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Cortical hand bone loss in rheumatoid arthritis

Evaluating digital X-ray radiogrammetry as outcome measure of disease activity, response variable to treatment and predictor of bone damage

Thesis for the degree of Philosophiae Doctor

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Kortikalt bentap i hender ved revmatoid artritt.

Evaluering av digital X-ray radiogrammetry som mål for sykdomsaktivitet, responsvariabel ved behandling og prediktor for benødeleggelse.

Revmatoid artritt (RA) er en inflammatorisk sykdom som angriper ledd. Sykdommen har varierende forløp, og det er viktig å velge ut pasienter med dårlig prognose tidlig i sykdomsforløpet. Det finnes i dag legemidler kan bremse benødeleggelse i ledd (erosjoner), og de mest brukte medikamentene hemmer tumor nekrose faktor-α (anti-TNF terapi). Det eksisterer ingen enkel blodprøve eller test som kan forutsi prognose. Gullstandarden er å bedømme erosjoner på røntgenbilder av hender.
Avkalkning av leddet, eller leddnær osteoporose, kan imidlertid sees før erosjoner, men kan ikke bedømmes kvantitativt ved å se på røntgenbilder.
Formålet med denne avhandlingen var å vurdere nytten av en målemetode som måler leddnær osteoporose i hender for bedømming av sykdomsaktivitet og prognose ved RA.

Det er brukt en databasert metode, digital X-ray radiogrammetry (DXR) som måler kortikal bentetthet (DXR-BMD) og kortikal ratio (DXR-MCI) fra røntgenbilder av hender.

Hovedfunn
Artikkel 1 inkluderte 215 RA pasienter som ble fulgt i 2 år. Her ble DXR sammenlignet med den mest brukte metoden for bentetthetsmåling: Dual energy X-ray absorptiometry (DXA). Tap av ben målt ved DXR ble påvirket av sykdomsaktivitet, mens dette ikke kunne vises ved DXA. DXA tap ble bare funnet hos pasienter som hadde kort sykdomsvarighet.
Artikkel 2 var en 10-års oppfølgingsstudie på 136 RA pasienter med sykdomsvarighet ≤4 år. De som tapte DXR-BMD etter ett år hadde betydelig større røntgenødeleggelse både etter 5 og 10 år, selv etter justering for de mest brukte prediktorer for fremtidig leddødeleggelse slik som positiv revmafaktor i blod (anti-CCP), høy inflammasjon (målt med CRP) og erosjoner på røntgenbilder i tidlig sykdomsfase.
Artikkel 3 var en 2 års dobbeltblind, randomisert studie på 768 pasienter med RA hvor effekten av behandling med anti-TNF terapi ble vurdert. Pasientene var inndelt i 3 behandlingsarmer: Methotrexate (MTX), anti-TNF terapi (adalimumab) eller en kombinasjon av disse. Kombinasjonsgruppen mistet minst ben målt med DXR, de som bare fikk anti-TNF terapi mistet mer mens MTX gruppen mistet mest.
Artikkel 4 vurderte presisjonen av DXR. Studien viste at DXR hadde en svært god presisjon (CV %) på 0.14-0.46 % avhengig av røntgenmaskin.

Konklusjon
DXR er en enkel og nøyaktig målemetode for å påvise kortikalt bentap i hender. DXR påvirkes av sykdomsaktivitet, behandling med anti-TNF terapi og kan prediktere senere leddødeleggelse.
DXR synes å ha de forutsetninger som skal til for å kunne bli et hjelpemiddel i vurdering av behandling og prognose hos den enkelte leddgiktpasient.

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LIST OF PAPERS

Paper 1
Mari Hoff, Glenn Haugeberg, Tore K. Kvien
Hand bone loss as an outcome measure in established rheumatoid arthritis. 2-year observational study comparing cortical and total bone loss
Arthritis Research and Therapy 2007; 9(4):R81.

Paper 2
Mari Hoff, Glenn Haugeberg, Sigrid Ødegård, Silje W. Syversen, Robert Landewé, Désirée van der Heijde, Tore K. Kvien
Cortical hand bone loss after 1-year in early rheumatoid arthritis predicts radiographic hand joint damage at 5-year and 10-year follow-up
Annals of the Rheumatic Diseases 2009; 68(3):324-329.

Paper 3
Mari Hoff, Tore K. Kvien, Johan Kälvesten, Aake Elden, Glenn Haugeberg
Adalimumab therapy reduces hand bone loss in early rheumatoid arthritis: explorative analyses from the PREMIER study
Annals of the Rheumatic Diseases 2009; 68(7):1171-1176.

Paper 4
Mari Hoff, Alvilde Dhainaut, Tore K. Kvien, Kristina Forslind, Johan Kälvesten, Glenn Haugeberg
Short-time in-vitro and in-vivo precision of direct digital X-ray radiogrammetry
Journal of Clinical Densitometry 2009; 12(1):17-21.

ABBREVIATIONS AND DEFINITIONS

ACR	American college of rheumatology
ASBMR	American Society for Bone and Mineral Research
anti-CCP	Anti cyclic citrullinated peptide
BMC	Bone mineral content
BMD	Bone mineral density (g/cm²)
BUA	Broadband ultrasound attenuation
c	Constant
CAD	Computer-aided diagnosis
CT	Computer tomography
CR	Computer radiography
CRP	C-reactive protein
CV (%)	Coefficient of variation
DAS	Disease activity score
DAS28	Disease activity score for 28 joint
DIP	Distal interphalangeal joint
DKK1	Dickkopf 1
DMARD	Disease-modifying anti-rheumatic drug
DR	Direct radiography
DXA	Dual X-ray absorptiometry
DXR	Digital X-ray radiogrammetry
DXR-BMD	Bone mineral density (g/cm²), estimated by digital X-ray radiogrammetry
DXR-MCI	Metacarpal index, estimated by digital X-ray radiogrammetry
dxr-online	Digital X-ray radiogrammetry – online. The brand name is written with lower case letters
ESR	Erythrocyte sedimentation rate
EULAR	The European League Against Rheumatism
EURIDISS	European Research on Incapacitating Disease and Social Support
F	Female
FFD	Film focus distance
HAQ	Health assessment questionnaire
IgM RF	Immunoglobulin-M rheumatoid factor
IL	Interleukin
IQR	Interquartile range
JSW	Joint space width
K	Kappa
kV	Kilo Volt
l	Length
LSC (%)	Least significant change
M	Male
mAs	MilliAmpere second
MCI	Metacarpal cortical index
MCP	Metacarpophalangeal joint
M-CSF	Macrophage colony stimulating factor
MHAQ	Modified health assessment questionnaire
MTP	Metatarsophalangeal joint
MTX	Methotrexate
NA	Not available

NS	Not significant
NSAID	Non-steroidal anti-inflammatory drug
ODF	Osteoclast differentiation factor
OPG	Osteoprotegerin
OPGL	Osteoprotegerin ligand
p	Porosity
PIP	Proximal interphalangeal joint
PREMIER	A **p**rospective multi-centre, **r**andomized, double-blind active comparator-controlled, parall**e**l-groups study comparing the fully human **m**onoclonal anti-TNF-α antibody Adalimumab given every second week with MTX given weekly and the combination of MTX and Adalimumab administered over 2 years **i**n patients with **e**arly **r**heumatoid arthritis.
PTPN 22	Protein pyrophosphate N22
qCT	Quantitative computer tomography
R / r	Radius
RA	Rheumatoid arthritis
RANK	Receptor activator of nuclear factor kappa
RANKL	Receptor activator of nuclear factor kappa ligand
RF	Rheumatoid factor
ROI	Regions of interest
SD	Standard deviation
SD_{diff}	Standard deviation difference
SDC	Smallest detectable change
SDD	Smallest detectable difference
SOS	Sound of speed
SPSS	Statistical Package for the Social Sciences
TNF	Tumour necrosis factor
TNF- α	Tumour necrosis factor alpha
TRANCE	TNF induced activation induced cytokine
TRAP	Tartrate-resistant acid phosphatase
vdH Sharp score	van der Heijde modification of the Sharp score
VPA	Volume per area
US	Ultrasound
W	Bone width

SUMMARY IN ENGLISH

Cortical hand bone loss in rheumatoid arthritis
Evaluating digital X-ray radiogrammetry as outcome measure of disease activity, response variable to treatment and predictor of bone damage

Background and objective

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease characterised by destruction of joints. The outcome in RA is heterogeneous, and it is important to select the patients with high risk for serious bone damage in the joints early in the disease course. New treatment with biologic agents has the ability to halter this damage, the most used agents block anti-tumour necrosis factor alpha (anti-TNF therapy). There exist no simple tests that can predict the progression in the individual patient. The gold standard is to evaluate the joint destruction (erosions) on radiographs of the hands. Periarticular osteoporosis is a sign that may appear before the erosions, however can not be quantified based on the visual impressions seen on radiographs.
The objective of this doctoral thesis was to evaluate the value of a new method of measuring periarticular osteoporosis to assess disease activity and prognosis in RA patients.

Methods

Periarticular osteoporosis was measured by digital X-ray radiogrammetry (DXR) which measures cortical bone mineral density (BMD) and cortical ratio (MCI) from radiographs of the hands. The computer calculates DXR in a defined area in the metacarpal bones 2-4.
This thesis consists of four papers: Two longitudinal observational studies (Paper 1 and 2), one blinded randomised study (Paper 3) and one study evaluating the precision of the DXR method (Paper 4).

Results

Paper 1 included 215 patients followed for two years. In this study DXR was compared to the gold standard for measuring BMD: Dual energy X-ray absorptiometry (DXA). Loss of cortical bone measured by DXR was influenced by disease activity, while DXA-BMD loss was not. DXA-BMD loss was only found in a subgroup with short disease duration (<3years).
Paper 2 was a 10-year observational study in 136 RA patients with disease duration ≤4 years. The patients who lost DXR the first year of follow-up had a greater joint damage on radiographs both after 5 and 10 years, even when corrected for the present most used

predictors: Positive rheumatoid factor (anti-CCP), high inflammation measured by C-reactive protein and presence of erosions on radiographs.
Paper 3 was a 2-year double blind, randomised study of 768 patients with RA evaluating the effect of anti-TNF therapy on hand bone. The patients were divided in three treatment groups: Methotrexate (MTX), anti-TNF therapy (adalimumab) or a combination of these. The combination group lost less hand bone, the anti-TNF therapy monotherapy group lost more and the MTX group lost most hand bone. The order of hand bone loss across the three treatment groups was similar to the order of radiographic progression.
Paper 4 evaluated the precision of DXR. A satisfying precision of 0.14-0.46 was found dependent of the radiographic equipment.

Conclusions

Hand bone loss measured by DXR is a feasible and precise method. It is influenced by disease activity, treatment with anti-TNF therapy and can predict subsequent radiographic bone damage. This thesis support that DXR has the potential to be a useful tool evaluating the disease severity in the individual RA patients.

1. INTRODUCTION

1.1 Background

1.1.1 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease characterised by synovitis and destruction of cartilage and bone in joints, especially the small joints of the hands and feet (1;2). The prevalence is about 0.5-1.0 % (3;4) with an annual incidence of 25-50/100.000 (4;5). This makes RA to one of the most frequent inflammatory rheumatic diseases. The morbidity is not only limited to the joint manifestations. Other organs which can be affected by the disease is skeleton, lung, kidney, vascular system, bone marrow and eyes (6). In RA mortality has also been found to be increased (6). For the individual patient the course of the disease can be devastating and for the health care system the economic burden may be considerable.

The aetiology is unknown, but according to our current understanding RA is an autoimmune inflammatory disease. In the majority of RA patients autoantibodies are detectable in serum (7). Autoantibodies against cyclic citrullinated peptide (anti-CCP) and immunoglobulin-M rheumatoid factor (IgM RF) are shown to be of diagnostic value and to predict poor outcome in terms of radiographic progression, physical function and mortality (7-9). In addition to autoantibodies (7) both genes (10) and environmental factors are considered to be central in the pathogenesis. The main genetic risk of RA comes from specific alleles at the HLA-DRB1 locus encoding the shared epitope (11;12), as well as the protein tyrosine phosphate gene N22 (PTPN 22) which both has been found to influence the course of the disease (13). Cigarette smoking is the major known environmental risk factor for RA (14), and the fact that RA is 2-4 times more common in females suggests an influence of sex-hormones (1;15).

The most frequent used treatment for RA is still disease modifying anti-rheumatic treatment (DMARDs), especially methotrexate (MTX). However, during the last decade biologic treatment targeting specific cytokines or molecules involved in the RA disease process has become available for use in clinical practice (9). The current commercially available biologic treatment consists of anti-TNF therapy (e.g. antibodies against tumour necrosis factor-alpha (TNF-α) or soluble TNF-α receptor antagonist); antibodies against CD-20 antigen expressed on B-lymphocytes; antibodies against interleukin (IL) 1 and 6; and T-

cell modulators. This new paradigm in treatment has improved the prognosis and outcome for RA patients (9).

1.1.2 Bone involvement in rheumatoid arthritis

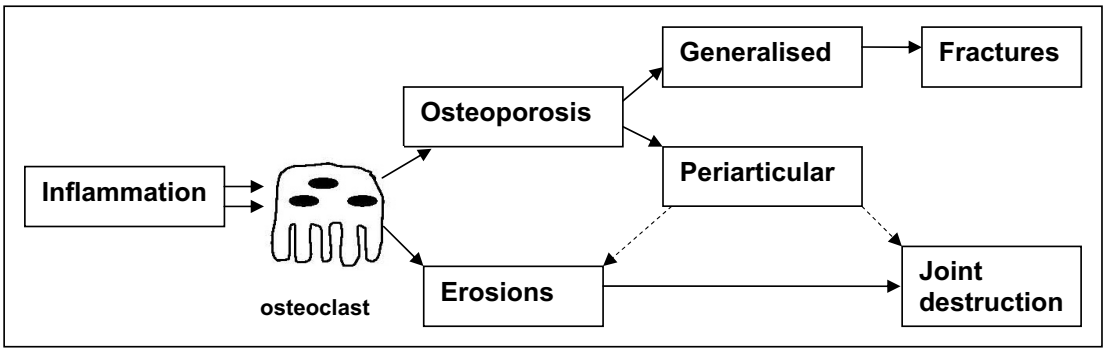
While the disability in early RA is driven by inflammation, the destruction of bone is the main reason for disability in established RA (2;9;16). Bone involvement in RA presents as erosions, generalized osteoporosis and periarticular (juxtaarticular) osteoporosis (Figure 1). Erosions and periarticular bone loss are both characteristic features on radiographs in RA and are included in the 1987 American College of Rheumatology (ACR) revised criteria of RA (17). Erosions on radiographs are specific for RA. However, the disadvantage is that joint damage may not appear on the radiographs early in the disease process. Periarticular osteoporosis is a typical radiographic finding in RA and may occur before the erosions are visible (18;19). It is considered as a hallmark of RA and may distinguish RA from other rheumatic diseases (20;21). However, hand bone loss can not be quantified on radiographs and it is estimated that bone loss less than 20-40 % can not be detected on plain radiographs (22;23). Quantitative hand bone measurements which capture periarticular osteoporosis have therefore been proposed as outcome measures in early RA (21;24).

An increased risk of general osteoporosis in RA is well known, and it is considered as an important extra-articular complication (25;26). Prevalence data on bone mineral density (BMD) reduction in RA has shown a 2-fold increase in osteoporosis for both women (26) and men (27). An increased risk for both vertebral (28) and hip fractures (29;30) has also been reported. In addition to the risk factors for primary osteoporosis as age, gender, menopause and peak bone mass, additional risk factor for osteoporosis in RA are immobilisation, medication and inflammation (27).

Use of corticosteroids has been considered as a major risk factor for osteoporosis, but MTX has also been related to low bone density (31). Corticosteroids has been shown to influence the calcium metabolism by reducing uptake from the intestines and augment the urinary excretion; to stimulate the bone resorptive cells (osteoclasts); to influence the function and lifespan of bone building cells (osteoblast); and to increase apoptosis of the cells imbedded in the bone (osteocyte, which is a mature osteoblast that no longer secretes bone matrix, but maintains bone metabolism) (32;33). Concerning MTX, a clinical syndrome called “MTX osteopathy” has been described (31). This syndrome is characterized by stress fractures, diffuse bone pain and osteoporosis in children treated for malignancies. In animal studies high dose MTX has been shown to induce apoptosis in osteocytes and suppress

proliferation of the cells in the growth plate (chondrocytes) as well as the osteoblasts and preosteoblasts (34). However, low-dose MTX (5-20 mg/week) both in cross-sectional (35;36) and longitudinal studies (37) has not indicated any negative effect on bone in adults.

Figure 1. Bone involvement in rheumatoid arthritis.



1.1.3 Mechanism for bone involvement in rheumatoid arthritis

As mentioned, bone involvement in RA presents as erosions, generalized osteoporosis and periarticular osteoporosis. The osteoclast is the main cell for bone resorption and an increase in the osteoclast activity causes osteoporosis (38), and osteoclasts have also been identified as responsible for bone resorption in RA (39). Substantial evidence from animal studies has shown that osteoclasts are essential for the development of joint destruction in arthritis (40;41). In one animal study transgenic mice that expressed human TNF-α and who developed a severe destructive arthritis were crossed with mice lacking osteoclasts. The resulting mutant mice developed arthritis, but were fully protected against bone destruction (40). Further, suppression the osteoclast by the potent bisphosphonate zoledronic acid, has indicated a reduction of erosions in animal studies (41-43).

Osteoclasts are multinucleated cells of hemapoetic origin from the monocytic cell line. Monocytes are entering the inflamed joint space and receive signals for further differentiation into osteoclasts (44). Expression of cathepsin K and tartrate-resistant acid phosphatase (TRAP) are early differentiation markers for osteoclastogenesis indicating formation of osteoclast precursors, while expression of calcitonin receptor is a late differentiation marker only found at sites of bone resorption or inflammation (45).

The activation and development of osteoclasts depends on stimulation from receptor activator of nuclear factor-κ ligand (RANKL). RANKL is also known as osteoclast

differentiation factor (ODF) (46), osteoprotegerin ligand (OPGL) and TNF-related activation-induced cytokine (TRANCE) (45). The American Society for Bone and Mineral Research (ASBMR) president's committee on nomenclature has recommended that this factor should be designated as "RANKL" (45).

RANKL is a member of the TNF ligand superfamily of cytokines and bind to the receptor activator of nuclear factor-κ (RANK) (45). The interaction between this receptor-ligand pair is essential for osteoclastogenesis (47). Mice with a serum transfer model of arthritis that were lacking RANKL were protected against bone destruction due to no stimulation of the osteoclasts (48). RANKL are predominantly expressed by activated T-lymphocytes and synovial fibroblast-like cells (45). Osteoprotegerin (OPG) is a naturally occurring decoy receptor for RANKL. It prevents the binding of RANKL to RANK and inhibit the biological activity of RANKL. The ratio of RANKL/OPG is determining the degree of osteoclast mediated bone resorption (45;49).

The expression of RANKL is stimulated by pro-inflammatory cytokines as TNF-α, IL-1, IL-6, IL-16, IL-17 and macrophage colony-stimulating factor (M-CSF) (49). It has also been suggested that TNF-α have the ability to bind directly to osteoclasts precursor through TNF-α receptor and stimulate the osteoclast formation (44;50). This gives TNF-α a dual effect on osteoclast formation.

The important role of the osteoclast in the development of erosions in RA has also recently been confirmed in human studies (51;52). The bisphosphonate zoledronic acid and the new antibody against RANKL, denosumab, are both potent suppressors of the osteoclast, and have been found to reduce the development of erosions in RA (51;52). Neither of the drugs did influence on disease activity.

Recently, a growing interest for the osteoblast has developed in inflammatory arthritis. An increased bone resorption should normally be coupled to an increased bone formation by the osteoblast, but this is not the case in RA. Studies suggest that the inflammation may suppress the bone formation activity of the osteoblast. The osteoblast is activated by the Wnt (wingless protein) pathway which also leads to an induction of OPG and thus reduces the activity of the osteoclast (53;54). TNF-α seems to induce Dickkopf 1 (DKK1) which inhibit Wnt. This further leads to a down-regulation of both the osteoblast and OPG, resulting in an inhibition of the bone formation. In this manner RA inflammation also seems to inhibit the osteoblast (53) which gives an additive negative effect of inflammation on bone.

1.1.4 Measurements of bone density

Bone strength is dependent of many factors such as BMD (55), bone architecture (56), bone quality and bone geometry (57). Several devices have been developed for quantitative assessments of BMD (58), including quantitative ultrasound (US) (59), quantitative computer tomography (qCT) (60), dual energy X-ray absorptiometry (DXA) (61) and radiogrammetry (62).

Ultrasound measures velocity (speed of sound, SOS) and frequency-dependent attenuation (broadband ultrasound attenuation, BUA). The major advantage for US is that it does not involve use of ionizing radiation. Further SOS and BUA are supposed to provide information about bone structure in addition to bone density (59;63). So far US has mainly been used for heel and finger assessment, but a method that can be used on femoral bone has recently been developed (64;65). However, ultrasound is lacking a validated reference system for use in the clinic and the precision is highly operator dependent (66). For hand phalanx US the interobserver reproducibility expressed as coefficient of variance (CV %) has been reported to be 0.9-2.8 % for SOS and 0.7-1.4 % for BUA (67).

Quantitative computer tomography (qCT) has the ability to determine the volumetric density three-dimensionally (g/cm^3) and it can distinguish between cortical and trabecular bone. Disadvantages for this method are the high radiation dose and the high cost. Furthermore, the precision is poorer than for DXA (58;60). The CV % for qCT in the ultradistal radius was found to be 1.18 % for total bone, 1.29 % for BMD trabecular bone and 1.67 % for cortical bone (68).

Dual energy X-ray absorptiometry (DXA) is considered as the gold standard for detection and management of osteoporosis (55;69). The main sites for measuring DXA is hip and spine, however every part of the skeleton can theoretically be measured by DXA. In the beginning of the 1990s a method for measuring DXA in hands was developed in a machine originally designed to measure DXA in hip and spine. The hand was positioned on a built-up plate consisting of sheets of perspex and aluminium (70). This method has been further developed, and software to measure DXA-BMD hand is now commercially available. In addition to measure whole hand, this software also have the possibility to measure regions of interest around the joints by a manual procedure (71). The bone density measured by DXA is both given as bone mineral content (BMC) and BMD. BMD is dependent on the area. In RA patients with severe deformities having difficulties to stretch their hand flat, it has been shown that the precision for BMD is dependent of the position of the hand while the precision of the

BMC is not (70). However, in most studies the precision is found to be better for DXA-BMD (coefficient of variation, CV % = 0.8-3.2 %) than for DXA-BMC (CV %=1.4-3.3 %), which has contributed to the selection of DXA-BMD as the most widely used method (24;72) (Table 3).

In this study the main focus was to investigate cortical BMD in hands with a modernised version of the radiogrammetry method called digital X-ray radiogrammetry (DXR). This DXR method is a development of the classical radiogrammetry proposed by Barnett and Nordin in 1960 (73). Radiogrammetry was originally developed to detect generalised osteoporosis. On plain radiographs geometrical measures were used to calculate cortical ratio defined as the ratio of cortical bone divided by total bone. The method was used e.g. in the metacarpals, the ulnae, the radius, the femur and the spine (73). The major limitation of the manual method was the poor precision, mainly due to the indistinct endosteal margin (74). Measurements of metacarpal cortical index (MCI) gave an intra-observer error (CV %) up to 8-10 % (75). With the introduction of DXA the use of radiogrammetry became limited. The digitised version of radiogrammetry, DXR, has improved the precision and the feasibility. The first version (Pronosco X-posure version 1.0) was measuring cortical thickness in the second to fourth metacarpals as well as distal ulna and radius on conventional radiographs (76), but the method has been further developed to only include the second to fourth metacarpals (version 2.0) (77). DXR was approved by the US Food and Drug Administration in 1999 as a clinical method for estimating BMD substantially equivalent to DXA (76). More technical details on both DXA and DXR are given in chapter 2.2.2.

1.1.5 Radiographs as outcome measure to detect bone damage in rheumatoid arthritis

Radiographs are the most frequent used imaging methods to assess joint damage in RA. Different scoring methods to obtain joint damage have been developed, e.g. the Steinbrocker score, the Larsen score and the Sharp score (78;79). The Steinbrocker method was developed in 1949 and gave a global assessment of the patient (80). The grade (range I-IV) was determined by the worst change in any joint and was therefore biased toward the most severely affected joint. This method has therefore been replaced by the works of Larsen and Sharp which give a continuous scale of more than 100 units (78).

The Larsen score was developed in 1974 and gives an overall score from 0-5 for both erosions and joint space narrowing (JSN) (81). This method has been modified several times. The most frequently used modification is from 1995 and includes 4 proximal interphalangeal

joints (PIP) and 4 metacarpophalangeal joint (MCP) from each hand, 4 regions from each wrist and 4 metatarsophalangeal joints (MTP) from each foot (82).

In 1971 Sharp developed his method, which originally included only the hands and wrists (83). In this method JSN and erosions were scored separately, 29 regions for each hand and wrist were scored for presence of erosions and 27 regions in each hand and wrist for JSN. Several modifications of this method have been proposed (84-86), also including the feet (87-89). In this thesis, two different modification of the Sharp method was applied; see also chapter 2.2.3 "Radiographic analyses" and Table 5. In Paper II the modification by van der Heijde (vdH Sharp score) was used which includes 16 regions for erosions and 15 regions of JSN in each hand and wrist as well as 6 joints for both erosions and JSN in each foot (87;89). In Paper III the applied modification of the Sharp method included 17 regions for erosions and 16 regions for JSN of each hand and wrist and 6 regions for erosions and 5 joints for JSN in each foot (88). No consensus exists on which modification of the Sharp score that should be applied (79).

1.1.6 Rheumatoid Arthritis and periarticular osteoporosis

As mentioned in chapter 1.1.2 periarticular osteoporosis is a typical early radiographic finding in RA and may occur on radiographs before the erosions are visible (18;19).

Studies support the fact that hand bone loss measured by DXA and DXR takes place in early RA (20;21;24), even in the undifferentiated stage of the RA disease process (20;21). Patients with RA have significantly lower DXA-BMD hand compared to healthy controls (71) and patients suffering from psoriatic arthritis (90). In longitudinal studies, RA patients has been found to lose more hand BMD both compared to patients with other rheumatic diseases (20;21;72) and healthy controls (91) (Table 1).

1.1.6.1 Periarticular versus generalised osteoporosis

The small joints in hands and feet are the most frequently involved joints in the inflammatory disease process in RA (17). Thus, bone density measures of the hand are most frequent used for assessment of periarticular osteoporosis in RA whereas bone density measures at e.g. spine and hip are measurement sites for generalised osteoporosis. Studies indicate that hand bone loss in early RA occur more rapidly than bone loss at hip and spine (21;92;93). Radiographic joint damage has been shown to be more strongly correlated with low hand DXR-BMD than DXA-BMD at hip and spine (77;94). These studies suggest that whereas the prolonged course of RA including immobility and the use of corticosteroids may be more

associated to generalized bone loss (26), the effect of inflammation may have a greater impact on hand bone loss.

1.1.6.2 The magnitude of hand bone loss

Age related changes in DXR-BMD and DXR-MCI in healthy adults have to our knowledge only been examined in cross-sectional studies (95-98).

Calculations from cross-sectional studies have revealed an annual rate of bone loss for both DXR-BMD ad DXR-MCI of 0.4 % to 0.9 % in healthy individuals (95-98). Hand bone loss observed in patients with RA or other inflammatory joint disorders is depicted in Table 1.

Assessments of hand DXA-BMD and DXA-BMC have mainly been performed in RA patients (Table 1). One year change in BMC among 37 healthy individuals (28 women, 14 postmenopausal) was found to be 0.9 % for men and -0.9 % for women (91). Patients with other rheumatic diseases than RA have been found to lose less DXA-BMD (21;72) and DXA-BMC (72) than patients with RA (Table 1). No reference population regarding age related loss of hand DXA-BMD or DXA-BMC exists.

The peak bone mass for DXA hip and spine has been reported to occur approximately around 35-40 years (99), while the peak bone mass for DXR-BMD has been reported to occur between age 40-49 years (96;97).

1.1.6.3 Predictors of hand bone loss and the association to inflammation

The understanding of hand bone loss as outcome measure in RA is limited both due to lack of data from longitudinal studies and the small number of patients included in previous studies (21;24;91).

Only few studies have examined associations between disease factors and hand bone loss in RA, but most of these studies have focused on early disease (21;24;91). The change in DXA-BMD hand has been found to be inversely correlated to baseline C-reactive protein (CRP) (21;91). However, in a 2-year longitudinal study a significant correlation (correlation coefficient, r=0.3) between CRP at baseline and change in DXA-BMC was seen only in patients with early RA defined as disease duration less than two years, but not in patients with longer disease duration (91). These findings were replicated in another longitudinal study (24), suggesting that whole hand DXA-BMD loss only takes place in the first two-three years of the RA disease process. This may limit the use of hand DXA-BMD as outcome measure in established disease (24;91). A study of patients with early arthritis followed for 12 months, found CRP and rheumatoid factor (RF) to be independent factors for DXA hand bone loss

Table 1. Longitudinal studies on hand bone mass measurement in patients with rheumatic diseases. Numbers in parenthesis.

	Diagnosis Study duration	Disease duration	Disease activity	DXR-BMD % change	DXR-MCI % change	DXA-BMC % change	DXA-BMD % change
Deodhar et al 1995 (91)	RA (81) 1 yr Healthy (37)	<2 yr (42)/ >2 yr (39)	CRP: 17 HAQ: 1.4			M (33): -3.3 -5.3/ -2.2 F (48): -1.5 -2.1/ 0.1 M (9): 0.9 F (28): -0.9	
Daragon et al 2001 (72)	RA (15)/ ORD (15) 1yr	<0.5 yr	Active arthritis			-2.2/ -0.3	-2.6/ -0.4
Deodhar et al 2003 (24)	RA (29) 5yr	<2 yr	CRP: 44 HAQ: 1.1			1 yr: -5.5 2 yr: -7.5 3 yr: -9.8 5 yr: -10.0	
Jensen et al 2004 * (20)	RA (51)/ ORD (21) 2yr	2 yr	CRP: 95 ESR: 14	-5.0/ -2.0			NS
Stewart et al 2004 (100)	RA (24) Erosive/ non-erosive 4yr	<1 yr	CRP: 16 ESR: 36 DAS: 3.3 HAQ: 1.1	1 yr: -7.1/ -0.2	1 yr -8.1/ -1.0		1 yr -5.4/ -1.0
Haugeberg et al 2005 (101)	RA (95) Prednisolone users/ non-users 2yr	<2 yr	CRP: 33 HAQ: 1.3	 -3.6/ -7.1			
Böttcher et al 2005 (102)	RA (258) 6yr	<1 yr	CRP>25 ESR>20	1 yr:-10.7 6 yr:-32.1	1 yr:-14.3 6 yr:-33.3		
Haugeberg et al 2006 (21)	RA (13)/ ORD (19)/ non-inflammatory (42) 1yr	<1 yr	CRP: 5 HAQ: 0.6				-4.3/ -0.5/ -0.9
Böttcher et al 2006 (23)	RA (313) 3yr	0.5-44 yr	NA	-22.3	-23.3		

*: median change, otherwise mean change

DXR= Digital X-ray radiogrammetry; BMD= Bone mineral density; MCI= metacarpal cortical index; DXA= Dual energy X-ray absorptiometry; BMC= Bone mineral content; yr= years; RA= rheumatoid arthritis; ORD= other rheumatic diseases; yr= years; CRP= C-reactive protein; ESR= Erythrocyte sedimentation rate; HAQ= Health assessment questionnaire, range 0-3; DAS= disease activity score; NA= Not available; NS= non-significant; M= Male; F= Female; N= Number

(21). In another study baseline number of swollen joint, HAQ-score and RF was also shown to be associated with change in DXA-BMC (24).

Cross-sectional studies have also demonstrated that hand BMD is lower in RA patients with high disease activity both for DXR (68) and DXA (92). Inflammation of the joints is, however, not restricted to the early phase of the RA disease, but may be present during the entire disease course (103). Thus, hand bone loss could theoretically be used as an outcome measure also in longstanding established RA.

1.1.6.4 Hand bone loss as response measure to treatment

The ability of inflammatory treatment to reduce hand bone loss in RA has been demonstrated in a 2-year double blind study comparing prednisolone 7.5 mg /day with placebo (101). The prednisolone group had less hand BMD-DXR loss at one and two years suggesting that the potent anti-inflammatory effect of prednisolone exceeded its negative effect on bone (101). This findings was confirmed in a very recent study published in 2008 where RA patients treated with high dose prednisolone or anti-TNF therapy had lower rate of hand bone loss than patients treated with conventional DMARDs (93).

Suppressing the inflammation by anti-TNF therapy has been demonstrated to significantly reduce the progression of radiographic joint damage in RA patients (88;104-108). It is suggested that anti-TNF therapy may prevent general bone loss (109-111). RA patients treated with anti-TNF therapy have been shown to have lower rate of bone loss at spine and hip than at hand (93;110), suggesting that the inflammation have a more pronounced effect on hands than on generalised bone.

1.1.6.5 Hand bone loss measured by DXA-BMD and DXR-BMD

Only a few studies have compared hand BMD measured by DXA-BMD and DXR-BMD in RA (20;112). In a cross sectional study DXR-BMD was significantly correlated to radiographic damage while the association to DXA-BMD was borderline (112). Further, disease duration was significantly correlated with DXR-BMD but not with DXA-BMD. A 2-year longitudinal study of patients with early RA and unclassified polyarthritis, found that DXR-BMD was associated to disease activity and decreased significantly through the study, while no changes in DXA-BMD were observed (20).

1.1.6.6 Hand bone loss and radiographic damage

As previously described, both periarticular osteoporosis and erosions are known as radiographic hallmarks of RA (17). Despite this fact, there is a lack of data on the relationship between hand bone loss and radiographic damage. Studies with conventional radiographs have supported the idea that bone loss precedes the development of erosions (18;19). The cross-sectional correlation between DXA-BMD and radiographic damage has previously been examined in three small studies. Two of these studies demonstrated a significant correlation (r) of 0.24 - 0.69, respectively (70;113), whereas no correlation between erosions and DXA was seen in the third study (90). Four longitudinal studies have been performed examining radiographic changes and DXA changes. Two studies revealed no significant correlation (24;72), while in a 2-year longitudinal study including 43 patients a significant correlation (r= -0.55) was found (114). A study published in 2007 reported that the number of RA patients with early disease loosing hand DXA-BMD defined by the smallest detectable change (SDC) at 24 weeks was significantly higher than the number of patients with an increase more than SDC in the vdH Sharp score at 48 weeks (115). They concluded that DXA-BMD was a more sensible method to detect bone damage in early RA patients than conventional hand radiographic scores.

For the DXR-method, several cross-sectional studies have found DXR-BMD to be lower in patients with high scores of radiographic joint damage than in patients with a low radiographic damage score(23;67;68;77;94;102;116;117). For assessment of radiographic joint damage different scoring methods has been applied including modifications of the Sharp method (23;67;102;116;117), modifications of the Larsen method (23;77;94;102;117), the Ratingen score (68) and the Steinbrocker score(67;77;102). In all these studies the correlation coefficient (r) between radiographic damage and DXR-BMD ranged from -0.42 to -0.66.

In one longitudinal pilot study including 24 patients the data indicated that DXR-BMD loss the first year of follow-up in early RA (<1 year disease duration at inclusion) could predict erosions at 4-year follow-up (100).

1.1.7 Personal background

During my work as a clinician I had become aware of how important it was to identify RA patients with high risk for severe bone destruction early in the disease course to improve their outcome. The interplay between the immune system and the bone fascinated me: Why did some patients with high inflammation not get any joint damage, while others apparently having less inflammation got destructions very fast? For a long time periarticular osteoporosis

has been known as an early feature on radiographs in RA patients (18) and has been recognised as a hallmark of RA (17). However, the benefit of using measurement of periarticular osteoporosis in RA as an outcome measure, and how to use these measures in clinical practice, had not been thoroughly examined. Diakonhjemmet hospital had access to a Pronosco X-posure 2.0 machine for measuring DXR from conventional hand X-rays. Some preliminary interesting work using this machine had already been done by my main supervisor Glenn Haugeberg (101;118). When I got the possibility to do my thesis on this project I was happy to assess the value of cortical hand bone loss in RA by using DXR as outcome measure of bone involvement and as a predictor of joint damage.

During the last few years, the knowledge in this field has increased substantially, but when this study started in 2005, only small longitudinal studies with few patients had been published assessing hand bone loss and its association with disease activity and the influence of anti-inflammatory treatment on hand bone. A few double blind randomised clinical trials had shown that anti-TNF therapy could halt erosions (88;104-106), and this fact was supported by other studies in 2006 (107;108). The effect of anti-TNF therapy on periarticular and generalised osteoporosis had not been examined. Further the role of the osteoclast in bone damage in RA was not fully understood (44;119) and the focus on the osteoblast (53;54) is so new that it is not even discussed in my papers.

1.2 General Aim and specific research questions

1.2.1 General aim

The general aim of this thesis was to examine the potential role of quantitative measurements of hand BMD as assessed by DXR as outcome measure and as predictor of radiographic progression in patients with RA.

1.2.2 Specific research questions

- Does hand bone loss have a value as a disease outcome measure in early and established RA (Paper I and III)?
- Which markers of the inflammatory disease process and disease severity are associated with hand bone loss in RA (Paper I and III)?
- Is cortical hand bone loss, assessed with DXR-BMD, an early marker of bone involvement in RA (Paper II)?
- Does cortical hand bone loss in early RA predict subsequent radiographic joint damage (Paper II)?
- What is the clinical value of DXR-BMD compared to other predictors of radiographic damage as e.g. presence of erosions, CRP, anti-CCP and RF IgM (Paper II)?
- Does potent suppression of inflammation with anti-TNF therapy prevent DXR hand bone loss in RA (Paper III)?
- Does hand bone loss in RA as assessed by DXR compared with scoring of radiographic joint damage support the hypothesis of a common cellular mechanism of bone erosions and bone loss (Paper II and III)?
- The precision for DXR-BMD assessed with Pronosco X-posure has been found to be superior to DXA-BMD hand. What is the precision of the new direct DXR method (dxr-online) (Paper IV)?

2. MATERIAL AND METHODS

2.1 Study design and study population

This thesis consists of two longitudinal observational studies; one double blind randomised study and one cross-sectional study. The DXR-method, which calculates BMD from radiographs, allowed us to use data from previously conducted studies with radiographs available.

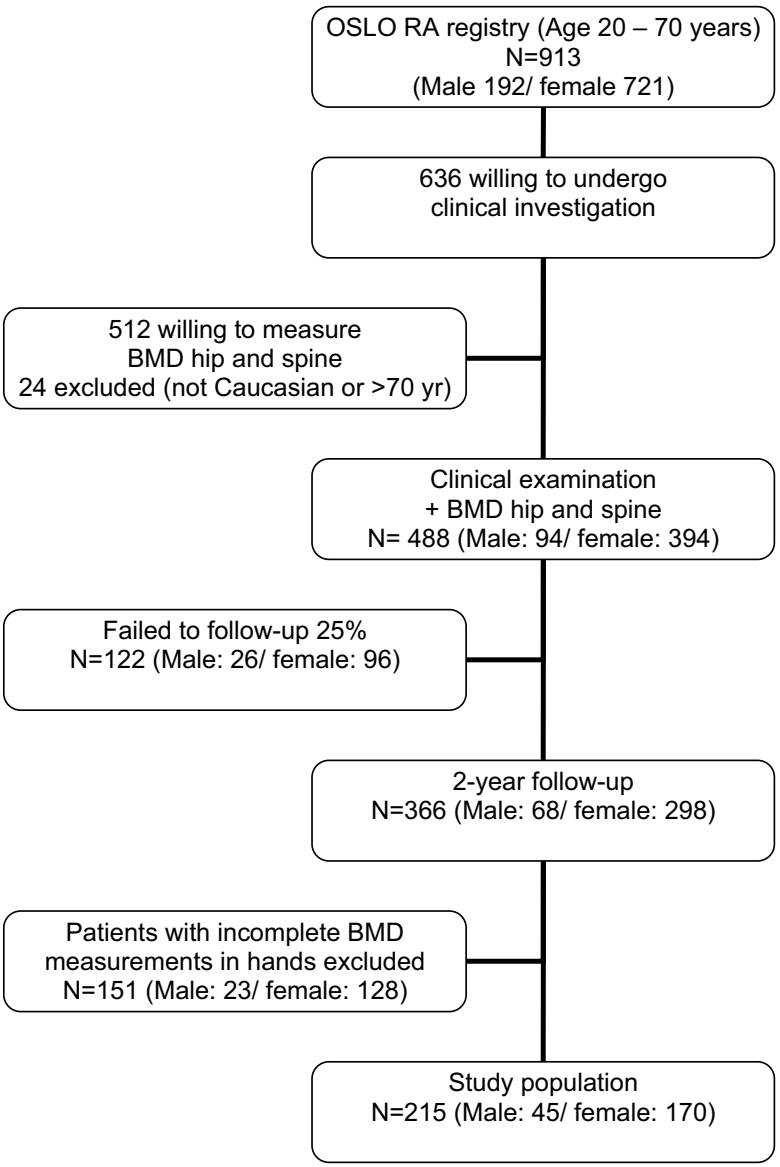
2.1.1 Oslo Rheumatoid Arthritis Register (Paper I)

The cohort in Paper I includes 215 patients (45 males and 170 females) from the Oslo RA-register. The Oslo RA-register was established during the period 1991-1994 by Kvien and colleagues (3). To be included in the RA registry the patients had to fulfil the RA criteria (17) and have a residential address in Oslo. Disease onset was recorded as the date when at least four out of seven classification criteria of RA was fulfilled (17). Patients with juvenile RA were not included in the cohort. The completeness of the register has been validated and was found to be 85 % for patients aged 20-79 years (3). The register is continuously updated with new cases and withdrawals due to death or new address outside Oslo.

From this register Haugeberg and colleagues described 2 year changes in hip and spine BMD in 366 patients (120) (Figure 2). The inclusion criteria were: Diagnosis of RA, age 20-70 years, and Caucasian. Clinical, laboratory and radiographic data were collected at baseline and after 2 years follow-up. During the observation period, patients were treated according to clinical judgement by their rheumatologist.

In the present study only patients with hand radiographs and measurement of DXA-BMD hand at baseline and 2 year follow-up from both hands were included. We used the mean of both hands to avoid bias regarding dominant and non-dominant hand. This approach has also been shown to improve precision for BMD assessment (115). We excluded 151 patients who missed at least one BMD measurement.

Figure 2. Flowchart of the OSLO rheumatoid arthritis registry.



2.1.2 The EURIDISS study sample (Paper II)

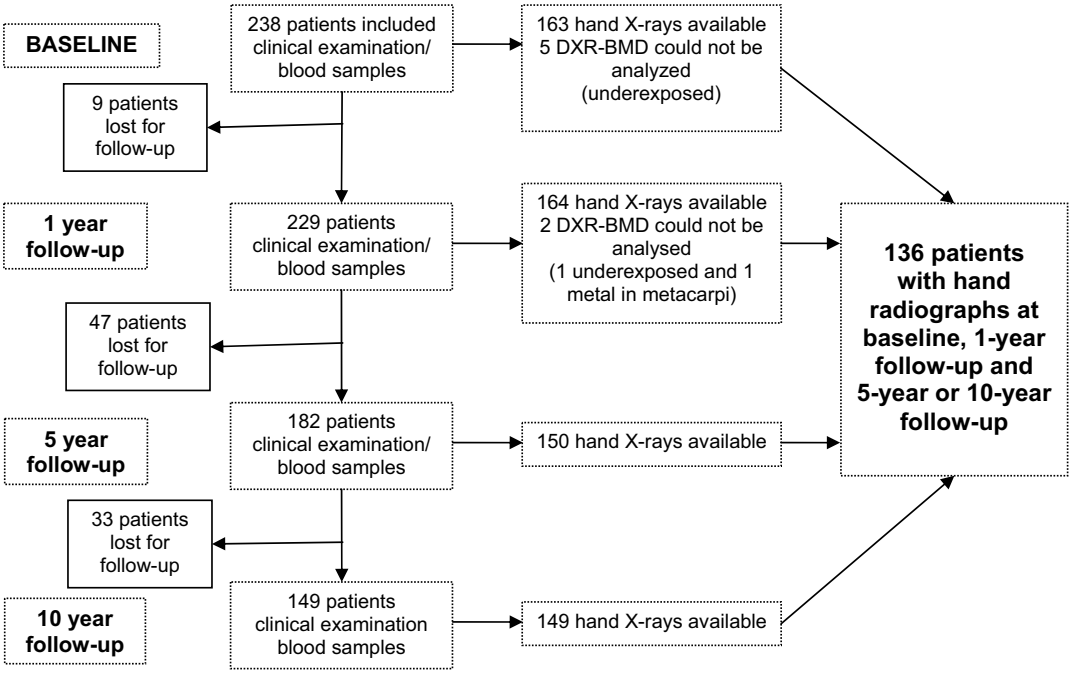
The RA patients in Paper II were recruited from the European Research on Incapacitating Disease and Social Support (EURIDISS) longitudinal observational study. The EURIDISS study was a European multi-centre study and the participating centres were Nancy in France, Groningen in the Netherlands and Oslo in Norway. The original intension was to study the effects of social network and social support on chronic diseases, and RA patients with disease duration four years or less were selected as a model for chronic disease. The Norwegian patients were mainly recruited from the Department of Rheumatology at Diakonhjemmet hospital in Oslo. Some patients were also recruited from the Department of Rheumatology at the Martina Hansen Hospital (121;122).

Inclusion criteria were diagnosis of RA (17), disease duration four years or less and age 20-70 years. At baseline in 1992 the Norwegian patient sample comprised 238 RA patients with mean disease duration of 2.3 years. All living patients who participated in the baseline visit (n=203) were asked to participate in the 10-year follow-up examination, resulting in 149 completers (Figure 3).

Clinical, laboratory and radiographic data were collected at baseline and at 1, 2, 5 and 10 years. Sera from the baseline visit were stored at -70 degrees Celsius for later analysis of micro-CRP, anti-CCP and IgM RF (8). During the observation period, patients were treated according to clinical judgement by their rheumatologist.

Patients with hand radiographs at baseline, 1 year follow-up and either 5 or 10 year follow-up were included in the present analyses. The number of patients with baseline radiographs available for scoring were 163, and 15 of these were excluded due to missing radiographs both at 5 and 10 year. Of the remaining 148 patients, baseline radiographs from five patients could not be analysed for DXR-BMD because the radiographs were underexposed and seven patients were excluded at the one year follow-up (five radiographs were missing, one underexposed and one patient had surgical material in the metacarpal bone). Finally, 136 patients were included in the analyses. The EURIDISS cohort has also been used to investigate other predictors of radiographic damage such as anti-CCP, IgM RF, erythrocyte sedimentation rate (ESR), (8), as well as the shared epitope and the PTPN22 gene (13).

Figure 3. Flowchart of the EURIDISS rheumatoid arthritis cohort.



2.1.3 The PREMIER study sample (Paper III)

The PREMIER study included 799 patients from 133 investigational sites. The main objective was to compare the efficacy and safety of adalimumab plus MTX versus adalimumab monotherapy and MTX monotherapy. The radiographic and clinical data from this 2-year, multi-centre, double-blind, randomised controlled study has previously been described in detail (108). Inclusion criteria were disease duration less than 3 years and aggressive RA (≥8 swollen joint; ≥10 tender joints; ESR of ≥28 or CRP ≥1.5 mg/dl; erosions or RF positive). Patients who previously had been treated with MTX, cyclophosphamide, cyclosporine, azathioprin or more than two other DMARDS were excluded. The combination group received adalimumab 40 mg subcutaneously every other week plus weekly oral MTX (rapidly increased to 20 mg/week), and the monotherapy groups received either adalimumab 40 mg sc every other week plus placebo or weekly oral MTX plus placebo. Clinical, laboratory and radiographic data were collected at baseline and at 26, 52, and 104 weeks of follow-up (108).

In this study our initial intension was to use DXR-BMD as primary outcome. However, many radiographs could not be analysed for BMD because of unknown image

resolution. The calculation of DXR-BMD is based volume per area and requires a known resolution. The DXR device does also calculate DXR-MCI defined as combined cortical thickness divided on total width. DXR-MCI is therefore a relative measure, less dependent of image resolution, and was used as the primary outcome measure. The correlation between DXR-BMD and DXR-MCI has been found to be substantial (r >0.90), both cross-sectionally (77) and longitudinally (123). Further definition and information about DXR is described in paragraph 2.2.2.1.

Results for DXR-BMD were also presented. All images with unknown resolution were analyzed by assuming 254 dpi (the scanning resolution for the radiographs before scoring). Several of the radiographs were, however, clearly of another resolution, most likely because these radiographs had been printed in non-true size before scanning. Based on analyses from studies with a controlled resolution (123), a deviation from baseline width greater than 2 % was likely to indicate an incorrect value. By using this 2 % value as a cut-off, 23 % of the radiographs were excluded from further DXR-BMD analyses. For DXR-MCI 768 radiographs were analysed at baseline and 537 at 2-year (compared to 799 and 539 in the original PREMIER). For DXR-BMD the respective numbers were 765 and 369 (Figure 4).

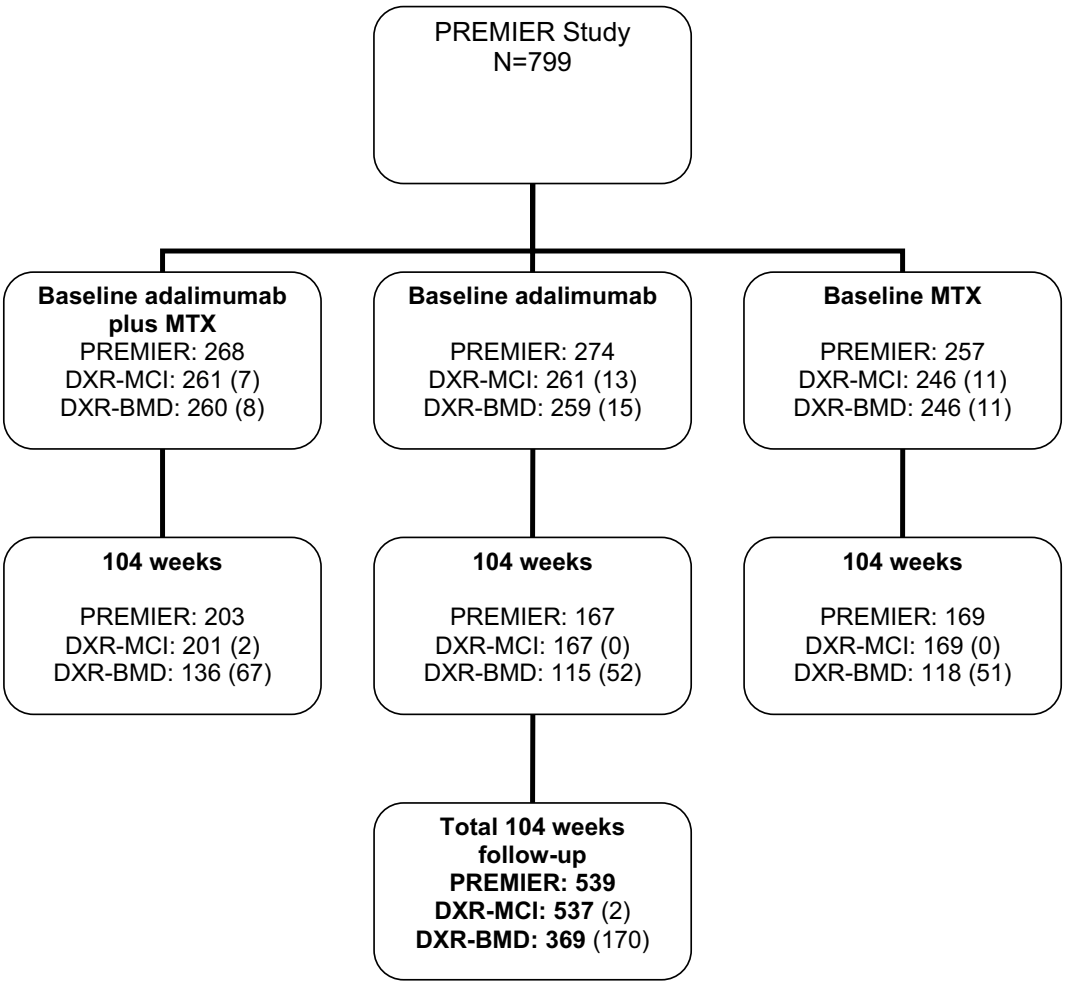
2.1.4 The precision study (Paper IV)

The precision study was duplex. The first part consisted of an in-vitro study where hand DXR-BMD was measured on the same cadaver forearm phantom 31 times with repositioning of the phantom between each radiograph, tested on four different standard X-ray equipments. All digitised radiographs were sent to Sectra and analysed with their new modified version of DXR the dxr-online software system (“dxr-online” is the brand name and written with lower case letters).

The second part consisted of an in-vivo precision study where the participants were recruited from consecutive individuals visiting an osteoporosis out-patient clinic. They were selected according to their total hip BMD values, 20 with osteoporosis and 20 with normal values. Unfortunately, one radiograph could not be analysed for DXR-BMD. Thus, 19 individuals with osteoporosis and 20 with normal DXA-BMD values were included. All participants underwent duplicate hand radiographs with reposition between each image. Because this study consisted of phantoms and healthy individuals, no demographic or clinical data were collected.

Figure 4. Flowchart of the PREMIER early rheumatoid arthritis cohort.

Numbers of missing X-rays compared with the original PREMIER study are provided in parentheses.



MTX= methotrexate; DXR= digital X-ray radiogrammetry; MCI= metacarpal cortical index; BMD= bone mass density.

2.2 Measures

2.2.1 Demographics and clinical measures

A broad spectre of variables was available from the different cohorts. Table 2 gives a summary of the different cohorts used in Paper I- III. Paper IV was a precision study of phantoms and healthy individuals and therefore no demographic or clinical data were included. I will emphasize that median values are stated in Table 1, Paper 1 and may therefore differ slightly from this Table.

Table 2. Baseline values of demographic and clinical measurements in the different papers. Mean (SD) for continuous variables, percentage for counts.

Domain	Variables	Paper I	Paper II	Paper III
Demographics	Age years	55.4 (11.1)	51.3 (12.1)	52.0 (13.6)
	Female, %	79	76	75
	BMI, kg/m^2	24.2 (3.7)		
	Smoke, %	31		
	Menopause, %	65		
Disease process	RA disease duration, years	11.0 (8.5)	2.2 (1.2)	0.75 (0.8)
	Rheumatoid factor, %	48	48	
	Anti-CCP, %		62	
	HAQ, 0-3		0.9 (0.6)	1.5 (0.6)
	MHAQ, 1-4	1.5 (0.4)		
	ESR, mm/hr	19.4 (14.7)	26.2 (20.9)	
	DAS28	4.1 (1.4)		6.3 (0.9)
	CRP, mg/l		9.4 (12.2)	40.0 (4.1)
	Swollen joint count			21.5
	Tender joint count			31.7
Bone involvement	DXA-BMD hand, g/cm^2	0.37 (0.07)		
	DXR-BMD, g/cm^2	0.51 (0.09)	0.55 (0.09)	0.57 (0.08)
	DXR-MCI	0.38 (0.09)		0.45 (0.09)
	Radiographic score		6.8 (12.2)	19.0 (20)
Medication	Current DMARDs, %		54	32
	Previous DMARDs, %	83		
	Current steroids, %	38	26	35
	Current anti-osteoporotic therapy, %	23		

RA= rheumatoid arthritis; Anti-CCP= anti-cyclic citrullinated peptide; CRP= C-reactive protein; HAQ= health assessment questionnaire; MHAQ= modified health assessment questionnaire; ESR= erythrocyte sedimentation rate; DAS28= disease activity score for 28 joints; DXA= dual X-ray absorptiometry; BMD= bone mineral density; DXR= digital X-ray radiogrammetry; MCI= metacarpal cortical index; DMARDs= disease modifying anti rheumatic drugs

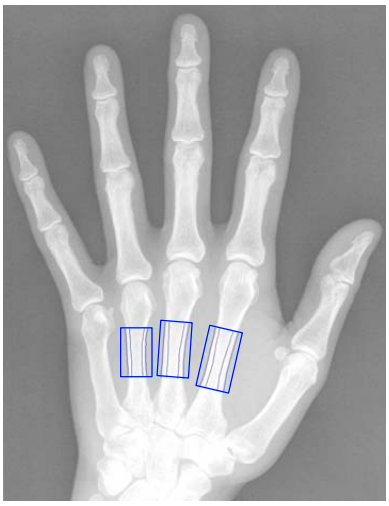
2.2.2 Bone Density measurements

2.2.2.1 Digital X-ray Radiogrammetry

DXR-BMD and DXR-MCI was in Paper I and II measured from standard, conventional radiographs by the Pronosco X-posure system TM, version 2.0 (Sectra, Linköping, Sweden) (62) and in Paper III and IV DXR was analysed by Sectra.

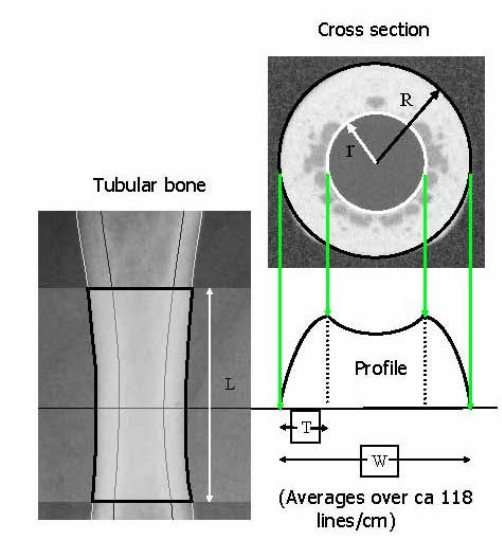
DXR is a computer version of the traditional technique of radiogrammetry (73). On standard hand radiographs the computer uses an active shape model (124) to recognize regions of interest around the narrowest part of the second, third and fourth metacarpal bone, (Figure 5). In each region cortical thickness, bone width and porosity is measured about 118 times per cm, (Figure 6).

Figure 5. Hand X-ray with regions of interest for DXR analyses. The boxes indicate the narrowest part of metacarpi 2-4 (photograph printed with permission from Sectra).



BMD is defined as: Bone mass divided by area.
Bone mass is defined as: Bone density multiplied by cortical volume corrected for porosity.
Density is defined by a constant (c).
In Pronosco X-posure system TM, version 2.0 c is determined such as DXR-BMD (measured in 562 women) on average is equal to the mid-distal forearm region of the Hologic QDR-2000 device. In the new direct DXR (dxr-online) c is adjusted by the calibration for the used radiographic equipment such that a set of five phantoms with a great range in bone density achieve the same BMD as in Pronosco X-posure.

Figure 6. Outline of principles for determination of the basic quantities for radiogrammetry (photograph printed with permission from Sectra).



R= radius of total bone

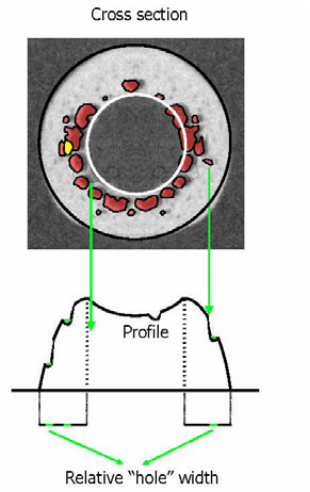
r= radius of trabecular bone

T= cortical thickness

W= width

Volume is cortical volume and given as volume per area (VPA). Assuming that the bone of interest is cylindrical the **VPA is defined as: π x (R^2-r^2)/ W,**
where R is the outer radius, r inner radius and W is the width, (Figure 6). VPA is calculated for the three metacarpi as a weighted average $VPA_{comb} = (VPA_2 + VPA_3 + 0.5\ VPA_4) / 2.5$, where VPA 2, 3 and 4 refer to VPA in metacarpus 2-4, respectively.
Porosity (p) is defined as the percent of cavities not occupied of mineral matter (Figure 7). Porosity for each of the bones is derived from the area percentage (ratio) of local intensity minima (holes) found in the cortical part of the bone relative to the entire cortical area.
The p is usually about 2 % (sometimes it can be a bit higher) and is corrected in the BMD estimate as 1-p. The final BMD estimate is defined as:
DXR-BMD= (density constant x volume x (1-porosity)) / area = c x VPA_{comb} x (1-p) (62;76).

Figure 7. Measurement of porosity
(photograph printed with permission from Sectra).



DXR-BMD requires a known resolution, because the equation for DXR-BMD is based on volume per area and a distance in a digitized radiograph cannot be measured when the resolution is unknown.

DXR-MCI is defined as the combined cortical thickness divided by the bone width and represents a relative bone measure which is less dependent of bone size and bone length than DXR-BMD (98;125). From Figure 6 the equation is:

DXR-MCI = (2 x (R-r)) / (2 x R).

The DXR method has improved the precision of MCI for diagnosing cortical bone loss (73;75;125).

Direct DXR (or "dxr-online") is a further development of the Pronosco X-posure system and has the advantage of analysing digitised radiographs. The direct DXR is calculated from the same formula and uses the same algorithm as the original DXR, except from the previously mentioned minor differences concerning the calculation of c. Digitised radiographs, from either computed radiography (CR) using a phosphorous plate, or digital radiography (DR), are sent online or as CD and is analysed at Sectra (Sectra Linköping, Sweden). This method was used in Paper IV.

2.2.2.2 Dual X-ray absorptiometry

In Paper I, standardised DXA-BMD measurements for left and right hand, total hip and spine (L2-4) were performed. The DXA is considered as the "gold standard", and the method is based on the known differences in absorption of high energy and low energy X-rays by bone and soft tissue. The relative attenuation of two different energy levels can be used to subtract the soft tissue component making it possible to calculate the mineral density of the bone.

The same DXA equipment Lunar Expert (Madison, Wisconsin) was used both at baseline and follow-up for all measurements. This software offers a mode for hand measurements and do not need a built-up plate consisting of sheets of perspex and aluminium as in the first measurements of DXA hand (70). We have chosen to use DXA of the whole hand. Even though measures of bone loss around the joints shown larger values of bone loss

the method for measuring whole hand is more feasible and the precision is considerably better than for regions around the finger joints (Table 3) (71;72).

All technical procedures were in accordance with the manufacturer's standardized procedures. (For hand: Mode: 1mA fast. Field: length: 23 cm height: 14.4 cm. Exposure factor: time (sec): 18.9, voltage (kVp): 134.0, Current (mA): 1.0).

2.2.2.3 Precision

Table 3 gives a review over selected published precision data for DXR and DXA. For DXR-BMD most previous studies have used non-dominant hand (20;62;76;98;102), while for DXA measures there are no consistence and mean of both hands (21;71;114;115;126), right hand (70;72;91;127) and non-dominant hand (20;90) have all been used. Most of the precision data for DXR were performed on the Pronosco X-posure version 1.0 system that also included ulna and radius as well as metacarpi 2-4. Prior to this study (Paper IV) no precision data for direct DXR had been published.

Table 3. Data from selected studies on precision (expressed as percentage coefficient of variation, CV %) for dual energy X-ray and digital X-ray radiogrammetry.

All studies performed with reposition between measurements
Pronosco 1.0: DXR calculated from 2-4 metacarpi, distal ulnae and distal radius
Pronosco 2.0: DXR calculated from 2-4 metacarpi
*^{n}: one hand, n: r= right, l= left, nd= non-dominant, **= mean of both hands, $^{r+l}$= right and left hands
DXR= digital X-ray radiogrammetry; BMD= bone mineral density; MCI= metacarpal cortical index; DXA= dual energy X-ray absorptiometry; BMC= bone mineral content; CV= coefficient of variation; PreM= pre menopausal; PostM= post menopausal; RA= rheumatoid arthritis; NA= not available

Study	Method	Localisation	DXR-BMD	DXR-MCI	DXA-BMD	DXA-BMC
Deodhar et al 1994 (70)	8 healthy 3 times	Whole hand*[r]				2.3
Alenfeld et al 2000 (71)	5 healthy 5 times	Whole hand** Joint			0.9 2.7-3.2	
Daragon et al 2001 (72)	15 healthy 2 times	Whole hand*[r] Joint			1.35 1.6-4.5	3.3
Harrison et al 2002 (90)	10 arthritis 4 times	Joint*[nd]			0.9-1.6	
Berglin et al 2003 (114)	16 healthy NA	Whole hand**			1.1	
Haugeberg et al 2007 (115)	81 healthy 2 times	Whole hand** Hip Spine			0.82 2.33 2.75	
Murphy et al 2008 (128)	7 patients 14 hands 3 times	Joint [r+l]			0.89-2.37	1.38-3.26
Jørgensen et al 2000 (76)	40 healthy 3 times 20 preM 20 postM	Pronosco 1.0*[nd]	 0.68 0.61			
Rosholm et al 2001 (62)	11 healthy 13 osteopor. 2 times	Pronosco 1.0*[nd]	0.59 0.59			
Hyldstrup et al 2001 (98)	24 healthy 2 times	Pronosco 1.0*[nd]		0.64		
Böttcher et al 2005 (102)	1radiograph 10 times	Pronosco 2.0	0.19	0.24		
Böttcher et al 2005 (129)	1 phantom 10 times Conventional/ printouts	Pronosco 2.0	0.33-0.49/ 0.33-1.50	0.50/ 0.78-1.28		
Hoff et al 2008 (126)	28 healthy 37 RA 2 times	Pronosco 2.0**	0.28 0.47	0.31 0.55		

2.2.3 Radiographic analyses

Technical conditions for the radiographs used in the different studies in this thesis (Paper I-IV) are depicted in Table 4.

Table 4. Technical conditions for radiographic analyses.

X-ray equipment	Conventional/ film Computed radiography (CR) Digital radiography (DR)	Film focus distance (FFD, cm)	Tube voltage (kV)	Exposure dose (mAs)	Paper
Siemens Multix	Conventional/ AGFA Crurix film	100	55	6	I, II
Different equipments*	Single emulsion/ mammography film	100	50-55	8	III
Agfa ADC Compact plus	CR	100	50	5	II, IV
Fuji FCR Profect	CR	100	40	8	IV
Fuji FCR XG1	CR	100	50	5	IV
Sectra MicroDose	DR	”built in”	35	10	IV

cm= centimetre; kV= kilo volt; mAs= milliAmpere second
*The PREMIER study was performed in 133 investigational sites on different radiographic equipments and tube voltage in accordance to this, but with a defined film, FFD, and exposure dose.

In Paper II the van der Heijde modification of the Sharp score was performed (87;89), while in Paper III another modification of the Sharp method for detecting radiographic damage was used (84;88;108). Table 5 summaries the differences between the two scoring methods.

Table 5. The differences between the two modifications of Sharp score.

	Erosions	Joint space narrowing	Max score
	vdH Sharp score		
Hands/ wrists			
Numbers of joints (score)	16 (0-5)	15 (0-4)	280
Feet			
Numbers of joints (score)	6 (0-10)	6 (0-4)	168
Total			448
	Mod Sharp score		
Hands/ wrists			
Numbers of joints (score)	17 (0-5)	16 (0-4)	298
Feet			
Numbers of joints (score)	6 (0-5)	5 (0-4)	100
Total			398

2.3 Statistical analyses

The statistical analyses were carried out using the Statistical Package for the Social Sciences for Windows (SPSS Inc. Chicago, Il, USA), version 13 for Paper I and version 14 for Paper II-IV. In addition Excel (Microsoft Office) was used for precision calculations in Paper IV. Two tailed p-values of 0.05 or less were considered statistically significant. Bone loss over time was expressed as negative values. Because of skewed data, non-parametric tests were used in Paper I-III.

2.3.1 Descriptive statistics

Descriptive statistics were applied to calculate mean values and standard deviation (SD) or median values and interquartile range (IQR, 25-75 percentiles) when appropriate, and as numbers and percentages for counts (Paper I-III).

2.3.2 Group analyses

Wilcoxon test for two related groups were used to evaluate changes within groups during the observation time (Paper I, II, III). For comparison between groups Mann-Whitney U test were used for two independent samples (Paper I, II, III) and Kruskall-Wallis tests for more than two independent samples (Paper I and III). In Paper III comparisons of changes in DXR were conducted using methodologies employed in the original PREMIER study (108). Instead of using three group comparison between the three treatment groups, two groups were compared in a hierarchical order with the Mann-Whitney U test, i.e. two-sided comparison of the combination group vs. MTX, followed by two-sided comparisons between the adalimumab monotherapy and MTX monotherapy treatment arms, and finally two-sided comparisons between the adalimumab monotherapy and the combination group. Each pair-wise comparison was completed only if the previous comparison was statistically significant. This approach was applied in the original PREMIER study because the main objective was to look for differences between the MTX group and the combination group. Chi square was used in Paper III to examine differences between categorical variables.

2.3.3 Correlation

Bivariate correlations using Spearman's correlation were used in Paper I-III, both to examine the correlation coefficient (r) between DXR and other variables and to select independent variables for the multivariate models.

2.3.4 Multivariate analyses

Multivariate linear regression analyses were used in Paper I-III. In Paper I DXA-BMD, DXR-BMD and DXR-MCI was used as dependent variables, in Paper III DXR-MCI, while change in vdH Sharp score was set as dependent variable in Paper II. Independent variables were either selected from the bivariate correlation analyses or by clinical judgement.

In Paper II multivariate logistic regressions were used because the dependent variable was dichotomised (increase in radiographic damage versus no increase in radiographic damage). From this logistic regression model a probability score was calculated to assess the risk of radiographic progression dependent on the combination of hand BMD loss, early radiographic damage and anti-CCP.

2.3.5 Precision

Differences between precision for the four digitised equipments were calculated according to Levene's test for variances in the in-vitro study. Bonferroni approach was used to adjust for multiple comparisons. Standard deviation (SD) and coefficient of variation (CV %) defined as (SD/ mean) x 100 was analysed. The measurement error was calculated using Bland Altman 95 % limits of agreement method (130). This gives an absolute and metric estimate of random measurement error, also called smallest detectable difference (SDD). Most disagreements between measurements are expected to be between these "limits of agreements" defined as $d \pm z_{(1-\alpha/2)} \times SD$, where d is the mean difference between the measurements and $z_{(1-\alpha/2)}$ is the $100(1-\alpha/2)^{th}$ centile of the normal distribution. The mean difference (d) is expected to be 0 because we do not assume a true change in BMD to occur between the measurements (130;131).

In the in-vivo study the same parameters were calculated, but the standard deviation difference (SD_{diff}) was used because only two measurements of several different patients were performed. The other calculations are based on SD_{diff} . We also calculated the least significant change (LSC %) defined as the smallest percentage change that can be considered to be statistically significant in an given individual (132;133).

Equations used in the precision study (Paper IV):

SD= $\sqrt{\sum_{i=1}^{nj} \frac{\left(x_{ij} - \bar{x}\right)^2}{n_j - 1}}$, or with 2 repeated measures in several patients

$SD_{diff} = \sqrt{\frac{\sum \left(a_i - b_i\right)^2}{2n}}$

CV %= (SD/ mean) x 100, or with 2 repeated measures in several patients:

CV % = (SD_{diff}/ mean) x 100 = $\left(\sqrt{\frac{\sum \left(a_i - b_i\right)^2}{2n}} / \left(\left(M_a + M_b\right)/2 \right) \right) X100$

SDD= $d \pm z_{(1-\alpha/2)}$ x SD

LSC %:= $z_{(1\div\alpha/2)} \times \sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2} \right)} \times CV\% = z_{(1\div\alpha/2)} \times \sqrt{2} \times CV\%$

n= number; d= difference; x_{ij}= measurement i of n=j; $\bar{x}$ = mean of the measurements
$z_{(1-\alpha/2)}$= 100(1-α/2)th centile of the normal distribution
a_1= measurement 1, a_2= measurement 2 in the same patients
M_a= mean of the first measurements, M_b= mean of the second measurements

2.4 Ethical aspects

Study I and II and the in-vivo part in study IV were approved by the regional committee for ethics and medical research. The Norwegian Data Inspectorate has approved the registry of RA patients in Oslo. Concerning the PREMIER study this was approved by a central institutional review board and independent ethics committee at each participating site (108).

3. SUMMARY OF RESULTS

3.1 Paper I:

Hand bone loss as outcome measure in established rheumatoid arthritis. A two-year observational study comparing cortical and total bone loss

The objective of this two year longitudinal observational study was to explore hand bone loss as disease outcome measure in established RA.

Hand bone loss was measured by both DXA and DXR. DXR was used to measure cortical hand BMD and MCI, whereas DXA was used to assess whole hand BMD. Only patients with performed DXA and DXR from both hands were included. 215 patients from the Oslo RA register (170 women and 45 men) with median disease duration of 9 years were included. This cohort was a part of a previous study (120), which examined 2-year bone loss at hip and spine in 366 RA patients.

This study applying two different quantitative bone measure methods had two main findings: First, total hand bone loss measured by DXA-BMD seemed to occur only in the first years of the RA disease (-0.96 % for patients with disease duration three years or less vs. 0.24 % in patients with disease duration over three years, $p<0.01$), whereas DXR-BMD measured cortical hand bone loss occurred both in early as well as late stages of the disease (-0.46 % vs. -0.93 %, $p=0.76$). Change in DXR-MCI was highly correlated to DXR-BMD ($r=0.94$, $p<0.001$). Second, disease activity expressed as DAS28 independently predicted loss of DXR-BMD but not changes in the DXA-BMD hand in the multivariate analysis. MHAQ and use of DMARD, prednisolone or anti-resorptive osteoporosis treatment did not influence the change of either DXA-BMD or DXR-BMD.

This was the first study to compare DXR-BMD and DXA-BMD loss in RA patients with both early and longstanding disease.

The conclusion was that DXA-BMD can only be used as an outcome measure in early RA, whereas DXR-BMD may be appropriate as a marker for disease activity during the whole disease course.

3.2 Paper II:

Cortical hand bone loss after 1-year in early rheumatoid arthritis predicts radiographic hand joint damage at 5-year and 10 year follow-up

The objective of this 10-year longitudinal study was to examine one year hand bone loss in early RA as a predictor of radiographic damage at 5 and 10 years follow-up.

A total number of 136 RA patients with early RA (disease duration 0-4 years, mean 2.2 years) were followed with clinical data and hand radiographs. Joint damage was scored according to the van der Heijde modification of the Sharp method (vdH Sharp score), and hand BMD was assessed by DXR-BMD.

Patients with DXR-BMD loss at one year (exceeding the measurement error of the method) had a significant higher median increase in vdH Sharp score compared to patients without loss at both 5 (12 vs. 2 units) and 10 years follow-up (22 vs. 4 units). Hand DXR-BMD loss was an independent predictive factor for radiographic damage when adjusted for other known predictors such as CRP, anti-CCP, IgM RF and baseline radiographic damage, both measured as absolute bone loss and dichotomised as hand bone loss versus not hand bone loss. An algorithm was made for the three risk factors: Anti-CCP, radiographic damage at baseline and DXR-BMD loss the first year of follow–up. If one risk factor was present the probability for radiographic damage at 10 years was 30-34 % and if two risk factors were present the respective value increased to 60-64 %. The presence of all three risk factors had a probability of subsequent radiographic progression of 87 % at 10 years.

The conclusion of this study was that early hand bone loss measured by DXR-BMD was an independent predictor of subsequent radiographic damage. The predictive power of DXR-BMD was comparable to other biomarkers that are well known predictors of radiographic joint damage as e.g. anti-CCP and CRP. Our findings support that quantitative hand bone loss in RA precedes radiographic joint damage and may be used as a tool for assessment of bone involvement, especially in early RA.

3.3 Paper III:

Adalimumab therapy reduces hand bone loss in early rheumatoid arthritis: Explorative analyses from the PREMIER study

The objective of this study was to examine the effect of anti-TNF therapy on hand osteoporosis and to identify predictors of hand bone loss. The basis for these analyses was the PREMIER study which was a 2-year year longitudinal double blind randomised clinical trial. The effect of adalimumab on hand bone loss was examined and related to radiographic joint damage in the three treatment arms of the PREMIER study: Adalimumab plus MTX, adalimumab and MTX monotherapy. A total of 768 patients were included at baseline and 537 completed 2 years. The included patients had high disease activity (inclusion criteria: ≥8 swollen joints; ESR ≥28 mm/h or CRP ≥1.5 mg/dl; presence of erosions or RF), disease duration less than three years and they had never received MTX. Hand bone loss was assessed by DXR on the same hand radiographs which had been scored with modified Sharp score at baseline, 26, 52 and 104 weeks. DXR-MCI was chosen as the main bone measure, due to the technical challenges with the analyses of DXR-BMD (described in detail in section 2.1.3 The PREMIER study sample).

The main finding in this study was that percentage hand bone loss both at 26, 52 and 104 weeks follow-up was lowest in the combination group (-1.15; -2.16; -3.03 at 26, 52 and 104 weeks) and greatest in the MTX group (-1.42; -2.87; -4.62) with figures in between for the adalimumab group (-1.33: -2.45; -4.03). The order of hand bone loss across the three treatment arms was similar to the order of radiographic progression. In multivariate analyses older age, elevated CRP, and non-use of adalimumab were independent predictors of hand bone loss.

We concluded that the results supported a similar pathogenic mechanism for hand bone loss and erosions in RA. The combination of adalimumab and MTX seemed to arrest hand bone loss less effectively than radiographic joint damage. Thus quantitative measures of osteoporosis may be a more sensitive tool for assessment of inflammatory bone involvement in RA.

3.4 Paper IV:

Short-time in-vitro and in-vivo precision of direct digital X-ray radiogrammetry

The aim of this study was to examine in-vitro and in-vivo precision for the new direct digital version of DXR, a development of the conventional DXR.

This study consisted of both an in-vitro and an in-vivo part. The in-vitro precision was tested on four different X-ray equipments, based on 31 radiographs of the same phantom. The in-vivo precision was based on duplicate hand radiographs from both hands in 39 individuals.

The in-vitro precision ranged from 0.14-0.30 % expressed as coefficient of variations (CV %) and from 0.0012-0.0028 g/cm^2 expressed as smallest detectable difference (SDD). The precision and the resolution of the radiographic equipment was strongly correlated ($r=0.95$, $p=0.05$). The corresponding values for the in-vivo precision for mean values of both hands were 0.46 % for CV %; 0.0046 g/cm^2 for SDD and 1.28 % for LSC %. The precision was better when the mean of both hands was used than the non-dominant hand alone.

The conclusion of this study was that the precision for direct DXR was highly satisfactory both in-vitro and in-vivo. Another important observation was that DXR-BMD values may differ between the X-ray equipments. Follow-up measurements in single individuals are therefore recommended to be performed with the same X-ray equipment to achieve the best precision.

4. DISCUSSION

The methodological strengths and limitations of this thesis are discussed in the first part of the discussion. The second part addresses the interpretations of the main results.

4.1 Methodological issues

4.1.1 Study design

This thesis consists of two longitudinal observational studies (Paper I and II), one double blind randomised study (Paper III) and one cross-sectional study (Paper IV).

Paper I-III had a retrospective longitudinal study design. The research questions were addressed after the data collection had been performed, and as a consequence, other variables than those chosen for the original studies were not available. The limitation of a retrospective study design also affected the hand bone density measurements, the primary outcome in this thesis, since the technical condition for the radiographs used for the DXR-BMD measurements had not been predefined - as will be further discussed below.

However, the hypotheses for the different papers were set as for a prospective study, and the calculations for treatment effects in Paper III were performed blinded. The use of the DXR method allowed us to study hand bone density on hand radiographs despite that hand bone density was not part of the initial study design or data collection in Paper II and III. This advantage of the DXR method gave us the opportunity to use data from previously conducted studies and to obtain quick answers on interesting research questions without building up a new longitudinal cohort.

In paper IV both the in-vitro and in-vivo short-time precision for direct DXR (dxr-online) were analysed. The dxr-online method is a further development of the Pronosco X-posure system. Because the clinical use of DXR-BMD is based on changes over time, we have also initiated a long-term precision study.

4.1.2 Bias

4.1.2.1 Selection bias

Selection biases are distortions that result from procedures used to select subjects and from factors that influence study participation (134). Such biases may have had an impact on all four Papers in this thesis. In Paper I the 215 participants were recruited from a cohort of 366 RA patients (120), Figure 2. Although the 366 patients from the original cohort was found to

be representative for the underlying RA population in Oslo (120), the 215 included patients in the present study had shorter disease duration, lower disease activity measured by DAS28, lower global assessment and used less prednisolone compared with those who were not included. In addition a conservative inclusion was performed in the study. Only patients with hand radiographs and measurement of DXA-BMD hand at baseline and 2-year follow-up for both hands were included. Another approach could have been to include patients with radiographs from one hand missing and used the available hand at all time points (this approach was used in Paper III). The approach in Paper 1 could have led to an exclusion of the most severely diseased patients with metal implants in hands or severely deformed hands – which would not be eligible for analyses by DXR. Further, patients older than 70 years were excluded. All this examples of selection bias may have influenced the relative small changes observed in Paper I.

In Paper II the major limitation was that only 163 of the 238 included patients had radiographs at baseline. Further, patients older than 70 years, and patients classified as stage IV according to Steinbrocker's functional class (80) were excluded from recruitment to the study. Due to the long observational time, 89 patients were lost from baseline to follow-up due to death (N=35), illness, reluctance to participate and moving out of the area. Further, seven radiographs could not be analysed for DXR-BMD (six were underexposed and one had metal in the metacarpal bone).

Loss of patients at follow-up was also a problem in Paper III. Compared to the original PREMIER study we missed 31 patients at baseline and two at 2-year follow-up due to lack of X-rays. In the original PREMIER study there were 260 withdrawals (77 due to an adverse effect; 111 due to lack of effect; and 72 patients dropped out without known reason).

In Paper IV precision was calculated only in healthy individuals and not in RA patients, which may be a limitation as it is suggested that the precision of DXR may be poorer in RA patients due to deformities and inflammation of the hand (126;135). However, for the DXR Pronosco X-posure system we have previously presented at the EULAR 2008 conference precision data calculated from RA patients (126) which did not differ substantially from previous precision reports on this method.

4.1.2.2 Diagnostic bias

To be included in Paper I, II and III the patients should fulfil the ACR criteria of RA (17). This may have led to an exclusion of patients with undifferentiated arthritis not yet fulfilling the ACR criteria for RA (136). In addition, inclusions based on classification criteria do not

have 100 % specificity. As a consequence, patients not having RA and patients with self-limited RA may have been included. However, patients in the cohorts from the Oslo RA register and the EURIDISS study were examined by a rheumatologist before inclusion.

The concept of early RA has changed since the inclusion of the patients in Paper II and III. When the EURIDISS study started in 1992 disease duration of four years or less was considered as early RA and according to the PREMIER study (with the protocol written in 2000) a disease duration less than three years was defined as early RA. Focus is now on treatment of patients with very early RA (137) and probable RA (138) as well as identifying patients with risk of developing RA (139). However, in the PREMIER study the mean disease duration for the participants were only 9.1 months, which in our opinion justify using the term "early RA".

4.1.2.3 Confounding bias

Confounding may be considered as a confusion of effects (134). For example high inflammation at baseline leads to increased radiographic damage in joints, but it also leads to increased bone loss. It is therefore important to adjust for high inflammation if we examine the effect of early bone loss on radiographic damage. In Paper II we made a multivariate model for radiographic damage, and in Paper I and III we made multivariate models for factors affecting bone loss. We have adjusted for confounding factors by using correlation analyses and clinical judgement. None of our models are fully explainable which means that there are unmeasured and unknown factors affecting bone loss that we have not revealed (residual confounding).

4.1.3 Representativeness

A central question in this thesis is if the results can be generalised and are valid for the RA population. The three different cohorts differed with regards to disease activity and disease duration (Table 6). The cohort in Paper I was from the Oslo RA register. A population study from 1994 supported that the Oslo RA register had an 85 % completeness (3;140) and that the register was representative of the entire RA population in Oslo. As discussed in 4.1.2.1 the patients in the present cohort had shorter disease duration and lower disease activity than the original cohort, but are most likely representative for the general RA population.

The patients included in the EURIDISS cohort were recruited from ordinary out-patients' clinics at the Diakonhjemmet Hospital and Martina Hansen Hospital with no strict inclusion criteria except that disease duration should be 4 years or less and the patients had to

fulfil the ACR classification criteria for RA (17). The patients were consecutively included and it is likely that also this cohort represent a broad spectrum of rather unselected RA patients. In this study 62 % were positive for anti-CCP, while 48 % were positive for RF. The proportion of RF positive patients correspond to the Oslo RA register, which also supports that EURIDISS cohort represent a broad spectre of the RA population (141).

The patient cohort in the PREMIER study (Paper III) included selected MTX naïve adult RA patients (>18 years) with very high baseline disease activity and short disease duration (<3 years). To meet these inclusion criteria, 133 investigational sites in Europe, North America and Australia were involved to include a sufficient number of patients. This cohort reflects the most severe patients with early RA. A similar cohort could probably not be established now in Western Europe because of the improved access to modern, effective therapies.

Due to the different patient cohorts in this thesis as described above, the patients may not reflect the entire RA population and thus the results from this thesis may not be universal for all RA patients. However, high disease activity was a predictor for DXR-BMD and DXR-MCI loss in both the cohort from the Oslo RA register and the PREMIER cohort. This finding suggests that the inflammatory disease process in RA is an important factor for hand bone loss in RA patients across different levels of disease activity and disease durations.

4.1.4 Bone measurements and imaging

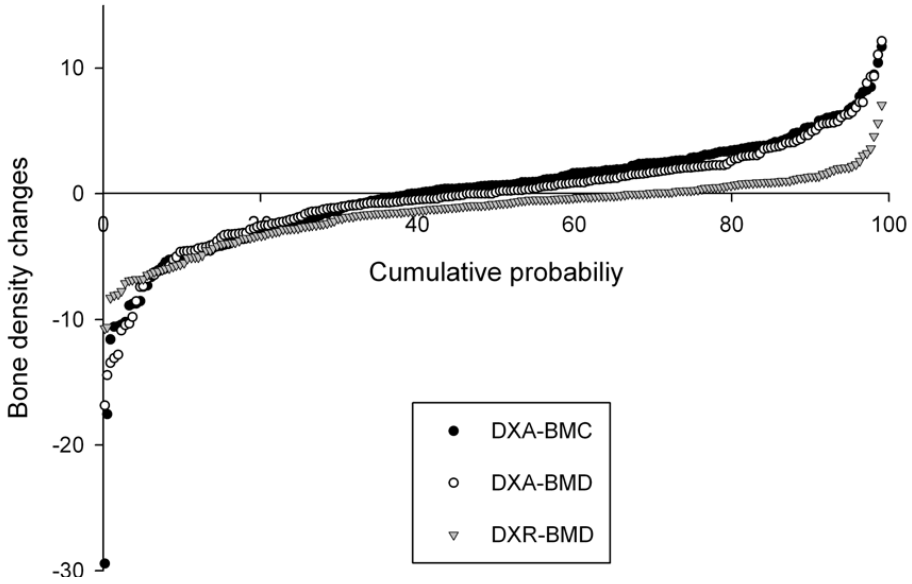
The main method for bone measurements in this thesis was the DXR method, and DXR was compared with DXA only in Paper I. The examinations of patients in the Oslo RA register in 1996-97 and 1998-99 had a focus on bone measures and included DXA measurements at various sites (120;142). DXA measures total bone and is based on X-ray absorptiometry, whereas DXR is based on geometric measures and linked to DXA through a density constant (see also part 2.2.2.1 and 2.2.2.2). We did not have the possibility to examine DXA-BMD hand in Paper II and III, since DXA was not available at Diakonhjemmet Hospital when the EURIDISS cohort was established and was not used in the PREMIER study. However, access to DXA measures would have been particularly interesting in these cohorts with shorter disease duration than the patients in Paper I, since DXA hand bone loss has been shown to be most pronounced in the first 2-3 years of the inflammatory disease process in RA (24).

4.1.4.1 DXA

DXA is considered as the gold standard among the bone density measurements, however the method do have limitations. DXA can not separate cortical and trabecular bone. The measure is planar (even if it is expected to give information about a volumetric measure), it is sensitive to calcium only, and the method does not give any information about the bone architecture or the collagen quality (143). The DXA bone density result is also influenced by soft tissue and marrow fat. Use of corticosteroids has been shown to increase fracture risk very rapidly after initialisation, before changes are measurable with DXA (144). This observation suggests that DXA does not capture the whole spectrum of bone strength. However, DXA is still considered as the best BMD measurement due to low radiation dose, feasibility, relative good precision and a good prediction of fractures (145).

As mentioned in chapter 2.2.2.2, DXA-BMD can be measured either around selected finger joints or by using whole hand. We chose to measure whole hand because this is more feasible in addition to reflect the total inflammation at the hands (146). Another objection may be that bone mineral content (BMC) should have been presented. BMC is not dependent of the area. Among RA patients with hand deformities the precision of BMC has not been found to be influenced by the position of the hand, in opposite to BMD (70;143). In figure 8 the cumulative probability plot of DXA-BMD, DXA-BMC and DXR-BMD percentage change from the patients in the Oslo RA register is presented (Paper 1).There is nearly a complete overlap between DXA-BMC and DXA-BMD which suggests that DXA-BMC does not give any major additional information on hand bone than DXA-BMD.

Figure 8. Cumulative probability plot of bone density percentage changes in Paper I.



4.1.4.2 DXR

The major limitation for DXR is that only cortical bone is measured. Further, the method can not recognise the metacarpals in patients with severe deformations or in patients with metallic implants, which may represent a bias by excluding the most disabled patients.

One main advantage of the DXR-method is that BMD and MCI is measured on hand radiographs which make it possible to do research on existing cross sectional and longitudinal cohorts. The DXR method is also very simple to use and have a good precision. In addition the Pronosco X-posure system used in Paper I and II is robust. Less than 1 % of the images analysed in Paper II were missed due to underexposed radiographs, metal in the metacarpal bone or deformities.

The calculation of DXR–BMD is based on volume per area and requires a known resolution, since a distance in a digitized radiograph cannot be measured when the resolution is unknown. This turned out to be a major challenge in Paper III, as mentioned in section 2.1.3. Ideally, DXR-BMD was intended to be the main outcome measure, but because of unknown resolution of many radiographs, DXR-MCI was used as the major hand bone measurement outcome. DXR-MCI is based on the ratio and not on absolute measures. By using DXR-MCI instead of DXR-BMD we lost the opportunity to correct for porosity.

Further DXR-BMD can be calibrated for blurring and particular qualities of the different radiographic measurement equipment. An old study from 1969 has even indicated that the correlation between ash mineral content and cortical area was better than between ash mineral content and cortical ratio indicating that DXR-BMD reflects bone mass better than DXR-MCI (147). However, DXR has improved the precision of MCI and a highly significant and strong correlation between DXR-MCI and DXR-BMD in cross-sectional studies has been found ($r=0.90$, $p<0.01$) (77). In Paper I we found a highly significant and strong correlation for 2-year change between DXR-MCI and DXR-BMD ($r=0.94$, $p<0.001$) (123). Both DXR-BMD and DXR-MCI were greatly correlated to DXA-BMD hand (123). On the basis of these observations we consider DXR-MCI to be a valid surrogate measure of hand bone mass change.

DXR measures the narrowest part of the metacarpal bone and not exactly the periarticular bone. This may theoretically be a limitation of the DXR-BMD method's ability to assess bone changes directly related to synovitis in the joints (62). However, the metacarpals will be influenced by the inflammation both in the MCP-joints and the wrist. Thus, the cortical bone loss may be considered to reflect overall joint inflammation in the hand.

4.1.4.3 Radiographs

Radiographs have traditionally been used to detect joint damage in RA and are considered as the gold standard for imaging in RA (148). However, recent studies have demonstrated that both US and magnetic resonance imaging (MRI) are more sensitive modalities in detecting erosions (148;149). Based on these facts, it would have been of interest to compare DXR with US and MRI. When the EURIDISS study started in 1992 conventional X-rays was the available method for assessing damage and this method was also applied for the 10-year follow-up. For the PREMIER study, radiographs were also used for assessment of joint damage. The use of US and MRI in clinical trials has been limited, mainly due to lack of validated scoring methods and due to feasibility.

The radiographic scorings methods were different in Paper II and III, which is not optimal, but the radiographs in Paper III (PREMIER) were already scored when we got the opportunity to analyse hand bone with DXR (Table 5). In Paper II only radiographic scoring results of hands were used because only hand radiographs were available. In Paper III combined results from hands and feet were used, which may have influenced the correlation (r) between change in DXR and radiographic damage. In Paper II the correlation (r) between

change in DXR and radiographic damage was -0.35 at 1 year and -0.47 at 2 year whereas in Paper III the corresponding numbers were -0.23 at 1 year and -0.32 at 2 year. The fact that DXR-BMD was used in Paper II while DXR-MCI was used in Paper III, in addition to higher radiographic damage score at baseline and shorter disease duration among the patients in Paper III, may also have influenced the correlation.

4.1.5 Clinical measures

The collected disease variables in Paper I-III differed and were collected at different time points. Thus, we have limited opportunities to compare the cohorts in this thesis. One example of inconsistencies in the data collection is that swollen joint count was not recorded at the baseline and 1-year follow-up visit in the EURIDISS cohort (Paper II) which excluded calculation of DAS28. CRP and ESR were therefore used as markers of inflammation and disease activity. Further, two different forms of HAQ questionnaires were used. In Paper II and III the HAQ ranging from 0-3 was performed (108;150), while in Paper I the modified HAQ (MHAQ, range 1-4) was used (26). Both the HAQ and MHAQ measure the extent of disability within 8 components of daily living, but in the MHAQ the number of item is reduced from 20 to 8 (151).

4.2 Results: Interpretations and comparison with other studies

4.2.1 The magnitude of bone loss: Comparison of the different cohorts (Paper I, Paper II, Paper III)

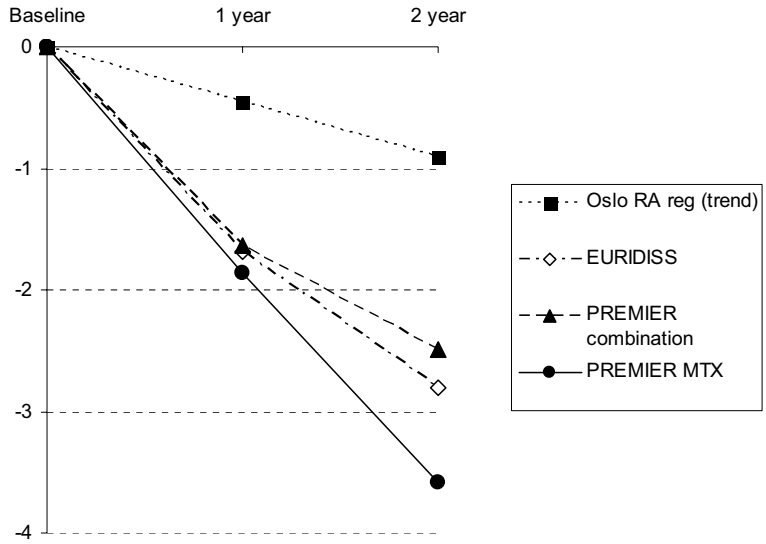
Bone loss over two year is depicted in Figure 9 in the different cohorts, both as DXR-BMD (Figure 9a) and DXR-MCI (Figure 9b). Both DXR-BMD loss and DXR-MCI loss were greatest in the group receiving MTX in the PREMIER cohort.

In Paper I the changes in DXR-BMD and DXR-MCI were small, both compared to the other cohorts in this thesis and previous studies (Table 1) (20;102). The main reason for this observation is most likely due to differences between patients included in the various studies with regards to disease activity and disease duration. The cohort in Paper I from the Oslo RA register was representative of the underlying patient population, and patients with mild RA disease dominated the number of included patients in this study. In addition, some of the most diseased patients may have been excluded, as previously discussed in section 4.1.2.1. Further, the disease duration was prolonged (median 9 years, mean 12 years), and both DXA-BMD

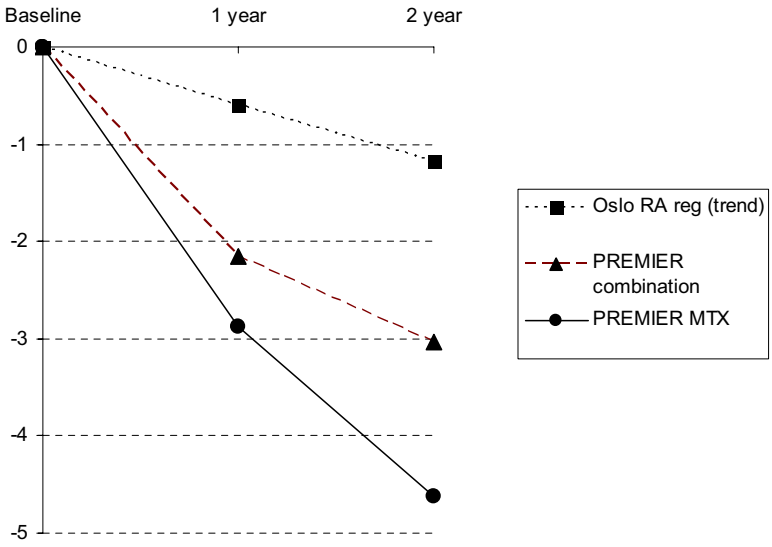
(24) and DXR-BMD (102) changes have been found to be greatest early in the disease. Böttcher et al reported annual DXR-BMD loss in the first six years of the disease to be as high as 11 %, with a subsequent decline to 3-4 % over the next years (102).

Figure 9. 2-year changes in DXR in the different rheumatoid arthritis cohorts.

a) Changes in DXR-BMD.



b) Changes in DXR-MCI.



To evaluate so small changes as in Paper I, the influence of normal bone loss, which takes place also in healthy adult subjects, is important. Normal bone loss for DXR-BMD has only been examined in cross-sectional studies showing an annual rate of bone loss between 0.4 and 0.9 % (95-98). In a 4-year longitudinal study comparing cross-sectional versus longitudinal evaluation of bone loss (measured by DXA-BMD), cross-sectional studies were overestimating the longitudinal loss in hip and spine, but not in the distal ulna and radius (152). The hand was not included in this analysis. However, age was not a significant predictor for hand bone loss over 2 year, neither for DXR-BMD nor DXA-BMD, in the multivariate model in Paper I (data not shown). The age of the cohort ranged from 22-70 year and it is difficult to estimate a mean normal bone loss for a so heterogeneous cohort with regard to age. A peak bone mass for DXR-BMD and DXR-MCI is found to be reached in age group 40-49 years (96;97). Thus the youngest age group should normally still have gained bone (95-97). Further a 10-year longitudinal study has suggested that the menopause, and not age per se, determines the start of a period with increased rate of cortical bone loss (153). We had information of menopausal status in Paper I, but it did not influence the multivariate model (data not shown). In Paper II and III we did unfortunately not have information regarding menopausal status.

The DXR loss in Paper II (the EURIDISS cohort) and Paper III (the PREMIER cohort) was more comparable to the loss in other studies (20;93;100;101). The patients in these two studies did also have high disease activity and shorter disease duration.

4.2.2 The precision of DXA and DXR (Paper I, Paper II, Paper III Paper IV)

With the relative small DXR-BMD changes, especially in Paper I, in addition to the technical challenges with the measurements as reported in Paper III, a precision study for DXR was considered to be of importance. A premise to use DXR on the individual level is that the technique has a sufficient precision to capture small changes and that these changes can be considered as true (i.e. the change in the individual has to be greater than the measurement error for the method).

In Table 3 precision from previous studies are depicted. The precision (CV %) of DXA-BMD has been found to be about 0.8-1.4 % for whole hand (71;72;114;115) and 0.9-4.5 around joints (71;72;90;128). For DXA-BMC the respective values are 2.3-3.3 % (70;72) and 1.4-3.3 (127).

Most of the precision data for DXR has been performed on Pronosco X-posure version 1.0 that included ulnae and radius as well as metacarpi 2-4. We calculated the precision data

on Pronosco X-posure version 2.0 in 28 healthy individuals and 37 RA patients (each individual performed two radiographs with reposition) (126) and reported results both for DXR-BMD and DXR-MCI. The precision for Pronosco X-posure version 2.0 was satisfactory and better than the precision both for DXA-BMD whole hand and DXA-BMD around single joints (Table 3). The precision increased when using mean of both hands instead of non-dominant hand and it seemed that the precision was poorer in patients with RA than healthy individuals. These data have not been published in a peer-reviewed journal due to the fact that the Pronosco X-posure system is no longer available, but has been replaced by the dxr-online system.

Table 6. The precision of Pronosco X-posure version 2.0 (own data (126)).

	Healthy individuals (N=28)			
	DXR-BMD (g/cm^2)		DXR-MCI (ratio)	
	Non-dominant hand	Mean both hands	Non-dominant hand	Mean both hands
Mean (SD)	0.601 (0.065)	0.612 (0.067)	0.479 (0.057)	0.481 (0.057)
SD difference	0.0025	0.0017	0.0024	0.0015
SDD	±0.0048	±0.0033	±0.0047	±0.0030
CV %	0.41	0.28	0.50	0.31
LSC %	1.13	0.78	1.39	0.87
	Rheumatoid Arthritis patients (N=37)			
Mean (SD)	0.512 (0.109)	0.515 (0.107)	0.380 (0.084)	0.379 (0.080)
SD difference	0.0030	0.0024	0.0025	0.0021
SDD	±0.0059	±0.0047	±0.0050	±0.0041
CV %	0.59	0.47	0.67	0.55
LSC %	1.64	1.30	1.86	1.53

N= number; DXR= digital X-ray radiogrammetry; BMD= bone mineral density; MCI= metacarpal cortical index; DXA dual energy X-ray absorptiometry; BMC= bone mineral content; SD= standard deviation; CV= coefficient of variation; SDD= smallest detectable difference; LSC %= least significant change.

For dxr-online, no precision data had been published prior to this thesis, except data available from the manufacturer. In the frame of this thesis we therefore conducted a short-time precision study. The results from this study (Paper IV) showed that the precision for direct DXR was at least as good as for the Pronosco X-posure system with a CV % ranging from 0.14 % to 0.30 %. An important message from this study was that the same radiographic equipment for follow-up should be used because of possible differences in bone density

measurements between the radiographic equipments. In our study the DXR-BMD for one of the equipment differed 1.1 % from the mean DXR-BMD, which may be due to the different ability among the radiographic equipments to recognize porosity due to resolution capacity.

4.2.3 DXR and DXA as outcome measure in rheumatoid arthritis (Paper I, Paper III)

The most surprising finding in Paper I was that DXA-BMD loss in hands only occurred in the first years of the disease, but not in patients with established disease, whereas a significant bone loss was seen for DXA-BMD at hip and spine and DXR-BMD, independent of disease duration (120;123). A discrepancy in loss of DXA-BMD hand between early and long-standing disease has previously been suggested based on the results of two longitudinally studies (24;91). Degenerative bone changes and increased inflammation in the small joints of the hand in the first years of the disease has been suggested partly to explain this finding (70). As DXA measures both trabecular and cortical bone a third explanation could be that the rate of trabecular and cortical bone loss is different in early versus late stages of the disease. The fact that the two methods for bone measurements are based on completely different techniques and that the precision for the DXR-method (76;126;154) is superior to the DXA method (71;72;115) may also contribute to the explanation. In another 2-year longitudinal study of early RA no changes in DXA-BMD hand was found (20).

The study reported in Paper I is the first study to evaluate DXA-BMD and DXR-BMD loss as outcome measures in RA with both early and established disease. In the few previous studies which have compared DXR and DXA hand in early disease the authors have concluded that changes in DXR is more sensitive than DXA to disease activity (20;112).

DXR is evaluated as an outcome measure both in early RA in Paper III (DXR-MCI) and in established RA in Paper I (DXR-BMD and DXR-MCI). DXR-MCI was found to be highly correlated to DXR-BMD in Paper I ($r=0.86$ at baseline and $r=0.94$ for percentage change over 2-year) (123). DXR turned out to be dependent of disease activity in both stages of the disease duration. DAS28 was the strongest predictor for DXR-BMD and DXR-MCI loss in Paper I, while in Paper III CRP together with non use of adalimumab and older age predicted DXR-MCI loss and DAS28 was borderline significant. These similar findings observed in two different cohorts support that DXR hand bone loss reflects the extent of the inflammatory activity in RA and thus may be a promising outcome measure of bone involvement in RA.

4.2.4 The effect of anti-TNF therapy on bone loss (Paper III)

Anti-TNF therapy has been found to significantly reduce the progression of radiographic joint damage in RA patients (88;104-108). Studies have also suggested that anti-TNF therapy may prevent general bone loss (109-111;155). Further, RA patients treated with anti-TNF therapy has been shown to have a lower rate of bone loss at spine and hip than at hand (93;110). Hand bone loss did also precede generalised bone loss (93), as has also been observed in RA patients not treated with anti-TNF therapy (21;92).

In a 2 year longitudinal treatment strategy study (the BeST study), RA patients treated with anti-TNF therapy or high dose prednisolone was shown to have a lower rate of bone loss at hand than patients treated with conventional DMARDs (93). Use of anti-TNF therapy had a positive effect on periarticular bone in another study which employed quantitative ultrasound (QUS) (155). Our findings support that treatment with anti-TNF therapy reduces hand bone loss. Further, the order of hand bone loss across treatment arms was similar to the order of radiographic progression support that erosions and bone loss are caused by the same cellular mechanism involving the osteoclast activated by the inflammatory RA disease process. Recent results from a study of the RANKL inhibitor denosumab further support this mechanistic hypothesis since this drug inhibited erosions (52) and hand DXA-BMD loss (156), but not cartilage destruction (52).

As described in chapter 1.1.3, TNF-α stimulates RANKL by cytokines in addition to stimulate the osteoclast formation directly (44;49;50). This may explain why TNF-α can reduce bone damage even if the disease activity remain moderate to high in the patients. This phenomenon is called “uncoupling”, i.e. some cytokines trigger the inflammation, while others seem to be more important for bone destruction. Further TNF-α seems to hamper the osteoblast activity (53). Blocking TNF-α will therefore decelerate bone destruction by influencing both the osteoclast and the osteoblast.

4.2.5 DXR-BMD as a predictor for radiographic damage (Paper II)

As previously stated periarticular osteoporosis has been known as a hallmark for RA and it is included as one of the ACR criteria of bone involvement beside erosions in RA (17). Further, periarticular osteoporosis on radiographs has been observed to precede the development of erosions (18;19) When this thesis started, the results from only one longitudinal pilot study had indicated that DXR-BMD loss in RA patients the first year of follow-up was a predictor of subsequent radiographic damage (100). The result from Paper II has confirmed the result from this pilot study. In our study DXR-BMD loss the first year of follow up was an

independent predictor for subsequent radiographic damage at 5 and 10 year, even when adjusted for other known predictors of radiographic progression as e.g. baseline radiographic damage, anti-CCP and markers of inflammation.

General bone loss has also been found to be influenced by disease activity (157), and it would have been of interest to examine if the loss in DXR-BMD was a part of the general bone loss or if it was specific for the hand. Unfortunately, DXA hip and spine measurements were not available in the EURIDISS cohort. As described in chapter 1.1.6.1, hand bone loss in early RA has been shown to occur more rapidly than bone loss in hip and spine (21;92;93). Hand BMD loss has also been found to be greater than BMD loss in hip and spine (93) and radiographic joint damage has been shown to be more strongly correlated with low hand DXR-BMD than DXA-BMD at hip and spine (77;94). All this findings suggest that hand bone loss is more influenced by inflammation, while generalised bone loss may be more associated with the prolonged course of RA, including use of corticosteroids and immobility (26).

In the multivariate linear regression model in Paper II the absolute DXR-BMD loss at one year was an independent predictor for radiographic damage at 5 and 10 year. The absolute vdH Sharp score at baseline was the most important predictor for the damage status at 5 and 10 year, i.e. if the radiographic damage is high at baseline it will also be high after 5 and 10 year. If the model were performed without anti-CCP or DXR-BMD respectively, the adjusted R square would have decreased about 2 % both at 5 and 10-years. Anti-CCP and DXR-BMD loss gave approximately the same contribution to the model.

These findings emphasise that the degree of radiographic damage is still the best predictor for subsequent radiographic damage. But with the new possibilities for treatment it will be important with predictors that can give information before the radiographic damage have occurred. For this purpose both DXR-BMD and anti-CCP are important. They are both more feasible to use than radiographic scoring and DXR may be more sensitive to change than radiographic joint progression. Further, both the level of DXR-BMD loss (as shown in Paper II) and high level of anti-CCP has been demonstrated to increase the risk of radiographic damage(8).

Only few of the patients in the EURIDISS cohort (Paper II) (N=7) did not loose DXR-BMD the first year of follow-up, but still had a considerable increase in radiographic damage. The reason for this discrepancy is not clear. Subanalysis of these patients showed that five were women, mean age was 45.7 year, six had positive anti-CCP (five with a high level) and four had radiographic damage at baseline. Six of these patients had a significant bone loss at

two year suggesting a late bone loss. These observations emphasise that RA is a heterogeneous disease and there is a need to combine several predictors to give the opportunity for personalized treatment based on individual prognostic factors.

5. CONCLUSION AND CLINICAL IMPLICATIONS

5.1 Answer to research questions

We were able to provide answers to the research questions presented in section 1.2.2

- Measurements of DXR-BMD and DXR-MCI can be used as an outcome measure both in early and established RA (Paper I and III), while DXA-BMD hand seems to be a valuable outcome measure in early RA only (Paper I).
- In early RA DXR-MCI loss was predicted of CRP, older age and the none-use of adalimumab, while high DAS28 was borderline significant. In established RA DXR-BMD loss and DXR-MCI loss was predicted by high disease activity (DAS28). Our results suggest that DXR-BMD is influenced by inflammation both in early and established RA and may be used in RA patients independent of disease duration. The only predictor for DXA-BMD hand loss in established RA was short disease duration. Disease activity did not influence the DXA-BMD loss in established RA (Paper I and III).
- Cortical hand bone loss was an early marker of bone involvement in RA (Paper II). At one year 67 % of patients had a DXR-BMD loss more than LSC (the measurement error for the method), while 46 % had an increase in vdH Sharp score more than 1 unit.
- Cortical hand bone loss predicted subsequent radiographic damage both at 5 years and 10 years follow-up. Both 1-year absolute change in DXR-BMD and 1-year change more than LSC were significant predictors of subsequent radiographic damage (Paper II).
- In our material the absolute value of DXR-BMD was a predictor for radiographic damage and was comparable to anti-CCP and inflammation measured by CRP, but not as good as the absolute radiographic damage (vdH Sharp score) at baseline. When dichotomised into bone loss vs. no bone loss, radiographic damage vs. no damage and anti-CCP positive vs. negative, the three predictors were comparable in strength (Paper II).
- Anti-TNF therapy, in our study adalimumab, reduced the rate of cortical hand bone loss. Patients who received adalimumab and MTX in combination therapy lost significantly less bone compared with patients who received MTX monotherapy. Use

of adalimumab was also a protector for bone loss when adjusted for disease activity, age, gender, disease duration, radiographic damage and HAQ.

- This thesis supports the hypothesis that erosions and osteoporosis are caused by the same cellular mechanism. Both the findings that cortical hand bone loss predicted subsequent radiographic damage combined with the fact that the order of hand bone loss across the three treatment arms (MTX vs. adalimumab vs. combination of MTX and adalimumab) was similar to the rate of radiographic progression support a common cellular mechanism, involving the osteoclast cell. (Paper II, III).
- The short time precision (CV %) for the new direct DXR which calculated DXR-BMD from digitized radiographs of the same phantom (31 radiographs with reposition) ranged from 0.14-0.30 in four different radiographic equipments. This is considerable better than for DXA-hand where the CV % is about 1 %. For healthy individuals (39 individuals with 2 radiographs with reposition) the CV % was 0.46. The reason for the discrepancy may be due to that the phantom is fixed in one position in addition to the different numbers of radiographs taken. However, the precision both in-vivo and in-vitro was highly satisfactory (Paper IV).

5.2 Clinical implications

In this thesis we have examined utilities for the DXR-BMD method that might be of clinical interest. We have shown that DXR-BMD hand bone loss was associated with markers of inflammation both in the early and established RA. These results suggest that DXR may be a possible outcome measure during the whole disease course in RA. In contrast, DXA-BMD of the hand seems to be valid as an outcome measure only in early RA. Further, our results have shown that hand bone loss in early RA was a predictor of subsequent radiographic damage and that the predictive power was comparable in strength to other predictors of joint damage as e.g. anti-CCP and CRP. Our study did also provide evidence that DXR-BMD could be used as response variable of bone involvement for potent anti inflammatory treatment. The predictive value of DXR combined with the ability of anti-TNF therapy to reduce both erosions and bone loss, suggest that DXR-BMD can be applied as a clinical tool to identify patients in need of potent biologic treatment. This thesis does also support the hypothesis of a common cellular pathway between radiographic erosions and bone loss, the two features of bone involvement in RA patients. We have also documented a highly satisfactory precision of the DXR method; both used on conventional and digitised radiographs.

Based on the findings from this thesis together with other studies on DXR published over the last years(20;93;94;100-102;116) , the manufacturer Sectra has developed dxr-online as a prediction tool for joint damage in RA.

However, there are still some questions to be answered before the DXR-BMD method can be recommended for use in daily clinical care. First, the DXR-BMD measurements are based on changes over time and the patients need to take two DXR-BMD measurements to calculate the hand bone loss. There is no cut-off value defined as pathological which is a limitation compared with the scoring of radiographic joint damage. Thus more research to evaluate a clinical cut-off value is of importance. Second, the Pronosco X-posure system has been replaced by dxr-online which calculates DXR-BMD only, without providing other parameters as MCI, cortical thickness, bone width and porosity. It is not clear if DXR-BMD can substitute all these measurements which may be a limitation for the dxr-online system compared with the Pronosco X-posure system. Further, several small studies suggest that the DXR-BMD loss is higher in patients with RA than other rheumatic diseases (20;21;72), but this has not been validated in larger cohorts and should be examined. At last, there is a lack of data understanding the natural rate of DXR hand bone loss in the normal population which is a major limitation of the method.

There is an increased focus on bone damage in RA among rheumatologists leading to an increased search for new tools for recognising bone involvement and therapy to reduce bone damage. We believe that this thesis increase our understanding and illuminates the possibilities of cortical hand bone loss, assessed by the DXR method, as a feature of inflammatory bone involvement in RA.

6. ERRATA

PAPER III

Concerning the multivariate model in Paper III, the variable "Treatment group" were actually coded as a ordinal explanatory variable with the rank MTX - adalimumab - combination therapy and not as a dummy variable as stated in the paper. A multivariate model calculated with dummy variables is therefore presented in Table 7. There were no differences between using the variable "treatment group" as an ordinal explanatory variable or as a dummy variable with MTX as the reference value.

Table 7. Predictors for percentage DXR-MCI loss at 104 weeks follow-up in 515 rheumatoid arthritis patients explored by multivariate linear regression model.

	DXR-MCI percentage change at 104 weeks	
	Beta	p-value
Age, years	-0.25	<0.001
Female gender	-0.04	0.38
Disease duration, years	0.06	0.12
C-reactive protein, mg/l	-0.23	<0.001
DAS 28	-0.09	0.07
Treatment group*		
• **Adalimumab vs. MTX**	0.06	0.18
• **Combination vs. MTX**	0.18	<0.001
R^2, adjusted	0.19	

* MTX used as reference treatment group. MCI baseline, Sharp score baseline and HAQ did not influence the model.
DXR= Digital X-ray radiogrammetry; MCI= Metacarpal cortical index; HAQ= Health Assessment Questionnaire; DAS28= 28-joint disease activity score.

7. APPENDIX

PAPER III

In Paper III calculations were performed for different parameters of DXR: BMD, cortical thickness (CT), width (W) and MCI were all analysed for the subgroup which had DXR-BMD measures (Table 8). DXR-CT followed the same pattern as DXR-MCI and DXR-BMD while DXR-W was stable for all time points and where not influenced by treatment. As this table in our opinion did not provide substantial and additional new information (and due to the maximal number of tables and figures allowed in Paper III) these data were published as a supplementary table for electronic publishing only.

The values for DXR porosity index are not included in the table. This DXR parameter has in previous studies been shown to have considerable poorer precision than DXR-MCI and DXR-BMD (158) Thus, within the methodological limitations in this study, we found these data to be inconclusive.

Table 8. The effect of methotrexate monotherapy, adalimumab monotherapy and adalimumab combined with methotrexate on different DXR parameters. Calculations are performed on the subgroup were DXR-BMD could be analysed.

		MTX Median (mean) percentage change	**Adalimumab** Median (mean) percentage change	**Combination** Median (mean) percentage change
DXR-MCI	26 weeks	-1.36(-2.04)	-1.16 (-1.83)	-1.11 (-1.66)
	52 weeks	-2.70 (-3.65)	-2.91 (-3.61)	-2.16 (-2.89)
	104 weeks	-4.50 (-5.67) *	-4.35 (-5.23)	-3.60 (-4.26)
DXR-BMD	26 weeks	-1.20(-1.73)	-0.96 (-1.46)	-1.06 (-1.25)
	52 weeks	-1.86 (-2.77)	-1.97 (-2.70)	-1.63 (-2.11)
	104 weeks	-3.58 (-4.22) **	-2.40 (-3.70)	-2.49 (-3.07)
DXR-cortical thickness	26 weeks	-1.53 (-2.14)	-1.12 (-1.78)	-1.34 (-1.80)
	52 weeks	-2.81 (-3.61)	-2.55 (-3.51)	-2.22 (-2.76)
	104 weeks	-5.02 (-5.46)***	-3.92 (-4.92)	-3.38 (-3.99)
DXR-bone width	26 weeks	-0.13 (-0.11)	-0.10 (-0.01)	-0.22 (-0.19)
	52 weeks	-0.04 (0.07)	0.01 (0.09)	0.07 (0.13)
	104 weeks	0.18 (0.13)	0.30 (0.14)	0.17 (0.17)

* At 104 weeks the MTX group lost more DXR-MCI than the combination group (p=0.04)
** At 104 weeks the MTX group lost more DXR-BMD than the combination group (p=0.049)
*** At 104 weeks the MTX group lost more DXR cortical thickness than the combination group (p=0.04)
DXR= digital X-ray radiogrammetry; BMD= bone mineral density; MCI= metacarpal cortical index; MTX= methotrexate

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Paper I

Research article

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Hand bone loss as an outcome measure in established rheumatoid arthritis: 2-year observational study comparing cortical and total bone loss

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Abstract

The aim of this 2-year longitudinal observational study was to explore hand bone loss as a disease outcome measure in established rheumatoid arthritis (RA).

A cohort of 215 patients with RA (170 women and 45 men, aged 20–70 years) were recruited from the Oslo RA registry and studied for changes in hand bone mass during a 2-year follow-up. Digital X-ray radiogrammetry (DXR) was used to measure cortical hand bone mineral density (BMD) and metacarpal cortical index, whereas dual-energy X-ray absorptiometry (DXA) was used to assess whole hand BMD, which measures total cortical and trabecular bone. DXA-BMD total hip and spine and informative data for disease and therapy were also collected.

Hand bone loss could be revealed over a 2-year follow-up measured by DXR-BMD (-0.90%, $P < 0.01$), but not by DXA-BMD (0.00%, $P = 0.87$). DXA-BMD hand bone loss was only observed in patients with disease duration ≤3 years and not in patients with longer disease duration (-0.96% versus 0.24%, $P < 0.01$), whereas loss of DXR-BMD was independent of disease duration. Disease activity (measured by the disease activity score including 28 joints) independently predicted loss of DXR-BMD but not changes in the DXA-BMD hand in the multivariate analysis. The change in DXR metacarpal cortical index was highly correlated to DXR-BMD ($r = 0.94$, $P < 0.001$).
These data suggest that DXR-BMD may be a more appropriate technique to identify RA-related bone involvement in hands compared with DXA-BMD measurement, but further studies are needed to explore this hypothesis.

Introduction

Periarticular bone loss and erosions on radiographs are characteristic features of bone damage in rheumatoid arthritis (RA) [1], and both features are caused by joint inflammation [2]. Substantial data suggest a common cellular pathway for both periarticular bone loss and erosions involving the osteoclast cell [3,4]. In active RA there is an excess production of proinflammatory cytokines (for example, IL-1 and TNFα), which stimulates receptor activator of nuclear factor kB ligand (RANKL) to activate the osteoclast cell [3-5].

Because periarticular bone loss is an early finding and may also precede erosions on radiographs [6], quantitative hand bone measurements that capture periarticular osteoporosis have been proposed as outcome measures in early RA [7,8]. Inflammation of the joints, however, is not restricted to the early phase of the RA disease, but may be present during the entire disease course [9]. Hand bone loss could therefore potentially be an outcome measure in RA patients with prolonged disease.

AOT = antiresorptive osteoporotic treatment; BMD = bone mineral density; DAS 28 = disease activity score including 28 joints; DMARD = disease-modifying antirheumatic drugs; DXA = dual-energy X-ray absorptiometry; DXR = digital X-ray radiogrammetry; IL = interleukin; MCI = metacarpal cortical index; MHAQ = Modified Health Assessment Questionnaire; RA = rheumatoid arthritis; TNF = tumor necrosis factor.

Several devices for quantitative bone measurements have been developed [10] – for example, quantitative computer tomography, measuring cortical and trabecular bone separately; quantitative ultrasound, providing measures that may reflect bone quality; dual-energy X-ray absorptiometry (DXA), which measures total cortical and trabecular bone; and digital X-ray radiogrammetry (DXR), which measures cortical bone only. DXA is considered the gold standard among bone measurement devices for assessment of bone density at the hip and the spine. DXA has not, however, been shown to be superior to other bone measure devices, such as DXR, in the hand [11]. DXR, which is a further development and digitalized version of the conventional radiogrammetry [12], is a new promising method for assessment of cortical hand bone loss [13].

The understanding of hand bone loss as an outcome measure in RA is mainly limited both due to lack of data from longitudinal studies and due to the small number of patients included in previous studies. Only a few studies have examined associations between disease factors and hand bone loss in RA, and most of them have focused on patients with early disease [6-8,11,14,15]. Data from two longitudinal studies by Deodhar and colleagues suggest that whole hand DXA bone mineral density (BMD) loss only takes place in the first 2–3 years of the RA disease process, which may limit the use of hand DXA-BMD as an outcome measure in prolonged disease [7,15]. Only a few studies have compared hand DXA-BMD with hand cortical bone DXR-BMD in RA [11,16].

The aim of the present study was to explore hand bone loss as a disease outcome measure in established RA assessed by DXR and by DXA and to compare the two methods.

Materials and methods

Patients

The 215 RA patients (45 males and 170 females) included in the present study were recruited from a longitudinal cohort of 366 RA patients (aged 20–70 years) [17], all patients fulfilling the American College of Rheumatology (ACR) criteria and enrolled in the Oslo RA register [18]. Two-year changes in generalized bone loss at the hip and the spine from this original cohort have previously been described in detail [17]. In the present study, only patients with hand radiographs and DXA-BMD measurement of the hand at baseline and 2-year follow-up were included; 151 patients missed at least one BMD measurement and were excluded. There were no other exclusion criteria.

Demographic and clinical variables

The demographic and clinical characteristics of the patients (Table 1) were recorded by a combination of self-reported questionnaires, interview and clinical investigation, as previously reported [17]. In short, the clinical examination included 28-swollen and tender joint counts as well as routine laboratory tests. The disease activity score including 28 joints (DAS28) was computed based on the erythrocyte sedimentation rate [19]. Patients with a titer ≥64 of the Waaler–Rose reaction were classified as rheumatoid factor-positive. The physician's global assessment of disease activity was measured on a visual analogue scale (0–100 mm). Use of antiresorptive osteoporotic treatment (AOT) with bisphosphonates or hormone replacement therapy, prednisolone and disease-modifying antirheumatic drugs (DMARD) was recorded. Physical disability was measured by the Modified Health Assessment Questionnaire (MHAQ) (eight items; range of scores 1–4) [20].

Bone mineral density measurements

The DXR-BMD and the DXR metacarpal cortical index (MCI) was measured by the Pronosco X-posure system™ (version 2.0; SECTRA, Linköping, Sweden) [13], a computer version of the traditional technique of radiogrammetry [12]. The computer automatically recognizes, on standard radiographs, regions of interest around the narrowest part of the second, third and fourth metacarpal bones of the hand. In each region, the cortical thickness, bone width and porosity is measured 118 times per centimeter. The final BMD estimate is defined as: DXR-BMD = $c \times VPA_{comb} \times (1 - p)$, where c is a constant (determined such that DXR-BMD on average is equal to the mid-distal forearm region of the Hologic QDR-2000 device (Hologic Inc., Bedford, MA, USA)), VPA is the volume per area and p is the porosity. The DXR method has previously been described in detail [13,21]. The MCI is defined as the combined cortical thickness divided by the outer cortical diameter and is a relative measure independent of bone size or bone length [22,23]. The DXR method has improved the precision of MCI for diagnosing cortical bone loss [12,23]. All radiographs of the hand were acquired by a Siemens Multix Polymat equipment (Siemens AG, Erlangen, Germany) (AGFA Curix film; film focus distance, 1 m; X-ray tube voltage, 55 kV; exposure dose, 6 mAs).

Standardized BMD measurements for the left and right hands and the total hip and spine (L2–L4) were performed using the same DXA equipment (Lunar Expert; Lunar Corporation, Madison, WI, USA) both at baseline and follow-up. All procedures were in accordance with the manufacturer's standardized procedures for hand BMD measurements.

For the DXR-BMD most previous studies have used the nondominant hand [11,14], while for DXA measures there is no consistency and both hands [8], the right hand [15,24] and the nondominant hand [11] have all been used. To avoid bias regarding dominant and nondominant hands and to achieve better precision, we used the mean of both hands. Only patients who had complete measurements for both DXA-BMD and DXR-BMD in both hands were therefore included.

Table 1

Patient characteristics at baseline and at 2-year follow-up

Variable	*n*	Baseline	At 2-year follow-up
Demographic			
Age (years)	215	57.4 (49.1–64.7)	
Female	215	170 (79.0%)	
Menopause	170	111 (65.3%)	
Body mass index (kg/m^2)	215	23.9 (21.3–26.2)	24.0 (21.5–26.2)
Smoker	210	65 (31.0%)	67 (31.9%)
Disease			
Disease duration (years)	215	9 (4–16)	
Age at disease onset (years)	215	45.0 (33.0–53.0)	
Rheumatoid factor-positive	202	97 (48.0%)	
Physician's global assessment score (visual analogue scale, 0–100 mm)	203	19.0 (8.0–39.8)	17.6 (8.5–30.0)
Modified Health Assessment Questionnaire (range 1–4)	214	1.50 (1.13–1.75)	1.50 (1.13–1.87)
Erythrocyte sedimentation rate (mm/hour)	210	16 (9–27)	14 (8–27)
Disease activity score including 28 joints	202	4.04 (3.17–4.96)	4.26 (3.36–5.06)
Medication			
Ever user of disease-modifying antirheumatic drugs	213	177 (83.1%)	177 (83.1%)
Corticosteroids	208	79 (37.9%)	85 (40.9%)
User of corticosteroids in the 2-year period	208		93 (44.7%)
Antiresorptive osteoporosis treatment	209	47 (22.5%)	68 (32.5%)
Ever user of antiresorptive osteoporosis treatment	209		92 (44.0%)
Calcium and/or vitamin D	210	113 (53.8%)	155 (73.8%)

Data presented as the median (interquartile range) or as the absolute value (%).

Precision of bone mineral density measurements

Short-time precision was calculated from the material of 28 healthy individuals who underwent duplicate hand BMD measurements and duplicate hand radiographs of both hands with repositioning of the hand between each assessment. Short-time precision based on the duplicate measurements, expressed as the percentage coefficient of variation, was 0.28% for the DXR-BMD hand and was 0.76% for the DXA-BMD hand. Long-time precision for DXR-BMD based on daily measurement of one hand radiograph was 0.25%, and long-time precision for the DXA-BMD hand based on daily measurements of the aluminum spine phantom supported by the Lunar Expert (Lunar Corporation) was 0.80%

Ethics and legal aspects

The study was approved by the regional committee for ethics and medical research.

The Norwegian Data Inspectorate approved the registry of RA patients in Oslo.

Statistical analysis

The statistical analyses were carried out using the SPSS program, version 13 (SPSS Inc., Chicago, IL, USA). Nonparametric tests were used for comparisons between groups (Mann–Whitney and Kruskal–Wallis tests) and within groups (Wilcoxon test) because of a skewed distribution of data. Results are presented as the median and interquartile range (25th–75th percentiles). Bivariate correlations were tested using Spearman's correlation.

Bone loss over time was expressed as a negative value. Changes of BMD measurements were compared across groups according to the disease duration (cut-off 3 years), baseline DAS28 (<3.2, low disease activity; 3.2–5.1, moderate disease activity; >5.1, high disease activity) and baseline MHAQ score (<1.50, 1.50–1 99, ≥2). The 3-year cut-off value for disease duration was chosen for pragmatic reasons due to a low number of included patients with short disease duration and reports in the literature suggesting hand bone loss only takes place in the first 3 years of disease duration [7].

The predictive values of disease duration, DAS28 and MHAQ score were also tested in a multiple linear regression model, with the change of hand BMD as the dependent variable and with adjustments for age, gender, rheumatoid factor and use of medication (AOT, prednisolone and DMARD). Enter and stepwise procedures were used. According to inspection of Q–Q plots, the distribution of residuals showed acceptable fit to the normal distribution regarding hand DXR-BMD, whereas one outlier was identified in the analysis with hand DXA-BMD as the dependent variable. This analysis was therefore performed both with and without the outlier.

Two tailed *P* values of 0.05 or less were considered statistically significant.

Results

Patient characteristics at baseline and at follow-up are presented in Table 1. The 215 examined patients in this study had shorter disease duration (9 years versus 15 years, $P < 0.01$), lower disease activity measured by the DAS28 (4.00 versus 4.62, $P < 0.01$), lower global assessment (19 versus 30, $P < 0.01$) and used less prednisolone (37% versus 54%, $P < 0.01$) compared with those who were not included ($n = 151$) from the original cohort ($n = 366$). The two groups were similar regarding age, gender, body mass index, smoking habits, rheumatoid factor, age of disease onset, erythrocyte sedimentation rate, menopause in women and use of DMARD and AOT.

Change in bone mineral density

In the entire group, a significant loss in hand BMD was seen at 2 years as measured by DXR-BMD (-0.90%) and DXR-MCI (-1.18%), but not as measured in the DXA-BMD hand (0.00%) (Figure 1). A significant bone loss was also observed for the DXA-BMD in the total hip (-0.72%) and in the spine L2–L4 (-0.78%) (Figure 1).

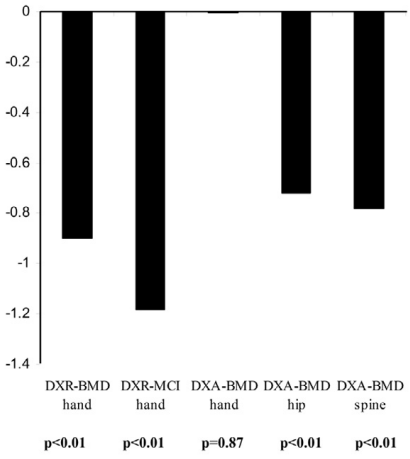
The correlation (*r* value) between the DXR-BMD hand and the DXA-BMD hand was 0.88 ($P < 0.001$) for baseline values and was 0.35 ($P < 0.001$) for 2-year BMD changes. Correlations between the change in the DXA hand and in the DXA total hip and spine were 0.35 ($P < 0.001$) and 0.18 ($P = 0.01$), whereas correlations between the change in the DXR hand and DXA total hip and spine were 0.23 ($P = 0.001$) and 0.10 ($P = 0.16$), respectively. The DXR-MCI was highly correlated with the DXR-BMD both at baseline ($r = 0.86$, $P < 0.001$) and as the percentage change over 2 years ($r = 0.94$, $P < 0.001$).

Association between disease duration and bone loss

At baseline 37 patients had a disease duration of 3 years or less and 178 patients had a disease duration longer than 3 years. DXA-BMD hand bone loss was only observed in patients with disease duration less than 3 years and not in patients with longer disease duration (-0.96% versus 0.24%, $P < 0.01$) (Table 2), whereas loss of DXR-BMD (-0.46% versus -0.93%, $P = 0.76$) as well as loss of DXR-MCI (-0.89 versus -1.29, $P = 0.66$), of the DXA-BMD total hip (-0.26% versus -0.76%, $P = 0.51$) and of the DXA-BMD spine (-0.71% versus -0.82%, $P = 0.64$) occurred independent of disease duration. The changes in BMD in the subgroups (according to disease duration) were all significant except for the DXA-BMD hand patients with disease duration longer than 3 years ($P = 0.26$) and the DXA-BMD spine ($P = 0.60$) and DXA-BMD total hip patients with disease duration less than 3 years (borderline significant, $P = 0.06$).

The patients with short and long disease duration were comparable with regard to demographic variables, disease activity and treatment with DMARD and corticosteroids, but AOT was used less frequently by patients with short disease duration than by patients with long disease duration (16.1% versus 35.5%, $P = 0.04$). The difference in DXA hand bone loss across patients with short and long disease duration, however, was also significant in the subgroup not using AOT (-1.41%

Figure 1



DXR-BMD hand	% change BMD	-0.90 (-2.79; 0.25)
	Baseline median BMD g/cm^2	0.52 (0.45; 0.57)
DXR-MCI hand	% change BMD	-1.18 (-3.24; -0.09)
	Baseline median MCI	0.38 (0.32; 0.36)
DXA-BMD hand	% change BMD	0.00 (-2.02; 2.08)
	Baseline median BMD g/cm^2	0.37 (0.32; 0.42)
DXA-BMD total hip	% change BMD	-0.72 (-3.20; 1.62)
	Baseline median BMD g/cm^2	0.90 (0.80; 0.99)
DXA-BMD spine (L2-L4)	% change BMD	-0.78 (-5.03; 3.20)
	Baseline median BMD g/cm^2	1.13 (0.98; 1.26)

Values are expressed in median and interquartile range (25-75 percentile).

Bone loss in 215 rheumatoid arthritis patients followed for 2 years. Bone loss assessed by digital X-ray radiogrammetry (DXR) bone mineral density (BMD) and metacarpal cortical index (MCI) of the hand, and by dual-energy X-ray absorptiometry (DXA) BMD of the hand, total hip and spine (L2–L4).

Table 2

Comparison of the baseline and the change in hand bone mineral density

	Disease duration			DAS28 at baseline				MHAQ at baseline			
	≤3 years	>3 years	*P* value*	<3.2	3.2–5.1	>5.1	*P* value	<1.50	1.50–1.99	≥2.0	*P* value
n	37	178	<0.01	55	103	44	<0.01	102	78	34	<0.01
Age (years)	55.4 (43–62)	58.0 (50–65)	0.10	53.5 (39–61)	55.8 (49–64)	62.2 (57–67)	<0.01	53.6 (41–64)	58.9 (52–64)	61.2 (54–67)	<0.01
DXA-BMD (g/cm^2)	0.39 (0.34–0.43)	0.36 (0.31–0.41)	0.04	0.40 (0.36–0.43)	0.38 (0.32–0.42)	0.33 (0.28–0.38)	<0.01	0.38 (0.33–0.43)	0.37 (0.31–0.41)	0.34 (0.30–0.39)	0.13
DXA-BMD change (%)	-0.96 (-4.4 to 1.5)	0.24 (-1.4 to 2.1)	<0.01	-0.40 (-2.4 to 1.8)	0.26 (-1.3 to 2.2)	0.04 (-3.4 to 2.2)	0.40	0.11 (-2.5 to 2.1)	0.0 (-1.2 to 2.0)	-0.12 (-4.1 to 2.2)	0.75
DXR-BMD (g/cm^2)	0.57 (0.50–0.61)	0.51 (0.44–0.56)	<0.01	0.56 (0.50–0.61)	0.53 (0.45–0.58)	0.46 (0.38–0.52)	<0.01	0.54 (0.49–0.59)	0.49 (0.44–0.57)	0.50 (0.40–0.53)	<0.01
DXR-BMD change (%)	-0.46 (-3.6 to 0.2)	-0.93 (-2.8 to 0.3)	0.76	-0.29 (-1.6 to 0.7)	-1.13 (-3.2 to 0.1)	-1.03 (-4.3 to 0.5)	0.03	-0.80 (-2.6 to 0.1)	-0.94 (-2.8 to 0.5)	-0.81 (-3.7 to 0.5)	0.90
DXR-MCI	0.40 (0.37–0.49)	0.37 (0.31–0.45)	<0.01	0.41 (0.34–0.48)	0.39 (0.33–0.46)	0.32 (0.27–0.38)	<0.01	0.40 (0.33–0.48)	0.37 (0.31–0.43)	0.33 (0.29–0.41)	<0.01
DXR-MCI change (%)	-0.89 (-5.5 to 0.0)	-1.29 (-3.1 to -0.1)	0.66	-0.76 (-1.8 to 0.3)	-1.34 (-3.4 to -0.4)	-1.13 (-5.2 to -0.2)	0.06	-1.33 (-3.1 to -0.3)	-1.20 (-3.2 to 0.3)	-0.71 (-5.0 to 0.0)	0.74

Digital X-ray radiogrammetry (DXR) bone mineral density (BMD), DXR metacarpal cortical index (MCI) and dual-energy X-ray absorptiometry (DXA) BMD assessed for levels of disease duration, for disease activity (disease activity score including 28 joints (DAS28)) and for physical function (Modified Health Assessment Questionnaire (MHAQ)) in rheumatoid arthritis patients. Data presented as the medians (interquartile range). **P* values between subgroups.

versus 0.11%, $P = 0.02$). These findings are consistent in a linear regression model adjusted for other variables that may influence hand bone loss (Table 3). The analysis was performed both with and without the outlier, with the same results.

Association between disease activity score and hand bone loss

At baseline 55 patients had low disease activity, 103 patients had moderate disease activity and 44 patients had high disease activity. Bone loss changes, as measured by DXR-BMD, differed across patients with different levels of disease activity (low, -0.29%; moderate, -1.13%; and high, -1.03%; $P = 0.03$), and were borderline significant for DXR-MCI (-0.76, -1.34 and -1.13, $P = 0.06$) (Table 2). No significant difference in DXA-measured hand BMD change was found for the low, moderate and high levels of disease activity (-0.40% versus 0.26% versus 0.04%, respectively; $P = 0.40$). Hand BMD baseline values, however, were significantly lower in the group with high disease activity in both the DXR-BMD and the DXA-BMD (Table 2).

Table 3

Risk factors for hand bone loss in a multivariate linear regression model

	DXA-BMD hand percentage change		DXR-BMD hand percentage change		DXR-MCI percentage change	
	B (standard error)	*P* value	*B* (standard error)	*P* value	*B* (standard error)	*P* value
Disease activity score including 28 joints	0.09 (0.25)	0.73	-0.47 (0.16)	0.003	-0.47 (0.18)	0.009
Disease duration <3 years	-2.84 (0.88)	0.001	0.46 (0.55)	0.40	0.45 (0.63)	0.47
Baseline BMD (g/cm^2)/MCI	-9.70 (5.01)	0.05	-3.80 (2.51)	0.13	-5.79 (2.81)	0.04
Prednisolone during 2-year follow-up (no/yes)	0.44 (0.69)	0.53	-0.03 (0.43)	0.95	-0.41 (0.49)	0.40
Ever disease-modifying antirheumatic drug user (no/yes)	-0.31 (0.90)	0.73	-0.58 (0.55)	0.30	-0.56 (0.63)	0.38
Ever antiresorptive osteoporosis treatment user (no/yes)	0.78 (0.70)	0.27	0.03 (0.42)	0.95	-0.05 (0.47)	0.91
R^2	0.11		0.05		0.06	

B values are unstandardized coefficients. Age, gender, rheumatoid factor and the Modified Health Questionnaire were also tested, but did not influence the results. DXA, dual-energy X-ray absorptiometry; DXR, digital X-ray radiogrammetry; BMD, bone mineral density; MCI, metacarpal cortical index.

The correlation (*r* value) between the DAS28 at baseline (continuous scale) and the hand DXR-BMD change was -0.19 ($P = 0.01$), between the DAS28 and the DXR-MCI change was -0.16 ($P = 0.03$), and between the DAS28 and the hand DXA-BMD change was 0.08 ($P = 0.27$). Patients in the group with high disease activity were significant older than the group with lowest disease activity. In a multivariate model, however, disease activity was independently associated with the percentage change in DXR-BMD ($B = -0.47$, $P < 0.01$) (Table 3) and with the DXR-MCI ($B = -0.47$, $P < 0.01$), after adjusting for other variables that could influence hand bone change as well as age.

Association between functional disability (MHAQ score) and hand bone loss

At baseline, 102 patients had a MHAQ score less than 1.50, 78 patients a score between 1.50 and 1.99, and 34 patients had a MHAQ score of two or more. The patient with highest MHAQ score was older than patients with lower MHAQ scores. Regarding correlation between the MHAQ score at baseline and the change in hand DXR-BMD, the DXR-MCI hand and the DXA-BMD hand were nonsignificant both for continuous values ($r = 0.00$, $P = 0.96$; $r = 0.03$, $P = 0.70$; and $r = -0.05$, $P = 0.51$) and for groups ($r = 0.02$, $P = 0.82$; $r = 0.05$, $P = 0.47$; and $r = -0.02$, $P = 0.82$) for the MHAQ score ranges <1.5, 1.50–1.99 and ≥2, respectively. There were no differences in the change in hand BMD dependent on the MHAQ group either in the DXR-BMD hand, the DXR-MCI hand or the DXA-BMD hand. Baseline values, however, were significantly higher in the group with the lowest MHAQ score with regards to DXR-BMD and DXR-MCI (Table 2). No such findings were seen regarding DXA measurements.

Associations between treatment and hand bone loss

At follow-up 33% of the patients were current users of AOT (88% used hormone replacement therapy and 12% used bisphosphonates) and 44% were ever users. A significant difference in DXA-BMD hand change was found between users and nonusers of AOT (0.44% versus 0%, $P = 0.04$). No such difference was seen for DXR-BMD (-1.01% versus -0.66%, $P = 0.54$) or DXR-MCI (-1.14 versus -1.19, $P = 0.60$) in users versus nonusers of AOT. Use of AOT, however, was not significantly associated with the change in DXA-BMD in the multivariate analyses (Table 3).

No significant difference in hand bone change was seen between ever users (83%) and never users (17%) of DMARD regarding the DXR-BMD hand (-0.90% versus -0.85%, $P = 0.29$), the DXR-MCI hand (-1.19 versus -0.78, $P = 0.17$) or the DXA-BMD hand (0.27% versus -0.34%, $P = 0.22$). During the 2-year follow-up 45% of patients had used prednisolone and 41% were current users at follow-up with a mean dose of 5.7 mg. No significant difference in change of hand BMD was observed between users and nonusers of prednisolone regarding DXR-BMD (-0.94% versus -0.66%, $P = 0.19$) or DXA-BMD (0.62% versus 0%, $P = 0.17$), but a group difference between users and nonusers was observed for DXR-MCI (-1.42 versus -0.98, $P = 0.05$). Prednisolone users, however, had a significantly higher disease activity than nonusers (data not shown) and the significant association between prednisolone and the change in DXR-MCI disappeared in the multivariate analysis (Table 3).

Discussion

The present study had two main findings. First, total hand bone loss measured by DXA-BMD seems to occur only in the first years of RA disease, whereas DXR-BMD-measured cortical hand bone loss occurs both in early stages as well as late stages of the disease. Second, patients with high disease activity at baseline lost more DXR-BMD and DXR-MCI than patients with low disease activity. In the present study there were only marginally differences between DXR-BMD and DXR-MCI, and our main focus in the discussion will therefore be on DXR-BMD.

A discrepancy in loss of DXA-BMD hand between early disease and long-standing disease has previously been suggested based on the results of two longitudinal studies [7,15]. Hand bone loss was only observed in the first 3 years and then stabilized over the next 2 years in a longitudinal study of 29 patients with RA [7]. Degenerative bone changes and increased inflammation in the small joints of the hand in the first years of the disease has been suggested partly to explain this finding [25]. As DXA-BMD measures both trabecular and cortical bone, a third explanation could be that the rate of trabecular and cortical bone loss is different in early stages versus late stages of the disease. Even if DXR-BMD hand bone loss occurs during the whole RA disease course, the bone loss has been shown to be more rapid in early disease compared with more prolonged disease [14]. Böttcher and colleagues reported annual DXR-BMD loss in the first 6 years of the disease to be as high as 11%, with a subsequent decline to 3–4% over the next years [14].

Interestingly, changes in the DXA-BMD in the total hip and spine were independent of the disease duration. There are few studies that have compared periarticular and generalized osteoporosis among RA patients [8,26-28]. Hand bone loss in early RA has been shown to occur more rapidly than bone loss in the hip and the spine [8,28]. Radiographic joint damage has been shown to be more strongly correlated with low hand DXR-BMD than DXA-BMD at the hip and the spine [26,27]. In a randomized, placebo-controlled trial among early RA patients, use of prednisolone reduced hand bone loss [29]. These data suggest that the effect of inflammation on hand bone in RA may be greater than the effect on other bones (for example, spine and hip). The generalized bone loss may be more associated with the prolonged course of RA, including the use of corticosteroids and immobility [30].

The other main finding in the present study is that patients with high disease activity at baseline lost more DXR-BMD than patients with low disease activity. Surprisingly, this association was not found between DXA-BMD hand bone loss and baseline disease activity, and this lack of association was consistent in both patients with short and long disease durations (data not shown). Some previous studies in early RA, however, have shown that disease activity is associated with both DXA-BMD-measured generalized bone loss [31] as well as localized bone loss [8]. Gough and colleagues [31] found that early RA patients with active disease (defined as mean C-reactive protein >20 mg/l over 12 months) showed greater generalized bone loss at the hip and the spine compared with patients with lower disease activity. Haugeberg and coworkers [8] found that C-reactive protein independently predicted hand BMD loss in patients with early undifferentiated arthritis who, during a 12-month follow-up, developed RA. Explanations for contradictory findings between these two studies and our study may be differences in disease activity and disease duration in the examined study cohorts.

The association between disease activity and DXR-BMD hand bone loss in our study was shown when dichotomizing the patients into groups based on disease activity (Table 2) and in linear multivariate analyses (Table 3). These consistent associations combined with the demonstration of bone loss independent of disease duration (Table 3) suggest that DXR-BMD is a robust outcome measure in RA, reflecting the inflammatory disease process in early stages as well as late stages of the disease. Only a few previous studies have been carried out with DXR-BMD loss as the key outcome measure [11,32]. Jensen and colleagues [11] found in patients with early RA (<2 years) that DXR-BMD was more strongly associated with disease activity than hand DXA-BMD. In a cross-sectional study, Böttcher and colleagues found that DXR-BMD was negatively correlated with disease activity measured by the DAS28 [32].

In the present study the hand bone loss measured by both DXR-BMD and DXA-BMD was less than that reported by other workers. Jensen and colleagues [11] found a loss of DXR-BMD of 5% over 2 years in an early RA disease group, and Haugeberg and colleagues found that the DXA-BMD hand loss was reduced by 4.3% in early RA disease patients [8]. One explanation for the lower rate of hand bone loss in the present study may be that our cohort was obtained from an observational study of patients with different levels of disease activity and duration. The recruitment of these patients from a validated RA register is also a strength of the present study as the results provide insight into what takes place in the real world of RA patients regarding hand bone loss [18]. Another reason for the less bone loss may be that the DXA-BMD hand was assessed as a whole hand and not around selected finger joints, which according to the cross-sectional study by Alenfeld and colleagues [33] has been suggested to be the best site to capture periarticular bone loss in RA. There are disadvantages using periarticular regions compared with the whole hand, however, which include poorer precision and poorer feasibility [33]. Because of skewed data, median values were used instead of mean values, neutralizing the effect of extremes on the BMD results.

The limitations of the present study were that relatively few patients had short disease duration. The effect of medication on the bone was also difficult to evaluate because patients had no standardized treatment but were treated according to clinical judgment. Adjusting for medication use in the multivariate analyses had no significant effect on BMD change either on the DXR-BMD hand or the DXA-BMD hand. A study with a randomized controlled design would give stronger evidence for the effects of medication.

Onepotential limitation using quantitative bone measures as an outcome measure in RA is the influence of normal bone loss, which also takes place in healthy adult subjects. Normal bone loss for DXR-BMD has only been examined in cross-sectional studies reporting an annual rate of bone loss between 0.4% and 0.9% [22,34-36]. For DXA-BMD hip and spine bone loss, using cross-sectional data has been shown to overestimate the rate ofnormal bone loss compared with longitudinal studies [37]. In the multivariate model, however, age was not a significant predictor for hand bone loss over 2 years either for DXR-BMD or for DXA-BMD (data not shown).

Conclusion

We suggest that hand DXA-BMD can only be used as an outcome measure in RA in the first years of the disease, whereas DXR-BMD may be used as a marker for disease activity and bone loss during the whole disease process, both in early disease as well as prolonged disease. The reason for this discrepancy is not clear and additional studies are warranted to further explore this hypothesis.

Competing interests

The authors declare they have no competing interests.

Authors' contributions

MH analyzed the data, performed the statistical analyses and prepared the manuscript.

TKK and GH designed the study, organized the data collection and contributed substantially to the drafting of the manuscript. All authors read and approved the final manuscript.

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Paper II

Cortical hand bone loss after 1 year in early rheumatoid arthritis predicts radiographic hand joint damage at 5-year and 10-year follow-up

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ABSTRACT

Objective: To examine 1-year hand bone loss in early rheumatoid arthritis (RA) as a predictor of radiographic damage at 5-year and 10-year follow-up

Methods: A total of 136 patients with RA (disease duration 0–4 years) were followed for 10 years with clinical data and hand radiographs. Joint damage was scored according to the van der Heijde modification of the Sharp method (vdH Sharp score) and hand bone mineral density (BMD) was measured by digital *x* ray radiogrammetry (DXR). Group comparisons, correlation analyses and multivariate analyses were performed to evaluate the relationship between hand bone loss and radiographic joint damage.

Results: Patients with hand BMD loss at 1 year had a higher median increase in vdH Sharp score compared to patients without loss at 5 years (12 vs 2, $p = 0.001$) and 10 years (22 vs 4, $p = 0.002$). In a linear regression model adjusting for age, gender, baseline C-reactive protein (CRP), anti-cyclic citrullinated peptide (CCP), IgM rheumatoid factor (RF) and radiographic damage, absolute hand DXR-BMD loss at 1 year was an independent predictor of radiographic outcome at 5 years ($p<0.01$) and 10 years ($p = 0.02$). In a logistic regression model the odds ratio (95% CI) for radiographic progression among patients with hand BMD loss was 3.5 (1.4 to 8.8) and 3.5 (1.4 to 8.4) at 5 and 10 years, respectively.

Conclusion: Early hand bone loss measured by DXR-BMD is an independent predictor of subsequent radiographic damage. Our findings support that quantitative hand bone loss in RA precedes radiographic joint damage and may be used as a tool for assessment of bone involvement in RA.

Periarticular osteoporosis and joint erosion are both known as radiographic hallmarks of rheumatoid arthritis (RA).[1] Evidence from animal[2 3] and human studies[4] support that erosions and osteoporosis are caused by an increased activation of osteoclasts. Hand bone loss has been shown to take place in early RA,[5] even in the undifferentiated stage of the RA disease process.[6 7] On hand radiographs periarticular osteoporosis has been shown to precede the development of erosions.[8]

Measures of quantitative hand bone loss eg, by dual energy *x* ray (DXA) and digital *x* ray radiogrammetry (DXR) in early RA have been proposed as an outcome measure for bone involvement.[9–11] However, there is a lack of data on the relationship between hand bone loss and radiographic joint damage. Cross-sectional studies have shown a moderate correlation between low hand bone mineral density (BMD) and radiographic damage[10 12–17] and two small longitudinal studies have indicated that early hand bone loss may predict subsequent radiographic joint damage.[18 19]

Thus, the objective of this study was to examine if cortical hand bone loss in early RA, as assessed by DXR in the first year of follow-up, could predict radiographic joint damage at 5-year and 10-year follow-up.

MATERIALS AND METHODS

Study design and study population

The current analysis is a part of the European Research on Incapacitating Disease and Social Support (EURIDISS) longitudinal observational study. The patients and methods have previously been described in detail elsewhere.[20 21] In short, all patients had disease duration of maximum 4 years at inclusion, were aged 20–70 years and fulfilled the American College of Rheumatology (ACR) criteria for RA.[1] Clinical, laboratory and radiographic data were collected at baseline and at 1, 2, 5 and 10 years. During the observation period, patients were treated according to clinical judgement by their rheumatologist. Sera from the baseline visit were stored at −70 degrees for later analysis of micro-C-reactive protein (CRP) (Dade Behring, Newark, New Jersey, USA), anti-cyclic citrullinated peptide (CCP) (ELISA, Inova Diagnostics, San Diego, California, USA) and rheumatoid factor IgM (IgM RF) (in-house ELISA).[22]

Patients with hand radiographs at baseline, 1-year follow-up and either 5-year or 10-year follow-up were included in the present analyses. The number of patients with baseline radiographs available for scoring were 163, and 15 of these were excluded due to missing radiographs at 5 and 10 years. Of the remaining 148 patients, baseline radiographs from 5 patients could not be analysed for DXR-BMD because the radiographs were underexposed and 7 patients were excluded at the 1-year follow-up (5 radiographs were missing, 1 was underexposed and 1 patient had surgical material in the metacarpal bone). A total of 136 patients were included in the final analyses.

Missing radiographic scores at 5 years ($n = 11$) were replaced by a projected score based on the radiographic progression from baseline to the 10-year assessment (horizontal imputation). Any 10-year missing data ($n = 18$) were replaced by last observation carried forward[23] to avoid overestimation of the radiographic damage. Missing DXR-BMD values were not imputed. To test the

robustness of the results, all analyses were repeated without imputation.

Radiographic analyses

The radiographs were scored according to the van der Heijde modification of the Sharp score (vdH Sharp score),[24] and were read in known time order by one experienced reader.[21] In all, 16 joint areas for erosions (0–5) and 15 for joint space narrowing (JSN) (0–4) were evaluated in each hand, and the maximum score was 280. Conventional radiographs were available at baseline, 1, 2 and 5 years, while radiographs at 10 years were digitised. Scoring of digitised and conventional radiographs have been shown to yield similar results.[25]

Analyses were performed for continuous and dichotomised data. Cut-off for an important increase in vdH Sharp score was defined as an increase of 1 unit per year (ie, 5 units at the 5-year and 10 units at the 10-year follow-up assessment).[22] The smallest detectable change (SDC) in radiographs read in known time order has been found to be 2.9 units.[26] The conventional radiographs were acquired by a Siemens Multix Polymat equipment (film: AGFA Curix (AGFA, Mortsel, Belgium); film focus distance (FFD): 100 cm; *x* ray tube voltage: 55 kV; exposure dose: 6 mAs). The digital radiographs were acquired by an AGFA ADC Compact (computed radiography) and a Siemens (Erlangen, Germany) tube (AGFA ADCC HR image plate, *x* ray tube voltage 50 kV, FFD 100 cm, exposure dose 5 mAs).

Cortical hand bone density

The conventional hand radiographs used for radiographic scoring of joint damage was also used for hand BMD measures at 1, 2 and 5 years. Cortical hand BMD was measured by DXR Pronosco X-posure system, V. 2.0 (Sectra, Linköping, Sweden),[27] which is a development of the traditional technique of radiogrammetry.[28] On hand radiographs the computer automatically recognises regions of interest around the narrowest part of the second, third and fourth metacarpal bone. In each region, cortical thickness, bone width and porosity is measured 118 times per cm. The BMD estimate is defined as: BMD-DXR = $c \times VPA_{comb} \times (1-p)$, were c is a constant (empirically determined so DXR-BMD on average was equal to the mid-distal forearm region of the Hologic QDR-2000 densitometer; Hologic, Bedford, Massachusetts, USA); VPA is volume per area and p is porosity. This method has been described in detail elsewhere.[27 29]

Table 1 Baseline characteristics in 136 included patients with rheumatoid arthritis (RA) and the 102 non-participants (mean (SD) values for continuous variables (also median and range for vdH Sharp score and DXR-BMD), percentages for counts)

	Patients included in study (n = 136)	Patients not included in study (n = 102)*
Age (years)	51.3 (12.1)	52.8 (14.2)
Female	76	71
Positive anti-CCP	62	59
Positive IgM RF	48	48
Disease duration (years)	2.2 (1.2)	2.4 (1.1)
CRP (mg/litre)	9.4 (12.2)	11.0 (13.4)
ESR (mm/h)	26.2 (20.9)	25.5 (18.3)
HAQ score (scale 0–3)	0.9 (0.6)	1.0 (0.7)
Current users of corticosteroids	26	29
Current users of DMARDs	54	49
vdH Sharp score	6.8 (12.2)	NA
	2.0 (0–69.0)	
Hand DXR-BMD (g/cm^2)	0.55 (0.09)	NA
	0.55 (0.33–0.77)	

*All group comparisons p>0.05.
CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; DXR-BMD, digital *x* ray radiogrammetry bone mineral density; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; NA, not available; RF, rheumatoid factor; vdH Sharp, van der Heijde modification of the Sharp score.

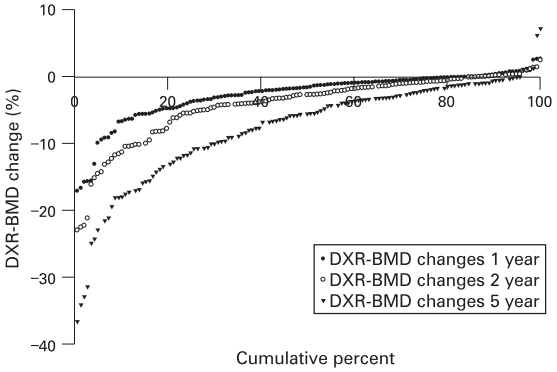

Figure 1 Cumulative probability plot of digital x ray radiogrammetry bone mineral density (DXR-BMD) percentage change at 1-year, 2-year and 5-year follow-up in patients with rheumatoid arthritis (RA).

Precision was calculated from 28 healthy individuals who underwent duplicate hand radiographs with repositioning of the hand between each measure. The coefficient of variation (CV%) was found to be 0.28%, and least significant change (LSC) was 0.78%[30]

$$(LSC\% = z_{(1 \div \alpha/2)} \times \sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)} \times CV\%)$$

The LSC was used as cut-off to define a DXR-BMD loss exceeding the measurement error on the individual level. We applied the mean values of both hands to avoid bias regarding dominant and non-dominant hand, and this approach has been shown to improve precision.[19]

Statistics

The statistical analyses were performed using SPSS V.14 (SPSS, Chicago, Illinois, USA).

Because of skewed data, non-parametric tests were used. Hand BMD loss was tested as a continuous and a dichotomised variable. The following methods were used: Spearman correlation, group comparisons using the Mann–Whitney U test and multivariate analyses (linear and logistic regression analyses).

A linear regression model was developed to investigate if absolute hand BMD loss during the first year could predict subsequent radiographic outcome (dependent variable). The model was adjusted for age and gender as well as for other potential predictors of radiographic damage: anti-CCP (cut-off: 25 U/ml), IgM RF (cut-off: 25 U/ml), CRP, Health Assessment Questionnaire (HAQ) and baseline vdH Sharp score. Separate models for 5 and 10 years were developed.

In a logistic regression model patients were dichotomised as progressors and non-progressors of radiographic damage with an annual increase of 1 unit as cut-off value (dependent variable) and stratified into patients with and without DXR-BMD loss with LSC as the cut-off value (independent variable). This

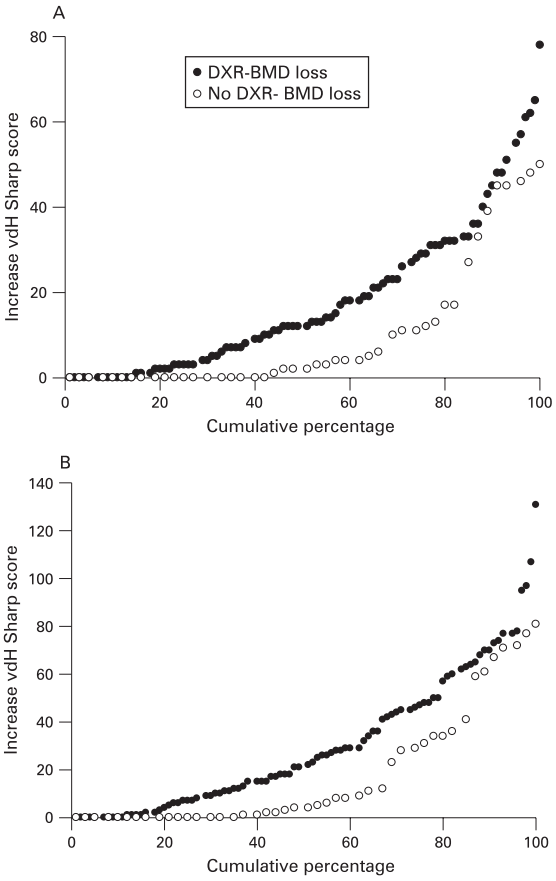

Figure 2 Change in van der Heijde Sharp score at 5 years (A) and 10 years (B) in patients with rheumatoid arthritis stratified for hand bone mineral density (BMD) loss at 1 year (cut-off >least significant change (LSC)). Presented as a cumulative probability plot were each symbol represent a patient (hand BMD loss n = 91, no hand BMD loss n = 45). (A) 5-year change in vdH Sharp score dependent on DXR-BMD loss or not at 1 year. p = 0.001 (group comparison Mann–Whitney U). (B) 10-year change in vdH Sharp score dependent on DXR-BMD loss or not at 1 year. p = 0.002 (group comparison Mann–Whitney U).

model was adjusted for the variables that were statistically significant in the linear regression model together with age and gender. For CRP, we used a cut-off of 10 mg/litre. From the logistic regression model a probability score was calculated to assess the risk of radiographic progression dependent on the combination of hand BMD loss, early radiographic damage and anti-CCP. All tests were two-sided and conducted at the p = 0.05 significance level.

Ethics and legal aspects

The study was approved by the regional committee for ethics and medical research and data collection was approved by the Data Inspectorate.

RESULTS

Patient characteristics at baseline are shown in table 1. No significant differences were observed between the 136 examined patients in the present analyses and the 102 patients who were excluded. At 5 years none of the patients were using anti-tumour necrosis factor (TNF)α therapy and 55% were using disease-modifying antirheumatic drugs (DMARDs); at 10-year follow-up the corresponding figures were 12% and 50%, respectively.

BMD change and radiographic damage

The median loss in hand DXR-BMD expressed in absolute values (g/cm^2) were 0.009 after 1 year, 0.016 after 2 years and 0.031 after 5 years. The percentage changes of hand DXR-BMD are displayed as a cumulative probability plot in fig 1. The hand bone change was similar in males and females (data not shown).

The mean (median) change in the vdH Sharp score were 3.6 (1.0), 7.1 (3.0), 15.5 (10.0) and 26.4 (16.0) units after 1, 2, 5 and 10 years, respectively. The correlation coefficient (r) between change in DXR-BMD and vdH Sharp score increased from −0.35 (p<0.01) at the first year, to −0.47 (p<0.01) at the second year and −0.56 (p<0.01) at the fifth year.

On the individual level, 67% of the patients had a hand BMD loss exceeding LSC in the first year of follow-up. The proportions of patients with radiographic progression in the hands (defined as 1 unit per year) were 46% after 1 year and 60% after 5 and 10 years. The radiographic progression (median values) was significantly higher in patients who lost DXR-BMD vs patients who did not lose DXR-BMD at 5 (12 vs 2, p = 0.001) and 10 years (22 vs 4 p = 0.002). These results are shown as cumulative probability plots in fig 2.

Multivariate linear regression models

Change at 1 year in DXR-BMD was a significant and independent predictor of vdH Sharp score at 5 and 10 years, adjusting for other relevant factors as baseline vdH Sharp score, anti-CCP, IgM RF, baseline micro-CRP, HAQ-score, age and gender (table 2). In this model a loss of 0.1 g/cm^2 would on average give an increase in vdH Sharp score of 20 units after 5 years and 28 units after 10 years (B values, not shown). Other significant predictors of radiographic damage were anti-CCP, radiographic damage at baseline and inflammatory activity assessed by CRP (the latter only at 5 years) (table 2).

Multivariate logistic regression models

Logistic regression models were created to see whether DXR-BMD loss could be used as a predictor in a clinical situation. The models were adjusted for the baseline variables that turned out to be statistically significant in the linear regression model, ie, baseline vdH Sharp score (cut-off 1 unit), anti-CCP (cut-off 25 U/ml) and CRP (cut-off 10 mg/litre) together with age and gender.

Loss of hand DXR-BMD exceeding the LSC during the first year of follow-up was an independent risk factor for radiographic progression at 5 and 10 years with an odds ratio (95% CI) of 3.5 (1.4 to 8.8) and 3.5 (1.4 to 8.4), respectively (table 3).

Radiographic damage at baseline and positive anti-CCP were also significant risk factors for subsequent radiographic damage (table 3). For these three predictors the probability for radiographic damage was calculated based on the odds ratios (OR). The probability of developing radiographic progression (for 5 years) was calculated as follows: log (p/1–p) = −1.61 (constant)+1.26 DXR-BMD loss+1.60 Radiographic damage at baseline+1.44 anti-CCP positive, where p is the probability and each variable is entered as a categorical variable (0 or 1) weighted by the importance of each variable. From this

Table 2 Digital x ray radiogrammetry bone mineral density (DXR-BMD) as a predictor of subsequent radiographic damage in patients with rheumatoid arthristis (RA) at 5-year and 10-year follow-up tested in multivariate linear regression models (n = 136)

	Radiographic outcome at 5 years (vdH Sharp score)		Radiographic outcome at 10 years (vdH Sharp score)	
Variable	**β**	**p Value**	**β**	**p Value**
Hand DXR-BMD change at 1 year (g/cm²)	−0.15	0.005	−0.15	0.02
Baseline vdH Sharp score	0.68	<0.001	0.56	<0.001
Positive anti-CCP	0.14	0.02	0.17	0.02
Positive IgM RF	0.09	0.11	0.09	0.17
CRP	0.15	0.005	0.11	0.09
HAQ	−0.05	0.30	−0.10	0.11
Age (years)	−0.08	0.11	−0.06	0.36
Female gender	0.02	0.75	0.07	0.25
R^2, adjusted	0.69		0.53	

Use of disease-modifying antirheumatic drugs and/or corticosteroids at baseline did not influence the model.
CCP, cyclic citrullinated peptide; CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; RF, rheumatoid factor; vdH Sharp, van der Heijde modification of the Sharp score.

algorithm the probability of radiographic damage at 5 years could be calculated as 94% for patients with bone loss, radiographic damage and positive anti-CCP and as 17% for a patient with none of these risk factors. These results, together with the 10-year data are depicted in fig 3. For the 5-year and 10-year data, hand bone loss adds additional information to the subsequent risk for radiographic damage.

Robustness

The multivariate models were also tested without imputation of missing values. The number of available patients at 5-year and 10-year follow-up was 126 and 118, respectively. In the linear regression model a loss of 0.1 g/cm² DXR-BMD would give an increase in vdH Sharp score of 19.8 units (p = 0.01) at 5 years and 23.2 units (p = 0.07) at 10 years. In the logistic regression model the OR (95% CI) for radiographic damage among those with hand bone loss were 3.0 (1.2 to 7.7) at 5 years and 2.9 (1.1 to 7.4) at 10 years. All group comparisons and correlation analyses remained statistically significant.

DISCUSSION

This study shows for the first time that hand bone loss is an independent predictor of progression of radiographic joint damage and that the predictive power is comparable to biomarkers that are well known predictors of radiographic joint damage as anti-CCP and CRP.

Algorithms have been proposed to identify patients with poor prognosis by the presence of various predictors.[22 31 32] Up to now early hand bone loss as a risk factor has not been included in any of these algorithms. The two characteristics of the DXR-BMD method, high sensitivity to change[29] and ability to predict joint damage, suggest that hand DXR-BMD may be used in prediction models of poor outcome in patients with early RA. In the multivariate analysis and in our algorithm based on presence or absence of the three risk factors, anti-CCP, erosive disease at baseline and 1-year change in bone loss, we showed that hand DXR-BMD gives additionally information to the other risk factors for predicting radiographic progression.

As illustrated in fig 3, a patient with radiographic damage at baseline, but negative anti-CCP and no hand bone loss during the first year will have a 34% probability of an important radiographic progression after 10 years, whereas a patient with erosions and hand BMD loss has a probability of 64%. With additional presence of anti-CCP this probability increases to 87% after 10 years. DXR-BMD has most additional predictive value in patients with only one risk factor (either baseline radiographic damage or anti-CCP). These data illustrate the potential of DXR-BMD as a predictive tool, but we find it important to recommend that our results also should be confirmed in patients with very early RA (ie, in patients without radiographic erosions).

The fact that bone loss in early RA occurs more rapidly than the development of erosions is not only supported by the

Table 3 Digital x ray radiogrammetry bone mineral density (DXR-BMD) as a predictor for subsequent increase in vdH Sharp score in patients with rheumatoid arthritis (RA) at 5-year and 10-year follow-up in multivariate logistic regression models

	Logistic regression analyses			
	Increase ⩾5 units at 5 years		Increase ⩾10 units at 10 years	
	β	**Odds ratio (95% CI)**	**β**	**Odds ratio (95% CI)**
DXR-BMD loss>LSC at 1 year	1.26	3.52 (1.42 to 8.75)	1.24	3.46 (1.43 to 8.35)
Baseline vdH Sharp score ⩾1	1.60	4.94 (2.01 to 12.10)	1.45	4.27 (1.78 to 10.27)
Positive anti-CCP	1.44	4.20 (1.77 to 9.98)	1.28	3.59 (1.54 to 8.38)
Elevated CRP (cut-off 10)	0.98	2.66 (0.92 to 7.72)	0.45	1.57 (0.59 to 4.19)
Age	−0.02	0.98 (0.94 to 1.02)	−0.01	0.99 (0.95 to 1.03)
Female gender	0.34	1.41 (0.52 to 3.86)	0.84	2.32 (0.88 to 6.10)
Constant	−1.61		−2.11	
Goodness of fit (Nagelkerke R square)	0.44		0.39	

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; vdH Sharp, van der Heijde modification of the Sharp score.

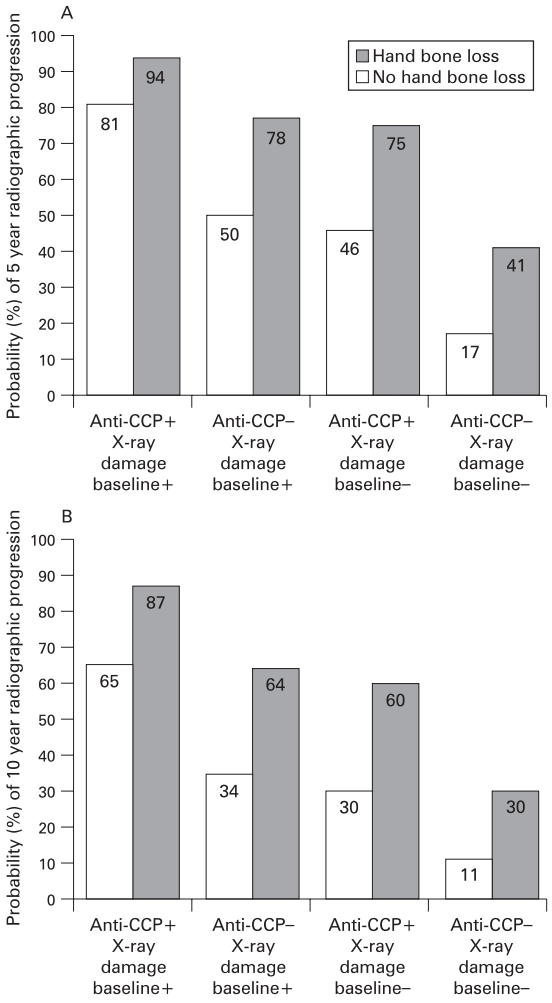

Figure 3 Probability of radiographic progression in patients with rheumatoid arthritis at 5 (A) and 10 years (B). The probability (%) is based on the odds ratio from the logistic regression model according to the combined presence of hand bone loss at 1 year, radiographic damage and anti-cyclic citrullinated peptide (CCP) at baseline.

multivariate analysis but also from correlation analysis. The correlation coefficient between hand bone change and radiographic damage increased from −0.35 at the first year to −0.56 at the fifth year. However it is important to emphasise that erosions are pathognomonic for bone involvement in RA, while bone loss may also occur in normal individuals. This limitation may, however, be a minor problem in studies with short observation time.[19] DXR-BMD values for the 10-year follow-up are not presented for methodological reasons as these radiographs were digitised and DXR-BMD assessed from digital printouts has been shown to have a poorer precision than conventional radiographs.[33] Further, the precision regarding direct analysing of digitised DXR-BMD has not been fully investigated.

One limitation of this study is that data on generalised bone loss was not available. From the literature we know that generalised bone loss has also been found to be associated with disease activity[34] and radiographic damage.[35 36] Few studies have compared periarticular and generalised osteoporosis among patients with RA.[7 13 17 37] Hand bone loss in early RA has been shown to occur more rapidly than bone loss in hip and spine.[7 37] Radiographic joint damage has been shown to be more strongly correlated with low hand DXR-BMD than DXA-BMD at hip and spine.[13 17] In a randomised placebo controlled trial among patients with early RA, use of prednisolone reduced hand bone loss.[38] These studies suggest that the effect of inflammation on hand bone in RA may be greater than the effect on other bones (eg, spine and hip). In addition the precision of BMD hand, and particularly DXR-BMD, is better than the precision of DXA measurements of the hip and spine. This difference also indicates that a longer observation period may be required to detect a true loss of bone in hip and spine.[19 29]

This study examined the relation between early hand bone loss and radiographic progression at 5 and 10 years. Horizontal imputations were used for the missing 5-year data and last observation carried forward used for the missing 10-year data. This approach was chosen because the literature is not consistent as to whether radiographic damage increases in a linear fashion during the first year of the disease and declines afterwards,[39] or increases linearly over a period of 10 years.[40 41] By using this conservative imputation the radiographic damage was not overestimated. No imputations regarding DXR-BMD were performed. All analyses were also repeated without imputations and hand bone loss turned out to be a significant predictor of radiographic outcome in all analyses except for the linear regression model at 10 years, which showed a borderline significance.

Although DXR-BMD shows convincing result as a predictor for radiographic damage, there are a few patients with no DXR-BMD bone loss who have a high increase in radiographic damage (fig 2). The reason for this is not clear. One explanation may be that RA is a heterogenic disease. However, this emphasise the importance of further research on predictors for radiographic damage.

In conclusion, we have shown that hand bone loss measured by DXR-BMD is an independent predictor of subsequent radiographic damage in patients with RA. Our findings support that quantitative hand bone measures may be a complimentary approach in the study of changes in bone involvement in RA and may be an important tool in the daily clinical work together with anti-CCP, markers of inflammation and radiographs to identify patients at high risk of developing progression in radiographic joint damage.

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Paper III

Adalimumab therapy reduces hand bone loss in early rheumatoid arthritis: explorative analyses from the PREMIER study

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ABSTRACT

Objective: The effect of adalimumab on hand osteoporosis was examined and related to radiographic joint damage in the three treatment arms of the PREMIER study: adalimumab plus methotrexate, adalimumab and methotrexate monotherapy. Predictors of hand bone loss were also searched for.

Methods: 768 patients (537 fulfilled 2 years) with rheumatoid arthritis (RA) for less than 3 years, never treated with methotrexate, were included. Hand bone loss was assessed by digital *x* ray radiogrammetry (DXR) on the same hand radiographs scored with modified Sharp score at baseline, 26, 52 and 104 weeks. For DXR, metacarpal cortical index (MCI) was the primary bone measure.

Results: At all time points the rate of percentage DXR–MCI loss was lowest in the combination group (−1.15; −2.16; −3.03) and greatest in the methotrexate monotherapy group (−1.42; −2.87; −4.62), with figures in between for the adalimumab monotherapy group (−1.33; −2.45; −4.03). Significant differences between the combination group and the methotrexate group were seen at 52 ($p = 0.009$) and 104 weeks ($p<0.001$). The order of hand bone loss across the three treatment arms was similar to the order of radiographic progression. Older age, elevated C-reactive protein and non-use of adalimumab were predictors of hand bone loss.

Conclusion: This study supports a similar pathogenic mechanism for hand bone loss and erosions in RA. The combination of adalimumab and methotrexate seems to arrest hand bone loss less effectively than radiographic joint damage. Quantitative measures of osteoporosis may thus be a more sensitive tool for assessment of inflammatory bone involvement in RA.

In rheumatoid arthritis (RA), bone damage on radiographs presents not only as erosions but also as periarticular osteoporosis.[1] Hand bone loss in early RA has been shown to occur more rapidly than bone loss at the hip and spine[2–4] and also predicts radiographic joint damage.[5]

Inflammatory activation of the osteoclast is involved in both features. Studies support that cytokines, eg, tumour necrosis factor (TNF) alpha, macrophage colony-stimulating factor and receptor activator of nuclear factor kappa ligand (RANKL), activate the osteoclast that causes osteoporosis (localised and generalised) and erosions.[6–8]

Anti-TNF therapy has been shown to reduce the progression of radiographic joint damage significantly in RA patients.[9–11] A few studies have also suggested that anti-TNF therapy may prevent general bone loss.[12–14]

Quantitative hand bone measures have been recommended for their sensitivity to assess inflammatory bone involvement in early RA.[15] However, only a few studies have examined the effect of anti-inflammatory treatment (including anti-TNF therapy) on hand bone loss in RA.[4 14 16 17] Furthermore, only one randomised controlled trial has been conducted in which the anti-inflammatory effects of prednisolone (7.5 mg daily) compared with placebo were shown to reduce significantly not only the rate of radiographic joint damage, but also the rate of hand bone loss.[17]

The primary objective of this analysis was to examine cortical hand bone loss in the three arms of the PREMIER study: adalimumab plus methotrexate versus adalimumab monotherapy versus methotrexate monotherapy and to evaluate associations between hand bone loss and radiographic progression. Our second objective was to identify potential predictors of hand bone loss.

METHODS

Study sample and design

The radiographic and clinical data from this 2-year, multicentre, double-blind, randomised controlled study (PREMIER) have previously been described in detail.[11] In short, the efficacy and safety of adalimumab plus methotrexate was compared with adalimumab monotherapy and with methotrexate monotherapy in 799 adult patients with early (<3 years, mean disease duration 9.1 months), aggressive RA (inclusion criteria: ⩾8 swollen joints; erythrocyte sedimentation rate ⩾28 or C-reactive protein (CRP) ⩾1.5 mg/dl; erosions or rheumatoid factor positive), who previously had not been treated with methotrexate, cyclophosphamide, cyclosporine, azathioprine or more than two other disease-modifying antirheumatic drugs (DMARD) (table 1).[11] The combination group received adalimumab 40 mg subcutaneously every other week plus weekly methotrexate by mouth (rapidly increased to 20 mg/week), and the monotherapy groups received either adalimumab 40 mg subcutaneously every other week plus placebo or weekly methotrexate by mouth plus placebo. Radiographs from hands and feet were scored according to the modified Sharp score (range 0–398).[11]

From this study, we present hand bone loss data at 26, 52 and 104 weeks of follow-up. To maintain the original study design of a blinded randomised controlled trial, the treatment code was kept secret for one of the authors who analysed the data (MH).

DXR hand bone measure

Digital *x* ray radiogrammetry (DXR; Sectra, Linköping, Sweden) was used to measure hand bone mineral density (BMD) and the metacarpal cortical index (MCI) on the same digitised hand *x* rays used for the assessment of radiographic joint damage. DXR is a computer version of the traditional radiogrammetry technique[18] and the method has previously been described in detail.[19–21] On hand radiographs, the computer automatically recognises regions of interest around the narrowest part of the second, third and fourth metacarpal bone and measures cortical thickness, bone width and porosity 118 times per centimetre. DXR–BMD is defined as: $c \times VPA_{comb} \times (1 - p)$, where c is a density constant, VPA is volume per area and p is porosity. DXR–MCI is defined as the combined cortical thickness divided by the bone width and is a relative bone measure independent of bone size and bone length.[21 22] In the literature short-time in-vivo precision (CV%) has been reported to range from 0.28% to 0.59% for DXR–BMD and from 0.31% to 0.64% for DXR–MCI.[19 21 23]

DXR–BMD was intended to be the main outcome measure in this study. However, many radiographs could not be analysed for BMD because of unknown image resolution. The equation for DXR–BMD is based on volume per area and requires a known resolution, because a distance in a digitised radiograph cannot be measured when the resolution is unknown. Therefore, DXR–MCI, which is a relative measure less dependent on image resolution, was used as the primary outcome measure. The correlation between DXR–BMD and DXR–MCI has been shown to be substantial ($r > 0.90$), both cross-sectionally[24] and longitudinally.[25]

For comparison, we also present results for DXR–BMD. All images with unknown resolution were analysed by assuming 254 dpi (the scanning resolution for the radiographs before scoring). Several of the radiographs were, however, clearly of a resolution other than 254 dpi, most likely because these radiographs had been printed in non-true size before scanning. We analysed all available images for DXR–BMD at baseline, as well as 26, 52 and 104 weeks and calculated DXR width. Based on analyses from studies with a controlled resolution,[25] a deviation from baseline width greater than 2% was likely to indicate an incorrect value. By using this 2% value as a cut-off, 23% of the radiographs were excluded from further DXR–BMD analyses. The flow chart in fig 1 illustrates the patients who were included in the DXR–MCI and DXR–BMD analyses.

To avoid bias regarding dominant and non-dominant hand and to achieve better precision, we employed mean value measurements from both hands.[15] If the radiograph from one hand could not be analysed, we used the radiograph from the available hand for all analyses at all time points.

The radiographs were acquired by a single emulsion mammography film; film focus distance 100 cm; tube voltage 50–55 kV according to the radiographic equipment and the exposure dose was 8 mAs.

Statistical analysis

As the data were skewed, non-parametric analyses were conducted. No imputations were performed. Baseline values were compared between treatment groups with the Kruskall–Wallis method for continuous variables and the χ^2 method for categorical variables. Comparisons of changes in DXR were conducted using methodologies employed in the original PREMIER study.[11] Two groups were compared in a hierarchical order with the Mann–Whitney U test, ie, two-sided comparison of the combination group versus methotrexate, followed by two-sided comparisons between the adalimumab monotherapy and methotrexate monotherapy treatment arms and finally two-sided comparisons between the adalimumab monotherapy and the combination group. Each pair-wise comparison was completed only if the previous comparison was statistically significant. Bone loss over time was expressed as a negative value.

A linear regression model was developed to search for predictors of hand BMD loss at 26, 52 and 104 weeks.

Table 1 Baseline characteristics for early RA patients in PREMIER*

	Adalimumab + methotrexate (N = 261)	Adalimumab monotherapy (N = 261)	Methotrexate monotherapy (N = 246)
Demographic characteristics			
Age, years	52.2 (13.8)	51.9 (13.7)	51.9 (13.3)
Female, no (%)	187 (71.6)	205 (78.5)	181 (73.6)
Clinical characteristics			
Disease duration, years	0.7 (0.8)	0.7 (0.8)	0.8 (0.9)
Previously taken DMARD, no (%)	84 (32.2)	87 (33.3)	78 (31.7)
Previously taken corticosteroids, no (%)	92 (35.2)	94 (36.0)	85 (34.6)
Tender joint count, 0–66	31.1 (14.1)	31.7 (13.5)	32.2 (14.3)
Swollen joint count, 0–66	21.2 (11.1)	21.7 (10.2)	21.6 (11.3)
C-reactive protein, mg/l	39.5 (42.4)	40.7 (38.6)	40.6 (41.2)
HAQ, 0–3	1.5 (0.6)	1.6 (0.6)†	1.5 (0.7)
DAS28	6.3 (0.9)	6.4 (0.9)	6.3 (0.9)
Image analysis			
Modified TSS			
Mean	18.1 (20.3)	18.4 (18.2)	21.5 (21.8)
Median (25–75th percentile)	12.8 (6.0–24.0)	13.5 (5.1–25.5)	15.5 (7.5–28.5)
DXR–MCI	0.45 (0.09)	0.45 (0.09)	0.46 (0.08)
DXR–BMD, g/cm^2	0.57 (0.08)	0.57 (0.08)	0.58 (0.08)

*Except where indicated results are given in mean (standard deviation) for continuous variables and numbers and percentages for categorical variables. †Significantly higher values in the adalimumab group compared with both the methotrexate and the combination group. BMD, bone mineral density; DAS28, 28-joint disease activity score; DMARD, disease-modifying antirheumatic drugs; DXR, digital *x* ray radiogrammetry; HAQ, health assessment questionnaire; MCI, metacarpal cortical index; RA, rheumatoid arthritis; TSS, total Sharp score.

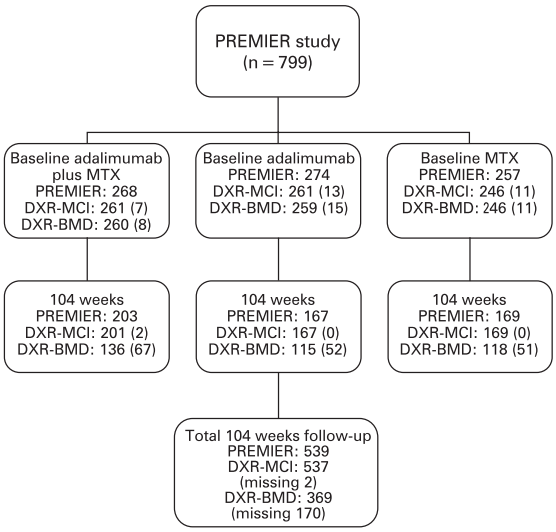

Figure 1 Flow chart of the examined patients with early rheumatoid arthritis in the present analysis. Numbers of missing *x* rays compared with the original PREMIER study are provided in parentheses. BMD, bone mineral density; DXR, digital *x* ray radiogrammetry; MCI, metacarpal cortical index; MTX, methotrexate.

Spearman correlation analyses were conducted in an attempt to correlate changes in DXR–MCI with the following baseline variables: disease duration; disease activity measured by 28-joint disease activity score (DAS28);[26] CRP; disability index of health assessment questionnaire (HAQ) scores;[27] previous use of DMARD and cortisone; radiographic joint damage; randomised treatment arm and absolute DXR–MCI value. The variables with a p value less than 0.15 were included in the multivariate model, which was also adjusted for age and gender. Treatment arm was coded as a dummy variable (methotrexate as 0, adalimumab as 1 and combination group as 2).

The PREMIER study was approved by a central institutional review board and independent ethics committees at each participating site.[11]

RESULTS

Baseline DXR–MCI values were available for 768 of the 799 patients enrolled in the PREMIER study and DXR–MCI values were missing for two of 539 patients who completed the study (fig 1). The corresponding numbers for available DXR–BMD data (based on the cut-off values for image resolution described in the Methods section) were 765 and 369, respectively (fig 1). Demographics and baseline clinical characteristics for the whole group were comparable between the three treatment arms (table 1).

The only statistically significant difference between treatment arms was a slightly greater mean HAQ score for the adalimumab monotherapy group. Before enrollment, corticosteroids had been used in 35% of the patients (mean daily dosage of prednisolone was 6.6 mg) and 32% had been treated with traditional DMARD other than methotrexate. The baseline radiographic damage scores were similar across treatment groups, with a median (mean) Sharp score of 14.0 (19.3) (table 1).

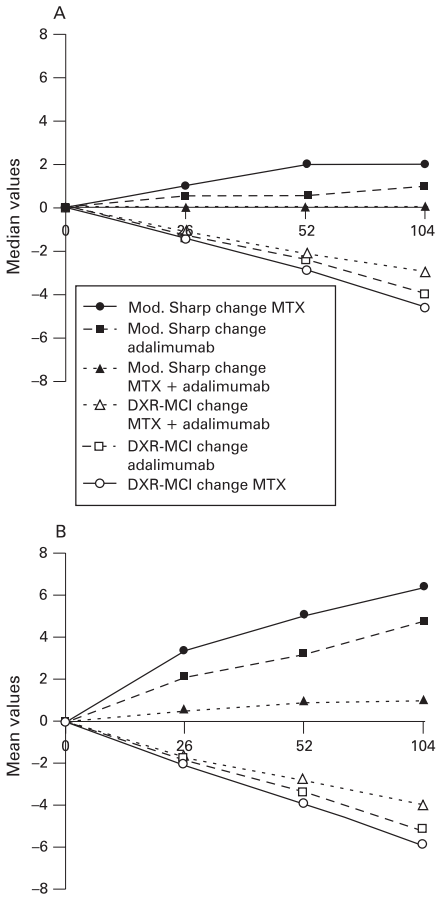

Figure 2 Changes in DXR–MCI (percentage) and modified Sharp score (units) over time in the three treatment groups of PREMIER (A, median values; B, mean values). DXR, digital *x* ray radiogrammetry; MCI, metacarpal cortical index; Mod Sharp, modified total Sharp score; MTX, methotrexate.

Median percentage DXR–MCI changes for all patients were −1.29, −2.45 and −3.72 at 26, 52 and 104 weeks. Corresponding values for DXR–BMD were −1.07%, −1.72% and −2.63%. Changes from baseline in DXR–MCI and DXR–BMD were significant for all subgroups at all time points during follow-up ($p<0.001$ for all). The use of corticosteroids or DMARD did not affect hand bone loss (data not shown).

Correlation coefficients (r) between the DXR–MCI and the DXR–BMD changes were 0.88, 0.93 and 0.94 at 26, 52 and 104 weeks ($p<0.001$ for all).

DXR–MCI changes between treatment arms

At 26, 52 and 104 weeks follow-up median percentage DXR–MCI changes were −1.15, −2.16 and −3.03 for the adalimumab plus methotrexate combination group, −1.33, −2.45 and −4.03 for the adalimumab monotherapy group and −1.42, −2.87 and −4.62 for the methotrexate monotherapy group (fig 2).

The rate of DXR–MCI loss was significantly greater for the methotrexate group compared with the combination group at

Table 2 Predictors for percentage DXR–MCI loss at 104 weeks follow-up in 515 RA patients explored by a multivariate linear regression model

	DXR–MCI percentage change at 104 weeks	
	Beta	p Value
Age, years	−0.25	<0.001
Female gender	−0.04	0.36
Disease duration, years	0.06	0.11
C-reactive protein, mg/l	−0.23	<0.001
DAS28	−0.09	0.07
Treatment group*	0.16	<0.001
R^2, adjusted	0.19	

*Treatment groups coded as a dummy variable: 0, methotrexate; 1, adalimumab; 2, adalimumab plus methotrexate. MCI baseline, Sharp score baseline and HAQ did not influence the model. DAS28, 28-joint disease activity score; DXR, digital *x* ray radiogrammetry; HAQ, health assessment questionnaire; MCI, metacarpal cortical index; RA, rheumatoid arthritis.

52 weeks (p = 0.009) and 104 weeks (p<0.001) and the same trend was also observed at 26 weeks (p = 0.19). DXR–MCI reduction in the adalimumab monotherapy group was numerically lower than in the methotrexate group at 104 weeks (p = 0.10).

DXR–BMD changes between treatment arms

The median DXR–BMD percentage changes at 26, 52 and 104 weeks were, respectively, −1.06, −1.63 and −2.49 in the combination group, −0.96, −1.97 and −2.40 for the adalimumab group and −1.20, −1.86 and −3.58 for the methotrexate group. The median DXR–BMD loss in the adalimumab group was numerically slightly less than in the combination group both at 26 and 104 weeks. However, the mean loss in the adalimumab group was greater at all time points (see supplementary table available online only). A significant difference between the DXR–BMD change in the methotrexate group and the combination group at 104 weeks (p = 0.049) was observed and a trend at 52 weeks (p = 0.10). A trend towards a difference between the methotrexate and adalimumab groups was observed at 104 weeks (p = 0.16).

Analyses on DXR–cortical thickness (DXR–CT) and DXR–bone width (DXR–W) were also performed on the same subgroups that were analysed for DXR–BMD. DXR–CT showed the same pattern of bone loss as DXR–BMD and DXR–MCI. DXR–W was stable at all time points and was not influenced by treatment (see supplementary table available online only).

DXR–MCI and radiographic damage

The median (mean) radiographic changes in modified Sharp score at 26, 52 and 104 weeks, respectively, were 0 (0.5), 0 (0.9) and 0 (1.0) for the combination group, 0.5 (2.1), 0.5 (3.3) and 1.0 (4.8) for the adalimumab monotherapy group and1.0 (3.4), 2.0 (5.1) and 2.0 (6.4) for the methotrexate monotherapy group (fig 2). The discrepancy in the results of this analysis compared with findings of the original PREMIER study is probably a result of the slight differences in the number of study participants (fig 1) and the fact that no imputations were conducted in the present study. The correlations (r) between DXR–MCI change and change in Sharp score at 26, 52 and 104 weeks were r = −0.12 (p = 0.001), r = −0.23 (p<0.001) and r = −0.32 (p<0.001). Comparable r values for correlations between DXR–BMD and Sharp score changes were −0.15, −0.23 and −0.33, respectively (p<0.001 for all).

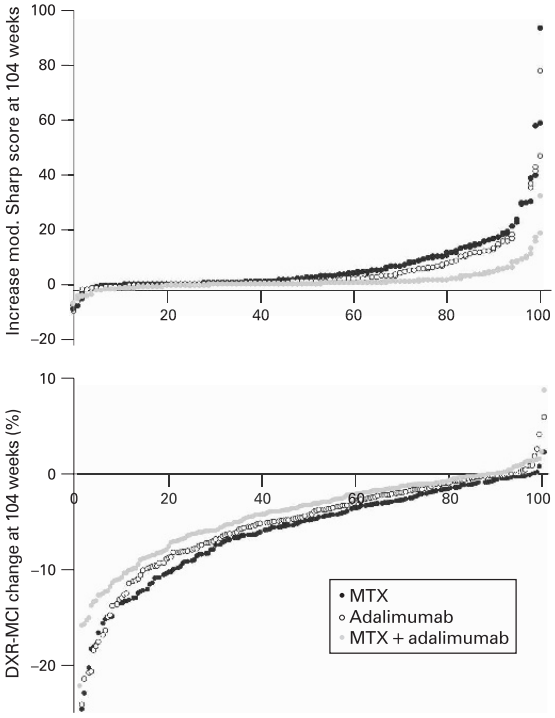

Figure 3 Cumulative probability plot: changes in DXR–MCI and radiographic scores at 104 weeks in PREMIER. DXR, digital *x* ray radiogrammetry; MCI, metacarpal cortical index; mod Sharp score, modified total Sharp score; MTX, methotrexate.

Multivariate model

At all time points the variables included in the final multivariate model were baseline values of disease duration, DAS28 score, CRP, DXR–MCI, HAQ, radiographic damage and treatment group (dummy variable), together with age and gender.

At 52 and 104 weeks, older age, greater CRP and non-use of adalimumab turned out to be independent predictors for cortical hand bone loss. At 26 weeks, female gender and greater CRP were predictors of cortical hand bone loss. The model for 104 weeks is depicted in table 2.

DISCUSSION

The key finding of this analysis was that anti-TNF therapy with adalimumab in combination with methotrexate provided better bone protection than either adalimumab or methotrexate monotherapies in patients with early, aggressive RA. The order of hand bone loss across the three treatment arms was the same as has been observed for overall radiographic damage in the PREMIER study (fig 2). Furthermore, the results from the multivariate model highlight the importance of inflammation (assessed with CRP) as the driving force for bone damage in active RA and the importance of TNF involvement in this process.

The present analysis adds evidence to the hypothesis that both erosions and osteoporosis are a result of the same pathophysiological mechanism, which includes activation of the osteoclast cell. This hypothesis is based on findings from both animal[6 28] and human[7] studies.

Convincing data exist that the suppression of inflammation is important to avoid bone damage in RA. Anti-TNF therapy has in several clinical trials been shown to reduce the progression of joint erosion.[9–11] Furthermore, there is also evidence that anti-TNF therapy reduces osteoporosis at the hip and spine.[4 12–14]

The ability of anti-inflammatory treatment to reduce hand bone loss in RA has been demonstrated in a double-blind study comparing oral prednisolone 7.5 mg/day for 2 years with placebo. The prednisolone group had less hand BMD loss at 1 and 2 years, suggesting that the potent anti-inflammatory effect of prednisolone exceeded its negative effect on bone.[17] With respect to the effects of anti-TNF therapy on hand bone loss in RA, only a few studies have been conducted. RA patients treated with anti-TNF therapy have been shown to have a lower rate of bone loss at the spine and hip than at the hand.[4 14] In a 2-year longitudinal treatment strategy study (the BeST study), RA patients treated with anti-TNF therapy or high-dose prednisolone were shown to have a lower rate of bone loss at the hand than patients treated with conventional DMARD.[4] Furthermore, in a study employing quantitative ultrasound, the use of anti-TNF therapy had a positive effect on periarticular bone.[16] The beneficial effect of anti-TNF treatment on inflammatory-related hand bone loss in RA is supported by our observations.

Methotrexate has been reported to have negative effects on bone and the term "methotrexate osteopathy" has been used to describe a clinical syndrome characterised by stress fractures, diffuse bone pain and osteoporosis in children treated for malignancies.[29] In animal studies high-dose methotrexate has been shown to induce apoptosis and suppress proliferation of the growth plate chondrocytes as well as proliferation of the osteoblasts and preosteoblasts.[30] However, low-dose methotrexate (5–20 mg/week) both in cross-sectional[31 32] and longitudinal[33] studies has not shown any negative effect of methotrexate on bone.

Despite the fact that bone loss was considerably lower in the combination group than in the methotrexate group, these patients were still losing hand bone. This loss may have been a result of the substantial disease activity in the PREMIER RA patients and their poor prognosis in terms of bone damage (rheumatoid factor-positive and erosive disease).[34]

The positive effects of anti-TNF therapy seemed to be more pronounced for radiographic joint damage than for hand bone mass (fig 2). One explanation for this may be that conventional radiographs are not sensitive enough to detect bone damage. Both ultrasound and magnetic resonance imaging (MRI) have been demonstrated to be more sensitive than radiographs in detecting erosions.[35] Furthermore, MRI can detect erosions years before they become visible on radiographs[36] and MRI synovitis has been detected in RA patients in both clinical and radiographic remission.[37] Although MRI and ultrasound are sensitive to detect erosions, there are still some limitations for clinical use due to availability and the lack of validated scoring systems.

Hand bone loss assessed by dual *x* ray absorptiometry has also been shown to be a more sensitive marker for bone damage than conventional radiographs.[15] Therefore, the combination of ever-present inflammation in patients with greater disease activity, as well as the ability of DXR to detect small changes in bone mass, may explain the ongoing loss of hand bone. It is also important to note the influence of normal age-related bone loss that takes place in healthy adults, especially postmenopausal women. Normal bone loss for DXR–MCI has only been examined in cross-sectional studies reporting an annual rate of bone loss between 0.7% and 0.9%.[21 38 39]

When this analysis was planned, we intended to analyse radiographs primarily for DXR–BMD, but for reasons described in the Methods section, there were difficulties in analysing a large percentage of the radiographs for DXR–BMD. By using the relative DXR–MCI measure instead of the absolute measure of BMD, we lost the opportunity to correct for porosity. Furthermore, DXR–BMD, as opposed to DXR–MCI, is calibrated for blurring and particular qualities of the different radiographic measurement equipment. However, DXR has improved the precision of MCI[22] and there is a strong correlation between DXR–BMD and DXR–MCI ($r > 0.9$).[24 25] DXR–MCI and DXR–BMD have also been found to be greatly correlated with dual *x* ray absorptiometry–BMD.[25] On the basis of these facts, we believe DXR–MCI to be a valid surrogate measure of hand bone mass.

Another limitation was our inability to retrieve information on the use of bisphosphonates. This may be of importance as treatment with bisphosphonates increases bone density. For the potent bisphosphonate, zoledronic acid, the suppression of osteoclast activity has even been shown to reduce the progression of erosions both in animal[40] and human[7] studies. Anti-TNF therapy inhibits the osteoclast by suppressing inflammation and decreases the RANK/RANKL pathway, while the aminobisphosphonate zoledronic acid acts directly on osteoclasts. However, we believe that the study design of a double-blind, randomised controlled trial has minimised the effect of this potential bias. In addition, zoledronic acid was not on the market for osteoporosis treatment when the PREMIER study was conducted. Furthermore, in an observational study the positive effect of infliximab on bone was found to be independent of bisphosphonate use.[12]

In conclusion, our analysis of data from PREMIER provides evidence that potent anti-TNF therapy not only reduces the risk of developing erosions, but also reduces the rate of inflammatory-related hand bone loss in RA. This study also suggests that the bone damage disease process is still present in RA patients treated with TNF antagonists, even if radiographic joint damage on radiographs is apparently arrested. Based on the findings from the present and previous studies, quantitative measures of hand bone loss in RA patients can be recommended as outcomes for future clinical trials to detect ongoing bone damage.

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Ethics approval: The PREMIER study was approved by a central institutional review board and independent ethics committees at each participating site.

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Paper IV

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Original Article

Short-Time In Vitro and In Vivo Precision of Direct Digital X-ray Radiogrammetry

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Abstract

Digital X-ray radiogrammetry (DXR) calculates peripheral bone mineral density (BMD) from hand radiographs. The aim of this study was to examine in vitro and in vivo precision for the new direct digital version of DXR, a development of the conventional DXR. The in vitro precision for direct DXR was tested on 4 different X-ray equipments, based on 31 radiographs of the same phantom. The in vivo precision was based on duplicate hand radiographs from both hands in 39 individuals. For the 4 X-ray equipments, in vitro precision ranged from 0.14% to 0.30%, expressed as coefficient of variations (CV%) and from 0.0012 to 0.0028 g/cm^2, expressed as smallest detectable difference (SDD). The precision was correlated to the resolution of the radiographic equipment ($r = 0.95$, $p = 0.05$). The corresponding values for the in vivo precision for mean values of both hands were: CV% = 0.46%; SDD = 0.0046 g/cm^2, and least significant change (LSC%) = 1.28%. The DXR-BMD for 1 of the X-ray equipments differed 1.1% from the overall mean. The precision for direct DXR was highly satisfactory both in vitro and in vivo. DXR-BMD values may differ between the radiographic equipments, and follow-up measurements should be performed with the same X-ray equipment.

Key Words: Digital X-ray radiogrammetry; Hand bone mineral density; Periarticular osteoporosis; Precision; Rheumatoid arthritis.

Introduction

Dual-energy X-ray absorptiometry (DXA) measurements at hip and spine are considered the gold standard for detection and management of osteoporosis *(1–3)*. Because DXA is considered as a resource-demanding method, there is a need for less expensive and more feasible methods to measure bone mineral density (BMD). In the past decade, several peripheral BMD measurements have been developed *(4)*, including digital X-ray radiogrammetry (DXR) *(5)*. DXR calculating BMD at metacarpal hand bones on radiographs is a further development of the classic radiogrammetry method first described in 1960 *(6)*. DXR-BMD has been shown to be a good predictor of fractures *(7)* and radiographic joint damage in rheumatoid arthritis (RA) *(8,9)*, and is also a response variable for anti-inflammatory treatment *(10,11)*.

For the DXR equipment that assesses bone density from scanned conventional hand radiographs (Pronosco X-posure, Sectra, LinKöping, Sweden), the short-time precision is shown to be superior compared with DXA assessed at hand, hip, and spine *(12,13)*. The DXR method has been further developed, and DXR-BMD can now also be analyzed directly from digital hand radiographs (direct DXR).

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The precision for direct DXR has not been fully investigated, and the objective of this study was to examine both in vitro and in vivo reproducibility for the direct version of DXR.

Methods

Direct DXR (Sectra, Linköping, Sweden), a further development of the DXR method developed for BMD calculations on conventional hand radiographs, was used to measure hand BMD on radiographs made in digitized format. DXR is a computer version of the traditional radiogrammetry technique *(6)*, and has been described in detail *(5,14)*. The computer automatically recognizes regions of interest around the narrowest part of the second, third, and fourth metacarpal bone on hand radiographs and measure cortical thickness, bone width, and porosity 118 times per cm. DXR-BMD is defined as: $c \times VPA_{comb} \times (1 - p)$, where c is a density constant, VPA is volume per area, and p is porosity.

In the in vitro study, hand DXR-BMD was measured on the same cadaver forearm phantom 31 times with repositioning of the phantom between each radiograph, tested on four different standard X-ray equipments. All digitized radiographs were sent to Sectra and analyzed with the same software. The four X-ray equipments (computed radiography (CR)/digital radiography (DR); film focus distance (FFD); tube voltage; exposure dose) were: Fuji FCR XG1 (Fujifilm Corporation, Tokyo; Japan) (CR; 100 cm; 50 kV; 5mAs), Fuji FCR Profect (Fujifilm Corporation, Tokyo; Japan) (CR; 100 cm, 40 kV; 8mAs), Agfa ADC Compact plus (Afga-Gevaert N.V., Mortsel, Belgium)(CR; 100 cm; 50 kV; 5mAs) and Sectra MicroDose D40 (Sectra, Linköping, Sweden)(DR; built-in; 35kV; 10mAs) (Table 1).

The participants for the in vivo precision study were recruited from consecutive individuals visiting an osteoporosis outpatient clinic, and were selected according to their BMD total hip values, 20 with osteoporosis and 20 with normal values. Unfortunately, 1 radiograph could not be analyzed for DXR-BMD. Thus, 19 individuals with osteoporosis and 20 with normal DXA-BMD values were included. All participants underwent duplicate hand radiographs with reposition between each image. The radiographs were acquired with Fuji FCR XG1 (CR; FFD = 100 cm; tube voltage = 50 kV; exposure dose = 5 mA).

The study was based on the current International Society for Clinical Densitometry recommendation doing precision analyses with at least 30 degrees of freedom *(13)* (http://www.iscd.org).

Statistics

Regarding the in vitro study, differences between precision for the four digitized equipments were calculated according to Levene's test for variances. Bonferroni approach was used to adjust for multiple comparisons. Standard deviation (SD) was calculated as $SD = \sqrt{\sum_{i=1}^{nj}(x_{ij}-\bar{x})^2/n_j-1}$*(15)*, and coefficient of variation (CV%) was defined as (SD/mean) × 100. The measurement error was calculated using Bland Altman 95% limits of agreement method *(16)*. This gives an absolute and metric estimate of random measurement error, also called smallest detectable difference (SDD). Most disagreements between measurements are expected to be between limits called "limits of agreements," defined as: $d \pm z_{(1-\alpha/2)} \times SD$, where d is the mean difference between the measurements and $z_{(1-\alpha/2)}$ is the $100(1-\alpha/2)$th centile of the normal distribution. The mean difference (d) is expected to be 0, because we do not assume a true change in BMD to occur between the measurements *(16,17)*.

In the in vivo study with 2 observations for each subject, the SD of the difference $(SD_{diff}) = \sqrt{(\sum(a_i-b_i)^2)/2n}$ estimated the within variability of the measurements. This gives a $CV\% = (\sqrt{((\sum(a_i-b_i)^2)/2n)})/((M_a+M_b)/2) \times 100$ (where a_i and b_i are the 2 measures from the same individual [i], M_a and M_b are mean values of a and b, and n is the number of paired observations) *(15,17)*. SDD was calculated in the same way as in the in vitro study, but SD_{diff} was used instead of SD ($SDD = d \pm z_{(1-\alpha/2)} \times SD_{diff}$) *(15)*. The least significant change (LSC%), the percentage change in BMD in an individual, which is considered to be statistically significant, was also calculated ($LSC\% = z_{(1-\alpha/2)} \times \sqrt{2} \times CV\%$) *(15,18)*.

Statistical analyses were performed with SPSS version 14 (SPSS inc., Chicago, IL) and Excel (Microsoft Office, Microsoft Corporation).

Table 1
Precision Data for the In Vitro Digital Radiographs for DXR-BMD Tested in 4 Different X-ray Equipments

X-ray equipment center	Resolution (mm/pixel)	Mean BMD value (g/cm^2)	Variance	CV%	SDD (g/cm^2)
Fuji FCR XG1, Kristiansand	0.100	0.471	1.0E-06	0.22	0.0020
Agfa ADC Compact plus, Trondheim	0.114	0.472	2.1E-06[b]	0.30	0.0028
Fuji FCR Profect, Helsingborg	0.050	0.472	6.1E-07	0.16	0.0015
Sectra MicroDose, Helsingborg	0.049	0.465[a]	3.9E-07	0.14	0.0012

Abbr: DXR, digital X-ray radiogrammetry; BMD, bone mineral density; CV%, coefficient of variation; SDD, smallest detectable difference.

[a]Differ by 1.1% from the overall mean.

[b]Fuji FCR Profect and Sectra MicroDose had a significantly better variance than the Agfa ADC Compact plus equipment.

Ethics and Legal Aspects

The in vivo study was approved by the regional committee for ethics and medical research.

Results

In Vitro

The in vitro precision data (variance, CV%, and SDD) for the 4 X-ray equipments are shown in Table 1. The DXR-BMD mean for all measurements was 0.470 mg/cm^2. The DXR-BMD mean for the most diverging X-ray equipment, the Sectra MicroDose, differed 1.1% from this overall mean (Table 1 and Fig. 1).

The precision for the 4 X-ray equipments tested ranged from 0.14% to 0.30% for CV% and from 0.0012 to 0.0028 g/cm^2 for SDD (Table 1). The resolution and the precision of the equipment correlated significantly ($r = 0.95$, $p = 0.05$), that is, high resolution resulted in high precision. Fuji FCR Profect and Sectra MicroDose had a significantly better variance than the Agfa ADC Compact plus ($p < 0.001$).

In Vivo

The in vivo precision results for direct DXR-BMD are displayed in Table 2. A Bland Altman plot for direct DXR is depicted in Fig. 2. The CV% for DXR-BMD was 0.61 for the nondominant hand, and improved to 0.46 using mean DXR-BMD of both hands. The mean DXR-BMD for the osteoporotic group was 0.435 mg/cm^2, whereas the mean DXR-BMD for the individuals with no osteoporosis was 0.596 mg/cm^2. There was no significant difference for the CV% between these 2 groups (0.45% for both; $p = 0.36$ between variances).

Table 2
Precision Data for the In Vivo Digital Radiographs (Fuji FCR XG1)

	DXR-BMD (g/cm^2)	
Precision Data	Nondominant hand	Mean of both hands
Mean (SD)	0.506 (0.108)	0.510 (0.110)
SD difference	0.003	0.002
SDD, 95% limits of agreement	0.0061	0.0046
CV%	0.61	0.46
LSC%	1.71	1.28

Abbr: DXR, digital X-ray radiogrammetry; BMD, bone mineral density; SD, standard deviation; SDD, smallest detectable difference; CV%, coefficient of variance; LSC%, least significant change.

Discussion

The short-time precision, both in vitro and in vivo, for direct DXR-BMD was highly satisfactory. The utility of DXR-BMD in research and clinical practice has been demonstrated in several studies *(7,8,19,20)*, and our observation supports the fact that DXR-BMD is a robust measure.

The in vitro precision for the 4 tested X-ray equipments expressed as CV% ranged from 0.14% to 0.30%. The reproducibility was dependent on the resolution capacity of the tested X-ray equipments as shown in Table 1.

The in vivo precision for direct DXR-BMD was comparable to that reported for scanned DXR-BMD (CV%: 0.28−0.68%) *(5,14,21)*. The new direct DXR is more feasible to use than scanned DXR, as it does not require any manual

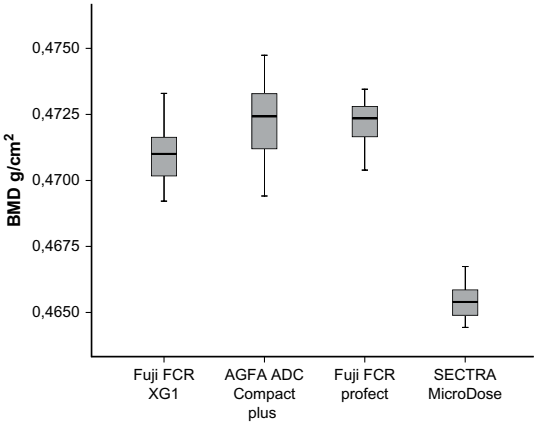

Fig. 1. Cadaver phantom bone mineral density values and variance in the 4 tested X-ray equipments (Box-and-whisker plot: the marked line shows the median, the outer linings of the box express the interquartile range, and the whiskers express the 95 percentage central range).

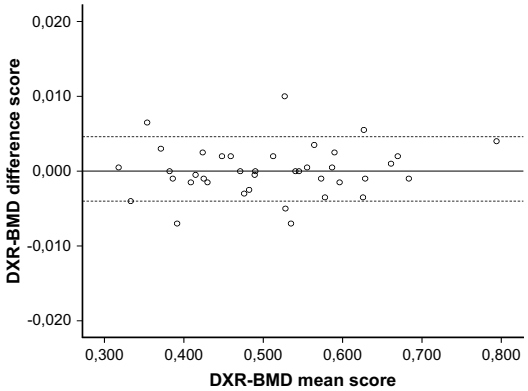

Fig. 2. Graph of the difference score (g/cm^2) against the mean score (g/cm^2) of the 2 digital X-ray radiogrammetry-bone mineral density measurements in the in vivo study (Bland Altman plot). The dashed lines represent the 95% limits of agreement.

scanning of the radiographs. The precision improved when using mean values of both hands. In this study, the CV% was reduced from 0.61% for BMD values of the nondominant hand to 0.46% for the mean BMD values of both hands. Thus, X-ray equipments with high resolution and mean BMD values of both hands should be used to obtain the best precision for DXR-BMD measurements. For use on the individual level, a change of 1.28% (=LSC%) in DXR-BMD by the use of mean BMD values of both hands is required to identify true bone loss exceeding the measurement error.

It appeared that the precision in the in vitro study was better than that in the in vivo study. One reason may be that the phantom in the in vitro study is fixed in one position, whereas the human hands may vary in position. This may especially be of importance in patients with RA, who might have deformities or inflammation in their hands. Another reason may be the difference in the number of images. Only 2 radiographs were performed on humans because of the radiation exposure, whereas there were 31 images of the phantom at each center.

The reproducibility for DXR was better than previous precision calculations for DXA measurements. The CV% for DXA is reported to be 0.80−1.00% (or an LSC% of 2.22−2.77) at whole hand *(22−24)*, 0.88−1.59% for total hip, and 0.88−2.00% for lumbar spine *(17,25,26)*. Further, the precision for DXA is shown to be influenced by the BMD in the examined patients, that is, low BMD values are reported to have poorer precision than high BMD values *(25,26)*. Subanalysis in the present study suggests that this is not the case with DXR-BMD, but further studies including more patients are needed to clarify this (Fig. 2).

Another important observation was that the X-ray equipments measured different values for the DXR-BMD. The Sectra MicroDose differed 1.1% from the overall mean BMD value (Fig. 1 and Table 1). The most likely explanation may be the measurements of porosity. The X-ray equipment that takes a sharply defined picture may detect porosity better. The BMD value will then be lower for high-porosity bones and higher for low-porosity bones. This may explain why the Sectra MicroDose, which is a high-resolution mammography system, measured a different BMD value than the other equipments. Other reasons for the differences in BMD may be different spectra of the X-rays or blurring of the radiographs. These observations support the fact that the same X-ray equipment should be used for longitudinal follow up of individuals. Regarding the analyses of conventional X-rays, the precision does not seem to be influenced either by film brand, FFD, or exposure level, but tube voltage may have an influence *(27,28)*. To our knowledge, there is a lack of data on long-term reproducibility for both scanned and direct DXR.

The ability of DXR to detect even small changes in BMD makes the method suitable for assessment at the individual level. In a study of osteoporotic women, annual DXR-BMD loss was found to be 0.004 g/cm^2 or 0.8% *(29)*. In RA patients, the annual loss of DXR-BMD has been shown to range from 1.7% (or an absolute median value of 0.009 g/cm^2) *(8)* to as much as a mean loss of 10% in patients with a high disease activity *(19)*. This means that the DXR method has the capacity to capture a significant DXR-BMD change over a short period of time. When combining these results with the LSC% of 1.28%, a significant DXR-BMD loss can be detected at 1.5 months in RA patients with high disease activity (monitoring time interval = LSC%/annual percentage loss) *(30)*. Early loss of DXR-BMD in RA patients is shown to predict subsequent radiographic damage, and a rapid loss of hand DXR-BMD may, therefore, be a candidate predictor for poor prognosis *(8,9)*.

A limitation of this study is that different methods were used in the in vitro and in vivo part of the study. However, to expose patients with radiation with the same number of X-rays as we did with the phantom were considered as unethical. Another limitation for the in vivo precision study was that no patients with RA were included.

In conclusion, the direct DXR method had highly satisfactory precision, and may, thus, be a potential important research and clinical tool for short-time follow-up of both osteoporotic and RA patients. Concerning the RA patients, it is an advantage that DXR-BMD can be analyzed on the same radiographs used for follow-up destruction, making the method feasible. It is important that follow-up radiographs are taken with the same X-ray equipment. Long-time precision studies are warranted to further validate the method.

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40. Kjell-Arne Rein: THE EFFECT OF EXTRACORPOREAL CIRCULATION ON SUBCUTANEOUS TRANSCAPILLARY FLUID BALANCE.
41. Arne Kristian Sandvik: RAT GASTRIC HISTAMINE.
42. Carl Bredo Dahl: ANIMAL MODELS IN PSYCHIATRY.

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43. Torbjørn A. Fredriksen: CERVICOGENIC HEADACHE.
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46. Nils Petter Jørgensen: DRUG EXPOSURE IN EARLY PREGNANCY.
47. Johan C. Ræder: PREMEDICATION AND GENERAL ANAESTHESIA IN OUTPATIENT GYNECOLOGICAL SURGERY.
48. M. R. Shalaby: IMMUNOREGULATORY PROPERTIES OF TNF-α AND THE RELATED CYTOKINES.
49. Anders Waage: THE COMPLEX PATTERN OF CYTOKINES IN SEPTIC SHOCK.
50. Bjarne Christian Eriksen: ELECTROSTIMULATION OF THE PELVIC FLOOR IN FEMALE URINARY INCONTINENCE.
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55. Eva Hofsli: TUMOR NECROSIS FACTOR AND MULTIDRUG RESISTANCE.
56. Helge S. Haarstad: TROPHIC EFFECTS OF CHOLECYSTOKININ AND SECRETIN ON THE RAT PANCREAS.
57. Lars Engebretsen: TREATMENT OF ACUTE ANTERIOR CRUCIATE LIGAMENT INJURIES.
58. Tarjei Rygnestad: DELIBERATE SELF-POISONING IN TRONDHEIM.
59. Arne Z. Henriksen: STUDIES ON CONSERVED ANTIGENIC DOMAINS ON MAJOR OUTER MEMBRANE PROTEINS FROM ENTEROBACTERIA.
60. Steinar Westin: UNEMPLOYMENT AND HEALTH: Medical and social consequences of a factory closure in a ten-year controlled follow-up study.
61. Ylva Sahlin: INJURY REGISTRATION, a tool for accident preventive work.
62. Helge Bjørnstad Pettersen: BIOSYNTHESIS OF COMPLEMENT BY HUMAN ALVEOLAR MACROPHAGES WITH SPECIAL REFERENCE TO SARCOIDOSIS.
63. Berit Schei: TRAPPED IN PAINFUL LOVE.
64. Lars J. Vatten: PROSPECTIVE STUDIES OF THE RISK OF BREAST CANCER IN A COHORT OF NORWEGIAN WOMAN.

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65. Kåre Bergh: APPLICATIONS OF ANTI-C5a SPECIFIC MONOCLONAL ANTIBODIES FOR THE ASSESSMENT OF COMPLEMENT ACTIVATION.
66. Svein Svenningsen: THE CLINICAL SIGNIFICANCE OF INCREASED FEMORAL ANTEVERSION.
67. Olbjørn Klepp: NONSEMINOMATOUS GERM CELL TESTIS CANCER: THERAPEUTIC OUTCOME AND PROGNOSTIC FACTORS.
68. Trond Sand: THE EFFECTS OF CLICK POLARITY ON BRAINSTEM AUDITORY EVOKED POTENTIALS AMPLITUDE, DISPERSION, AND LATENCY VARIABLES.
69. Kjetil B. Åsbakk: STUDIES OF A PROTEIN FROM PSORIATIC SCALE, PSO P27, WITH RESPECT TO ITS POTENTIAL ROLE IN IMMUNE REACTIONS IN PSORIASIS.
70. Arnulf Hestnes: STUDIES ON DOWN´S SYNDROME.
71. Randi Nygaard: LONG-TERM SURVIVAL IN CHILDHOOD LEUKEMIA.
72. Bjørn Hagen: THIO-TEPA.
73. Svein Anda: EVALUATION OF THE HIP JOINT BY COMPUTED TOMOGRAMPHY AND ULTRASONOGRAPHY.

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74. Martin Svartberg: AN INVESTIGATION OF PROCESS AND OUTCOME OF SHORT-TERM PSYCHODYNAMIC PSYCHOTHERAPY.
75. Stig Arild Slørdahl: AORTIC REGURGITATION.
76. Harold C Sexton: STUDIES RELATING TO THE TREATMENT OF SYMPTOMATIC NON-PSYCHOTIC PATIENTS.
77. Maurice B. Vincent: VASOACTIVE PEPTIDES IN THE OCULAR/FOREHEAD AREA.
78. Terje Johannessen: CONTROLLED TRIALS IN SINGLE SUBJECTS.
79. Turid Nilsen: PYROPHOSPHATE IN HEPATOCYTE IRON METABOLISM.
80. Olav Haraldseth: NMR SPECTROSCOPY OF CEREBRAL ISCHEMIA AND REPERFUSION IN RAT.
81. Eiliv Brenna: REGULATION OF FUNCTION AND GROWTH OF THE OXYNTIC MUCOSA.

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82. Gunnar Bovim: CERVICOGENIC HEADACHE.
83. Jarl Arne Kahn: ASSISTED PROCREATION.
84. Bjørn Naume: IMMUNOREGULATORY EFFECTS OF CYTOKINES ON NK CELLS.
85. Rune Wiseth: AORTIC VALVE REPLACEMENT.
86. Jie Ming Shen: BLOOD FLOW VELOCITY AND RESPIRATORY STUDIES.
87. Piotr Kruszewski: SUNCT SYNDROME WITH SPECIAL REFERENCE TO THE AUTONOMIC NERVOUS SYSTEM.
88. Mette Haase Moen: ENDOMETRIOSIS.
89. Anne Vik: VASCULAR GAS EMBOLISM DURING AIR INFUSION AND AFTER DECOMPRESSION IN PIGS.
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91. Kjell Å. Salvesen: ROUTINE ULTRASONOGRAPHY IN UTERO AND DEVELOPMENT IN CHILDHOOD.

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92. Nina-Beate Liabakk: DEVELOPMENT OF IMMUNOASSAYS FOR TNF AND ITS SOLUBLE RECEPTORS.
93. Sverre Helge Torp: *erb*B ONCOGENES IN HUMAN GLIOMAS AND MENINGIOMAS.
94. Olav M. Linaker: MENTAL RETARDATION AND PSYCHIATRY. Past and present.
95. Per Oscar Feet: INCREASED ANTIDEPRESSANT AND ANTIPANIC EFFECT IN COMBINED TREATMENT WITH DIXYRAZINE AND TRICYCLIC ANTIDEPRESSANTS.
96. Stein Olav Samstad: CROSS SECTIONAL FLOW VELOCITY PROFILES FROM TWO-DIMENSIONAL DOPPLER ULTRASOUND: Studies on early mitral blood flow.
97. Bjørn Backe: STUDIES IN ANTENATAL CARE.
98. Gerd Inger Ringdal: QUALITY OF LIFE IN CANCER PATIENTS.
99. Torvid Kiserud: THE DUCTUS VENOSUS IN THE HUMAN FETUS.
100. Hans E. Fjøsne: HORMONAL REGULATION OF PROSTATIC METABOLISM.
101. Eylert Brodtkorb: CLINICAL ASPECTS OF EPILEPSY IN THE MENTALLY RETARDED.
102. Roar Juul: PEPTIDERGIC MECHANISMS IN HUMAN SUBARACHNOID HEMORRHAGE.
103. Unni Syversen: CHROMOGRANIN A. Phsysiological and Clinical Role.

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104. Odd Gunnar Brakstad: THERMOSTABLE NUCLEASE AND THE *nuc* GENE IN THE DIAGNOSIS OF *Staphylococcus aureus* INFECTIONS.
105. Terje Engan: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF PLASMA IN MALIGNANT DISEASE.
106. Kirsten Rasmussen: VIOLENCE IN THE MENTALLY DISORDERED.
107. Finn Egil Skjeldestad: INDUCED ABORTION: Timetrends and Determinants.
108. Roar Stenseth: THORACIC EPIDURAL ANALGESIA IN AORTOCORONARY BYPASS SURGERY.
109. Arild Faxvaag: STUDIES OF IMMUNE CELL FUNCTION *in mice infected with* MURINE RETROVIRUS.

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110. Svend Aakhus: NONINVASIVE COMPUTERIZED ASSESSMENT OF LEFT VENTRICULAR FUNCTION AND SYSTEMIC ARTERIAL PROPERTIES. Methodology and some clinical applications.
111. Klaus-Dieter Bolz: INTRAVASCULAR ULTRASONOGRAPHY.
112. Petter Aadahl: CARDIOVASCULAR EFFECTS OF THORACIC AORTIC CROSS-CLAMPING.
113. Sigurd Steinshamn: CYTOKINE MEDIATORS DURING GRANULOCYTOPENIC INFECTIONS.
114. Hans Stifoss-Hanssen: SEEKING MEANING OR HAPPINESS?
115. Anne Kvikstad: LIFE CHANGE EVENTS AND MARITAL STATUS IN RELATION TO RISK AND PROGNOSIS OF CANCER.
116. Torbjørn Grøntvedt: TREATMENT OF ACUTE AND CHRONIC ANTERIOR CRUCIATE LIGAMENT INJURIES. A clinical and biomechanical study.
117. Sigrid Hørven Wigers: CLINICAL STUDIES OF FIBROMYALGIA WITH FOCUS ON ETIOLOGY, TREATMENT AND OUTCOME.
118. Jan Schjøtt: MYOCARDIAL PROTECTION: Functional and Metabolic Characteristics of Two Endogenous Protective Principles.
119. Marit Martinussen: STUDIES OF INTESTINAL BLOOD FLOW AND ITS RELATION TO TRANSITIONAL CIRCULATORY ADAPATION IN NEWBORN INFANTS.
120. Tomm B. Müller: MAGNETIC RESONANCE IMAGING IN FOCAL CEREBRAL ISCHEMIA.
121. Rune Haaverstad: OEDEMA FORMATION OF THE LOWER EXTREMITIES.
122. Magne Børset: THE ROLE OF CYTOKINES IN MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO HEPATOCYTE GROWTH FACTOR.
123. Geir Smedslund: A THEORETICAL AND EMPIRICAL INVESTIGATION OF SMOKING, STRESS AND DISEASE: RESULTS FROM A POPULATION SURVEY.

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124. Torstein Vik: GROWTH, MORBIDITY, AND PSYCHOMOTOR DEVELOPMENT IN INFANTS WHO WERE GROWTH RETARDED *IN UTERO*.
125. Siri Forsmo: ASPECTS AND CONSEQUENCES OF OPPORTUNISTIC SCREENING FOR CERVICAL CANCER. Results based on data from three Norwegian counties.
126. Jon S. Skranes: CEREBRAL MRI AND NEURODEVELOPMENTAL OUTCOME IN VERY LOW BIRTH WEIGHT (VLBW) CHILDREN. A follow-up study of a geographically based year cohort of VLBW children at ages one and six years.
127. Knut Bjørnstad: COMPUTERIZED ECHOCARDIOGRAPHY FOR EVALUTION OF CORONARY ARTERY DISEASE.
128. Grethe Elisabeth Borchgrevink: DIAGNOSIS AND TREATMENT OF WHIPLASH/NECK SPRAIN INJURIES CAUSED BY CAR ACCIDENTS.
129. Tor Elsås: NEUROPEPTIDES AND NITRIC OXIDE SYNTHASE IN OCULAR AUTONOMIC AND SENSORY NERVES.
130. Rolf W. Gråwe: EPIDEMIOLOGICAL AND NEUROPSYCHOLOGICAL PERSPECTIVES ON SCHIZOPHRENIA.
131. Tonje Strømholm: CEREBRAL HAEMODYNAMICS DURING THORACIC AORTIC CROSSCLAMPING. An experimental study in pigs.

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132. Martinus Bråten: STUDIES ON SOME PROBLEMS REALTED TO INTRAMEDULLARY NAILING OF FEMORAL FRACTURES.
133. Ståle Nordgård: PROLIFERATIVE ACTIVITY AND DNA CONTENT AS PROGNOSTIC INDICATORS IN ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK.

134. Egil Lien: SOLUBLE RECEPTORS FOR **TNF** AND **LPS**: RELEASE PATTERN AND POSSIBLE SIGNIFICANCE IN DISEASE.
135. Marit Bjørgaas: HYPOGLYCAEMIA IN CHILDREN WITH DIABETES MELLITUS
136. Frank Skorpen: GENETIC AND FUNCTIONAL ANALYSES OF DNA REPAIR IN HUMAN CELLS.
137. Juan A. Pareja: SUNCT SYNDROME. ON THE CLINICAL PICTURE. ITS DISTINCTION FROM OTHER, SIMILAR HEADACHES.
138. Anders Angelsen: NEUROENDOCRINE CELLS IN HUMAN PROSTATIC CARCINOMAS AND THE PROSTATIC COMPLEX OF RAT, GUINEA PIG, CAT AND DOG.
139. Fabio Antonaci: CHRONIC PAROXYSMAL HEMICRANIA AND HEMICRANIA CONTINUA: TWO DIFFERENT ENTITIES?
140. Sven M. Carlsen: ENDOCRINE AND METABOLIC EFFECTS OF METFORMIN WITH SPECIAL EMPHASIS ON CARDIOVASCULAR RISK FACTORES.

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141. Terje A. Murberg: DEPRESSIVE SYMPTOMS AND COPING AMONG PATIENTS WITH CONGESTIVE HEART FAILURE.
142. Harm-Gerd Karl Blaas: THE EMBRYONIC EXAMINATION. Ultrasound studies on the development of the human embryo.
143. Noèmi Becser Andersen:THE CEPHALIC SENSORY NERVES IN UNILATERAL HEADACHES. Anatomical background and neurophysiological evaluation.
144. Eli-Janne Fiskerstrand: LASER TREATMENT OF PORT WINE STAINS. A study of the efficacy and limitations of the pulsed dye laser. Clinical and morfological analyses aimed at improving the therapeutic outcome.
145. Bård Kulseng: A STUDY OF ALGINATE CAPSULE PROPERTIES AND CYTOKINES IN RELATION TO INSULIN DEPENDENT DIABETES MELLITUS.
146. Terje Haug: STRUCTURE AND REGULATION OF THE HUMAN UNG GENE ENCODING URACIL-DNA GLYCOSYLASE.
147. Heidi Brurok: MANGANESE AND THE HEART. A Magic Metal with Diagnostic and Therapeutic Possibilites.
148. Agnes Kathrine Lie: DIAGNOSIS AND PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL INTRAEPITELIAL NEOPLASIA. Relationship to Cell Cycle Regulatory Proteins and HLA DQBI Genes.
149. Ronald Mårvik: PHARMACOLOGICAL, PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STUDIES ON ISOLATED STOMACS.
150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

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158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING CLINICAL AND EXPERIMENTAL STUDIES
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160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.
161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.
162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.

163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.
168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

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178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT
192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS

193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM
198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

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201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

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216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.
217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN

218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES
223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

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235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
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