

Structural joint damage and hand bone loss in patients with rheumatoid arthritis

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THE 4 ORIGINAL PAPERS ARE

1. Ørnbjerg LM, Østergaard M, Bøyesen P, Krogh NS, Thormann A, Tarp U, Poulsen UE, Espesen J, Ringsdal VS, Graudal N, Kollerup G, Jensen DV, Madsen OR, Glinborg B, Christensen T, Lindegaard H, Dencker D, Hansen A, Andersen AR, Hetland ML.
Impact of tumour necrosis factor inhibitor treatment on radiographic progression in rheumatoid arthritis patients in clinical practice: results from the nationwide Danish DANBIO registry.
Annals of the Rheumatic Diseases. 2013 Jan;72(1):57-63
2. Ørnbjerg LM, Østergaard M, Bøyesen P, Krogh NS, Thormann A, Tarp U, Poulsen UE, Espesen J, Schlemmer A, Graudal N, Kollerup G, Jensen DV, Madsen OR, Glinborg B, Christensen T, Lindegaard H, Bøhme W, Hansen A, Andersen AR, Hetland ML.
Which factors influence radiographic progression during treatment with tumor necrosis factor inhibitors in clinical practice? Results from 930 patients with rheumatoid arthritis in the nationwide Danish DANBIO registry.
The Journal of Rheumatology. 2014 Dec;41(12):2352-60
3. Ørnbjerg LM, Østergaard M, Jensen T, Hyldstrup L, Bach-Mortensen P, Bøyesen P, Thormann A, Tarp U, Bøhme W, Lindegaard H, Poulsen U, Schlemmer A, Graudal N, Rødgaard A, Espesen J, Kollerup G, Glinborg B, Madsen OR, Jensen DV, Hetland ML.
Establishment of age and sex adjusted reference data for hand bone mass and investigation of hand bone loss in patients with rheumatoid arthritis treated in clinical practice. Results from the Copenhagen Osteoarthritis Study and the DANBIO registry.

4. Arthritis Research & Therapy. 2016 Feb 24;18:53
Ørnbjerg LM, Østergaard M, Jensen J, Hørslev-Petersen K, Stengaard-Pedersen K, Junker P, Ellingsen T, Ahlquist P, Lindegaard H, Linauskas A, Schlemmer A, Dam MY, Hansen I, Lottenburger T, Ammitzbøll CG, Jørgensen A, Krintel SB, Raun J, ML Hetland.
Hand bone loss in early rheumatoid arthritis during a methotrexate-based treat-to-target-strategy with or without adalimumab - A substudy of the Optimized treatment algorithm in Early RA (OPERA) trial.
Clinical Rheumatology. 2017 Apr;36(4):781-789

ABBREVIATIONS (Alphabetic order)

ACR	American College of Rheumatology
AIC	Akaike Information Criterion
ANOVA	Analysis of variance
Anti-CCP	anti Cyclic Citrullinated Peptide
bDMARD	biological Disease Modifying Anti-Rheumatic Drug
BMD	Bone Mineral Density
c	constant
CCHS	Copenhagen City Heart Study
CI	Confidence Interval
COS	Copenhagen Osteoarthritis Study
CRP	C-reactive protein
CV	Coefficient of Variation
DAS28	Disease Activity Score 28 joints
DEXA	Dual Energy X-ray Absorptiometry
DICOM	Digital Imaging and Communication in Medicine
DRS	Danish Society for Rheumatology
DXR	Digital X-ray Radiogrammetry
dxr-online	Digital X-ray Radiogrammetry – software, online version.
ES	Erosion Score
ESR	Erythrocyte sedimentation rate
HAQ	Health Assessment Questionnaire
HBL _{6months}	0-6 months change in DXR-BMD
i.a.	intra-articular
ICC	Intra-class Correlation Coefficient
IgM-RF	IgM Rheumatoid Factor
IL-1	Interleukin 1
IL-6	Interleukin 6
i.m.	intra-muscular
IQR	Inter Quartile Range
JSN	Joint Space Narrowing Score
MCP	Meta Carpo Phalangeal
MMP	Matrix Metalloproteinases
MRI	Magnetic Resonance Imaging

MTX	Methotrexate
OPERA	Optimized Treatment Algorithm in Early RA
OR	Odds Ratio
P	porosity
pQCT	peripheral Quantitative Computer Tomography
QMUS	Quantitative Multisite Ultrasound
RA	Rheumatoid arthritis
RADS	Danish Council for the use of expensive hospital medicines
RANK	receptor activator of nuclear factor-kappa B
RANK-L	receptor activator of nuclear factor-kappa B ligand
RCT	Randomized Controlled Trial
ROI	Regions of Interest
SD	Standard Deviation
SDC	Smallest Detectable Change
SDD	Smallest Detectable Difference
sDMARD	synthetic Disease Modifying Anti-Rheumatic Drug
SvdH	Sharp/van der Heijde
T	cortical thickness
TNF	Tumour necrosis factor alpha
TSS	Total Sharp Score
US	Ultrasound
VAS	Visual Analogue Scale
VPA	Volume per area
W	Bone width
X-rays	conventional radiographs
28SJC	28 Swollen Joint Count
28TJC	28 Tender Joint Count

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease leading to disability, increased morbidity and mortality with potential large socio-economic consequences for the individual patient and the society. In the first years after diagnosis pain and joint swelling are the primary causes of disability, while destruction of joint bone and cartilage (visualized on x-rays as structural joint damage and hand bone loss) is a main driver of disability in established disease. Prevention of joint destruction is thus an important treatment goal. However, RA is a heterogeneous disease with large variations in disease course as some patients rapidly develop progressive joint destruction while the joints of other patients remain intact throughout the disease course. Identification of predictive markers for joint destruction could aid the clinician and patient in making tailored treatment choices. In clinical practice increasing numbers of patients are treated with biological therapies, including inhibitors of tumor necrosis factor alpha (TNF), to avoid development or worsening of joint destruction although the efficacy of biological therapies to halt joint destruction outside of randomized controlled trials (RCTs) has largely not been studied.

The focus of the present thesis is assessment of TNF-inhibitor treatment effectiveness on structural joint damage and hand bone loss in clinical practice and identification of variables predictive of and associated with structural joint damage and hand bone loss during TNF-inhibitor treatment.

AIMS

The overall aim of this study was to assess and predict structural joint damage and hand bone loss in patients with early and established RA treated with synthetic Disease Modifying Anti Rheumat-

ic Drugs (sDMARDS) and TNF-inhibitors. The overall aim was investigated in two cohorts:

Cohort A: an observational, nationwide, longitudinal cohort study of established RA patients treated in clinical practice (DANBIO X-ray cohort)

Cohort B: a randomized placebo-controlled clinical trial of early RA patients (OPERA cohort)

Structural joint damage was assessed with the Sharp/van der Heijde (SvdH) method and hand bone loss was assessed with Digital X-ray Radiogrammetry (DXR).

The overall aim involved the following specific aims:

Specific aims

In patients with established RA treated with TNF-inhibitors after failure of sDMARD treatment in clinical practice (Cohort A):

- To investigate the impact of TNF-inhibitor treatment on structural joint damage progression (Paper I)
- To assess whether conventional clinical and laboratory baseline variables can predict structural joint damage progression (Paper II).
- To assess whether TNF-inhibitor switching, treatment withdrawal and inflammatory activity during treatment are associated with structural joint damage progression (Paper II).
- To investigate the impact of TNF-inhibitor treatment on hand bone loss (Paper III).
- To assess whether conventional clinical and laboratory baseline variables can predict hand bone loss (Paper III).
- To assess whether inflammatory activity is associated with hand bone loss (Paper IV).

In early RA patients treated with methotrexate (MTX) and intra-articular triamcinolone with or without adalimumab in a randomized placebo-controlled trial (Cohort B):

- To investigate the impact of addition of adalimumab on hand bone loss (Paper IV)
- To assess whether conventional clinical and laboratory baseline variables can predict hand bone loss (Paper IV).
- To assess whether inflammatory activity during treatment is associated with hand bone loss (Paper IV)
- To investigate whether hand bone loss in the first 6 months of treatment is independently associated with structural joint damage progression after 24 months (Paper IV)

In the general population:

- To establish a reference material for normal hand bone mass in order to estimate age- and sex-related hand bone loss (Paper IV).

BACKGROUND

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease affecting approximately 1% of the adult Danish population(1, 2). The etiology is largely unknown though both genetic and environmental factors have been linked to an increased risk of developing RA(3). The disease primarily affects women (female/male ratio 2-3:1) and disease incidence peaks between 45 and 75 years of age(4-6). RA is characterized by inflammation of the synovial tissue typically in a symmetrical pattern involving the small joints of the hands and feet though all synovial joints can be affected. In addition extra-articular and systemic manifestations may be present as RA is a systemic disease(7, 8). Impaired physical func-

tion is a prominent feature caused by swollen and tender joints in addition to joint destruction(9). While the contribution of joint swelling to decreased functional capacity may be reversed by targeted treatment, destruction of joint bone and cartilage contributes irreversibly to impaired physical function(10, 11).

Patophysiology

Synovial inflammation

Inflamed synovial tissue from RA patients is characterized by the presence of inappropriately activated and interacting immune cells(7). In short, antigen-presenting cells communicate with T-cells through T-cell receptor-MHC interaction inducing T-cell activation in the presence of co-stimulatory signals mediated by the CD28-B7 receptor family. The antigen-presenting B-cells also function as antibody-producing cells and the produced antibodies lead to immune complex formation in the joints. Monocytes and macrophages activated by T-cell signaling and immune complexes produce pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF), interleukin 1 (IL-1) and interleukin 6 (IL-6), all of which stimulate further cytokine production and expression of cell-adhesion molecules. This inflammatory cascade results in synovial hyperplasia, increased synovial vascularity and fibrinous deposits(7). The inflammatory process affects all joint structures, including the bone at the joint margins, the articular cartilage and the periarticular bone(12).

Structural joint damage

As part of the inflammatory cascade, monocytes (osteoclast precursors) are recruited to the inflamed synovium where TNF induces expression of surface receptors necessary for differentiation to osteoclasts(13). Activated TH1 and TH17-cells express receptor activator of nuclear factor-kappa B ligand (RANK-L) that bind to RANK molecules on the osteoclast precursors stimulating fusion of the osteoclast precursors to become pre-osteoclasts and mature osteoclasts. Pro-inflammatory cytokines (TNF, IL-1 and IL-6) drive osteoclast formation by inducing RANK-L expression in T-cells. The large number of mature osteoclasts results in an imbalanced bone remodeling at the synovium-bone interface and the increased bone resorption leads to marginal joint erosions(13-16).

Synovial inflammation causes cartilage destruction through release of matrix-degrading metalloproteinases (MMP) from synovial monocytes and macrophages. Furthermore, TNF and IL-1 produced in the synovium stimulate the chondrocytes to release MMPs while suppressing the formation of new matrix(12).

Hand bone loss

The periarticular osteoporosis caused by a local loss of bone mineral density (BMD) in RA is most apparent in the cortical parts of the bone due to increased endosteal resorption(17). Bone loss at the metacarpal sites is thought to be caused by reduced mechanical loading and relative immobilisation of the adjacent joints. Cytokine signaling from the inflamed synovium or bone marrow may contribute to increased osteoclast-mediated bone loss at the metacarpal sites by a local or systemic effect(16).

Disease manifestations

Clinical assessment of RA patients

Swollen, tender joints and increased laboratory measures of inflammation (C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR)) are the manifestations of synovial inflammation in

clinical practice. Joint involvement is assessed by a physician with a systematic examination of 28 or 44 joints for swelling (28/44SJC) and tenderness (28/44TJC)(18). An overall disease activity score (DAS28) is calculated based on a 28 Joint Count, CRP/ESR and the patients' global assessment of disease activity on a Visual Analogue Scale (VAS Global)(19). Pain and fatigue are monitored by VAS pain and VAS fatigue scores. Functional capacity is measured by the Health Assessment Questionnaire (HAQ)(20).

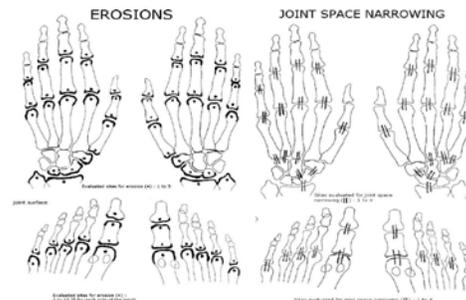
Assessment of bone involvement in RA patients

In clinical practice, progression of bone and cartilage damage (structural joint damage) and periarticular osteoporosis (hand bone loss) are assessed by conventional radiographs (x-rays) qualitatively evaluated by a radiologist. For research purposes several quantitative scoring systems for structural joint damage have been validated, the Sharp/van der Heijde method (SvdH) being the most sensitive and widely used scoring system(21-23). The assessment of quantitative hand bone loss has been done by multiple methods including Dual Energy X-ray Absorptiometry (DEXA), Quantitative Multisite Ultrasound (QMUS), peripheral Quantitative Computer Tomography (pQCT) and Digital X-ray Radiogrammetry (DXR). The DXR method has shown superior sensitivity to DEXA and QMUS for detection of RA-related hand bone loss(24-26).

Structural joint damage

The SvdH scoring system includes separate evaluation of 16 areas in each hand for erosions and 15 areas in each hand for joint space narrowing, while 6 areas in each foot is evaluated for erosions and joint space narrowing (Figure 1). If erosions are present, each erosion is assigned an erosion score (ES) ranging from 0 to 3 (Table 1). The maximal ES per joint in the hands is 5, while maximal ES per joint is 10 in the feet. Each area is assigned a joint space narrowing score (JSN) ranging from 0-4 (Table 1). Maximal JSN per joint is 4 in hands and feet. All ES and JSN scores are summed to a Total Sharp Score (TSS).

Figure 1: Evaluated sites for erosions and joint space narrowing in the Sharp/van der Heijde method.



	Joint Space Narrowing Score	Erosion Score
0	Normal	No erosion
1	Focal or doubtful JSN	Discrete erosion
2	Generalized JSN with > 50% of joint space left	Larger erosion not extending the imaginary middle of the

		bone
3	Generalized JSN with > 50% of joint space left OR subluxation	Larger erosion extending the imaginary middle of the bone
4	Bony ankylosis OR complete luxation	-
5	-	Complete collapse of bone

Hand bone loss

Digital X-ray Radiogrammetry is based on an automated segmentation of the diaphysis into cortical and medullar regions. To locate the bones in the x-ray, the DXR technology applies a model-based algorithm adapted to find the diaphysis of metacarpals II, III and IV. After each diaphysis has been identified, regions of interest (ROI) are placed automatically for the three metacarpals (Figure 2).

Figure 2: Hand x-ray with the three regions of interest used for estimation of DXR-BMD.



Image printed with permission from Scandinavian Journal of Rheumatology.

The algorithm places the three ROI's in a coupled fashion by sliding them in a partly fixed configuration along the bone shafts to a position identified by the minimum combined bone width. The heights of the ROIs are fixed to 2.0 cm, 1.8 cm, and 1.6 cm for the 2nd, 3rd, and 4th metacarpals, respectively. Within each ROI, the endosteal (inner) and periosteal (outer) edges are automatically found, thereby segmenting the bone into two cortical regions and one endosteal region. The average cortical thickness (T_i) and bone width (W_i) are determined for each metacarpal i , accumulating over 90-204 measurements per centimeter bone. This implies that a total of 300-700 measurements contribute to the average cortical thickness of a single bone.

A bone volume per projected area (VPA, cm) is computed for each metacarpal assuming a cylindrically shaped bone:

$$VPA_i = \pi * (R_i^2 - r_i^2) / W_i \pi * T_i * (1 - T_i / W_i)$$

The total VPA for the metacarpals is defined as a weighted average:

$$VPA_{mc} = (VPA_2 + VPA_3 + 0.5 VPA_4) / 2.5$$

The fourth metacarpal bone is given a lower weight mainly due to a lower precision. The final DXR-BMD is then calculated as:

$$DXR-BMD = c * VPA_{mc} * (1-P)$$

The scaling constant c is determined so that DXR-BMD on the average is equal to that of the mid-distal forearm region of the Hologic QDR 2000 densitometer (Hologic, Waltham, MA, USA).

The constant adapts VPA to both the volumetric mineral density of compact bone and the typical shape characteristics of the involved bones. P is an estimated three-dimensional porosity, aimed to be the fraction of the cortical bone volume that is not occupied by bone(27).

Version 1.02 of the DXR software, X-posure System™, applied the analysis to the second, third and fourth metacarpals, the radius and the ulna, while version 2.0 limited the analysis to the second, third and fourth metacarpals. The modification was introduced to allow standard hand x-rays to be used in the routine measurement of DXR-BMD.

Treatment

Treatment strategies

In Denmark, early RA patients initiate treatment with a synthetic Disease Modifying Anti-Rheumatic Drug (sDMARD), preferably Methotrexate (MTX) if not contraindicated, escalated to full therapeutic dosage (20-25mg/week) within one-two months(28, 29). As bridging therapy either intra-articular (i.a.) or intramuscular (i.m.) glucocorticoids are frequently used. If active disease ($DAS28 > 3.2$) is present after 3-4 months, treatment will be adjusted by switching to or adding one or two synthetic sDMARD (sulphasalazine, hydroxychloroquine or leflunomide). In year 2000 (slightly revised in 2002) the Danish Society for Rheumatology (DRS) published national guidelines for treatment with biological DMARDs (bDMARDs) stating that in patients with active disease (> 6 swollen joints) despite treatment with two different sDMARDs for at least 4 months bDMARDs could be considered. Awaiting an update from the DRS in 2006, the steering committee of the Danish nationwide DANBIO registry suggested a modification of the guidelines (active disease, $DAS28 > 3.2$ or progressive structural joint damage or prednisolone treatment ≥ 7.5 mg/day despite sDMARDs), which were in general use until new national guidelines were issued by the Danish Council for the use of expensive hospital medicines (RADS) and DRS in 2012(30, 31). The TNF-inhibitor infliximab was marketed in 2000, followed by etanercept (2000) and adalimumab (2003)). All bDMARD treatment is preferably administered with concomitant sDMARDs according to the decision of the treating rheumatologist at the Departments of Rheumatology(32).

DANBIO

In the DANBIO registry (www.danbio-online.dk), disease characteristics and outcomes are reported prospectively among patients with inflammatory rheumatic disease treated in clinical practice. DANBIO covers > 90% of all adult RA patients treated with biological bDMARDs(33). DANBIO is an important clinical tool for treating rheumatologists providing an excellent overview of disease activity and disease course facilitating patient participation in

treatment choices. In addition DANBIO is a powerful research database and a national quality registry. It is recommended by DRS that all RA patients should have x-rays of hands and feet performed upon treatment initiation and yearly thereafter(32).

Effectiveness of TNF-inhibitor treatment on structural joint damage and hand bone loss - status by year 2011

Structural joint damage

By year 2011, large RCTs had convincingly documented that TNF-inhibitors reduce or halt progression of structural joint damage in both early and established RA(34-38). In contrast, only two studies had investigated the effectiveness of TNF-inhibitor treatment in clinical practice. In 99 patients from the Czech National Registry infliximab reduced progression of structural joint damage assessed with SvdH(39). However, generalizability of the results were limited as the study population far from reflected patients treated in routine care as all patients had high disease activity (DAS28>5.1) and extensive radiographic damage(40). A study by Finckh et al. included all patients in the Swiss Registry that had received infliximab or etanercept for at least 10 months without major interruption (< 4 months) and found equal effectiveness for the two TNF-inhibitors with regards to suppression of progression of structural joint damage(41). These results could not be compared to findings from RCTs as a different scoring method for structural joint damage was used. Thus, the results from RCTs required confirmation from a large cohort of RA patients treated in clinical practice with structural joint damage assessment by SvdH-score.

Hand bone loss

By 2011, two post-hoc analyses from RCTs had investigated the efficacy of TNF-inhibitors on hand bone loss in early RA and had found that patients treated with infliximab or adalimumab in combination with MTX had a lower hand bone loss than patients treated with MTX monotherapy(42, 43). No studies had investigated the effectiveness of TNF-inhibitor treatment on hand bone loss neither in established RA patients nor in RA patients treated in clinical practice. A common definition of increased hand bone loss including the normal age- and sex-related hand bone loss had not been established, since no studies had investigated whether TNF-inhibitor treatment normalizes hand bone loss in the individual patients.

Predictors of structural joint damage and hand bone loss - status by year 2011

Structural joint damage

Post hoc analyses of RCTs had shown that IgM-Rheumatoid Factor (IgM-RF) or anti Cyclic Citrullinated Peptide (anti-CCP) positivity, elevated inflammatory markers (CRP or ESR), many swollen joints and existing erosions were predictors of structural joint damage progression during treatment with TNF-inhibitors. However, the results had limited generalizability due to selection bias of patients(44, 45). Observational studies from clinical practice were lacking.

Hand bone loss

High disease activity (CRP or DAS28) in addition to older age and post-menopausal status had been shown to predict a higher rate of hand bone loss in RCTs of infliximab and adalimumab(42, 43). Observational studies from clinical practice were lacking.

PATIENTS AND METHODS

Cohorts and study design

The present PhD thesis incorporates data from two longitudinal RA patient cohorts and a cross-sectional reference cohort from the general population. Cohorts and study designs are briefly described below and in more detail in the enclosed papers (Appendices I-IV).

DANBIO X-ray study

The DANBIO X-ray study was an observational retrospective longitudinal study of the course of structural joint damage and hand bone loss in RA patients registered in DANBIO. All Danish Departments of Rheumatology were invited to participate and 17 of 25 Departments did (Figure 2). Included were all patients in DANBIO with RA who 1) were TNF-inhibitor naïve, 2) started treatment with adalimumab, etanercept or infliximab before July 1st 2007 and 3) had at least two relevant sets of hand x-rays (baseline and follow-up). A baseline x-ray had to precede the initiation of TNF-inhibitor treatment by less than 3 months (preferably 0-3 months after start of a TNF-inhibitor), while the follow-up x-ray had to be obtained more than 6 months after the baseline X-ray (preferably 2 years after TNF-inhibitor start). If available, a pre-baseline x-ray preceding both TNF-inhibitor start and baseline X-ray with > 6 months (preferably 2 years prior to TNF-inhibitor initiation) were collected.

In paper I, the 517 patients with three relevant x-rays constituted the study population in Paper I (Specific aim 1), while these 517 patients in addition to 413 patients who had two relevant x-rays constituted the study population (n=930) in Paper II (Specific aim 2 and 3) (Figure 3).

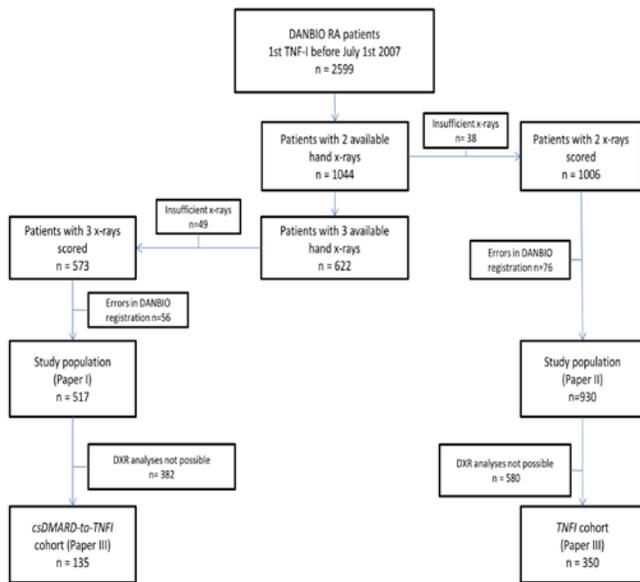
Figure 2: Participating Departments of Rheumatology in the DANBIO X-ray study



All x-rays from the DANBIO X-ray study were sent to the manufacturer of dxr-online, Sectra, for DXR-analysis. DXR-BMD could not be analysed in a substantial number of x-rays due to technical problems (underexposure, insufficient positioning of hands, type

of acquisition modality or equipment changed between x-rays, large change in image post processing) or disease related factors (prostheses or severe bone damage). If a patient only had DXR-BMD of one hand at any one timepoint, all analyses were based on that hand. In paper III, the 135 patients with three DXR-BMD measurements constituted the csDMARD-to-TNFI cohort (Specific aim 4), while the 350 patients with two DXR-BMD measurements constituted the TNFI-cohort (Specific aim 5 and 6) (Figure 3).

Figure 3: Flowchart of the DANBIO X-ray cohorts (Paper I,II and III)

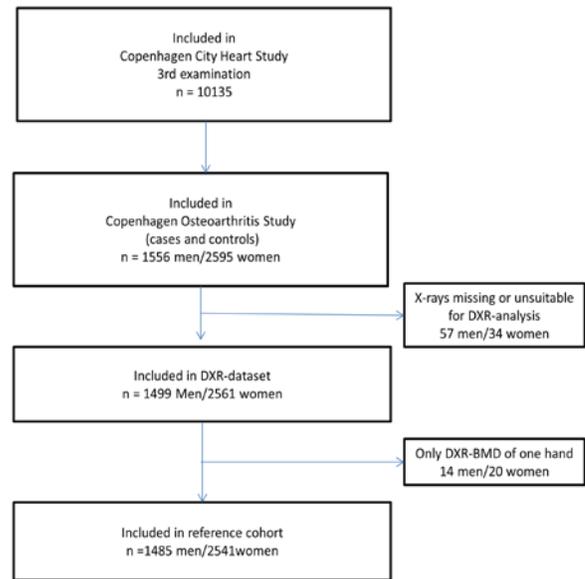


Copenhagen Osteoarthritis Cohort

The Copenhagen City Heart Study (CCHS) was a prospective population study started in 1976. The participants have been followed since and re-examined with regard to primarily cardiovascular risk factors(46). Copenhagen Osteoarthritis Study (COS) was a cross-sectional sub-study initiated at the third examination in the years 1991-1994. From the CCHS cohort of 10135 individuals, 2,949 (1,023 men/1,926 women) subjects were selected for radiography of the pelvis, lumbar spine, hand and distal forearm (economic considerations were prohibitive for complete inclusion of the cohort)(47). Inclusion criteria into the radiography protocol were positive answers in four or more of 50 main questions with up to 5 sub-questions in a questionnaire covering musculoskeletal disorders. In addition, 1,202 subjects (533 men/669 women), with three or fewer positive answers were selected as controls. Individuals with previous surgery of the lower extremities or the spine, a history or radiographs suggesting childhood hip disorders, or a history of inflammatory joint disease were excluded. Standardized radiographs of the pelvis, lumbar spine and of the hand and distal forearm were obtained. Hand x-rays were analysed with DXR. The mean of the right and left hand was calculated to offset the difference between the dominant and non-dominant hand.

In paper III, the 4151 individuals (1485 men/2541 women) with available DXR-BMD measurements of both right and left hand constituted the reference cohort for normal hand bone mass measured with DXR (Specific aim 11) (Figure 4).

Figure 4: Flow-chart of the Copenhagen Osteoarthritis Cohort (Paper III)



Optimized Treatment Algorithm for Patients with Early RA (OPERA) cohort

The OPERA study was an investigator-initiated, randomized, double-blind, placebo-controlled, two armed, parallel-group, multicentre one year trial with an open one year extension study. One-hundred-and-eighty sDMARD-naïve patients with early RA (disease duration <6 months) according to the American College of Rheumatology (ACR) 1987 classification criteria for RA and active disease (DAS28>3.2) were included between August 2007 and January 2010 at 15 Danish Departments of Rheumatology. Main in- and exclusion criteria are presented in Table 2. All patients received oral MTX (increased to 20 mg/week over 2 months) in combination with placebo-adalimumab or adalimumab 40 mg subcutaneously every other week. In case of DAS28 > 3.2 after three months of treatment sulphasalazine 2g/day and hydroxychloroquine 200 mg/day were added. After 12 months adalimumab/placebo-adalimumab was withdrawn. Patients were followed up with monthly visits for the first 3 months and visits after 6, 9, 12, 16, 20 and 24 months thereafter. At all visits, swollen joints were injected with triamcinolone hex-acetonide (20 mg/mL, 0.5–2 mL/joint, maximally four joints and 4 mL per visit).

Of the 180 included patients 7 patients did not have a baseline x-ray performed, while 14 patients did not have a two-year x-ray performed. As shown in Figure 5, DXR-BMD analysis was not possible in a substantial number of radiographs due to technical issues. Both hands were measured if possible and the mean used for analyses. If a patient only had DXR-BMD of one hand at any one timepoint, all analyses were based on that hand. In paper IV, the 70 patients with DXR-BMD measurements at baseline and after 12 months of follow-up constituted the OPERA-HBL_{one-year} cohort (Specific aim 7,8 and 9). The 90 patients with DXR-BMD measurements at baseline and after 6 months in addition to two-year radiographic data constituted the OPERA-HBL_{6months} prediction cohort (Specific aim 10).

Table 2**Main in- and exclusion criteria in the OPERA study****Inclusion criteria:**

Rheumatoid arthritis according to the ACR 1987 classification criteria for RA

Diagnosis < 6 months

Active disease (DAS28 >3.2)

Written informed consent

Exclusion criteria:

Latent tuberculosis

Active or recurrent infections or severe infection in the past 30 days.

Positive serology for hepatitis B or C indicating active infection.

Medical story with positive HIV-status.

Medical history with histoplasmosis or listeriosis.

Previous malignant disease (except radically treated malignancies after 5 years without relapse).

Previous diagnosis or signs of demyelinating disease in the central nervous system.

Renal insufficiency (creatinine clearance < 35 ml/min - nomogram).

Affected liver function: Liver enzymes > 2 upper normal limits.

Clinically significant alcohol or drug abuse in the past year.

Uncontrolled diabetes, unstable ischemic heart disease (NYHA III-IV), active chronic inflammatory bowel disease, recent cerebral apoplexia, chronic leg ulcer and any other condition imposing an increased risk to the subject if he/she participates in the protocol, as judged by the investigator.

Anticoagulant therapy.

Pregnancy or breast-feeding.

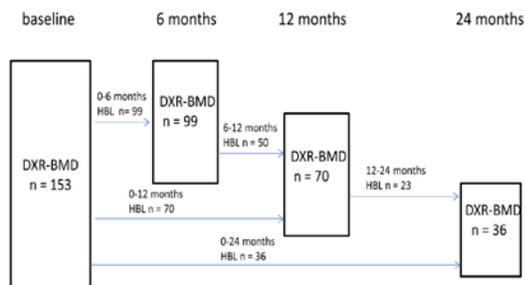
Other inflammatory rheumatologic diseases.

Active or recent infection with Parvovirus B19.

Previous sDMARD treatment.

Glucocorticoid treatment with the past 4 weeks (except nasal and inhalation steroids).

Any contraindication for study medicine.

Figure 5: Flowchart of the OPERA-HBL cohorts (Paper IV)

In 124 patients one or more radiographs were discarded due to change in acquisition modality (from analogue to digital or from one type of x-ray equipment to another) between baseline and follow-up. In 10 patients one or more radiographs were discarded due to differing post-acquisition magnifications between baseline and follow-up radiographs.

Clinical assessment**DANBIO X-ray cohort**

A 28SJC, 28TJC and CRP from the clinical visits closest in time to the pre-baseline x-ray (*pre-baseline visit*) and follow-up x-ray (*follow-up visit*) were obtained from DANBIO or the patient files. For baseline x-ray the clinical visit (*baseline visit*) closest to the date of TNF-inhibitor initiation was used. As data on patient's VAS Global was missing in many patients at the *pre-baseline visit* a

DAS28 based on 3 variables was calculated for all visits(48, 49). HAQ scores (Danish version without correction for devices), VAS pain and VAS fatigue were also collected if available.

OPERA cohort

At all clinical visits a 28SJC, 28TJC, CRP, Physician VAS Global, HAQ (Danish version without correction for devices), Patients VAS Global, pain and fatigue were registered. A DAS28 based on 4 variables were calculated at all visits(48).

Assessment of radiographs**Structural joint damage - Sharp van der Heijde method**

The relevant x-rays (analogue and digital) were collected from 17 (DANBIO X-ray) and 15 (OPERA) Departments of Radiology. Analogue x-rays were digitized and anonymized. Reading was done without knowledge of the chronology of images according to the Sharp/van der Heijde method. In the DANBIO X-ray cohort only hand x-rays were read, and consequently maximum TSS score was 280. In the OPERA cohort both x-rays of hands and feet were read, why maximum score was 448.

X-rays from DANBIO X-ray cohort were read by Pernille Bøyesen, Diakonhjemmet Hospital, Norway. In the first reading 573 patients with three available x-rays were read (Paper I). The intraobserver reliability of this reading was estimated from reevaluation of the x-rays of 61 patients representing high and low baseline-scores as well as high and low progression rates. The intraobserver intra-class correlation coefficient (ICC) (one-way random effects model) for status scores at baseline was 0.95 while the ICC for TSS change in the TNF-inhibitor period was 0.34. The smallest detectable change (SDC) for TSS change was 4.9 TSS units/year (Paper I). In a later reading x-rays from 471 additional patients with two available x-rays were read. In this reading intraobserver reliability was estimated from re-evaluation of 57 patients. ICC for status scores at baseline was 0.96, ICC for TSS change was 0.20. SDC was 2.86 TSS units/year. Combining all re-evaluations resulted in overall ICC for TSS change 0.35 and SDC 3.9 Sharp units/year (Paper II and III). Annual structural joint damage progression rates for the individual patients before baseline (DMARD period) were calculated by subtracting TSS at pre-baseline x-ray from TSS at baseline x-ray and dividing the change in TSS with the number of days between the two x-rays and multiplying by 365 days. In a similar manner annual structural joint damage progression rates between baseline x-ray and follow-up x-ray (TNF-inhibitor period) were calculated.

X-rays from the OPERA cohort were read by Lykke Midtbøll Ørnbjerg. Intra-observer reliability was estimated from re-evaluation of x-rays from 18 patients, representing high and low progression rates. ICC for status scores at baseline was 0.88, ICC for TSS change was 0.88, while SDC for two-year TSS change was 1.8 TSS units (Paper IV). Two-year structural joint damage progression was calculated by subtracting TSS at baseline x-ray from TSS at two-year x-ray.

Hand bone loss - DXR**Copenhagen Osteoarthritis Study**

X-rays were placed on a flat-bed scanner and digitized. Analyses were performed by Trine Jensen and Pernille Bach-Mortensen. Both version 1.02 and version 2.0 of the X-posure System™ were used to estimate DXR-BMD from hand x-rays in the Copenhagen Osteoarthritis Study as version 2.0 was introduced while analyses were taking place.

A linear conversion equation for X-posure System™ v 1.02 to X-posure System™ v 2.0 was calculated based on 5 radiographs of the hand of men and women:
New estimate DXR-BMD (version 2.0) = a (1.04882) x old estimate DXR-BMD (version 1.02) + b (-0,043321). All DXR-BMD results from X-posure System™ v 1.02 were converted to X-posure System™ v 2.0 according to this equation.

DANBIO X-ray and OPERA cohorts

Digitized and anonymised hand x-rays from the DANBIO X-ray and OPERA cohorts were transferred in an electronic format (Digital Imaging and Communication in Medicine (DICOM-files)) to Sectra, Sweden where DXR-analysis was performed by Johan Kalvesten with the dxr-online™ system, a fully automated version of the DXR software. The algorithm from the X-posure System™ v.2.0 is applied by the dxr-online™ system(50). Study-specific reproducibility assessments were not performed. The in vivo short-term coefficient of variation (CV) has previously been shown to be 0.46% and Smallest Detectable Difference (SDD) 0.0046 g/cm²(51).

Ethics

DANBIO X-ray cohort

According to the Danish laws, no ethical approval or informed consent is needed for the publication of research data that are based on routine collection of data.

Copenhagen Osteoarthritis cohort

The Danish ethics committee for the City of Copenhagen and Frederiksberg approved the Copenhagen City Heart Study (100.2039/91). All individuals signed an informed consent form.

OPERA cohort

The OPERA study was approved by the Regional Ethics Committee, Denmark (VEK-20070008). All patients signed an informed consent form.

Statistical analysis

The main statistical analyses used for each of the specific aims are presented in this paragraph. Further details are found in the original papers I-IV.

All statistical analyses were carried out with the statistical software R (version 2.9.0 in Paper I, version 2.1.3.0 in Paper II, version 2.15.3 in paper III and version 3.2.0 in paper IV), R foundation for Statistical Computing, Vienna(52). Analyses were two-sided and p-values of less than 0.05 were considered statistically significant. Descriptive statistics were used to calculate mean (standard deviation (SD)) for normally distributed continuous variables and median (Inter Quartile Range (IQR)) for non-normally distributed continuous variables. Categorical variables are presented by frequencies or percentages. Comparisons between groups and over time were performed with parametric (Students t-test and paired t-test) or non-parametric (Mann-Whitney and Wilcoxon signed rank test) according to the distribution of data. Chi-square test was used to the test differences between groups for categorical variables. In accordance with the recommendations by Van der Heijde et al. rates of structural joint damage progression were analysed with both parametric and non-parametric analyses(53).

Structural joint damage progression was primarily defined as a change in TSS > 0 (Paper I, II, III and IV), secondarily as a change

in TSS > study specific SDC (Paper I, II and IV)(54). Hand bone loss is presented as absolute (mg/cm²) and relative (%) change in DXR-BMD (Paper III and IV). Inflammatory activity over time was estimated by calculating time-averaged CRP or DAS28 from all available measurements during follow-up (Paper I,II,III and IV)(55).

To investigate the impact of TNF-inhibitor treatment on structural joint damage progression in clinical practice (Specific aim 1) annual progression rates during sDMARD-treatment and subsequent TNF-inhibitor treatment were compared. As sensitivity analyses we performed separate analyses on the 84 patients who started TNF-inhibitor treatment before 1 January 2003 (at which time adalimumab was marketed) in order to address the problem of confounding by chronology, and analyses of patients who had a disease duration of more than 10 years (n=160) versus less than 10 years to address the problem of channelling bias(56).

Logistic regression analyses were used to identify baseline predictors of structural joint damage progression during TNF-inhibitor treatment in clinical practice (Specific aim 2). Baseline variables were analysed with univariate analyses, and significant variables (p < 0.10) were included in a multivariable logistic regression analysis with backwards selection. The following variables were tested as categorical variables: sex, type of TNF-inhibitor, concomitant MTX, concomitant prednisolone, TNF-inhibitor monotherapy, IgM-RF positivity, anti-CCP positivity and current smoking (yes/no). The following variables were tested as continuous variables: baseline DAS28, CRP, SJC, TJC, VAS Global, HAQ, age, disease duration, number of previous sDMARDs, calendar year of treatment initiation, and TSS. In the multivariable analysis, IgM-RF but not anti-CCP was included because of missing anti-CCP data. A separate multivariable analysis including only patients with anti-CCP data was performed as well as an analysis using structural joint damage progression > SDC = 3.9 as the definition of progression.

Univariate logistic regression analyses were used to investigate the association between inflammatory activity during TNF-inhibitor treatment (assessed with the continuous variables time-averaged-CRP, DAS28, 28SJC and 28TJC), switching or stopping biological treatment (assessed as a dummy variable with continued treatment as reference) and structural joint damage progression in RA patients treated in clinical practice (Specific aim 3). In addition progression rates were compared between patients who continued 1st TNF-inhibitor, switched biological treatment or stopped biological treatment with parametric and non-parametric analyses.

To investigate impact of TNF-inhibitor treatment on hand bone loss in clinical practice (Specific aim 4), hand bone loss was compared between DMARD and TNF-inhibitor periods by non-parametric analyses due to a skewed distribution of hand bone loss. Increased hand bone loss in an individual patient was defined as a negative annual hand bone loss exceeding the lower 95% Confidence Interval (CI) of the normal annual hand bone loss for the matching sex and year of age. The proportion of patients with increased hand bone loss in the DMARD and TNF-inhibitor periods were compared with Chi-square test.

To identify baseline predictors for increased hand bone loss during TNF-inhibitor treatment in clinical practice (Specific aim 5) potential baseline predictors were analysed with univariate logistic regression (increased hand bone loss +/- as dependent variable) and significant variables (p<0.10) included in a multiple logistic regression analysis with backward's selection. Tested categorical variables were: sex, type of TNF-inhibitor, concomi-

tant MTX, concomitant prednisolone, TNF-inhibitor monotherapy, IgM-RF positivity and current smoking. Tested continuous variables were: baseline DAS28, CRP, SJC, TJC, VAS Global, HAQ, age, disease duration, number of previous sDMARDs, calendar year of treatment initiation and DXR-BMD.

Univariate logistic regression analyses were used to analyse the association between inflammatory activity (assessed with time-averaged-CRP, DAS28, 28SJC and 28TJC) and hand bone loss during TNF-inhibitor treatment in clinical practice (Specific aim 6). In addition hand bone loss was compared between patients who were in time-averaged remission during follow-up (DAS28 < 2.6) and patients who were not.

To evaluate the impact of addition of adalimumab on hand bone loss in early RA patients (Specific aim 7) one-year hand bone loss was compared between patients treated with MTX+adalimumab and MTX+placebo by Mann-Whitney test. In addition, a linear mixed effects model were used to examine hand bone loss over time (0-6 months, 6-12 months and 12-24 months) including all available measurements of hand bone loss. Time, treatment group and the interaction between treatment group and time were tested while adjusting for age and sex. Analyses were conducted as intention-to-treat.

Potential baseline predictors for hand bone loss (Specific aim 8) in the early RA patients treated with MTX +/- adalimumab (*OPERA-HBL_{one-year}* cohort) were initially tested in univariate linear regression analyses (one-year hand bone loss as dependent variable). Tested categorical baseline variables were: sex, IgM-RF positivity and anti-CCP positivity. Tested continuous baseline variables were: DAS28, CRP, SJC, TJC, VAS Global, VAS Pain, VAS Fatigue, Physician VAS Global, HAQ, age, disease duration and DXR-BMD. Variables with p < 0.10 were subsequently entered into a multivariable linear regression model. Similarly, univariate logistic regression analyses were performed with increased vs. normal hand bone loss (according to reference values defined in Paper III) as the dependent variable.

The association between inflammatory activity (assessed with time-averaged-CRP and DAS28) and hand bone loss in early RA patients (Specific aim 9) were investigated with linear regression analyses.

To investigate whether 0-6 months hand bone loss (*HBL_{6months}*) was independently associated with two-year structural joint damage progression (Specific aim 10) a multiple linear (change in TSS from baseline to 24 months as dependent variable) and logistic regression(a) change in TSS > 0 and b) change in TSS >SDC = 1.8 as dependent variable) analyses were performed in the *OPERA-HBL_{6months}* prediction cohort. Initially, variables were tested in univariate analysis and variables with p < 0.10 were subsequently entered into multivariable models. Tested categorical baseline variables were: sex, IgM-RF positivity and anti-CCP positivity. Tested continuous baseline variables were: DAS28, CRP, SJC, TJC, VAS Global, VAS Pain, VAS Fatigue, Doctor VAS, HAQ, age, disease duration and TSS. In addition to variables assessed at baseline, 0-6 months change in DAS28, ES and DXR-BMD (*HBL_{6months}*) were tested.

To establish a reference material for hand bone mass measured by DXR-BMD (Specific aim 11) mean (SD) DXR-BMD was calculated for 13 age-groups (10-year intervals in the lowest and highest age-groups, remaining age-groups 5-year interval) for men and women separately. To estimated normal age- and sex-related HBL/year two linear regression models for the relation between age and DXR-BMD were fitted for men and women

separately. Model fits were compared with the Akaike Information Criterion (AIC) for non-nested models and ANOVA for nested models. Standard graphical tests of model assumptions were performed (plots inspected for linearity, homoscedasticity and normally distributed residuals). From the final models estimated mean annual change in DXR-BMD were calculated for all year of ages from 18 to 89 in both sexes. The derived estimates constituted reference values for normal HBL/year.

SUMMARY OF MAIN RESULTS

RA study cohorts

Table 3 summarizes selected baseline characteristics of the cohorts us in the present thesis.

Name of cohort in paper	Study population	Study population	csDMARD-to-TNFI cohort	TNFI cohort	OPERA-HBL _{one-year} cohort	OPERA-HBL _{6months} prediction cohort	Reference cohort
Paper	I	II	III	III	IV	IV	III
Specific aim	1	2,3	5	6,7	8,9,10	11	4
No. of participants	517	930	135	350	70	90	4151
Women	394 (76%)	697 (75%)	72 (85%)	277 (79%)	46 (66%)	63 (70%)	2618 (63%)
Age, years	57 (48-63)	57 (48-64)	57 (47-64)	56.6 (47-64)	53 (41-63)	56 (45-63)	64 (54-72)
IgM-RF positive (%)	406 (80%)	704 (79%)	96 (71%)	266 (79%)	48 (69%)	60 (66%)	
Anti-CCP positive (%) ^a	151 (65%)	275 (64%)	42 (52%)	110 (60%)	45 (64%)	53 (59%)	
Disease duration (years)	5 (1-13)	9 (4-16)	6 (4-12)	8 (4-14)	0.2 (0.1-0.4)	0.2 (2.1-0.4)	
Current smoking (%)	171 (40%)	294 (38%)	45 (35%)	119 (34%)	18 (26%)	25 (28%)	
No. of previous sDMARDs	3 (2-4)	3 (2-4)	3 (3-4)	3 (2-4)	0 (0-0)	0 (0-0)	
DAS28	5.0 (4.2-5.7)	5.0 (4.3-5.8)	5.3 (4.4-6.1)	5.3 (4.5-6.1)	5.7 (5.0-6.2)	5.4 (5.0-6.2)	
CRP (mg/L), mean (SD)	31 (39)	31 (37)	32 (18)	29 (40)	35 (41)	32 (38)	
Type of TNF-I							
INF	319 (59%)	546 (59%)	101 (75%)	218 (62%)	-	-	
ETAN	62 (12%)	171 (18%)	17 (12%)	68 (20%)	-	-	
ADA	76 (15%)	122 (13%)	18 (13%)	64 (18%)	33 (47%)	49 (54%)	
MTX treatment during follow-up (%)	435 (84%)	745 (80%)	116 (86%)	289 (83%)	70 (100%)	90 (100%)	
MTX dosage in patients taking MTX (mg/week)	15 (10-20)	15 (10-20)	17.5 (12.5-20)	15 (10-20)	-	-	
Glucocorticoid treatment during follow-up (%)	370 (72%)	555 (60%)	103 (76%)	224 (64%)	70 (100%)	90 (100%)	
Time-averaged dose of prednisolone in patients receiving glucocorticoids* (mg/day)	1.5 (0.4-5.0)	2.2 (0.5-3.2)	1.3 (0.4-3.8)	2.0 (0.5-4.6)	0.4 (0.3-0.5)	0.5 (0.3-0.6)	
HAQ	1.250 (0.750-1.750)	1.250 (0.750-1.825)	1.250 (0.750-1.750)	1.250 (0.750-1.750)	1.125 (0.625-1.750)	1.125 (0.750-1.625)	
Total Sharp Score, mean (SD)	26 (33)	31 (40)	19 (24)	22 (27)	4 (6)	5 (7)	
Total Sharp Score	13 (2-41)	15 (3-45)	10 (1-29)	12 (3-35)	2 (1-6)	3 (1-6)	
Erosive disease (%)	400 (77%)	765 (82%)	103 (76%)	284 (81%)	41 (59%)	49 (54%)	
DXR-BMD (g/cm ²), mean (SD)	-	-	0.506 (0.10)	0.502 (0.10)	0.590 (0.08)	-	0.547 (0.09)

Values are median (InterQuartileRange) unless otherwise stated.

Percentage of patients with available data on anti-CCP status ranged from 46 (TNFI-cohort) to 100 (OPERA-HBL cohorts) * All administrations of glucocorticoids (peroral, intramuscular, intraarticular and intravenous) converted to corresponding prednisolone dosages)

Impact of TNF-inhibitor treatment on structural joint damage progression (Specific aim 1, Paper I)

In the DMARD-period, the median TSS increased from 7 (pre-baseline x-ray) to 13 (baseline x-ray) ($p = 0.0005$, Wilcoxon Signed Rank). At the end of the TNF-inhibitor period (follow-up x-ray) the median TSS was 14 (baseline TSS vs. follow-up TSS, $p = 0.53$, Wilcoxon Signed Rank). The annual rate of structural joint damage progression decreased from 0.7 (2.1) TSS units/year (median (mean)) in the DMARD- period to 0 (0.7) TSS units/year in the TNF-inhibitor period ($p < 0.0001$, Wilcoxon signed rank, (paired t-test)). Significant decreases of progression rates in ES (mean (SD) 10. (1.8) vs. 0.4 (1.4), $p < 0.001$, paired t-test) and JSN (1.1 (2.6) vs. 0.3 (1.2), $p < 0.001$, paired t-test) were also found. The majority of patients (305 (59%)) experienced structural joint damage progression (change in TSS > 0) in the DMARD-period, while 158 patients (31%) progressed in the TNF-inhibitor period ($p < 0.0001$, Chi-Square). A total of 71 patients had a high progression rate (i.e. $> \text{SDC}$ (4.9 TSS units/year) in the DMARD-period compared to 22 patients in the TNF-inhibitor period ($p < 0.0001$, Chi-square).

Baseline predictors of structural joint damage progression during TNF-inhibitor treatment (Specific aim 2, Paper II)

In univariate analyses concomitant treatment with prednisolone, IgM-RF positivity, anti-CCP positivity, age, CRP-level and TSS predicted structural joint damage progression. In a multivariable analysis including IgM-RF (but not anti-CCP) while adjusting for gender, concomitant treatment with prednisolone (Odds Ratio (OR) 1.39 (95%CI 1.03-1.89, $p = 0.03$), IgM-RF positivity (OR 1.75 (1.16-2.60), $p = 0.008$), increasing age (OR 1.30 (1.14-1.48)/10 year increase, $p = 0.001$) and baseline TSS (1.05 (1.01-1.09)/10 units increase, $p = 0.02$) remained independent predictors of structural joint damage progression. Type of TNF-inhibitor did not predict structural joint damage progression. Including anti-CCP (replacing IgM-RF), positive anti-CCP (OR) 1.72 (95% (1.06-2.87)) and age (OR 1.36 (1.12-1.66)/10 year increase) were independent predictors of structural joint damage progression ($n=432$). The fraction of explained variation (Nagelkerkes R^2) in the two models were 0.07 and 0.06, respectively. When both IgM-RF and anti-CCP were included in the model IgM-RF was an independent predictor of structural joint damage progression while anti-CCP was not (data not shown).

With progression $> \text{SDC}$ (3.9) as dependent variable IgM-RF positivity (OR 11.2 (2.4-198.5)) and CRP-level (OR 1.007 (1.001-1.011)/mg/L increase) predicted structural joint damage progression in univariate and multivariable analyses. Nagelkerkes R^2 was 0.06.

Association of TNF-inhibitor switching, treatment withdrawal and inflammatory activity during treatment with structural joint damage progression (Specific aim 3, Paper II)

Time-averaged-CRP and DAS28 during TNF-inhibitor treatment were associated with structural joint damage progression (OR 1.02 (1.01-1.03)/mg/L increase and OR 1.3 (1.14-1.50)/unit increase, both $p < 0.0001$). Time-averaged 28SJC and TJC was not associated with structural joint damage progression. Patients who switched TNF-inhibitor treatment or withdrew from biological treatment during follow-up had a higher risk of structural joint damage progression than patients who continued their initial TNF-inhibitor, OR 1.62 (1.17-2.22) ($p = 0.003$) and 1.75 (1.08-2.78) ($p = 0.02$), respectively. In a model that included both the baseline predictors and time-averaged CRP both switching

and withdrawal from biological treatment remained independently associated with structural joint damage progression (OR 1.68 and 2.06, respectively, $p < 0.001$). Patients that continued treatment had an annual progression rate of median (IQR)/mean(SD) 0 (0-0)/0.3 (1.6) TSS Units/year. This was lower than in patients who switched treatment (0 (0-0.8), $p < 0.001$ (Mann-Whitney)/1 (3.6), $p = 0.002$ (t-test)) or stopped treatment (0 (0-1), $p = 0.006$ (Mann-Whitney)/0.8 (2.0), $p = 0.02$ (t-test)).

Impact of TNF-inhibitor treatment on hand bone loss (Specific aim 4, Paper III)

In the 135 patients in the *csDMARD-to-TNFI*-cohort, pre-baseline median (IQR) DXR-BMD was 0.545 (0.474-0.597) g/cm² decreasing to 0.516 (0.441-0.578) g/cm² at baseline ($p < 0.001$, Wilcoxon Signed Rank). At follow-up, DXR-BMD had further decreased to 0.504 (0.424-0.557) g/cm² ($p < 0.001$, Wilcoxon Signed Rank). Hand bone loss was significantly lower in the TNF-inhibitor period (-0.0051 g/cm²/year and -1.15%/year) compared to the DMARD-period (-0.0082 g/cm²/year and -1.55%/year), $p < 0.001$ for both comparisons (Wilcoxon Signed Rank). In the DMARD-period 101 (75%) patients had increased hand bone loss while increased hand bone loss was found in 79 (59%) patients in the TNF-inhibitor period ($p = 0.17$, Chi-Sq). Thirty-eight patients had an increased hand bone loss in the DMARD-period that normalised in the TNF-inhibitor period, while 16 patients had a normal hand bone loss in the DMARD-period but increased hand bone loss in the TNF-inhibitor period.

Baseline predictors of hand bone loss during TNF-inhibitor treatment (Specific aim 5, Paper III)

In univariate logistic regression analyses of the *TNFI*-cohort high baseline DXR-BMD, longer disease duration, IgM-RF-positivity and high DAS28 predicted increased hand bone loss, while age, HAQ-score, CRP, sex, smoking status, type of TNF-inhibitor, calendar year of treatment initiation and concomitant treatment with prednisolone and MTX did not. In the multivariable model, DXR-BMD (OR 1.005/mg increase (95% CI 1.003-1.008)) and DAS28 (OR 1.43/unit increase (95%CI 1.15-1.81)) were independent predictors of increased hand bone loss. Nagelkerkes R^2 was 0.12. In a model including time-averaged-DAS28 during TNF-inhibitor treatment baseline DXR-BMD and DAS28 remained independent predictors of increased hand bone loss, R^2 increased to 0.20.

Association between disease activity during TNF-inhibitor treatment and hand bone loss (Specific aim 6, Paper III)

Increased hand bone loss during TNF-inhibitor treatment was associated with time-averaged-DAS28 (OR 1.69 (1.34-2.15)/unit increase) $p < 0.001$ and all of its components: time-averaged-28SJC (OR 1.29 (1.15-1.46)/joint increase), $p < 0.001$, time-averaged-28TJC (OR 1.11 (1.05-1.19)/joint increase), $p < 0.001$ and time-averaged-CRP (OR 1.02(1.005-1.04)/(mg/L) increase), $p = 0.02$).

During TNF-inhibitor treatment 81 patients were in time-averaged remission (time-averaged-DAS28 < 2.6) and 42 (52%) of these patients had a normal hand bone loss. In contrast, 98 (39%) of the 254 patients not in time-averaged remission had a normal hand bone loss (Chi-sq, $p = 0.04$). Patients in time-averaged remission had a lower hand bone loss than patients who were not (-0.0032 vs. -0.0058 g/cm²/year), $p = 0.003$ (Mann-Whitney).

Impact of adalimumab treatment on hand bone loss (Specific aim 7, Paper IV)

In the OPERA-HBL_{one-year} cohort mean (standard deviation (SD)) DXR-BMD at baseline was 0.594 (0.08)/0.586 (0.08) g/cm² decreasing to 0.582 (0.08)/0.575 (0.08) g/cm² after 12 months in the placebo- and adalimumab groups, respectively (p < 0.001, paired t-test for both comparisons). After 12 months of follow-up patients had a median (IQR) hand bone loss of -11 (-20 – -1.7)mg/cm² /-1.9 (-3.3 - -0.26) % in the placebo-group compared to -11 (-21 – 0.38)mg/cm²/-1.8 (-3.6 – 0.06) % in the adalimumab-group, p = 0.865/0.98, Mann-Whitney. In the placebo-group 26 of 37 (70%) patients had increased hand bone loss compared to 23 of 33 (70%) patients in the adalimumab-group, p = 1, Chisq. These findings were confirmed by the linear mixed effects model showing that neither time, treatment group or their interaction were significantly associated with HBL.

Baseline predictors of hand bone loss (Specific aim 8, Paper IV)

In univariate linear regression analyses baseline age, HAQ and TSS at baseline were borderline predictive of subsequent higher hand bone loss (absolute and relative) (all p < 0.10), while neither sex, anti-CCP, IgM-RF, baseline DXR-BMD, DAS28, CRP, Patient VAS Global, 28SJC, 28TJC or days from symptoms till diagnosis predicted hand bone loss. In a multivariable linear regression analysis baseline age (-0.35 mg/cm²/year increase, p = 0.005) and HAQ (-5.9 mg/cm²/unit increase, p = 0.04) were independent predictors of hand bone loss. No baseline variables were associated with increased hand bone loss in logistic regression analyses.

Association between disease activity during treatment and hand bone loss (Specific aim 9, Paper IV)

Time-averaged-DAS28 was significantly associated with one-year hand bone loss (β-coefficient -7.2 mg/cm²/unit increase, p = 0.04), while time-averaged-CRP was not ((β-coefficient -0.45 mg/cm²/unit increase, p = 0.09)) in the OPERA-HBL_{one-year} cohort. The association between hand bone loss and time-averaged-DAS28 was numerically larger in the placebo-group (β-coefficient -8.6 (95% Confidence Interval (CI) -18;1.7)mg/cm²/unit increase) compared to the adalimumab-group (β-coefficient -5.4 (95%CI -14;4.0) mg/cm²/unit increase). However, no interaction between treatment and time-averaged-DAS28 on hand bone loss was found (p = 0.665).

Early hand bone loss and prediction of structural joint damage progression (Specific aim 10, Paper IV)

In univariate linear regression analyses high HBL_{6months} and placebo treatment were associated with a larger increase in TSS from 0-24 months, while IgM-RF positivity and high baseline HAQ were borderline predictors. In the multivariable linear regression analysis high HBL_{6months} (β = -0.086 TSS unit/mg/cm² increase, p = 0.006) and placebo treatment (β = 1.61 TSS units, p = 0.02) were independently associated with a larger two-year change in TSS, while high HAQ (β = 0.88 TSS units/unit increase, p = 0.07) and IgM-RF positivity (β = 1.45, p = 0.055) were borderline predictive.

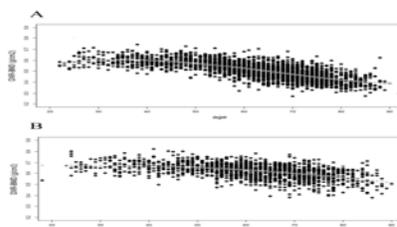
In univariate logistic regression with structural joint damage progression (change in TSS > 0) as dependent variable, HBL_{6months} and IgM-RF (OR 0.96, p = 0.08 and OR 2.3, p = 0.07) were borderline associated with an increased risk. In a multiple logistic regression model, no variables were independently associated with radiographic progression. In univariate logistic regression analyses

of radiographic progression > SDC, HBL_{6months} were associated with an increased risk (OR 0.96, p = 0.04) while IgM-RF were borderline predictive (OR 2.6, p = 0.06). In a multiple logistic regression model, no variables were independently associated with progression > SDC.

Reference material for normal hand bone loss (Specific aim 11, Paper III)

Distribution of DXR-BMD in women (A) and men (B) in the reference cohort (Copenhagen Osteoarthritis Cohort) are presented in Figure 5. In women, the best fitting model was DXR-BMD = 0.020 x age – 0.00040 x age² + 0.0000021 x age³ (R²= 0.54); in men DXR-BMD = 0.0018 x age – 0.0000371 x age² (R²=0.27). Estimated mean annual changes in DXR-BMD (ie. normal annual hand bone loss) in men and women derived from the models are presented in Table 4A and B averaged over 5-year age intervals (10-year intervals in the lowest and highest age groups). In men, the model estimated an increasing annual hand bone loss from 35 years onwards reaching a maximum of -0.0047 g/cm²/-0.8 % per year at the age of 85. In women, an annual increase in DXR-BMD till 35 years was estimated followed by a continuous hand bone loss, accelerated between 55 and 70 years (>-0.0050/-1.0 %).

Figure 5: Distribution of DXR-BMD (g/cm²) in the reference cohort of A: 2541 Danish women and B: 1485 Danish men



Bone Mineral Density in hands - men		Absolute annual change (g/cm ²)			Relative annual change (%)
Age (years)	n	BMD (g/cm ²), mean (SD)	Estimate	95% confidence interval	
18-29	32	0.666 (0.06)	-0.00012	-0.00095 - 0.00071	- 0.02
30-34	35	0.671 (0.06)	-0.00060	-0.00128 - 0.00007	- 0.09
35-39	45	0.679 (0.05)	-0.00098	-0.00153 - 0.00042	- 0.1
40-44	71	0.660 (0.05)	-0.00135	-0.00179 - 0.00090	- 0.2
45-49	98	0.668 (0.05)	-0.00172	-0.00206 - 0.00138	- 0.3
50-54	133	0.653 (0.05)	-0.00202	-0.00235 - 0.00184	- 0.3
55-59	209	0.636 (0.05)	-0.00247	-0.00269 - 0.00225	- 0.4
60-64	198	0.623 (0.06)	-0.00284	-0.00309 - 0.00259	- 0.5
65-69	211	0.602 (0.06)	-0.00321	-0.00354 - 0.00288	- 0.5
70-74	213	0.594 (0.06)	-0.00358	-0.00401 - 0.00316	- 0.6
75-79	140	0.572 (0.06)	-0.00396	-0.00450 - 0.00342	- 0.7
80-84	68	0.563 (0.06)	-0.00432	-0.00499 - 0.00367	- 0.8
85-93	32	0.534 (0.08)	-0.00470	-0.00548 - 0.00392	- 0.8
Total	1485	0.619 (0.07)			

Table 4 B
Age-related absolute and relative changes of DXR-BMD in 2541 Danish women

Bone Mineral Density in hands – women			Absolute annual change (g/cm ²)		Relative annual change (%)
Age (years)	n	BMD (g/cm ²), mean (SD)	Estimate	95% confidence interval	
18-29	41	0.597 (0.05)	0.00416	0.00268 - 0.00564	0.7
30-34	51	0.599 (0.04)	0.00123	0.00031 – 0.00213	0.2
35-39	84	0.601 (0.05)	- 0.00066	-0.00122 - - 0.00009	-0.1
40-44	116	0.589 (0.04)	- 0.00224	-0.00260 - - 0.00189	-0.4
45-49	131	0.592 (0.05)	-0.00351	-0.00379 - - 0.00323	-0.6
50-54	185	0.569 (0.05)	-0.00437	-0.00470 - - 0.00411	-0.8
55-59	322	0.541 (0.05)	-0.00513	-0.00543 - - 0.00483	-0.9
60-64	311	0.513 (0.06)	-0.00548	-0.00575 - - 0.00521	-1.0
65-69	430	0.486 (0.06)	-0.00552	-0.00577 - - 0.00527	-1.1
70-74	394	0.460 (0.05)	-0.00525	-0.00559 - - 0.00492	-1.1
75-79	307	0.440 (0.05)	-0.00468	-0.00525 - - 0.00410	-1.0
80-84	123	0.411 (0.05)	-0.00380	-0.00472 - - 0.00288	-0.9
85-93	46	0.400 (0.06)	-0.00261	-0.00397 - - 0.00124	-0.7
Total	2541	0.505 (0.08)			

DISCUSSION

The first part of the discussion reviews methodological considerations of the thesis. In the second part the main results of the thesis are discussed.

Methodological considerations

Study design: RCTs vs. observational studies

In the present thesis structural joint damage and hand bone loss were assessed and predicted in two methodologically very different study designs: an RCT (OPERA), and an observational study (DANBIO X-ray study). The RCT design has the strong advantage of randomization enabling a difference in outcome (ex. hand bone loss) to be attributed to treatment. During the conduction of a RCT large efforts are made to ensure complete data collection, e.g. all patients in the OPERA study had anti-CCP analyses performed, VAS Global registered etc. However, the strict in- and exclusion criteria, selecting for patients with high RA disease activity and limited comorbidity, result in low external validity and generalizability of the obtained results(57). The observational study, on the other hand, represents the large heterogeneous population of RA patients treated outside of trials that include patients who differ with respect to disease activity, disease course, comorbidity and compliance from patients eligible for the RCTs (40, 58). A general weakness of the observational design is that no conclusions with regards to causality can be made. In addition, missing data is an inherent challenge. In the DANBIO x-ray study a review of all patient files were performed. Despite this only ≈50% of patients had an anti-CCP measurement, while 8

patients had HAQ or VAS Global score registered two years prior to TNF-inhibitor treatment. The RA diagnosis itself exemplifies the heterogeneity vs. homogeneity of the study populations. In the DANBIO X-ray study all patients registered in the DANBIO database with the diagnosis RA were eligible for inclusion, while patients included in the OPERA study had to fulfill the ACR 1987 classification criteria for RA(15).

The two study designs provide complementary information and therefore the inclusion of structural joint damage and hand bone loss data from both study designs may be considered a strength of the thesis(59).

Patient selection

To be included in the DANBIO X-ray study patients had to have at least two x-rays performed at relevant timepoints, which may have selected a study population, who had been more closely monitored than the general DANBIO-registered RA patient. However, no clinically relevant differences in baseline characteristics known to influence structural joint damage progression were found between the study populations (Paper I and II) and the patients registered in DANBIO without relevant x-rays. In the first years of TNF-inhibitor postmarketing use, many of the patients who were selected for TNF-inhibitor treatment had longstanding disease, which may influence the effectiveness of the treatment. To address this potential bias, sensitivity analysis of patients initiating treatment before vs. after 2003 was performed showing similar effectiveness of TNF-inhibitor treatment with regards to structural joint damage progression in the groups. Consequently, we consider the results from Paper I and II as representative of all Danish RA patients treated with TNF-inhibitors.

In paper III, all x-rays from the DANBIO X-ray study were sent for DXR-analysis but technical conditions or disease-related factors as severe bone damage or inserted prostheses in MCP-joints, hindered DXR-analysis of many x-rays. While the technical challenges are unlikely to bias the selection of patients, the disease-related factors hindering DXR-analysis, introduces a selection bias in the *csDMARD-to-TNFI*-cohort and the *TNFI*-cohort (Paper III) as patients with extensive structural joint damage were excluded. This bias is evident by the higher TSS in the patients with x-rays not suitable for DXR-analysis (median 17 vs. 12 TSS units).

The individuals included in the overall CCHS were randomly selected from the urban county of Østerbro through a social security number algorithm(60). In contrast, the individuals subsequently included in the COS were selected based on their answers to an extensive questionnaire on musculoskeletal symptoms(61). Theoretically, the reference cohort in Paper III could carry a higher burden of pain than the general population. Unfortunately, we did not have access to the questionnaires and were thus not able to analyse cases and controls separately. Since a recent study from Korea found no association between musculoskeletal pain and BMD and since the prevalence of musculoskeletal pain is substantial in the Danish population, we consider the COS cohort a suitable reference cohort for our purpose(62, 63). The large size of the reference cohort allows rather precise estimation of age- and sex-related hand bone loss on a group level, which is a strength compared to previously published reference materials(64-67).

In paper IV, technical conditions hindered hand bone loss assessment in many patients as the DXR technology requires x-rays to be obtained with the same image acquisition modality (analogue vs. digital) and equipment if DXR-BMD measurements

are to be compared and a hand bone loss calculated. Unfortunately, the OPERA protocol for x-ray acquisition did not include a specific DXR-BMD protocol, so less than 50% patients could be included in the *OPERA-HBL_{one-year}* cohort, thereby decreasing the statistical power considerably. In contrast to Paper III, no x-rays were excluded due to structural joint damage (since the patients had early RA) and no differences between patients with and without hand bone loss data were found. Therefore, we consider the *OPERA-HBL_{one-year}* cohort as representative of the entire OPERA-cohort.

Structural joint damage

In this thesis structural joint damage was assessed with the SvdH method by a single observer blinded for patient identity, chronology of the x-rays and treatment. Optimally, two observers would have read all x-rays and the mean score used as outcome in subsequent analyses(53). As both studies were investigator-initiated with limited funding this was not feasible. However, both readers had received training and calibration sessions in the SvdH method from an expert reader (Annelies Boonen, Maastricht University Hospital, Holland) and showed good intra-observer agreement (SDC 1.8-4.9 TSS units) comparable to the intra-reader agreement of experienced readers, so we find the validity of the structural joint damage data satisfactory(68).

In the DANBIO X-ray study knowledge of the x-ray chronology would undoubtedly have biased the reader as all patients received sDMARD-treatment between the two first x-rays and TNF-inhibitor treatment between the second and third (Paper I). In contrast, the decision to read x-rays from the OPERA study (Paper IV) blinded for chronology can be debated. Traditionally, RCT x-rays have been read in this manner but recent data suggest that reading with known time sequence is preferable due to increased precision translating into higher statistical power from the same sample size(69).

The choice of cut-off value for progression in structural joint damage is another debated subject. Throughout the papers I-IV we have adhered to the recommendations by van der Heijde et al citing “ After much discussion, consensus was reached that both the percentage of patients with progression > 0.5 (for two readers or 0 for one reader) and the percentage of patients with progression > SDD should be presented”(53). A later publication by the group of van der Heijde recommends the use of SDC above SDD(54). From a clinical and statistical perspective SDC is the most relevant cut-off level, as this is the smallest change that can reliably be discriminated from measurement error. As few patients experienced progression >SDC and to enable comparison with previous studies the primary analyses were performed with 0 as the cut-off and sensitivity analyses performed with SDC as the cut-off.

When planning the DANBIO X-ray study assessment of both hand and feet x-rays was the goal, but during the retrospective collection it became clear that in clinical practice few patients had x-rays of the feet performed on a routine basis. To maximize patient inclusion we chose to limit analyses to hand x-rays. This may have influenced the results in Paper I as structural joint damage progression in the hands and feet are only moderately correlated(70). In Paper II, the low level of structural joint damage progression resulted in few patients labelled as progressors, which limited the statistical power for identification of predictors. According to a study by Knevel et al, inclusion of feet x-rays could

have identified an additional 31% of patients with progression in structural joint damage, thereby increasing statistical power(70).

Hand bone loss

In this thesis hand bone loss assessment was performed with the dxr-online system by the manufacturer Sectra. Though the DXR-analysis is operator-independent the blinding of patient identity, x-ray chronology and treatment was kept. Apart from the technical challenges described above the value of hand bone loss assessment as outcome measure has been limited by the lack of a meaningful common definition of increased hand bone loss. This is evident from the different attempts to define increased hand bone loss that has been suggested in the literature: hand bone loss above the median in the study population, the SDD of hand bone loss or cut-off values provided by the manufacturer Sectra based on the hand bone loss in two early RA cohorts(71-75). None of these definitions consider the normal hand bone loss that varies considerably with age and sex. One of the specific aims of the current PhD project was to establish a reference material that took the age- and sex-related normal hand bone loss into account. The reference values were estimated from the largest published reference material for DXR-BMD and the estimates used as cut-off for increased vs. normal hand bone loss. The main limitation of our approach is the cross-sectional design of the reference cohort resulting in estimated mean values of hand bone loss, not true hand bone loss measured in individuals. This approach is an established method when no longitudinal studies are available, but should ideally be supplemented with longitudinal data(76). Melton et al. compared rates of DEXA BMD loss estimated from cross-sectional baseline data with rates obtained from longitudinal assessment and found that cross-sectional data overestimated BMD loss in some skeletal sites (hip and spine) and underestimated the loss in others (radius and ulna)(77). The background for these findings is unclear and their relevance for DXR is not known.

Discussion of the main results

TNF-inhibitor effectiveness on structural joint damage and hand bone loss

In RCTs of patients with insufficient response or adverse events to sDMARD treatment, TNF-inhibitors have been shown to halt structural joint damage progression compared with MTX monotherapy, with mean progression rates during TNF-inhibitor treatment between -0.7 and 1.6 TSS units/year(34, 38, 78). In paper I the mean structural joint damage progression rate decreased from 2.1 to 0.7 TSS units/year after TNF-inhibitor initiation suggesting that the benefit of TNF-inhibitors in patients treated in clinical practice corresponds to that reported in RCTs. In the Czech National Registry, infliximab did not slow the annual progression rate as effectively (from 8.56 (estimate based on disease duration) to 2.0 TSS/units year) which probably reflect that the 99 patients in the study were highly selected with high disease activity (DAS28 >5.1) and extensive radiographic damage (mean TSS 90.1)(39).

In Paper IV, structural joint damage progression was numerically, but not statistically, different between the treatment groups in the OPERA-study(79). This finding differs from the PREMIER study, where a clear benefit with regards to structural joint damage progression from addition of adalimumab to MTX in early RA patients (two-year change in TSS 1.9 in the MTX+adalimumab arm vs. 10.4 in MTX monotherapy arm) was found. The progression

rate in the monotherapy arm of the OPERA-HBL_{one-year} cohort (mean two-year change in TSS 2.4) was very low, and similar to the progression rate of 1.9 TSS units after two years in the combination arm in PREMIER. This striking difference may be partly explained by more severe disease at baseline in PREMIER (inclusion criteria included erosions or IgM-RF positivity), but could also be ascribed to the aggressive OPERA treat-to-target strategy aiming at remission with the use of intra-articular injections and MTX(80).

In Paper III, a decrease in hand bone loss (from -1.55%/year to -1.15%/year) was observed after initiation of TNF-inhibitor treatment in the *csDMARD-to-TNFI* cohort. The latter is considerably higher than hand bone loss in the combination treatment arms in BEST (-0.6 and -0.9%/year), but lower than the -1.63%/year in the combination arm of PREMIER, probably reflecting a more selected patient population in PREMIER than in BEST and the observational DANBIO X-ray study. In Paper IV, a hand bone loss of -1.8%/year irrespective of treatment was found in the OPERA-HBL_{one-year} cohort. The magnitude of this hand bone loss was unexpected as OPERA had inclusion criteria similar to BEST in addition to a lower rate of structural joint damage progression (mean one-year TSS change 3.9 vs. mean two-year TSS change 1.6). As treatment with intra-articular methylprednisolone tended to protect against hand bone loss measured by DEXA in a study by Haugeberg et al, we find that intra-articular triamcinolone is an unlikely cause for the higher hand bone loss(81). An explanation for the considerably higher hand bone loss is thus not obvious.

Despite TNF-inhibitor treatment increased hand bone loss compared to the general population was observed in 59% of patients in the *csDMARD-to-TNFI* (Paper III) and 70 % of the adalimumab-treated patients in the OPERA-HBL_{one-year} cohort (Paper IV). These data indicate that the well-established ability of TNF-inhibitors to prevent structural joint damage progression is less convincing in terms of hand bone loss. This could be explained by a higher sensitivity of hand bone loss assessment for RA bone involvement compared to x-ray evaluation. However, 40 patients in the *csDMARD-to-TNFI* cohort and 8 patients in the OPERA-HBL_{one-year} cohort had structural joint damage progression but normal hand bone loss, which suggest a dissociation between the two types of bone damage. This was also found in the EURIDISS cohort and indicates differing pathophysiological mechanisms despite the common role of the osteoclast(16, 72). A hypothetical explanation could be that TNF-inhibitor treatment normalizes the imbalanced bone remodelling and thus halt structural joint damage progression while low levels of joint inflammation continue to cause a relative inactivity of the joints resulting in a lower but continued hand bone loss.

The findings suggest that TNF-inhibitors are effective in reducing structural joint damage progression and hand bone loss in patients with insufficient clinical response to sDMARD-treatment, while the treatment strategy rather than the treatment itself may be more important in the effort to prevent structural joint damage and hand bone loss in sDMARD-naïve patients.

Predictors of structural joint damage and hand bone loss

Numerous studies from the pre-TNF-inhibitor era have identified baseline structural joint damage, IgM-RF positivity and high disease activity (DAS28, CRP, ESR or 28SJC) as predictors of structural joint damage progression irrespective of treatment(82, 83). Post-hoc analyses of the ASPIRE and BEST trials to a large extent confirmed these findings in TNF-inhibitor treated patients from

RCTs with the addition of older age as negative predictor in ASPIRE(44, 45, 84). In Paper II, older age, IgM-RF positivity and existing structural joint damage predicted structural joint damage progression in TNF-inhibitor treated patients from clinical practice, confirming the predictive potential of these variables. In contrast, high disease activity did not predict structural joint damage progression in our observational study, while prednisolone treatment did. Most likely prednisolone therapy serves as a surrogate marker for high disease activity reflecting that patients with active disease initiate prednisolone (leading to a lower DAS28).

In Paper IV, IgM-RF positivity was borderline associated with structural joint damage progression in line with Paper II and the literature. It is note-worthy that IgM-RF positivity is a consistent predictor of structural joint damage progression in TNF-inhibitor treated patients, but seems to be of less importance regarding the achievement of clinical treatment response indicating that pathways leading to joint inflammation and joint damage are not identical(85-88).

In addition to IgM-RF, placebo treatment and hand bone loss in the first 6 months were independently associated with two-year change in TSS in linear regression analysis and borderline associated with radiographic progression in logistic regression analysis in Paper IV. The findings add to the notion that early hand bone loss may have a role in identification of patients with aggressive disease as suggested by previous studies(72, 73, 89, 90). An inherent limitation of hand bone loss assessment as a predictor of disease course is the need for serial x-rays. Other imaging modalities with known predictive value (Ultrasound (US) and Magnetic Resonance Imaging (MRI)) have the obvious advantage of providing predictive information at baseline allowing earlier prognostication of patients(91). Whether hand bone loss can provide additional predictive information compared with these modalities for structural joint damage progression on x-rays is not known, but a Norwegian study of 84 patients found that grey scale synovitis on US and bone marrow edema on MRI were significant predictors of one-year MRI erosive progression while 0-3 months hand bone loss were only borderline significant(92).

In paper IV age was an independent predictor of hand bone loss in linear regression analyses which is in accordance with both BEST and PREMIER. When hand bone loss was dichotomized into normal vs. increased values based on age- and sex-related reference values, no association between age and hand bone loss was found in either Paper III or IV, suggesting that the association found in linear regression analyses may, at least in part, be explained by the age-related physiological hand bone loss. In the large *TNFI* cohort (n=350) in Paper III, DAS28 was a predictor of increased hand bone loss in logistic regression analysis in line with previous studies(24, 42, 43, 50), while no association between DAS28 and hand bone loss was found in the OPERA-HBL_{one-year} cohort (n=70) in Paper IV. Instead, high HAQ-score was a predictor of hand bone loss in linear regression analyses as previously seen in the CIMESTRA trial probably reflecting the contribution of joint immobility to hand bone loss(74).

The explained fraction of variation (R^2) was low in all of the prediction models in the thesis, ranging from 5 to 20%. This is only slightly lower than R^2 reported in the prediction models for structural joint damage based on ASPIRE and BEST data ($\approx 15\%$ vs. $\approx 30\%$), but considerably lower than R^2 of a prediction model for 5-year structural joint damage progression constructed in the EURIDISS (sDMARD-treated patients with disease duration < 4

years) cohort that identified one-year hand bone loss as an independent predictor (69%) (44, 45, 72). To some extent, this discrepancy may be caused by differing treatment and disease duration in the study populations. The low fractions of explained variation in our studies could – at least partly - be explained by the lack of data on variables known to predict structural joint damage progression such as MRI findings, US findings, anemia, body mass index and genetic markers(93-97). The findings stress that while conventional clinical and laboratory variables may provide predictive information in groups of patients, the ongoing search for predictors of disease course or treatment response in the individual patient (e.g. circulating biomarkers or improved imaging modalities) is highly relevant.

Time-averaged inflammatory activity and its association with structural joint damage progression and hand bone loss

In Paper II, inflammatory activity during TNF-inhibitor treatment (assessed with time-averaged CRP and DAS28) was associated with structural joint damage progression in RA patients with established disease treated in clinical practice. This association has previously been reported in sDMARD-treated patients with early RA(98, 99), but the debate regarding the concept of uncoupling between inflammation and structural joint damage progression during TNF-inhibitor treatment has questioned whether the same association is present in TNF-inhibitor treated patients(100-102). Our findings suggest that inflammation is a main driver of structural joint damage progression, also in patients treated with TNF-inhibitors.

While high disease activity is a consistent baseline predictor of hand bone loss, few studies have investigated the association between disease activity over time and hand bone loss. In Paper III and IV we report a consistent association between time-averaged disease activity and hand bone loss regardless of treatment. These findings contrast with findings from PREMIER, where an association between disease activity and hand bone loss only was present in the monotherapy group and not in patients treated with adalimumab(103). The time-averaged disease activity measures in Paper III and IV were based on a median of 7 measurements compared to three in the PREMIER study, which may explain some of the discrepancy(104).

The findings suggest that a treatment strategy with suppression of inflammation as a goal is important to avoid structural joint damage progression and hand bone loss in all patients irrespective of treatment modality.

CONCLUSIONS

After conduction of the work presented in the thesis the following can be concluded with respect to the specific aims:

In patients with established RA treated with TNF-inhibitors after failure of sDMARD treatment in clinical practice (Cohort A),

- Structural joint damage progression was significantly lower during two years of TNF-inhibitor treatment compared to the previous two years of sDMARD-treatment.
- Existing structural joint damage, older age, IgM-RF positivity and concomitant treatment with prednisolone were independent predictors of progression in structural joint damage.
- Treatment switching and withdrawal from TNF-inhibitor treatment were associated with progression in structural joint damage as was high inflammatory activity during treatment.

- Hand bone loss was significantly lower during two years of TNF-inhibitor treatment compared to the previous two years of sDMARD-treatment, but a normal hand bone loss was only achieved in a minority of patients.
- Hand bone mass and DAS28 at initiation of TNF-inhibitor treatment were independent predictors of increased hand bone loss.
- High inflammatory activity during treatment was associated with increased hand bone loss and patients who were in sustained remission had a lower hand bone loss than patients who were not.

In early RA patients treated with methotrexate (MTX) and intra-articular triamcinolone with or without adalimumab in a randomized placebo-controlled trial (Cohort B),

- Addition of adalimumab neither reduced hand bone loss nor increased the proportion of patients who achieved a normal hand bone loss.
- Older age and high HAQ-score were independent predictors of hand bone loss.
- Hand bone loss was associated with inflammatory activity during treatment assessed with time-averaged DAS28, but not with time-averaged CRP.
- Early hand bone loss in the first 6 months of treatment was independently associated with the change in structural joint damage scores after two years and borderline associated with structural joint damage progression per se.

In the general population,

- Normal hand bone loss estimated from a reference material for hand bone mass varied considerably with age and sex

To conclude on the main aim of the thesis, overall structural joint damage progression was low and the majority of established and early RA patients treated with sDMARDs and TNF-inhibitors did not show any progression of structural joint damage. In contrast, hand bone loss was increased compared to the general population in the majority of patients in both cohorts irrespective of treatment. Independent predictors of structural joint damage progression and hand bone loss were identified in both cohorts, but the explained fraction of variation was low. The findings highlight that the disease course in RA can be modified by targeted treatment. However, the factors that predict response and disease course are complex and only incompletely explained by conventional clinical and laboratory assessments.

ENGLISH SUMMARY

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by pain, swelling and progressive destruction of the joints leading to loss of function and invalidity. The bone destruction in RA is characterised by two distinct features: structural joint damage and hand bone loss, and their prevention is an important treatment goal. Inhibitors of tumour necrosis factor alpha (TNF-inhibitors) have markedly improved the treatment options in RA patients who fail treatment with conventional synthetic Disease Modifying Anti Rheumatic Drugs (sDMARDs), but their effectiveness with regards to structural joint damage and hand bone loss, predictors thereof and the association with disease activity during treatment have mainly been investigated in randomized con-

trolled trials (RCTs) with limited generalizability due to strict inclusion and exclusion criteria.

The main aim of the PhD thesis was to assess and predict structural joint damage and hand bone loss in patients with early and established RA treated with sDMARDs and TNF-inhibitors. This was investigated in two cohorts: A) The "DANBIO X-ray study": an observational, nationwide, longitudinal cohort study of established RA patients treated in clinical practice who initiated TNF-inhibitor treatment after failure of sDMARDs and B) The "OPERA study": a randomized controlled trial of sDMARD-naïve patients with early RA treated with methotrexate (MTX) and intraarticular glucocorticoid injections in combination with adalimumab or placebo-adalimumab. Structural joint damage progression was assessed with the Sharp/van der Heijde radiographic method and hand bone loss was assessed with Digital X-ray Radiogrammetry. From the studies presented in the PhD thesis the following was concluded:

- Structural joint damage progression and hand bone loss were significantly lower during two years of TNF-inhibitor treatment compared to the previous two years of sDMARD-treatment in the DANBIO X-ray Study. The majority of patients had no progression of structural joint damage during two years of TNF-inhibitor treatment, while hand bone loss remained increased compared to reference values from the general population in the majority of patients. Adalimumab had no impact on hand bone loss in the OPERA study.
- Existing structural joint damage, older age, IgM-Rheumatoid factor positivity and concomitant treatment with prednisolone were independent predictors of progression in structural joint damage in the DANBIO X-ray cohort, while high hand bone loss in the first 6 months of treatment and placebo treatment were independently associated with increase in structural joint damage scores in the OPERA study. A high hand bone mass and disease activity were independent predictors of increased hand bone loss in the DANBIO X-ray study, while older age and high functional disability predicted hand bone loss in the OPERA study.
- High disease activity during treatment was associated with structural joint damage progression during TNF-inhibitor treatment in the DANBIO X-ray study and with hand bone loss in the DANBIO X-ray and OPERA studies.

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