Prescribed doses were the maximum recommended, but not adjusted to individual weight (Table **1**). Hematopoietic, renal and hepatic function, as well as CRP and ESR, were monitored monthly with blood analysis.

Table 1. Antibiotics and doses used for oral treatment.

Antibiotic	Dose
rifampin	300 mg / 8h
ciprofloxacin	750 mg / 12h
levofloxacin	500 mg / 12h
fosfomicin	1000 mg / 8h
doxycycline	100 mg / 12h
linezolid	600 mg / 12h
amoxicillin-clavulanic acid	875 mg / 8h

2.7. Second Stage (Reimplantation)

This was delayed until clinical and serological normalization (healing of wound, no inflammatory signs, ESR<30 mm/h and CRP<0.8 mg/L). At the beginning of this surgery, three to eight tissue samples were obtained for microbiological culture from those areas with the worst macroscopic aspect (including at least one synovial sample and one sample from each interphase). A second debridement was performed, and a new prosthesis was implanted with Copal cement [2].

2.8. Cultures in Reimplantation Surgery

The cases were divided into two groups. Patients with positive cultures are those who presented the same microorganism isolate in at least two intraoperative cultures from the reimplantation surgery (even if it grew in enrichment media or if it is considered habitual contaminant); patients with contaminated cultures were those with any microorganism growing in only one of the intraoperative cultures. All these micro-organism patients (those with positive cultures and those with contaminated ones) were treated with the second cycle of combined oral antibiotics, selected according to the new antibiogram, for six months, following the same criteria described above [2].

2.9. Clinical Series

From 2002 to 2017, 64 patients were treated with a two-stage protocol in our hospital. Of these 64, nine were excluded because the reimplantation culture results were lost (change from paper to digital history). Among the 55 included patients (Table **2**), 28 showed all cultures as negative in their reimplantation surgery and were not considered for this study. On the contrary, 14 were considered as patients with positive cultures (at least two similar microbiological isolations), and 13 as patients with contaminated cultures (only one positive culture). Patients were followed in the outpatient clinic for clinical, radiographic and serological (CRP and ESR) monitoring at 4, 8, 12, 16, 20 and 24 weeks post-second surgery. Afterward they continued clinical, radiographic and serological controls every six months until the time of writing or their death. No patient has been lost to follow-up.

2.10. Infection Eradication

This was defined as the absence of any clinical, radiographic or serological signs of infection during the entire follow-up. Clinical signs and symptoms taken as suggestive of infection were chronic severe pain, persistent This was defined as the absence of any clinical, radiographic or serological signs of infection during the entire follow-up. Clinical signs and symptoms taken as suggestive of infection were chronic severe pain, persistent

Table 2. Epidemiological characteristics of patients.

Patient	Age	Sex	Joint	Culture 1st Stage	Culture 2nd Stage	Group
1	73	Male	Hip	*Pseudomonas aeruginosa + Micrococcus sp*	Negative	Healed
2	72	Male	Knee	*Enterobacter cloacae+ Strenotrophomona+ Acinetobacter baumanii*	Negative	Healed
3	63	Female	Knee	MRSA	MRSE+ *Enterococcus faecalis*	Positive
4	73	Female	Knee	MSSE	MSSE	Contaminated
5	82	Female	Hip	*Staphylococcus hominis*	Negative	Healed
6	79	Female	Knee	*Enterobacter agglomerans*	Negative	Healed
7	78	Female	Hip	*E.coli* ESBL+ *Micrococcus sp*	Negative	Healed
8	70	Female	Hip	MRSE	Negative	Healed
9	73	Female	Knee	MSSA	MSSE	Contaminated
10	68	Female	Knee	MRSE	MRSE	Positive
11	57	Female	Knee	*Klebsiella pneumoniae*+ MSSE+ *Corynebacterium difteriae*	*Klebsiella pneumoniae*	Positive
12	59	Female	Hip	*Candida parapsilosis*	Negative	Healed
13	77	Male	Hip	MSSE	Negative	Healed
14	78	Female	Hip	*Enterobacter aerogenes* ESBL+ MSSE	Negative	Healed
15	78	Female	Hip	MRSE	Negative	Healed
16	78	Male	Hip	MSSA	MSSE	Contaminated
17	81	Female	Knee	MSSA	MRSE	Positive
18	62	Female	Knee	*Candida parapsilosis+ Streptococcus agalactiae+ Corynebacterium*	MRSE	Positive
19	71	Female	Knee	*Enterococcus faecium*	Negative	Healed
20	79	Female	Knee	*Pseudomonas aeruginosa*	Negative	Healed
21	66	Female	Hip	MRSE	MSSE	Contaminated
22	73	Female	Knee	MRSA+ *Bacillus sp*	MSSE+ *Staphylococcus auricularis*	Positive
23	77	Female	Knee	MRSA	MRSA	Positive
24	74	Male	Knee	MSSE	Negative	Healed
25	47	Female	Hip	MRSA	MSSE	Contaminated
26	57	Female	Knee	MRSE	MRSE	Positive
27	84	Male	Hip	*Escherichia coli* ESBL+ MRSE	Negative	Healed
28	79	Female	Hip	MSSA+ *Klebsiella pneumoniae* ESBL	*Corynebacterium difteriae*	Contaminated
29	79	Female	Knee	MSSE	Negative	Healed
30	77	Female	Knee	*Enterobacter cloacae+ Streptococcus agalactiae+ Providencia stuartii*	*Enterococcus faecium*	Positive
31	65	Male	Knee	*Staphylococcus warnerii*	S. coagulasa negative	Contaminated
32	76	Male	Hip	MSSA	Negative	Healed
33	50	Male	Knee	MRSA+ *Escherichia Coli+ Peptostreptococcus magnus+ Bacteroides fragilis*	Negative	Healed
34	75	Male	Knee	MSSA	MSSE	Contaminated
35	76	Male	Knee	MSSA	MSSA	Positive
36	62	Male	Hip	*Streptococcus agalactiae*	Negative	Healed
37	74	Male	Knee	MRSE+ *Serratia marcescens*	Negative	Healed
38	71	Female	Knee	*Klebsiella pneumoniae*	Negative	Healed
39	57	Female	Knee	MRSE+ atypical *Mycobacterium*	MRSE+ *Cutibacterium*	Contaminated
40	61	Female	Knee	MSSA+ *Staphylococcus lugdonensis*	MRSE	Contaminated
41	75	Female	Knee	*Cutibacterium acnes+ Enterococcus faecium*	Negative	Healed
42	78	Female	Hip	*Pseudomonas aeruginosa*	Negative	Healed
43	69	Female	Hip	MRSE	*Escherichia coli*	Contaminated

(Table 2) contd.....

Patient	Age	Sex	Joint	Culture 1st Stage	Culture 2nd Stage	Group
44	64	Female	Knee	MSSE	MSSE	Positive
45	71	Female	Hip	MSSE	Negative	Healed
46	79	Female	Hip	MSSE	Negative	Healed
47	66	Male	Hip	MSSA	Negative	Healed
48	70	Female	Hip	*Pseudomonas aeruginosa*	*Streptococcus agalactiae*	Contaminated
49	72	Female	Knee	*Serratia marcescens*	Negative	Healed
50	56	Male	Hip	MRSE	MSSE	Contaminated
51	75	Male	Knee	MRSA	Negative	Healed
52	77	Female	Knee	MSSE	MRSE	Positive
53	81	Female	Knee	*Pseudomonas aeruginosa*+ *Enterobacter cloacae*+ MRSE+ *Micrococcus sp*	MRSE	Positive
54	77	Female	Knee	MSSA	Negative	Healed
55	63	Male	Hip	MRSE	MRSE	Positive

ESBL: Extended-spectrum beta-lactamase
MS: methicillin-sensitive; MR: methicillin-resistant
SE: *Staphylococcus epidermidis;* SA: *Staphylococcus aureus*

2.11. Statistical Analysis

A prior calculation of sample size was not performed so as to include as many patients as possible. Independent variables were age, sex, joint, cultures in the first stage surgery, and cultures in the second stage surgery. Dependent variables were infection recurrence by original bacteria, new infection by different bacteria, functional orthopaedic result evaluated by Harris Hip score (HHS) [16] or Knee Society Clinical Rating Score (KSCRS) [17], and radiographic loosening. Pearson and Fisher's tests were applied for qualitative variables and Student's t-test for quantitative variables. Calculations were done using IBM-SPSS Statistics v.24 software. Significance was established at 0.05% ($p \leq 0.05$), and the odds ratios were calculated.

3. RESULTS

The average age of patients with positive cultures (those with at least two positive cultures of the same microorganism in reimplantation surgery) was 69.7 +/- 8.6 years, while that of patients with contaminated cultures (those with only one positive culture) was 66.8 +/- 9.5. This difference was not significant (p= 0.420). The group of patients with positive cultures was formed by 12 females and two males, while those with contaminated cultures were nine females and four males. Again the difference was not statistically significant (p=0.385). On the contrary, PJI was located in the knee in 13/14 positive cultures, but in only 6/13 contaminated cultures; this difference was statistically significant (p= 0.013).

Methicillin-resistant *Staphylococcus Epidermidis* (MRSE) was the predominant microorganism in patients with positive cultures, being isolated in 7/14 (50%) cases (Table **3**). Meanwhile, the predominant bacteria in patients with contaminated cultures were Methicillin-Sensitive *Staphylococcus Epidermidis* (MSSE), cultured in 6/13 (46.2%) (Table **3**). This difference is also statistically significant (p=0.019). On the contrary, there were no significant differences in the prevalence of polymicrobial cultures between patients with positive cultures (5/14) and those with contaminated ones (4/13) (p=0.785).

The bacteria isolated in reimplantation surgery were the same as those identified in the first surgery (persistence of infection) in 9/14 (64.3%) patients with positive cultures and in only 5/13 (38.5%) with contaminated cultures, but this difference was not significant (p=0.257).

There have been three infection recurrences in the entire series. All of them appeared in patients with positive cultures in reimplantation surgery, all developed in knee infections, two were in females and one in a male. No infection recurrence has presented during follow-up in patients with contaminated cultures. This difference is statistically significant (p=0.013).

Table 3. Results of intraoperative cultures in reimplantation surgery.

	Microorganism	Absolute Number	%
Positive cultures	*Enterococcus faecium*	1	7,1
	Klebsiella pneumoniae	1	7,1
	MRSA	1	7,1
	MRSE	7	50,0
	MRSE+ *Enterococcus faecalis*	1	7,1
	MSSA	1	7,1
	MSSE	1	7,1
	MSSE+ *Staphylococcus auricularis*	1	7,1

(Table 3) contd.....

	Microorganism	Absolute Number	%
Contaminated cultures	*Corynebacterium difteriae*	1	7,7
	Escherichia coli	1	7,7
	Streptococcus piogenes	1	7,7
	MRSE	1	7,7
	MRSE+ *Cutibacterium acnes*	1	7,7
	MSSE	6	46,2
	Staphylococcus auricularis	1	7,7
	Staphylococcus hominis	1	7,7

MS: Methicillin-sensitive; MR: Methicillin-resistant
SE: *Staphylococcus epidermidis;* SA: *Staphylococcus aureus*

There have been no radiological loosenings in any of the groups over the course of the whole follow-up.

We have not found significant differences between patients with positive cultures when compared with those with contaminated cultures when functional orthopaedic results are analyzed at the end of follow-up (as evaluated by HHS and KSCRS scores) (p=0.411). Interestingly, patients with polymicrobial cultures presented lower HHS and KSCRS scores than those infected by only one bacteria and this result appeared in patients with positive cultures as well as in those with contaminated ones, but the differences were not significant for either groups (p=0.094 and p=0.186).

4. DISCUSSION

This study has some limitations. The first comes from its retrospective design resulting in the loss of some data (culture results records), but no patient has been lost during the entire follow-up. The second limitation derives from the small number of cases, so the statistical significance is sometimes not reached when data are compared. PJI is a low-frequency complication (0.3% to 2.22% in primary arthroplasties and 5.9% in revision surgery) [18] and is treated with different protocols, so the number of PJI treated with a two-stage protocol is very small. Moreover, even nowadays multiple microbiological sampling in reimplantation surgery is not a frequent practice.

The definition of the infected patient when reimplantation surgery is performed (persistence of previous infection or a new infection) is highly controversial in the literature. We have found 14/55 patients with positive cultures and 13/55 with contaminated cultures (Table **2**), which is a high rate. Published figures are highly variable, ranging from 0% in old papers [10] to 50% in some recent ones [9, 12]. All these results must be analyzed cautiously because of four great differences between published studies. The first is the different definition of the positive *vs.* contaminated case: this has evolved over the years [19], especially after the development of new microbiological techniques such as sonication [12, 20], as well as methods of data interpretations [9]. The second difference between studies is the type of spacer used, or even not used as is the case for the hips in our series. The third difference is derived from the different techniques employed in each local Microbiology Department (for example, use or not of enrichment media). The fourth, and possibly the most important, arises from the number and type of microbiological samples obtained and the location from which they are obtained. The patients analyzed in the present series present a high number of samples (up to eight in the most recent cases) and always have bone and synovium samples, as well as sonication results. Other studies include articular fluid and synovial membrane [9], tissue and sonication [11, 12], only sonication [13], swabs from tissues [5] or cement [10]. Perhaps in the future bacterial 16s RNA detection [21] may become standard. Other confounding factors could also influence culture results in reimplantation: aseptic preparation of the surgical field, plastic drapes, double gloves, or the duration and difficulty of the surgery [22].

As far as is known to authors, no previous study has compared the epidemiological characteristics of reimplantation patients with positive cultures *vs.* those with contaminated cultures. Our positive culture cases were most frequent in knee surgeries, and this was statistically significant, while another study [13] reports a greater incidence in hips (70% *vs.* 62%) but without statistical difference. Similarly, our cases with contaminated cultures appear with more frequency in hips (7/13), and again this was significant. A plausible explanation for this difference may come from the use of spacer, a foreign body that liberates high doses of antibiotics over the first days, but afterward may be colonized by a new biofilm composed of resistant organisms [23]. With this hypothesis, not using a spacer (as done in our hips) may actually be a protective factor.

We have also observed a significant difference in the bacteria isolated in reimplantation cultures: MRSE is predominant in patients with positive cultures, while MSSE is predominant in those with contaminated cultures. The same bacteria were cultured in the first and the second stages in 9/14 cases with positive cultures and in 5/13 with contaminated ones: this is clearly a persistence of infection. Different microorganisms were isolated in 5/14 positive cultures and 8/13 contaminated ones: this result may be interpreted as a new infection. Few papers have differentiated persistence and new infection, but the rate of persistence is low (25% to 36,4%) [9, 13].

We have diagnosed three infection recurrences in patients with positive cultures (3/13, 21%) and not one in those with contaminated cultures. Again, no previous study had compared the clinical evolution of patients with positive *vs.* contaminated cultures in reimplantation. Published results are limited to reporting recurrence in patients with positive cultures. Some papers report a low risk of infection recurrence, from 0% to 9% [9 - 11, 24], while others report a very high risk, 24% [5], 50% [12], or up to 63% [13]. This high variability may be explained

by the different treatments applied to these patients. Mariconda *et al*. [11] performed surgical debridement and antibiotics, Cabo *et al*. [9] prescribed six weeks of antibiotics, while Sorlí *et al*. [13] did not apply a protocolized therapy and did not put all their patients on antibiotics, while Nelson *et al*. [12] did not prescribe any treatment except in cases with obvious symptoms.

Another relevant difference between studies is the follow-up period: from one year in some papers [13] to ten years in our series. Patients with a polymicrobial infection have a worse clinical evolution. This is not statistically significant in our series, but has been previously noted [25, 26].

CONCLUSION

Two or more positive cultures in reimplantation surgery appeared in 25% of patients treated for a PJI with a two-stage exchange. Additionally, another 25% of patients presented contaminated cultures because they present only one positive culture in reimplantation surgery. Patients with positive cultures were more frequently women and have suffered a knee infection. The most frequent bacteria in cases with positive cultures were methicillin-resistant *Staphylococcus epidermidis*, while the most frequent in cases with contaminated cultures were methicillin-sensitive *Staphylococcus epidermidis*. With the treatment protocol applied, there were no infection recurrences in patients with contaminated cultures, but those with positive cultures presented a risk of over 20%.

LIST OF ABBREVIATIONS

CRP = C reactive protein

ESR = Erytrocite sedimentation rate

HHS = Harris Hip Score

KSCRS = Knee Society Clinical Rating Score

MSSE = Methicillin-sensitive *Staphylococcus epidermidis*

MRSE = Methicillin-resistent *Staphylococcus epidermidis*

PIJ = Prosthetic joint infection

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study has been approved by the Local Ethics Committee with the number 3261 on December 14th, 2017.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

All patients included gave written and oral Informed Consent Term for the inclusion of their clinical data (not personal ones) for the study. Confidentiality for personal data has been maintained along all the study.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

Eduardo Garcia-Cimbrelo is on the editorial board of the Open Orthopaedics Journal.

No more conflict of interest. No Financial disclosure.

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