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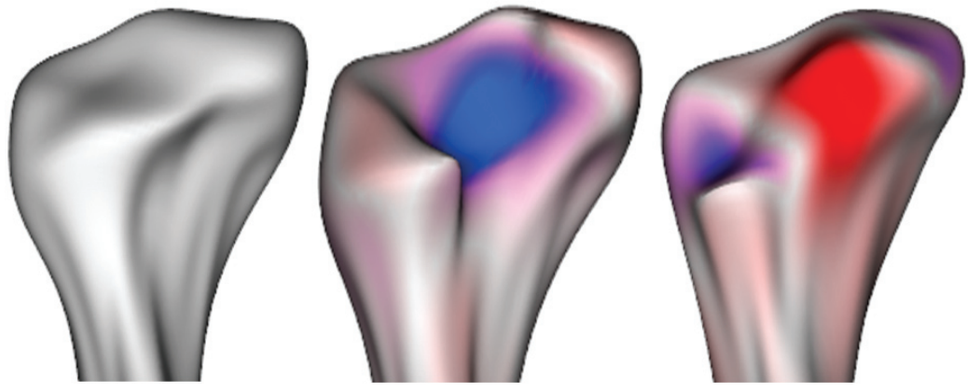

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Cover image

Three-dimensional renderings of a temporomandibular joint condyle processed from cone-beam computerized tomography scans at the time for RA diagnosis and at one-year and two-year follow-up. The first illustration is a rendering of the condyle at the time for diagnosis and the others are visualizations of bone tissue changes showing bone resorption in blue, bone formation in red and no change in white. In this particular condyle bone tissue resorption was seen after one year and new bone formation was identified between the one-year and two-year examinations.

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To Alexandra and Reza

How success is measured is different for each of us.
The first and most important step is realizing that the secret of success is inside.

Garry Kasparov

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Contents

Summary

The general aim was to investigate the occurrence and development of TMJ bone tissue resorption and to identify predictors for progression during the two first years with rheumatoid arthritis.

The thesis comprises one cross-sectional part and one longitudinal part. The aim of the cross-sectional part was to investigate whether there was an association between the radiographic sign (TMJ erosions) or the clinical sign (crepitus) of TMJ bone tissue resorption and glutamate and if that relationship was influenced by serotonin, tumor necrosis factor, interleukin-1β, interleukin-6, vascular endothelial growth factor, C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor or sex hormones. The first aim of the longitudinal part was to investigate whether TMJ erosions or crepitus, glutamate, inflammatory mediators and markers changed over time and if the change in TMJ erosions was related to these factors. The second aim was to investigate the predictive value of TMJ erosions, glutamate and crepitus as well as the inflammatory mediators and markers for change in TMJ erosions over two years after diagnosis.

Both parts of the thesis comprised the same group of patients with recent RA diagnosis. They were 47 at first visit and 41 attended the two year follow-up. Similar examination protocols were used for the cross-sectional and the longitudinal part. The subjects were examined for TMJ erosions on cone-beam computerized tomographs and the clinical examination included the articular sound of crepitus. Blood samples were obtained and analyzed for glutamate, serotonin, tumor necrosis factor, interleukin-1β, interleukin-6 and vascular endothelial growth factor, C-reactive protein, erythrocyte sedimentation rate and rheumatoid factor, as well as for estradiol and testosterone.

Significance of univariate correlations was calculated with Spearman rank correlation coefficient. To test the significance of the differences between visits the Friedman ANOVA on ranks was used. Multivariate regression was used to investigate the relative influence by independent variables on the presence, development or prediction of the TMJ erosions. Interaction analysis was performed by including an interaction term in addition to the main effects in the multiple linear regression model. The significance of the differences between groups was calculated with Fisher´s exact test for variables on nominal scale and Mann-Whitney

U-test for variables on ordinal scale. A probability level of less than 0.05 was considered significant in all analyses.

Seventy-two percent of the patients showed TMJ erosions at the beginning of the study. TMJ erosion score was positively correlated to both glutamate and crepitus. There was also a positive correlation between erosions and C-reactive protein in the patients with estradiol levels ≥ 65 pmol/L. Multiple stepwise regression showed that TMJ crepitus ($p < 0.001$), glutamate ($p = 0.005$) and erythrocyte sedimentation rate ($p = 0.010$) explained 41% of the variation in the dependent variable TMJ bone erosion score ($p < 0.001$). Glutamate was positively correlated to TMJ erosion score in the patients without TMJ crepitus, with C-reactive protein < 3mg/L, C-reactive protein < 3 mg/L and estradiol < 50 pmol/L as well as C-reactive protein < 3 mg/L and testosterone ≤ 1.2 pmol/L. Progression of the erosion score was found in 29% of the patients and 43% showed regression during the first two years after RA diagnosis. The progression of erosion score was explained to 34% by testosterone ($p = 0.012$) and interleukin-1β ($p = 0.024$). Patients with higher than median level of glutamate at the first visit showed more reduction in erosion score than those with lower level ($p = 0.035$). TMJ erosion score at the first visit was negatively correlated to progression of the score ($r_s = -0.64$, $p < 0.001$). The multivariate analysis showed that erosion score was the only significant predictive factor that explained 53% of the regression ($p < 0.001$).

TMJ bone tissue resorption was found to be present in a majority of patients with early RA. Glutamate modulates TMJ bone tissue resorption in the early phase of disease with absence of crepitus and under the influence by systemic inflammation and sex hormones. Regression in TMJ bone tissue resorption was the most frequent finding over a two-year period directly after diagnosis. Progression however seems to be associated with both inflammatory and non-inflammatory mechanisms while progression of crepitus is associated with inflammation when glutamate is increased. Regression of TMJ bone tissue resorption could be predicted by early TMJ erosions and glutamate. The results indicate that TMJ bone tissue resorption can be expected early in RA.

Keywords

Arthritis, rheumatoid; Bone tissue resorption; Cone-beam computed tomography; C-reactive-protein; Erythrocyte sedimentation rate; Estradiol; Glutamate; Interleukin-1β; Interleukin-6; Rheumatoid factor; Serotonin; Temporomandibular joint; Testosterone; Tumor necrosis factor; Vascular endothelial growth factor.

Preface

This thesis is based on the following manuscripts, which will be referred to in the text by their Roman numerals:

I. Endogenous glutamate in association with inflammatory and hormonal factors modulates bone tissue resorption of the temporomandibular joint in patients with early rheumatoid arthritis.

Hajati A-K, Alstergren P, Näsström K, Bratt J and Kopp S.

J Oral Maxillofac Surg. 2009 Sep;67(9):1895-903.

II. Temporomandibular joint bone tissue resorption in patients with early rheumatoid arthritis can be predicted by joint crepitus and plasma glutamate level.

Hajati A-K, Alstergren P, Näsström K, Bratt J and Kopp S.

Mediators of Inflammation. 2010:627803.

III. Bone tissue resorption in the temporomandibular joint and its relation to systemic inflammation and crepitus during the first two years of rheumatoid arthritis.

Hajati A-K, Alstergren P, Näsström K, Bratt J and Kopp S.

Manuscript.

IV. Temporomandibular joint bone tissue resorption and plasma glutamate predict regression of articular bone tissue resorption in two years for patients with early diagnosed rheumatoid arthritis.

Hajati A-K, Alstergren P, Näsström K, Bratt J and Kopp S.

Manuscript.

Abbreviations

5-HT	Serotonin (5-hydroxytryptamine)
BARFOT	Better Anti-Rheumatic Farmacotherapy
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ANOVA	Analysis of variance
CT	Computerized tomography
DAS28	Disease activity score 28
DMARD	Disease modifying anti-rheumatic drug
EIA	Enzyme immuno-assay
GC	Glucocorticosteroids
VEGF	Vascular endothelial growth factor
NMDA	N-methyl-D-aspartate
NSAID	Non-steroidal anti-inflammatory drug
OPG	Osteoprotegerin
Glu	Glutamate
IL-1β	Interleukin-1beta
IL-6	Interleukin-6
TNF	Tumor necrosis factor
RA	Rheumatoid arthritis
RANK	Receptor activator of nuclear factor κ B
RANKL	Receptor activator of nuclear factor κ B Ligand
TMJ	Temporomandibular joint

Introduction

RHEUMATOID ARTHRITIS

RA is one of the most common systemic inflammatory conditions with a worldwide prevalence of 0.5-1% (Kvien et al., 1997; Alamanos et al., 2006; Englund et al., 2010). It is more common in women with a female: male ratio of 3:1 (Symmons, 2002). The etiology of RA is still partially unknown but knowledge of the pathogenetic processes is increasing.

RA is mainly characterized by progressive articular cartilage degradation and bone tissue resorption as well as pain and loss of function in the musculoskeletal system. Damage to the articular cartilage and bone by the disease process accounts for a considerable part of the disability in RA (Scott et al., 2000). If untreated, it leads to severe disability as well as poor quality of life for most patients (Yelin et al., 1987; Hashimoto et al., 2001; Sokka, 2003).

The disease process starts years before the clinical onset and certain RA-specific autoantibodies, e.g. autoantibody against cyclic citrullinated peptide, are present in the blood years before the symptoms start (Aho et al., 1991; Aho et al., 1993; Rantapaa-Dahlqvist et al., 2003). Both experimental and clinical studies confirm that a subclinical phase precedes the clinically manifest arthritis (Kraan et al., 1998; Hayer et al., 2007). Hayer et al. (2007) showed that synovitis as well as osteoclast formation and activation is established in the articular synovium and underlying bone before the clinical symptoms appear.

Current diagnostic criteria for RA (Arnett et al., 1988) are mainly based on clinical findings typical for the chronic phase and have limitations regarding the possibilities to identify early phases of RA and to predict early disease development. Early diagnosis of RA has been considered important to identify individuals who will develop severe destructive disease, so that effective treatment can be initiated before irreversible damage occurs (van Aken et al., 2004).

The radiographic sign of erosion is generally considered to be an indicator of bone tissue resorption in RA. The presence of erosions in joints from hands and feet may serve as an estimate of RA activity (Forslind et al., 2009) and severity as it predicts for progression and poor prognosis when detected early in the disease (van der Heijde et al.,

1992). About 70 % of RA patients show erosions on plain radiographs of the small joints in hands and feet already at the time of diagnosis (van der Heijde, 1995; Goekoop-Ruiterman et al., 2005).

Temporomandibular joint rheumatoid arthritis

TMJ involvement is common in the late phase of RA with a prevalence varying from 5-86% depending on patient selection, diagnostic criteria and techniques used (Syrjanen, 1985; Tegelberg and Kopp, 1987; Lin et al., 2007). The most important clinical consequences of late phase TMJ RA are pain and functional disability, e.g. pain upon mouth opening and chewing or anterior open bite due to TMJ bone tissue resorption, which have a significant impact on the patients' daily activities and quality-of-life (Voog et al., 2003b).

The severity of TMJ RA is well correlated to the severity of general disease. Markers of inflammation such as C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor and thrombocyte particle count have all been associated with the severity of TMJ RA (Goupille et al., 1993; Nordahl et al., 2001; Voog et al., 2003a; Lin et al., 2007). The destructive process does not necessarily correlate to local clinical signs and symptoms as radiographic signs of bone tissue resorption can be observed in a large proportion of asymptomatic patients (Goupille et al., 1990; Goupille et al., 1993; Celiker et al., 1995). RA involvement of the TMJ may therefore be undiagnosed and untreated early in the process. The question is to what extent radiographic signs of TMJ RA occur regardless of clinical signs and symptoms of the TMJ.

Radiographic examination of erosions in TMJ RA is difficult owing to the location of the joint and its relation to other cranial structures. However, when detected on conventional tomograms erosions in the late phase of TMJ RA have a frequency of about 50% (Gynther et al., 1996; Voog et al., 2003a) and progress in 19%-33% of the patients over one to four years (Nordahl et al., 2001; Voog et al., 2003a). It has been shown that the severity of radiographic changes in late TMJ RA is comparable to those of the metacarpophalangeal and metatarsophalangeal joints of the hands and feet (Akerman et al., 1991). The frequency and progression of TMJ bone tissue resorption in early RA is unknown as well as its predictive value for progression.

Crepitus is a common clinical sign of late phase TMJ RA with a reported frequency of 75% with a disease duration of more than five years (Tegelberg and Kopp, 1987). Crepitus is associated with cartilage and

bone tissue destruction in late phase TMJ RA (Kopp and Rockler, 1979; Akerman et al., 1988; Wiese et al., 2008). However, the relationship between crepitus and radiologic changes of the TMJ in RA patients is weak as 80% of joints with crepitus have shown evidence of TMJ bone tissue resorption whereas only 47% of joints with radiographic erosions had crepitus (Akerman et al., 1988). This indicates that articular cartilage and bone destruction may develop by different mechanisms in late phase TMJ RA. This relationship is not yet elucidated in recently diagnosed RA.

MECHANISMS OF BONE TISSUE RESORPTION

Bone is a dynamic tissue with continuous resorption and formation to adapt to its functional role. The bone turnover and remodeling is accomplished by coordinated intercellular signals between osteoblasts and osteoclasts which is dependent on the RANKL/RANK/OPG system under influence of mechanical loading and locally produced cytokines, growth factors and neuroregulators as well as hormones. For example, proinflammatory mediators as well as sex hormones influence the osteoclast differentiation and the osteoclastic bone resorptive activity (Takahashi et al., 1988; Lacey et al., 1998; Shevde et al., 2000; Michael et al., 2005; Galal et al., 2008).

RANKL modulates osteoclast differentiation and activity via the RANK receptor on the osteoclasts or pre-osteoclasts whereas OPG acts as a competitive antagonist to RANKL (Simonet et al., 1997). In patients with early RA the relation between RANKL and OPG predicts articular bone tissue resorption in hands and feet (Geusens et al., 2006). Activated T cells as well as synovial fibroblasts in active synovitis express more RANKL compared to those from patients with less active disease or from patients with osteoarthritis (Kong et al., 1999; Gravallese et al., 2000; Takayanagi et al., 2000; Kotake et al., 2001; Crotti et al., 2002; Haynes et al., 2003). This increase in RANKL expression is accompanied by a decrease in OPG expression in patients with active disease in comparison to patients with less active disease or healthy individuals (Haynes et al., 2003). The imbalance in RANKL, RANK and OPG expression in the synovial tissues probably favors local differentiation and activity of osteoclasts (Ainola et al., 2008). There is, however, only limited knowledge available about the influence by glutamate, serotonin, proinflammatory cytokines and hormones on the modulation of early TMJ RA bone tissue resorption.

Glutamate

Glutamate is an excitatory amino acid which is involved in peripheral mediation of inflammation and bone tissue remodeling in physiologic as well as in pathologic processes. Glutamate is also considered to be the mediator of load-related bone tissue remodeling (Skerry, 2002; Turner et al., 2002; Spencer and Genever, 2003; Mason, 2004). Glutamate is involved in bone resorption and formation through stimulation of N-methyl-D-aspartate (NMDA) receptors on osteoclasts and osteoblasts (Reynolds and Miller, 1988; Hollmann and Heinemann, 1994; Spencer et al., 2007). Glutamate activation of the NMDA receptor expressed on osteoclasts or osteoblasts regulate their expression of RANKL and RANK (Chenu et al., 1998; Peet et al., 1999; Genever and Skerry, 2001; Itzstein et al., 2001; Gu et al., 2002; Mentaverri et al., 2003; Ho et al., 2005; Spencer et al., 2007; Lin et al., 2008). A potential source of glutamate is the nerve fibers in the bone microenvironment (Chenu, 2002a; Chenu, 2002c). In RA.the sources of glutamate are activated thrombocytes, lymphocytes, macrophages, neutrophils, fibroblasts, local nervfibers in the synovium as well as osteoblasts and activated osteoclasts in the bone tissue (Chenu et al., 1998; Lawand et al., 2000; Chenu, 2002b; Gravallese, 2002; Aliprandi et al., 2005; Tremolizzo et al., 2006; Spencer et al., 2007).

Local concentrations of glutamate are significantly elevated in acute and chronic inflammation in both animal models and humans (Omote et al., 1998; Lawand et al., 2000; McNearney et al., 2000). Elevated levels have also been reported from synovial tissues in RA (Lawand et al., 1997; McNearney et al., 2004; Tremolizzo et al., 2006). Glutamate is also elevated in plasma possibly partly as a result from activation of thrombocytes which release glutamate ex vivo when challenged with aggregating stimuli such as the rheumatoid factor (Trang et al., 1985; Aliprandi et al., 2005). Continuous thrombocyte stimulation with ensuing release of glutamate into the plasma may also be accomplished by proinflammatory cytokines such as TNF and IL-1β since thrombocytes express cytokine receptors that reduce thrombocyte reuptake of glutamate (Loppnow et al., 1998; Aliprandi et al., 2005). There are thus reasons to believe that glutamate and the NMDA receptor are involved in the articular bone destruction occurring in RA.

MEDIATORS OF INFLAMMATION

Cytokines and serotonin are mediators involved in processes like inflammation and modulation of bone remodeling and turnover. TNF, IL-1β, IL-6 and VEGF levels are increased both systemically in the blood and locally in the synovium and synovial fluid in RA where they have been associated with articular bone and cartilage destruction by enhancing RANKL expression and production of cartilage-destructive enzymes (Duff, 1994; Lacey et al., 1998; Ballara et al., 2001; Kwan Tat et al., 2004; Walsh and Gravallese, 2004).

TNF has the most prominent role in orchestrating the inflammatory process and immune response and is primarily produced by activated T-cells and macrophages in the inflamed synovium (MacNaul et al., 1990; Danning et al., 2000). TNF has the capacity to regulate osteoclast differentiation and function directly or indirectly, independent of its role in inflammation (Kobayashi et al., 2000; Komine et al., 2001).

TNF levels are increased in the synovial fluid and the synovial membrane of patients with late phase RA (Buchan et al., 1988; Saxne et al., 1988). There is also an increase in plasma and synovial fluid levels of cytokines in early-phase RA (Raza et al., 2005; Kokkonen et al., 2010). The TNF levels in plasma as well as in the TMJ synovial fluid are related to local TMJ bone tissue resorption (Nordahl et al., 1998; Nordahl et al., 2000; Voog et al., 2003a). Anti-TNF treatment of RA results in retardation of radiographic progression (Walsh and Gravallese, 2004).

IL-1β is produced in high levels by activated macrophages and synovial fibroblasts but also by monocytes and osteoblasts during inflammation but not in healthy individuals where IL-1β is undetectable in synovial fluid from the TMJ as well as in plasma (Eastgate et al., 1988; MacNaul et al., 1990; Dinarello, 1996; Alstergren et al., 1999; Danning et al., 2000). IL-1β has strong bone-resorbing effects via receptors on preosteoclasts, osteoclasts and osteoblasts inducing osteoclast differentiation and activation (Tunyogi-Csapo et al., 2008; Jung et al., 2009). Similar to TNF, IL-1β can indirectly regulate osteoclastogenesis by upregulating RANKL expression in osteoblasts (Hofbauer et al., 1999). IL-1β can also promote the survival and function of mature osteoclasts (Jimi et al., 1999; Kobayashi et al., 2000; Suda et al., 2001).

Presence of IL-1β in plasma and in the TMJ synovial fluid is related to TMJ destruction, expressed as anterior open bite, in patients with chronic inflammatory disorders (Alstergren et al., 1998). Neutralization of TNF and

IL-1β by soluble IL-1β and TNF receptors decreases the osteoclast formation and bone loss in experimentally induced arthritis (Walsh and Gravallese, 2004). In bone tissue resorption inhibition of IL-1β by the IL-1β receptor antagonist leads to decreased progression of radiographically assessed bone loss (van den Berg and Bresnihan, 1999).

IL-6 has a prominent role in inflammation-induced bone tissue resorption and is primarily produced by osteoblasts, which also express receptors for IL-6 (Bellido et al., 1996). IL-6 induces RANKL mRNA expression in murine osteoblastic cell lines (Nakashima et al., 2000). It is possible that IL-6 stimulates osteoblastic RANKL production and exerts an indirect effect on osteoclasts via the RANKL/RANK/OPG system (Kwan Tat et al., 2004). IL-6 synthesis in osteoblasts is also upregulated by decreased estrogen levels indicating a role in RA osteoporosis (Girasole et al., 1992; Poli et al., 1994).

VEGF plays a major role in RA angiogenesis by stimulating proliferation and migration of endothelial cells to form new blood vessels and also increases the permeability of the vessels (Brown et al., 1997; Jackson et al., 1997; Achen and Stacker, 1998; Paleolog, 2002). The source of VEGF includes fibroblasts, osteoblasts, macrophages, thrombocytes and lymphocytes (Brown et al., 1997). The expression is upregulated upon hypoxia mainly by macrophages and fibroblasts in the synovial membrane of RA patients and VEGF is therefore present in the synovial fluid of RA patients (Shweiki et al., 1992; Fava et al., 1994; Koch et al., 1994; Nagashima et al., 1995; Harada et al., 1998; Paleolog and Miotla, 1998).

Serum level of VEGF is elevated in patients with active RA compared to healthy individuals and it is related to disease activity (Nagashima et al., 1995; Harada et al., 1998; Ballara et al., 2001; Lee et al., 2001; Sone et al., 2001). Serum VEGF level in early RA was predictive of progression of bone tissue resorption after one year (Ballara et al., 2001). It was previously reported from a study of an arthritis model in mice that inhibition of angiogenesis also reduced the destruction of cartilage and bone (de Bandt et al., 2000; Miotla et al., 2000). Osteoclasts express VEGF receptors and stimulate osteoclastic bone tissue resorption in vitro and may therefore contribute to joint destruction by direct stimulation of osteoclasts and osteoclast precursors or indirectly by contributing to synovial hyperplasia (Nakagawa et al., 2000; Pap and Distler, 2005).

Peripheral serotonin is an endogenous mediator of inflammation which is involved in bone remodeling (Herbert and Schmidt, 1992; Pierce et al., 1995; Warden et al., 2005; Bliziotes et al., 2006; Modder et al., 2010). Serotonin has

a key role in bone formation via the 5-HT_{2B} receptor on osteocytes and osteoblasts (Bliziotes et al., 2001; Westbroek et al., 2001; Collet et al., 2008). Serotonin is involved in osteoblast induced inhibition of osteoclast differentiation by increasing OPG and decreasing RANKL secretion from osteoblasts (Gustafsson et al., 2006). Activation of serotonin receptors on osteoblasts, preosteoclasts and osteoclasts leads to bone tissue resorption and osteoclastogenesis (Battaglino et al., 2004; Yadav et al., 2008; Modder et al., 2010). Serotonin is mainly released from activated thrombocytes (Endresen, 1989; McNicol and Israels, 1999; Battaglino et al., 2004). It is present in bone marrow, synovial membrane, synovial fluid and plasma of patients with late phase RA (Alstergren and Kopp, 1997; Bliziotes et al., 2001; Kopp and Alstergren, 2002; Battaglino et al., 2004; Seide et al., 2004).

High plasma level of serotonin has been positively associated with progression of bone tissue resorption indicating an influence by serotonin on local bone tissue resorption. In addition high serum levels of serotonin were associated with regression while low levels were associated with progression in the late phase of TMJ RA (Voog et al., 2003a; Voog et al., 2004). The exact nature of the serotonin contribution to bone tissue resorption and the influence of sex is still unclear.

SYSTEMIC MARKERS OF DISEASE AND INFLAMMATORY ACTIVITY

Recent data from early undiagnosed arthritis show that solely presence of erosions is not predictive for development of RA. Patients who developed RA were more often positive for rheumatoid factor and had higher systemic disease activity than those who did not develop the disease (Thabet et al., 2009). This indicates that early detection of the disease needs to combine several predictors accurately.

C-reactive protein and erythrocyte sedimentation rate, together with rheumatoid factor and anti-citrullinated cyclic peptide, comprise the basis for estimation of systemic inflammatory activity in RA. These markers have all been associated with occurrence, progression and prediction of articular bone tissue resorption in RA (Amos et al., 1977; Larsen et al., 1977; van Leeuwen et al., 1993; Combe et al., 1995; Jansen et al., 2001; Syversen et al., 2008). C-reactive protein was found to be related to radiographic signs as well as progression of TMJ bone tissue resorption in RA (Celiker et al., 1995; Nordahl et al., 2001; Voog et al., 2004). C-reactive protein and erythrocyte sedimentation rate are up regulated as a result of elevated cytokine expression. However, C-

reactive protein is a better marker of inflammation and joint damage while erythrocyte sedimentation rate adds information reflecting disease severity (Bull et al., 1989; Wolfe, 1997; Jansen et al., 2001).

The rheumatoid factor is a well accepted predictor of disease activity in RA and poor prognosis (van der Heijde et al., 1992; van der Heijde, 1995; van Leeuwen et al., 1995; Uhlig et al., 2000; Vittecoq et al., 2003). Rheumatoid factor has been found to be related to radiographic signs of TMJ bone tissue resorption in the late phase (Celiker et al., 1995). How these systemic markers reflect TMJ articular bone tissue resorption in early RA is not known.

SEX HORMONES

The influence by sex hormones on the disease activity of RA is an interesting topic since the frequency of RA is higher in fertile women and sex hormones have been suggested to be responsible for the sex-related differences in RA (Masi et al., 1999; Dao and LeResche, 2000; Kopp, 2001; Cutolo et al., 2004; Cutolo et al., 2006).

Both estradiol and testosterone are known to have immunomodulating effects but different roles with estradiol enhancing humoral immunity and androgens as natural immune suppressors (Masi et al., 1999; Cutolo and Wilder, 2000; Khosla et al., 2001; Riggs et al., 2002; Cutolo et al., 2004; Cutolo et al., 2006; Cutolo, 2009). Estrogen and testosterone have direct and indirect inhibitory effects on osteoclast formation and bone tissue resorption. Estrogens inhibit the release of TNF and IL-1β by monocytes and T-cells and IL-6 by stromal cells and stimulate the secretion of OPG by osteoblasts. Testosterone enhances bone formation through its antiapoptotic effect on osteoblasts (Shevde et al., 2000; Riggs, 2002; Michael et al., 2005; Galal et al., 2008). Reduction of estrogens or androgens increases the rate of bone remodelling and also causes a focal imbalance between resorption and formation by prolonging the lifespan of osteoclasts and shortening the lifespan of osteoblasts (Vanderschueren et al., 2004).

Estrogen deficiency in post-menopausal women seems to be responsible for the major part of the differences in prevalence of RA between men and women (Straub, 2007). The increased bone tissue resorption in postmenopausal women is clearly related to estrogen deficiency (Riggs, 2002). In addition, estrogen and inflammation contribute additively to osteoporosis (Jochems et al., 2005). Testosterone levels have been

inversely related to clinical severity in RA (Gordon et al., 1988; Tengstrand et al., 2009). Low systemic and local levels of androgens, including testosterone, have been observed in both women and men with RA (Cutolo et al., 2005; Cutolo, 2009). Surprisingly, men with RA were found to have higher estradiol levels than healthy men and the levels were related to indices of inflammation (Tengstrand et al., 2003).

Aims

GENERAL AIM

The general aim of this thesis was to investigate the occurrence and development of the radiographic sign of TMJ bone tissue resorption in patients with early RA as well as to identify predictors for the development over the first two years of disease.

SPECIFIC AIMS

Cross-sectional part (Study I and II)

The aims of the cross-sectional part were to investigate:

- if the plasma level of glutamate is related to TMJ bone tissue resorption (Study I)
- if the relation between TMJ bone tissue resorption and glutamate is influenced by systemic inflammatory activity as well as levels of the sex hormones estradiol and testosterone (Study I)
- if TMJ bone tissue resorption is related to TMJ crepitus as a clinical sign of articular cartilage and bone damage (Study II)
- if TMJ bone tissue resorption in patients with or without crepitus is related to glutamate, serotonin, cytokines (TNF, IL-1β, IL-6, VEGF), C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor, estradiol or testosterone (Study II)
- if these relationships are influenced by sex (Study II)

Longitudinal part (Study III and IV)

The aims of the longitudinal part were to investigate

- whether TMJ bone tissue resorption, glutamate, crepitus of the TMJ, circulating inflammatory mediators (serotonin, TNF, IL-1β, IL-6, VEGF) and markers (C-reactive protein, erythrocyte sedimentation rate) change over time (Study III)
- whether longitudinal changes in TMJ bone tissue resorption are related to changes in glutamate or TMJ crepitus (Study III)
- whether longitudinal changes in TMJ bone tissue resorption are related to change in inflammatory mediators (serotonin, IL- 1β),

markers (C-reactive protein, erythrocyte sedimentation rate) or estradiol and testosterone (Study III)

- whether progression of TMJ bone tissue resorption can be predicted by glutamate, joint crepitus or serotonin (Study IV)
- whether progression of TMJ bone tissue resorption can be predicted by the circulating inflammatory mediators (IL-1β, TNF, IL-6, VEGF) or inflammatory markers (C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor) (Study IV)

Materials and methods

PATIENTS

Between May 2004 and May 2006, 160 out of approximately 380 patients recently diagnosed with RA were ivited to participate in the present study. They were diagnosed at the Department of Rheumatology, Karolinska University Hospital in Huddinge, Sweden. A total of 47 patients (29 women and 18 men; 28 (60%) seropositive) accepted to participate and 41 (26 women and 15 men) of them fulfilled the two-year longitudinal follow-up. The complete numbers of patients included in the study are presented in Fig 1.

Six patients did not attend any follow-up examination, due to death (1), health reasons (2), unknown reasons (2) and panic in the CT (1). At the one-year follow-up one patient declined to participate because she did not experience any symptoms and another did not accept the CT examination due to pregnancy. At the two-year follow-up three patients dropped out for health or other personal reasons and one CT scan failed even after rescan. Out of 47 patients 38 fulfilled the longitudinal part, 35 of those went through all examinations at all three visits. The baseline characteristics of the 41 patients represented a typical RA patient population sample. A comparison with 379 recently diagnosed RA patients included in a Swedish multicenter survey, Better Anti-

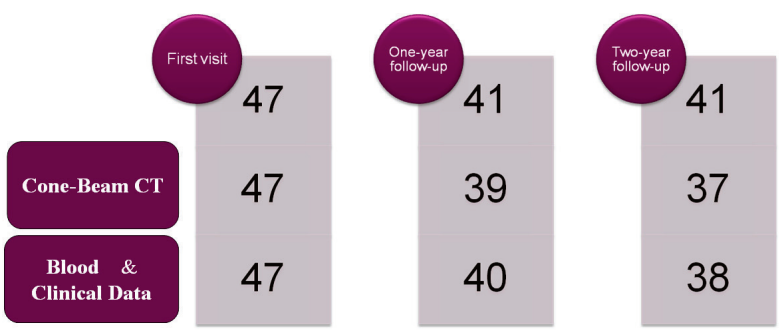

Figure 1. Number of patients and examinations at first visit as well as at the one-year and two- year follow-up.

Table 1. Baseline characteristics of 41 patients in the current TMJ study and a RA patient sample of 379 patients from the BARFOT study.

	TMJ study	n	**BARFOT study**	n
Age (years)	62 (53-67)	41	55 (45-67)	379
Sex (female)	71%	41	65%	379
Disease duration (months)	7 (4-10)	41	6 (4-8)	379
Anti-cyclic citrullinated peptide (IU/mL)	52%	33	54%	374
C-reactive protein (mg/L)	0 (0-18)	41	19 (6-43)	364
Erythrocyte sedimentation rate (mm/h)	22 (17-32)	41	29 (14-50)	378
Rheumatoid factor (IU/mL)	53%	40	61%	373
DAS28 (0-10)	6 (5-6.5)	38	5.1 (4.2-5.8)	371
General pain (NRS 0-10)	2 (0-5)	41	4 (3-7)	367
TMJ pain (NRS 0-10)	0 (0-0)	41		

Values are median (25th to 75th percentile) or percentage. NRS = numerical rating scale. The following values were considered positive: Anti-cyclic citrullinated peptide ≥25 U/mL, C-reactive protein ≥ 3 mg/L, erythrocyte sedimentation rate > 24 mm/h, rheumatoid factor ≥ 20 IU, and DAS28>3.2. DAS28 = 28 joint disease actvity score, n = number of patients .

Rheumatic Farmacotherapy (BARFOT), is shown in Table 1 (Forslind et al., 2004). BARFOT is an observation study with the intention to follow disease status and treatment in newly (maximum duration 12 months) diagnosed RA patients longitudinally over 15 years. Eighty-five percent (35/41) of the patients in the current project were also included in the BARFOT study.

The patients entered this study at a median (25^{th} – 75^{th} percentiles) of 23 days after diagnosis of RA. They were diagnosed with RA according to the revised American College of Rheumatology (ACR) criteria (Arnett et al., 1988), age above 18 and verbal consent. Exclusion criteria were current malignancies, TMJ surgery or trauma within two years, recent intra-articular glucocorticoid injection in the TMJ (within 1 month) as well as diseases other than RA as a cause of craniofacial pain. They were assigned to a brief questionnaire in order to evaluate if they fulfilled the following inclusion criteria; RA diagnosis according to the ACR criteria, duration less than one year and age more than eighteen years. The exclusion criteria were; current malignancies,

Table 2. Clinical and serological data at RA diagnosis and start of this study. 47 patients participated in the cross-sectional part (I and II) and 41 patients in the longitudinal part (III and IV).

Study I and II (n = 47)	**Diagnosis (%)**	n	**Study start (%)**	n
General Pain	100	45	83	47
Pain of the Temporomandibular Joint			13	47
C-reactive protein	100	45	43	47
Erythrocyte sedimentation rate	63	46	36	47
Rheumatoid factor	60	47	57	47
Study III and IV (n = 41)	**Diagnosis (%)**	n	**Study start (%)**	n
General Pain	100	41	71	41
Pain of the Temporomandibular Joint			12	41
C-reactive protein	100	39	37	41
Erythrocyte sedimentation rate	58	40	33	40
Rheumatoid factor	59	41	53	40

Values in percent abnormal. The following values were considered abnormal: C-reactive protein ≥ 3 mg/L, Erythrocyte sedimentation rate > 24 mm/h and Rheumatoid factor ≥ 20 IU. n = number of patients

TMJ surgery or trauma within two years, recent, less than one month, intra-articular glucocorticoid injection in the TMJ. Table 2 presents the variation in median (25^{th}-75^{th} percentile) values and frequency of clinical and serological data over 23 days after diagnosis.

TREATMENTS

The individual medication was based on the general clinical and serological status. The distribution of the principal treatments given is illustrated in Fig. 2. A majority of the patients started with a single DMARD, where the first choice was methotrexate but also salazopyrine or gold salt injections were given to a minority of the patients. At the three-month follow-up non-responders had changed treatment to either a combination of DMARDs, including methotrexate, sulfasalazine and hydroxychloroquine, or anti-TNF in combination with methotrexate.

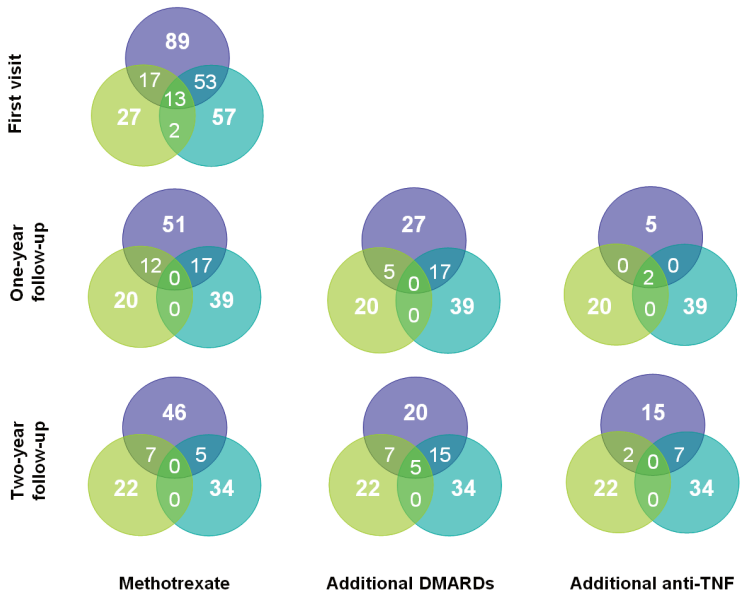

Figure 2. Venn-diagrams showing the distribution (%) of major anti-rheumatic therapy as well as the combination with additional anti-inflammatory drugs at: first visit (47 patients), one-year follow-up (41 patients) and two-year follow-up (41 patients). Violet = anti-rheumatic therapy, green = low dose glucocorticoids, blue = non-steroidal anti-inflammatory drugs.

Glucocorticoids, either by oral or intra-articular administration were given as additional treatment to 20-27% of the patients. The need of supplemental NSAIDs, which were administered on a regular basis or in case of need, was reduced from 57% to 34% during the study period.

If a patient showed clinical symptoms of TMJ RA or extended radiographic signs of TMJ bone tissue resorption intra-articular administration with 0.5 mL methylprednisolone and lidocain (Depo-Medrol cum Lidocaine 40 mg/mL + 10 mg/mL) was performed.Three patients received bilateral local glucocorticoids at first visit and seven at the one-year follow-up (3 bilaterally and 4 unilaterally).

ETHICAL CONSIDERATIONS

All invited patients received verbal and written information about the project and time to think before deciding about participation. The study design, methods used and patient selection was approved by the ethical

committee at Karolinska Institutet, Stockholm, Sweden (03-204 & 452/03-204) and by the local radiation committee at Karolinska University Hospital in Huddinge, Sweden (19/03). All participating patients gave their informed consent according to the Declaration of Helsinki before inclusion.

RADIOGRAPHIC EXAMINATION

Radiographic examination of the TMJ region was performed with a craniofacial cone-beam CT (NewTom QR DVT mod 9000; QR s.r.l; Verona, Italy; Fig. 3A). The cone-beam technology obtained all the necessary data for the volumetric region desired by a complete rotation of the X-ray source and detector around a fix axis. The acquisition of the digital volume was carried out with the patient placed on an adjustable bench in a supine position with the TMJ located centrally in the region of interest (Fig. 3B). A reconstruction volume of 130x130x130 mm was performed and the mean scan time 75 seconds.

The acquired volume, the so-called raw data of the exam, were then able to be reconstructed in any plane desired giving planar images for analysis by the NewTom software (NewTom 3G). The reconstructions were processed by the same operator.

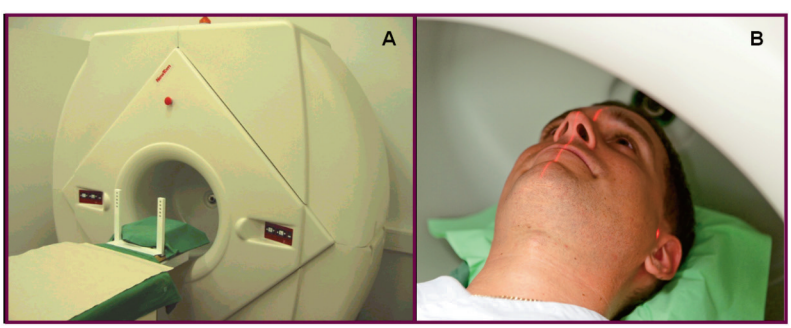

Figure 3. A) Cone-beam computerized tomograph, NewTom Model QR-DVT 9000, at the Department of Oral radiology, Karolinska Institutet, Huddinge. B) Patient adjustment, with the TMJ centrally in the region of interest, showing two laser indicators, a vertical line on the facial midline and a point over the TMJ lateral pole, used to precisely define the patient orientation.

Reconstruction procedure

The reconstruction procedure was performed in four steps: 1) Determination of the axial inclination 2) “Primary reconstructions”, the reconstruction of axial views 3) Determination of the trans-axial plane 4) "Secondary reconstructions”, the reconstruction of the trans-axial views used for the final analyses. The serial axial sections were obtained at 0.3mm (large field) and trans-axial sections at 0.5mm (35mm width) intervals.

Determination of the axial plane

The first step aimed to define a reproducible orientation of serial sections from the raw data. In order to control the orientation between three examinations reference planes were determined on a lateral cephalogram taken at every visit (Planmeca ProMax, Helsinki, Finland; Fig. 4a). The cephalogram was taken of the patient in a reliable natural head position keeping the mandible in exactly the same position as during the cone-beam CT scan with the help of an occlusion index (Lundstrom et al., 1995). As the digital volume was processed separately for each component of the TMJ, one reference plane was used for the temporal part and one for the condylar part. For the temporal component a reference plane parallel to the hard palate was used (Fig. 4A) and for the condylar component the reference plane was perpendicular to the mandibular ramus (Fig. 4B). The exact superior-inferior position of both reference planes was located across a constructed point 1cm inferior to the posterior nasal spine, at a line along the axis of the pterygo palatinal fossa. In order to control the magnitude of the linear measurement on the lateral cephalogram a gauge was used.

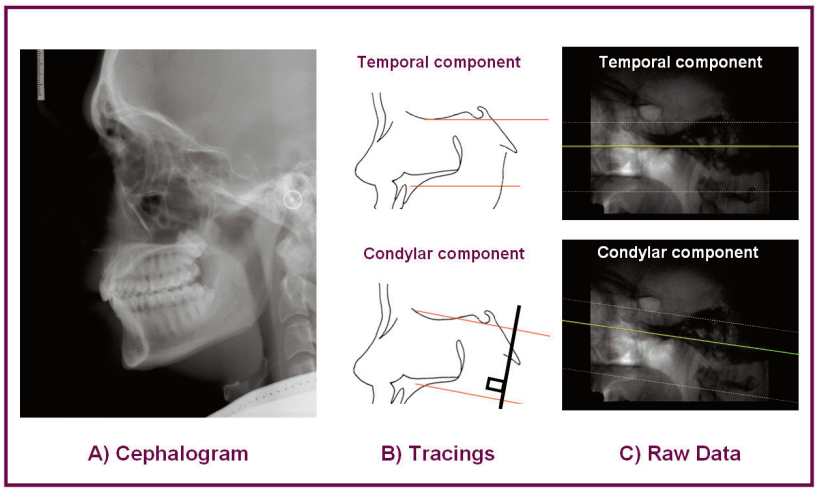

Figure 4. A) A lateral cephalogram with the patient in the natural head position. B) Showing orientation and extension of the serial axial sections defined and transferred on tracings; temporal component (upper) and condylar component (lower). C) Showing orientation and extension of the serial axial sections from the raw data perspective; temporal component (upper) and condylar component (lower).

Primary reconstructions

The second step aimed to define a reproducible volumetric extension of serial axial sections, from the raw data, which originated from the predetermined reference planes. Therefore the reference planes and anatomic structures of interest were first traced at the lateral cephalogram on a transparent film, transferred and superimposed on the raw data image in the New Tom software. The superimposition was performed after synchronizing the magnification of the raw data view (Fig. 4C). The primary reconstruction could then be processed with the axial section of the volume at first visit through the reference plane and the last through the tangent of the most inferior point of the Turkish saddle. The total amount of section from first examination was saved as a reference for the follow-up exams and reconstructions.

Determination of the trans-axial plane

The fourth step aimed to define a reproducible orientation of serial trans-axial sections from the primary reconstruction. In order to do so a

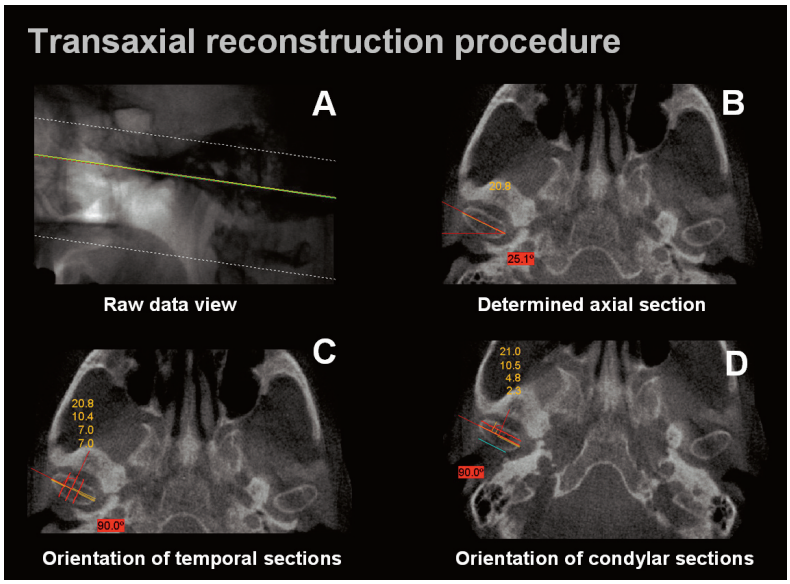

Figure 5. A) Raw data view showing the extension of the serial axial sections and the level of axial section selected for the trans-axial reconstruction procedure. B) NewTom software tool showing the section level at maximum condylar width and long-axis inclination of the condylar head. C) Axial view showing the orientation of trans-axial sections used for analysis patients right temporal part. D) Axial view showing the orientation of trans-axial sections used for analysis of a patient's right condyle.

reference axial section incorporating the maximum condylar medio-lateral width was selected for each side of the temporal and condylar reconstructions and saved as a reference for the follow-up exams. The registered width (mm) and angulation of condylar axis was also saved for the repeated reconstructions. For every single patient the secondary reconstructions were processed four times; the right and left temporal part as well as on the right and left condylar part. The settings of trans-axial reconstructions were 35mm in width and 0,5mm thickness.

Secondary reconstructions

Allowing identification of the exact localization of the trans-axial sections of the temporal and condylar components the secondary reconstructions was defined in relation to the condylar long axis, the angulation and maximum width (Fig. 5B). That reference for the serial trans-axial sections was used and identical for the condylar part as well as the temporal part.

The trans-axial temporal sections were posteriorly-anteriorly oriented perpendicular to the long condylar axis and initiated two mm medially of the medial pole through all condyle to the lateral pole. Three specific sections were determined labeled with a marker for the final analysis, one in the middle of the long axis and the other two one third of the distances to the medial and lateral poles (Fig. 5A). The section numbers as well as distances were saved as a reference for repeated reconstructions. In order to reconstruct an identical section for all three cone-beam CT exams the reconstructions and measurements were performed from the medial pole to the lateral. The number of the three sections of interest from first examination was saved as a reference for the follow-up reconstructions.

The trans-axial condylar sections were medially-laterally oriented parallel to the condylar long axis and initiated two mm posteriorly, to a tangent through the most posterior condylar border and parallel to the long axis. Two specific sections were determined labeled with a marker for the final analysis, one through the long axis and the other at the middle of the distance between the long axis and the tangent of the anterior border of the condyle (Fig. 5B). The section numbers as well as distances were measured saved as a reference for repeated reconstructions. In order to reconstruct an identical section for all cone-beam CT exams the reconstructions and measurements were performed from the posterior to anterior. The number of the two sections of interest from first examination was saved as a reference for the follow-up reconstructions.

Analyses of radiographs

The cone-beam CT trans-axial sections were finally evaluated for presence or absence of erosions. Erosion was defined as a local area with decreased density of the cortical joint surface sometimes including the adjacent subcortical bone (Akerman, 1987). The planar images were viewed from the frontal aspect of the condyle and from the sagital aspect of the temporal component. The two corresponding sections of the condyle, one central (the section with the largest condylar width) and one anterior were analyzed in the lateral, central and medial parts. The extension of erosive changes was expressed as a score ranging from 0 to 24 for each patient (Fig. 6). The sum of the score of the right and left sides

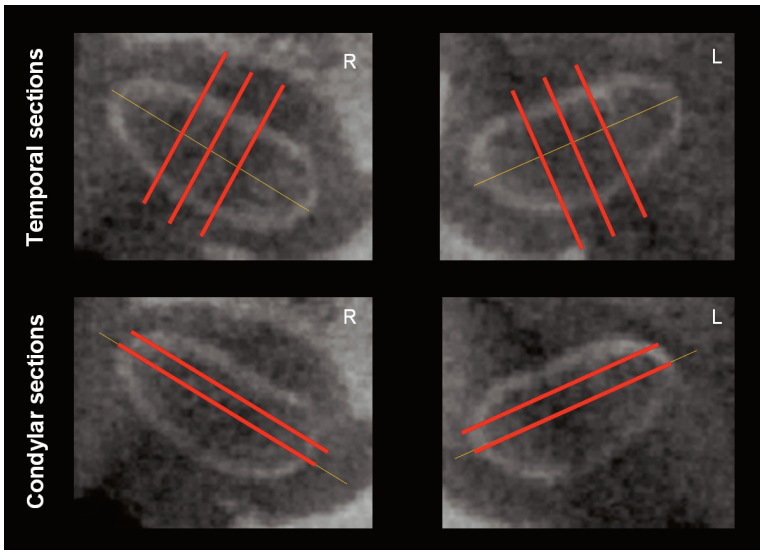

Figure 6. Red markers illustrate the orientation and precise position of the transaxial sections from an axial view of the right and left temporomandibular joint. The readings of the temporal component were performed on three lateral sections perpendicular to the condylar axis, a lateral, central and medial, and of the condylar component two anterior sections parallel to the condylar axis, a central and anterior, were used.

for each individual was used in the analysis. The development over time was expressed as the difference between the final score at two-year follow-up and the first visit.

The reading of the erosions was made by one skilled radiologist. When erosions were assessed, their presence was verified in the frontal, sagittal and axial views. Intra observer consistency between repeated readings of erosions was tested by a randomized selection of 20 joints from 10 individuals. The scoring system was tested in a blinded fashion by the same radiologist two to three years apart. On a patient level, the repeated readings showed a difference of one score unit between the two occasions in three patients, whereas the readings of the other seven patients showed no differences. The relative reproducibility of detection of erosive changes varied between 70 and 100% for the different areas where the medial part of the anterior frontal section of the condyle showed the lowest degree of reproducibility and regions on the temporal part the highest.

The longitudinal development of two patients seen in the frontal view of the central sections through the condylar axis is illustrated in Fig. 7. They demonstrated advanced erosions at first visit or at the one-year follow-up.

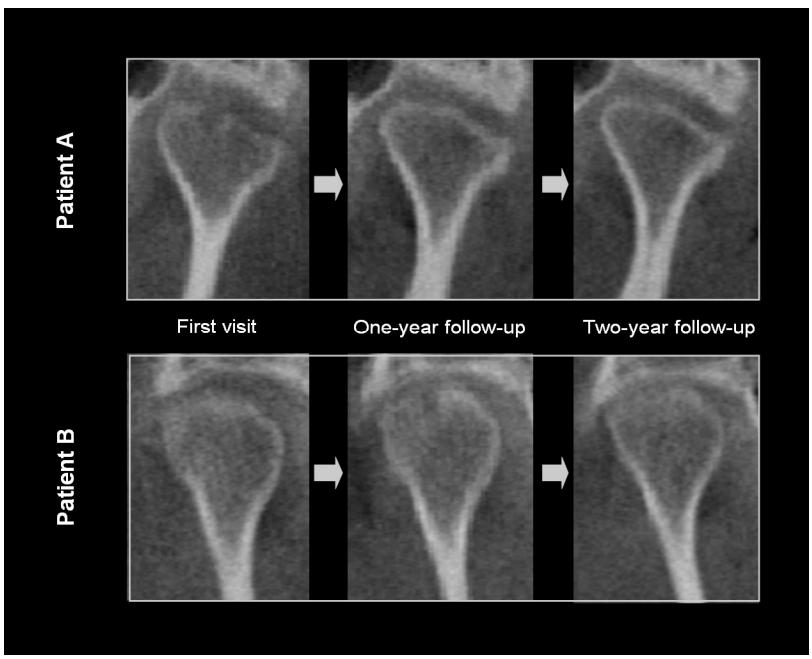

Figure 7. Frontal views of cone-beam computerized tomographs, showing the two-year development of a temporomandibular joint in two patients with TMJ erosions. Patient A showed TMJ erosion at first visit and received intra articular injection of glucocorticoids. At the one-year follow-up the erosion was absent and the new bone stayed in remission at the two-year follow-up. Patient B developed TMJ erosion during the first year after diagnosis which was treated with intra articular glucocorticoid injection at the one-year follow-up. New bone formation was observed at the two-year follow-up.

CLINICAL EXAMINATION

General clinical variables

A disease-activity score based on 28 joint counts (DAS28, 0-10) was assessed by a rheumatologist when the diagnosis was determined at the Department of Rheumatology, Karolinska University Hospital, Huddinge, Sweden.

Number of involved joints was a summation variable used as a reference for the general clinical status. Patient reported pain, tenderness, stiffness or swollenness from nine joint regions (neck, upper and lower back, shoulders, elbows, hands, hips, knees and feet), giving a maximum score of 9.

Clinical signs of TMJ bone tissue resorption

TMJ crepitus was assessed bilaterally as a clinical sign of cartilage and bone tissue resorption. The degree of crepitus was recorded during mandibular rotational and translational movements using a score (0-4), where 1 = palpable and 2 = audible.

Blood sampling and laboratory procedure

Venous blood samples were collected the same day as the clinical and radiographic examinations. In order to assess the inflammatory activity the inflammatory markers erythrocyte sedimentation rate and C-reactive protein as well as the disease marker rheumatoid factor were analyzed in serum. Blood samples were also taken to determine the serum levels of estradiol and testosterone. All these analyses except for erythrocyte sedimentation rate were performed with standard procedures at the Department of Laboratory Medicine (Clinical Chemistry and Immunology), Karolinska University Hospital, Huddinge, Sweden. Determination of the erythrocyte sedimentation rate was performed according to the Westergren procedure. Venous blood sampled in EDTA tubes was immediately cooled and centrifuged (1500 *g* for 10 min at +4ºC) for analysis of glutamate, TNF, IL-1β and IL-6 in plasma. Other venous blood samples were stored without additives in room temperature for 1 hour to coagulate and thereafter centrifuged (1500 *g* for 30 min at +4ºC) for analyses of serotonin serum levels. VEGF was analysed in lyzed whole blood. After preparation the supernatants were pipetted into polypropylene tubes except for serum samples that were stored in polystyrene tubes in -80ºC until analysis. The concentrations of

all investigated mediators were determined by commercially available assays at the Department of Dental Medicine Laboratory. Glutamate in plasma was analysed by the Amplex® Red Glutamic acid/glutamate oxidase assay (Amplex, Invitrogen, Eugene, USA). This glutamate assay had a detection limit of 10 μmol/L according to the manufacturer. TNF, IL-1β and IL-6 in plasma were analysed with the high-sensitivity human cytokine Lincoplex kit for simultaneous multianalyte detection with Luminex technology and instrumentation (Lincoplex kit, Linco Research, St Charles, USA). The assay minimum detection concentration is 0.05 (TNF), 0.06 (IL-1β) and 0.10 (IL-6) pg/mL, respectively. The intraassay coefficients of variation are 3.5% (TNF), 3.1% (IL-1β) and 3.5% (IL-6) and the interassay coefficient are 3.8% (TNF), 2.2% (IL-1β) and 4.5% (IL-6). VEGF was analysed by a commercially available enzyme-linked immunoassay kit (Quantikine Human Immunoassays, R&D Inc, Minneapolis, USA) in lysed whole blood. Serotonin was analyzed by a commercially available competitive enzyme immunoassay kit (EIA-kit. No 0642, Immunotech International, Marseille, France). The kit has a detection limit and sensitivity of 0.5 nmol/L. The intraassay coefficient of variation is less than 9.4% and the interassay coefficient of variation less than 9.9%, according to the manufacturer.

STATISTICAL ANALYSES

Median, min, max and the 25th and 75th percentiles as well as mean and standard deviation were used for descriptive statistics. Both parametric and nonparametric methods were used when applicable. Normality, constant variance and power were tested for each analysis. A probability level of less than 0.05 was considered significant.

Cross-sectional part (study I and II)

Significance of univariate correlations was calculated with Spearman rank correlation coefficient (r_s). Correlations between the variables of interest were tested in all patients and the significance of the correlations between TMJ erosion score and glutamate was tested in prespecified subgroups according to C-reactive protein, erythrocyte sedimentation rate, crepitus, estradiol and testosterone as well as sex. The significance of the differences between groups was tested with the Mann-Whitney U-test. In order to select the strongest independent variables explaining TMJ erosion score a stepwise linear multiple regression analysis was performed. Independent variables (Sex, serotonin, TNF, IL-1β, IL-6, VEGF,

estradiol, testosterone, C-reactive protein, erythrocyte sedimentation rate and rheumatoid factor) were included in the multivariate regression to test for the dependent (parametric) variable TMJ erosion score in order to investigate their relative importance to explain erosions. Interaction analysis was performed by including an interaction term in addition to the main effects in a multiple linear regression model.

Longitudinal part (study III and IV)

The significance of the differences between visits was tested by the Friedman ANOVA on ranks. Change of independent variables (joint crepitus, glutamate, serotonin, TNF, IL-1β, IL-6, VEGF, C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor, estradiol and testosterone) between first visit and the two-year follow-up were tested for multivariate regression to the dependent variable change in TMJ erosion score in order to investigate their relative influence. The significance of univariate correlations in change between the variables was tested in all patients as well as in prespecified subgroups regarding sex, TMJ crepitus and glutamate by the Spearman rank correlation coefficient (r_s). In order to select the strongest independent predictors to change in TMJ erosion score a stepwise linear multiple regression analysis was performed. Independent variables (initial TMJ erosion score, sex, joint crepitus, glutamate, serotonin, TNF, IL-6, IL-1β, C-reactive protein, erythrocyte sedimentation rate and rheumatoid factor) at the start of the study were tested for multivariate regression to the dependent (parametric) variable change in TMJ erosion score in order to investigate their relative importance to predict the changes of erosions. The significance of the differences between groups was calculated with Fisher´s exact test for variables on a nominal scale (radiographic progression, regression) and Mann-Whitney U-test for variables on an ordinal scale. Interaction analysis was performed by including an interaction term in addition to the main effects in a multiple linear regression model.

Results and discussion

CROSS-SECTIONAL PART (STUDY I AND II)

TMJ bone tissue resorption is common in early RA

A total of 72% of the patients had TMJ bone tissue resorption with a median TMJ erosion score (25^{th} - 75^{th} percentile) of 2 (0 - 4). There was a great inter-individual variation with a maximum score of 9, which was found in one patient.

A large proportion of the patients, all with a disease duration less than one year, showed signs of TMJ bone tissue resorption. The extension of the resorption was small in general with a median score of 2 on a 0-24 scale. This finding, nevertheless, indicates that the TMJ is commonly and very early involved in RA, which has not been known previously. In two earlier studies, 50-52% of RA patients with a disease duration of 9-14 years had radiographic signs of TMJ bone tissue resorption (Gynther et al., 1996; Voog et al., 2003a). The considerably higher frequency reported in our study can be explained by the use of a different and more sensitive technique with computer tomography.

TMJ bone tissue resorption is related to glutamate and crepitus and inflammatory activity

TMJ erosion score was positively correlated to glutamate ($r_s = 0.30$, $n = 47$, $p = 0.043$) and TMJ crepitus ($r_s = 0.38$, $n = 47$, $p = 0.008$). Multiple stepwise regression showed that TMJ crepitus ($p < 0.001$) together with glutamate ($p = 0.005$) and erythrocyte sedimentation rate ($p = 0.010$) were positively related to the erosion score and explained 41% of its variation ($p < 0.001$). Sex, serotonin, TNF, IL-1β, IL-6, VEGF, C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor, estradiol, progesterone and testosterone were also included into the stepwise regression but did not significantly add to the ability of the model to predict erosion score. In the patients without TMJ crepitus, bone erosion score was positively correlated to glutamate ($r_s = 0.45$, $n = 35$, $p = 0.007$). The corresponding correlation in the patients with crepitus was negative but did not reach statistical significance ($r_s = -0.35$, $n = 12$, $p = 0.264$). Interaction analysis with multiple linear regression showed that there was a different relation ($p = 0.050$) between bone erosion score and glutamate level in the patients without crepitus compared to those with

crepitus. In a subgroup of patients with estradiol levels ≥ 65 pmol/L there was a positive correlation between erosion score and C-reactive protein (r_s = 0.68, n = 16, p = 0.004). The erosion score was higher in patients with C-reactive protein levels ≥3mg/L and estradiol ≥ 65 pmol/L than those with lower C-reactive protein and estradiol levels (p = 0.025, n = 16).

These results agree with the hypothesis that glutamate is associated with bone tissue resorption of the TMJ RA as assessed by radiographic erosions. It seems that glutamate is released in the bone microenvironment by osteoblasts, activated osteoclasts and peripheral nerves as part of normal bone remodeling but also by inflammatory cells in the inflamed synovium (Lawand et al., 2000; Genever and Skerry, 2001; Chenu, 2002b; Gravallese, 2002; Staud et al., 2003; Aliprandi et al., 2005; Tremolizzo et al., 2006; Spencer et al., 2007). In turn, activation of NMDA receptors stimulates osteoblast precursor proliferation, NFκB-mediated osteoclast differentiation and inhibition of osteoclast apoptosis and the resulting net signaling effect modulates changes in bone mass (Spencer et al., 2006). The mature resorbing osteoclast is a target for glutamate by the NMDA receptor, which when activated results in bone tissue resorption in the healthy organism (Saika et al., 2001; Chenu, 2002a; Spencer et al., 2007). Fibroblast-like synoviocytes from patients with active RA express glutamate receptor mRNA for the glutamate NMDA, kainate and AMPA receptors, where activated kainate/AMPA receptors increase the release of proinflammatory cytokine IL-6 and subsequently matrix metalloproteinase 2, an enzyme highly involved in matrix degradation (Flood et al., 2007). The findings indicate that glutamate modulates bone tissue resorption, which is an intriguing pathophysiologic mechanism in RA as previously suggested by Flood et al. (2007). Flood and coworkers also found that glutamate receptor activation may contribute to the synovial hyperplasia and thus pannus development by stimulation of synoviocyte proliferation. Whether glutamate regulates release of cytokines from synoviocytes or other synovial cells remains to be investigated.

Glutamate had a stronger correlation with TMJ bone tissue resorption when crepitus was absent. The correlation could not be found when crepitus was present. One possible explanation might be that the crepitus in this study was mainly palpable (21%) and infrequently audible (2%), which indicates a lower degree of destruction in agreement with the short disease duration. The frequency of crepitus was also lower (26%)

than in a previous study of TMJ RA where 75% of both men and women were reported to have crepitus (Tegelberg and Kopp, 1987). However, the majority of these patients had a disease duration of more than 5 years. The relation between crepitus and TMJ bone tissue resorption was significant. Another study reported a weak but statistically significant relationship between crepitus and radiologic changes of the TMJ in RA patients, where 80% of joints with crepitus showed evidence of bone tissue resorption while only 47% of the joints with bone tissue resorption had crepitus (Akerman et al., 1988). This latter finding is in agreement with ours and indicates that articular cartilage and bone tissue destruction develop with different mechanisms in TMJ RA.

The regression model also included erythrocyte sedimentation rate as a predictor of bone tissue resorption when the other variables were accounted for. Neither C-reactive protein, erythrocyte sedimentation rate nor rheumatoid factor was significantly correlated to the bone erosion score in the non-parametric univariate analysis. The multivariate relationship between bone tissue resorption and erythrocyte sedimentation rate should therefore be interpreted with caution. However, such a relation can be expected for erosions of systemic inflammatory nature and a strong association between serial measurements of erythrocyte sedimentation rate and radiographic joint destruction has been found (Graudal, 2004). TMJ bone tissue resorption was thus not directly related to systemic inflammatory activity in the univariate analysis but there was a correlation found between TMJ bone tissue resorption and C-reactive protein which depended on the level of estradiol. Estradiol has been found to increase the expression of osteoprotegrin via the estrogen receptor α (Yoneda et al., 2004). Osteoprotegrin reduces osteoclastic bone tissue resorption by binding to RANKL. Estradiol can also decrease proinflammatory cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages (Kotake et al., 2001). These results are consistent with the finding of no erosions in the RA patients with estradiol levels ≥ 65 pmol/L combined with low systemic inflammatory activity. However, the erosion score correlated to C-reactive protein in the patients with estradiol level ≥ 65 pmol/L, i.e. a combination of high levels of C-reactive protein and estradiol resulted in a high score of erosions. In previous studies of progression of radiographic changes in RA, time-averaged C-reactive protein levels were correlated to increase in Larsen score and regarding the TMJ, progression during a period of 25-46 months was

related to C-reactive protein level (Plant et al., 2000; Voog et al., 2004). The influence of estradiol was not investigated in those studies. The short disease duration as well as the cross-sectional character of the present study may also influence the relation between systemic inflammatory activity and erosions.

Glutamate modulates TMJ bone tissue resorption at low systemic inflammation and low levels of sex hormones

Glutamate levels in plasma correlated to TMJ erosion score in the subgroup of patients with C-reactive protein levels of < 3 mg/L (r_s = 0.46, n = 27, p = 0.017) or to testosterone levels ≤ 1.2 nmol/L (r_s = 0.56, n = 21, p = 0.008). There was stronger positive correlations between glutamate and erosions in the patients with C-reactive protein < 3 mg/L and testosterone levels ≤ 1.2 pmol/L (r_s = 0.84, n = 14, p < 0.001) as well as in the patients with C-reactive protein < 3 mg/L and estradiol levels < 50 pmol/L (r_s = 0.63, n= 13, p= 0.022). The results suggest an influence on the relationship between plasma glutamate and TMJ bone tissue resorption by testosterone since this relation was considerably stronger in the patients with testosterone levels ≤ 1.2 nmol/L. In addition, plasma glutamate was associated with TMJ bone tissue resorption particularly in patients with low systemic inflammatory activity, a factor that in combination with estradiol and testosterone seems to influence the glutamate modulation of TMJ bone tissue resorption.

Glutamate may thus be a modulator of connective tissue cell function regarding bone tissue resorption in joints with RA especially in individuals with low systemic inflammatory activity and low levels of sex hormones. Circulating glutamate may activate locally expressed receptors on e.g. osteoclasts or fibroblast like synoviocytes and modulate bone tissue resorption in patients with low systemic inflammation in combination with low estradiol or testosterone and thereby represent a bone resorbing mechanism independent on systemic inflammation. Estrogen deficiency plays a crucial role in acceleration of bone tissue resorption in autoimmune arthritis associated with RANKL-mediated osteoclastogenesis in a murine model for rheumatoid arthritis (Yoneda et al., 2004). This finding may explain the bone tissue resorption in the patients with estradiol levels <50 pmol/L in combination with high systemic inflammatory activity. RANKL mRNA and protein have been identified in cultured synovial fibroblasts from patients with RA (Khalkhali-Ellis et al., 2000). Glutamate may modulate the bone tissue

resorption via the RANKL/RANK/OPG system under influence of estradiol and cytokines.

The influence by testosterone in bone tissue resorption is more unclear in RA (Cutolo, 2009). However, low testosterone levels may be expected during inflammation since systemic proinflammatory cytokines suppress the hypothalamic-pituitary-gonadal axis (Jones and Bhalla, 1993). In testosterone deficiency the inhibiting influence on osteoclast formation and activity is reduced (Michael et al., 2005). Androgens including testosterone are down-regulated in active RA synovial tissues and systemic levels are reduced in both women and men in RA and inversely related to clinical severity (Gordon et al., 1988; Cutolo et al., 2005; Cutolo, 2009; Tengstrand et al., 2009). As androgens exert anti-inflammatory activities in RA synovium one may hypothesize that low androgen levels may facilitate local inflammation (Cutolo et al., 2007).

Serotonin mediates TMJ bone tissue resorption in men

The TMJ bone erosion score was positively correlated to serotonin level in serum ($r_s = 0.54$, $n = 16$, $p = 0.030$) in men. Interaction analysis showed a significant ($p = 0.014$) difference between the sexes.

The relationship between bone tissue resorption and serum level of serotonin seems to be positive and specific for men since there was a significant sex difference regarding this relationship. Serotonin may modulate osteoblast bone formation via the 5-HT_{2B} receptor and the osteoclast differentiation through its transporter 5-HTT (Trang et al., 1985; Westbroek et al., 2001; Battaglino et al., 2004; Collet et al., 2008). In this study the observed bone tissue resorption cannot be considered as normal remodeling due to the combination with active RA. However, serotonin may play an important role for articular bone tissue remodeling in both healthy individuals and in patients with RA since osteocytes and osteoblasts express receptors for serotonin and 5-HTT (Bliziotes et al., 2006). A longitudinal study of mainly women with late phase RA showed that low serum levels of serotonin were associated with progression of erosions, while high levels were associated with regression during a 25 – 46 month interval (Voog et al., 2004). Whether serotonin in serum is associated with continuing bone tissue resorption in the early stage of RA needs further studies. The exact nature of the serotonin contribution to bone tissue resorption and the influence of sex is, however, still unclear.

Change in TMJ bone tissue resorption is inconsistent

The TMJ erosion score for all patients was reduced from a median (25^{th} - 75^{th} percentile) of 2 (0 - 4) to 0(0 - 2). The reduction was not significant through the two first years after diagnosis ($p = 0.488$). The change in erosion score varied inconsistently over time as 29 % (11/37) showed progression of erosion score, 43 % (16/37) showed regression and 28% (10/37) showed the same erosion score at the two-year follow-up as at study start.

The results show that a substantial portion of patients can be expected to show a regression or no change in TMJ bone tissue resorption over the first two years after diagnosis of RA. The progression of TMJ bone tissue resorption was less marked than the regression over the two years after RA diagnosis. The TMJ progression rate was similar to the 19-33% found in patients with late phase and symptomatic TMJ RA in well-controlled disease and general duration of about 9-14 years (Nordahl et al., 2001; Voog et al., 2004). Regression in TMJ erosions was also seen in 31% of the patients in the study by Voog et al. (2004). The progression was less marked than that reported from hand and foot joints on plain radiographs, where erosion score progressed in 63.6% of the patients over three years despite early treatment with conventional DMARDs (Machold et al., 2006). The lower progression rate in the TMJ in the current study may be explained by the early general treatment given. The group of patients who showed progression of TMJ bone tissue resorption may belong to a subgroup who does not respond to the anti-inflammatory treatment or that the bone tissue resorption may include a non-inflammatory osteoporosis-like resorption (Machold et al., 2007; Clarke and Khosla, 2010).

TMJ bone tissue resorption is modulated by inflammatory and non-inflammatory mechanisms

The erythrocyte sedimentation rate decreased ($p = < 0.001$, $n = 33$) but not C-reactive protein ($p = 0.540$, $n = 36$) during the study period. Multiple stepwise regression showed that change of the independent variables IL-1β ($p = 0.024$) and testosterone ($p = 0.012$) were positively related to the change in erosion score ($p = 0.010$, $n = 21$) and explained 34% of that change over two years. IL-1β ($p = 0.027$, $n = 36$) as well as testosterone ($p = 0.048$, $n = 32$) increased during the two years period.

Progression of TMJ bone tissue resorption can be expected to occur especially in patients who show an increase of testosterone and IL-1β in the blood during the first two-year of disease. IL-1β is involved in the mechanisms behind destruction of bone and cartilage in RA. IL-1β acts through two different pathways; by differentiation and activation of osteoclasts by upregulating RANKL expression and by triggering production of proteolytic enzymes from synoviocytes and inflammatory cells in the inflamed synovium (Rengel et al., 2007; Schett, 2007). Increase of IL-1β appeared in this study as the most important inflammatory factor explaining progression of bone tissue resorption. The weak relation between the systemic markers of inflammation and progression of TMJ bone tissue resorption is in contrast to earlier studies where radiographic progression in hands and feet as well as the TMJ was related to C-reactive protein levels (Plant et al., 2000; Voog et al., 2004). One explanation may be diverging treatment strategies or the time between start of treatment and first visit in the current study.

The influence of testosterone on bone tissue resorption is unclear in RA (Cutolo, 2009). Testosterone has immunosuppressive properties (Masi et al., 1999). In addition, the testosterone level increases when the disease activity decreases in men (Gordon et al., 1988; Tengstrand et al., 2009). The testosterone levels increased during the two-year period in our study and the systemic inflammation decreased. Our results showed that the increase of testosterone is positively associated with progression of bone tissue resorption during the first two years of disease, independently of IL-1β. The positive relationship between the increase of testosterone and TMJ bone tissue resorption is in contrast to earlier studies where testosterone has been related to inhibition of osteoclast formation and activity (Riggs, 2002; Michael et al., 2005). In addition, we have also shown that testosterone was associated with TMJ bone tissue resorption in RA patients shortly after diagnosis (Study I). The influence by testosterone on articular bone tissue resorption needs further evaluation, especially regarding character of bone tissue resorption.

Progression of TMJ cartilage destruction is influenced by glutamate and systemic inflammation

Glutamate increased from a median(25^{th}-75^{th} percentiles) of 3.7(1.0-7.3) mmol/L to 9.1(5.8-17.5) mmol/L during the two-year period ($p < 0.001$). In a subgroup with glutamate increase more than our median value ≥ 3.4 mmol/L between first visit and two-year follow-up progression of

crepitus was positively correlated to increase in erythrocyte sedimentation rate (r_s = 0.59, n = 17, p = 0.012) while patients with less than median increase of glutamate (< 3.4 mmol/L) showed a negative correlation (r_s = -0.77, n = 16, p < 0.001). Interaction analysis with multiple regression showed that the difference between these groups was significant (p < 0.001). TMJ crepitus increased (p = 0.020) but that change was not correlated to TMJ erosion score. The serum level of serotonin decreased (p = 0.003, n = 36) over the two-year period. The reduction in serotonin was larger in the patients who had TMJ crepitus after two years (median = -406, 25^{th}/75^{th} percentile = -202/-505) than those without (median = -137, 25^{th}/75^{th} percentile = -391/5.3; p = 0.045). The thrombocyte particle count decreased during the two years after RA diagnosis (p < 0.001, n = 34).

The subgroup with increase of plasma glutamate of more than 3.4 mmol/L showed a positive correlation between change in TMJ crepitus, a clinical sign of cartilage or bone damage and change in the inflammatory marker erythrocyte sedimentation rate. This finding is in agreement with earlier studies by Flood et al. (2007) who showed that elevated glutamate in synovial fluid may influence destruction of cartilage by stimulating synovial fibroblasts to express proMMP-2 in the synovial tissues (Flood et al., 2007). Development of crepitus from cartilage or bone degradation may thus be due to release of glutamate in the inflamed synovium over the first two years after diagnosis. However, there was a positive relationship between glutamate and TMJ erosion score at the first visit in the absence of crepitus, which may be due to not yet sufficiently developed crepitus at this early stage. Glutamate may partly be involved in the cartilage degradation and partly in the bone tissue resorption through different mechanisms where the cartilage degradation is a slower process and dependent on the local inflammation (Study II). TMJ crepitus increased during the two-year period, indicating escalated articular cartilage damage. However, this change was not related to the change in the TMJ erosion score, which further suggests that articular cartilage and bone tissue degradation are mediated by at least partly independent mechanisms (Schett, 2007). Crepitus reflects the inflammatory event which leads to expression of matrix-degrading enzymes and their destruction of the surface cartilage (Pap et al., 2001).

Serotonin was reduced and the reduction was more pronounced in patient with crepitus at the two-year follow-up indicating that the reduction of serotonin was more pronounced in patients with

destructive changes of the articular surface of the TMJ. The osteoblast has receptors for serotonin (Bliziotes et al., 2001; Westbroek et al., 2001; Collet et al., 2008) and the biological effect of these receptors may be reduced by reduction of serotonin and thereby modulate bone turnover.

LONGITUDINAL PART - PREDICTION (STUDY IV)

Regression of TMJ bone tissue resorption is predicted by the early extent of TMJ bone tissue resorption

The change of TMJ erosion score was negatively correlated with erosion score at the first visit (r_s = -0.64, n = 38, $p < 0.001$) i.e. regression of bone tissue resorption was associated with high initial erosion score. Multiple stepwise regression showed that initial erosion score was the only significant and a negative predictor explaining 48% of the variation of the change in erosion score after two years ($p< 0.001$, n = 35). However, univariate analysis showed that patients with joint crepitus at the first visit more frequently had a decrease in erosion score (7/10 = 70%) than the patients without (9/28 = 32%; p = 0.044).

The association between presence of early erosions and regression of erosions may be due to the phenomenon called “regression towards the mean”, which means that spontaneous remission of abnormal values, unrelated to treatment, is likely to occur if many patients show abnormal values before treatment. However, in this case the decrease in TMJ bone tissue resorption is likely to be mainly due to the treatment since the RA disease normally progresses regarding articular tissue destruction. Another possibility is that there was an observer change in the reading of the radiographic data between visits, i.e. that a more cautious registration was made of erosions at follow-up. This is a possibility since the time period between registrations was about two years. However, the change in erosion score was associated with changes in e.g. IL-1β, TNF, VEGF and testosterone, which rather indicate that the change in regression was related to biologic processes (Study III).

Patients with presence of crepitus at the first visit showed a decrease in TMJ erosion score more frequently than patients without crepitus suggesting that patients where the surface cartilage is damaged have a better response to anti-inflammatory treatment. In turn, this indicates that crepitus in RA is a sign of inflammatory cartilage and bone tissue destruction. Crepitus is a clinical sign of cartilage destruction that may

not be present when RA starts but develops gradually and later becomes a common feature (Tegelberg and Kopp, 1987; Akerman et al., 1988; Wiese et al., 2008). However, the mechanisms behind articular cartilage and bone tissue resorption may differ in the early disease process (Appel et al., 2006; Schett, 2007; Appel et al., 2009; Study II; Study III). In RA, cartilage destruction is probably a direct consequence of the synovial inflammation with cytokines and other inflammatory factors that elicit production and release of proteolytic enzymes (Pap et al., 2001). Bone tissue resorption is related to an unbalance in the RANKL/RANK/OPG system most likely caused by synovial inflammation but also by non-inflammatory osteoporosis (Clarke and Khosla, 2010).

Regression of TMJ bone tissue resorption is predicted by high plasma level of glutamate

Patients with plasma glutamate at median level or above (≥ 3.7 mmol/L) at first visit showed a more frequent decrease (11/18 = 61%) in erosion score than patients with a level below the median (< 3.7 mmol/L; 5/19 = 26%; p = 0.035, n = 37; Table 3) over two years. The group with higher plasma level of glutamate showed a median decrease in TMJ erosion score of -1 compared to no change in the group with lower level.

High plasma level of glutamate (≥ 3.7 mmol/L) early after diagnosis seems to predict decrease of TMJ bone tissue resorption during the first two years of RA. It seems that glutamate modulates synovial inflammation but also non-inflammatory bone tissue resorption (Chenu et al., 1998; Flood et al., 2007). Glutamate was related to bone tissue resorption in patients with low systemic inflammatory activity, which indicates a role of glutamate in non-inflammatory bone tissue resorption (Study I). In addition, glutamate was associated with bone tissue resorption selectively in patients without joint crepitus (Study II). It is therefore possible that glutamate has a dual role in bone turnover by regulating both bone tissue resorption and bone formation giving the possibility of glutamate to promote bone repair in RA (Chenu et al., 1998). The glutamate NMDA receptor on preosteoclasts and osteoclasts stimulates differentiation and activation, i.e. bone tissue resorption, while it also prevents bone tissue resorption by activating osteoblasts (Chenu et al., 1998).

Regression of TMJ bone tissue resorption is predicted by serotonin in men

In the men, the change in erosion score was negatively correlated to serum serotonin level at the first visit (r_s = -0.57, n = 14, p = 0.034), which means that the extent of TMJ bone tissue resorption decreased in men with high initial levels of serotonin. Interaction analysis with multiple linear regression showed a significant (p = 0.034) interaction between sex and serotonin. This implies a significant difference in relation between TMJ bone tissue resorption and serotonin in men compared to women.

In a cross-sectional study high serum serotonin was positively associated with TMJ bone tissue resorption in males with early RA (Study II). In the present study, early and high serotonin levels in serum predicted regression of TMJ bone tissue resorption after two years in the male RA patients. During the first two years in RA disease serotonin decreased significantly in men (Study III). Our results are in agreement with a previous study where the serum level of serotonin also predicted regression of TMJ bone tissue resorption in a chronic phase of RA (Voog et al., 2004). High serotonin levels in TMJ synovial fluid have been found to predict a positive treatment response on TMJ pain by intra articular anti-inflammatory treatment in patients with chronic RA, suggesting that serotonin is involved in the inflammatory process (Fredriksson et al., 2005). These results indicate that serotonin contribute to inflammatory bone tissue resorption.

Inflammatory markers do not predict TMJ bone tissue resorption

None of the inflammatory markers C-reactive protein, erythrocyte sedimentation rate or rheumatoid factor did significantly add to the ability of the multiple regression model to predict TMJ erosion score when included in the stepwise regression.

This result differs from previous studies in local as well as general involvement of RA (Celiker et al., 1995; Uhlig et al., 2000; Jansen et al., 2001; Nordahl et al., 2001; Voog et al., 2003a; Syversen et al., 2008; Thabet et al., 2009) where C-reactive protein, erythrocyte sedimentation rate as well as rheumatoid factor were related to articular bone tissue resorption. The reason for why the current study was not able to identify any predictive inflammatory markers for progression of TMJ bone tissue resorption is unclear but may be due to dual character of bone tissue

resorption, i.e. inflammatory versus non-inflammatory. Another explanation may be the general treatment before the first visit.

General discussion

The main finding of this study is that TMJ bone tissue resorption is present early after diagnosis of RA. The high frequency of TMJs with bone tissue resorption indicates that a subclinical process is already present at diagnosis of the general disease. The etiology of the bone tissue resorption may comprise both inflammatory and non-inflammatory components. Another major finding is that a large proportion of the patients showed regression of TMJ bone tissue resorption during the first two years of RA, which may be due to early and aggressive treatment but differs from earlier studies.

Articular tissue destruction in RA comprises both cartilage degradation and bone tissue resorption. In the present study, the early glutamate-dependent bone tissue resorption was found when TMJ crepitus was absent, indicating a process underneath the articular cartilage (Appel et al., 2006; Appel et al., 2009). The cartilage degradation is most probably of inflammatory nature whereas the bone tissue resorption may also be due to non-inflammatory processes like periarticular osteopenia, which in turn may be due to a deficiency in sex hormones (Gough et al., 1994; Sambrook, 2000; Vanderschueren et al., 2004Appel et al., 2006; Jimenez-Boj et al., 2007; Schett, 2008; Study I; Schett and Sieper, 2009).

The positive relationship between glutamate and TMJ erosion score at the early stage was found in patients without crepitus, i.e. with intact cartilage. Glutamate may also be involved in bone tissue resorption through systemic inflammatory mechanisms, since increased crepitus was associated with increased erythrocyte sedimentation rate only in patients with markedly increased glutamate. There are indications of a dual role of glutamate regarding bone tissue turnover as glutamate regulates both bone tissue resorption and formation but this study was solely concerned with bone resorption. The biologic mechanisms are scarcely known but the glutamate NMDA receptor on preosteoclasts stimulates osteoclastogenesis, i.e. bone tissue resorption (Chenu et al., 1998). The findings in the current project indicate that glutamate modulates bone tissue resorption and probably cartilage degradation but through different mechanisms in early RA.

Regression of articular bone tissue resorption, as assessed by radiography, has not been shown for other RA joints. One explanation may be differences in radiographic methodology. The current study aimed at register erosions and did not include assessment of total bone

tissue loss or other morphological changes. This means that while the erosions decreased, probably as a consequence of the early anti-inflammatory treatment, a progression of the total bone loss might still have occurred.

Regression of TMJ bone tissue resorption was more frequent (43%) in the current study than in a previous study of late phase TMJ RA (24%; Nordahl et al., 2001). In that study, the follow-up interval was one year whereas it was two years in the current project. Another explanation to the high regression frequency in the current study may be the early and aggressive treatment. Patients who failed to respond to methotrexate monotherapy, as judged by disease activity (DAS≥3.2), were given additional sulfasalazine and hydroxychloroquine or anti-TNF therapy as early as three months after diagnosis. Synovitis in a particular joint is associated with progression of erosions and joint space narrowing but not in patients treated early with infliximab (Klarenbeek et al., 2010). Anti-TNF therapy is generally associated with inhibition of joint destruction in RA (Aletaha et al., 2007; Tanino et al., 2009) and reduction in disease activity (Aletaha et al., 2007; van der Heijde et al., 2007). In the current study 15% of the patients were on treatment with infliximab or etanercept at the 2 year follow-up. Another explanation to the regression found in the current study, although of less importance, may be that 7% of the patients with clinical signs of TMJ arthritis or radiographic erosions at the first visit and 17% at the one-year follow-up received intra-articular injection of glucocorticoid. The individual reduction in erosion score over time may therefore, at least partly, be explained by the response to the early and specific treatment (Quinn and Emery, 2003; Nell et al., 2004; van Vollenhoven et al., 2009).

The high frequency of TMJ bone tissue resorption (72%) found in Study I compared to other studies may be explained by the advanced radiographic technique used. Erosions may be detected earlier by CT because of its ability to clearly delineate cortical bony margins (Perry et al., 2005). CT has been considered valuable for detecting and monitoring articular bone erosions in RA as the sensitivity and reproducibility is higher than plain radiographs for detecting bone erosions in metacarpophalangeal joints as well as in the TMJ (Goupille et al., 1990; Larheim and Kolbenstvedt, 1990; Dohn et al., 2007). Cone-beam CT was chosen because of its low exposure of radiation (Mozzo et al., 1998; Danforth et al., 2003; Hashimoto et al., 2003; Ludlow et al., 2003; Mah et al., 2003; Scarfe and Farman, 2008). Cone-beam CT is at least as reliable

as conventional CT in diagnostics of TMJ bone tissue changes but provide superior reliability and greater accuracy than conventional tomograms in detecting TMJ erosions (Honda et al., 2006; Honey et al., 2007).

The patients included were diagnosed with RA shortly before their first visit in the current project; most of them were examined for this study within less than one month after diagnosis and about 6 months after their report of debut of symptoms. Most patients began treatment with DMARDs promptly after diagnosis, i.e. before the start of this investigation, and many of these also used NSAIDs. A reduced systemic and local inflammatory activity by this early medication is likely. However, the influence of the medication on the results from the first visit regarding radiographic signs of bone tissue resorption can be expected to be minor or even insignificant.

The level of plasma glutamate was lower in the patients at the first visit than in healthy individuals, which may be due to the early treatment. However, higher levels of glutamate with a median 7.0 mmol/L were found in patients with low C-reactive protein and estradiol, in whom glutamate was most strongly associated with TMJ bone tissue resorption. One explanation may be that glutamate participates in local rather than systemic inflammatory mechanisms of bone tissue resorption. However, it has been shown that the glutamate level in TMJ synovial fluid is associated with local inflammation, as expressed by local TNF concentration, despite low systemic inflammatory activity (Alstergren et al., 2007; Clarke and Khosla, 2010).

The patients of our study must be considered as representative for the current RA patient population in Sweden since 85% of the patients were included in BARFOT, a Swedish multicenter registry project aimed to observe clinical, serological, radiographic and treatment data longitudinally. The BARFOT project includes all newly diagnosed RA patients from six rheumatologic centers in Sweden and should therefore be a suitable reference population. However, the systemic inflammatory activity, as assessed by C-reactive protein, was lower in our patient sample. The probable reason for this difference is the time of a median of 23 days from the start of medication to the first visit. The frequency of TMJ pain at rest was only 13% in our study which may partly explain why rather few of the invited patients, 47 out of 380, were willing to participate in this particular study.

Conclusions

Cross-sectional part (I and II)

This part of the study indicated that

- a majority of patients with early RA present with radiographic signs of bone tissue resorption of the TMJ (Study I)
- circulating glutamate is associated with the extent of TMJ bone tissue resorption (Study I)
- the relationship between glutamate and TMJ bone tissue resorption seems to be modulated by systemic inflammatory activity, estradiol or testosterone levels (Study I)
- presence of TMJ bone tissue resorption can be explained by a combination of TMJ crepitus, elevated plasma level of glutamate and systemic inflammatory activity (Study II)
- the relation between glutamate and TMJ bone tissue resorption is different in patients with or without crepitus indicating that glutamate has a particular role in joints without cartilage destruction (Study II)
- bone tissue resorption at this early stage is related to the inflammatory mediator serotonin in men but not in women (Study II)

Longitudinal part (III and IV)

The results of this part of the study showed that during the first two years of RA

- TMJ bone tissue resorption can be expected to regress in most patients, however, progression can be expected in a considerable number (Study III)
- progression of TMJ bone tissue resorption seems to be associated with IL-1β related inflammatory mechanisms as well as non-inflammatory mechanisms involving testosterone (Study III)
- progression of TMJ crepitus seems to be associated with inflammation (increase of erythrocyte sedimentation rate) under influence of glutamate (in patients with high increase of glutamate levels) (Study III)

- regression of TMJ bone tissue resorption is predicted by early high TMJ erosion score (Study IV)
- regression of TMJ bone tissue resorption is predicted by TMJ crepitus (Study IV)
- regression of TMJ bone tissue resorption is predicted by high glutamate levels in plasma and in the men by high levels of serotonin (Study IV)

Populärvetenskaplig sammanfattning

Ledgångsreumatism är en kronisk, systemisk, inflammatorisk sjukdom som angriper och bryter ner kroppens leder. Sjukdomen förekommer hos ca 0,5-1 % av befolkningen och drabbar oftare kvinnor än män. Orsaken till reumatism är inte helt känd men sjukdomen kan bli väldigt handikappande med trötthet, smärta och nedsatt rörlighet som vanliga kännetecken när den är etablerad. Många faktorer har nämnts som orsak däribland genetiska och miljöfaktorer. Sannolikt är det en kombination av flera. Man tror även att könshormoner inverkar eftersom sjukdomen främst drabbar kvinnor efter klimakteriet.

Diagnosen ledgångsreumatism ställs utifrån kliniskt ledstatus, röntgen och blodprov som visar särskilda sjukdomsmarkörer. Tidiga tecken på bennedbrytning i lederna på röntgenbilder anses vara en indikation på dålig prognos. Därför utgör ledröntgen en viktig del i diagnostiken men även i uppföljningen sjukdomen. Idag vet man att tidigt fastställd diagnos och insatt behandling (både lokal i den enskilda leden och generell) är avgörande för det fortsatta sjukdomsförloppet. Ju tidigare och effektivare behandling desto större är chansen att bromsa och i bästa fall stanna upp ledernas fortsatta nedbrytning.

Ungefär 50 % av reumatikerna drabbas av reumatism i käkleden med smärta och funktionsnedsättning som följd. Käkledsengagemanget är handikappande av många skäl då det påverkar tuggning och tal. Ny kunskap har visat att leddestruktion kan fortgå trots att man inte har några symptom eller kliniska tecken på inflammation. Även om det inte finns några entydiga resultat så har det har visat sig från andra leder att flera inflammatoriska ämnen eller markörer kan vara av diagnostisk och prognostisk betydelse för den fortsatta utvecklingen. Vi har studerat glutamat, serotonin, TNF, Il-1β, IL-6, VEGF, C-reaktivt protein, sänkningsreaktionen, reumatoid faktor samt könshormonerna östradiol och testosteron.

Det övergripande målet med denna avhandling var att ta redan på i vilken utsträckning det förekommer röntgenförändringar i käkleden tidigt i sjukdomsförloppet, hur dessa utvecklas över tid och om de indikerar en sämre prognos.

Avhandlingen består av två delar: en tvärsnitts studie och en longitudinell studie. Målet med tvärsnittsstudien var att relatera röntgenfynden till inflammatoriska ämnen och hormoner i blodet samt

kliniska tecken på nedbrytning i käkled. Målen med den longitudinella studien var att reda ut hur förändringar på röntgen kan relateras till förändringar avseende inflammatoriska ämnen och hormoner samt kliniska tecken.

Den första delen av avhandlingen omfattade 47 patienter och den andra 41, som undersöktes med avseende på röntgenförändringar, käkledsljud, inflammatoriska ämnen i blod och könshormoner. Den första undersökningen genomfördes ca tre veckor efter fastställande av diagnosen ledgångsreumatism. Därefter upprepades denna undersökning efter ett och två år. Analys av röntgenförändringar, s.k. erosioner, utfördes på bilder tagna med dator tomografi. Resultaten från första delen visade att 72 % av patienterna uppvisade erosioner på röntgen kort efter att diagnosen ställts. Erosionerna var relaterade till det inflammatoriska ämnet glutamat och krepitationer men också till markörer för inflammatorisk aktivitet. Sambandet mellan glutamat och erosioner var starkare vid frånvaro av krepitationer, vid låg inflammatorisk aktivitet och låga nivåer av kvinnligt eller manligt könshormon. Resultaten från andra delen visade att 29 % av patienterna hade fortsatt nedbrytning av leden medan 43 % av patienterna uppvisade en minskning. Fortsatt nedbrytning kunde delvis förklaras med ökningen av en inflammations substans som är vanligt förekommande i samband med inflammatoriskt betingad bennedbrytning, interleukin-1β. Den förklarades också av en ökning av det manliga könshormonet, testosteron. Det tycks vara så att tidigt uppkomna erosioner eller högre glutamat nivåer leder till en minskad nedbrytning. Sannolikt är dessa fynd relaterade till tidigt insatt och effektiv behandling.

Sammanfattningsvis indikerar resultaten från denna avhandling att tidiga tecken på bennedbrytning i käkleden hos reumatiker är vanligt förekommande. Bennedbrytningen tycks dock kunna minska om den upptäcks och behandlas tidigt. Tidiga röntgen tecken tycks vara relaterade till glutamat förekomst särskilt vid låg inflammatorisk aktivitet och i frånvaro av kliniska tecken. Däremot tycks tidiga röntgen tecken eller glutamat vara positivt för en återgång under de två första åren efter diagnosen ledgångsreumatism.

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