



## Microbiological investigation of the mandibular condyle in patients with advanced osteoarthritis of the temporomandibular joint

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### ABSTRACT

**Purpose:** The aim of this study was to investigate the presence of bacteria in samples of the temporomandibular joint taken from patients suffering from advanced osteoarthritis of the temporomandibular joint (TMJ).

**Materials and methods:** 25 fresh mandibular condyle samples were taken from 17 consecutive patients undergoing mandibular condylectomy (8 bilateral) for advanced TMJ osteoarthritis (Dimitroulis Category 5 joints). The joint samples were stained and cultured for the presence of microorganisms following a standardised joint culture protocol.

**Results:** No evidence of bacteria was found on staining or solid culture mediums. Late growth (day 12) of commensal skin organisms (*P. Acnes*, *S. Epidermitis*, *S. Capitis*) were identified in enriched broth samples in 5 joint samples. No statistically significant associations were noted between positive broth samples and age or previous joint intervention ( $p > 0.05$ ).

**Conclusions:** Within the limitations of this study, we have failed to identify meaningful bacterial growth in tissues (i.e. condylar head) of the TMJ that would suggest a contributory bacterial pathogenesis for arthritis of the TMJ.

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## 1. Introduction

Osteoarthritis of the TMJ has been classically defined as a progressive low-inflammatory disorder, characterised by degeneration and abrasion of the articular cartilage with a simultaneous remodelling of the underlying bone (Mercuri, 2008; Tanaka et al., 2008). The clinical presentation of this pathophysiological process may be characterised by joint pain, loss of function, crepitus, and in later stages retrognathia or apertognathia (Mercuri, 2008; Dimitroulis, 2013).

The classification and aetiology of osteoarthritis can be divided into primary osteoarthritis, idiopathic due to the absence of identifiable local, or systemic causes or secondary osteoarthritis. Causes for secondary osteoarthritis include trauma, iatrogenesis, and infection of the joint (Kang et al., 2007; Tanaka et al., 2008).

Treatment options for osteoarthritis can be divided into non-invasive modalities (such as non-steroidal anti-inflammatory medications, occlusal splints, physiotherapy) minimally invasive

(arthroscopy, arthrocentesis) and surgical modalities (arthroplasty, condylectomy) (de Souza et al., 2012). While the majority of TMJ arthritis may be managed with non-invasive or minimally invasive modalities, surgical modalities are often necessary in patient with late stage disease who have failed to improve with these, and have significant pain and loss of function (Dimitroulis, 2005; Mercuri, 2008).

Several studies have explored the role of chronic bacterial infection of the joint space in the pathogenesis of advanced internal joint derangement and arthritis with varying conclusions (Henry et al., 2000; Kim et al., 2003; McIntosh and Dimitroulis, 2012; Lund et al., 2015; Olsen-Bergem et al., 2016). A MEDLINE search found no current published studies exploring the presence of microbes from direct sampling of articular tissues of the joints in individuals diagnosed with osteoarthritis of the TMJ.

The identification of a clear aetiology or contributory mechanisms of TMJ osteoarthritis is complicated by a polymodal pathogenesis, as well as varying symptoms and an often insidious onset. While significant late-stage osteoarthritic disease of the TMJ carries significant decrease in quality of life often necessitating surgery (Dimitroulis, 2005; Tanaka et al., 2008), several studies have

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demonstrated discrepancies between osteoarthritic findings and clinical symptomology (Brooks, 1992; Sano et al., 2008). Early identification of TMJ arthritis and establishing contributory pathways of pathogenesis has implications in isolating factors that may halt progression of the pathophysiological process and potential surgery (de Souza et al., 2012).

The aim of this present study is to investigate the presence of bacteria and other micro-organisms in fresh tissue samples of human mandibular condyles taken from patients suffering from advanced osteoarthritis of the TMJ.

## 2. Material and methods

This study was approved by the St Vincent's Hospital Human Research Ethics Committee (QA 018/15).

### 2.1. Study design

A prospective case series of patients undergoing mandibular condylectomy. The study inclusion criteria were:

- (1) Evidence of Dimitroulis category 5 joint disease (Dimitroulis, 2013) on magnetic resonance imaging
- (2) Pain localised to the affected TMJ and loss of function
- (3) Failure of conservative measures (splint therapy, physiotherapy, medication) for at least 6 months
- (4) Performed by a sole operator (senior author GD)

Exclusion criteria for recruitment included:

- (1) History of rheumatoid arthritis or juvenile rheumatoid arthritis
- (2) Previous open fracture of mandible
- (3) History of Septic arthritis

All patients provided informed written consent to allow for microbiological analysis of a small tissue sample taken from the condyle, which is routinely sent for histopathology.

### 2.2. Surgical procedure

Following general anaesthetic induction via naso-endotracheal intubation, patients were prepared following strict sterile theatre conditions. A prophylactic antibiotic dose of 1 g Cefazolin was administered at the time of induction. The TMJ was approached via a pre-auricular endaural incision. Following identification of the lateral capsule, the capsule was sharply dissected and discal tissues (if present) were removed. The unsalvageable condylar head was then resected with a reciprocating saw (Fig. 1).

Following condylectomy, the lateral pole of the mandibular condyle was excised and placed into a sterile container to be sent fresh to the microbiology laboratory. The remaining specimen was sent in formalin for histopathology. The surrounding fossa and eminence were debrided and the joint was reconstructed with a prosthetic total joint replacement system (i.e. stock TMJ Biomet – Jacksonville, FL). Alternatively, in cases of total edentulous patients, an abdominal dermis-fat graft was placed in the resultant cavity with no reconstruction (i.e. condylectomy alone). Layered closure was then performed with interrupted deep and superficial sutures.

### 2.3. Specimen handling and processing

The fresh specimens were transported to an independent laboratory for microbiological analysis, to be processed within 2 h of



Fig. 1. Example of a resected arthritic TMJ specimen. The lateral pole was subsequently removed from this specimen.

procurement. This laboratory had been utilised in a previous study by the senior author (McIntosh and Dimitroulis, 2012).

A haematoxylin and eosin (H-E) Gram stain was performed immediately to assess for bacteria, tissue cells, polymorphs and mycotic elements. The laboratory was blinded to the nature and purpose of the study, however a standardised joint culture protocol (utilised for all joint cultures) was followed, involving both broth and solid culture mediums (Table 1).

Specimens were preliminarily reported at 24 and 48 h as per protocol. Mediums were also reviewed at day 5 and day 12. If there was growth in the enrichment broth as indicated by turbidity in the broth, a gram stain and culture onto solid media would then be carried out to determine the morphology of the organism in question so the appropriate sensitivity testing can take place.

## 3. Results

Fresh mandibular condylar samples were taken from 25 joints in 17 consecutive patients who underwent mandibular condylectomy for advanced TMJ osteoarthritis by the senior author (GD).

The average age of the subjects involved was 53 years. The range was between 20 and 76 years. All subjects were female. Nine subjects underwent unilateral condylectomy, and eight subjects underwent bilateral condylectomy. In addition to Dimitroulis classification category 5 joint disease in all patients, three patients identified had a pre-surgery diagnosis of juvenile idiopathic osteoarthritis, and 14 patients had a pre-surgery diagnosis of osteoarthritis. Nine patients had previous surgical interventions to the joint. The demographic data and previous surgical interventions are outlined in Table 2.

All 25 specimens submitted for H-E gram staining were negative for any micro-organisms, however two samples were noted to have polymorphs. No growth of micro-organisms was noted in any of the solid culture mediums at 5 days or at final review.

There was growth in five condylar specimen broth samples on final review at day 12. These samples were initially negative for growth of organisms at day 2 and day 5. *Propionibacterium acnes* was identified in all five positive broth samples. *Staphylococcus capitis* was additionally identified in 1/5 positive broth samples, *Staphylococcus epidermis* was additionally identified in 1/5 positive broth samples. Of the joints with positive broth samples, 4/5 have had previous surgical interventions. A summary of the positive samples and previous interventions are outlined in Table 3. All

**Table 1**  
Standard protocols for joint culture mediums.

Media	Atmosphere	Time for reading	Temperature
Blood agar (BA)	CO <sub>2</sub>	24 and 48 h	35 °C
Chocolate again (CA)	CO <sub>2</sub>	24 and 48 h	35 °C
Anaerobic agar (AA) pre –reduced	AnO <sub>2</sub>	48 h	35 °C
Thioglycollate broth	O <sub>2</sub>	7 days	35 °C
Additional media is used if the specimen is identified			
Purulent specimen			
• Saponin Lysed BA (SAP)	CO <sub>2</sub>	24 and 48 h	35 °C
Intracellular organisms seen in polymorphs on Gram stain			
• Saponin Lysed BA (SAP)	CO <sub>2</sub>	24 and 48 h	35 °C
Gram negative bacilli seen on Gram stain			
• MacConkey Agar (MC)	O <sub>2</sub>	24 h	35 °C
Gram negative bacilli mixed with Gram positive organisms			
• Blood nalidixic acid colistin agar (BNC)	CO <sub>2</sub>	24 and 48 h	35 °C
Fungi			
• Sabouraud's agar (F)	O <sub>2</sub>	7 days	30 °C
Actinomyces			
• Nalidixic acid Tween agar (Nat) + MTZ5 disc	AnO <sub>2</sub>	7 days	35 °C

(Note Joint culture protocols differ from normal culture protocols).

these samples were reported as isolated growth. No significant correlation was found between presence of bacteria and previous joint intervention ( $P = 0.078$ ) or age ( $P = 0.91$ ).

#### 4. Discussion

The potential for bacteria to induce arthritic changes is well-documented in literature concerning septic arthritis. As the synovial membrane has no limiting basement membrane, haematogenous seeding and subsequent colonization of bacteria in the joint space can occur, inducing pro-inflammatory cytokines such as IL-6, TNF- $\alpha$  and IL-1 $\beta$ . This leads to release of metalloproteinases and other cartilage degrading enzymes, causing damage and destruction of the articular surfaces of the joint space (Shirliff and Mader, 2002).

Several studies have identified a wide variety of bacteria in samplings of synovial fluid. Kim et al. detected bacteria via PCR testing in the synovial fluid in the majority of patients with disc

displacement of the TMJ and healthy controls, with an increased prevalence of *Staphylococcus aureus* in the non-healthy joints (Kim et al., 2003). Another study involving PCR synovial fluid sampling of 30 patients with TMD and 5 controls found mixed bacterial presence in the majority of patients with TMD, and no bacteria in the control group (Sun et al., 2008). The significance of bacterial presence in synovial fluid sampling is unknown, and has been questioned in the past, as findings of bacteria in synovial fluid, a dialysate of plasma may be systemic rather than localised to the TMJ (McIntosh and Dimitroulis, 2012). Neither of these studies involved the drawing of serum samples to confirm or refute the presence of bacteria.

The link between sexually transmitted infections associated with reactive arthritis and TMD has also been explored in several studies (Henry et al., 2000, 2007). In the most recent series of studies concerning chlamydial DNA in retrodiscal tissue of the TMJ, Henry et al. identified higher levels of TNF-alpha and IL-6 in samples positive for chlamydia trachomatis DNA via immunostaining compared to samples which tested negative for chlamydia. A similar more recent study by Lund et al. also examined discal tissue in patients undergoing discectomy for TMJ dysfunction, and failed to identify the presence of chlamydial DNA in any samples via PCR testing (Lund et al., 2015). *Chlamydia trachomatis*, as an obligate intracellular anaerobe is unlikely to be cultured in our selected mediums and as such we cannot shed any further light on these conflicting results.

Within our current case series, all joint cultures failed to grow bacteria within the 5 day period. With extended testing, only 5 samples were positive for any bacterial presence, and in all of these samples isolated growths were only identified in enriched broth mediums, identified at day 12 and beyond with no corresponding growth on any aerobic or anaerobic solid culture mediums. This is in keeping with a previous study (McIntosh and Dimitroulis, 2012) where fresh joint tissue specimens taken from the retrodiscal tissues of the TMJ in 12 consecutive patients failed to find any bacterial growth.

The organisms identified within these positive samples were *Staphylococcus Capitis*, staphylococcus epidermis and Propionibacterium acnes. All three cultured organisms are commensal skin organisms commonly found on the scalp, face and neck with low pathogenicity. While all three organisms have been implicated in the development of septic arthritis in case reports, this is exceedingly rare in native non-prosthetic joints (Tevell et al., 2017). One recent study found a positive correlation between presence of Propionibacterium in TMJ synovial fluid samplings of patients with

**Table 2**  
Summary of patient demographics, joints involved and previous interventions.

Patient	Age	Sex	TMJ site	diagnosis	Previous joint intervention
1	52	F	L	OA	Nil
2	64	F	L	OA	Arthroscopy, discectomy
3	60	F	L	OA	Nil
4	73	F	R	OA	Nil
5	31	F	L	JIA	Nil
6	68	F	L	OA	Nil
7	27	F	R	JIA	Discectomy
7	27	F	L	JIA	Discectomy
8	84	F	L	OA	Nil
9	45	F	L	OA	Nil
9	45	F	R	OA	Nil
10	47	F	R	OA	Nil
10	47	F	L	OA	Nil
11	45	F	R	OA	Nil
12	20	F	R	JIA	Nil
12	20	F	L	JIA	Nil
13	62	F	R	OA	Arthroscopy
13	62	F	L	OA	Nil
14	72	F	L	OA	Disc plication, arthrocentesis
14	72	F	R	OA	Disc plication, arthrocentesis
15	76	F	R	OA	Arthrocentesis
15	76	F	L	OA	Arthrocentesis
16	44	F	L	OA	Arthrocentesis
17	43	F	R	OA	Arthroscopy
17	43	F	L	OA	Arthroscopy
18	56	F	L	OA	Arthroscopy, eminectomy
19	21	F	L	JIA	Nil

OA: Osteoarthritis, JIA: Juvenile Idiopathic Arthritis.

**Table 3**  
Summary of positive broth samples.

Patient	TMJ Site	Previous intervention	Gram Stain	Polymorphs present	Day 2 Growth (Solid or broth)	Solid Culture Growth (Day 14)	Broth Growth (Day 14)
11	R	N	Nil	Y	N	N	<i>Staphylococcus capitis</i> , <i>Propionibacterium acnes</i> (both isolated)
14	R	Y	Nil	N	N	N	<i>Propionibacterium acnes</i> (Isolated)
16	L	Y	Nil	N	N	N	<i>Propionibacterium acnes</i> (isolated)
17	R	Y	Nil	N	N	N	<i>Staphylococcus epidermis</i> (Isolated), <i>Propionibacterium acnes</i> (isolated)
18	L	Y	Nil	Y	N	N	<i>Propionibacterium acnes</i> (Isolated)

JIA or rheumatoid arthritis and pain on palpation of the TMJ; however the conclusions drawn from the remainder of the study were mixed, as overall presence of bacteria was also linked to less jaw pain and better function (Olsen-Bergem et al., 2016).

The significance of bacterial presence from direct sampling of joint tissues remains controversial. One likely explanation is contamination, despite stringent efforts to maintain sterility in both theatre and lab processing. This would be a reasonable explanation in the present case series, as all organisms grown were isolated growths of commensal skin organisms. With studies utilising PCR for diagnosis, the possibility of contamination may also arise from the detection of non-viable bacterial DNA that may still be present following antiseptic preparation of the harvest site. The significance of non-commensal bacterial organisms detected by previous studies utilising a molecular diagnosis is also difficult to ascertain, as studies involving direct sampling of tissues are hampered by a lack of a control group, due to obvious ethical implications, and heterogeneous methodology (Henry et al., 2007; Lund et al., 2015). This may also explain why there is such a wide range of bacterial species identified in the temporomandibular joint, potentially affecting the external validity of studies.

A potential limitation within our current case series may be the utilisation of a culture technique rather than molecular analysis. PCR techniques overcome some limitations of bacterial culture – especially with regards to the identification of difficult-to-grow obligate intracellular species such as *Chlamydia trachomatis* with specific PCR assays. However, the additional diagnostic benefit of universal PCR assays when compared to more traditional culture techniques in joint disease has not been demonstrably proven. Within the orthopaedic literature, Bemer et al. noted a lower sensitivity rate with the use of broad range PCR 16S rRNA assays when compared to tissue culture in known cases of prosthetic joint infection (Bemer et al., 2014). A more recent prospective cohort study by Larsen et al. compared the diagnostic utility of culture and 16S RNA sequencing in 42 patients with working diagnoses of prosthetic joint infection. Within this series of patients, only one was diagnosed by 16S rRNA sequencing alone, while five diagnoses were confirmed by culture samples alone (Larsen et al., 2018).

Further studies would benefit from a standardised approach towards identification of bacteria within the joint space. This may include measures such as a standardised protocol for sampling, as well as concurrent standardised culture and PCR methods to provide comparable conclusions regarding the significance of presence of bacteria within the joint space.

## 5. Conclusion

Osteoarthritis of the TMJ is a polymodal pathogenic process. Within the limitations of this study, we have failed to identify meaningful bacterial growth in tissues (i.e. condylar head) of the TMJ that would suggest a contributory bacterial pathogenesis for arthritis of the TMJ.

## Conflicts of interest

The authors have no conflicts of interest to declare.

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