Implant sonication versus intraoperative tissue sample cultures for Periprosthetic Joint Infection (PJI) of Shoulder Arthroplasty

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Abstract. Introduction: Periprosthetic joint infection (PJI) is the most problematic complications after shoulder arthroplasty. Many diagnostic tools have been identified to find infection, such as hystopatologic examination of tissue sections or cultures of intraoperative tissue. Implant sonication fluid culture showed good results in order to enhance diagnostic accuracy, but literature results are still controversial. Aim of our study is to compare the results of sonication with intraoperative tissue sample cultures. Patients and Methods: From February 2016 to January 2018 we performed 102 revisions of Total Shoulder Arthroplasty (TSA) for suspected PJI. Sixty - five patients respected the criteria for admission to the study and were enrolled. In each case periprostethic specimens were collected and explanted prosthesis were put inside sterile fluid, sonicated and then placed under culture. Results: Among the sixty-five patients, 36 were considered as possible, probable or certain infection. Tissue cultures were positive for infection in thirty - four cases (52,3%) and in nineteen cases was found the positivity for Cutibacterium acnes. Sonication fluid cultures were positive in forty cases (61,5%), with a positivity for Cutibacterium acnes in twenty - seven cases. The sensitivities of sonication and tissue cultures for the diagnosis of shoulder PJI were 83.3% and 88,9% (P = 0,08); the specificities were 65.5% and 93,1% (P < 0.01) respectively. Conclusion: Our results suggest that sonication technique had not shown a clear advantage in postoperative shoulder PJI diagnosis, but it's a real aid to detect Cutibacterium acnes. In any case, sensitivity and mostly specificity were higher with tissue cultures. (www.actabiomedica.it)

Key words: sonication, tissue culture, periprosthetic shoulder infection, Cutibacterium acnes

Introduction

The number of shoulder arthroplasties is constantly increasing worldwide (1). Periprosthetic joint infection (PJI) is one of the most scared complications for physicians after shoulder replacement surgery; the incidence has been reported to be 1,1%(2), increasing up to 15,4% if we consider revision surgery (3). Furthermore it is well known this complication to be a relevant and heavy economic burden, due to the cost of treatments and long hospitalization (4,5). Cutibacterium acnes has certainly a key role inside the matter of shoulder PJI. This Gram- Positive anaerobe is a commensal of the pilosebaceus follicles and it can frequently colonize the deep layers of the skin next to the neck, chest, upper limb and mostly in the axillary region. It is estimated that around 56% of shoulder infections after orthopedic implant involve Cutibacterium acnes, with a greater frequence in male gender (6,7). This bacterium can adhere over the orthopedic implants by the production of a biofilm layer, which make it difficult to eradicate: a combined

approach of prolonged antibiotic therapy followed by a further surgical treatment is often needed (8). It is widely accepted that the only medical management brings poorer results; this approach should be strictly reserved if the patient is unoperable due to an unacceptable surgery risk or his comorbidities (9-12). To diagnose a PJI caused by Cutibacterium acnes is still challenging: its low virulence often causes a subtle presentation, patients' symptoms can be limited to pain or be absent (13), furthermore the negativity for inflammation marker can endure for two years after surgery (14,15). A promptly recognition of PJI due to Cutibacterium acnes becomes crucial for the correct management of the prosthetic implant failure. In order to diagnosticate a PJI caused by Cutibacterium acnes, culture examination of periprosthetic tissue collected intraoperatively is considered to be the gold standard. This methodic implies the risk of false positives (contaminants from the skin) or false negatives (insufficient time for culture). During the years the number of diagnosis for this bacterium have been probably understimated; nowadays it is common opinion that Cutibacterium acnes requires at least 2 weeks of culture to exclude a false negative (16-21). Implant sonication fluid culture emerged as a promising diagnostic tool over the infection cases of orthopedic implants. Despite many authors suggest the use of sonication as a common practice when PJI is suspected (22-26), there is still controversy about this topic.

The aim of our study is to clarify wheter sonication brings benefit in sospicious cases of shoulder PJI through comparison with intraoperative tissue samples cultures.

Patients and Methods

We performed a retrospective case-control study reviewed surgery data of 102 Total Shoulder Arthroplasty Revision from February 2016 to January 2018, with diagnosis of probable infection of prosthetic implant. All patients underwent sonication of implant removed and intraoperative withdrawal of sample tissues. Samples was collected in sterile screwcapped container after sterile gloves changing.

Inclusion criteria were the availability of sonication of the implant, 5 intraoperative periprosthetic sample tissues, no contralateral shoulder prosthesis. Exclusion criteria were: less then two weeks wash out from antibiotics therapy, less than 5 intraoperative periprosthetic samples tissues, no sonication.

Sixty-five patients respected inclusion criteria and were enrolled for the study. The diagnosis of infection has always been carried out using the criteria that have been confirmed for years by the Committee on Periprosthetic Shoulder Infections of ICM (27).

The presence of one of the following major criteria poses a certain diagnosis of infection:

Presence of a sinus tract

Presence of intra-articular pus.

Presence of 2 positive cultures with phenotypically identical virulent organisms.

The presence of minor criteria (Table 1) allow to stratify the risk of infection in the absence of the feedback of the main criteria. Based on the score obtained by the positivity for the individual tests:

6 or greater with identified organism indicates probable PJI

Minor Criteria	Weight
Single positive tissue culture with virulent organism	3
Single positive tissue culture with low-virulence organism	1
Second positive tissue culture (identical low- virulence organism)	3
Unexpected wound drainage	4
Positive frozen section (5 PMNs in 5 high-power fields)	2
Positive preoperative aspirate culture (low or high virulence)	2
Elevated synovial neutrophil percentage (>80%)	2
Elevated synovial WBC count (>3000 cells/mL)	2
Elevated ESR (>30 mm/h)	2
Elevated CRP level (>10 mg/L)	2
Elevated synovial a-defensin level	2
Cloudy fluid	2
Humeral loosening	3

Table 1. Minor criteria for definition of shoulder PJI. PMN, polymorphonuclear leukocyte; WBC, white blood cell; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

6 or greater without identified organism indicates possible PJI

Fewer than 6:

- Single positive culture with virulent organism indicates possible PJI

- 2 positive cultures with low-virulence organism indicates possible PJI

 Negative cultures or only single positive culture with low- virulence organism indicates an unlikely PJI.

During revision surgery, at least 5 tissue specimens were collected, taken from different sites and with greatest suspicion for a local infection. The prosthetic implant, once explanted, was placed inside a sterile continent and subsequently subjected to sonication. The administration of perioperative antibiotics has always been carried out after tissue samples have been taken.

Sonication method

The prosthesis sample, for proper sonication, must be completely plunged in the Brain Heart Infusion broth (BHI) or physiological liquid. The following steps will be carried out in the laboratory:

Incubate the material at 37 °C for at least 30 minutes;

Check that the caps of the containers are tightly closed

Seal the caps of the containers with parafilm

Sonication for 5 minutes with preset program

Vortex for at least 30 seconds

To sow with the 10 μ L loop

Aerobic culture: BHI enrichment broth and agar blood (COS) incubated in aerobiosis, chocolate agar (PVX) in CO2. Anaerobic culture: Thioglycollate enrichment broth, blood agar (COS), Schadler agar (SCS) and Schadler agar+ antibiotic mixture (SNVS) incubated in anaerobiosis.

After 24/48 hours and daily up to 7 days of incubation, all enrichment broths and plates are evaluated, if there is bacterial growth, identification and antibiogram of the colonies are carried out and the microorganisms will be reported with semi-quantitative charge (positive after enrichment only if the growth has occurred from BHI broth).

On the 14th day if no growth is highlighted on the plates or clouding of the enrichment broth a subculture from the BHI broth is carried out on a blood agar plate. In the absence of growth, the sample is reported negative.

Statistical Analisis

Descriptive statistical analysis was performed through simple and double frequency tables. Paired dichotomous variables (positive/negative outcome of the two methods) were compared with the McNemar test, while concordance was calculated with Cohen's kappa. The quantitative variables (number of bacteria detected with the two methods) were compared with the Wilcoxon test for paired data. For all tests the significance threshold was 0.05. The scan was performed with the STATA 14.2 software.

Patients considered as a certain, probable or possible infection were placed within a single group of patients (PJI), patients with an unlikely diagnosis of periprosthetic infection were placed in the group of the no infected (NPJI). The results of colture sample tissues was used to define the state of infection, as indicated by the criteria listed by the MCA. Sensitivity and specificity were derived from tissue culture data and sonication fluid culture inserted into a table 2-by-2 contingency tables. To assess sensitivity and specificity obtained for each method we performed the Chi-quadro test.

Results

Among the 102 revisions of total shoulder arthroplasty, 65 patients satisfied the criteria for eligibility during the period under examination.

Tirty-six patients (55.4%), according to the MCA criteria for the diagnosis of prosthetic joint, were considered as possible, probable or certain infections. In Twenty-nine patients (45.6%) the diagnosis of infection was excluded and considered unlikely. Within the group of patients diagnosed with infection, 88.9% (32/36) have a positive result in sample tissue culture and 83.3% (30/36) positive sonication for at least one bacterium. Considering all the elegible patients, the sample tissue culture was positive for 52.3% (34/65), while sonication was positive for 61.5% (40/65) (Table 2).

Contincency table	Positive tissue coltures exam	Negative tissue coltures exam	Positive fluid sonication exam	Negative fluid sonication exam
РЈІ	32 (49,2%)	4 (6,2%)	30 (46,2%)	6 (9,2%)
NPJI	2 (3,1%)	27 (41,5%)	10 (15,4%)	19 (29,2%)

Table 2. Summary of sonication and tissue cultures within 2-by-2 respective contingency tables. PJI group considers certain, possible and probable diagnosis of infection; NPJI group considers unlikely diagnosis of infection.

Cutibacterium acnes was the most frequently isolated pathogen for both the methodics esaminated. More specifically it was identified by sample tissue culture in 55.9 % (19/34). Others patogens were: Staphilococcus epidermidis (13/34, 38.2%), Staphilococcus aureus (5/34, 14.7%), Enterococcus faecalis (3/34, 8.8%), Peptostreptococcus magnus (2/34, 5.9%), Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa (1/34, 2.9%). Cutibacterium acnes is identified by sonication in 67.5% (27/40). Others were Staphilococcus epidermidis (13/40, 32.5%), Staphilococcus aureus (4/40, 10%), Staphilococcus warneri (3/40, 7.5%), Enterobacter cloacae, Escherichia coli, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa and Streptococcus parasanguinis (1/40, 2.5%) (Table 3).

Cutibacterium acnes has been identified in twenty-seven patients through sonication (66.7% in PJI group) and in nineteen patients through samplet tissues culture (100% in PJI group). In PJI group, Cutibacterium acnes was identified in 63.9% cases.

Polymicrobial infections were identified in sixteen patients (24.6%), 9 through Sonication, 10 through sample tissues culture; no method was simultaneously negative. In seven cases, sonication has identified at least one pathogen more than the culture; in eight cases, sample tissues culture has identified al least one pathogen more than sonication.

The sensitivity of culture examination from sonication fluid was 83.3%; in standard sample tissues culture examination was 88.9% (P=0.08). Specificity 65.5% for sonication and 93.1% for sample tissue culture (P<0.01). Matching the two methodics in the diagnosis of infection, sensitivity was 94.4% while specificity was 58.6% (Table 4).

The results of sensitivity, specificity, positive and negative predictive value and accuracy of the different methods are summarized in Table 5.

Discussion

The Second International Consensus Meeting on Muscoloskeletal Infection (ICM 2018) discussed the role of sonication for the diagnosis of shoulder PJI: only two studies regarding this topic have been identified (28,29). Because of the contrasting results of this very limited literature, the ICM definied as "unclear" the usefulness of sonication during shoulder PJI process. A special focus is given to the Cutibacterium acnes due to its frequent involvement in shoulder infections. A missed diagnosis can lead to the relapse of the infection or the failure of a new implant; these problematic situations bring to high cost for national healthcare, long therapies for patients and to uncertain results (4). These knowledges have convinced us to use sonication as a tool that could improve our ability to make diagnoses. Our results don't suggest an advantage compared to the sample tissues culture examination (gold standard (27)).

The results of this study clearly show that sonication and tissue cultures are not interchangeable, even if we had similar number of positive cases: not infrequently one diagnostic tool identified at least one more pathogen than the other exam. The specificity of the tissue culture examination showed a statistically significant superiority than sonication, as well as the sensitivity slightly higher. The possible utilisation of sonication as an additional tool in the diagnosis of periprosthetic shoulder infection, despite a slight increase in sensitivity, considerably reduces diagnostic specificity: the risk of improperly treating as infected aseptic failures of prosthetic implants is possible, obtaining a dangerous outcome.

Within the poor literature on this specific topic, studies such as that of Grosso et al. (28) or, more recently, Doruk Akgün et al. (31) affirmed the absence of benefits in use of sonication applied to periprosthetic shoulder infections. Likewise our work does not

Case number (total:65)	Group	Tissue cultures	Sonication cultures	
1	PJI	C. acnes	C. acnes, Staphilococcus epidermidis	
2	NPJI	Negative	Negative	
3	NPJI	Staphilococcus aureus	Negative	
4	NPJI	Negative	Negative	
5	NPJI	Negative	Negative	
6	PJI	C. acnes, Staphilococcus epidermidis	Staphilococcus epidermidis	
7	NPJI	Negative	Negative	
8	NPJI	Negative	Negative	
9	PJI	C. acnes	Negative	
10	NPJI	Negative	C. acnes	
11	NPJI	Negative	Negative	
12	PJI	Staphilococcus epidermidis	Staphilococcus epidermidis	
13	PJI	C. acnes	C. acnes, Staphilococcus warneri	
14	NPJI	Negative	Negative	
15	NPJI	Negative	Negative	
16	NPJI	Negative	C. acnes	
17	PJI	Negative	C. acnes	
18	PJI	Negative	C. acnes	
19	NPJI	Negative	Negative	
20	NPJI	Negative	Negative	
21	PJI	C. acnes	C. acnes Enterobacter cloacae	
22	PJI	C. acnes	C. acnes, Staphilococcus epidermidis	
23	NPJI	Negative	Negative	
24	PJI	C. acnes	C. acnes, Staphilococcus Epidermidis	
25	NPJI	Negative	C. acnes	
26	PJI	Pseudomonas aruginosa	Pseudomonas aeruginosa	
27	NPJI	Negative	Negative	
28	PJI	C. acnes	C. acnes	
29	PJI	Staphilococcus epidermidis	C. acnes, Staphilococcus epidermidis, Staphilococcus aureus	
30	NPJI	Negative	C. acnes	
31	PJI	Staphilococcus epidermidis	C. acnes	
32	NPJI	Negative	Staphilococcus epidermidis	
33	NPJI	Negative	Negative	
34	PJI	Proteus mirabilis	Proteus mirabilis	
35	PJI	Negative	Negative	
36	PJI	C. acnes	Negative	
37	PJI	Staphilococcus aureus, Peptostreptococcus magnus	Staphilococcus aureus	
38	NPJI	Negative	Negative	

Table 3. Summary of the isolated pathogens from tissue cultures and sonication cultures.

(continuous)

Case number	_			
(total:65)	Group	Tissue cultures	Sonication cultures	
39	NPJI	Negative	Negative	
40	PJI	C. acnes, Staphilococcus epidermidis	C. acnes	
41	PJI	C. acnes, Staphilococcus epidermidis	C. acnes, Staphilococcus epidermidis, Streptococcus parasanguinis	
42	PJI	Staphilococcus epidermidis	Staphilococcus epidermidis	
43	NPJI	Staphilococcus aureus	Negative	
44	PJI	C. acnes	C. acnes, Staphilococcus epidermidis, Staphilococcus warneri	
45	PJI	C. acnes, Staphilococcus epidermidis, Enterococcus faecalis	C. acnes, Staphilococcus epidermidis	
46	NPJI	Negative	C. acnes	
47	PJI	Staphilococcus aureus	Staphilococcus aureus	
48	PJI	C. acnes	Staphilococcus warneri	
49	NPJI	Negative	C. acnes	
50	NPJI	Negative	Negative	
51	PJI	C. acnes, Staphilococcus epidermidis	C. acnes	
52	PJI	C. acnes	C. acnes	
53	PJI	C. acnes, Staphilococcus epidermidis, Enterococcus faecalis	C. acnes	
54	PJI	Escherichia coli	Escherichia coli	
55	PJI	Staphilococcus epidermidis	Negative	
56	NPJI	C. acnes	Negative	
57	PJI	C. acnes, Staphilococcus epidermidis	C. acnes, Staphilococcus epidermidis	
58	NPJI	C. acnes	Negative	
59	NPJI	C. acnes	Negative	
60	NPJI	Negative	Negative	
61	PJI	Staphilococcus epidermidis	Staphilococcus epidermidis	
62	PJI	Enterococcus faecalis, Peptostreptococcus magnus	Enterococcus faecalis	
63	PJI	C. acnes	Negative	
64	PJI	Staphilococcus aureus	Staphilococcus aureus	
65	PJI	Negative	Negative	

Table 4. Results of combined sonication and tissue cultures within 2-by-2 of	contingency tables
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Contingency table Sonication or tissue cultures positive exam		Sonication and tissue cultures negative exam		
РЈІ	34 (52,3%)	2 (3,1%)		
NPJI	12 (18,5%)	17 (26,2%)		

show any statistically significant benefit with use of sonication in the diagnosis of shoulder PJI.

The work of Piper et al. (29) suggested the usefulness of the method and is cited by the ICM; it should be emphasized, however, that among the criteria used to consider an infected patient within that study there is cultural examination only for a period of up to 7 days: the literature agreed on an average time of two

Diagnostic tool	Sensibility	Specificity	PPV	NPV	Accuracy
Cultural tissues	88,9%	93,1%	94,1%	87,1%	90,8%
Sonication	83,3%	65,5%	75%	76%	75,4%
Cultural e sonication	94,4%	58,6%	73,9%	89,5%	78,5%

Table 5. Summary of sensibility, specificity, PPV (positive predictive value), NPV (negative predictive value) and accuracy from eachdiagnostic tool.

weeks (at least eleven days) to exclude the numerous false negatives due to Cutibacterium acnes, indolent pathogen with long incubation times (16-21).

Kadler et al. have stated that Cutibacterium acnes is the main pathogen in cases of shoulder PJI (6,7); our study confirms this figure (involved in 63.9% of cases), but even in this case sonication has not been higher than in sample culture, reporting in 33.3% a false positive; the sample tissue culture has always been associated with a certain diagnosis of infection within our case studies: we obviously do not consider this figure to be certain, due to the limited number of cases considered within the cohort of patients, but still indicative of greater accuracy of this methodic. The absence of a clear advantage was clear in polymicrobial infections where we have not identified one methodic superior to the other.

Conclusions

An accurate diagnosis is crucial for detection of shoulder PJI. Every tool useful to improve our diagnostic accuracy should be considerated and used. Implant sonication fluid culture proved to be a valid help during shoulder PJI diagnosis but does not replace tissue sample cultures. Cutibacterium acnes confirmed being the most represented pathogen in shoulder PJI and tissue culture has greater specificity rather than sonication in its diagnosis. Major limitation of this study is the relative small cohort of patients, due to the specific topic considered: we believe a wider sample can confirm statystical significance of gathered datas.

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Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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- Accepted: 17 May 2021
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