Diagnostic and Prognostic Potential of Joint Imaging in Patients with Anti-Citrullinated Protein Antibodies

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Cover/picture/Illustration/Design: Ultrasound image of an inflamed joint taken by the author

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Isidor's idea of TIRx /T-Rex

To

Edna,

Isidor & HugoMax

ABSTRACT

The introduction of novel therapeutic strategies set new goals for the patients' outcome, which aims to achieve remission. This goal requires early diagnosis of RA and prompt efficient pharmacotherapy. The introduction of anti-citrullinated protein antibodies (ACPA) two decades ago allowed an earlier RA diagnosis. However, there are indications that ACPA positivity is still associated with higher rates of radiographic damage. As the small joints in hands and feet commonly are the first involved sites of inflammation, the role of different imaging modalities were studied regarding their diagnostic and prognostic impact for assessment of arthritis in RA. Further, ultrasound (US) and radiography were used to study the association between RA-specific antibodies and the occurrence of arthritis and joint damage in systemic lupus erythematosus (SLE).

The use of US allows assessment of soft tissue like joint capsules, tendons and bursae. Used for a live scanning, it is easy to detect effusions and edema. Doppler indicates vasoproliferation where inflammation is present. Also, US seems to be more sensitive than radiography to detect minimal structural changes located at bone surfaces. We wanted to investigate whether US findings in a pre-RA stage can predict development of arthritis.

Digital X-ray radiogrammetry (DXR) is a technique based on computerized analyses of standard hand radiographs to calculate peripheral bone mineral density (BMD) of the three middle metacarpal bones (DXR-BMD). In order for early treatment decisions, we aimed to study whether changes in DXR-BMD loss after 3 months can predict radiographic damage in early RA.

In conclusion, the studies showed that ACPA-positivity is still associated with a higher risk of radiographic damage regardless of early treatment decisions. Therefore, close radiographic monitoring and readiness to intensive treatment is warranted in ACPA-positive patients. This thesis also shows that erosions detected by US in ACPA-positive patients with arthralgia predict development of clinical arthritis. Also, the magnitude of DXR-BMD loss helps identify patients at higher risk for future radiographic damage, and may therefore help to improve early treatment decisions. Finally, US and radiography confirm a higher rate of arthritis and erosions also in SLE patients who are positive for RA-specific antibodies.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Reumatoid artrit (RA) är en kronisk inflammatorisk ledsjukdom. Inflammationen kan i sin tur orsaka urkalkning av bentätheten med benskörhet och lefskada som följd. Typiska symtom är, svullnad, värmeökning och funktions nedsättning i lederna. Första symtomet är dock oftast värk som kan finnas redan långt innan en patient visar typiska fynd som svullnad i lederna eller förhöjda inflammatoriska värden. Förekomst av autoantikroppar som reumatoid faktor (RF) eller antikroppar mot citrullinerade proteiner (ACPA) kan hjälpa till att identifiera patienter med ökad risk att insjukna. Trots förbättrade behandlingsmöjligheter med nya antireumatiska läkemedel finns det i våra undersökningsresultat fortfarande tecken på lefskada (erosioner) på röntgen hos en del av patienterna. Dessa erosioner är vanligare hos patienter som är positiva för ACPA. Således finns det behov av att hitta undersökningsmetoder som kan hjälpa att diagnosticera RA ännu tidigare så att lämplig behandling med bromsmediciner kan initieras så tidigt som möjligt.

Vi ville därför undersöka om nyare undersökningsmetoder som ultraljud (UL) och digital X-ray radiogrammetry (DXR) kan hjälpa att identifiera patienter med ledvärk och ACPA positivitet. UL är en enkel undersökningsmetod som används på reumatologkliniker för bedömning av inflammation i leder, senor och slem säckar. Vi fann att inflammation i lederna av patienter med värk och ACPA positivitet är mycket vanligare jämfört med friska personer. De ACPA positiva patienter som redan hade små erosioner vid första UL-undersökningen hade klart högre risk att utveckla ledinflammationer senare i förloppet.

DXR är en automatiserad metod som beräknar benförlust i metacarpalbenen (mellanhandsbenen) 2-4 utav digitala röntgenbilder. Vi kunde se, att en högre benförlust under den första 3 månader var kopplat till ökad förekomst av röntgenologiskt påvisbara lefskador efter 1 år.

Våra studieresultat visar att UL kan bidra att identifiera patienter med ledvärk och ACPA som har hög risk att utveckla inflammatorisk ledsjukdom. Tidig behandling och rätt val av antireumatisk behandling hos dessa patienter kan förbättra prognosen. DXR kan hjälpa reumatologen att identifiera patienter som inom närmaste året kommer utveckla lefskada, men bidrar inte med prognostisk information på längre sikt.

PAPERS IN THE THESIS

- I Michael Ziegelasch**, Myrthe A. M. van Delft, Philip Wallin, Thomas Skogh, César Magro-Checa, Gerda M. Steup-Beekman, Leendert A. Trouw, Alf Kastbom and Christopher Sjöwall
Antibodies against carbamylated proteins and cyclic citrullinated peptides in systemic lupus erythematosus: results from two welldefined European cohort.
Arthritis Research & Therapy (2016) 18:289
- II Michael Ziegelasch**, Kristina Forslind, Thomas Skogh, Katrine Riklund, Alf Kastbom and Ewa Berglin
Decrease in bone mineral density during three months after diagnosis of early rheumatoid arthritis measured by digital X-ray radiogrammetry predicts radiographic joint damage after one year
Arthritis Research & Therapy (2017) 19:195
- III Michael Ziegelasch**, Antonia Boman, Klara Martinsson, Ingrid Thyberg, Claudia Jacobs, Britt-Marie Nyhäll-Wåhlin, Anna Svärd, Ewa Berglin, Solbritt Rantapää-Dahlqvist, Thomas Skogh, Alf Kastbom
ACPA-associated radiographic damage in early RA uncoupled from inflammation and initial treatment response
Manuscript (submitted)
- IV Michael Ziegelasch**, Emma Eloff, Hilde Berner-Hammer, Jan Cedergren, Klara Martinsson, Åsa Reckner, Thomas Skogh, Mattias Magnusson , Alf Kastbom
Joint erosions visible on ultrasound predict arthritis development in patients with anti-citrullinated protein antibodies and musculoskeletal pain but no swollen joints
Manuscript

ABBREVIATIONS

ACPA – Anti-citrullinated peptide/protein antibodies
ACR – American college of rheumatology
bDMARD – Biologic disease modifying anti-rheumatic drug
BMD – Bone mineral density
CCP – Cyclic citrullinated peptides
CDAI – Clinical disease activity index
CRP – C-reactive protein
csDMARD – Conventional synthetic disease modifying anti-rheumatic drug
DAS28 – 28-joint Disease Activity Score
DMARD – Disease modifying anti-rheumatic drug
DXA – Dual energy X-ray absorptiometry
ESR – Erythrocyte sedimentation rate
EULAR – European league against rheumatism
GC – Glucocorticosteroids
GS – Grey scale
HAQ – Health assessment questionnaire
HLA – Human leukocyte antigen
IL - Interleukin
MSK – Musculoskeletal
MTX – Methotrexate
NSAID – Non steroidal anti-inflammatory drugs
PD – Power Doppler
RA – Rheumatoid arthritis
RF – Rheumatoid factor
SDAI – Simplified disease activity index
SJ – Swollen joints
SE – Shared epitope
SLE – Systemic lupus erythematosus
SLICC – Systemic Lupus International Collaborating Clinics
T2T – Treat to target
TJ – Tender joints
TNF- α – Tumor necrosis factor-alpha
US - Ultrasound
VAS – Visual analogue scale

1. INTRODUCTION

1.1. Rheumatic diseases and arthritis

Rheumatism is translated from old Greek and means “flow”. It was described already 1591 in *Liber de Rheumatismo et pleuritide dorsali* by Guillaume de Baillou. In his opinion, rheumatism was caused by any kind of cold secretion flowing from the brain down to the extremities. Accumulated in hands and feet it was associated with symptoms like swelling and tearing and pulling pains. One of the first described rheumatic disorders was gout. Today, more than 100 diagnoses belong to the rheumatic diseases. They are all inflammatory and may be localized in the skeleton, joints, muscles, connective tissue, vessels and other organ systems. Arthritis is a common feature in a row of rheumatic diseases such as rheumatoid arthritis (RA), but also in e.g. systemic lupus erythematosus (SLE). Autoimmune processes start prior to the development of overt RA, a period referred as ‘preclinical RA’ [1, 2].

1.2. The course of RA

1.2.1. Preclinical RA

The combination of genetic, autoimmune and environmental factors are important for susceptibility to RA [3]. In RA, the genetic contribution of human leucocyte antigen (HLA) has been estimated between 30-50 % [4]. In fact, class II HLA is directly involved in the pathogenesis RA [5]. The shared epitope (SE) hypothesis describes the presence of a specific amino acid sequence in the protein molecule of HLA-DRB1 which is thought to facilitate the presentation of arthritogenic peptides to T-cells [5]. In RA, SE alleles are risk factors primarily for RA if antibodies to citrullinated peptide/proteins (ACPA) are present [6].

ACPAs have proved to be powerful biomarkers with pathological effects allowing the prediction of RA in patients with musculoskeletal (MSK) pain (particularly arthralgia) but without clinical arthritis [7, 8]. ACPAs are

autoantibodies directed against citrullinated peptides and proteins. Typically, the process of citrullination starts during inflammation [9, 10]. Arginine deiminase converts arginine amino acid residues into citrulline residues in proteins such as vimentin, fibrinogen, filaggrin, alfa-enolase etc. The post-translational shapes can be seen as antigens generating immune responses which results in the production of ACPAs with $\geq 95\%$ specificity for RA [1]. Several studies confirm that the presence of ACPAs associates with accelerated bone erosions in RA [11-13]. To detect ACPAs, several assays have been developed, for instance employing filaggrin-derived peptides (CCP-assay), mutated citrullinated vimentin (MCV-assay), and viral citrullinated peptides (VCP-assay).

Recently, antibodies directed against carbamylated antigens (anti-CarP) were identified in RA patients as well as in healthy individuals before the onset of clinical symptoms [14]. These antibodies may be helpful to predict RA mainly in ACPA- and RF-negative cases [15-17]. Anti-CarP was found to have a high specificity of 89% and a sensitivity of 44% for RA [18]. Anti-CarP can also be detected in other rheumatic diseases, for instance SLE, where anti-CarP as well as anti-CCP associate with erosive joint disease [19].

Carbamylation is mediated by a chemical reaction of cyanate mainly with lysine residues in proteins [20]. Cyanate is present in the body in equilibrium with urea. Inflammation, smoking and renal failure have been reported to increase the non-enzymatic post-translational modification in which cyanate binds to molecules containing primary amine or thiol groups and forms carbamyl groups [20].

Environmental factors (smoking, periodontitis, gut microbiome) as well as coexisting conditions (gender, insulin dependent diabetes mellitus, education, overweight etc.) may finally trigger the onset of RA [21-23]. However, it can take years until a healthy ACPA positive person with a genetic disposition will develop overt RA [1] (**Figure 1**).

1.2.1. Clinical phase

Arthritis is defined as joint inflammation and is accompanied by typical joint symptoms such as skin redness over the joint (rubor), swelling (tumor), heat (calor), pain (dolor) and decreased joint function (functio laesa). The internal membrane of the joint capsule, the synovium, is the primary site of inflammation. Acute phase pro-inflammatory cytokines such as interleukin (IL) -1 and -6, Tumor necrosis factor (TNF), and interferons are raised and poured out with synovial fluid into the joint cavity [24]. Hyperemia in the synovium occurs due to neovascularization and vasodilatation (**Figure 2**). This results in development of the hyperplastic proliferative synovium [25]. If the inflammation lasts more than 6 weeks, it is regarded as chronic. An increased amount of local and circulating pro-inflammatory cells can be observed. Ongoing, the remodeling of the synovium leads to the formation of pannus, which is a layer of fibro-vascular tissue. In this phase, the pro-inflammatory cytokines mediate activation of cartilage- and bone-degrading cells, i.e.

chondroclasts and osteoclasts [26]. The first sign of this is periarticular bone loss [27], accompanied by development of erosions.

Peripheral arthritis is an obligatory part of the 1987 ACR classification criteria as well as the 2010 ACR/EULAR criteria for RA.

1.3. Treat to Target

1.3.1. The Treat to Target concept

The introduction of novel therapeutic strategies during the new millennium set new goals for the patients' outcome, where the "Treat to Target" (T2T) concept aims to achieve remission. This idea includes early diagnosis of RA and prompt efficient pharmacotherapy in order to successfully achieve remission, with the potential to prevent work disability and minimize socioeconomic consequences for the patients and their families. Despite earlier treatment and the introduction of biologic agents, the rate of disability pension results in high costs also in the new millennium [28, 29].

1.3.2. Arthritis in the diagnosis of rheumatic diseases

1.3.2.1. Classification criteria for rheumatoid arthritis

In 1937, the Norwegian blood group serologist Erik Waaler discovered a "serum factor" among patients with RA, and which caused agglutination of human IgG bound to sheep red blood cells. This factor, which was later designed as "Rheumatoid Factor" (RF), proved to be autoantibodies against human IgG-Fc among RA patients. Waaler's findings were published in 1940. In 1948, the American scientist Harry M Rose 'rediscovered' the same auto-antibody [30], which is still designated RF. The first classification criteria for RA were published in 1958, and contained RF as well as two histological factors among 11 components in total [31]. In 1987, a new set of modified RA criteria was launched (**Table 1**). However, it was later concluded that the performance of the 1987 criteria did not differ significantly from the 1958 criteria [32]. As radiographic damage is a sign of longstanding disease, the patients identified by these criteria were still at great risk to develop disabilities.

Table 1

The 1987 ACR classification criteria for rheumatoid arthritis

At least 4 of the following 7 criterias:

1. Morning stiffness >1 hour
2. Arthritis in ≥ 3 joint areas
3. Arthritis of hand joints
4. Symmetric arthritis
5. Rheumatoid nodules
6. Rheumatoid factor
7. Radiographic changes

The criteria 1-4 must have been present for ≥ 6 weeks

To reach the T2T aims, it was necessary to maximize the sensitivity for the diagnosis of RA, and new classification criteria were created [33]. Thus, in July 2010, the 2010 ACR/EULAR RA classification criteria were introduced [34] (**Table 2**). These new classification criteria include ACPA testing. Also, the 2010 criteria were modified regarding the classification of joint involvement in RA, contributing with up to 5 of the 6 needed points for RA diagnosis. Hence, the 2010 criteria are constructed to enable identification of earlier RA as compared to previous settings.

Table 2 ACR/EULAR 2010-criteria for RA		
Joint involvement	1 large joint	0
	2-10 large joints	1
	1-3 small joints	2
	4-10 small joints	3
	>10 joints (at least 1 small)	5
Duration of synovitis	< 6 weeks	0
	≥ 6 weeks	1
Serology	Neither RF nor anti-CCP positive	0
	At least one test low positive	2
	At least one test high positive	3
Acute phase reactants	CRP and ESR normal	0
	Abnormal CRP or ESR	1
≥6 points = RA		

1.3.2.2. Arthritis in other rheumatic diseases

Arthritis is a common feature also in a row of other rheumatic conditions. Secondary to arthrosis, arthritis can be caused by mechanical stress, trauma or osteoporosis and will then be termed “osteoarthritis”. Clinical arthritis may include gout, spondylarthritis (including psoriasis arthritis), reactive arthritis, sarcoidosis, Still’s disease, vasculitis syndromes and others with joint affection.

Furthermore, arthritis can be found as one criterion in the ACR-82 and 2012 revised SLICC-classification criteria for SLE [35, 36]. Articular involvement in SLE is common and has been reported to be present in up to 80 % of the cases [37]. However, the prevalence of erosive arthritis in SLE is low, but may complicate the distinction between SLE and RA [38]. “Rhupus” has been described as an overlap syndrome in which patients fulfill classification criteria for both conditions - SLE as well as RA [39, 40].

1.3.2.3. Diagnosis by clinical features and the role of imaging

Patients with MSK pain are commonly referred from primary health care to the rheumatology department if they test positive for ACPA. These patients may or may not have elevated inflammatory variables such as C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR). When clinical arthritis is present at the first visit to a rheumatologist, and the patient fulfills the ACR classification criteria for RA, anti-rheumatic therapy with corticosteroids and a disease modifying anti-rheumatic drug (DMARD) will be initiated.

Among imaging modalities, conventional radiographs of the hands and feet are still the first choice; first to have a baseline status for later monitoring of treatment efficacy, second to figure out information regarding the prognosis as erosions at baseline are predictive of further joint damage [41]. There are some known scoring instruments to describe the radiographic status. Sharp score modified by van der Hejde (SHS) and Larsen score are the most commonly used in clinical trials and research. In the SHS, a maximum score of 448 can be reached, consisting of an erosion score range from 0 to 280 and joint space narrowing score range 0 to 168 [42]. In Larsen score, 32 joints will be graded 0-5 regarding structural changes and can result in a maximum score of 160 [43]. However, these methods are difficult to apply in clinical practice as they are very time-consuming [44]. In an attempt to solve this, the author and coworkers developed a modified scoring system according to Larsen, suggesting assessment of present erosions (Yes/No) with a maximum score 32. However, when testing the suggested simplified Larsen erosion score among local radiologists, it was still found too time consuming (unpublished data). One way to circumvent this is to describe the findings, instead of counting erosions.

In clinical practice as well as in research, disease activity is commonly measured by the 28-joint count disease activity score (DAS28), including joint swelling, tender joints, ESR and patients global VAS [45]. To assess functional ability of the patients, the health assessment questionnaire (HAQ) is widely used [46].

1.3.3. Antirheumatic treatment

Non-steroidal anti-inflammatory drugs (NSAIDs) are often initiated by the general practitioner during the waiting period for the first rheumatologist consultation. Once the diagnosis of RA is confirmed by the rheumatologist, DMARDs should be started immediately [47]. Since DMARDs need weeks to months to reach effectiveness, glucocorticosteroids (GCs) are used to achieve rapid clinical improvement. When used systemically, GCs decrease the migration of macrophages, B- and T-cells into injured tissue and inhibit secretion of pro-inflammatory cytokines [48]. Already in low doses, GCs may have protective effects regarding radiographic progression [49]. Furthermore, GCs can be used for intraarticular injections. In these cases, GCs will be directly effective in the joint capsules' internal synovial membrane on the basis of inhibited expression of pro-inflammatory cytokines (TNF, IL-1, IL-6, and IFN) [50]. However, GCs used over a long time, have a risk of side effects such as diabetes mellitus, osteoporosis, hypertension, edema, and muscle atrophy. Osteoporosis prophylaxis with calcium, vitamin D, and bisphosphonate is recommended [51]. The rheumatologist aims to taper and discontinue the treatment with GCs as soon as the chosen DMARD takes over the control of the disease [47].

According to EULAR recommendations, methotrexate (MTX) should be part of the first line treatment strategies [47]. MTX is highly effective as monotherapy as well as in combination with other DMARDs. MTX in monotherapy has been shown up to 70 % improvement rates according to ACR criteria in DMARD-naïve patients [52]. Adding an adequate dose of folic acid, MTX is generally well tolerated [53]. However, hepatic, renal or pulmonary side effects can hinder the treatment with MTX. In these cases sulfasalazine or leflunomide should be considered as part of first treatment strategy.

Two decades ago, at the beginning of the new millennium, the first biologic (b)DMARDs were introduced and has since then revolutionized the treatment options for patients with highly inflammatory disease. Treatment with bDMARDs are most often initiated as a second or third option, in case of failures with conventional synthetic (cs)DMARD. At first, the tumor necrosis factor-alpha (TNF) inhibitor etanercept was introduced, and a little later even Infliximab and adalimumab. golimumab and certolizumabpegol followed a

couple of years later. Besides TNF inhibitors, also IL-1 and 6- inhibitors (anakinra and tocilizumab respectively), inhibitor of T-cell activation (abatacept) and depletion of CD20-positive B cells (rituximab) were introduced as treatment options. Recently, oral inhibitors of Janus kinases (JAK inhibitors baricitinib and tofacitinib) became further treatment options.

1.3.4. Monitoring

At RA onset and initiation of pharmacotherapy, a tight control of treatment according to the T2T strategy has shown to be most effective. As recommended in the T2T concept, the use of activity scoring instruments leads to more rapid DAS28 remission and to a higher percentage of remission compared to “usual care treatment” [54, 55].. DAS28 comprises inflammatory marker (ESR or CRP), swollen and tender joint count, and a visual analogue scale (VAS) to assess the patients’ general health. Initially, DAS included 44 joints recommended by Ritchie [56]. This was later modified and reduced to 28 joints defining the current DAS28 (or DAS28-CRP when CRP is used instead of ESR) [45, 57] (**Figure 3**).

Other scores in use are the “Simplified Disease Activity Index” (SDAI) and “Clinical Disease Activity Index” (CDAI) which are simpler instruments, but more stringent in defining remission [58, 59] (**Figure 3**).

According to EULAR, remission is defined as DAS28<2.6. DAS28≤3.2 stands for low, >3.2 - ≤5.1 for moderate and >5.1 for high disease activity. Response to treatment according EULAR is based on both the change in DAS28 and the absolute value at evaluation **Figure 4**).

Present DAS	DAS Improvement		
	> 1.2	> 0.6 and ≤ 1.2	≤ 0.6
≤ 3.2	Good response	Moderate response	No response
> 3.2 and ≤ 5.1	Moderate response	Moderate response	No response
> 5.1	Moderate response	No response	No response

Figure 4 EULAR response criteria for RA

1.3.5. Assessment of clinical remission

One of the ACR/EULAR definitions of remission in RA clinical trials is Boolean-based and another is based on SDAI which has been described above [60] . The Boolean based includes TJ count ≤1, SJ count ≤1, CRP≤1mg/dl, and patients global (PG) assessment ≤1 [60]. In SDAI, the patient is considered to be in remission when having a score of ≤3.3 [61]. The most used score for definition of RA remission in clinical practice is the DAS28<2.6. However, it can be difficult to reach the remission criteria as the PG assessment is often high, particularly among elderly patients [62]. On the other hand, the assessment of ankles and forefeet are partly limited. Tenderness and swelling in the feet have proved to be greater compared to other joints, thereby resulting in higher scores. Hence, it can be difficult to reach ‘true remission’, and discussions are ongoing whether or not assessment of the feet should be excluded when evaluating remission [60].

1.4. Alternatives of imaging for diagnosing and predicting arthritis

Despite new revolutionized treatment options, work disability remains high among patients with RA [63]. Disabilities are associated with functional impairment and higher mortality rates [64]. The right timing for starting of anti-rheumatic therapy is in a window of opportunity when clinical symptoms appear [65]. Synovitis is the primary site of pathology in RA; therefore it seems logical that imaging modalities to detect subclinical inflammation should be used to ensure good response to treatment with DMARDs in an early stage of the disease.

1.4.1. Ultrasound

Ultrasound (US) is defined as sound at frequencies above the audible frequency range of humans. Grey scale (GS) US is an imaging modality in medicine since a couple of decades. Doppler US is the compression or elongation of sound waves due to changes in the distance between the transmitter and receiver. The Doppler effect is named after the Austrian physicist Christian Doppler (1803-1853), who thought that the colour of the stars is based on the change of distance during light emission.

US has become a promising imaging tool in rheumatology. It allows the assessment of soft tissues like joint capsules, tendons and bursae. US is inexpensive and, used for real time scanning, it is possible to communicate with the patient and to focus directly on the site of illness. It can also be conveniently used as a bedside tool. Treatment strategies including intraarticular injections can be decided at once during the patient's consultation with the rheumatologist. Further advantages are no radiation, and it is easy to use for monitoring. However, limitations are that US cannot penetrate bone. The examiner has to understand the multiplane examination technique, and to be skilled in anatomy of the examined structures. Furthermore, the examiner has to be able to manage instrument settings, particularly of Doppler, as positive adequate power Doppler (PD) can crucially contribute to useful assessments of inflammation [66, 67].

Ultrasound detects effusions and oedema as signs of ongoing musculoskeletal (MSK) inflammation. GS ultrasound visualizes thickening of the synovial membrane in joints and tendons, effusions and structural bone changes, such as erosions. Addition of PD to GS findings allows detection of hyperemia, which is a sign of active inflammation [68] (**Figure 5**). Early diagnostic US to detect ongoing/progressive inflammation has been shown to predict progression to arthritis, both among ACPA-positive and/or RF-positive, and sero-negative arthralgia patients [69-72]. The risk of progression has been reported to be highest among patients with positive PD [73]. According to the 2010 criteria, US may be used to confirm the clinical findings [34]. A Japanese study showed that US may improve the performance of ACR-EULAR 2010 to identify patients who will need DMARD treatment [74], but general US screening to increase fulfilment of the 2010 criteria remains to be validated.

US is comparable with magnet resonance imaging (MRI) in terms of detecting synovial inflammation [75, 76]. Regarding structural changes as erosions, US is markedly site-dependent as it cannot penetrate bone. US is superior to X-ray in easily accessible joints, but the sensitivity for detecting bone erosions is low in anatomically complicated joints [77].

Besides the use of US as a diagnostic tool, it has discussed as an imaging modality to monitor remission. There is evidence that patients who were clinically assessed to be in remission, but where US actually showed signs of ongoing inflammatory activity, faced a significantly increased risk of radiographic progression [78, 79]. However, a randomized trial from Haavardsholm et al. showed no additional benefit of ultrasound monitoring in

RA treated intensively according to T2T [80]. There are also indications that patients in clinical remission with only minor signs inflammatory activity by PD remain inactive for a longer period compared to those with high PD activity [81]. Ultrasound used for follow up to assess remission and possibly to optimize treatment, should be considered with reservation.

1.4.2. Digital X-ray radiogrammetry

Bone loss is a common feature in RA. It can appear as systemic osteoporosis caused by treatment with GC’s, is influenced by sex and high age, and local as periarticular bone loss due to arthritis [82-84]. Currently, the indication for measurement of bone mineral density (BMD) is mostly to monitor systemic osteoporosis. Several non-invasive methods are in use, where dual energy X-ray absorptiometry (DXA) is considered the gold standard. Other methods include quantitative computed tomography (QCT), quantitative ultrasound (QUS), and radiographic absorptiometry (**Table 3**).

Table 3 Methods of bone mineral density assessments	
Method	Technique
DXA	Measures absorbtion of X-ray beams of the femoral neck and lumbal spine
QCT	Measures the central trabecular portion of the lumbar spine and hip
Radiographic absorptiometry	Compares the bone to be measured with a metal wedge by using ordinary X-ray
QUS	Measures speed of sound and broadband attenuation at the calcaneus or phalanges
DXR	Relates the sum of corticalis to the entire wdth of the bone by using digital X-ray rediographs
DXA Dual energy X-ray absorptiometry, DXR Digital X-ray radiogrammetry, QCT Quantitative computed tomography, QUS Quantitative ultrasound	

Digital X-ray radiogrammetry (DXR) is a technique to measure periarticular BMD of the metacarpal bones 2-4 in hands, i.e. in close proximity to 2 of the most affected joints in RA; MCP 2 and 3. Bone loss detected by this method has repeatedly been shown to predict radiographic joint progression in early RA. [85-88]. However, the majority of previous studies investigated 12-months

changes in DXR-BMD, and after this time, DXR-evaluation of joint damage was not superior to conventional radiography for prediction of further radiologic progression

Except QCT, which measures bone mineral mass per volume, the other methods measure per projected area.

2. AIMS OF THE THESIS

General aim

The overall aim of this thesis was to assess the diagnostic and prognostic potential of different joint imaging modalities to predict arthritis and clinical outcomes in patients with anti-citrullinated protein antibodies.

Specific aims

Paper I

To investigate the relationship between RA-related autoantibodies and arthritis in SLE.

Paper II

To evaluate whether BMD loss measured by DXR predicts radiographic joint damage in patients with early RA.

Paper III

To determine the predictive value of anti-CCP on disease activity and radiographic joint damage in contemporary early RA.

Paper IV

To investigate whether ultrasound findings predict arthritis development in patients with anti-CCP and pain but no clinical arthritis.

3. MATERIALS AND METHODS

3.1. Paper I – SLE study

In September 2008, the prospective follow-up program KLURING (a Swedish acronym for ‘Clinical Lupus Register in Northeastern Gothia’) at the Rheumatology Clinic, Linköping University Hospital, Sweden, started to include patients diagnosed with SLE according to ACR criteria and/or Systemic Lupus International Collaborating Clinics (SLICC) classification criteria [36, 37]. At the time-point of this study, 236 patients were included, of which 83 % were prevalent cases and 17 % had newly diagnosed SLE. The mean age was 54 years and 207/236 (88 %) of the patients were females.

16 of the 236 (7 %) KLURING patients were positive for ACPA. For the US assessment of arthritis, we developed a semi-graded protocol including 36 clinically relevant synovial small joints in hands and feet. Also, 6 tendons referred by Berner-Hammer et al. were subject of our examinations [89] (Table 4).

Table 4 Ultrasound protocol used in the studies of paper 1 and 4 including 26 joints + 2 tendons in hands and 10 joints + 4 tendons in feet		
Hands	Radiocarpal joint	Extensor carpi ulnaris
	Intercarpal joint	
	Distal radioulnar joint	
	MCP joints I-V	
	PIP joints I-V	
Feet	MTP I-V	Tibialis posterior
		Flexor digitorum longus
Max score	GS: 108	GS: 18
grading 0-3	PD: 108	PD: 18
GS Grey scale; MCP Metacarpophalangeal joints; MTP Metatarsophalangeal joints; PIP Proximal interphalangeal joints; PD Power Doppler		

The findings were assessed regarding synovial hypertrophy in GS and inflammatory activity with PD. The US examiner was blinded to the ACPA-status of the patients. To grade synovitis, the semi-quantitative scoring system by Szkudlarek et al. is commonly use [90]. Herein, synovial hypertrophy is graded 0–3 (0 = no thickening, 1=minimal thickening not bulging over the line linking the tops of periarticular bones, 2=thickening over but not extending the diaphysis, 3 = synovial thickening bulging over the tops of the periarticular bones and extension over the diaphysis of at least one side (**Figure 6**)) and perfusion 0–3 (0 = no flow in the synovium, 1=single vessel signals, 2=confluent vessel signals in less than half of the synovial area, 3 = confluent vessel signals in more than one half of the synovium (**Figure 7**)).

3.2. Paper II – DXR study

In this study, 176 patients from three Swedish centers were included (Helsingborg, Linköping and Umeå with 60, 48 and 68 patients respectively). All patients were >18 years old (range 19-87 years) and fulfilled the ACR/EULAR classification criteria for RA. ACPA and RF were assessed at baseline, and CRP, ESR DAS28, and HAQ were obtained at all scheduled visits (baseline, 3, 6, 12, 24 months). For the evaluation of BMD loss, radiographs of 129 patients were available from baseline, 1 and 2 years. The radiographs were assessed according to Larsen score by one reader at each participating center. In Larsen score, 32 joints are assessed; metacarpophalangeal joints II-V, proximal interphalangeal joints II-V, the wrists divided into four areas and the metatarsophalangeal joints II-V. Each joint was graded 0-5 regarding structural changes (**Table 5**) and could result in a maximum score of 160 [43].

Table 5 Grading of radiographic findings according to Larsen

Grade	Findings
0	Intact bony outlines and normal joint space
1	Erosions <1 mm in diameter or joint space narrowing
2	One or several small erosions (diameter >1 mm)
3	Marked erosions
4	Severe erosions: no joint space left, but partly preserved original bony outlines
5	Mutilating changes: The original bony outlines have been destroyed

The smallest detectable change (SDC) was calculated for the three readers individually according to the method of Bruynesteyn [91]. Radiographic progression was defined as a difference in Larsen score above the SDC of the corresponding reader. The intra-rater and inter-rater reliability of the readers was assessed by calculating the intraclass correlation coefficient (ICC). The ICC was 0.903.

BMD was estimated on hand radiographs of the second, third and fourth metacarpal bones using DXR (the online Pronosco X-posure System, SECTRA, Linköping, Sweden) [85]. DXR is a technique based on computerized analyses of standard hand radiographs to calculate peripheral bone mineral density (BMD) of the three middle metacarpal bones [91, 92] (**Figure 8**).

BMD loss/month	Change [mg/cm ²]
Normal	<0.25
Moderate	≥0.25 but <2.5
Severe	≥2.5

Figure 8 DXR Method Hand bone mineral density from the centres of the metacarpal bones II-IV are measured with DXR online method (dxr-online, Sectra AB, Linköping, Sweden), in which the sum of corticalis is related to the entire width of the bone. BMD bone mineral density; DXR Digital X-ray radiogrammetry

3.3. Paper III – TIRA study

Two multicenter prospective observational early RA cohorts denoted ‘TIRA’ (= a Swedish acronym for “early intervention in rheumatoid arthritis”) enrolled patients with recent-onset RA 10 years apart. Inclusions criteria were symptom duration (defined as first observed joint swelling <12 months), and either fulfilment of the 1987 ACR criteria [27] or suffering from morning stiffness >60 min, symmetrical arthritis, and small joint engagement.

TIRA-1 enrolled patients 1996-1999, i.e. in the “pre-ACPA era”. During this time, treatment with biologics was not used in clinical practice. After the millennium, TIRA-2 enrolled patients 2006-2009, i.e. when bDMARDs have been introduced and become routine in rheumatology care. However, patients in both TIRA cohorts were not treated with DMARDs prior to enrollment. The follow up visits were scheduled at 6, 12, 24 and 36 months recording DAS28, HAQ, and ongoing treatment. Laboratory variables were monitored according to national Swedish guidelines at all visits. Yearly radiographs of hands and feet were obtained up to 3 years in TIRA-2, and scored according to Larsen. To

confirm our findings, we used independent cohorts from Umeå in Northern Sweden (TRAM-1 and TRAM-2, respectively), which were similar regarding inclusion criteria, time-periods of enrollment, and follow-up procedures. Also, radiographs were scored according to Larsen in both TRAM cohorts.

3.4. Paper IV – TIRx study

The prospective observational study designated ‘TIRx’ (Swedish acronym meaning x-tra early intervention in RA) is a single center study conducted at the University hospital in Linköping, Sweden. 116 anti-CCP-positive patients with musculoskeletal pain and maximum 1 palpable synovitis were enrolled in this study. Eligible patients referred from primary care centers were screened by one of four participating rheumatologists between 2010 and 2013. Exclusion criteria were >1 clinical arthritis, corticosteroid therapy during the preceding 6 weeks, a prior diagnosis of inflammatory rheumatic disease or age <18. Due to various reasons, 12 subjects discontinued (**Figure 9**).

Ultrasound examinations were performed by the author. All patients were examined with the same settings for both B-mode (grey scale) and power Doppler (PD). To grade synovitis, we used the semi quantitative scoring system of Szkudlarek et al [90]. The rheumatologists as well as the patients were blinded to the ultrasonography results. IgG anti-CCP and IgM-RF were measured at baseline. DAS28 and Health HAQ were assessed at each visit. The patients were treated as suggested by the physician and the patients' acceptance. Radiographs of hands and feet were obtained at baseline, 3 months (hands only), and after 1 and 2 years. Hand bone mineral density (BMD) of the metacarpal bones II-IV was measured with DXR online method. The radiographs will be evaluated by the author according to the Larsen score, and the findings will be related to BMD loss measured by DXR in a future separate study.

Also, an age-matched control group consisting of 100 healthy blood donors (50 women and 50 men) was recruited for serum sampling and ultrasound examination identical to that of the patients. This allowed us to characterize to which extent ultrasound findings occur among healthy individuals, and interpret patient findings accordingly.

4. RESULTS

4.1. Paper I

Antibodies against carbamylated proteins and cyclic citrullinated peptides in systemic lupus erythematosus: results from two well defined European cohorts

4.1.1. Patient characteristics

16 ACPA positive and 16 ACPA negative patients from the KLURING cohort were examined with ultrasound regarding arthritis and tenosynovitis. The patients in both groups matched regarding age (median 58 years), gender (females n=14 and n=13 respectively), disease duration (median 10.5 years) and were very similar regarding prednisolone dose (2.5mg). 7 patients were positive for anti-CarP and 7 for RF.

4.1.2. Radiographic and ultrasound findings related to antibody status

Patients with ongoing clinical peripheral arthritis and/or MSK symptoms underwent radiographs (102/236 (43 %)). Erosions were identified in 10 patients (9,8 % of those with radiographs available). All 16 ACPA positive patients had radiographs available, 4 of whom had radiographic erosions compared to 6 of the 86 ACPA negative ($p<0.05$).

Among the patients who were examined with ultrasound regarding arthritis and tenosynovitis, none of the ACPA negative SLE controls (14/16 with radiographs available) compared to 4 of ACPA positives had radiographic erosions ($p=0.103$). Significantly more patients among the RF positives ($n=7$) vs. negatives ($n=23$) (3 vs. 1; $p=0.031$) and anti-CarP positives ($n=7$) vs. negatives ($n=23$) (4 vs. 0; $p=0.001$) had erosions.

The ACPA positive patients had significantly higher US mean sum scores both in GS and PD compared to ACPA negatives (11,5 vs. 4,1; $p=0.014$ and 4,7 vs.

0.4, $p=0.037$ respectively). The same trend with numerically higher mean sum scores in GS and PD among the antibody positive patients could be seen regarding RF (12.0 vs. 6.6; $p=0.32$ and 5.4 vs. 1.8; $p=0.30$ respectively) and anti-CarP (10.9 vs. 7.0; $p=0.29$ and 5.4 vs. 1.8; $p=0.30$ respectively) (**Figure 10**).

4.2. Paper II

Decrease in bone mineral density during three months after diagnosis of early rheumatoid arthritis measured by digital X-ray radiogrammetry predicts radiographic joint damage after one year

4.2.1. Patient characteristics at baseline

In this study, radiographs of 129 patients were available for scoring according to Larsen and DXR analysis. The median age of the included patients was 58 years and 64 % were females. Among patients with BMD loss, the portion of females was marginally higher compared to the patients without BMD loss (70.4 vs. 55.1 %; $p=0.05$). Also, at baseline, patients with BMD loss were

significantly older compared to the others (60 vs. 56 years; $p=0.042$), had significantly higher DAS28, (5.06 vs. 4.57; $p=0.023$), Larsen scores (4.7 vs. 3.2; $p=0.034$), but lower BMD values (565 vs. 661mg/ccm; $p= 0.008$).

4.2.2. Development of Larsen score in relation to bone loss during the first three months

In general, the higher the grade of BMD loss within the first 3 months, the higher the total Larsen score (mean) at baseline ($p=0.039$), after 1 year ($p=0.024$) and 2 years ($p=0.056$) (**Figure 11**). In simple regression analysis, sex, ACPA, and baseline values of ESR, DAS28, and Larsen score were significantly or trend-wise ($p<0.2$) associated with change in Larsen score greater than SDC. Multiple regression analysis adjusting for these variables showed a significant association between 3-month BMD loss and increase in Larsen score > SDC after 1 year ($p = 0.033$, adjusted R-squared = 0.069). However, 2-year increase in Larsen score was not significantly associated with 3-months BMD loss ($p= 0.626$, adjusted R-squared=0.034)

4.3. Paper III

ACPA-associated radiographic damage in early RA uncoupled from inflammation and initial treatment response

4.3.1. Patient characteristics

The patients in TIRA-1 and TIRA-2 had baseline characteristics rather typical for early RA cohorts. There were no significant differences between the 2 cohorts except for age (mean 55 vs. 59 years, $p=0.003$). ACPA was not used as a diagnostic biomarker in TIRA-1, but the proportion of patients testing positive were similar to TIRA-2 (65 vs. 68 % respectively; $p=0.44$).

4.3.2. Treatment and disease course according to baseline autoantibody status in TIRA-1 and TIRA-2

In the pre-millennium cohort TIRA-1, the baseline levels for ESR and CRP differed not significantly among ACPA-positives subjects compared to the –negatives. However, the RF-positives compared to –negatives had significant higher ESR (37 vs. 31 mm/h; $p=0.012$) and trend-wise higher CRP (31 vs. 25 mg/L; $p=0.054$) at baseline. Treatment with DMARDs was started in 54 % of the patients (44 % of the RF positives and 52 % of the ACPA positives (**Figure 12**)). ACPA associated with higher CRP and ESR during the follow-up period ($p=0.001$ and $p=0.002$, respectively).

In TIRA-2, ACPA positive patients had borderline lower ESR at baseline (31 vs. 35 mm/h; $p=0.096$), and significantly lower CRP (23 vs. 34 mg/L; $p=0.007$). Treatment with DMARDs was initiated in 93 % among the ACPA positive patients, and in 91 % among the ACPA negatives ($p=0.715$) (**Figure 12**). ESR and CRP did not differ significantly during follow-up according to baseline ACPA (Figure 13) or RF status in TIRA-2.

Figure 13 Disease activity and inflammatory markers over time in relation to baseline anti-citrullinated protein antibody status in early rheumatoid arthritis patients enrolled 1996-1998 (TIRA-1) and 2006-2009 (TIRA-2). CRP C-reactive protein; DAS Disease activity score; ESR Erythrocyte sedimentation rate

4.3.3. Radiographic outcome is associated with baseline ACPA status

In TIRA-2, baseline Larsen scores did not significantly differ between ACPA positive and ACPA negative patients (3.0 vs. 2.4, $p=0.28$). At 36 months, however, ACPA positive patients had significantly higher Larsen score than ACPA negative patients (mean 5.4 vs. 3.5, $p=0.027$ (**Figure 14**)).

Similar results regarding radiographic damage were seen in the confirmation cohorts from Northern Sweden, both in the historical and the modern cohort (TRAM-1 and TRAM-2 respectively). Testing positive for ACPA at baseline was associated with higher Larsen scores at baseline (TRAM-1; mean 5.8 vs. 2.7, $p=0.01$ and TRAM-2; mean 6.6 vs. 5.1, $p=0.03$) as well as follow up (TRAM-1; mean 11.3 vs. 6.4, $p=0.001$ and TRAM-2; mean 8.9 vs. 6.7, $p=0.009$ (**Figure 15**)).

In multivariable analysis, Larsen score at follow-up was independently associated with baseline Larsen score ($p < 0.001$), but also baseline ACPA status reached statistical significance ($p = 0.039$). In multivariable linear regression analysis in TIRA-2, baseline ACPA status remained associated with 3-year radiographic damage also after adjusting for EULAR response to treatment at 6 months ($p = 0.050$, regression coefficient = 1.045, 95 % CI 0.00-2.089). Also in the contemporary confirmation cohort TRAM-2, ACPA remained significantly associated with follow-up Larsen score after adjusting also for initial treatment response ($p = 0.027$, regression coefficient 1.109, 95 % CI 1.127-2.090).

4.4. Paper IV

Erosions on ultrasound predict arthritis development in patients with anti-citrullinated protein antibodies and musculoskeletal pain but no swollen joints

4.4.1. Patient characteristics

The included patients had a mean age 51.9 years (SD=15.1), and 82 (79.6 %) were females. Further baseline characteristics are listed in **Table 6**. As controls, we recruited 100 blood donors (50 females, 50 males; mean age 52 years) from

the Department of Transfusion Medicine at Linköping University Hospital. The controls underwent serum sampling and US examination once.

Table 6 Baseline characteristics of ACPA positive individuals		
Characteristic		N=104
Age (SD)		51.9
Gender, females		83 (79.8%)
Symptom duration	0-6 months	21 (20.2%)
	6-18 months	44 (42.3%)
	>18 months	39 (37.5%)
ACPA-level	Low	40 (38.5%)
	High	64 (61.5%)
RF	Negative	70 (67.3%)
	Positive	34 (32.7%)
CRP [mg/L] (SD)		7.3 (8.0)
ESR [mm/h] (SD)		12.9 (11.1)
Smoking	Nonsmoker	57 (54.8%)
	Ex-smoker	29 (27.9%)
	Current smoker	18 (17.3%)
DAS28 (SD)		
HAQ (SD)		0.38 (0.44)
ACPA Anti-citrullinated protein antibodies; CRP C-reactive protein; DAS Disease activity score; ESR Erythrocyte sedimentation rate; HAQ RF Rheumatoid factor; SD Standard deviation		

Among the 104 patients in the TIRx study, 22 patients had clinical arthritis at baseline.

The patients with clinical arthritis had significantly higher mean DAS28 compared to the 82 patients who had not (3.35 vs. 2.49; p=0.001). The other baseline characteristics did not differ between these groups.

4.4.2. Initiation of treatment

As clinical arthritis was the variable to predict by ultrasound, the remaining 82 patients without baseline arthritis were further evaluated. Among them, 35 (43 %) received treatment with oral GC and/or DMARD within the first 2 years, (29 (35 %) received GC, 29 (35 %) received DMARD, and 32 (31 %) received both. Seven out of the 35 patients initiated oral GC or DMARD therapy without presenting with clinical arthritis. The remaining 28 patients were diagnosed with clinical arthritis before or after starting treatment with GC and/or DMARDs. Follow-up in TIRx is ongoing, but in this study we evaluate follow-up until September 1st, 2017, resulting in a mean follow-up time of 68 months.

As expected, significantly more patients with clinical arthritis during follow-up received treatment with DMARDs and/or GC (28 vs. 11 %; $p<0.001$).

4.4.3. Ultrasound findings in ACPA positive individuals with arthralgia and healthy controls

At joint-level, significantly more MCP- and PIP-joints of the patients had signs of synovial hypertrophy ($GS\geq 1$) (5.2 vs. 2.5 %; $p<0.001$ and 6.6 vs. 1.5 %; $p<0.001$ respectively). In contrast, significantly more control MTP joints had synovial hypertrophy as compared to patients (48.5 vs. 23.9 %, $p<0.001$). In MTP joints, the GS findings were mostly seen in the MTP 1-4, especially among the controls, but PD was found significantly more in the patients MTP joints with synovial hypertrophy, as compared to control MTP joints (2.0 vs. 0.2 %; $p=0.001$). Therefore, we present MTP5 separately, and excluded the MTP 1-4 joints regarding GS analyses, but included MTP1-4 regarding PD. PD signals ($PD\geq 1$) occurred significantly more often also in the other joint regions among patients as compared to controls (wrists 7.9 vs. 2.0 %, $p<0.001$; MCPs 0.7 vs. 0 %, $p=0.026$; PIPs 1.7 vs. 0 %, $p=0.001$).

4.4.4. Ultrasound findings and arthritis development

13 (15.6 %) of the 82 patients had ≥ 1 erosions verified by US at baseline. Ten (77 %) of these patients developed clinical arthritis within the observation

period as compared to 29 (35 %) among those without US erosion ($p=0.032$). In cox regression analysis, adjusting for RF, age, sex, ACPA level, and baseline levels of ESR and CRP, US erosion predicted development of clinical arthritis (hazard ratio 4.2, 95 % CI 1.7-10.4, $p=0.002$) (**Figure 16**).

Figure 16. Survival plot illustrating progression to arthritis during follow-up in relation to the presence of ultrasound erosions at baseline among patients with anti-citrullinated protein antibodies and musculoskeletal pain.

5. DISCUSSION

Polyarthritis is the most typical clinical feature of RA, and is part of both the 1987 and 2010 ACR classification criteria for RA. Following the ‘treat to target’ (T2T) concept, the 2010 criteria, which weight arthritis and include ACPA, bring rheumatologists closer to an earlier diagnosis of RA than it was possible using the criteria from 1987. However, work in this thesis shows that ACPA is associated with arthritis and radiographic damage despite of earlier diagnosis and treatment decisions. We aimed to investigate whether the early use of different imaging modalities in ACPA positive patients can help to predict arthritis development and outcome.

5.1. Diagnostic potential of joint imaging in patients with anti-citrullinated protein antibodies

5.1.1. Ultrasound used in the earliest stage of ACPA positive arthralgia

In RA, US is already established as a clinical tool. Following the T2T concept, algorithms for the use of US to aid diagnosis, monitoring and assessment of remission of RA, has recently been elaborated [93]. However, the value of US before RA can be diagnosed by the ACR classification criteria, has been investigated in only a few studies which differ in methodology as well as antibody status of the included patients [70, 72, 73]. In **paper IV** we studied US findings in ACPA positive patients without clinical arthritis in a window between the preclinical and clinical phase of RA. Our results show that, compared to healthy blood donors, patients with MSK pain and ACPA positivity in a preclinical phase have significantly more signs of inflammatory joint changes detected by US. These findings are in line with the study from Nam et al [73]. Previous MRI studies by Hoving et al. showed similar results in a very early phase of RA development [94].

5.1.2. Arthritis confirmed by ultrasound more common in ACPA positive SLE-patients

To assess the occurrence of arthritis in ACPA positive SLE patients in **paper I**, US was used. Arthritis detected by ultrasound occurred significantly more often in ACPA positive SLE-patients as compared to ACPA negative. The same trend was observed also in anti-CarP and RF-positive patients with SLE. Three of the 32 examined patients were positive ACPA, RF and anti-CarP. These patients had significantly more US-verified arthritis and tenosynovitis as compared to those who were not. However, with only three triple-positive patients, it is not possible to draw any conclusions, although it is in line with a recent study by Verheul et al demonstrating a high specificity for RA in triple seropositivity in RA [95]

US is a valuable tool for the detection of arthritis, tenosynovitis and erosions in patients with SLE [96, 97]. It can be assumed, that ACPA-positivity in SLE indicates a phenotype characterized by arthritis. However, as there are only a few studies, which in addition differ in the methodology, further validation of US in SLE is necessary before it can be used as a tool in clinical practice.

5.1.3. Radiographic damage in rheumatoid arthritis is associated with ACPA positivity

In **paper III** we compared the association between ACPA and radiographic joint destruction in RA in two cohorts with very different prerequisites for early RA management. First, a pre-Millennium cohort (TIRA-1) enrolled patients before ACPA was introduced as diagnostic marker, and before the biologic DMARDs were introduced, targeting pro-inflammatory cytokines as TNF- α , IL-1, IL-6, to decrease T-cell activation or to destroy B-cells. The second cohort, TIRA-2, studied the 'ACPA era', where early aggressive treatment, T2T strategies, and bDMARDs were no longer controversial. Introduction of the 2010 ACR/EULAR criteria allowed earlier diagnosis of RA, thereby facilitating earlier treatment decisions.

In the pre-Millennium cohort TIRA-1, ACPA was analysed later from stored samples. ACPA was shown to associate with higher disease activity (DAS28, ESR, CRP, swollen joints) during follow up, as previously reported [98]. This

observation may definitely be a result of the low rate of patients treated with early DMARDs. Notable in TIRA-1 is, that the RF status obviously did not influence early treatment decisions as only 44 % of the RF positive patients (and 58 % of the RF negatives) started treatment with DMARDs at baseline.

Since the beginning of the new Millennium, when ACPA became used as a diagnostic tool and also became part of the new ACR classification criteria 2010, RA diagnosis can be made at an earlier stage, enabling earlier introduction of DMARDs including potent biologics. These facts have resulted in lower disease activity during follow up in the modern cohorts TIRA-2 and TRAM-2. However, despite significantly improved and early instituted modern anti-rheumatic pharmacotherapy, many of ACPA positive RA patients still face an increased risk of developing radiographic damage with high Larsen scores.

5.1.4. Higher rates of radiographic damage in ACPA- and anti-CarP positive SLE-patients

Arthritis also occurs in other rheumatic diseases including SLE, and when RA-related antibodies are present, an ‘overlap disease’ called “rhupus” may be suggested [39, 40]. It was recently shown that ‘triple positivity’ for the RA-related antibodies ACPA, RF and anti-CarP was highly specific for RA development [96]. In **paper I** we found, that all three RA-associated antibodies (ACPA, anti-CarP and RF) were significantly associated with radiographically confirmed erosions in SLE-patients. We hypothesize that pathogenetic mechanisms could be similar in RA and in a small group of patients with SLE defined by a clinical phenotype dominated by arthritis. Interestingly though, 60 % of the studied SLE-patients with radiology proven erosions were not identified by any of the antibodies.

5.2. Prognostic potential of joint imaging to predict arthritis and joint damage in patients with anti-citrullinated protein antibodies

5.2.1. Ultrasound detected erosions in ACPA positive patients are predictive for arthritis

In **paper IV** we also studied the value of US to predict arthritis in ACPA positive patients without clinical arthritis in a window between the preclinical and clinical phase of RA. In this study we failed to show significant predictive values at patient level of either GS or PD at baseline. However, erosions were not rare and could be detected in 16% of the ACPA positive patients with MSK symptoms, also after adjusting for potential confounders. The presence of erosions on US predicts arthritis development, also after adjusting for potential confounders.

These results are in line with the study by Tamas et al, where at least one erosion could be detected by US in 66.7 % of patients with early RA [99]. Interestingly, some studies have shown that erosions can easily be detected by US [100-102]. Also in the absence of radiographically detected erosions, US can identify erosions as shown in the study by Dohn et al [103]. Apart from our study, one more group (Nam et al) has described the predictive value of erosions detected by US to foresay arthritis [73]. The results regarding US erosions from this study are similar to our findings.

Only the presence of US erosions predicts arthritis development in our study, despite the lack of predictive value concerning GS and PD scoring. Taken together, US is a valuable tool to risk-stratify ACPA-positive patients with MSK pain, but future studies will address whether US erosions also predict long-term progression of joint damage as determined on conventional radiographs.

5.2.2. Digital X-ray radiogrammetry works as a prognostic tool for radiographic damage in newly diagnosed rheumatoid arthritis

In **paper II** we showed that BMD loss over 3 months in new onset RA measured by DXR is predictive for radiographic damage after 1 year. To our knowledge, this is the first study measuring BMD loss within such a short interval. Using DXR as a prognostic tool, treatment decisions to avoid radiographic damage and disability could be done in an earlier stage of the disease. However, in our study we did not observe a significant difference in the DXR value at baseline in the ACPA-positive compared with the ACPA-negative patients. This fact is contrary to the findings in other studies in which BMD loss was significantly more widespread in ACPA-positive patients [104-106]. Furthermore, Forslind et al. showed that patients with early RA, who were on a daily low dose of GC in addition to conventional DMARDS, had significantly less DXR-BMD loss as compared with patients with RA who were not receiving GC [85]. This finding was attributed to the anti-inflammatory effect of prednisolone, hampering osteopenia induced by inflammation. Since treatment with GC parallel to DMARDs at the time point of RA diagnosis is common, the value of the DXR method to predict radiographic damage has to be justified with more studies.

6. CONCLUSIONS

ACPA is associated with arthritis and is associated with higher risk for radiographic damage not only in RA but also in SLE.

ACPA and anti-CarP positivity in SLE patients are associated with an arthritic phenotype in SLE and correlate significantly with development of erosions.

ACPA in the era of treatment with biologics is still associated with increased risk for radiographic damage.

US detected erosions in an early stage of RA development predict development of arthritis. However, we could not confirm a clinical value of GS and PD in non-arthritic patients with ACPA and MSK pain (**Figure 16**).

DXR-BMD loss in newly RA diagnosed patients predicts radiographic damage after 1 year (**Figure 17**).

7. FUTURE PLANS

Does early introduction of treatment with biologics really decrease radiographic changes? ACPA as a specific biomarker for RA and associate with radiographic damage. In paper III, the collected TIRA data still provides a lot of potential information to be evaluated. One project in planning is to read and score radiographs in TIRA-2 after 10 years from inclusion with the aim to evaluate radiographic changes and their relation to antibody status over a long time.

Do US findings, DXR-BMD loss and/or radiographic erosions in a preclinical stage indicate a possibly more aggressive course of RA? And are these imaging modalities associated with radiographic outcome after 10 years? All patients in the TIRx study (paper IV) were examined regarding radiographic changes and underwent DXR to measure BMD-loss. These data have to be assessed. Further, we plan new X-rays in all patients after 10 years.

Can the combination of anti-CarP antibodies, ACPAs, and RF precede arthritis in a near future? Triple seropositivity has a very high specificity for the identification of patients with early RA. In order to further investigate whether triple autoantibody positivity in arthralgia patients can precede arthritis and in which period of time, a prospective study using ultrasound to detect early signs of joint inflammation would be a suitable option.

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¹⁾ The mentioned 'old-fashioned' ultrasound machine was without Doppler and of course not the used equipment in my research.

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