THE STAPHYLOCOCCUS WARNERI BIOFILM FORMATORY STRAIN ISOLATION IN THE PERIPROSTHETIC HIP INFECTION BY THE SONICATION TECHNIQUE: CASE REPORT

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ABSTARCT
WE REPORT THE CASE OF A 71 YEARS OLD WOMAN WITH RIGHT PRIMARY OSTEOARTHRITIS OF THE HIP SURGED WITH CEMENTED HIP ARTHROPLASTY WICH THREE YEARS LATER ACCUSES PAIN AT THE PROSTHESIS HIP WITH NONSPECIFIC INFLAMMATORY BLOOD TESTS FOR INFECTION AND WITH NEGATIVE CULTURES. IN THE ABSENCE OF A BACTERIA GROWTH THE PROSTHETIC IMPLANT WAS SENT TO A SPECIALIZED LAB IN ORDER TO SONICATE IT. AFTER ONLY 3 DAYS AFTER THIS PROCEDURE THE ISOLATION OF STAPHYLOCOCCUS WARNERI STRAIN SUCCEEDED AND 4 MOUNTHS AFTER THE REMOVAL OF THE HIP PROSTHESIS A REVISON PROSTHESIS WAS INSTALLED. AFTER THE ARTHROPLASTY REVISION , THE PATIENT HAD A FAVOURABLE EVOLUTION AT GENERAL AND LOCAL LEVEL AND ALSO A GOOD SOCIAL INTEGRATION AT 4 MONTHS AFTER THE SURGERY, NEVER AGAIN PRESENTING ANY SIGNS OF INFECTION.

KEY WORDS: SONICATION, PERIPROSTHETIC INFECTION, BACTERIAL CULTURE

INTRODUCTION:

Adhesion of bacteria to human tissue surfaces and implanted prosthesis surfaces is an important step in the pathogenesis of periprosthetic infection having as a result the impossibility of their issolation and identification through the standard cultures.

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CASE REPORT:

We report the case of a 71 years old woman with right primary osteoarthritis of the hip surged with cemented total hip arthroplasty 3 years ago, with a good post-surgery evolution for the next 2 years when the patient, after suffering a trauma by falling from her own height is presenting with a periprosthetic fracture (Weber b) in which fracture reduction and the osteosynthesis with Dall Miles plate was performed. One year later the patient accuses pain at the prosthesis hip (in the absence of a fistula) accompanied by the relative functional impotence. At the local examination no changes at post surgery scar are observed. The X Ray it reveals precocious *loosening* signs (figure 1). The results of the inflammatory blood tests are not suggestive as for the septic phenomenon:

The leukocytes values (8.80 x10^3/µL) and fibrinogen (392 mg/dl) within normal limits.

The protein C (7,87 mg/l) and the erythrocyte sedimentation rate (18 mm/h) slightly increased.

Setting the periprosthetic hip infection diagnosis at this stage is difficult, but the debut of the inexplicable pain and the specific images suggest a possible septical evolution**.** In order to sustain this diagnosis a puncture aspiration is done from the periprosthetic fluid in order to do the cultures and the antibiogram on conventional smears (Gram / Giemsa for bacteria cultures as well as for fungus), the aspirate being collected into sterile tubes and then sent to the lab. The result was read after a period of 5 days (the standard time for bacteria growth).[5]

Preoperative X-Ray –prosthesis loosening
Figure 1

Microbiological culture
Figure 2

After this incubation period the bacteria result was negative, presenting some segments of fibrin, some rare inflammatory cells in the absence of a growing bacteria or fungus. Appearance of a fistula during hospitalization (while keeping negative cultures) lead to the decision of prosthesis removal and mounting a cement spacer (figure 3). The surgery of prosthesis removal consisted of chemical debridement (8 liters of saline *solution* were used)

[5] Osmon D.R, Berbari E.F Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of America., Clin Infect Dis;56:e1–25, 2013

and mechanical debridement. It was done the complete removal of the whole prosthetic implant and the Dall Miles plate and mounting an antibiotic loaded cement spacer. In the moment of the surgery 6 tissue biopsies were collected from the periprosthetic with specific inflammatory look which were inoculated in agar environment and incubated at 37 degrees for 5 days. In the absence of a bacteria growth until this moment, the prosthetic implant was sent to a specialized lab in order to sonicate it.

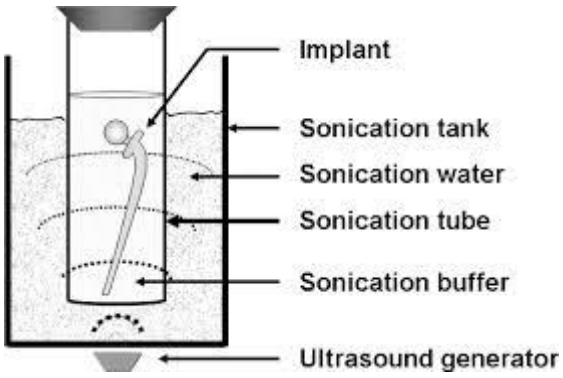
Preoperative X-Ray cement spacer
Figure 3

Revision Arthroplasty
Figure 4

The sonication process[6] consisted of adding Ringer solution (500 ml) under sterile conditions in the recipient (figure 5) containing the implant, then processing by vortexing for 30 seconds, followed by sonication (figure 6). for 1 minute, and the resulted liquid was poured on the Agar motherboards for the aerobic and anaerobic cultures.

The values needed for diagnosing an infection after the sonication process were considered as below[7]:

1. >50 CFU/ml (Colony Forming Unit)
2. 10-40 CFU/ml in the presence of the clinical symptoms and the increased inflammatory blood tests.
3. Presence under 10 CFU/ml infirm the microbiological diagnosis of infection.



sterile recipient containing the implant
Figure 5

sonication system
Figure 6

[6] Trampuz A, Piper KE, Jacobson MJ. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med;357:654, 2007

[7] Osmon D.R, Berbari E.F Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of America., Clin Infect Dis;56:e1–25, 2013

After only 3 days after this procedure the isolation of staphylococcus warneri strain succeeded (figure 2). This strain has the capacity of forming a biofilm on the surface of the implant followed by cell agglomeration in multiple layers secreting matrix formatting substances in which infection agents can be found.[8]

Because of the changes in the cell metabolism settled by complex signal paths from the biofilms, staphylococcus warneri passes to a dormant phase, a phase of minimum cell proliferation which makes also impossible its isolation by conventional cultures. The periprosthetic infections with this type of bacteria strain biofilm formation are very hard both to treat and diagnose, the new formed matrix ensuring a real barrier against cells lines of defense and to the diffusion mechanism of the antibiotics. The low level of cell proliferation affects dramatically the bactericide action of the antibiotics as well as the cultures in vitro in the scope of the bacteriological diagnosis. Forming the biofilms is a slow process, this being a specific of the late infections. The bacteria able to form this biofilm has a low virulence compared to the strains able to generate early infections, as in our case, a late infection accompanied by a little clinical signs making it hard to differentiate from the aseptic implant failure. After identifying the responsible germ for the infection, the antibiogram was done (after 18 hours [Tabel 1]) which revealed a high resistance to penicillin and gentamicin with sensitivity to vancomycin and clindamycin.

Selected Organism : Staphylococcus warneri

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Erythromycin	<= 0.25	S
Benzylpenicillin	0.25	R	Clindamycin	<= 0.25	S
Ampicillin			Linezolid	4	S
Oxacillin	TRM		Teicoplanin	2	S
Imipenem			Vancomycin	1	S
Gentamicin High Level (synergy)			Tetracycline	2	*R
Streptomycin High Level (synergy)			Tigecycline	TRM	
Gentamicin	4	*R	Fosfomycin	>= 128	
Ciprofloxacin	<= 0.5	S	Fusidic Acid		
Moxifloxacin	<= 0.25	S	Rifampicin	<= 0.5	*I
Inducible Clindamycin Resistance	NEG	-	Trimethoprim/Sulfamethoxazole	<= 10	S

Confidence:	Consistent	
Phenotype:	BETA-LACTAMS	MODIFICATION OF PBP (mecA)

Antibiogram result at 18 hours-
Table 1

After the surgery the patient received intravenous antibiotics (20 days) , as per the result of the antibiogram with cephalosporins 3rd generation (cefort 1g at 12 hours) and vancomycin (1g at 12 hours). The intravenous antibiotherapy was followed by oral antibiotherapy with fluorochinolone for 30. The clinical and laboratory reevaluations (blood test for inflammation) at the 6 weeks control indicate a general and local favourable evolution with the normalization of all inflammatory samples; 4 months after the removal of the hip prosthesis the cement spacer ablation was done and a revision prothesis was installed (figure 4).

[8] Scorzolini L, Lichtner M, Iannetta M, Mengoni F, Russo G, Panni AS, Vasto M, Bove M, Villani C, Mastroianni CM, Vullo V. Sonication technique improves microbiological diagnosis in patients treated with antibiotics before surgery for prosthetic joint infections. New Microbiol. 2014 Jul;37(3):321-8. Epub 2014 Jul 1.

During the surgery revision 6 tissular byopsies and periarticular fluid were taken and being sent to the microbiologically lab in order to identify if any latent bacteria still exists. After the arthroplasty revision, the patient had a favourable evolution at general and local level and also a good social integration 4 months after the surgery, never again presenting any signs of infection.

CONCLUSION:

We stress out the importance of the ultrasound procedure of separation and bacteria identifying in this case report after only 3 days as against to conventional cultures, because of a little bacteria quantity (able to cause a late infection) as well as bacteria collonizing at the prosthetic implant level with forming a biofilm at its surface[9] having as a result the impossibility of their issolation and identification through the standard cultures.

As a conclusion, staphylococcus warneri as well as other strains able to form a biofilm on the prosthetic implant by using the sonication technique make possible their isolation and identification[10] contributing so at forming a complete bacteriological diagnosis (including antibiogram), and, consequently, at a correct therapy with substantial improvement of the treatment in periprosthetic infections.

[9] Lass R, Giurea A, Kubista B, Hirschl AM, Graninger W, Presterl E, Windhager R, Holinka J Bacterial adherence to different components of total hip prosthesis in patients with prosthetic joint infection.. Int Orthop. 2014 Aug;38(8):1597-602. doi: 10.1007/s00264-014-2358-2. Epub 2014 May 10

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