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BRIEF REPORT

Are *Cutibacterium acnes* present at the end of primary shoulder prosthetic surgeries responsible for infection? Prospective study

Carlos Torrens[1] · Beatriz Bellosillo[2,3] · Joan Gibert[2,3] · Albert Alier[1] · Fernando Santana[1] · Nuria Prim[4] · Stéphane Corvec[5]

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Abstract

The purpose of this study was to investigate if the *C. acnes* present at the end of a primary shoulder arthroplasty could be responsible for shoulder arthroplasty infection. Prospective study includes patients undergoing primary shoulder arthroplasty from January 2015 until December 2018. From all the patients included, 5 to 12 tissue samples were obtained and were specifically cultured to detect the presence of *C. acnes.* DNA was extracted from the *C acnes* isolated colonies and Whole Genome Sequencing (WGS) analysis was done. A cohort of 156 patients was finally included. In twenty-seven patients, the *C. acnes* was present at the end of the primary surgery. Two of these patients developed a *C. acnes* periprosthetic shoulder infection at 6 and 4 months after the primary surgery. WGS of *C. acnes* isolated colonies showed that all the revision-surgery isolates clustered near to the corresponding primary-surgery isolates compared to the other independent bacterial colonies. (99.89% of similarity). *C. acnes* present at the end of the primary surgery can be the cause of early or delayed periprosthetic joint infections in shoulder arthroplasty.

Keywords Shoulder prosthesis · *Cutibacterium acnes* · Whole Genome Sequencing · Bone and joint infection

Introduction

Cutibacterium acnes (*C. acnes*) is the organism most frequently implicated in periprosthetic shoulder infections [1]. Recently, it has been suggested that molecular typing of multiple isolates is essential to diagnose *C. acnes* device-related-infections [2]. Moreover, it has been demonstrated that at the end of a primary shoulder arthroplasty replacement, nearly 20% of the patients present *C. acnes* positive cultures, belonging mostly to phylotypes IB and II which have been frequently involved in implant-associated infection [3, 4]. Nevertheless, the clinical significance of this persistence of *C. acnes* on the skin surface and in the deep layers during shoulder arthroplasty surgery remains still unknown.

In the current study, we have assessed the clinical meaning of shoulder prosthesis seeding by *C. acnes*.

Materials and methods

Study design and inclusion criteria

All patients undergoing primary reverse shoulder arthroplasty from January 2015 until December 2018 were prospectively included and followed for a minimum of 2 years (2–5 years). The study ends in December 2020 after completion of follow-up. In all of them, 5 to 12 cultures were obtained and analyzed. Exclusion criteria included active infection, invasive shoulder treatment and/or invasive imaging exploration during the last 6 months. All the patients included signed informed consent to participate in this study, approved by the Parc de Salut Mar Ethical Committee

✉ Carlos Torrens
86925@parcdesalutmar.cat

1 Orthopaedic Department, Hospital del Mar, Passeig Marítim 25-29, 08003 Barcelona, Spain

2 Pathology Department, Hospital del Mar, Barcelona, Spain

3 Cancer Research Program, Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

4 Microbiology Service, Laboratori de Referència de Catalunya, Hospital del Mar, Barcelona, Spain

5 Service de Bactériologie Et Des Contrôles Microbiologiques, Université de Nantes, CHU Nantes, CRCINA U1232, 44000 Nantes, France

(2014/5996/I and 2020/9141/I). The study was performed at a single tertiary University Hospital.

Bacterial procedures

The number of cultures obtained per patient ranged from 5 to 12, including in all the cases skin and deep tissue cultures. Each tissue sample was individually homogenized and used to inoculate a Chocolate PolyVitex agar plate (bioMérieux, Marcy-l'Etoile, France) and a Schaedler agar plate (bioMérieux), doing the same in a thioglycolate broth. These cultures were incubated for 7 days at 37 °C aerobically (with 5% CO_2) and anaerobically for 14 days. Because the study was designed to determine the presence of *C. acnes* at the end of primary surgery (not to determine infection), a culture was considered positive for *C acnes* when two or more colonies were observed.

Whole Genome Sequencing analysis

DNA was extracted from the *C acnes* isolated colonies with the QIAsymphony DSP Virus/Pathogen Midi Kit (Qiagen, Hilden, Germany). Libraries were prepared using Nextera XT kit (Illumina) and sequenced in an Illumina MiSeq sequencer producing around 300.000 2 × 250 bp reads per sample.

Sequencing files were preprocessed using The Microbial Genome Atlas pipeline [5]. Isolate nucleotide distances were calculated using Genome-based distance matrix calculator from the enveomics collection. Data analysis and plotting were performed using R 3.6.3.

Results

Patient's characteristics

A cohort of 156 patients with a mean age of 75 years-old (56–89) were included in the study. In 27 patients, positive cultures for *C. acnes* were found at the end of the primary surgery (17%). Among them, there were 14 males and 13 females. Two of these 27 patients developed a periprosthetic shoulder infection at 6 and 4 months after primary surgery. Both were 75-year-olds and males, yielding an infection rate of 7.7% for male. For the first patient, 12 cultures were obtained at the index surgery and eight of them turned to be positive for *C. acnes*. At revision surgery, nine cultures were obtained and eight were positive for *C. acnes*. For the second patient, five cultures were obtained at the index surgery and only one turned to be positive for *C. acnes.* At revision surgery, seven cultures were obtained and four were positive for *C. acnes* (Fig. 1). In both patients, the only microorganism present in all cultures was the *C. acnes.* None of the patients with initial negative cultures developed prosthetic joint infection.

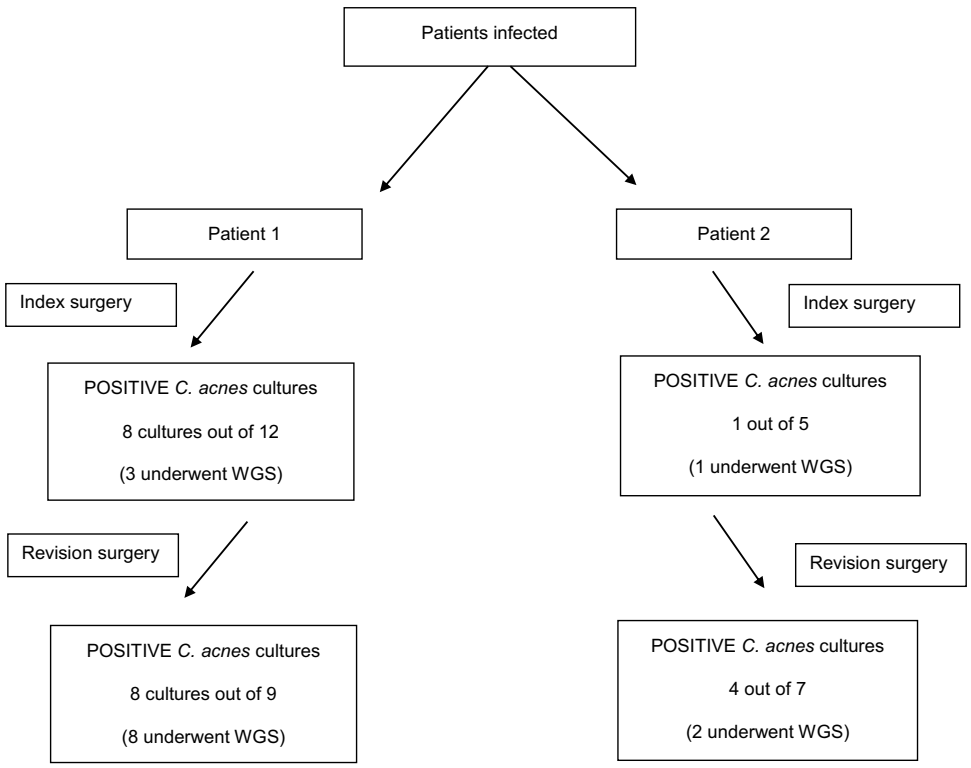

Fig. 1 Flow chart of the cultures of the infected patients

Cutibacterium acnes genomic analysis

To assess if the microorganism present during the revision surgery samples was the same or related to the one of the primary surgery, we performed Whole Genome Sequencing (WGS) analysis after DNA extraction from colonies. After reseeding, we obtained enough DNA from 3 index surgery isolates for the first patient, and 1 index surgery isolate for the second patient, and from 8 and 2 revision surgery isolates, respectively. In addition, we sequenced 35 isolates obtained at the time of the index surgery from 19 patients who did not suffer post-surgery infection and 7 isolates from 3 patients who were treated because of infection. After quality control, sequence analysis was feasible for 50 out of the 57 samples (24 patients). The *C. acnes* identified phylotypes for the 50 samples corresponded to phylotype II ($n=23$), IB ($n=17$), and IA ($n=10$).

Average Nucleotide Identity (ANI) value was assessed, measuring the nucleotide-level genomic similarity between two genomes (Fig. 2). As shown, we found a clear ANI clustering in two major groups which seem to correspond, mainly, to its associated phylotype (97–98% ANI). Moreover, when analyzing both isolates that developed a periprosthetic shoulder infection (PE10 and PE27), we found that all the revision-surgery isolates cluster nearer to their corresponding primary-surgery isolates compared to the other independent bacterial ones (Fig. 2).

Discussion

C. acnes is commonly isolated both in superficial and deep tissues after primary shoulder arthroplasty although the clinical significance of these positive cultures is yet to be defined [1, 3]. The results of this study show that the *C. acnes* present at the end of the primary arthroplasty is potentially responsible for the development of a delayed-periprosthetic joint infection. Genotyping of multiple isolates at the time of implantation during shoulder surgery can be a means of assessing the *C. acnes* burden inoculated by the surgeon. *C. acnes* can create microcolonies and participate in the race to the surface implant with biofilm formation leading to arthroplasty failure months or years after, as in the two patients reported in this study [6, 7].

Antibiotic prophylaxis and standard skin preparation in shoulder surgery seem to be suboptimal to eradicate *C. acnes* and avoid infection. The effectiveness of skin preparation solutions in shoulder surgery seems to be better for the 2% chlorhexidine (CHG) gluconate and 70% isopropyl alcohol, but in only 71.4% of the patients' eradication of *C. acnes* from the skin is achieved [8]. Indeed, Nakase et al.

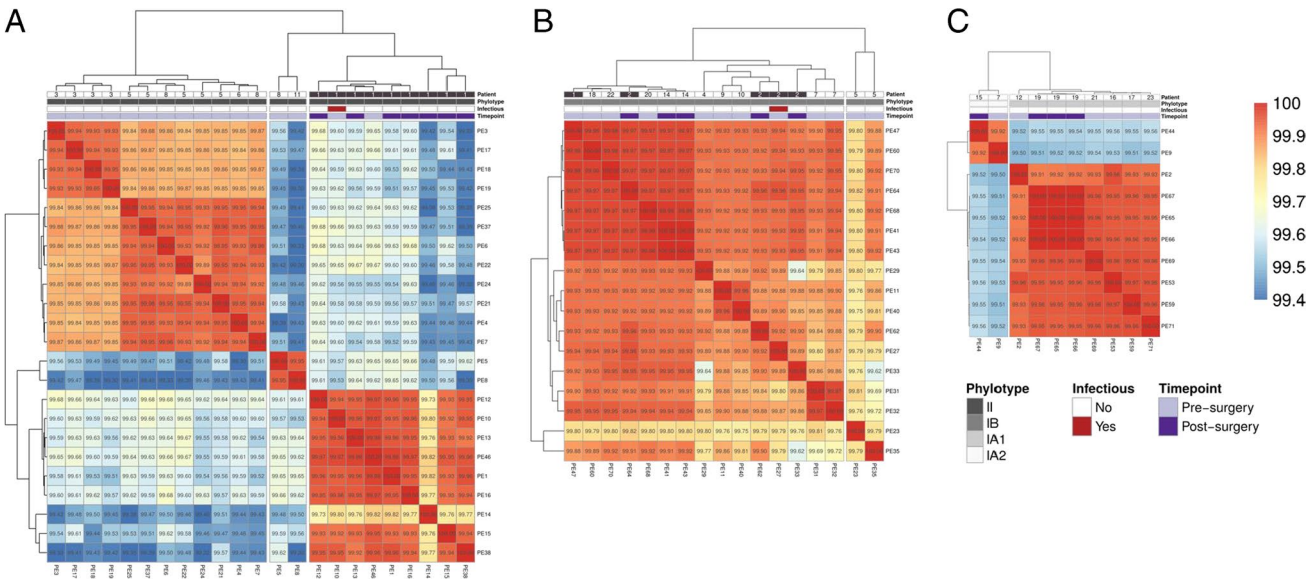

Fig. 2 Average Nucleotide Identity (ANI) in sequenced samples. Heatmaps comparing the 50 *C acnes* isolates (23 Phylotype II—A, 17 Phylotype IB—B and 10 Phylotypes IA1/IA2—C) from 24 patients that were finally evaluable using paired genetic distances. Similarities are plotted from the closest (red) to the furthest (blue). The samples are grouped according to the hierarchical clustering (top panels). Samples from different phylotypes shared ~ 97% similarity and comparisons are not depicted. The heatmap indicates that the relative genetic distances are small among isolates from the same patient and cluster, but between isolates from different clusters the genetic distances are large. The samples (PE10 and PE27), obtained in the primary surgery of the two patients (patients 1 and 2, respectively) who developed periprosthetic infections, are indicated as infectious. Patient identification and time of sampling are also depicted. Mean ANI distance between technical replicates is 99.97%

have demonstrated that *C. acnes* strains had high CHG minimal bactericidal concentrations [9]. In the same manner, standard antibiotic prophylaxis with cefazolin is of limited value in reducing or eliminating *C. acnes* colonization in the deep layer of the skin [1].

Consequently, nearly 20% of primary shoulder arthroplasties end with the presence of *C. acnes* both in superficial and in deep tissues. Shoulder prosthesis infection is in fact a skin ecosystem disease, especially in young men [10]. The genomic proximity between primary-surgery and revision-surgery isolates suggests that most of periprosthetic shoulder infection would be from preexisting bacteria in the host rather than from contamination after surgery or selection of resistant strains. We demonstrated by WGS analysis of *C. acnes* isolates that the way of contamination leading to the infection is linked to the primary surgery incision of the skin. Indeed, here for both patients, the same *C. acnes* strain was identified at the end of the primary surgery and during the revision surgery due to infection signs. Recently, the use of WGS was highlighted to allow unambiguously the recognition of *C. acnes* diversity [2]. Shoulder prosthesis infections belong to the true homotypic group proposed by El Sayed with a single pathogenic clone as we demonstrated for these patients [2]. Finally, as performed in this study, it may be necessary and better in routine practice to test multiple isolates from different samples from a patient rather than assuming that only one isolate is representative of the whole [11]. The availability in the next future of this innovative tool will allow distinguishing infection, polyclonal infection from contamination.

To date, the specific way of contamination for shoulder arthroplasty infection due to *C. acnes* is clearly identified. *C. acnes* present at the end of the primary surgery can be the cause of early or delayed periprosthetic joint infections in shoulder arthroplasty. Efforts need to be done to better address skin decontamination before surgery to more efficiently avoid prosthesis seeding with *C. acnes*.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by [Carlos Torrens], [Beatriz Bellosillo], [Joan Gibert], [Albert Alier], [Fernando Santana], [Nuria Prim], and [Stéphane Corvec]. The first draft of the manuscript was written by [Carlos Torrens] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability Supplementary material will be available at reasonable request.

Declarations

Ethics approval All the patients included signed informed consent to participate in this study, approved by the Parc de Salut Mar Ethical Committee (2014/5996/I and 2020/9141/I).

Consent to participate All the patients included signed informed consent to participate in this study.

Consent for publication All the patients included signed informed consent to participate in this study and to consent publication.

Competing interests The authors declare no competing interests.

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