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Association between *Propionibacterium acnes* and frozen shoulder: a pilot study

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Abstract

Background: Frozen shoulder has not previously been shown to be associated with infection. The present study set out to confirm the null hypothesis that there is no relationship between infection and frozen shoulder using two modern scientific methods, extended culture and polymerase chain reaction (PCR) for bacterial nucleic acids.

Methods: A prospective cohort of 10 patients undergoing arthroscopic release for stage II idiopathic frozen shoulder had two biopsies of tissue taken from the affected shoulder joint capsule at the time of surgery, along with control biopsies of subdermal fat. The biopsies and controls were examined with extended culture and PCR for microbial nucleic acid.

Results: Eight of the 10 patients had positive findings on extended culture in their shoulder capsule and, in six of these, *Propionibacterium acnes* was present.

Conclusions: The findings mean that we must reject the null hypothesis that there is no relationship between infection and frozen shoulder. More studies are urgently needed to confirm or refute these findings. If they are confirmed, this could potentially lead to new and effective treatments for this common, painful and disabling condition. Could *P. acnes* be the *Helicobacter* of frozen shoulder?

Keywords

adhesive capsulitis, frozen shoulder, *Propionibacterium acnes*

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Introduction

Frozen shoulder was defined by Codman in 1934[1] but continues to be one of the most enigmatic conditions in orthopaedic surgery. Over the past decade, the enigma has begun to unravel.[2] The capsule of the shoulder joint becomes fibrosed[3–7] with Type I and Type III collagen.[6,8] There is cellular proliferation of fibroblasts and myofibroblasts[8] and the finding of mast cells in the synovium would suggest some inflammatory trigger.[9,10]

Many questions remain to be answered about the pathology and aetiology of frozen shoulder. Prime amongst these is what is the trigger that leads to the early findings of angiogenesis and inflammatory change, the consequent fibrosis of the joint and the final remodelling of the fibrosed capsule?

No trigger has as yet been found for this condition. Minor trauma has been implicated and there are known associations with diabetes[11] and Dupuytren's contracture.[12] There is also some evidence of a genetic predisposition.[13,14]

An infective trigger had been considered as early as 1934 by Codman when he compared frozen shoulder to tuberculosis of the shoulder.[1] The early symptoms of pain and stiffness were similar yet tuberculosis left the joint destroyed but the joint surface was never destroyed in frozen shoulder.[1] Over the next 80 years, speculation about an infective trigger was dismissed

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because no organism was ever grown from a frozen shoulder.

However, consequent upon the management of prosthetic joint infections, we have recently discovered that there are many forms of low grade organism that are difficult to culture, such as *Propionibacterium acnes*.[15] We have also discovered pathological organisms within the human body where no organisms could be thought to exist or survive, such as *Helicobacter* in peptic ulceration[16] and *P. acnes* in the intervertebral discs of patients with low back pain.[17]

Propionibacterium acnes is an aerotolerant anaerobic gram positive bacterium that is a common skin commensal found on the face, shoulders and upper trunk in particular.[18] It can cause skin infections such as *Acne vulgaris*.[19] *Propionibacterium acnes* is difficult to culture and its role in infections such as may occur after shoulder joint replacement has only recently been recognized.[15]

The present pilot study aimed to investigate whether an infective agent such as *P. acnes* could be a trigger for the onset of this common, painful and disabling condition? The null hypothesis being that there is no relationship between infection and frozen shoulder.

Materials and methods

This was a prospective cohort study and potential patients were identified in the clinic by a consultant shoulder surgeon prior to surgery. Strict inclusion and exclusion criteria were used. To be included in the study, patients had to be diagnosed with stage II idiopathic frozen shoulder according to Codman's criteria, which had failed conservative treatment. As such, they had severe pain, night pain and stiffness. They had a normal appearance of the glenohumeral joint on anteroposterior and axillary radiographs and were over 18 years of age and able to provide their informed consent.

Patients with cognitive impairment, a history of cancer, previous shoulder surgery, previous fracture of the shoulder, previous dislocation of the shoulder or a body mass index above $35\,kg\,m^{-2}$ were excluded. Patients found to have abnormal intra-articular pathology at the time of diagnostic arthroscopy were also excluded (e.g. rotator cuff tears, osteoarthritis or biceps pathology).

All patients underwent an arthroscopic capsular release with a standardized anaesthetic technique of an interscalene block and general anaesthesia. An examination under anaesthetic was performed to confirm the diagnosis and the range of movement was documented.

The skin was prepared with 2% chlorhexidine gluconate solution in 70% alcohol and standard exclusion drapes were applied. A stab incision was made on the posterior aspect of the shoulder. The first subcutaneous fat sample was then taken using clean instruments before the glenohumeral joint was penetrated. This was labelled FAT 1. All samples were placed in separate pots containing Robertson's cooked-meat broth culture medium. A sterilized 30° arthroscope was then placed into the glenohumeral joint. A standard diagnostic arthroscopy was performed to confirm the diagnosis of frozen shoulder and to document any abnormal pathology. Patients with abnormal pathology were excluded from the study and no further samples were taken. For those patients who were not excluded, an anterior incision was made and the second subcutaneous fat sample was taken with clean instruments and labelled FAT 2. An arthroscopic duckbill punch was then inserted to take a sample from the thickened medial glenohumeral ligament and the sample was labelled CAPSULE 1. A second clean punch was then introduced and a sample taken from the capsule and labelled CAPSULE 2. The arthroscopic capsular release was then completed using a radiofrequency device from the anterior portal. Once this was completed, all of the arthroscopy equipment was removed and a third piece of subcutaneous fat was taken from the posterior portal with clean equipment to act as another control. This was labelled FAT 3.

These samples were transferred immediately to the Exeter Microbiology laboratory where they were incubated at 37 °C. After 7 days, broths were terminally subcultured onto chocolate agar (incubated in CO_2), blood agar (incubated in CO_2) and fastidious anaerobic agar (incubated anaerobically). Organisms were identified by standard methods and matrix-assisted laser desorption/ionization. Antibiotic sensitivities were determined according to the British Society of Antimicrobial Chemotherapy National Standard Method. In addition, samples of the Robertson's cooked-meat broth were aliquoted at 7 days, and sent to Micropathology Ltd (University of Warwick Science Park, Venture Centre, Coventry, UK) for 16S RNA detection. RNA was extracted and amplified using a validated in-house assay. Any product amplified from the unknown sample was sequenced and compared to known sequences available in Genbank[20] using the BLAST tool.[21]

Results

Twelve consecutive patients were recruited into the present study. However, two were excluded as a result of intra-articular pathology at the time of diagnostic arthroscopy (one full thickness supraspinatus tear and one partial thickness rotator cuff tear). Therefore, 10 patients underwent complete sampling. Six were

female and four male. The mean (range) age was 51.3 years (28 years to 70 years). All had a clinical diagnosis of Stage II idiopathic frozen shoulder with an angiogenic capsule, thickened middle glenohumeral ligament and capsule and reduced volume found at diagnostic arthroscopy.

The results are summarized in Table 1. Eight patients had positive findings on extended culture of their rotator interval capsule. Six patients had evidence of *P. acnes* from the biopsies of the shoulder joint capsule.

Discussion

The null hypothesis for the present study was that infection is not associated with frozen shoulder; no researcher had ever grown any organism in the 80 years subsequent to this disease being described. However, the results of this pilot study were remarkable because eight of the 10 consecutive patients had a positive culture. In six patients, this was *P. acnes*, one carried a *Bacillus* and, in the eighth, it was a coagulase negative *Staphylococcus*. Thus, the null hypothesis had to be rejected.

Koch stated in 1877 that four criteria needed to be fulfilled to establish a causal relationship between a causative microbe and disease.[22] The first postulate is that a microbe must be found in abundance in all organisms suffering disease but should not be found in healthy organisms. In the present study, we failed to isolate any organisms in two patients with the condition. This could be for a number of reasons. An infection was not present and there may be more than one trigger for frozen shoulder. An infection was present but not detected. The frozen shoulder is a sequalae of an infection, which may now not be present. Because this was a small pilot study, we cannot answer all of these questions.

The second postulate states that a microbe must be isolated from the diseased organism and grown in pure culture which we achieved.

Koch himself abandoned the universal requirement of the third postulate when he found that not all organisms exposed to an infectious agent will acquire the infection. For example, not everybody exposed to tuberculosis or cholera developed the disease and not everyone exposed to polio becomes paralyzed. Non-infection may be a result of general health, proper immune functioning, acquired immunity from previous infection or vaccination, or genetic immunity (e.g. resistance to malaria in sickle disease).

It is possible that some of these positive results were contaminants from the skin because *P. acnes* is a common skin commensal of the face, neck and shoulder region, particularly in adolescents.[23] However, the skin was prepared with chlorhexidine in all cases, which is bactericidal.[24] Five separate freshly autoclaved duckbill resectors were used in each patient so that there could be no cross-contamination between biopsies.

The high level of positives in the subcutaneous fat for the controls probably reflects that *P. acnes* is a common skin commensal around the shoulder throughout life. If this is the trigger for frozen shoulder, it could be postulated that this accounts for frozen shoulder being more common than arthrofibrosis of other joints.

Stirling et al. recently reported that tissue from the intervertebral discs of patients with disc herniation and low back pain was infected with *P. acnes* and *Corynebacterium* in 53% of cases.[25] They then went on to perform a study with control patients from other spinal disorders such as scoliosis and found infection in 37% of herniated discs and 0% of other conditions, showing that the positive findings were not contamination from the skin. Further studies are now required in the shoulder to make stronger conclusions.

Minor trauma to the disc was considered to be part of the aetiology in Modic 1 changes and low back pain. It is then assumed that the traumatized disc is seeded with *P. acnes* or *Corynebacterium* through haematogenous spread, possibly from the mouth or teeth.[17] Interestingly, minor trauma is often reported in patients at the onset of their frozen shoulder.[26]

One criticism of studies on infection of intervertebral herniated discs was that a number of the patients had undergone previous spinal surgery, and it is possible that infection could have been consequent upon the surgery. None of our patients had had any previous surgery to the shoulder. However, five had had a previous injection to that shoulder, although this did not correlate with infection. Of the two patients who showed no sign of shoulder infection, one had had a previous injection to the shoulder. Because all of these injections had been performed in primary care, it is likely that none had been intra-articular. It is sensible to suggest that further studies should exclude any previous injection, just as we excluded previous surgery.

The results of the present study are extremely interesting and do not answer all of the questions regarding frozen shoulder. However, it could be postulated that the reason why frozen shoulder is common around the age of 50 years is because immune function decreases with increasing age, namely so-called immunosenescence.[27] Alternatively, at this age, dental caries and gum disease allows anaerobic mouth and skin commensal organisms to gain access to the shoulder during normal bacteraemias. It is possible that frozen shoulder is more common in diabetics because of their reduced resistance to infection. And it is also possible that the reason frozen shoulder never recurs is a result of acquired immunity.

Table 1 Summary of results.

Study number	Fat 1	Fat 2	Cap 1	Cap 2	Fat 3
1	*Bacillus*	Negative	Negative	Negative	*Propionibacterium acnes*
2	*Propionibacterium acnes*	*Propionibacterium acnes*	*Propionibacterium acnes*	*Propionibacterium acnes* *Staphylococcus warneri*	*Propionibacterium acnes*
3	Negative	Negative	Negative	Negative	Negative
4	*Bacillus*	*Propionibacterium acnes* *Bacillus*	*Bacillus*	Negative	Negative
5	*Bacillus*	*Propionibacterium acnes*	*Staphylococcus hominis*	*Propionibacterium acnes* *Staphylococcus epidermidis*	Negative
6	*Propionibacterium acnes*	*Propionibacterium acnes*	*Propionibacterium acnes*	*Propionibacterium acnes*	*Propionibacterium acnes*
7	*Bacillus*	*Bacillus*	Coagulase negative *Staphylococcus*	Coagulase negative *Staphylococcus*	*Bacillus*
8	*Propionibacterium acnes* *Bacillus*	*Propionibacterium acnes* *Bacillus*	*Propionibacterium acnes*	*Propionibacterium acnes* Coagulase negative *Staphylococcus*	*Propionibacterium acnes* *Staphylococcus epidermidis*
9	Negative	*Staphylococcus epidermidis*	*Propionibacterium acnes* *Corynebacterium*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis* *Staphylococcus haemolyticus*
10	*Propionibacterium acnes*	Negative	Negative	*Propionibacterium acnes* *Staphylococcus epidermidis*	Negative

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Author contributions

TBD devised the project and helped write the paper. MB obtained ethics approval, created the study paper work and wrote the paper. SG assisted in the theatre and collated results. CRA advised on microbiological processing and helped write the paper. JK recruited patients and operated on them. CDS recruited patients, operated on them, helped write the paper and was the principle investigator

Declaration of conflicting interests

None

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Ethical permission

Permission was granted from NRES Committee South West – Cornwall & Plymouth.

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