Year : 2018

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Mariaux Sandrine, 2018, Diagnosis of subclinical infection in prosthetic two-stage exchange: Evaluation of the effect of sonication on antibiotic release from bone cernent spacers and the utility of molecular diagnostics (PCR)

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive http://serval.unil.ch
Document URN : urn:nbn:ch:serval-BIB_6DBBA98A8FCB9

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UNIVERSITÉ DE LAUSANNE - FACULTÉ DE BIOLOGIE ET DE MÉDECINE
Département de l'appareil locomoteur
Service d'Orthopédie et traumatologie

Diagnosis of subclinical infection in prosthetic two-stage exchange: Evaluation of the effect of sonication on antibiotic release from bone cement spacers and the utility of molecular diagnostics (PCR)

THESE

préparée sous la direction du Professeur Olivier Borens

et présentée à la Faculté de biologie et de médecine de l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

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Originaire de Vionnaz (VS)

Lausanne
2018

UNIL | Université de Lausanne

Faculté de biologie
et de médecine

Ecole Doctorale
Doctorat en médecine

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Directeur de thèse	*Monsieur le Professeur* ***Olivier Borens***
Co-Directeur de thèse	
Expert	*Monsieur le Professeur* ***Gilbert Greub***
Vice-Directeur de l'Ecole doctorale	*Monsieur le Professeur* ***John Prior***

la Commission MD de l'Ecole doctorale autorise l'impression de la thèse de

Madame Sandrine MARIAUX

intitulée

Diagnosis of subclinical infection in prosthetic two-stage exchange: Evaluation of the effect of sonication on antibiotic release from bone cement spacers and the utility of molecular diagnostics (PCR)

Lausanne, le 4 mai 2018

pour Le Doyen
de la Faculté de Biologie et de Médecine

Monsieur le Professeur John Prior
Vice-Directeur de l'Ecole doctorale

Diagnostic d'infection persistante lors de changement de prothèses en 2 temps:

- **Evaluation de l'effet de la sonication sur le relâchement des antibiotiques à partir des espaceurs en ciment**
- **Analyses PCRs du liquide de sonication des espaceurs en ciment**

Sandrine Mariaux, Ulrika Furustrand Tafin, Olivier Borens

Introduction: Lorsqu'une infection de prothèse est traitée par un changement en 2 temps, un espaceur en ciment chargé d'antibiotiques est utilisé durant l'intervalle entre l'ablation de la prothèse et la réimplantation d'une nouvelle prothèse. Selon notre expérience, les cultures du liquide de sonication des espaceurs sont le plus souvent négatives. Les 2 objectifs de notre étude sont :

- évaluer si la sonication cause une élution des antibiotiques, menant à une concentration élevée d'antibiotiques dans le liquide de sonication, pouvant inhiber la croissance des bactéries
- investiguer si les analyses PCR améliorent la détection des bactéries dans le liquide de sonication des espaceurs

Méthode: Une étude prospective monocentrique a été effectuée de septembre 2014 à janvier 2016. Les critères d'inclusions étaient les infections de prothèses traitées en 2 temps et l'accord du patient à participer à l'étude. Les espaceurs ont été fabriqués à partir de ciment contenant déjà de la gentamycine, dans lesquels de la tobramycine et de la vancomycine ont été ajoutées. La concentration des différents antibiotiques a été effectuée par spectrométrie de masse. En plus des prélèvements de tissus profonds peropératoires pour analyse microbiologique et de la sonication de l'espaceur, des analyses de PCR à large spectre, spécifique à S. aureus et une analyse multiplex Unyvero ont été effectuées sur le liquide de sonication des espaceurs.

Résultats: 30 patients ont été identifiés (15 prothèses de hanches, 14 prothèses de genoux, 1 prothèse de cheville). Lors de la réimplantation, les cultures des prélèvements de tissus profonds et la sonication de l'espaceur étaient toutes négatives.

La concentration moyenne d'antibiotiques était de 13.2µg/ml, 392 µg/ml et 16.6 µg/ml pour la vancomycine, la tobramycine et la gentamycine, respectivement. D'après le comité européen sur les tests de sensibilité aux antibiotiques (EUCAST), ces concentrations sont plus élevées que les concentrations minimales inhibitrices (CMIs) pour la plupart des bactéries responsables des infections prothétiques.

Les PCRs pan-bactériennes étaient toutes négatives. Les analyses PCR spécifiques à S. aureus étaient positives dans 5 cas. Néanmoins, aucun des 5 cas n'a présenté de persistance d'infection cliniquement en postopératoire. Les analyses PCR multiplex Unyvero étaient positives chez 6 patients. Néanmoins, la quantité de bactéries était très faible. Aucun de ces 6 patients n'a présenté d'infection persistante après réimplantation. Sur 30 patients, nous avons eu 2 cas d'infections persistantes et 4 cas de récurrences d'infections (une bactérie différente de l'infection initiale dans 3 cas et une infection hématogène lié à une infection d'un ulcère diabétique causé par la même bactérie que l'infection initiale).

Conclusion: Les cultures du liquide de sonication des espaceurs sont toutes stériles dans notre série. Les concentrations d'antibiotiques élevées relâchées durant la sonication peuvent expliquer ces cultures négatives (potentiels faux négatifs).

Néanmoins, 3 types de PCR n'ont pas permis de détecter des bactéries lorsque la sonication de l'espaceur était négative. Dans notre étude, les PCRs ne permettent pas d'améliorer la détection de bactéries et n'aide pas à la prédiction de quel patient présentera une infection persistante ou récurrente. Le changement de prothèse en 2 temps avec intervalle court, associé à un espaceur en ciment charges d'antibiotiques est un traitement efficace qui permet d'éradiquer l'infection. En effet, les cultures et les analyses ne permettent pas de détecter des bactéries dans le liquide de sonication des espaceurs.

IVYSPRING INTERNATIONAL PUBLISHER

Journal of Bone and Joint Infection
2018; 3(1): 37-42. doi: 10.7150/jbji.23668

Research Paper

Diagnosis of Persistent Infection in Prosthetic Two-Stage Exchange: Evaluation of the Effect of Sonication on Antibiotic Release from Bone Cement Spacers

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Received: 2017.11.05; Accepted: 2018.01.31; Published: 2018.03.07

Abstract

Introduction: When treating periprosthetic joint infection with a two-stage procedure, antibiotic-impregnated spacers can be used in the interval between prosthetic removal and reimplantation. In our experience, cultures of sonicated spacers are most often negative. The objective of the study was to assess whether that sonication causes an elution of antibiotics, leading to elevated antibiotic concentrations in the sonication fluid inhibiting bacterial growth and thus causing false-negative cultures.

Methods: A prospective monocentric study was performed from September 2014 to March 2016. Inclusion criteria were a two-stage procedure for prosthetic infection and agreement of the patient to participate in the study. Spacers were made of gentamicin-containing cement to which tobramycin and vancomycin were added. Antibiotic concentrations in the sonication fluid were determined by mass-spectometry (LC-MS).

Results: 30 patients were identified (15 hip and 14 knee and 1 ankle arthroplasties). No cases of culture positive sonicated spacer fluid were observed in our serie. In the sonication fluid median concentrations of 13.2µg/ml, 392 µg/ml and 16.6 µg/ml were detected for vancomycin, tobramycin and gentamicin, respectively. According to the European Committee on antimicrobial susceptibility testing (EUCAST), these concentrations released from cement spacer during sonication are higher than the minimal inhibitory concentrations (MICs) for most bacteria relevant in prosthetic joint infections.

Conclusion: Spacer sonication cultures remained sterile in all of our cases. Elevated concentrations of antibiotics released during sonication could explain partly negative-cultured sonicated spacers. Indeed, the absence of antibiotic free interval during the two-stages can also contribute to false-negative spacers sonicated cultures.

Key words: Infection, Two-Stage Exchange, Sonication, Spacer

Introduction

Periprosthetic joint infection is a common complication following joint arthroplasty, estimated at 1 and 2% for total hip arthroplasty and total knee arthroplasty, respectively. As the incidence of prosthetic joint replacements increases, the infection problem is going to remain an important issue in the future.

One of the possible options for the treatment of periprosthetic joint infections is a two-stage exchange procedure. During the interval between removal of the prosthesis and reimplantation, antibiotic-impregnated cement spacers can be used. They have

the advantages of local antibiotic release, dead space management and prevention of soft tissue retraction. However, the spacer can also act as a foreign body and thus be colonized by biofilm forming microorganisms. In the literature, most studies report cases of spacer infection at the second-stage procedure [1-4].

Sonication is a method to take off the bacterial biofilm containing adherent microorganisms on a prosthetic implant by ultrasound. By this process, the bacteria return to a planktonic state, can be incubated and analyzed. It increases significantly the sensitivity of bacterial cultures from 61% for standard cultures to 79% after sonication [5]. This is thus a useful method for diagnosis of periprosthetic infection. However, its use for diagnosis of persistent infection at the spacer stage is unclear so far.

According to Nelson et al [1], 50% of their removed and sonicated spacers were infected at the time of the second stage procedure. In the group with positive sonication results, 50% of patients had a re-infection at 2 years follow-up. In our experience, cultures of sonicated spacers were always negative. However, in their series, the interval during the two-stages was longer and antibiotics were suspended 6 weeks before reimplantation. Those two reasons could increase the probability to identify persistent infection.

The objective of the study was to assess whether that spacer sonication causes an elution of antibiotics, leading to elevated antibiotic concentrations in the sonication fluid inhibiting bacterial growth and causing false-negative cultures.

Method

A prospective monocentric study was performed from September 2014 to March 2016 at the Lausanne University Hospital (CHUV). Inclusion criteria were patients who were operated for a periprosthetic joint infection treated with two-stage exchange and who gave their informed consent to the study. The study was approved by the local ethical committee.

The diagnosis of infection was confirmed either by multiple positive periprosthetic cultures and/or, sonication of the prosthesis at the first stage of the procedure. The threshold of ≥50 CFU was defined as positive cultures, being a sign of infection [5]. Moreover, patients with fistula were considered infected even if all cultures samples were negative.

30 consecutive patients were included: 15 total hip arthroplasty (THA), 14 total knee arthroplasty (TKA), 1 total ankle arthroplasty (TAA). 8 patients were female and 22 were male. 8 patients were diabetic (26.6%). Mean age was 66 years old (range 28-85). The bacteria identified were *Staphylococcus epidermidis (8), S. aureus (7), S. capitis (3), Streptococcus dysgalactiae (4), S. milleri (2), S. pneumoniae (1), S. salivarius (1), Enterococcus faecalis (1), Cutibacterium acnes (1), Clostridium celecrescens (1)* and *Campylobacter fetus (1).*

At the first stage of the procedure, the prosthesis was removed and was sent for sonication to the laboratory of microbiology [5-6], Wide debridement was performed collecting at least 2-3 periprosthetic tissues samples which were sent for culture. Then a handmade spacer was formed. For the production of the spacer 40g of the shelf cement containing 0,5gr of gentamycin (Palacos R+G, Hereaus Medical, Berlin, GER) were handmixed with supplemental 1.2g tobramycin and 2g vancomycin. Empiric intravenous antibiotics were administrated postoperatively followed by specific intravenous antibiotics, once the susceptibility tests were available. Rifampin was not introduced before the second stage was completed, in order to avoid development of rifampin-resistant bacteria. Indeed, Achermann et al proved that PJI with high initial bacterial load, inappropriate initial debridement and length of intravenous antibiotics shorter than 2 weeks were risk factors to develop resistance to rifampin. Moreover, in presence of wound discharge or sinus tract, the use of rifampin could select rifampin-resistant skin micro-organisms and could cause surinfection. They also attested that even if rifampin is postponed for several days, it does not alter survival rate of the prosthetic implant. For those reasons, we chose to introduce rifampin only after the second stage, when wound was calm [7].

A short interval from 2 to 4 weeks was chosen for each case; the best time of reimplantation being decided depending on local status (acceptable quality of bone or soft tissue at the time of implant removal), pathogen involved (absence of difficult-to-treat microorganisms such as rifampicin-resistant staphylococci, ciprofloxacin-resistant gram-negative bacteria, fungi) and decreasing of C-reactive protein (CRP) and white cell count, without any strict cut-off value. No antibiotic free period was performed between the 2 stages.

At the second stage, the spacer was removed, a wide debridement was performed and the new prosthesis was implanted. At this stage, cultures of 2-3 samples were done and the spacer was sonicated. For the purpose of the study, concentration of each antibiotic in the sonication fluid was measured.

Our protocol of sonication consists in two minutes at 40kHz using sonication device Bactosonic (Bandelin GmbH, Berlin, Germany). It was based on the protocol published by Trampuz et al, and adapted according to the Microlabs standard operating procedures [5]. A minimum of phosphate buffered

saline (PBS) fluid was poured in the sterile container containing the spacer. The quantity of fluid was depending on the size of the spacer. Unfortunately, the quantity could not be standardized for every case and was not measured systematically. Therefore, the concentration of antibiotics is only indicative for the purpose of the study. It does not imply that the antibiotic concentrations are efficient enough to treat locally the infection. Indeed, these are *ex vivo* antibiotic concentrations and do not represent the local concentration of antibiotics around the spacer in the patient.

After sonication, a sample of sonication fluid was collected under laminar flow for measurement of antibiotic concentration. Antibiotic concentrations in the sonication fluid were determined by liquid chromatography associated with mass-spectometry (LC-MS).

The analysis was performed during the first 3-4 days after sonication and samples were kept at -80 Celsius degrees between the different stages of the procedure.

Results

At reimplantation, cultures of tissue samples and spacer sonication fluid were all negative.

At a mean follow-up of 12.8 months (range from 1 to 24 months), we had two persistent infections: one patient infected with *S. epidermidis* and one patient infected with *methicilin-resistant S. aureus (MRSA).* Four patients had a re-infection (13.3%): one hematogenous THA infection by *S. aureus* caused by diabetic foot ulcer 9 months later, one hematogenous THA infection by *S. aureus* 5 months later and two cases of persistent serous discharge of wound 1 month after reimplantation (1 THA infection by *E. faecalis* and 1 TKA infection by *E. cloacae).* As the bacteria identified were different from the first stage procedure, they were treated by debridement, changing of the mobile part and implant retention. Re-infection appeared between 1 and 8 months after reimplantation (mean: 3.5 months).

In the sonication fluid, median concentrations of 13.2µg/ml (min. 1.4 µg/ml, max 49.2 µg/ml), 39.2 µg/ml (min. 0 µg/ml, max 1068,8 µg/ml), and 16.6 µg/ml (min 0 µg/ml, max 169.7 µg/ml) were detected for vancomycin, tobramycin and gentamicin, respectively (Table 1). The detailed antibiotic concentrations are listed in Table 2. According to the European Committee on antimicrobial susceptibility testing (EUCAST), these antibiotic concentrations released from cement spacer during sonication are higher than the minimal inhibitory concentrations (MICs) for most bacteria relevant in PJI (Table 3). Only one case (Case 7) showed antibiotic concentration for *S. aureus* lower than MIC. Moreover, despite standardized protocol and operative report, tobramycin and vancomycin were not mixed to the Palacos R+G cement.

Table 1. Mean antibiotic concentrations in spacer sonicated fluid

	Gentamicin mg/l	Tobramycin mg/l	Vancomycin mg/l
Median	16.6	392	13.2
Min; Max	2.2; 169.7	4.7; 1068	1.4; 49.2

Discussion

In our serie, the survival rate free-of infection was 80 % at a mean follow-up of 12.8 months. Those results are similar to other studies where survival rates were between 67% and 94% [1],[9],[10],[8],[11]. Four on six infections in our serie were newly acquired infections with different germs compared to the initial infection in 3 cases. Two cases were persistent infections. We can conclude that 28 on 30 cases were true-negative spacer sonication cultures.

From the literature, we already know that bacteria can adhere on cement spacer despite a high load of antibiotics [12]. *In vitro* and *in vivo* studies have shown that antibiotics are released from cement spacers in high concentrations during the first few days after implantation [13-14]. After a peak of antibiotic levels during the few days of spacer implantation, a lower residual antibiotic concentration persists during the following weeks [15-16].

In our study and after explantation of the spacer, the concentrations of antibiotics in the spacer sonication fluid were sufficiently high for microbial growth inhibition of most bacteria responsible for prosthetic joint infection, even if important variability between patients was observed. However, the volume of fluid used in the sonication process was not standardized; those results do not represent the in vivo concentrations. Hendricks et al also demonstrated that sonication tends to increase antibiotic release *in vitro* [17]. The same results were found by Kummer et al. *In vitro* polymethyl methacrylate (PMMA) scaffolds containing antibiotics were stored in 37°C for up to 6 weeks. Sonication increased antibiotics elution, especially during the first 2 weeks. The release was more stable for vancomycin, in comparison with gentamycin that decreased over time. However, all concentrations were above MICs of microorganisms responsible for most frequent PJI infection [18]. Ensing et al added that increased antibiotic release from cement blocks by ultrasounds is active on bacteria in planktonic state as bacteria in biofilms. However, the efficiency of

antibiotics differs depending on the germs. Indeed, S. aureus and *Coagulase-negative staphylococcus* are more susceptible than *Pseudomonas* [19]. However, Clauss et al disagree with the results cited above. In their study *in vitro*, in which PMMA samples were exposed to bacteria for 1-2 days, bacterial growth was not altered by release of antibiotics. They found >500CFU/ml in sonication fluid when *S. aureus* and *E. faecalis* were tested. However, *C. acnes* was influenced by antibiotic release. That phenomenon increased by a longer interval between sonication and time of analysis [20]. This shows that the reaction is different depending on the bacteria involved.

Table 2. Detailed antibiotic concentrations

Patients	Implants	Primary infection	Antibiotics concentrations			Re-infection
			Gentamicin mg/l	Tobramycin mg/l	Vancomycin mg/l	
1	THR	*Streptococcus dysgalactiae*	8.12	64.98	19.216	None
2	TKR	*S. epidermidis*	39.49	185.064	89.733	Persistent infection with cutaneous fistula
3	THR	*methicilin-resistant S. epidermidis*	5.062	7.841	5.652	None
4	THR	*Cutibacterium acnes*	4.916	4.675	3.491	None
5	THR	*methicilin-resistant S. epidermidis*	9.893	16.879	7.148	None
6	THR	*S. aureus*	25.53	37.708	17.398	Re-infection by *S. aureus*
7	TKR	*S. aureus*	1.439	0	0	None
8	THR	*S. epidermidis*	16.134	76.711	81.183	None
9	THR	*methicilin-resistant S. epidermidis*	6.665	7.166	2.489	Re-infection by *Enterococcus faecalis*
10	TAR	*Staphylococcus capitis*	13.152	12.404	5.554	None
11	TKR	*Streptococcus pneumoniae*	8.2	13.9	3.3	None
12	THR	*Enterococcus faecalis*	33	66.6	17.7	None
13	THR	*S. aureus*	49.2	153.3	77.7	None
14	THR	*S. epidermidis*	17	15.6	2.5	None
15	TKR	*Streptococcus dysgalactiae*	22.92	99.721	169.686	Re-infection by *Staph aureus*
16	TKR	*methicillin-resistant S. aureus*	10.716	39.208	16.624	Re-infection by *Enterobacter cloacae*
17	THR	*Streptococcus milleri*	4.65	5.00	2.56	None
18	TKR	*Streptococcus salivarus*	10.36	5.89	2.30	None
19	TKR	*Streptococcus dysgalactiae*	43.42	884.94	285.92	None
20	THR	*Campylobacter fetus*	23.86	25.43	200.94	None
21	TKR	*Streptococcus milleri*	23.63	425.23	2.76	None
22	TKR	*methicillin-resistant S. aureus*	25.85	1068.61	371.35	None
23	TKR	*methicillin-resistant S. aureus*	24.36	751.26	8.99	Persistent infection with cutaneous fistula
24	TKR	*Streptococcus dysgalactiae*	9.79	6.20	213.85	None
25	THR	*S. epidermidis*	42.38	84.92	24.84	None
26	THR	*Staphylococcus capitis*	1.50	17.62	15.62	None
27	TKR	*Clostridium celerecrescens*	8.08	6.18	2.19	None
28	TKR	*methicilin-resistant S. epidermidis*	24.03	460.7	107.29	None
29	THR	*S. aureus*	9.12	16.08	6.53	None
30	TKR	*Staphylococcus capitis*	64.28	1068.8	225.93	None

THR= Total Hip Replacement; TKR= Total Knee Replacement; TAR= Total Ankle Replacement

Table 3. Minimal Inhibitory Concentration (MIC) breakpoint.

	Gentamycin (mg/l)	Tobramycin (mg/l)	Vancomycin (mg/l)
Staphylococcus aureus	1	1	2
Coagulase-Negative Staphylococcus	1	1	4
Streptococcus spp	-	-	2
Enterobacteriae	2-4	2	-
Pseudomonas spp	4	4	-
Enterococcus spp	-	-	4
Corynebacterium spp	1	-	2
Acinebacter spp	4	4	-
Clostridium difficile	-	-	2
Gram positive anaerobes	-	-	2
Cutibacterium acnes	-	-	-
Campylobacter spp	-	-	-

Spp= species; - = not available; Values from "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1. 2017. http://www.eucast.org.

Concerning *in vivo* studies, there is no consensus on the quantity and type of antibiotics needed in spacers. Corona et al. have tested spacers containing different loads of antibiotics, either gentamicin alone, or gentamicin and vancomycin [21]. However, there was no statistically significant difference between different groups in terms of re-infection and complication rate. Those results are similar to the study of Nettrour which showed 88% of survival free of infection, with no difference between groups (tobramycin, vancomycin or both of them; with dose of antibiotics contained in the cement either below or above 4g) [11]. Nevertheless, some authors published that association of antibiotics would be more efficient than one antibiotic alone due to synergic effect [13],[15]. The quantity of antibiotics is then at the discretion of the surgeon.

In our study, the combination of 3 antibiotics was chosen; vancomycin to cover Gram positive infection, tobramycin to cover Gram negative and gentamicin already present in the cement; to be active against all bacteria mostly responsible for prosthetic joint infection, even the more virulent ones. Based on our experience a short interval exchange, once the micro-organism is identified at the first stage procedure, is an acceptable option for eradication of infection. IV- antibiotics were continued during the whole interval between the two stages. From our point of view, this limits the risk of colonization of the cement spacer.

However, even with standardized protocols, local antibiotics concentrations have shown some discrepancy between individuals. Indeed, elution depends on surface area, that is different for each joint and each patient due to the centromedullary width and size of the bones; characteristics and quantity of antibiotics, and characteristics of bone cement (porosity and roughness) [3],[22]. The type of spacer used does not seem to interfere significantly with antibiotic release. In their review, Pivec et al. showed no difference in re-infection rate in articulating versus static spacers in TKA [23]. Moreover, handmade versus prefabricated spacers had similar re-infection rates. The only significant difference was a tendency of increased dislocation, and fracture rate for spacers made intraoperatively by the surgeon [24].

In conclusion, in our study of explanted spacers, antibiotic concentrations released from cement spacer during sonication are high enough to cause culture-negative spacer sonicated fluid. Therefore, sonication of cement spacers does not seem relevant for diagnostic purposes. Indeed, a negative spacer sonication does not confirm that periprosthetic infection is completely cured. However, the absence of an antibiotic free interval in our serie between the two-stages can also contribute to false-negative cultures.

Our study has some limitations. The number of patients is relatively small, although relatively high compared to most studies on spacers sonication. The difference of spacer size between patients and the absence of standardization of the quantity of liquid in sonication are also issues to measure precisely antibiotic concentrations. This explains the variation of antibiotic concentration between patients in our series and this is the reason why our results should not be compared with *in vivo* studies. Indeed, the antibiotic concentrations in our study are not representative of the local antibiotic concentration around the spacer in the patient. The length of follow-up of our study can also be considered too short to highlight low-grade bacteria such as *Cutibacterium acnes* and *Enterococci*. However, the aim of this study was to measure the elution of antibiotics out of the explanted spacer through sonication.

Conclusions

Spacer sonication cultures remained sterile in all of our cases. Elevated concentrations of antibiotics released during sonication could explain at least partly negative-cultured sonicated spacers. Therefore, sonication of cement spacers does not seem relevant for diagnostic purposes. Indeed, a negative spacer sonication does not confirm the absence of periprosthetic infection and does not help to predict which patients will suffer from a persistent infection.

Acknowledgements

This work was supported by RMS (Robert Mathys Stiftung Foundation, a nonprofit institute that support research in Switzerland) (grant of 42'000 Swiss francs) and AOTrauma foundation Switzerland (grant: 6'000 Swiss francs). Curetis provided the material to use the Unyvero system. However, they did not participate to our study.

IRB/Ethical Committee Approval

This study was approved by the independent local ethics committee (Commission cantonale (VD) d'éthique de la recherche sur l'être humain). Protocol 136/15 on 18th July 2014.

Competing Interests

Sandrine Mariaux and Ulrika Furustrand Tafin have no disclosures.

Here are disclosures of Prof. Olivier Borens: paid presentations for Zimmer-Biomet, Medacta, Lima and Heraeus. Unpaid consultant for Medacta, Lima and Zimmer-Biomet. Research support from Matthys, Bonesupport and Lima. Part of orthopaedic publications board of Journal of Bone and Joint Infection. Board member of Swiss orthopaedics, former Board member of European Bone and Joint infection society, President of AOTrauma Switzerland, Trustee AOFoundation.

References

[1] Nelson CL, Jones RB, Wingert NC, Foltzer M, Bowen TR. Sonication of Antibiotic Spacers Predicts Failure during Two-stage Revision for Prosthetic Knee and Hip Infections. Clin Orthop Relat Res 2014;472:2208–14. doi:10.1007/s11999-014-3571-4.

[2] Sorlí L, Puig L, Torres-Claramunt R, González A, Alier A, Knobel H, et al. The relationship between microbiology results in the second of a two-stage exchange procedure using cement spacers and the outcome after revision total joint replacement for infection. J Bone Jt Surg Br 2012;94B:249–53. doi:10.1302/0301-620X.94B2.27779.

[3] Mariconda M, Ascione T, Balato G, Rotondo R, Smeraglia F, Costa GG, et al. Sonication of antibiotic-loaded cement spacers in a two-stage revision protocol for infected joint arthroplasty. BMC Musculoskelet Disord 2013;14. doi:10.1186/1471-2474-14-193.

[4] Schmolders J, Hischebeth GT, Friedrich MJ, Randau TM, Wimmer MD, Kohlhof H, et al. Evidence of MRSE on a gentamicin and vancomycin

impregnated polymethyl-methacrylate (PMMA) bone cement spacer after two-stage exchange arthroplasty due to periprosthetic joint infection of the knee. BMC Infect Dis 2014;14:1–5. doi:10.1186/1471-2334-14-144.
[5] Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of Removed Hip and Knee Prostheses for Diagnosis of Infection. N Engl J Med 2007;357:654–63. doi:10.1056/NEJMoa061588.
[6] Borens O, Yusuf E, Steinrücken J, Trampuz A. Accurate and early diagnosis of orthopedic device-related infection by microbial heat production and sonication. J Orthop Res Off Publ Orthop Res Soc 2013;31:1700–3. doi:10.1002/jor.22419.
[7] Achermann Y, Eigenmann K, Ledergerber B, Derksen L, Rafeiner P, Clauss M, et al. Factors associated with rifampin resistance in staphylococcal periprosthetic joint infections (PJI): a matched case-control study. Infection 2013;41:431–7. doi:10.1007/s15010-012-0325-7.
[8] Mortazavi SMJ, Vegari D, Ho A, Zmistowski B, Parvizi J. Two-stage Exchange Arthroplasty for Infected Total Knee Arthroplasty: Predictors of Failure. Clin Orthop Relat Res 2011;469:3049–54. doi:10.1007/s11999-011-2030-8.
[9] Berbari EF, Marculescu C, Sia I, Lahr BD, Hanssen AD, Steckelberg JM, et al. Culture-negative prosthetic joint infection. Clin Infect Dis Off Publ Infect Dis Soc Am 2007;45:1113–9. doi:10.1086/522184.
[10] Kurd MF, Ghanem E, Steinbrecher J, Parvizi J. Two-stage Exchange Knee Arthroplasty: Does Resistance of the Infecting Organism Influence the Outcome? Clin Orthop Relat Res 2010;468:2060–6. doi:10.1007/s11999-010-1296-6.
[11] Nettrour JF, Polikandriotis JA, Bernasek TL, Gustke KA, Lyons ST. Articulating Spacers for the Treatment of Infected Total Knee Arthroplasty: Effect of Antibiotic Combinations and Concentrations. Orthopedics 2013;36:e19–24. doi:10.3928/01477447-20121217-13.
[12] Anagnostakos K, Fürst O, Kelm J. Antibiotic-impregnated PMMA hip spacers: current status. Acta Orthop 2006;77. doi:10.1080/17453670610012719.
[13] Anagnostakos K, Wilmes P, Schmitt E, Kelm J. Elution of gentamicin and vancomycin from polymethylmethacrylate beads and hip spacers in vivo. Acta Orthop 2009;80. doi:10.3109/17453670902884700.
[14] Bertazzoni Minelli E, Benini A, Samaila E, Bondi M, Magnan B. Antimicrobial activity of gentamicin and vancomycin combination in joint fluids after antibiotic-loaded cement spacer implantation in two-stage revision surgery. J Chemother 2015;27:17–24. doi:10.1179/1973947813Y.0000000157.
[15] Bertazzonni Minelli E, Benini A, Magnan B, Bartolozzi P. Release of gentamicin and vancomycin from temporary human hip spacers in two-stage revision of infected arthroplasty. J Antimicrob Chemother 2004;53. doi:10.1093/jac/dkh032.
[16] Fink B, Vogt S, Reinsch M, Büchner H. Sufficient release of antibiotic by a spacer 6 weeks after implantation in two-stage revision of infected hip prostheses. Clin Orthop Relat Res 2011;469. doi:10.1007/s11999-011-1937-4.
[17] Hendriks JGE, Ensing GT, van Horn JR, Lubbers J, van der Mei HC, Busscher HJ. Increased release of gentamicin from acrylic bone cements under influence of low-frequency ultrasound. J Controlled Release 2003;92:369–74. doi:10.1016/S0168-3659(03)00361-4.
[18] Kummer A, Tafin UF, Borens O. Effect of Sonication on the Elution of Antibiotics from Polymethyl Methacrylate (PMMA). J Bone Jt Infect 2017;2:208–12. doi:10.7150/jbji.22443.
[19] Ensing GT, Neut D, van Horn JR, van der Mei HC, Busscher HJ. The combination of ultrasound with antibiotics released from bone cement decreases the viability of planktonic and biofilm bacteria: an in vitro study with clinical strains. J Antimicrob Chemother 2006;58:1287–90. doi:10.1093/jac/dkl402.
[20] Clauss M, Laschkolnig E, Graf S, Kühn K-D. Influence of Sonication on Bacterial Regrowth from Antibiotic Loaded PMMA Scaffolds - An In-vitro Study. J Bone Jt Infect 2017;2:213–7. doi:10.7150/jbji.22382.
[21] Corona PS, Barro V, Mendez M, Cáceres E, Flores X. Industrially Prefabricated Cement Spacers: Do Vancomycin- and Gentamicin-impregnated Spacers Offer Any Advantage? Clin Orthop Relat Res 2014;472:923–32. doi:10.1007/s11999-013-3342-7.
[22] Jaeblon T. Polymethylmethacrylate: properties and contemporary uses in orthopaedics. J Am Acad Orthop Surg 2010;18:297–305.
[23] Pivec R, Naziri Q, Issa K, Banerjee S, Mont MA. Systematic Review Comparing Static and Articulating Spacers Used for Revision of Infected Total Knee Arthroplasty. J Arthroplasty. 2014 Mar;29(3):553-7.e1. doi: 10.1016/j.arth.2013.07.041. .
[24] Citak M, Masri BA, Springer B, Argenson J-N, Kendoff DO. Are Preformed Articulating Spacers Superior To Surgeon-Made Articulating Spacers in the Treatment Of PJI in THA? A Literature Review. Open Orthop J 2015;9:255–61. doi:10.2174/1874325001509010255.

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European Bone & Joint Infection Society EBJIS

Journal of Bone and Joint Infection
2017; 2(4): 218-223. doi: 10.7150/jbji.23078

Research Paper

Diagnosis Of Persistent Infection In Prosthetic Two-Stage Exchange: PCR analysis of Sonication fluid From Bone Cement Spacers

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Received: 2017.09.28; Accepted: 2017.10.26; Published: 2017.11.17

Abstract

Introduction: When treating periprosthetic joint infections with a two-stage procedure, antibiotic-impregnated spacers are used in the interval between removal of prosthesis and reimplantation. According to our experience, cultures of sonicated spacers are most often negative. The objective of our study was to investigate whether PCR analysis would improve the detection of bacteria in the spacer sonication fluid.

Methods: A prospective monocentric study was performed from September 2014 to January 2016. Inclusion criteria were two-stage procedure for prosthetic infection and agreement of the patient to participate in the study. Beside tissues samples and sonication, broad range bacterial PCRs, specific *S. aureus* PCRs and Unyvero-multiplex PCRs were performed on the sonicated spacer fluid.

Results: 30 patients were identified (15 hip, 14 knee and 1 ankle replacements). At reimplantation, cultures of tissue samples and spacer sonication fluid were all negative. Broad range PCRs were all negative. Specific *S. aureus* PCRs were positive in 5 cases. We had two persistent infections and four cases of infection recurrence were observed, with bacteria different than for the initial infection in three cases.

Conclusion: The three different types of PCRs did not detect any bacteria in spacer sonication fluid that was culture-negative. In our study, PCR did not improve the bacterial detection and did not help to predict whether the patient will present a persistent or recurrent infection. Prosthetic 2-stage exchange with short interval and antibiotic-impregnated spacer is an efficient treatment to eradicate infection as both culture- and molecular-based methods were unable to detect bacteria in spacer sonication fluid after reimplantation.

Key words: Infection, Two-Stage Exchange, Sonication, Spacer

Introduction

When treating periprosthetic joint infections with a two-stage procedure, antibiotic-impregnated spacers can be used in the interval between implant removal and reimplantation of a new prosthesis. The spacer provides local antibiotics, prevents soft tissues retraction and avoids formation of seroma in the dead space left by the removed prosthesis. However, it may also act as a foreign-body that can be colonized by microorganisms.

In the literature, most studies report cases of positive spacer sonication at the time of second stage procedure from 20% to 50% [1-4]. Nevertheless, according to our experience, cultures of sonicated spacers are always negative. Those results can be explained either by the absence of bacteria, or by the inhibition of bacteria by antibiotics eluted in the

sonication fluid. In our series, antibiotic concentrations of spacer sonicated fluid are high enough to prevent bacteria growth on cultures. The objective in this study was to investigate whether PCR analysis would improve the detection of bacteria in the spacer sonication fluid.

Methods

A prospective monocentric study was performed from September 2014 to March 2016 at the Lausanne University Hospital (CHUV), Switzerland. Inclusion criteria were patients who were operated for a periprosthetic joint infection treated with two-stage exchange and who gave their informed consent to participate in the study. The study was approved by the local ethical committee.

The diagnosis of infection was confirmed either by multiple positive periprosthetic cultures and/or, sonication of the prosthesis at the first stage of the procedure. The threshold of ≥50 CFU was defined as positive cultures, being a sign of infection [5]. Moreover, patients with fistula were considered infected even if all cultures samples were negative.

30 consecutive patients were included: 15 total hip arthroplasties (THA), 14 total knee arthroplasties (TKA), 1 total ankle arthroplasty (TAA). 8 patients were female and 22 were male. Mean age was 66 years old (range 28-85). The bacteria identified were *Staphylococcus epidermidis (8), S. aureus (7), S. capitis (3), Streptococcus dysgalactiae (4), S. milleri (2), S. pneumoniae (1), S. salivarius (1), Enterococcus faecalis (1), Propionibacterium acnes (1), Clostridium celerecrescens (1)* and *Campylobacter fetus (1)*.

At the first stage of the procedure, the prosthesis was removed and was sent for sonication to the laboratory of microbiology [5-6]. Wide debridement was performed collecting at least 2-3 periprosthetic tissues samples which were sent for culture. Then a handmade spacer was formed. For the production of the spacer 40g of the shelf cement containing 0.5g of gentamycin (Palacos G, Hereaus Medical, Berlin, Germany) were handmixed with supplemental 1.2g tobramycin and 2g vancomycin. Empiric intravenous antibiotics were administrated postoperatively followed by specific intravenous antibiotics, once the susceptibility tests were available. Rifampicin was not introduced before the second stage was completed, in order to avoid development of rifampicin-resistant bacteria. A short interval from 2 to 4 weeks was chosen for each case; the best time of reimplantation being decided depending on patient's health condition (for example, the day of the second-stage procedure would be postponed in case of cardiac or diabetic decompensation), local status (acceptable quality of bone or soft tissue at the time of implant removal), pathogen involved (absence of difficult-to-treat microorganisms such as rifampicin-resistant staphylococci, ciprofloxacin-resistant gram-negative bacteria, fungi) and decreasing of CRP and white blood cell count, without any strict cut-off value. There was no antibiotic-free period between the two stages.

At the second stage, the spacer was removed, a wide debridement was performed and the new prosthesis was implanted. At this stage, cultures of 2-3 samples were collected and the spacer was sonicated. For the purpose of the study, Gram strain and three types of PCR were done on sonication fluid on sonication fluid.

Our sonication protocol consists of two minutes at 40kHz using sonication device Bactosonic (Bandelin GmbH, Berlin, Germany). A minimum of phosphate buffered saline (PBS) fluid was poured in the sterile container containing the spacer. The quantity of fluid was depending on the size of the spacer and covered at least 90% of the spacer.

After sonication, a sample of sonication fluid was collected under laminar flow for PCR analysis. One portion of the liquid was centrifugated at a relative centrifugal force of 17.44 g or 10'000 rotations per minute (rpm/min). The pellet was resuspended in PBS fluid.

Unyvero Multiplex-PCRs (Curetis, Germany), broad range bacterial PCRs (16S), were performed on the sonicated spacer fluid. Then *Staphylococcus aureus* and *mecA gene* specific PCRs were used only on sonicated spacer fluid of patients who had primary infection with those specific bacteria (11 patients). The *mecA* gene is a specific gene found in bacteria, either in S. aureus or S. negative coagulase, that determines for resistance to methicilin.

Home-brew developed PCRs specific for *S. aureus* and *mec A gene* were performed in a second step on a subset of specimens that were negative with the broad range bacterial PCRs and the multiplex-Unyvero PCRs system but positive by culture for those specific bacteria in the primary infection.

The PCR analyses were performed during the first 3-4 days after sonication, except for Unyvero Multiplex-PCRs that were performed 2 to 4 weeks after sonication, with samples kept at -80°C between the different stages of the procedure.

Results

At a mean follow-up of 12.8 months (range from 1 to 24 months), we had two persistent infections: one patient infected with *S. epidermidis* and one patient infected with *methicilin-resistant S. aureus (MRSA)*. Four patients had a re-infection (13.3%): one

hematogenous THA infection by *S. aureus* caused by diabetic foot ulcer 9 months later, one hematogenous THA infection by *S. aureus* 5 months later and two cases of persistent serous discharge of wound 1 month after reimplantation (1 THA infection by *E. faecalis* and 1 TKA infection by *E. cloacae*). As the bacteria identified were different from the first stage procedure, they were treated by debridement, changing of the mobile part and implant retention. Re-infection appeared between 1 and 8 months after reimplantation (mean: 3.5 months).

At reimplantation, Gram strain, cultures of tissue samples and spacer sonication fluid were all negative. Table 1 describes the results obtained with the different PCR methods. Of culture-negative samples, the broad range bacterial PCRs were all negative. However, specific *S. aureus* PCRs (associated with analyses of *mecA* gene) were positive in 5 cases: for *methicilin-resistant negative coagulase Staphylococcus* in two cases, for *methicilin-resistant S. aureus* in two cases and for *methicilin-sensitive S. aureus* in one case. Concerning those five cases, in two cases of primary infection by *methicilin-resistant S. epidermidis*, specific *S. aureus* PCR was positive for the *methicillin-resistant negative coagulase Staphylococcus*. However, one patient did not present a re-infection during the follow-up time of the study and the other patient developed a re-infection at *E. faecalis*. In one case of primary infection by *methicilin-resistant S. epidermidis*, specific *S. aureus* PCR was positive for *methicilin-resistant S. aureus*. However, this patient did not develop a re-infection. In one case of primary infection at *methicilin-resistant S. aureus*, specific *S. aureus* PCR was positive for the same bacteria. However, this patient developed a re-infection at *E. cloacae*. In one case of primary infection by *methicilin-sensitive S. aureus*, specific PCR was positive for the same bacteria. However, this patient did not present a reinfection.

Table 1. Results of PCR analysis

Patients	Implants	Primary infection	At second stage of total arthroplasty replacement				Re-infection
			Tissue cultures	Broad range PCR	Specific S.aureus PCR	Multiplex Unyvero PCR	
1	THR	Streptococcus dysgalactiae	STERILE	NEGATIVE	NA	NEGATIVE	None
2	TKR	S. epidermidis	STERILE	NEGATIVE	NA	NEGATIVE	Persistent infection with cutaneous fistula
3	THR	methicilin-resistant S. epidermidis	STERILE	NEGATIVE	POSITIVE S.aureus / POSITIVE mecA	NEGATIVE	None
4	THR	Propionibacterium acnes	STERILE	NEGATIVE	NA	NEGATIVE	None
5	THR	methicilin-resistant S. epidermidis	STERILE	NEGATIVE	NEGATIVE S.aureus / POSITIVE mecA	NEGATIVE	None
6	THR	S. aureus	STERILE	NEGATIVE	NEGATIVE S.aureus / NEGATIVE mecA	NEGATIVE	Re-infection by S. aureus
7	TKR	S. aureus	STERILE	NEGATIVE	NA	NEGATIVE	None
8	THR	S. epidermidis	STERILE	NEGATIVE	NA	NEGATIVE	None
9	THR	methicilin-resistant S. epidermidis	STERILE	NEGATIVE	NEGATIVE S.aureus / POSITIVE mecA	NEGATIVE	Re-infection by Enterococcus faecalis
10	TAR	Staphylococcus capitis	STERILE	NEGATIVE	NA	NEGATIVE	None
11	TKR	Streptococcus pneumoniae	STERILE	NEGATIVE	NA	S.aureus (+)	None
12	THR	Enterococcus faecalis	STERILE	NEGATIVE	NA	NEGATIVE	None
13	THR	S. aureus	STERILE	NEGATIVE	POSITIVE S.aureus/ NEGATIVE mecA	S.aureus (+)	None
14	THR	S. epidermidis	STERILE	NEGATIVE	NA	S. negative coagulase (+)	None
15	TKR	Streptococcus dysgalactiae	STERILE	NEGATIVE	NA	NEGATIVE	Re-infection by Staph aureus
16	TKR	methicillin-resistant S. aureus	STERILE	NEGATIVE	POSITIVE S.aureus/ POSITIVE mecA	NEGATIVE	Re-infection by Enterobacter cloacae
17	THR	Streptococcus milleri	STERILE	NEGATIVE	NA	S. negative coagulase (+)	None
18	TKR	Streptococcus salivarus	STERILE	NEGATIVE	NA	S. negative coagulase (+) + P. acnes (+)	None
19	TKR	Streptococcus dysgalactiae	STERILE	NEGATIVE	NA	NEGATIVE	None
20	THR	Campylobacter fetus	STERILE	NEGATIVE	NA	NEGATIVE	None
21	TKR	Streptococcus milleri	STERILE	NEGATIVE	NA	NEGATIVE	None
22	TKR	methicillin-resistant S. aureus	STERILE	NEGATIVE	NEGATIVE S.aureus/ NEGATIVE mecA	NEGATIVE	None
23	TKR	methicillin-resistant S. aureus	STERILE	NEGATIVE	NEGATIVE S.aureus/ NEGATIVE mecA	NEGATIVE	Persistent infection with cutaneous fistula
24	TKR	Streptococcus dysgalactiae	STERILE	NEGATIVE	NA	S. negative coagulase (+)	None
25	THR	S. epidermidis	STERILE	NEGATIVE	NEGATIVE S.aureus/ NEGATIVE mecA	NEGATIVE	None
26	THR	Staphylococcus capitis	STERILE	NEGATIVE	NA	NEGATIVE	None

Patients	Implants	Primary infection	At second stage of total arthroplasty replacement				Re-infection
			Tissue cultures	Broad range PCR	Specific S.aureus PCR	Multiplex Unyvero PCR	
27	TKR	Clostridium celerecrescens	STERILE	NEGATIVE	NA	NEGATIVE	None
28	TKR	methicilin-resistant S. epidermidis	STERILE	NEGATIVE	NEGATIVE S.aureus/ NEGATIVE mecA	NEGATIVE	None
29	THR	S. aureus	STERILE	NEGATIVE	NEGATIVE S.aureus/ NEGATIVE mecA	NEGATIVE	None
30	TKR	Staphylococcus capitis	STERILE	NEGATIVE	NA	NEGATIVE	None

THR= Total Hip Replacement; TKR= Total Knee Replacement; TAR= Total Ankle Replacement; NA = Not applicable (specific PCR was only performed in the initial infection was caused by S.aureus); mecA= specific gene found in bacteria, either S. aureus or S. negative coagulase, that determines for resistance to methicillin

We also used Unyvero Multiplex-PCR, which is a new diagnostic system that allows PCR-based detection of implant and tissue infections. This system can detect 23 different pathogens simultaneously and the whole process takes 5 hours as compared to 72 hours with standard cultures. In studies using the Unyvero system, a sensitivity of 80.6% and a specificity of 96% were reported for prosthetic joint infections when compared to tissue cultures [7-8]. The main pathogens involved in tissue and implant infections are included in this commercial molecular system (including *Staphylococcus species (sp.), Streptococcus sp., Pseudomonas sp., Enterococcus sp., Propionibacterium sp., Escherischia sp.* and *Klebsiella sp.*), and it gives a positive result when $\geq 10^4$ pathogens are found per ml, depending on the pathogen. In this study, in order to increase the probability of finding pathogens, the spacer sonication fluid was centrifuged to concentrate the number of bacteria in the sample. The Unyvero system seemed an interesting tool as it can analyse simultaneously most frequent pathogens involved in PJI quite quickly, which would allow us to get results earlier than with standard cultures. We tested this system in our case of prosthetic joint infection to see if it could replace some of the current analyses we use.

The results of the multiplex Unyvero PCR were positive for six patients but showed only small quantity of bacteria. The Unyvero system does not indicate a precise number of pathogens but only an estimation. In two cases, the same bacteria that had caused the primary infection was detected. In four cases, a different bacterium than primary infection was detected. In all 6 cases, no re-infection has developed after the end of the antibiotic treatment for the prosthetic joint infection. This showed that this system was not suitable to exclude persistent infection in 2-stage exchange procedures.

Concerning the four cases of infection recurrence which were observed, none had positive PCR for the bacteria involved in their re-infection.

As said above, we have a mean follow-up of 12.8 months, which is a limitation in our study. However, as most infections were caused by Streptococci and Staphyloccoci, we would expect appearance of early infection rather than late infection.

Discussion

Sonication has proven its efficiency in diagnosis of prosthetic joint infection. Trampuz et al showed that sonication fluid cultures have a higher sensibility compared to standard cultures (78.5% versus 60.8%) [5]. However, the sensitivity of sonication fluid cultures decreases slightly to 75% for patients receiving antibiotics within 2 weeks prior to the surgical procedure [5].

Portillo [9] also confirmed the utility of PCR to differentiate prosthetic joint infection and aseptic loosening. However, uncertainty remains concerning the relevance in positive spacer sonication or positive PCR at second stage. Indeed, PCR cannot confirm the presence of viable bacteria in the samples [10]. As PCR is a very sensitive method, it can also show contamination [11].

Concerning spacer sonication, a few studies exist in the literature. In a study from Mariconda et al. 6 of 21 patients had positive sonication cultures, with the same bacteria as found in the first stage surgery. Of the 6 patients with positive sonication cultures, 3 had negative standard cultures [3]. In another study from Nelson et al., 18 of 36 patients had positive sonication cultures. The interval between the two operative stages was a minimum of 12 weeks; a minimum of 6 weeks with intravenous antibiotic administration followed by 6 weeks free of antibiotics. In their study, 11 patients had a re-infection. Of those 11 patients, nine had positive sonication cultures at second stage surgery (31%) but only four cases had positive standard cultures [1]. In a study of 55 patients, Sorli et al. showed 11 cases of subclinical infection at the time of reimplantation. They defined subclinical infection as a patient that showed no clinical signs of acute infection (satisfactory local status and normal CRP), but with either positive sonication or at least 2 positive tissues samples for the same bacteria. 18 patients developed re-infection at 12 months, including 8 of those with subclinical infection [2]. In the group with subclinical infection, they identified the same bacteria involved in the primary infection for 3 of 8 patients.

The cited studies seem to conclude that positive

spacer sonication could be predictive of long-term failure. However, in contrast to our short-interval two-stage exchange procedure, these studies [1-3] describe two-stage procedures with long interval, including a period free of antibiotics before reimplantation. The different treatment approach could explain our results showing only negative standard cultures of the sonicated spacers included in this study. In our experience, concentration of antibiotics present in the sonication fluid is high enough to inhibit the growth of bacteria on cultures. That could be an explanation why our spacer sonication cultures are negative. Based on this hypothesis, PCR analyses were done to increase the chances to detect the presence of bacteria with a non-culture based method.

In our study, all cases that developed a recurrence of infection had negative PCR results for the bacteria involved in the re-infection. Patients with positive PCR results were most often positive for the micro-organism responsible for the primary infection. Although the latter was detected by PCR, these patients did not present reinfection and did not need another surgical procedure. We can thus conclude that a few pathogens originated from the initial infection are not significant and cannot be interpreted as persistent infection in this study.

In our study, the different PCR analyses used did not help to predict which patients will develop a reinfection. PCR showed us either a very small quantity of the bacteria involved in the primary infection, or mixed flora that could be interpreted as contamination. The multiplex PCR system used in this study was faster and less labor-intensive to perform, but remained negative due to very small amount of microorganisms in the sonication fluid. The *S. aureus* specific PCR on the other hand may be too sensitive to diagnose subclinical infection as the low number of pathogens detected would not be clinically significant. With this thought, multiplex PCR, remaining negative, would give us the relevant clinical result, as no clinical persistent infection was detected in our study.

The management of the second step in two stage exchange surgery is still a matter of debate in the treatment of prosthetic joint infection. Currently, there is no consensus to determine the best timing for second stage. Different authors could not determine C-reactive protein (CRP) and ESR cut-off values, allowing for reimplantation, as CRP was even lower in some patients of the re-infection group, compared to the control group [12-14]. Likewise, spacer sonication and PCR at the second stage cannot predict recurrence of infection. Further investigations are needed to identify if PCR could be used to exclude persistence of infection at the second stage of the surgical procedure for prosthetic joint infection.

Conclusions

To our knowledge, this is the first study on relevance of PCR at second stage procedure in prosthetic joint infection. In our study, three different PCR analyses did not improve the bacterial detection and did not help to predict whether the patient will present a recurrence of infection. Prosthetic two-stage exchange with short interval and antibiotic-impregnated spacer is an efficient treatment to eradicate infection as both culture- and molecular-based methods were unable to detect bacteria in spacer sonication fluid after reimplantation.

Acknowledgement

This work was supported by RMS (Robert Mathys Stiftung Foundation, a non profit institute that support research in Switzerland) (grant of 42'000 Swiss francs) and AOTrauma foundation Switzerland (grant: 6'000 Swiss francs). Curetis provided the material to use the Unyvero system. However, they did not participate to our study.

IRB/Ethical Committee Approval

This study was approved by the independent local ethics committee (Commission cantonale (VD) d'éthique de la recherche sur l'être humain). Protocol 136/15 on 18th July 2014.

Competing Interests

Sandrine Mariaux and Ulrika Furustrand Tafin have no disclosures.

Here are disclosures of Prof. Olivier Borens: paid presentations for Zimmer-Biomet, Medacta, Lima and Heraeus. Unpaid consultant for Medacta, Lima and Zimmer-Biomet. Research support from Matthys, Bonesupport and Lima. Part of orthopaedic publications board of Journal of Bone and Joint Infection. Board member of Swiss orthopaedics, former Board member of European Bone and Joint infection society, President of AOTrauma Switzerland, Trustee AOFoundation.

References

[1] Nelson CL, Jones RB, Wingert NC, Foltzer M, Bowen TR. Sonication of Antibiotic Spacers Predicts Failure during Two-stage Revision for Prosthetic Knee and Hip Infections. Clin Orthop Relat Res 2014;472:2208–14. doi:10.1007/s11999-014-3571-4.

[2] Sorlí L, Puig L, Torres-Claramunt R, González A, Alier A, Knobel H, et al. The relationship between microbiology results in the second of a two-stage exchange procedure using cement spacers and the outcome after revision total joint replacement for infection. J Bone Jt Surg Br 2012;94B:249–53. doi:10.1302/0301-620X.94B2.27779.

[3] Mariconda M, Ascione T, Balato G, Rotondo R, Smeraglia F, Costa GG, et al. Sonication of antibiotic-loaded cement spacers in a two-stage revision protocol

for infected joint arthroplasty. BMC Musculoskelet Disord 2013;14. doi:10.1186/1471-2474-14-193.
[4] Schmolders J, Hischebeth GT, Friedrich MJ, Randau TM, Wimmer MD, Kohlhof H, et al. Evidence of MRSE on a gentamicin and vancomycin impregnated polymethyl-methacrylate (PMMA) bone cement spacer after two-stage exchange arthroplasty due to periprosthetic joint infection of the knee. BMC Infect Dis 2014;14:1–5. doi:10.1186/1471-2334-14-144.
[5] Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of Removed Hip and Knee Prostheses for Diagnosis of Infection. N Engl J Med 2007;357:654–63. doi:10.1056/NEJMoa061588.
[6] Borens O, Yusuf E, Steinrücken J, Trampuz A. Accurate and early diagnosis of orthopedic device-related infection by microbial heat production and sonication. J Orthop Res Off Publ Orthop Res Soc 2013;31:1700–3. doi:10.1002/jor.22419.
[7] Prieto-Borja L, Rodriguez-Sevilla G, Auñon A, Pérez-Jorge C, Sandoval E, García-Cañete J, et al. Evaluation of a commercial multiplex PCR (Unyvero i60(®)) designed for the diagnosis of bone and joint infections using prosthetic-joint sonication. Enferm Infecc Microbiol Clin 2017;35:236–42. doi:10.1016/j.eimc.2016.09.007.
[8] Hischebeth GTR, Randau TM, Buhr JK, Wimmer MD, Hoerauf A, Molitor E, et al. Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. J Microbiol Methods 2016;121:27–32. doi:10.1016/j.mimet.2015.12.010.
[9] Portillo ME, Salvadó M, Sorli L, Alier A, Martínez S, Trampuz A, et al. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. J Infect 2012;65:541–8. doi:10.1016/j.jinf.2012.08.018.
[10] Kobayashi H, Oethinger M, Tuohy MJ, Procop GW, Bauer TW. Improved Detection of Biofilm-formative Bacteria by Vortexing and Sonication: A Pilot Study. Clin Orthop 2009;467:1360–4. doi:10.1007/s11999-008-0609-5.
[11] Bereza P, Ekiel A, Augustciak-Duma A, Aptekorz M, Wilk I, Kusz D, et al. Comparison of cultures and 16S rRNA sequencing for identification of bacteria in two-stage revision arthroplasties: preliminary report. BMC Musculoskelet Disord 2016;17. doi:10.1186/s12891-016-0991-1.
[12] Kusuma SK, Ward J, Jacofsky M, Sporer SM, Della Valle CJ. What is the role of serological testing between stages of two-stage reconstruction of the infected prosthetic knee? Clin Orthop 2011;469:1002–8. doi:10.1007/s11999-010-1619-7.
[13] Ghanem E, Azzam K, Seeley M, Joshi A, Parvizi J. Staged Revision for Knee Arthroplasty Infection: What Is the Role of Serologic Tests Before Reimplantation? Clin Orthop Relat Res 2009;467:1699–705. doi:10.1007/s11999-009-0742-9.
[14] Hoell S, Moeller A, Gosheger G, Hardes J, Dieckmann R, Schulz D. Two-stage revision arthroplasty for periprosthetic joint infections: What is the value of cultures and white cell count in synovial fluid and CRP in serum before second stage reimplantation? Arch Orthop Trauma Surg 2016;136:447–52. doi:10.1007/s00402-015-2404-6.

Diagnosis of subclinical infection in prosthetic two-stage exchange: Evaluation of the effect of sonication on antibiotic release from bone cement spacers and the utility of molecular diagnostics (PCR)

Thèse de doctorat en médecine de Sandrine Mariaux préparée sous la direction du Professeur Olivier Borens

Annexe 1 : Types de PCR utilisées

3 types de PCR ont été effectués durant notre étude sur le liquide de sonication d'espaceurs en ciment :

- des **PCR à large spectre (eubactériennes)**, amplifiant le gène codant pour l'ARN ribosomal (sous-unité 16S), présent dans l'ensemble des espèces bactériennes
- des **PCR duplex de type taqMan** amplifiant le gène spécifique du *S. aureus* et le gène codant pour la résistance à la méticilline (présent chez les *S. aureus* et *S. epidermidis* résistant à la méticilline)
- ainsi que des **PCR multiplex Unyvero**, nouveau système diagnostique de la société Curetis pouvant identifier 23 bactéries différentes, impliquées habituellement dans les infections orthopédiques