Osteoarthritis in Temporomandibular Joint

Internal Derangement

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ABSTRACT

Internal derangement of the temporomandibular joint is the most common diagnosis leading to joint surgery. Two variants of internal derangement exist, painful clicking and chronic closed lock. Painful clicking is characterized by no or minor arthritic changes. Joints with chronic closed lock, on the other hand, frequently exhibit osteoarthritic degeneration of the disc and fibrocartilage. This study was done in order to elucidate some of the underlying biologic processes during disc displacement.

The occurrence of fibroblasts, chondrocytes and blood vessels was studied morphologically in the tissue of temporomandibular joint disc, the intermediate zone and the posterior disc attachment region in control autopsy specimens and compared with corresponding tissues from patients with temporomandibular joint internal derangement. The volume density of blood vessels was significantly higher in the posterior disc attachment region in patient specimens than in autopsy controls. Whether this reflects a role for the vessels in the pathogenesis of temporomandibular joint internal derangement or merely is a reaction to another type of injury remains to be settled. In both the autopsy control and the patient specimens, chondrocytes and fibroblasts were characteristic for disc respective posterior disc attachment region. Thus it appears that occurrence of these cells can be used to distinguish temporomandibular joint disc from posterior disc attachment in small biopsy specimens.

The glycosaminoglycans of the extracellular matrix were analysed by means of capillary zone electrophoresis in specimens from temporomandibular joint disc and posterior disc attachment in patients with internal derangement of the temporomandibular joint. There were significant differences in the amount of glycosaminoglycans between the two groups of patients with internal derangement – painful clicking and chronic closed lock. Values in patients with painful clicking were comparable to those of normal individuals, while patients having chronic closed lock showed significantly reduced values. Both groups showed higher values in the posterior disc attachment when compared to the disc and similar pattern of glycosaminoglycan sulphation. Based on the close correlation between the osteoarthritic associated degradation in the temporomandibular joint disc and in the posterior disc attachment, it is suggested, that once osteoarthritis develops in patients with painful clicking, the effects on the attachments will lead to chronic closed lock.

RT-PCR was used to analyze the expressions of a series of mRNAs coding for proteoglycans in specimens obtained during discectomy of the temporomandibular joint in patients with signs and symptoms of painful clicking and chronic closed lock. The degradation of matrix in patients with chronic closed lock of the temporomandibular joint seems not to be caused by a reduced synthesis and the degenerative process seen in these patients is one with low turnover similar to the situation in primary osteoarthritis of hyaline cartilage.

An indirect microimmunofluorescence test for detecting antibodies against Chlamydia trachomatis was used to analyse serum samples from a subgroup of patients with chronic closed lock. This subgroup of patients showed monoarthritis without any signs of degenerative joint disease. The occurrence of serum antibodies to Chlamydia trachomatis was significantly higher in patients than in controls. However, this occurrence did not correlate with severity of observed tissue changes.
List of original papers

The thesis is based on the following papers which will be referred to in the text by their Roman numerals (I-IV)

I Characterization of tissue components in the temporomandibular joint disc and posterior disc attachment region: Internal derangement and control autopsy specimens compared by morphometry.
Paegle DI, Holmlund AB, Reinholt FP.

II Matrix glycosaminoglycans in the temporomandibular joint in patients with painful clicking and chronic closed lock.
Paegle DI, Holmlund AB, Hjerpe A.

III Expression of proteoglycan mRNA in patients with painful clicking and chronic closed lock of the temporomandibular joint.
Paegle DI, Holmlund AB, Leonchiks A, Hjerpe A.

IV The occurrence of antibodies against Chlamydia species in patients with monoarthritis and chronic closed lock of the temporomandibular joint.
Paegle DI, Holmlund AB, Rotzén Östlund MD, Grillner L.
<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>CL</td>
<td>Chronic closed lock</td>
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<tr>
<td>CS</td>
<td>Chondroitin sulphate</td>
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<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
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<tr>
<td>GAPDH</td>
<td>Glyceraldehydes-3-phosphate dehydrogenase</td>
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<td>HAS-1</td>
<td>Hyaluronan synthase 1</td>
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<td>ID</td>
<td>Internal derangement</td>
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<td>IZ</td>
<td>Intermediate zone</td>
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<td>MA</td>
<td>Monoarthritis</td>
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<td>OA</td>
<td>Osteoarthritis</td>
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<td>PC</td>
<td>Painful clicking</td>
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<tr>
<td>PDA</td>
<td>Posterior disc attachment</td>
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<td>PG</td>
<td>Proteoglycan</td>
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<td>TMJ</td>
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THE TEMPOROMANDIBULAR JOINT

a. Anatomy, function and development

The temporomandibular joint (TMJ) is an articulation between the lower jaw and the base of the skull. The bone elements of this articulation are the mandibular condyles below and squamous temporal bones above. This joint is divided into upper and lower compartment by the TMJ disc. The upper compartment allows the gliding movement while the lower compartment functions primarily as a hinge joint. The TMJ articulating surfaces are lined by a cartilage with a fibrous surface that is dense and compact containing collagen and a few fine elastic fibers in the articular eminence and thin in glenoid fossa (Sharawy 2000).

During the development, the bones of this synovial joint are formed directly from intramembranous centers of ossification rather than by indirect bone formation, i.e. cartilage forming a backbone for the subsequent formation of bone tissue. Thus, there is no residual persistent layer of cartilage to cover the articular surface like in joints of long tubular bones. Instead, the bone tissue develops with surrounding fibrous periosteum that also covers the articular zone. The periosteum lining the articular surfaces is gradually transformed during its early development into the dense fibrous articular tissues of the TMJ, and articular forces acting through the TMJ play an important role in this gradual transformation (Hylander 1992).

The articular disc is derived from a mesenchymal tissue compartment that also gives raise to the capsule of the TMJ and the tendon of the lateral pterygoid muscles (Okeon JP 1998). In adults inferior lateral pterygoid muscle inserts primarily into the neck of the condyle while superior lateral pterygoid muscle inserts into the capsule, the disc and the neck of the condyle. The exact attachment of the superior lateral pterygoid muscle to the disc is somewhat debatable. Although some authors suggest no attachment (Wilkinson 1988), several studies reveal the presence of a
muscle-disc attachment (Carpentier & al 1988, Marguelles-Bonnet & al 1989). The majority of the fibers of the superior lateral pterygoid muscle attach to the neck of the condyle and only 30 to 40% attach to the disc. It is also important to note that the attachments are more predominant on the medial aspect than on the lateral. It is thus possible that superior lateral pterygoid muscle affects the position of the disc (Juniper 1984).

Anteriorly the disc is fused with the capsule and posteriorly it continues as the posterior disc attachment (PDA) inserting in the base of the skull and mandibular condyle. The disc is not attached to the capsule laterally or medially. Instead, it is tightly bound directly to the medial and lateral poles of the mandibular condyle thus moving with condyle.

The TMJ condyle and the temporal bone do not fit together in the absence of the disc. The disc fills the gaps created by the rounded bony edges of the joint and thus stabilizes the joint during rotation and translation. The maximal joint contact provided by the disc reduces the contact stress on the load-bearing surfaces of the joint. In addition to the shock-absorber function, the disc reduces the distance between the articular surfaces so that the remaining space can be filled with only the capillary film of synovial fluid that spreads covering the articular surfaces and maintains lubrication. Also, because of the attachment of the disc to the retro-discal tissue, the disc translation opens the vascularized retro-discal tissue and thereby increases the blood flow during jaw opening.

Also the evolutionary history of the TMJ clearly shows that this was not a transformation of a cartilaginous model precursor (Hylander 1992). This joint represents an area of contact between two previously unrelated membranous bones. It has been created by migrating muscles and ligaments in their attempt to allow for a greater range of functional movements of the tooth-bearing portion of the lower jaw (Noble & Cleanor 1992).
The manner in which the TMJ in this way has been evolutionary modified reflects the sensitivity of the tissues to functional stress. Also, the muscle attachments depend upon the functional activity of the individual for their development; that the muscle development can, within limits, reflect the exercise and activity that each muscle experiences. Thus, the TMJ in each individual responds to the presence or absence of functional stress and strains transmitted to it. The wide range of actions and rich variation in development of this joint during the evolution is a good indicator of the great adaptiveness of these tissues (Noble & Cleanor 1992 5). However, this evolutionary adaptation refers to long time frames, a single lifetime being too short to adjust for an ‘abnormal’ stress, i.e. a strain that results in dysfunction.

b. Cartilage composition and function

Cartilage is a unique group of tissues with an abundant extracellular matrix secreted by chondrocytes. These tissues are often classified in four different groups (Gardner 1992, Cormack 1987):

1) hyaline cartilage – the tissues from which the embryonic axial and peripheral skeleton is formed. It remains in the load-bearing surfaces of diarthrodial joints and epiphyseal growth plates. Hyaline cartilage is also found in other sites such as nasal septum and bronchi.

2) fibrocartilage – a cartilage with more apparent collagenous structure. This tissue is mainly found in intraarticular discs and menisci.

3) elastic cartilage – a cartilage containing elastine. It is found in tissues where flexibility is needed such as in the epiglottis and external ear.

4) amorphous cartilage – the gellike tissue that has no collagen network found in the nucleus pulposus of the intervertebral discs.
**Hyaline cartilage** is a tissue where the extracellular matrix is a highly hydrated gel that masks fibrous components of the tissue giving glass-like appearance. The main components in this matrix are proteoglycan (PG) and collagen. Typical for the cartilage is that this latter protein is mainly of type II collagen with small amounts of types VI, IX and XI also present. The collagen reinforces the gel and gives it mechanical stability.

The major PG of cartilage is a large aggregating molecule, aggrecan, with extensive substitutions of chondroitin sulphate (CS) and keratan sulphate chains as well as a low number of oligosaccharides. These constituents comprise about 80-90% of the molecular mass and dominate the properties of the PG.

The main glycosaminoglycan (GAG) in aggrecan, CS, contains 20-50 disaccharide units, which makes 40-100 negative charges in the form of carboxyl and sulphate groups. The aggrecan monomer carries in the order of 100 such chains, thus giving some $10^3$-$10^4$, negative charges per molecule. These charges dissociate in the presence of water, each dissociation immobilizing several water molecules. Also, GAG molecules do not fold but remain in a fairly extended form. Because of this they occupy a huge volume for a relatively small mass, an effect that is accentuated due to the large capacity of their charges to bind water. These aggrecans become anchored in tissue because of their ability to specifically interact with hyaluronic acid, thus forming huge complexes with further increased charge density. In fact, the charge density is so high that the water present, also when it constitutes 70-80% of the tissue weight, is not sufficient to hydrate all charges. This creates a substantial ‘swelling pressure’ in the tissue, a phenomenon that is important for the ability of the tissue to take up load (Gardner 1992).

However, the normal cartilage does not swell in solution, even when it is removed from the joint and cut into slices (Maroudas 1976). This implies that its swelling pressure is counteracted by
considerable inelastic constraints due to the collagen fiber network. Likewise, the treatment with enzymes that degrade PGs does not alter the gross appearance of cartilage even if it has been depleted of 90% of its total PG content. However, the ability to withstand load diminishes simultaneously with loss of PGs. Treatment with collagenase, on the other hand, completely solubilises the cartilage.

*Collagens* have other important functions apart from giving cartilage its form and tensile strength. Cells may communicate with one another over relatively long distances by applying tension or ‘sensing’ tension through the extracellular matrix. This intracellular signaling is mediated by specific transmembrane integrin receptors (Milam 2000). This form of intercellular communication may influence the metabolic activities of resident cell populations. For example, cells in muscle tendons subjected to tensile forces synthesize type I collagen and fibronectin. However, the same cell population subjected to compressive loads is induced to make type II collagen and proteoglycans (Vogel 1993).

*Fibrocartilage*

Fibrocartilage is found in TMJ. The articular surface of the condyle is covered by a thick layer of fibroelastic tissue containing fibroblasts and a variable number of chondrocytes. The condylar covering is sometimes classified as fibrocartilage, and its components vary with age and the region of the condyle – anterior, middle, or posterior (Sharawy 2000 b). At a young age, the deepest layer of the fibrocartilage is rich in small undifferentiated cells; this is called the reserve cell layer (Carlsson & Öberg 1974). Between the reserve cell layer and the subchondral bone in the young condyle, a hyaline cartilage exists. It appears that in the presence of cartilage, the condyle is able to adapt to excess loading by becoming hyperplastic (Carlsson & Öberg 1974).
The TMJ disc is an avascular noninnervated cartilage. At the electron microscopic level, the extracellular matrix consists primarily of collagens that run parallel to each other at the intermediate thin zone and in all directions at the anterior and posterior thick zones. In loaded areas, the diameter of collagen fibrils varies, a characteristic shared by tissues that are subjected to heavy loading (Ghadially 1983). The majority of cells are fibroblasts with some chondrocytes.

Fibrocartilage may also develop during osteoarthritis (OA) in hyaline articular cartilage. The penetration of blood vessels through the subchondral bone and calcified cartilage provides sites for microfractures extending into the cartilage (Sokoloff 1993). Fibroblast ingrowth occurs at these sites. These cells undergo cartilaginous metaplasia and elaborate a fibrous matrix containing type I collagen. Fibrocartilage is elaborated also in and above microfractures within the bony plate that comprises the articular surface at the sites that have been denuded of cartilage. Here, the collagen is organized with thicker fibers that often are oriented perpendicular to the surface (Grynpas & al 1980). Although fibrocartilage provides a less adequate articular covering than normal hyaline cartilage, it can provide a functionally acceptable articular surface in the joint. Indeed, with joint motion, fibrocartilage can assume many of the characteristics of hyaline cartilage (Kim & al 1991). Also, an adaptation to intermittent stress in tendon may encourage fibrocartilage differentiation (Giori & Carter 1992).

Fibrocartilage such as in menisci and discs contains principally type I collagen fibers (Messner & Gao 1998). Except at the margins, structures are avascular. In association with type I collagen there is relatively less PG present. As the consequence of the lower PG content the structure is that of an oriented, compact fibrous meshwork containing less water than hyaline cartilage.

In summary, the unique property of PGs in cartilage is thus their ability to make tissues resistant to load by facilitating compliance to compression. This seems to work also in lower total
concentrations, like in fibrocartilage, providing the compartment containing PGs is correspondingly smaller with maintained working concentration. The total concentration of PGs in tissue is therefore not congruent to this ability of PGs to maintain a swelling pressure at the same time as it allows a pulse dampened compression and the total composition of cartilage matrix must be considered to understand this function.

Collagens provide this restraint for changes in volume e.g. swelling of cartilage in normal circumstances can only be accomplished within the limits of collagen meshwork. At the same time the collagen provide strength to the tissue and limits the extent of swelling, maintaining the swelling pressure at a certain level.

**Osteoarthritis**

Osteoarthritis (OA) can – from our understanding of its etiology - be devided into primary and secondary forms. Primary OA is by definition a form without known etiology, while the secondary OA can be associated with known underlying abnormalities or injuries. The OA condition should rather be considered to be a common end stage in different processes that gives degeneration and losses of the articular cartilage.

OA can be defined in different ways, based on symptoms or histopathological findings. The pathology of osteoarthritis involves the whole joint in a disease process that includes focal and progressive losses of articular cartilage with concomitant changes in the underlying bone. This includes the development of cartilage lesions or erosions, and the increased thickness of the bony envelope (bony sclerosis). The soft-tissue structures in and around the joint are also affected. These changes involve the synovium, which may show modest inflammatory infiltrates; the ligaments, which are often lax and the bridging muscle, which becomes weak. However, many patients with pathologic and radiographic evidence of OA have no symptoms.
From a clinical perspective, the most compelling definition of this disease is one that combines its pathology with the symptoms, which in this condition includes pain that occurs with joint use. Unfortunately, the cause of pain in OA is unknown.

Prior to the onset of OA an initial insult or wear may alter the metabolic responses in chondrocytes, which attempt to adjust to the overload by increasing their anabolic activity e.g. enhanced synthesis of extracellular matrix components. This increased production of PGs accelerates water influx into the tissue, which results in a higher swelling pressure that may counteract the overload.

Although the cartilage may be able to withstand such an insult or overload, the tissue sooner or later reaches the point where the strain becomes too large. At this point cells disintegrate and release proteolytic enzymes. The onset of OA is then characterized by this release of synovial-derived and a cartilage-derived proteases initiating cartilage matrix degradation, with matrix metalloproteinases and aggrecanases currently attracting attention. Together, these proteases have the ability to degrade the major macromolecular constituents of the cartilage matrix, such as collagens, aggrecan, and other matrix proteins. The effect of this is a substantial loss of matrix components and matrix swelling pressure, which contrasts the preceding ‘pre-OA’ tissue response. At the same time, the remaining PGs may still attract substantial amounts of water, sometimes leading to oedema in the tissue. The pressure, however, is decreased and losses of other matrix components such as collagen, makes the tissue more fragile. Subsequently overload will then result in mechanical destruction of the tissue.

Ultimately, elevated matrix degradation results in a complete loss of the cartilage. Once this tissue degradation is initiated, this seems to be a ‘point of no return’, a vicious cycle is driving further tissue destruction. In hyaline cartilage destruction with tissue fibrillation and loss of
tissue fragments is common. In the fibrous cartilage of the TMJ disc, where structure is more similar to that of tendon, this initial lesion is followed by tissue destruction in the form of perforation.

OA is not classified as inflammatory arthritis. Neutrophils, the cellular hallmark of an acute inflammatory response, do not generally accumulate in OA synovial fluid. Classical signs of inflammation – heat, redness, swelling, and pain – are also not typically present. Moreover, the expression of cyclooxygenase-2, a marker of inflammatory events in tissue, is not a characteristic feature of OA synovium.

However, in selected patients inflammatory processes play a role in provoking signs and symptoms – and, although the data is preliminary, may contribute to the progression of disease (Spector & al 1997, Dieppe & al 1993, Ayral X & al 1996, 2001).

Arthroscopy has provided new insights to the presence of inflammation in OA. Work by Dougados and co-workers suggests that arthroscopic evidence of synovitis in OA is a risk factor for progressive cartilage degeneration (Ayral & al 1997, Ayral & al 2001).

It is interesting to note in this respect that in TMJ patients, arthroscopically, high frequencies of synovitis, degenerative changes and fibrosis were observed in patients with generalized osteoarthritis and rheumatoid arthritis. Histologic and immunohistochemical examinations showed similarly high frequencies of synovial inflammation in generalized osteoarthritis and rheumatoid arthritis patients, differing clearly from those in reference material. Although generalized osteoarthritis and rheumatoid arthritis may have different causes, the tissue reaction was similar (Gynther & al 1997).
**TMJ osteoarthritis**

Osteoarthritis is the commonest disease affecting the TMJ. Much controversy exists regarding the etiology of TMJ OA. Most theories about the etiology of OA are based on articular cartilage failure. Older theories have considered extra-cartilaginous factors (subchondral bone, synovial fluid, vascular changes) as the primary cause and OA as a secondary problem (Howell 1984). Later studies however have mainly focused on TMJ OA as being a result of ‘wear and tear’, and maladaptation to increased joint loading (Axelsson & al 1992, Axelsson 1993, Axelsson & al 1987, Westesson & Rohlin 1984, Stegenga & al 1992).

In general, the early stage of OA is subclinical, and signs and symptoms develop in the later stages of the disorder (Boering 1966). Therefore it may be difficult to assess the true prevalence of TMJ OA. Distinct radiographic signs are most clearly observed in the final stages.

Nevertheless, visible lesions of the disease have been demonstrated macroscopically in 22-38% of the TMJs in an autopsy material covering age groups between 20 to 90 years (Öberg & al 1971, Hansson & Öberg 1977, Nannmark & al 1990) and in about 80% in age groups between 60 to 80 years (Åkerman & al 1984). Similarly, radiographic studies of TMJs have shown OA signs in 10 to 40% of the joints examined (Eričson & Lundberg 1968, Madsen 1966, Carlsson & al 1968, Eckerdahl 1973, Lindvall & al 1976).

Macroscopic signs of OA in the disc include thinning or perforation often in the posterio-lateral part (Öberg & al 1971). Arthroscopic studies of OA in the TMJ disc also reveal fibrillation, perforation and sometimes fibrotic adherences between the disc and the bony parts of the joint (Holmlund & Helsing 1988, Holmlund & al 1989). Microscopically, the fibrous arrangement is disorganized during the development of OA (de Bont & al 1986), and the lesions also affect other matrix components. Decreased metachromasia have been observed in OA areas in human TMJ discs, as compared to unaffected tissue, indicating loss of PGs. Histologically, discs from
subjects with internal derangement (ID) also showed hyalinization (Isberg & Isacsson 1983, Scapino & al 1983), mineralization (Axelsson 1992) and ingrowth of vessels (Kurita & al 1989). Clinically, the diagnosis of OA is currently based on a combination of symptoms, clinical signs and radiological findings. Pain, related to load and motion is the dominating clinical problem. The most common clinical signs are restricted range of motion and, when bone erosion is present, crepitating sounds from the joint (Holmlund & Axelsson 1996). Typical radiographic changes are erosions, reduced joint space, osteophytes and sclerosis.

The diagnostic sensitivity has been increased with the use of new diagnostic tools. Arthroscopy would reveal the structural abnormality in patients with normal radiographs and provide the treatment option. In addition, molecular markers in synovial fluid, serum and cartilage may have a potential to monitor early cartilage lesions (Dahlgberg & al 1992, Belcher & al 1997, Neidhart & al 1997), although this has not yet reached general application in clinical routine.

**TMJ internal derangement**

Internal derangement of the TMJ, defined as a displacement of the disc to the condylar head and articular eminence, plays an important role in TMJ dysfunction, and as a clinical problem, was recognized over 100 years ago (Annandale 1887). Although OA may develop without disc displacement, internal derangement appears to be highly correlated with TMJ OA (Westesson PL & Rohlin M 1984). The precise causal relationship between disc displacement and OA has not yet been described but it seems that ID of the TMJ may progress to OA, analogous to such a progression in, for example, the dog knee after experimental section of a cruciate ligament.

Temporomandibular joint internal derangement as such has two clinical expressions – painful clicking (PC) and chronic closed lock (CL). In PC there is a temporary subluxation of the disc, often termed reducing disc displacement. CL is characterized clinically by a painful reduced
translation of the disc-condyle complex. There is a definite difference in both clinical and radiological findings in patients displaying PC compared to those having CL, chronic closed lock frequently exhibiting more symptoms and sometimes radiographic signs of OA.

The structure and biochemical composition of the contacting surfaces of the TMJ may be altered when a disc displacement occurs. Disc deformation or perforation, atypical cellular architecture, osteophyte formation, subchondral bone resorption, disruption of the physical continuity of the articular surface of the mandibular condyle, and adhesion formation have all been observed in studies of human cadaver TMJ with articular disc displacement (Castelli & al 1985, Nannmark & al 1990, Helmy & al 1989, Axelsson & al 1987, Brand & al 1989, Kurita & al 1989, Blackwood 1966). However, there is still considerable debate concerning the mechanisms involved in articular disc displacement and the significance of the phenomenon relative to the pathogenesis of TMJ OA.

The relationship between OA and displacement of the disc has been discussed extensively. However, the relationship is still unclear. One suggestion has been that during OA the roughness of the articular surfaces will affect the sliding properties of the joint. The displacement of the TMJ disc would then rather be a side effect of OA (Stegenga 1991 b).

Another explanation favors a connection between ID and OA in TMJ disorder, here, however, mainly relating to degenerative changes in the disc (Boering 1969). Boering distinguished three consecutive stages of the disorder, based on the clinical signs and the radiographic pictures. The initial stage was characterized by clicking and no or only slight radiographically visible degenerative changes. The intermediate stage was characterized clinically by pain and restriction of movement, and radiographically by progressive degenerative changes. The terminal stage was characterized clinically by crepitus, absence of pain, and little or no restriction of movement, and
radiographically by extensive degenerative changes. This assumption was also supported in a comprehensive study by Rasmussen in 1983 who also suggested the term “temporomandibular arthropathy” for this clinical course.

The observation that articular disc displacement can ultimately lead to degenerative joint disease was reported by Farrar and McCarty (Farrar & McCarty 1979, Farrar 1985) and later Wilkes developed a classification scheme (Wilkes 1989). This is also supported by animal studies that revealed significant biochemical and histologic changes in the mandibular condyle and temporal bone after discectomy (Yaillen & al 1979, Block & Bouvier 1990, Hinton 1992). Similar changes in humans were suggested by radiologic studies of patients who had undergone discectomy (Brown 1980, Eriksson & Westesson 1985, Tolvanen & al 1988) and in cadaver studies (Castelli 1985 & al, Helmy & al 1989). The findings of Eriksson (1985) and Holmlund (1989) also support progression since signs of OA, such as crepitation, structural hard tissue changes as well as perforation and deformation of the disc, were consistently found in patients showing CL but hardly ever in patients showing PC. The concept has led to the operations that aimed at repositioning of the disc. However, follow-up studies indicate lower success rates compared with discectomy (Holmlund & al 1993, Trumpy & Lyberg 1995).

The TMJ has a remarkable adaptive capacity (Block & Bouvier 1990, Meikle 1992). However, there are histologic and arthroscopic studies of human TMJs that have identified structural defects believed to represent failure in its adaptive mechanisms (Solberg & al 1985, Westesson & Rohlin 1984, Westesson & al 1985). Thus, some individuals are capable of mounting an adaptive response to an articular disc displacement while others are not. This may also explain why some patients with PC do not develop CL.
In patients with CL a subgroup has been identified, showing monoarthritis (MA) without any signs of generalized joint disease. During surgery, pronounced inflammatory changes and fibrotic bands were found in these patients contrasting to the other patients with TMJ CL. The picture much resembled that of arthritis of infectious origin. In CL there is a female predominance of approximately 3:1. In MA patients it appears that females are almost exclusively affected.

One cause for reactive arthritis can be an infection by Chlamydia trachomatis (Keat & al 1989). Such infections have increased in females during the last decade (Beagley & Timms 2000). Chlamydia trachomatis has also been associated with TMJ internal derangement (Henry & al 1999, 2000). This association was determined by detection of chlamydia antigens and genome in synovial tissue. Later the same author investigated the serology of patients with internal derangement and found a high frequency of serum antibodies to Chlamydia trachomatis in these patients (Henry & al 2001), although this was not correlated to the intra-articular pathology. A possible causative role of Chlamydia trachomatis infection in the development of OA and ID however necessitates the demonstration of this infection being more prevalent in patients compared to controls.
AIMS

To improve our understanding of the pathogenesis of OA in TMJ internal derangement, we studied human TMJ discs, with the aim to answer the following questions:

- How does ID affect the disc and PDA from a tissue point of view? In what way are the GAGs and the PGs affected in the different stages of ID?
- How do the different stages of ID relate to OA as reflected by changes in concentration of selected GAGs and PGs?

To improve our understanding of the factors related to progression of TMJ ID we correlated the aggressiveness of the condition with the presence of immune response to Chlamydia trachomatis, addressing the following questions:

- Is Chlamydia infection associated with TMJ internal derangement?
- Does exposure to Chlamydia trachomatis induce a more aggressive development of ID?
MATERIAL AND METHODS

Material

Autopsy material (I)

The specimens were harvested within hours after death to minimize autolytic changes in the tissue. At autopsy, the roof over the glenoid fossa was gently removed from middle cranial fossa with a chisel following a previously described procedure. The disc and anterior half of the PDA were then gently removed using a scalpel (Hellsing & Holmlund 1985).

- Patients (I, II, III)

Disc specimens were obtained during discectomy from consecutive patients with the clinical diagnosis of painful internal derangement.

Methods

Light microscopy (I)

Both autopsy and patient specimens included the TMJ disc and the anterior part of the PDA. They were oriented and cut sagittally in the central part and then fixed by immersion in 2% neutral-buffered formaldehyde solution. Subsequently they were embedded in paraffin wax, and 3-micrometer thin sections were cut and stained with hematoxylin and eosin and examined by light microscopy at a final magnification of x 100.

Stereology (I)

In the analysis of each histological section, the disc (D), the intermediate zone (IZ), the posterior disc attachment (PDA) were measured separately. The volume density was then calculated for chondrocytes, fibroblasts and blood vessels (Gundersen & al 1988). Disc was identified as fibrocartilage, PDA as areolar connective tissue, and intermediate zone as the area having the features of both D and PDA. Chondrocytes were defined as round cells with well-defined loose
pericellular matrix, fibroblasts as elongated or spindle-shaped cells embedded in fibrous connective tissue matrix, and blood vessels as structures lined by endothelial cells.

**Biochemistry (II)**

TMJ disc and posterior disc attachment were excised during TMJ disectomy. The disc and posterior disc attachment were separated immediately, snap-frozen in liquid nitrogen, and stored at -70°C until processed.

Tissues were solubilized with papain, then GAGs were precipitated by adding 4 volumes of ethanol. Following centrifugation (11 000 x g for 5 min) the pellets were digested overnight at 37°C (w/v) Tris, pH 7.5, the buffer also containing 100 U/l of each of chondroitinase AC and chondroitinase ABC. The digests were then chromatographed on a Spheri/5 amino MPLC column (30 mm x 4.6 mm i.d.; Brownlee Labs, Santa Clara, CA, USA). The eluates were monitored spectrophotometrically (A231) and the sulphated delta-disaccharides derived from CS were collected and pooled. The sulphation patterns in these pools were then analysed by capillary zone electrophoresis. Separation and analysis were carried out on an uncoated fused-silica capillary tube (75 μm I.D., 55 cm total length and 50 cm from the injection point to the detector) at 25°C. Before each run, the capillary tube was washed with 0.1 M NaOH, and then with the operating buffer (various sodium orthophosphate buffers at pH ranging from 2.55 to 5.00). For optimal separation, the electrophoresis was performed at 20 kV using 15 mM sodium orthophosphate buffer at pH 3.00 and reversing the electrodes so that the constituents to be analysed would migrate from the negative (cathode) to the positive (anode) electrode by electrophoretic mobility and against the electroosmotic flow of the buffer (Karamanos & al 1995).
**RT-PCR (III)**

TMJ disc and posterior disc attachment were excised during TMJ dissection. They were separated immediately and stored on RNeater™ (Ambion Inc, Austin, Texas, USA) at -20°C until processed.

**Preparation of total RNA.** Total RNA from cartilage and connective tissue was extracted using RNeasy Kit (QIAGEN Inc, Hilden, Germany). Thus, the samples were ground in liquid nitrogen, and total RNA was extracted according to the manufacturer’s instructions. The final RNA pellets were resuspended in 30 µl RNase-free water. The total RNA concentrations were measured spectrophotometrically at 260 nm. RNA was stored at -20°C until further analysis.

**RT-PCR.** 150 ng of total RNA from each sample were first reverse-transcribed into cDNA in a total volume of 20 µl, using SuperScript Reverse Transcriptase (Gibco BRL, Carlsbad, California, USA) and oligo(dT) primers pdT 15-18 (Pharmacia Biotech, Uppsala, Sweden). Optimal cycling parameters were determined in pilot experiments. Thus cycling parameters were varied to avoid saturation of either analyte or reference amplimer. The selected cycle numbers gave relative fluorescence in the 1:3 – 3:1 range. The PCR amplification of proteoglycan genes sequences was carried out for 35 cycles: 94°C for 30 sec, annealing temperature for 30 sec, 72°C for 1 min (Mastercycler Thermal Cycler, Eppendorf, Germany). cDNA sequences were analysed by simultaneously amplifying the glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene. This was obtained by adding 37 pmol of each primer for GAPDH to the reaction after the first 3-12 cycles of amplification.

**Serology (IV)**

Blood samples for serum analyses were collected after dissection, stored at -20°C and the serum analysed using an indirect microimmunofluorescence test for detecting antibodies against Clamydia trachomatis (Labsystems OY, Helsinki, Finland).
Statistics

Repeated analysis of variance (ANOVA) was used (I, III). Independent t-test and paired t-tests (II). Pearson’s Chi-Square test, Pearson’s correlation tests and paired t-tests (IV).
Results

Paper I. Morphometrical measurements showed that the disc, intermediate zone and posterior disc attachment were easily identified in the autopsy control material. Fibroblasts were mainly seen in the PDA region, diminishing toward the IZ and almost disappearing in the disc regions. Chondrocytes showed tendency to cluster in the disc region where most of them were found. However, they were not seen in the PDA region and were uncommon in IZ. No blood vessels were found in the disc region. They were present mainly in the PDA region with some extensions into the IZ. The findings were similar in both joints of a given person, regardless of age and gender. The volume densities of the fibroblasts, chondrocytes, and vessels differed significantly in the various tissue compartments of the controls.

All surgical specimens deviated more or less from the control material regarding the histologic findings. In the specimens from ID patients, the fibroblasts in the loose connective tissue showed a more variable nuclear morphology than those in the controls. Furthermore, the chondrocytes were more irregular in shape. However, the distributions of fibroblasts and chondrocytes were similar in both types of specimens. Blood vessels were absent in D regions, but many were found in the PDA region. They extended relatively frequently from the PDA region into the IZ but were fewer than in the PDA region. In ID patients the volume densities of the fibroblasts, chondrocytes, and vessels differed significantly in the various compartments. The volume density of blood vessels was significantly higher in ID patients than in controls.

Paper II. The total amount of CS in disc specimens from patients with PC was comparable to that of normal individuals. The levels of GAG concentration found in the posterior disc attachment were higher than in the disc.
Compared to PC patients CL patients showed a substantial reduction of GAG content in both the disc and the posterior disc attachment. The degree of reduction in the two tissues correlated closely.

The pattern of GAG sulphation was similar in disc and posterior disc attachment. The 4/6 -sulfation ratio was somewhat increased when comparing the disc attachment in PC and CL, while the value was unaffected in the corresponding disc tissue.

**Paper III.** In the disc there was a significant increase of aggrecan expression in the CL group when comparing tissues from PC and CL patients. For biglycan, fibromodulin and versican, there was a considerable inter-individual variability. In the PDA tissue the CL group had slightly increased expression of hyaluronan synthase (HAS-1) and biglycan although the differences were not significant. The relative fluorescence values for fibromodulin and versican varied considerably within the groups, a variability that did not seem to correlate to group identity.

**Paper IV.** No statistically significant differences were found between MA and CCL patients for any of the Chlamydia pneumoniae, Chlamydia psittaci and Chlamydia trachomatis. Significant differences were found between patients and controls for Chlamydia trachomatis only. As for titers to Chlamydia trachomatis, a tendency to higher titer levels were found in MA compared to controls and the CCL group.
DISCUSSION

Studies of specimens obtained at discectomy have certain advantages. Such specimens can be correctly oriented and provide optimal conditions for morphologic examination of all compartments, including the synovial lining (paper I), biochemistry (paper II) and molecular biology (paper III). These specimens can also be used as reference material for studies of TMJ disease (paper II).

Several histologic studies have been performed on the TMJ disc and synovial lining in disease (Isacsson & al 1986, Kurita & al 1989, Pereira & al 1996, Gynther & al 1994, 1997, Holmlund & al 1992, Hall & al 1984, Merrill & al 1990, Dijkgraaf & al 1999). However, the interpretation of data has been hampered by the lack of controls (Hall & al 1984, Merrill & al 1990, Dijkgraaf & al 1999) or availability of control specimens only from discrepant age groups (Isacsson & al 1986, Kurita & al 1989, Pereira & al 1996). Moreover, these analyses have been limited to descriptive estimates without objective measures. The use of age-matched control group solves the former problem. The reproducibility of morphometrical examination makes it possible to obtain reliable quantitative information from relatively small groups of samples (Weibel 1979, Romppanen & Collan 1983, Gundersen & al 1988).

It was possible to correctly identify the two studied tissue compartments (disc and PDA) in specimens from autopsy controls and patients. At difference with previous studies (Kurita & al 1989, Pereira & al 1996, Luder 1993) our material showed no signs of cartilage metaplasia in the PDA region. Therefore, the different tissue compartments can be identified by the presence of chondrocytes in disc (paper I).

The only parameter that differed between patients and controls was the volume density of the vessels (paper I). This agrees with the results of previous arthroscopic studies (Gynther & al
1994, Holmlund & Hellsing 1988, Holmlund 1989) and observations in previous histologic studies (Kurita & al 1989, Holmlund & al 1992). Blood vessels were infrequent in the IZ in the controls but common in patients (paper I). The presence of a vascular network close to D may indicate repair in the course of an inflammatory process, but the exact role of this increase of vessels in the pathogenesis of TMJ disc derangement remains to be settled. The effect of hormones on matrix regeneration is an interesting subject for further studies. It has been reported that males are 3 times more capable of regenerating matrix than females and they also regenerate their matrix more quickly (Ng & al 1999, Milam 2000).

The two patient subgroups (PC and CL) have distinctly different patterns for tissues to react (paper II). A direct trauma to the TMJ is supposed to contribute to destruction of normal tissue (Pullinger & Seligman 1991). There is plenty of evidence of the importance of load in animal OA models (Radin & al 1978, 1982, 1991, Eggli & al 1988). Our results suggest that an increased local load would contribute to the development of OA since CL was tightly associated with reduced concentration of GAGs paralleling the appearance of clinical symptoms (paper II).

When the increased load is confined to the disc margins, such as in PC, osteoarthritic degeneration can cause weakening of the PDA resulting in disc displacement and CL. When the patients just display the symptoms of PC, however, there are no degenerative changes in the tissue composition (paper II), indicating sufficient adaptive response, that will compensate for the increased load during disc displacement (Giori & Carter 1992, Milam 2000, Eriksson & al 1985, Holmlund & Axelson 1989). The total concentration of GAGs in disc specimens from these patients with PC is comparable to that of normal individuals (Axelson & al 1992 a). The levels found in PDA were higher than in the disc. They were also high when compared to other dense connective tissues such as tendons (Vogel & al 1993) and similar to those in loose
connective tissues such as in the gingival (Dahllöf & al 1986). Therefore, this patient group might represent a suitable reference material for studies of TMJ disorders.

The subluxation of the disc associated with disc displacement increases the point load on the disc, particularly in its margins. The tissue can, however, adapt to this, and patients who display the symptoms of PC may have deformities in the disc (Giori & Carter 1992, Milam 2000, Eriksson & al 1985, Holmlund & Axelsson 1989). There are, however, no signs of matrix degradation in these tissues (paper II), in fact, the concentration of GAGs in both the disc and PDA specimens from patients with PC is compatible with that of corresponding tissues of normal individuals (Axelsson & al 1992 b, Dahllöf & al 1986).

While the PG content in the disc matrix seemed unaffected in patients with PC, all patients with CL revealed biochemical signs of tissue degradation as measured by the GAG concentration. This shows the close correlation between TMJ disc OA and the appearance of CL, although in some patients with PC the symptoms will progress into CL even if such a sequence is found in a limited number of patients only indicating that OA may develop in other ways (Könönen M & al 1996, de Leeuw & al 1996).

Even the association between the presence of microorganisms and TMJ degenerative and inflammatory disease may exist (paper IV). The internal derangement might be associated with higher exposure to Chlamydia trachomatis (paper IV). Statistically significant differences were found for Chlamydia trachomatis only when comparing patients and controls. In CL group of patients it was detected in both males and females. MA group consisted of females only, as did control group. Interestingly so, more aggressive subgroup of patients – after the onset of the disease - could not be correlated to higher exposure to Chlamydia species. Probably, infectious agents might be a co-factor in the disease development while not contributing to its progression.
It is also interesting to see that in the group of ID patients there is the presence of a vascular network close to disc that may indicate repair in the course of an inflammatory process (paper I).

Similar to other joints the increased point load on the TMJ disc might initiate the development of OA. The pattern varies depending on the underlying cause for tissue degradation and infectious agents such as Chlamydia may promote this process. In case of patients with disc derangement, the TMJ OA would primarily affect areas close to the margins of the disc where this load is most extensive. When this happens, the degradation not only affects the disc fibrocartilage, but this process also seems to extend to the neighbouring attachments. The close correlation between the changes in GAG concentration seen in disc and PDA, respectively, indicates that this is a common event once OA develops, i.e., an OA degeneration of peripheral disc tissue will also affect the attachments.

Some, but not all patients with disc derangement will thus develop OA. The disc degeneration thus includes both disc tissue and adjacent tissues, which may affect the laxity of the disc attachment. The mobility of the disc may then be extended sufficiently to cause luxation, and because of this the patient may display symptoms of the more permanent CL, the OA process being the cause of altered pattern of symptoms (PC versus CL). The findings support this sequence of events in the development of TMJ OA associated with disc derangement.

The decrease in GAG concentration seen in both disc and PDA in patients with CL may be the result of either decreased synthesis and/or decreased turnover. In the present material it was not possible (with PC as control) to correlate concentration of GAG to a correspondingly decreased mRNA expression of any major PGs normally found in these tissues (paper III). Assuming that this expression pattern reflects the rates of synthesis, it seems probable that the decrease in
GAGs seen in CL relates to an increased turnover of matrix components rather than the decreased synthesis rate.
CONCLUSIONS

- The identification of compartments of the TMJ disc should focus on chondrocytes in the disc and fibroblasts and vascularity in the PDA. Patients and autopsy controls differed significantly in regard to volume density of vessels.

- Two clinical expressions of internal derangement represent two different ways for tissue to react. The tissues of PC patients are comparable to those of normal individuals indicating an adaptive response to the strains they are subjected to. The tissues of CL patients show reduced concentrations of GAGs in both disc and PDA.

- Development of OA in patients with PC is associated with the appearance of CL.

- The degradation of matrix in CL patients seems not to be caused by a reduced synthesis and the degenerative process seen in these patients is one with low turnover similar to the situation in primary osteoarthritis of hyaline cartilage.

- Chlamydia trachomatis infection may be associated with TMJ chronic closed lock. The occurrence of antibodies to Chlamydia trachomatis did not correlate with the severity of joint disease.
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