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The effect of surgical delay on bacterial colonization in proximal humeral fracture

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The effect of surgical delay on bacterial colonization in proximal humeral fracture

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Running title: bacterial colonization in humeral fractures

Abstract

Introduction: Postoperative infection is a severe complication after proximal humeral fracture surgical treatment. The aim of this study was to determine if the surgical delay could modify the number and type of bacteria on the surgical site.

Materials and methods: A two stages study was set up. In the first stage the effect of delay was simulated in 20 patients affected by proximal humeral fracture treated conservatively. In a second stage, the effect of delay was measured in 20 patients that underwent surgery. In stage 1, three skin culture swabs were taken in correspondence of the deltopectoral approach, the day of the fracture(day 0), the day after(day 1) and five days after fracture(day 5). In stage 2, skin swab cultures were taken the day of trauma and immediately before surgery, cultured on various media suitable for aerobic and anaerobic bacteria.

Results: The number of bacteria increased over the course of the study, from day 0 to day 5, both considering the total number of Colony-Forming Unit and individual species of pathogen bacteria. The second stage of the study confirmed these data. An increasing number of bacteria was observed in patients that underwent surgery later than 2 days from trauma.

Conclusions: The delay to surgery increased bacterial colonization of the skin in the deltopectoral approach area including common pathogenic bacteria such as *Staphylococcus aureus*, coagulase-negative staphylococci and *Propionibacterium acnes*. This might justify the correlation between delay to surgery and risk of infection.

Keywords: proximal humeral fracture, infection, propionibacterium acnes, skin swab cultures, delay of surgery.

Introduction:

Proximal humeral fractures (PHF) are one of the most common fractures as they represent 45% of the fractures of the upper limb[1]. Considering the increasing age of the population, the incidence of proximal humeral fractures is likely to increase[2]. Approximately 20% of these fractures require surgery, with the aim of restoring function and resolving pain[3]. Despite the good outcomes that have been reported after surgery, high complication rates have been described[4-7]. Postoperative infection is one of the most severe complications. The incidence varies from 0 to 8% depending on the studies, and the consequences can be devastating for function, patient quality of life and total costs[8,9].

Unlike the obvious relevance of this topic, very few articles have focused on the risk factors for the development of infection after surgical treatment for proximal humeral fractures. A recent multicenter study by Blonna *et al.* showed a potential correlation between acute infection and delayed surgery with a peak of infection at around 5 days post-trauma[10]. Although the explanation of this observation remains unclear, one of the hypotheses is that the delay to surgery determines an increasing number of bacteria on the surgical site. Shoulders affected by proximal humeral fracture have in fact some peculiarities that distinguish them from elective surgery. In the case of a fracture, patients commonly undergo surgery after some days of immobilization with a bandage or a sling, often without the possibility of washing the affected shoulder. This might determine an increase in the number of skin bacteria that could potentially affect the surgical site.

The objective of our study was to determine if the delay to surgery could modify the skin bacterial load and type on the surgical site.

Materials and Methods:

In light of the Italian law, no institutional review board approval was mandatory for this study. The study has been performed in accordance with the ethical standards in the 1964 Declaration of Helsinki and has been carried out in accordance with relevant regulations of the Italian National Health care System. An informed consent was obtained for all patients.

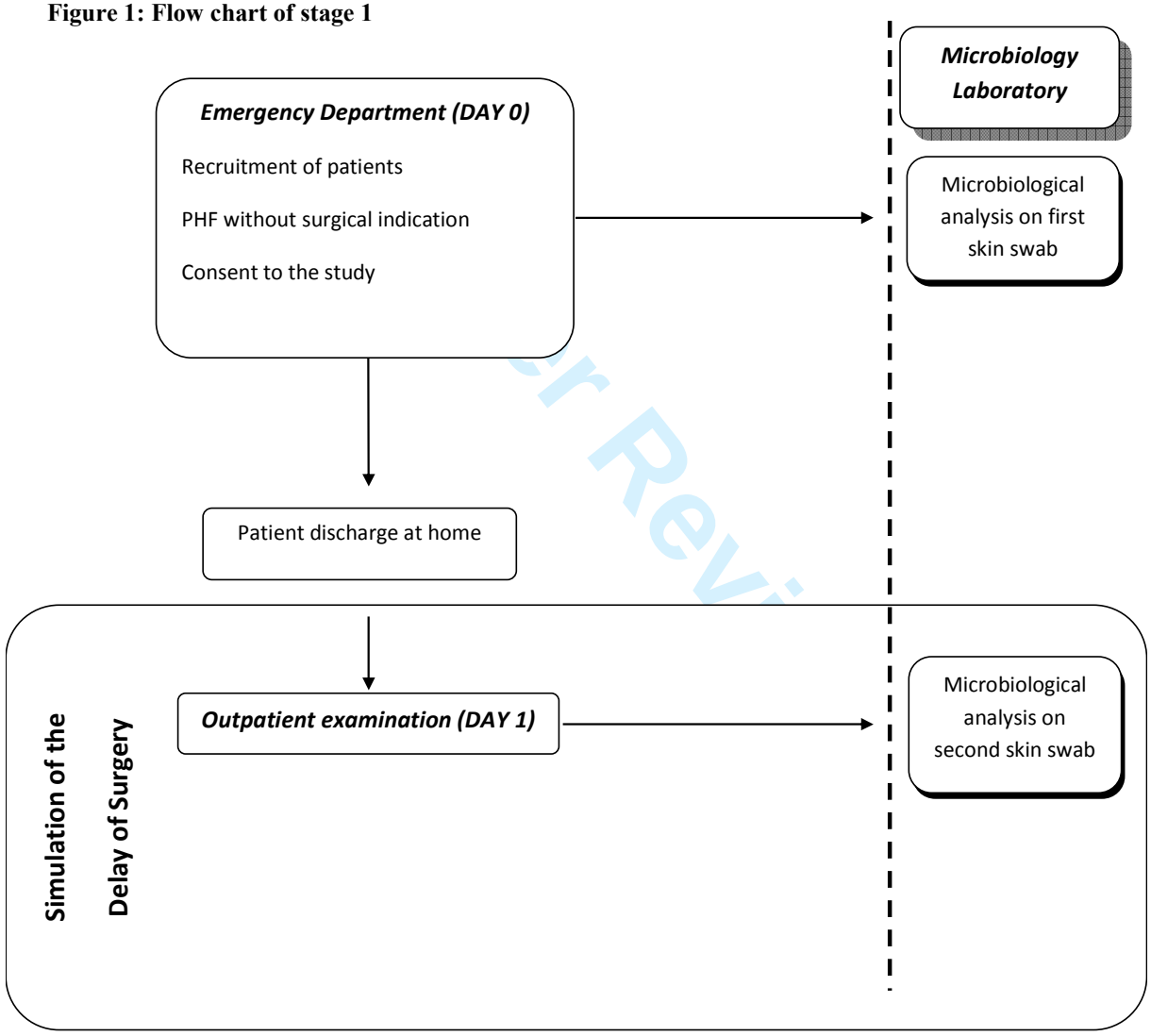
This study was set up in two stages. In the first stage, our hypothesis was tested in a model simulating the conditions of a proximal humeral fracture treated surgically. This model was designed to better control some of the variables such as delay to surgery and patients-related variables. In a subsequent stage our hypothesis was tested directly in patients that underwent surgery for treatment of proximal humeral fractures.

First stage.

Twenty-five consecutive patients affected by proximal humeral fracture were initially included in this study. All the patients were recruited in the Emergency Room of the Department of Orthopaedics and Traumatology, Mauriziano-Umberto I Hospital, University of Torino, the day of the trauma. The inclusion criteria were: a) consent to the study protocol, b) undisplaced proximal humeral fracture with indication to a conservative treatment. After inclusion in the study, patients were interviewed for comorbidities, demographic data were collected, and the first skin culture swab was taken from the area of the deltopectoral approach (day 0). The patients were managed in a way that simulated the management of patients treated surgically. In patients planned for surgery the affected shoulder is placed in a bandage for pain control and the patient is discharged at home until the day of surgery. In the first stage of the study, since the patients were treated conservatively, the delay of surgery was

simulated by assessing the same patient for an ambulatory visit in two different dates. The patient was scheduled to attend the outpatient clinic the day after the trauma (day 1, to simulate an acute surgery) and the fifth day after the trauma (day 5, to simulate a delay of 5 days). In each of the two examinations, a skin culture swab was taken from the area of the deltopectoral approach (Figure 1).

Figure 1: Flow chart of stage 1



Second stage.

In the second stage of the study, 20 consecutive patients affected by displaced proximal humeral fractures scheduled for surgical treatment were included. All the patients were recruited in the emergency room the day of the trauma, where the first skin culture swab was taken from the area of the deltopectoral approach. The patients were placed in a bandage. The second skin culture swab was collected the day of surgery in the operative room, immediately before skin preparation. The delay to surgery usually depended on the availability of CT scan, hospital bed and operative room. All the patients had an antibiotic prophylaxis immediately before surgery with a 2g dose of cefazolin. The patients were followed up at 1 week, 2 weeks, 4 weeks, 3 months, 6 months and 1 year after surgery for diagnosis of superficial or deep infection.

Bacterial isolation and identification

Skin swabs were immediately put into sterile culture swab tubes that contained Amies transport medium (Becton Dickinson Italia S.p.a., BD, Buccinasco, Milan, Italy) and brought within one hour to the Bacteriology and Mycology Laboratory of the Department of Public Health and Pediatrics, University of Torino. Serial 10-fold dilutions were prepared for each sample in saline solution (0.9% NaCl) so that the number of colony-forming units (C.F.U.)/mL was determined; 100 µL of each dilution were spread on various cultural media suitable for aerobic and anaerobic bacteria. The following media were used: Nutrient Agar (NA; Oxoid S.p.A., Milan) for aerobic bacteria; Mannitol Salt Agar (MSA; Merck Bracco, Milan for staphylococci; Schaedler Agar plus 5% blood (BD) for anaerobic bacteria.

Plates were incubated for 24-48 hours at 37°C under aerobic conditions for aerobic bacteria and for up 7 to 14 days at 37°C under strictly anaerobic conditions within an anaerobic system (Gaspak EZ anaerobe pouch system kit, BD) for obligate and facultative anaerobe bacteria. After incubation, the C.F.U. number -was recorded. All colonies with different morphologies, colors, sizes, and hemolytic reactions were selected so that as many of the predominant bacterial types as possible could be obtained. For morphologic analysis, Gram staining was performed and cellular morphologies were determined by light microscopy. Studies of enzymatic activities and fermentation of sugars were used to identify isolated facultative anaerobic/aerobic microorganisms. These biochemical tests were performed with commercially available API systems (BioMérieux, Rome, Italy) for aerobic bacteria (i.e. API Staph, API 20NE) and for anaerobic bacteria (i.e. API 20A), according to the manufacturers' instructions. For the count of the different bacterial species we used the higher number of species measured in each media.

Statistical analysis. The differences between day 0, day 1, day 5 (stage 1) and between trauma and surgery, were compared using the Wilcoxon test for paired samples. This test was preferred after the Kolmogorov-Smirnov test for normality revealed a not-normal distribution of the data.

Results:

First stage

Out of the 25 patients initially included in the first stage of the study, 5 were excluded because they did not attend one of the scheduled appointments therefore swab cultures could be collected. The remaining 20 patients formed the studied population.

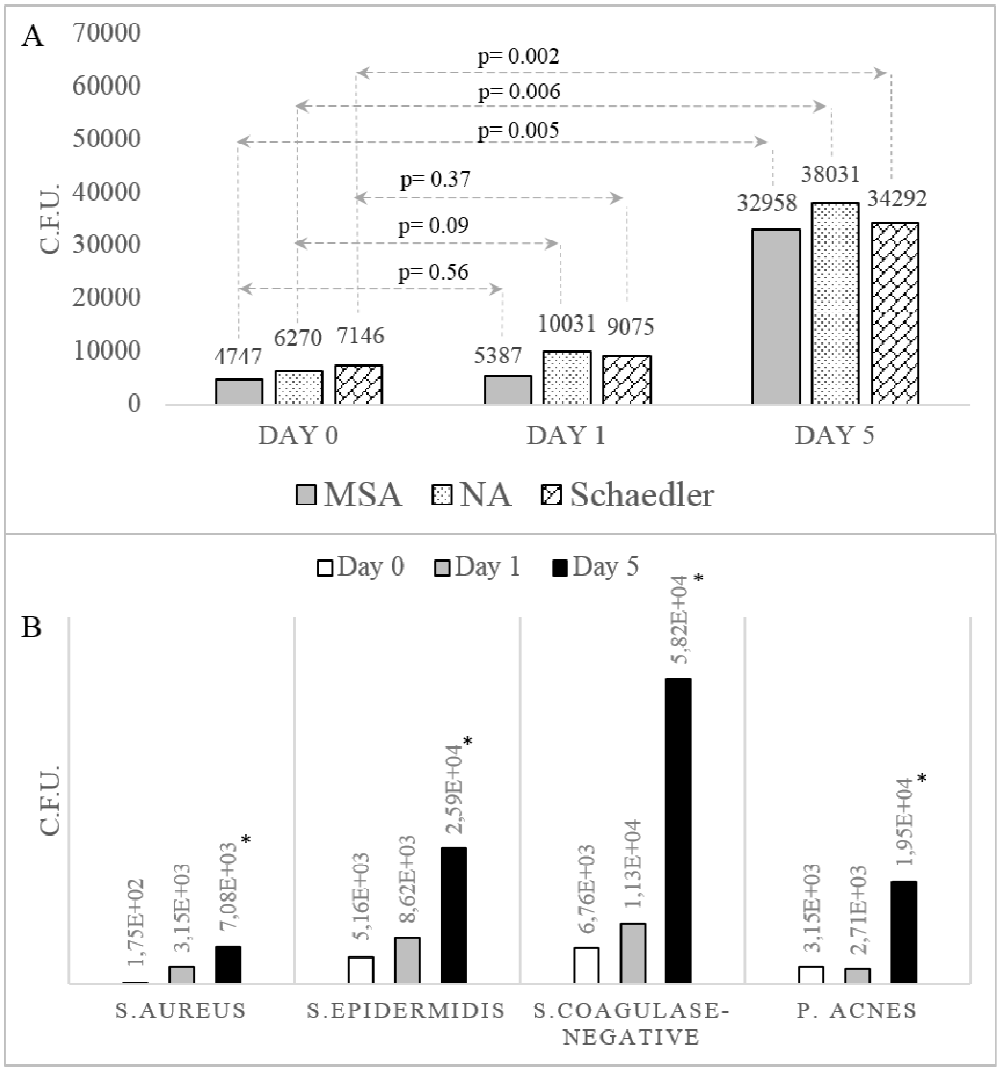
The average age of the patients was 67 years (range 50-85 years), 12 (60%) were female.

The number of bacteria on skin culture swabs increased over the course of the study, from day 0 to day 5, both considering the total number of C.F.U. on the individual cultural media (Figure 2A) and the C.F.U. of the individual species of bacteria (Figure 2B). The C.F.U. on MSA increased from $4.75*10^3$ C.F.U. the day of trauma to $5.39*10^3$ on day 1 (p= 0.56), to $3.30*10^4$ on day 5 (p= 0.005). The C.F.U. on NA increased from $6.27*10^3$ C.F.U. the day of trauma to $1.00*10^4$ on day 1 (p= 0.09), to $3.80*10^4$ on day 5 (p= 0.006). The C.F.U. on Schaedler Agar increased from $7.15*10^3$ C.F.U. the day of trauma to $9.08*10^3$ on day 1 (p= 0.37), to $3.43*10^4$ on day 5 (p= 0.002).

Considering the single pathogen bacteria, 3 patients had a positive culture for *Staphylococcus aureus* the day of trauma and 7 patients on day 5 post trauma. Twelve patients had a positive culture for *Staphylococcus epidermidis* the day of trauma and 13 on day 5 post trauma. Eleven patients had a positive culture for *Propionibacterium acnes* the day of trauma and 13 on day 5 post trauma. *S. aureus* increased from $1.75*10^2$ C.F.U. (day 0), to $3.15*10^3$ C.F.U. on day 1 (p= 0.14), and to $7.08*10^3$ C.F.U. on day 5 (p= 0.04). The *P. acnes* decreased from $3.15*10^3$

C.F.U. (day 0), to $2.71*10^3$ C.F.U. on day 1 (p= 0.05), but increased to $1.95*10^4$ C.F.U. on day 5 (p= 0.03). The coauglase-negative Staphylococci (CoNS) increased from $6.76*10^3$ C.F.U. on day 0, to $1.13*10^4$ C.F.U. on day 1 (p= 0.1), and to $5.82*10^4$ C.F.U. on day 5 (p= 0.002) (Figure 2B).

Figure 2A&B



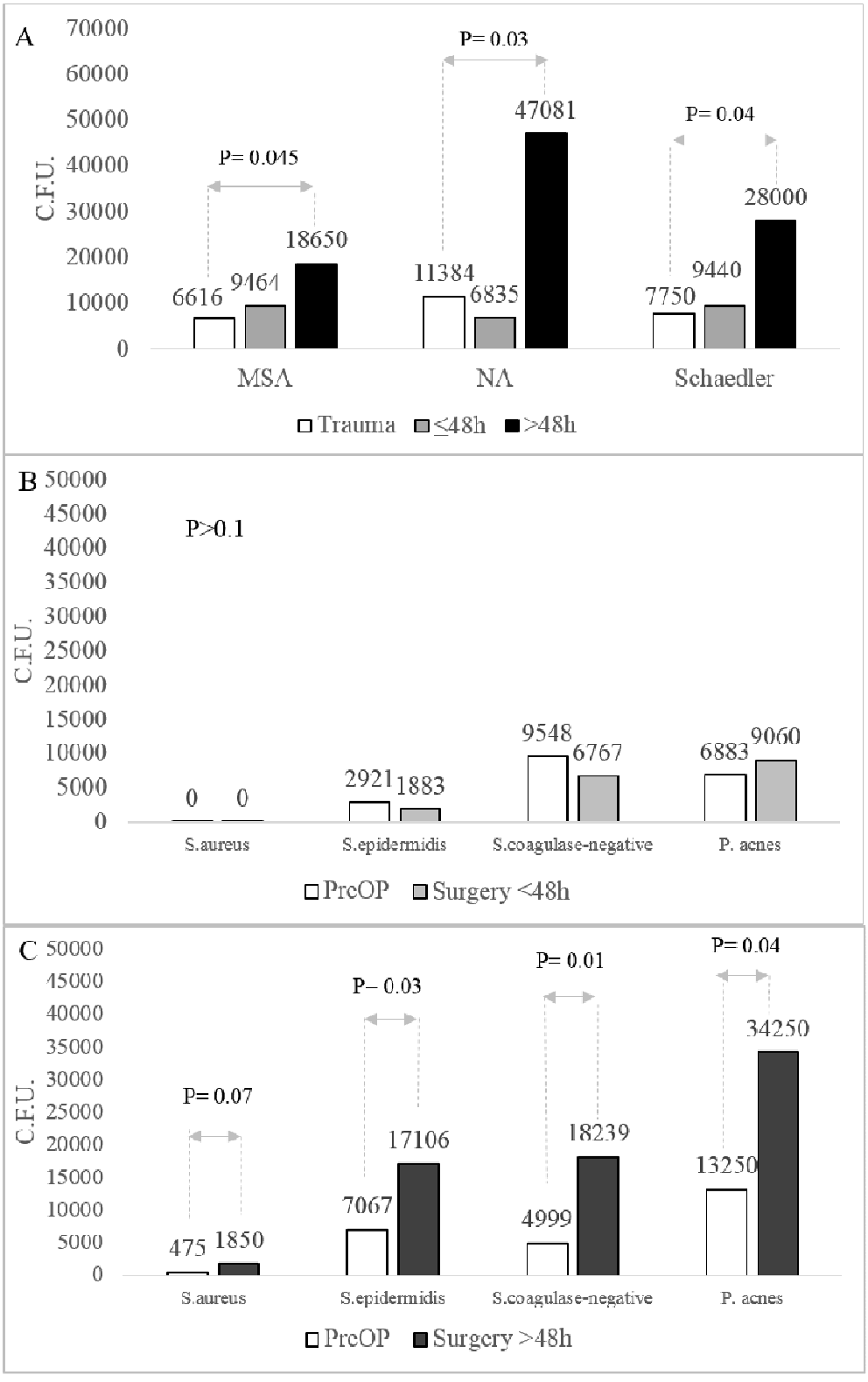
Second stage

The 20 patients included in the second stage of the study underwent surgery at an average of 4.4 days from trauma (range 0 to 7 days). The average age of the patients was 65 years (range 45-90 years), 14 (70%) were female. Seventeen patients underwent a reduction and fixation

using an external fixator[11-13], 2 a reverse arthroplasty and one emiarthroplasty[14]. For descriptive purpose patients were divided into two subgroups according to the delay to surgery. In the first group patients underwent surgery within 2 days from trauma (9 patients, average delayed 1.7 days), in the second group patients that underwent surgery after 2 days from trauma (11 patients, average delay 6.4 days).

The data in Figure 3A,B,C confirm the data observed in the first stage of the study. A bacterial increase was observed in the patients that underwent surgery after 2 days from trauma. The change of bacteria between trauma to surgery, in the patients that underwent surgery within 2 days from trauma, was not statistically significant ($p > 0.1$). The increase from trauma to surgery was significant in the subgroup of patients that underwent surgery after 2 days for all the variables ($p < 0.05$) except for the increase of *S. aureus* (Figure 3C).

Figure 3A,B,C


A
C.F.U.
70000
60000
50000
40000
30000
20000
10000
0
P= 0.045
P= 0.03
P= 0.04
6616
9464
18650
11384
6835
47081
7750
9440
28000
MSA
NA
Schaedler
Trauma
≤48h
>48h
B
C.F.U.
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
P>0.1
0
0
2921
1883
9548
6767
6883
9060
S.aureus
S.epidermidis
S.coagulase-negative
P. acnes
PreOP
Surgery <48h
C
C.F.U.
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
P= 0.07
P– 0.03
P= 0.01
P= 0.04
475
1850
7067
17106
4999
18239
13250
34250
S.aureus
S.epidermidis
S.coagulase-negative
P. acnes
PreOP
Surgery >48h

In the subgroup of 9 patients that underwent surgery within 2 days form trauma, one had a positive culture for *S. aureus*, 5 patients had a positive culture for *S. epidermidis* the day of trauma and at surgery, 8 patients had a positive culture for CoNS the day of trauma and at surgery, 5 patients had a positive culture for *P. acnes* the day of trauma and at surgery.

In the subgroup of 11 patients that underwent surgery after 2 days form trauma, 4 had a positive culture for *S. aureus* the day of trauma and 5 at surgery, 8 patients had a positive culture for *S. epidermidis* the day of trauma and 10 at surgery, 9 patients had a positive culture for CoNS the day of trauma and 10 at surgery, 5 patients had a positive culture for *P.acnes* the day of trauma and 7 at surgery.

One patient (5%) had a diagnosis of deep infection 6 months after surgery (shoulder hemiarthroplasty). He had persistent shoulder pain, radiographic signs of infection and positive bone scan. An arthrocentesis was performed for identification of pathogen bacteria. The culture was positive for *P.acnes*. This patient had a positive culture the day of trauma for *P.acnes* ($2.93*10^4$ C.F.U.). He underwent surgery 5 days from trauma when the number of *P.acnes* increased to $1.52*10^5$ C.F.U.

Discussion:

The aim of this study was to demonstrate if the delay to surgery increases the bacterial colonization of the shoulder skin at the level of the surgical approach. The outcomes confirm that the number of bacteria, including pathogen bacteria, increases on the skin surface during the time between trauma to surgery. Although this study clearly shows an increase of pathogen bacteria, this does not prove that the delay to surgery increases the rate of infection. The occurrence of infection is, in fact, multifactorial but we here analyzed only one of the potential factors. Other variables such us patient's immune status, type of antibiotic prophylaxis or type of skin preparation could affect the infection rate.

A recent study by Blonna *et al.* analyzed risk factors for acute infection after fixation for proximal humeral fracture[10]. This retrospective multicenter study analyzed 452 proximal humeral fractures with an infection rate of about 4%. The most common bacteria were *S. aureus* and CoNS. In the present study we showed that the number of *S. aureus* and CoNS increased significantly when the surgery is performed 5 days after trauma (in a simulated model) or after 2 days from trauma (in the "in vivo" model). Our report also evidenced that the *P. acnes* increased by 6 times 5 days after trauma. Interestingly the patient that, in our series, had a chronic *P. acnes* infection, underwent surgery 5 days after trauma and had an extremely high number of *P. acnes* at the level of deltopectoral approach.

Although these data alone do not prove the association between delay to surgery and infection rate, they strongly suggest that this topic deserves further studies. Recently the scientific community attention has been focused on *P. acnes* , since this bacterium is believed to be one of the major responsible of chronic infection in shoulder[15-18].

In our series, the delay to surgery increased significantly the number of *P. acnes* and the effect of the delay seemed to be more pronounced for this pathogen compared to other bacteria. The local condition of the skin under the bandage could be responsible of this increase.

Other studies have investigated the bacteria responsible for post operative shoulders infections. Athwal et al. reported 5 cases of acute shoulder infection in 259 patients that underwent open reduction and fixation of proximal humeral fractures. Polimicrobial infection was present in one case with the remaining cases presenting monomicrobial infection. CoNS species were the most common microorganism isolated, infecting 3 of 5 patients whereas *P. acnes* was isolated from 2 patient[8].

Smith et al.[19] analyzed early complications of proximal humeral fractures. Out of 22 hemiarthroplasty, 2 had a deep infection. One infection was due to *S. epidermidis* and the other to both *S. aureus* and *P. acnes*. Both were treated with surgical debridement, intravenous antibiotics, and retention of the components. Of the 82 shoulders that underwent surgery there was one acute deep infection (*S. epidermidis*) treated at 3 weeks with 2 surgical debridements, intravenous antibiotics, and retention of internal fixation with no recurrence of infection. Furthermore in this group one patient had his plate broken without healing of the fracture site. At the time of re-intervention he had a positive culture at the nonunion site (*P. acnes*)[19].

The direct correlation between bacterial load and type at the level of the deltopectoral approach and infection rate, although conceivable, has never been proved. However some studies analyzed the effect of shoulder skin preparation on bacteria, suggesting that the number of bacteria is proportional to the risk of infections[19-22]. Saltzman et al. showed that a 2% chlorhexidine gluconate and 70% isopropyl alcohol is more effective than povidone-iodine in eliminating overall bacteria from the shoulder region[22]. The results of this “in vitro"

study were furthermore confirmed by a clinical multicenterstudy that showed that washing the shoulder with 4% gluconate chlorhexidine and a subsequent preparation with iodopovidone, can significantly reduce the infection rate[10].

This study has at least three main clear limitations. The first one is that the number of patients included is not enough to be sure that delay of surgery is the main risk factor for infection after surgical treatment for proximal humeral fracture. In this series among these patients only one had a diagnosis of deep infection. A multicenter study would be advisable. The second limitation is that the effect of the delay was tested only considering 5 days after trauma. However we decided to focus on the first 5 days after trauma because this is the most common clinical condition. We cannot exclude that longer delay could influence the type of bacteria in an unexpected way. In other districts there are evidences suggesting that delaying surgery after 7 days can reduce the infection rate. Blonna *et al.* showed a slight reduction of infection after proximal humeral fracture when surgery was performed after one week[10]. This aspect needs to be investigated in further studies.

The last limitation, already reported above, is that an increased number of bacteria is not synonymous of infection because other variables such as the type of skin preparation could affect the rate of infection. In an ongoing study, we are analyzing the effect of skin preparation in relationship with the number and type of bacteria before skin disinfection. This would represent another step forward in the prevention of infection.

In conclusion this study demonstrates that delaying the surgical treatment of a proximal humeral fracture increases the number of skin pathogen bacteria above the deltopectoral sulcus, potentially affecting the infection rate.

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The authors have no conflicts of interest to declare

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Legends:

Figure 1: **Flow chart of stage 1 study**

PHF: Proximal Humeral Fracture

Figure 2A, B: **Bacterial increasing overtime in conservatively treated patients (stage 1)**

The number of bacteria on skin culture swabs increased over the course of the study, from day 0 to day 5, both considering the total number of C.F.U. on the individual culture media (A) and the C.F.U. of the individual bacterial species (Figure B).

C.F.U.= Colony Forming Unit

MSA= Mannitol Salt Agar

NA= Nutrient Agar

*= $p<0.05$

Figure 3A, B, C: **Bacterial increasing overtime in surgically treated patients (stage 2)**

In the patients that underwent surgery within 2 days from trauma the change of bacteria was not statistically significant ($p<0.1$, Figure A, B). An increase of bacteria was observed in the patients that underwent surgery after 2 days from trauma, both considering the total number of C.F.U. on the individual culture media (A, C).

C.F.U.= Colony Forming Unit

MSA= Mannitol Salt Agar

NA= Nutrient Agar