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Are changes in rheumatoid factor levels reflecting change in prognosis of rheumatoid arthritis? – A retrospective data analysis

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1 Zusammenfassung

Einführung Radiologische Progression bei rheumatoider Arthritis (RA) weist einen Zusammenhang mit dem Nachweis von Rheumafaktor (RF) und Antikörpern gegen zitrullinierte Peptide (ACPA) auf. In früheren Studien wurde bereits gezeigt, dass die Titer dieser beiden Autoantikörper unter Therapie veränderlich sein können.

Ziele In dieser Studie untersuchten wir, ob Veränderungen im Autoantikörpertiter mit einer Veränderung der Prognose der RA einhergehen.

Methoden Wir beobachteten eine Kohorte von 450 RA Patienten über eine dreijährige retrospektive Periode. Wir erhoben Röntgenbilder der Hände und Füße, die in diesem Zeitraum erstellt wurden, Testergebnisse für RF und ACPA, die innerhalb von drei Monaten vor, oder nach dieser Bildgebung erfolgt sind, sowie Krankheitsaktivitätsparameter. In einem logistischen Regressionsmodel untersuchten wir dann den Effekt von Antikörperveränderungen auf die radiologische Progression. Patienten mit unterschiedlichen Autoantikörper-Verläufen wurden statistisch gematcht. Abschließend wurden alle Ergebnisse in einem Matrix Model zusammengefasst.

Ergebnisse Krankheitsaktivität, bereits vorbestehende strukturelle Schäden, die Höhe des RF Titers zu Beginn des Beobachtungszeitraums, sowie der Titerabfall von RF über drei Jahre wurden als prognostisch bedeutsame Faktoren identifiziert. In multivariaten Modellen konnte gezeigt werden, dass ein größerer Abfall des RF Titers mit vermehrter radiologischer Progression einhergeht ($p=0.037$). Ein Matrix Model wurde erstellt um Risikopopulationen für radiologische Progression zu identifizieren.

Schlussfolgerung Eine mögliche Erklärung dieses Zusammenhangs kann sein, dass hohe Krankheitsaktivitäts-assoziierte RF Titer – die eben auch die Veränderlichkeit ausmachen – mit Progression einhergehen, während von der Krankheitsaktivität unabhängige (unter Therapie stabile) Titer weniger direkte strukturelle Konsequenzen tragen. Da ACPA Titer deutlich weniger Veränderungen aufweisen, lässt sich keine verlässliche Aussage zu einer möglichen Assoziation von Veränderungen mit radiologischer Progression treffen.

2 Abstract

Background The presence of rheumatoid factor (RF) and antibodies against citrullinated peptides (ACPA) is associated with progression in patients with rheumatoid arthritis (RA). RF and ACPA levels may change under therapy, but it has not been elucidated yet, if such changes in autoantibody levels reflect a true change in prognosis of RA.

Objective Here we aimed to investigate whether changes of RF and ACPA reflect a change in prognosis of RA in the longer term.

Methods For that purpose, a cohort of 450 RA patients was assessed over a three-year retrospective period. Radiographs of hands and feet within three years (30 to 42 months) and serological tests for RF and ACPA taken within three months prior or after the respective radiograph were obtained, and logistic regression analyses was used to examine the effect of RF and ACPA on damage progression. Within matching analyses of groups with different autoantibody courses we identified changes in RF levels as risk factor for radiographic progression ($p=0.037$). The results were combined into a matrix model that consisted of risk factors arranged in increasing risk of damage progression.

Results Disease activity, already existing radiographic damage and RF levels at baseline, as well as the decrease of RF over the three-year observational period were included as dichotomous variables. A matrix model was developed to identify subpopulations of RA patients at higher predicted risk for radiographic progression.

Conclusions With greater decline in RF levels comes greater amount of progression. One possible explanation for this effect might be, that high levels of disease activity related RF – which reflect in fact changeability – are associated with damage progression, whereas RF levels which are independent of disease activity (and which are steady during therapy) bear less structural consequences. Since ACPA levels seem to be much less reactive than RF, no reliable statement can be made whether or not there is an association with damage progression.

3 Scientific background

3.1 Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterised by a symmetric peripheral polyarthritis. It is the most common form of chronic inflammatory arthritis affecting approximately 0.5 to 1% of the adult population, concerning women two- to four times more frequently than men, and with increasing prevalence in age.[1] RA leads to inflammation of synovial tissue with symmetric involvement of peripheral joints, typically of the hands and feet, and to damage of cartilage and bone. It can also affect non-articular structures, such as tendons, ligaments, and fasciae. Because of its systemic nature RA involves other tissues as well, leading to extra-articular disease manifestations in up to 40%.[2] During the last two decades management of RA has been revolutionized. With efficacious therapeutics, substantial advances in outcomes measurement, as well as the advent of new classification criteria for RA, the outcome for many patients has been improved; so low disease activity and remission are now a feasible goal for a large proportion of patients.[3, 4] Besides joint involvement, duration of symptoms and acute-phase reactants, serological findings, meaning autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), are part of the classification criteria mentioned above. These autoantibodies do not only have diagnostic but also prognostic value, considering that seropositivity for RF and ACPA is associated with structural and functional deterioration. In case of RF, damage progression is related to higher levels of disease activity triggered by the autoantibodies, but might also be due to an independent effect of RF.[5] Investigations on ACPA showed similar results, so that patients with high titres were especially prone to damage progression.[6] It is also conceivable that higher inflammatory activity drives the production of more RF, thus increasing their levels. Indeed, recent findings showed that RF and ACPA decrease significantly under therapy[7], leading to the question, if with changing levels of autoantibodies prognosis changes too, or whether this is simply the aforementioned disease activity related part.

3.2 Pathogenesis

Research over the last two decades has contributed to important advances in our understanding of disease pathogenesis at a molecular and cellular level.

In 2012 the European League Against Rheumatism (EULAR) Study Group for Risk Factors for RA published a concept of the natural history of RA from being at risk because of a certain genotype until manifestation of disease.[8] Several genetic and environmental risk factors have been identified. Heritability is estimated to be up to 60%, with disease concordance rates for monozygotic twins of 15-30%.[9] About one-third of this genetic susceptibility is amounted to the major histocompatibility complex (MHC), respectively the human leukocyte antigen (HLA) system. Specific HLA-DRB gene polymorphisms encode a conserved amino acid sequence – the so-called 'shared epitope' (SE) – in the HLA-DR antigen-binding groove, which is strongly associated with RA as well as the occurrence of ACPA.[10, 11] The low concordance rates for twins mentioned above point to existence of environmental risk factors, among which cigarette smoking raises the risk the most by interacting with the SE, particularly in ACPA-positive patients.[12]

But how does it get from an increased risk to the development of disease? Multiple studies suggest that systemic autoimmunity precedes RA and that clinical onset very likely follows an inflammatory or stressful trigger.[13] This becomes apparent with the detection of RF and ACPA many years before first symptoms occur.[14]

This trigger may result from a defect in negative selection permitting the formation of autoreactive T cells in RA synovium.[15] This might be elicited by dendritic cells, presenting self-antigens, such as human cartilage derived glycoprotein 39 (HC gp39) and citrullinated peptides to T cells, and secreting chemokines in order to recruit monocytes, natural killer cells and other T cells and dendritic cells.[16]

T cells differentiate into Th1 and Th17 cells. Both of these populations produce proinflammatory cytokines, help B cells differentiating into autoantibody-producing plasma cells, stimulate osteoclastogenesis and lead to cartilage damage.[17]

Furthermore there is a third lineage of T cells in RA – regulatory T cells – whose task is to supress effector T cells. They are enriched in synovial fluids of RA patients and associated

with low disease activity.[18, 19] In Fig. 1 a schematic representation of this inflammation cascade is shown.

The role of B cells in RA is not only to produce autoantibodies, including RF and ACPA, further activating complement[20], but they also act as antigen-presenting cells as well as regulatory cells.[21] Th1 and Th17 cells also activate macrophages, major producers of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), Interleukin 1 (IL-1), and IL-6, all leading to joint damage, a hallmark of RA.[22] They induce production of nitric oxide and matrix metalloproteinases (MMP), resulting in loss of cartilage integrity and attachment of fibroblasts building pannus.[23] Further MMPs and proteinases of the ADAMTS (a disintegrin-like and metalloproteinase with trombospondin)-family lead to destruction of cartilage targeting native collagens and proteoglycans.[24]

Moreover bone erosions arise with the presence of RANKL (Receptor Activator of NF-κB Ligand), normally produced by osteoblasts, but in RA synovium also by fibroblasts and activated T cells.[25] Differentiation from osteoclast precursor cells to mature osteoclasts may be directly stimulated by ACPA, produced by plasma cells. Finally, the eroded (resorbed) bony tissue is replaced by inflamed synovial tissue, leading to further bone destruction.[26]

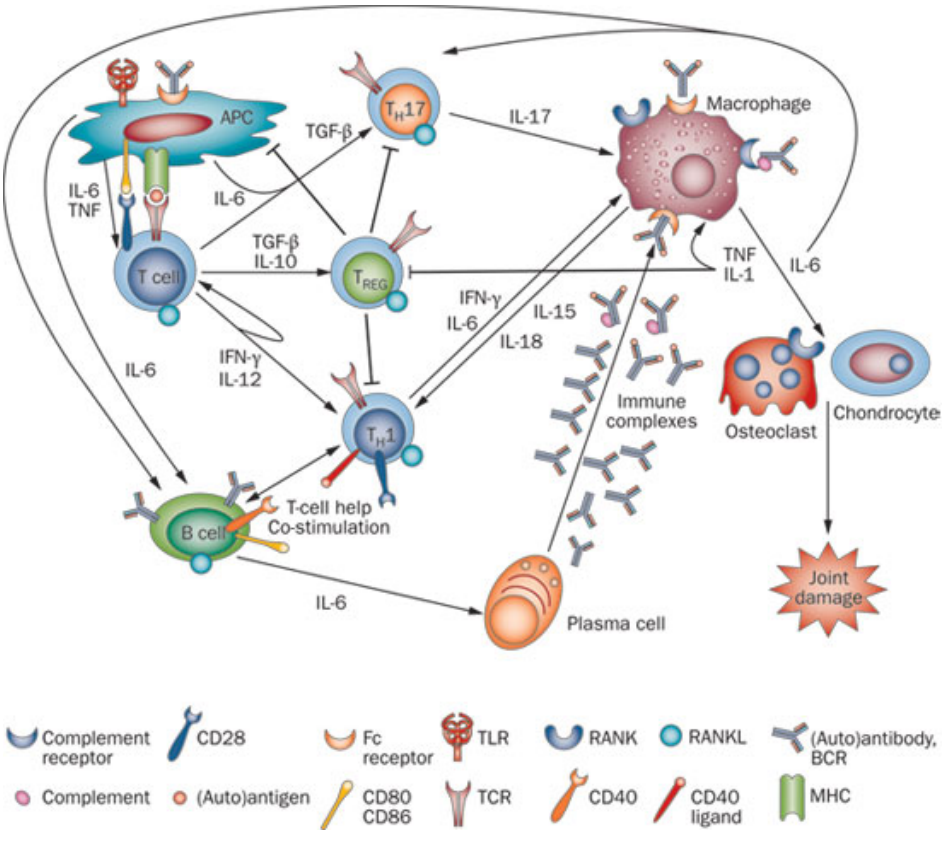

Fig. 1: Pathogenesis of RA.[27]

3.3 Diagnosis

With remission as desired goal and considering that joint damage is irreversible, it is important for patients' function and quality of life to diagnose RA early in order to treat it effectively from the start. The challenge of achieving this has less to do with the absence of effective drugs, but is rather a consequence of deliberations on potential undertreatment or overtreatment of the patient. Whereas in the former case there is the risk of structural damage, which cannot be reversed even by optimal treatment, the latter carries the risk of serious adverse events of the therapies employed.[28, 29]

But before discussing the diagnosis of RA, several terms need to be defined: the term 'early arthritis' refers to the duration of the symptom of arthritis and 'undifferentiated arthritis' relates to the absence of a specific diagnosis, despite diagnostic evaluation. It is obvious that these two terms overlap at some point, leading to the question, which specific steps need to be taken to call an early arthritis 'undifferentiated'. For that purpose, an algorithm on how to examine patients with new-onset arthritis was developed, which can either establish a specific diagnosis or result in the classification of 'undifferentiated'.[30] Starting with a physical examination and exclusion of trauma, the first rheumatologic question arises by subdividing in acutely inflamed (e.g. gout or septic arthritis) and sub-acute arthritis. Therefore, arthrocentesis may be essential: if the analysis of the synovial fluid results in abnormalities that are typical for a particular disease, such as the presence of a high leucocyte count and a positive Gram staining for septic arthritis or the detection of intracellular urate crystals for gout, it is possible to already make a reliable diagnosis at this point.

Thereafter, clinical, laboratory and imaging features will help making differential diagnoses, including viral polyarthritis, peripheral spondylarthropathy, Lyme arthritis, sarcoid arthritis, polymyalgia rheumatica and osteoarthritis as well as other systemic rheumatic diseases, such as systemic lupus erythematosus (SLE), Sjögren's syndrome, dermatomyositis and mixed connective tissue disease. An initial stratification according to the number or pattern of swollen joints may be helpful. Only if all these efforts are unsuccessful, the presentation should be characterised as 'undifferentiated arthritis' (shown as 'UPIA' for undifferentiated peripheral inflammatory arthritis in Fig. 2). This 'diagnosis' then needs to be re-evaluated periodically.

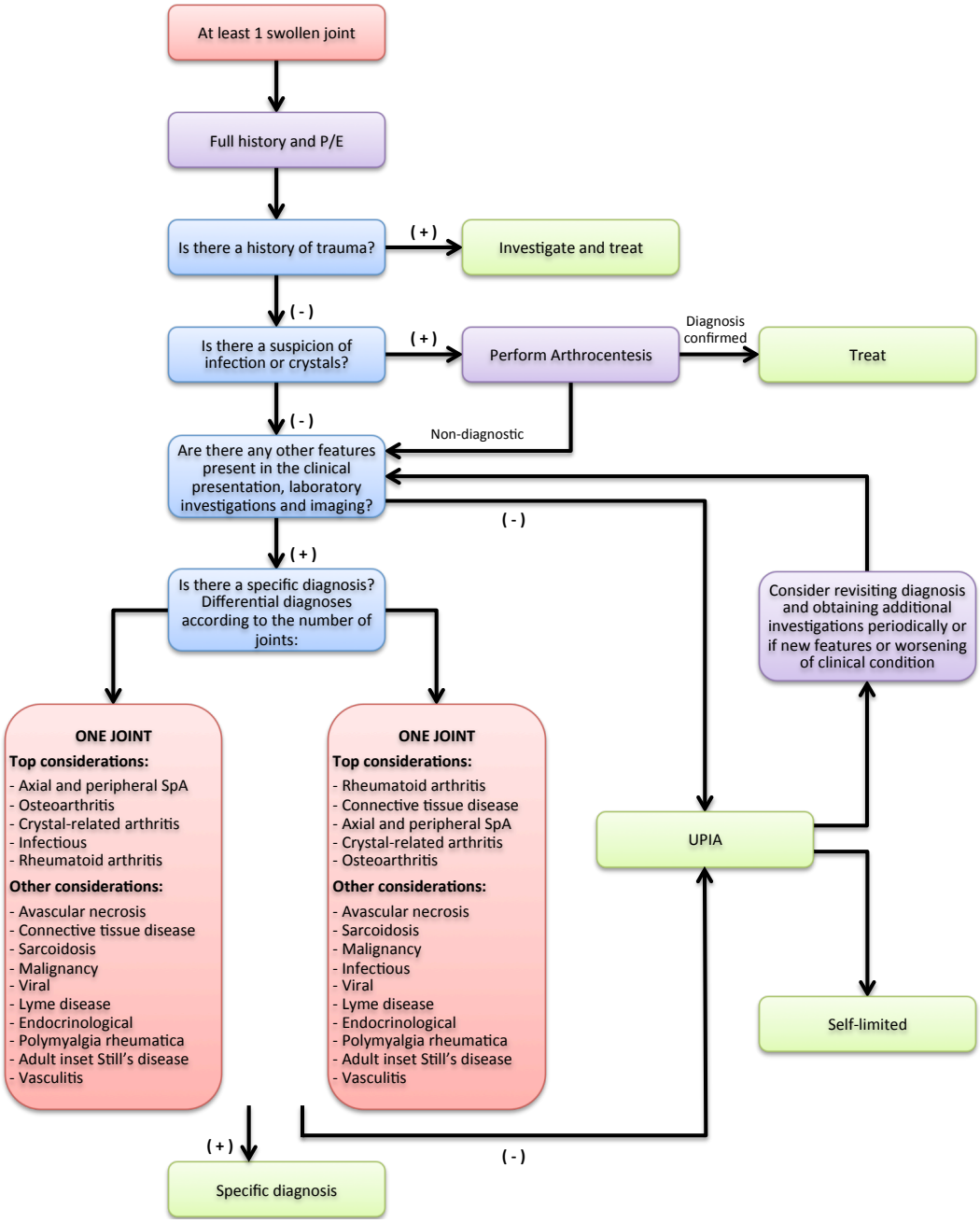

Fig. 2: Algorithm for examining patients with a new-onset arthritis. Adapted from Hazlewood G., et al.[30]

Although no diagnostic criteria exist, classification criteria are available and may be used as guidance for clinical diagnosis. Despite the fact that classification criteria are made for including most homogenous patient populations in trials while accepting the possibility of misclassification in the individual, they may help in establishing the diagnosis of RA in individual situations. Until 2010, the established classification criteria for RA were those by the American College of Rheumatology (ACR) dating from 1987.[31] They have been seen increasingly controversial due to their lack of sensitivity especially in early arthritis, given that they were developed from a cohort of mostly long-standing RA patients.[32] These criteria, for example, included erosions as a feature of diagnosis, which occur only in 10% of patients with new-onset RA, as well as rheumatoid nodules, which are seen even much less frequently in early disease.[33] Moreover they did not include testing for ACPA, which has entered daily clinical practice over the last decade.

Therefore a joint working group of the ACR and the European League Against Rheumatism (EULAR) was established in 2007 to develop new criteria for RA, which were published in 2010.[3] The new criteria target any patient who presents with at least one clinically swollen joint, for which no other disease is clearly responsible. For a definite classification of a patient as having RA a score ≥6/10 is then required (Tab. 1). Although radiographic examination is not compulsory in the new scoring system, patients presenting with joint destruction typical for RA may be classified directly (i.e. without applying the scoring system).[34] While classification criteria may provide help in diagnosing RA, it is important to always keep in mind that rheumatologists can overrule the classification result at any time based on the specific clinical presentation and their professional expertise.

Joint involvement (tender or swollen)[1]	
1 large joint	**0**
2 – 10 large joints	**1**
1 – 3 small joints (with or without involvement of large joints)	**2**
4 – 10 small joints (with or without involvement of large joints)	**3**
>10 joints (at least one small joint)	**5**
Serology[2] (at least one test result is needed for classification)	
Negative RF and negative ACPA	**0**
Low positive RF or low positive ACPA	**2**
High positive RF or high positive ACPA	**3**
Acute-phase reactants[3] (at least one test result is needed for classification)	
Normal CRP and normal ESR	**0**
Abnormal CRP or abnormal ESR	**1**
Duration of symptoms[4]	
<6 weeks	**0**
≥6 weeks	**1**

Tab. 1: 2010 ACR/EULAR classification criteria for RA.[3]

[1]Distal interphalangeal joints, first carpometacarpal joints and first metatarsophalangeal (MTP) joints are excluded from assessment. Categories of joint distribution are classified according to the location and number of the involved joints, with placement into the highest category possible based on the pattern of joint involvement. 'Large joints' refers to shoulders, elbows, hips, knees and ankles. 'Small joints' refers to the MTP joints, proximal interphalangeal joints, second to fifth MTP joints, thumb interphalangeal joints and wrists. 'Symmetric' is defined as bilateral involvement of at least one region. In the category '>10 joints,' at least one of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere.

[2]Negative refers to international unit (IU) values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but three of less times the ULN for the laboratory and assay; high-positive refers to IU values that are more than three times the ULN for the laboratory and assay. When rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low positive for RF.

[3]Normal/abnormal is determined by local laboratory standards.

[4]Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.

3.4 Clinical features

RA is the most frequent chronic systemic autoimmune disease with joint involvement, showing incidence of 41 per 100,000 people per year[35] with a prevalence of 0.5%.[36-38] It is characterised by a symmetric peripheral polyarthritis leading to pain and joint destruction with the consequence of disability, reduced quality of life and high levels of work instability.[39]

RA includes a broad spectrum of possible disease courses and related outcomes ranging from patients with almost self-limiting disease to severe, progressively destructive disease with increased morbidity and mortality.[40] Despite this wide range of possibilities, RA generally shows an insidious onset of mono-, oligo- or polyarthritis with tender or swollen joints, soft tissue swelling and long-lasting morning stiffness. In addition other less specific symptoms such as generalized weakness and fatigue are commonly seen. Laboratory markers such as RF and ACPA are important tools to distinguish RA from other forms of arthritides and elevated levels of acute-phase reactants, e.g. erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), can be detected even years before disease onset.[41, 42] Radiographic examinations to document erosions and progression of the disease as well as ultrasound including power Doppler examinations for visualization of the inflammatory soft tissue process should be used to confirm diagnosis.

The typical joints affected in RA include the small finger joints – the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints –, wrists and forefeet, concerning especially the metatarsophalangeal (MTP) joints. While distal interphalangeal (DIP) joints are hardly ever affected, involvement of large joints like shoulder, elbows, hip and knee joints are commonly seen in established disease. Especially in the past, in the absence of many effective therapeutic options, the dreadful result of irreversible cartilage and bone destruction became apparent in quite a number of patients with RA: ulnar deviation due to involvement of carpal bones and MCP joints, swan-neck deformity (hyperextension of PIP and flexion of DIP joints), boutonnière deformity (flexion of the PIP and hyperextension of DIP joints) and Z-deformity of the thumb (flexion of the first metacarpophalangeal joint and hyperextension of the interphalangeal joint) are all examples and consequences of soft tissue damage caused by inflammatory process of RA. (Fig. 3)

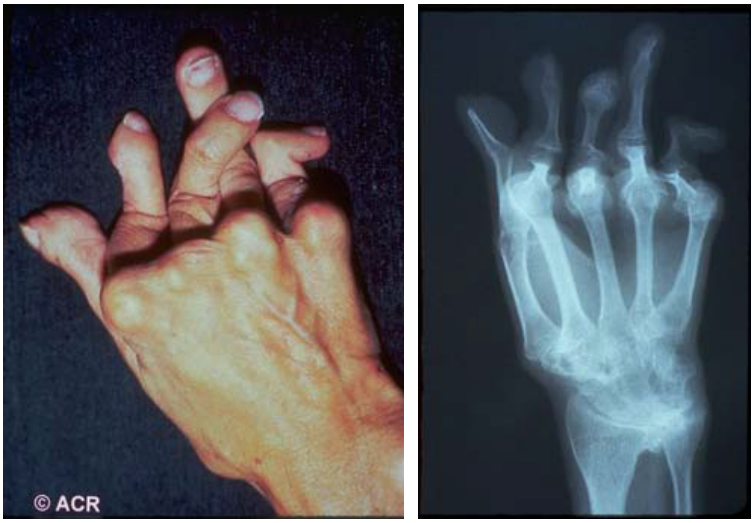

Fig. 3: Joint deformities in RA.
Courtesy of the American College of Rheumatology, taken from the Rheumatology Image Bank.

Due to its systemic nature, RA does not only involve the musculoskeletal system but potentially affects other organs as well. Despite their declining incidence, rheumatoid nodules are regarded as among the more frequent extra-articular manifestations. They primarily occur in seropositive patients with severe and active disease and usually present as subcutaneous nodules along the forearm and fingers, but in some cases can also affect internal organs. Another organ more commonly affected by RA is the eye. Eye involvement may span from mild symptoms e.g. related to episcleritis to potentially severely impairing complications, e.g. following scleritis and keratitis. Less commonly seen extra-articular manifestations include lung and cardiac involvement, e.g. pleuritis, interstitial lung disease and exudative pericarditis, as well as vasculitis, amyloidosis and Felty's syndrome.[2]

RA is also afflicted with a wide range of comorbidities: the incidence of severe infections is significantly increased due to immune-modulating effects of the disease itself as well as the application of immunosuppressants in therapy. In this context it should be especially mentioned, that the therapeutic blocking of TNF-α leads to an increased risk of opportunistic infections and above all reactivation of latent tuberculosis, so screening for tuberculosis is now standard before initiating therapy.[43] Other comorbidities include osteoporosis, especially in a context of steroid therapy, as well as cardiovascular disease, malignancies and overlap syndromes with other autoimmune disease such as polymyalgia rheumatica, mixed connective tissue disease, secondary Sjögren's syndrome and SLE, the latter then called "rhupus". All this illustrates the very heterogeneous disease pattern of RA requiring a lot of clinical knowledge and experience to achieve best outcome for the patient.

3.5 Management

With the revolution of RA therapy within the last 20 years, a paradigm shift from remission as almost fictional aspiration towards a reasonably achievable goal has been implemented. Therefore several management guidelines have lately been published outlining the state-of-the-art treatment of RA.[44, 45] (Tab. 2)

They agree that early diagnosis is key to achieve and maintain remission and to prevent bad long-term outcome.[4, 46] Thus it is important to refer patients as soon as possible to a specialist, start an early intensive therapy and suppress inflammation quickly. Subsequently patients should be seen regularly aiming for remission, or at least low disease activity, to effectively prevent further disease progression.

According to EULAR recommendations, first-line therapy should include methotrexate, a conventional synthetic DMARD (csDMARD), given its clinical and structural effectiveness, good long-term tolerability, adjustable dose, its orally and subcutaneously availability, and its reasonable costs compared to other therapeutic agents.[47, 48] If responding insufficiently to MTX or in case of contraindications, monotherapy with other csDMARDs or a combination therapy should be considered. (Tab. 3) Glucocorticoids are used as bridging therapy or when flares occur. They can be injected in inflamed joints and administered intramuscularly as well as orally. However, considering the broad spectrum of adverse effects, e.g. immunodeficiency, osteoporosis and Cushing's disease, the basic principle in the use of steroids should always be 'as short as possible, as long as necessary'[49], but ideally, they should not be given longer than 6 months.

With deeper understanding of underlying pathogenesis, new therapeutic agents, so-called biologicals, or biological DMARDs (bDMARDs), targeting specific cytokines or cells, entered the market. While efficacy in monotherapy was similar to MTX, there is an added benefit in combination with MTX. Due to their costs, they are restricted to patients who fail on at least one conventional DMARD. The currently available mode of actions of biological compounds includes inhibition of TNF-α and IL-6, targeting B lymphocytes, and interference with T cell co-stimulation. (Tab. 4) With Tofacitinib, a drug of the Janus kinase (JAK) inhibitor class, there is now the first targeted synthetic DMARD (tsDMARD) on the market, which is recommended after failure of at least on bDMARD.

EULAR recommendations for the management of RA
Overarching principles
A. Treatment of RA patients should aim at the best care and must be based on a shared decision between the patient and the rheumatologist
B. Rheumatologists are the specialists who should primarily care for RA patients
C. RA incurs high individual, societal and medical costs, all of which should be considered in its management by the treating rheumatologist
Recommendations
1. Therapy with DMARDs should be started as soon as the diagnosis of RA is made
2. Treatment should be aimed at reaching a target of remission or low disease activity
3. Monitoring should be frequent in active disease (every 1–3 months); if there is no improvement by at most 3 months after the start of treatment or the target has not been reached by 6 months, therapy should be adjusted
4. MTX should be part of the first treatment strategy in patients with active RA
5. In cases of MTX contraindications (or early intolerance), sulfasalazine or leflunomide should be considered as part of the (first) treatment strategy
6. In DMARD-naïve patients, irrespective of the addition of glucocorticoids, csDMARD monotherapy or combination therapy of csDMARDs should be used
7. Low-dose glucocorticoids should be considered as part of the initial treatment strategy (in combination with one or more csDMARDs) for up to 6 months, but should be tapered as rapidly as clinically feasible
8. If the treatment target is not achieved with the first DMARD strategy, in the absence of poor prognostic factors, change to another csDMARD strategy should be considered; when poor prognostic factors are present, addition of a bDMARD should be considered
9. In patients responding insufficiently to MTX and/or other csDMARD strategies, with or without glucocorticoids, bDMARDs should be commenced with MTX
10. If a first bDMARD has failed, patients should be treated with another bDMARD; if a first TNF inhibitor therapy has failed, patients may receive another TNF inhibitor or a biological agent with another mode of action
11. Tofacitinib may be considered after biological treatment has failed
12. If a patient is in persistent remission after having tapered glucocorticoids, one can consider tapering bDMARDs, especially if this treatment is combined with a csDMARD
13. In cases of sustained long-term remission, cautious reduction of the csDMARD dose could be considered, as a shared decision between patient and physician
14. When therapy needs to be adjusted, factors apart from disease activity, such as progression of structural damage, comorbidities and safety issues, should be taken into account

Tab. 2: EULAR recommendations for the management of rheumatoid arthritis: 2013 update.[50]

Drug name	Brand Name	Effect
Methotrexate	Ebetrexat®, Methotrexat Pfizer®	Inhibition of purine and pyrimidine synthesis
Leflunomide	Arava®	Inhibition of pyrimidine synthesis
Sulfasalazine	Salazopyrin®	Anti-inflammatory, mechanism not identified
Chloroquine	Resochin®	Anti-inflammatory, mechanism not identified
Gold	Tauredon®	Anti-inflammatory, mechanism not identified

Tab. 3: DMARDs in treatment of RA.

Drug name	Brand Name	Effect
Etanercept	Enbrel®	TNF-α receptor fusion protein
Infliximab	Remicade®	Chimeric monoclonal antibody to TNF-α
Adalimumab	Humira®	Fully human monoclonal antibody to TNF-α
Golimumab	Simponi®	Fully human monoclonal antibody to TNF-α
Certolizumab	Cimzia®	Humanized anti-TNFα antibody Fab fragment
Tocilizumab	RoActemra®	Humanized monoclonal antibody to the IL-6 receptor
Anakinra	Kinaret®	IL-1 receptor antagonist
Abatacept	Orencia®	T cell co-stimulation inhibitor
Rituximab	MabThera®	Chimeric monoclonal antibody to CD20
Tofacitinib	Xeljanz®	Janus kinase inhibitor

Tab. 4: Targeted therapies in treatment of RA.

3.6 Autoantibodies in RA

RF are autoantibodies recognizing the C-terminal domain of the constant region of the heavy chain in human IgG. It can be detected in 60 to 80% of RA patients as well as in 5% of healthy individuals, so neither does a negative result exclude the disease nor does a positive confirm it.[51] It is possible to assess RF in a number of different ways, including classic agglutination techniques such as the obsolete Waaler-Rose test, turbidimetric techniques such as laser nephelometry, which is currently standard at our hospital, and enzyme-linked immunosorbent assay (ELISA), which also allows determination of RF subtypes. Among these autoantibodies the IgM subtype is the most frequently found species and testing for IgM-RF has 69% sensitivity and 85% specificity. While IgG- and IgA-subtypes show higher specificity for the disease, due to their lack of sensitivity they do not provide any further diagnostic information.[52] The cut-off value for a positive test result is 12 IU/ml at our hospital, although higher cutpoints have been shown to have higher diagnostic and prognostic specificity for RA.[53, 54]

The role of RF in pathogenesis is not completely solved yet, but it has been described that RF-specific B cells may capture and present self-antigens to T cells by immune complex uptake. RF also activates complement by building immune complexes with autologous IgG.[55] These complexes activate complement, including C5a, which binds to C5a receptors on macrophages, which leads to their further activation. This contributes to the inflammatory cascade by, inter alia, releasing proinflammatory cytokines, leading to localized tissue damage.[20] (Fig. 4)

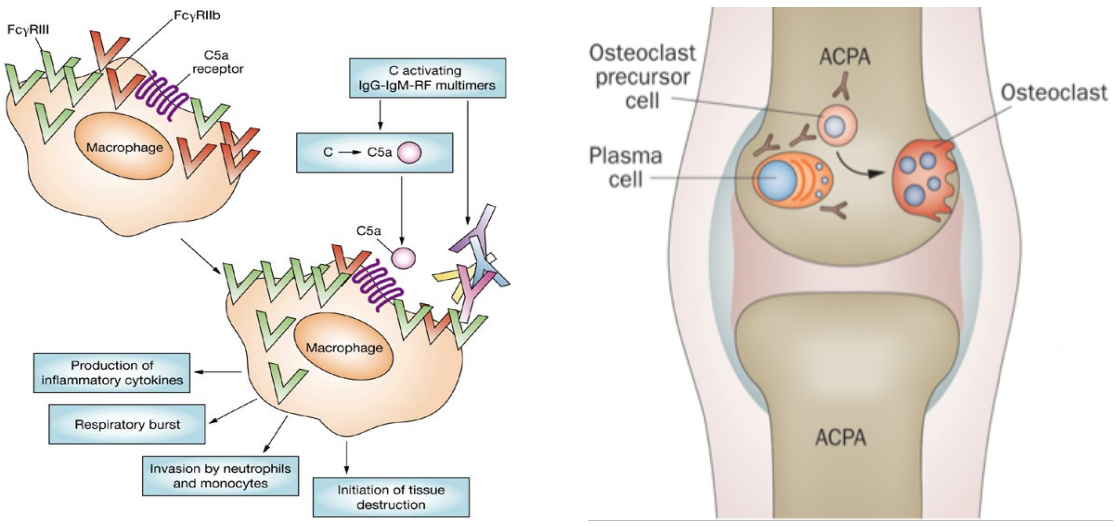

Fig. 4: RF and ACPA and their suspected role in pathogenesis of RA.[20, 26]

While RF is well known since the 1940s, ACPA were applied much later in clinical practice. They are characterised with specificity against post-translationally deiminated arginyl residues, so-called citrullinated proteins, such as vimentin, α-enolase and the α- and β-chains of fibrin.[56] ACPA shows only moderate levels of sensitivity and can be found in up to 65% of RA patients, though having very high specificity of 95%.[57] Because RA shares symptoms with other rheumatic disease conditions, ACPA is considered to be of particular diagnostic use in patients who are negative for RF.[58] The most frequently used ACPA test today is the second-generation CCP assay (CCP2), whose cut-off at our clinic is 10 IU/ml. Just like with RF, the presence of ACPA can be a powerful predictor of disease course. There is a strong association between ACPA and joint damage and it has been identified as a predictor of radiographic progression in patients with early RA. It is important to note that this association is not linked to the simultaneous presence of RF, and therefore both antibodies can be considered as predictors of erosiveness, and it has also been argued that the presence of ACPA may boost the effect of RF on disease progression.[6]

Generally, the pathogenetic mechanisms leading to progression are still poorly understood and it is not yet clear, whether, and if so, how ACPA is involved. Some studies suggest, that ACPA seems to play a role in the evolution of bone erosions in the course of RA stimulating osteoclast differentiation by binding on their surface and increasing autocrine stimulation of TNF production and thereby leading to initial bone loss.[26] (Fig. 4)

As previously mentioned, RF and ACPA may not be constant in RA, but rather have the potential to change under therapy. While RF declines faster, to a larger extent and in a greater number of patients than ACPA, these changes are seen mostly in patients who experience a clear reduction of disease activity.[7] It is not yet clear if and how these changes affect prognosis in RA.

3.7 Structural outcomes and their assessment

Joint damage is the hallmark of RA. Usually progressing over time, structural changes of bone and cartilage can be quantified by scoring plain radiographs for erosions and joint space narrowing (JSN).[59] Typical erosiveness of RA has recently been defined as erosions in more than three separate joints[34] and the following predictors for erosive disease have been identified so far: female gender, early detection of erosive changes, elevated acute-phase reactants (ESR and/or CRP) as well as seropositivity for RF and/or ACPA.[60]

Hands and feet are most frequently imaged, because these joint areas are affected in most patients with RA and thought to be also representative for large joint involvement.[61] There is a large number of different radiographic scores in use, but the most common method is the Sharp Score and its modifications. It assesses erosions reflecting bone damage, as well as joint space narrowing reflecting cartilage loss.[62] Originally evaluating only the joints of the hands and wrists it was later extended by the feet. In 1989 van der Heijde had modified the Sharp score once again by adding the metatarsophalangeal joints and the interproximal joints of the big toes, whereas some areas of the wrist were excluded due to difficulties assessing them.[63] In the resulting modified Sharp/van der Heijde Score (SvH, Fig. 5) erosions are counted in overall 44 joints, comprising from 32 joints of the hand and 12 of the feet.

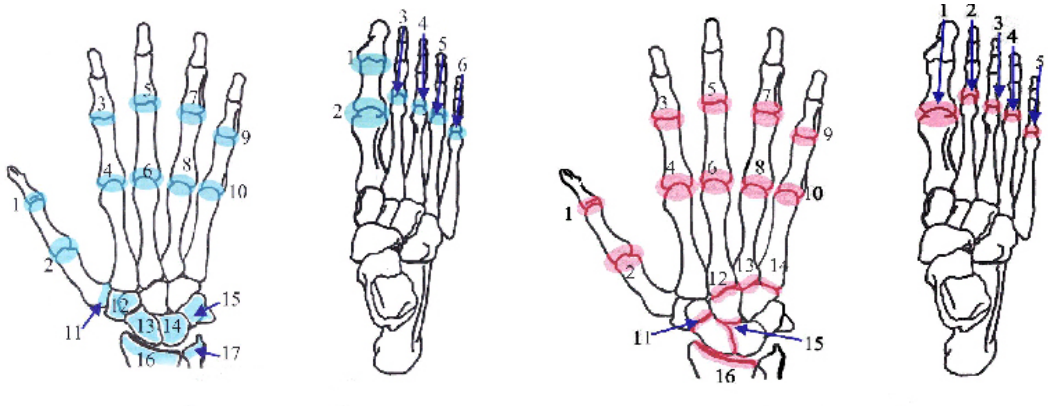

Fig. 5: A schedule of the joints that are included in the Sharp/van der Heijde scoring method (blue erosions, red JSN).[64]

They are scored 1 if there is a discrete interruption of the cortical surface. If there is a larger defect, a higher score is given according to the extent of damage. In the hands, the maximum erosion score is 5, in the feet it is 10, so the maximum number of erosions is 160 in the hands and 120 in the feet. (Fig. 6)

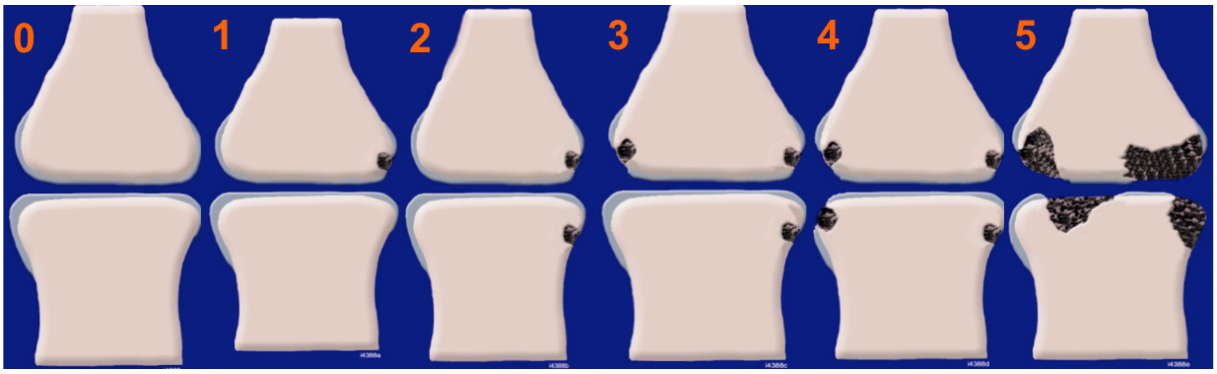

Fig. 6: Scoring system for erosions in the Sharp/van der Heijde scoring method.[65]

For JSN five grades are recognized, from 0 meaning normal to 4 corresponding to joint ankylosis. Giving 30 joints of the hands and again 12 of the feet are scored; the maximum scores for JSN are 120 and 48 respectively. (Fig. 7)

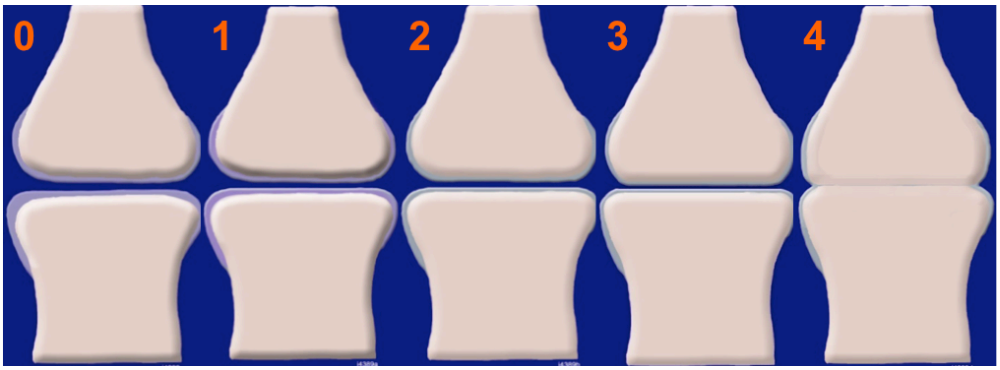

Fig. 7: Scoring system for JSN in the Sharp/van der Heijde scoring method.[65]

This results in a maximum total score of 448, although even aggressive disease rarely exceeds a score of 200.

4 Background to the current investigation

RF and ACPA are autoantibodies with clear prognostic implications[5, 6]. While in the past RF has been considered as a disease attribute (‘seropositive’ or ‘seronegative’ RA), its ability to change imposes a challenge to this concept. Indeed, in clinical practice patients who present to the rheumatologist may be diagnosed with seropositive RA, while after a course of effective treatment they test negative for RF. ACPA, being less sensitive for RA have been shown to be less changeable upon treatment[7], although seroconversions may still occur.

Current treatment concepts focus on treating RA to a target of remission or low disease activity; however, the presence or absence of autoantibodies may influence the choice of compound or the aggressiveness on the path to the target[50]. It remains unclear though, if an initially positive patient, who converts after a course of treatment, is prognostically different to a patient, in whom autoantibodies endure. Currently, strategies are based on the current level rather, but there is no additional consideration of changes in RF, which may have potential to determine the need for further therapy, or may conversely indicate a situation, in which therapy may be safely reduced.

In the present study we aimed to investigate the changeability of autoantibodies, their principal association with progression of RA, and whether seroconversion carries a specific prognostic implication. These points are more specifically spelled out in the following Objectives section.

5 Objectives

The main objective of the study is to analyse whether changes of RF and ACPA levels during the course of disease and treatment influence prognosis. Therefore we evaluate changes of RF and ACPA as predictive markers for radiographic progression, which is an accepted structural outcome in RA.

Detailed objectives:

1) **Association of autoantibody (AAB) levels and structure**

   To analyse AAB levels and their influence on radiographic progression over time

2) **Association of AAB seroconversion with structure**

   To compare structural progression in patients with constantly high, or constantly low RF levels to the progression of patients with initially positive RF levels, which convert to negative levels.

3) **Association of changes in AAB levels with structure**

   To investigate the structural changes associated with changes in AAB levels.

All analyses were initially done univariately, but then also controlling for the effect of disease activity and already existing structural damage on these findings.

6 Methods

6.1 Study design

We designed a study including patients from the routine outpatient clinic of the Division of Rheumatology, Medical University Vienna. Inclusion criteria were classifiable RA by the 1987 ARA criteria or the 2010 ACR/EULAR classification criteria[3, 31], two available radiographs of hands and feet about three years (30 to 42 months) apart, as well as serological tests for RF and ACPA taken within three months prior or after each radiograph. If more than one of such clinical interval was available, we included the first. Below, we consider the first time point for each patient as *baseline*, and the last as *endpoint*.

Radiographic images and the serological data were obtained prospectively, and were retrospectively scored by an experienced reader using the modified Sharp/van der Heijde (SvH) method (see above).

In the course of the present study, changes of RF and ACPA levels over a three-year period will be examined for their influence on radiographic progression. Results will also be adjusted for disease activity, expressed as Simplified Disease Activity Index (SDAI), in order to examine the independent effect of autoantibodies and their changes on structural outcomes. (Fig. 8)

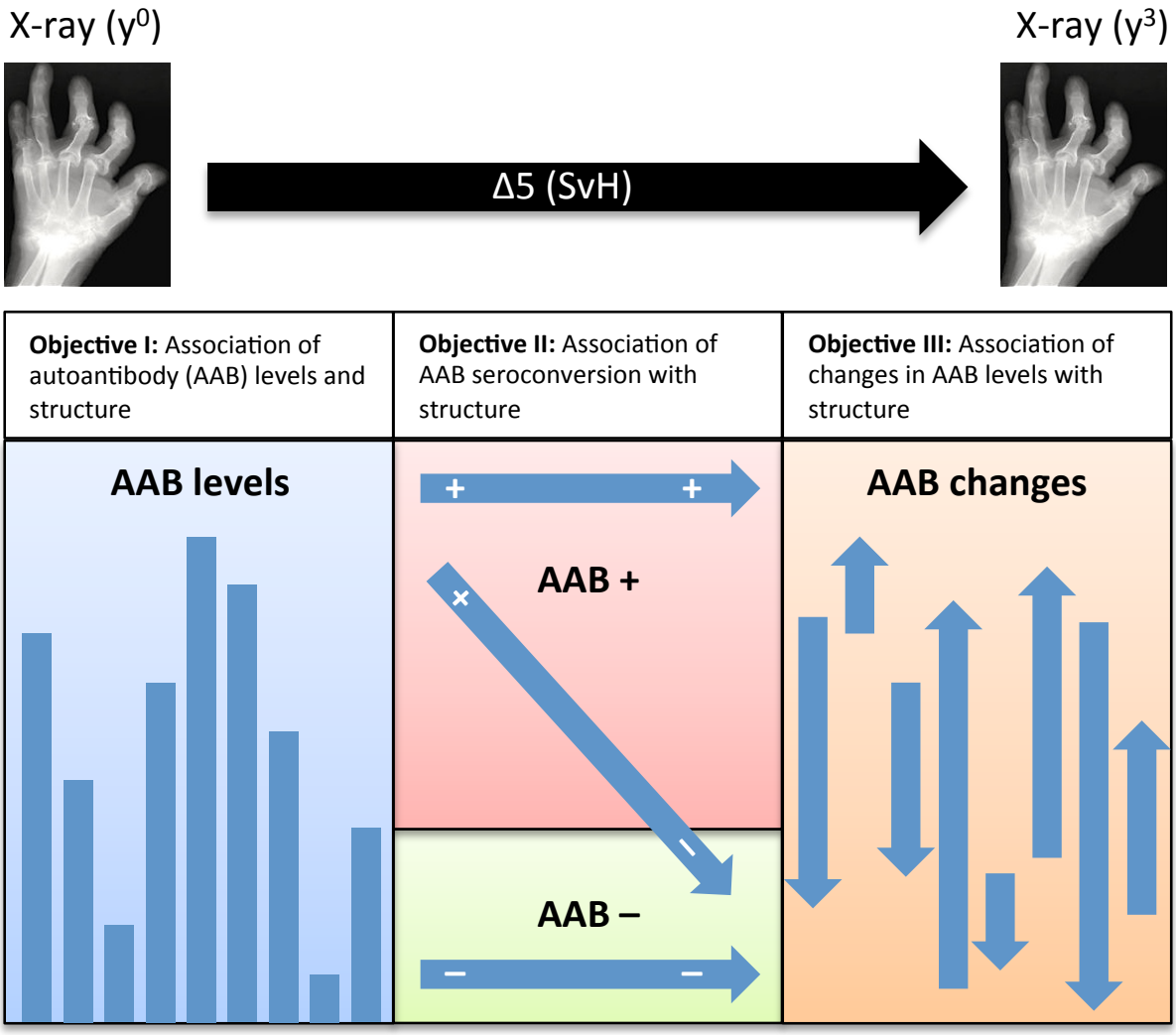

Fig. 8: Study design.
In three distinct analyses the association of serological findings (independent variables; bottom panels) with structural progression (Sharp van der Heijde radiographic score, SvH, with a change of 5 or more; dependent variable; top panel) will be analysed. The independent variables include the level of autoantibodies (AAB) (bottom left), the seroconversion status (bottom middle), and the changes of autoantibody levels (bottom right).

6.2 Patients and data

Data for this study were extracted from a clinical database, which had been established at the Division in 1998. Since then, laboratory and epidemiological data of RA patients who visited the outpatient clinic of the rheumatology department were prospectively documented at every visit, usually every three to four months. Data quality, integrity and accuracy are ensured by constant maintenance, periodical updates, and quality checks. Patients enrolled in this database are diagnosed with RA according the 1987 revised ACR criteria or (after their introduction) the 2010 RA classification criteria, as appropriate at the time.

Available data comprise patient demographics and disease characteristics, including disease duration, clinical assessments performed by a specialised team of biometricians (performance of joint counts, global scores, and pain scores), functional and quality of life assessments by questionnaires, as well as data on therapy. Radiographic images of the hands and feet were obtained on a circa annual basis for all patients with RA. The digitised images of these radiographs were scored by the modified SvH method, and the results were also entered into the database.

Patients were selected according to the aforementioned inclusion criteria (see 6.1). Autoantibodies levels at the beginning of the observation period (baseline) and after three years (endpoint) were obtained from the database.

6.3 Risks and benefit for the patient

Due to the retrospective study design, there is no expected risk or benefit for any patient. In the past several studies identified various predictors of radiographic progression, but never evaluated their possible changeability and its implications for prognosis. This study now will provide insight to the prognostic value of autoantibody measurement in relation to joint progression, help understand if autoantibodies are a disease feature, a disease activity related feature, or both, and how these components relate to progression of RA.

The study was approved by the Ethical Committee of the Medical University of Vienna.

6.4 Statistical analyses

The following statistical analyses were performed with IBM© SPSS© statistical software, version 21.0.0, p-values <0.05 were considered significant. In all analyses, structural progression was defined as an increase of 5 or more on the Sharp van der Heijde radiographic score (SvH; rand 0-448) over three years.

Analyses supporting objective 1:

- Logistic regression analysis was used to examine the effect of RF and ACPA levels (continuous scale) on damage progression. Results were adjusted for disease activity (SDAI).
- For the autoantibodies determined at the start of the observation period, the effect on damage progression was assessed prospectively, in other words looking at the effect on progression of damage over the *next* 3 years. For autoantibodies determined 3 years later, the effect was assessed retrospectively, in other words looking at the effect of the current autoantibody levels (or presence) on progression over the *past* 3 years

Analyses supporting objective 2:

- To compare conversion of autoantibody status and its effect on prognosis, patients were divided into groups according to their different courses of autoantibody levels (positive → positive, positive → negative, negative → negative, and negative → positive). RF positivity was defined using a threshold cut point of 12 IU/ml and ACPA positivity using a threshold cut point of 10 IU/ml.
- RF seroconverters (positive → negative) were matched with non-seroconverters (positive → positive) by k-nearest neighbors algorithm for disease activity (SDAI) and radiographic damage at baseline (SvH), and were compared regarding their radiographic progression using the Mann-Whitney-U test.
- In addition, RF seroconverters (positive → negative) were matched with non-seroconverters (positive → positive) by k-nearest neighbors algorithm for RF levels (IU/ml) and radiographic damage at baseline (SvH), and were compared regarding their radiographic progression using the Mann-Whitney-U test.

Analyses supporting objective 3:

- Patients with RF changes over time were matched by k-nearest neighbors algorithm for disease activity (SDAI) and radiographic damage (SvH) at baseline with those constantly high, or constantly low RF levels (using tertiles: high positive → high positive; high positive → moderate positive; high positive → low positive; high positive → negative; moderate positive → moderate positive; moderate positive → low positive; moderate positive → negative; low positive → low positive; and low positive → negative), and the associations with the outcome were compared regarding their radiographic progression using the Mann-Whitney-U test.
- In addition, Patients with RF changes over time were matched by k-nearest neighbors algorithm for RF levels (IU/ml) and radiographic damage (SvH) at baseline with those constantly high, or constantly low RF levels (using tertiles: high positive → high positive; high positive → moderate positive; high positive → low positive; high positive → negative; moderate positive → moderate positive; moderate positive → low positive; moderate positive → negative; low positive → low positive; and low positive → negative), and the associations with the outcome were compared regarding their radiographic progression using the Mann-Whitney-U test.
- RF tertiles were defined using a threshold cut point for high positive: RF level > 250.6 IU/ml; for moderate positive: RF level ≤ 250.6 IU/ml, > 67.3 IU/ml; and for low positive: RF level ≤ 67.3 IU/ml, > 12 IU/ml.
- Logistic regression analysis selecting baseline risk factors of RF level (≤120 IU/ml, >120 IU/ml), disease activity (SDAI ≤15, SDAI >15) and radiographic damage (≤20 in SvH, >20 in SvH), as well as decrease of RF over the three-year observational period (≤60 IU/ml, >60 IU/ml) was used to examine the joint effect of these predictors on radiographic progression. Results were combined into a matrix model and arranged in increasing predicted probability of radiographic progression.

7 Results

7.1 Patients

450 patients with 6512 clinical visits between 2001 and 2013 could be included in our study. For all of the 450 patients two radiographs of hands and feet about three years (30 to 42 months) apart, as well as serological data for RF and ACPA were available. 60% (60.2%) of them were positive for RF at baseline, and 62% (61.6%) for ACPA. At the first time point of the observation period defined for the study, patients had a mean age of 56 (55.7) years and suffered from a long standing RA with a disease history of about 7 (7.0) years. On average patients were in moderate disease activity by CDAI, SDAI and DAS28 and the mean radiographic score at baseline was 38 (38.0) points in the SvH Score with an average progression of 8 (8.0) points over the subsequent three-year observational period. (Tab. 5)

Baseline characteristics	Mean (SD)	Baseline characteristics	Mean (SD)
Age (years)	55.7 (12.5)	**CRP (mg/dl)**	1.4 (2.0)
RF (IU/ml)	183.6 (517.2)	**ESR (mm)**	26.4 (22.2)
RF Positivity (%)	60.2	**Patient Global Assessment**	34.2 (25.4)
ACPA (IU/ml)	187.2 (232.6)	**Evaluator Global Assessment**	22.0 (19.0)
ACPA Positivity (%)	61.6	**SDAI**	13.8 (11.3)
Disease Duration (years)	7.0 (9.3)	**CDAI**	12.4 (10.6)
HAQ	0.728 (0.756)	**DAS28**	3.7 (1.4)
Swollen Joint Count 28	3.3 (3.8)	**Baseline Damage (SvH)**	38.0 (56.1)
Tender Joint Count 28	3.6 (5.4)	**Progression after 3 years (SvH)**	8.0 (11.2)

Tab. 5: Baseline characteristics.
Abbreviations: SD, Standard deviation; HAQ, Health Assessment Questionnaire; CRP, C-reactive protein; SDAI, Simplified Disease Activity Index; CDAI, Clinical Disease Activity Index; DAS28, Disease Activity Score 28; SvH; Sharp/van der Heijde Score.

7.2 Autoantibody levels and radiographic progression (*objective 1*)

7.2.1 Effect of RF on radiographic progression

To evaluate a potential association between radiographic progression and the course of RF, we performed logistic regression analysis. First, we examined the link between the RF at the time of the first radiograph and radiographic progression over the next 3 years. Here we used the nearest available serological data for RF as a continuous variable and progression as dichotomous, defining an increase of five points or more in the SvH Score over three years as progression. There was a clear and significant trend of more future radiographic progression with higher RF levels at baseline (p=0.039). We performed the same analysis with RF at the time of the radiograph three years later, which showed that this association had been lost (p=0.515), i.e. the RF-level at the end of the observation period did not correlate anymore with the prior progression and, therefore, the reduction of the RF levels in the course of therapy had led to levels of RF that were not correlated with disease progression. The Wald score presented in Tab. 6 can be used a good reflection of the statistical strength of the association, from which the p-value is then derived. Higher Wald scores indicate stronger associations.

Baseline	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available RF (IU/ml) at first radiograph	0.001	0.000	4.250	1	0.039	1.001
Constant	-0.003	0.106	0.001	1	0.975	0.997

Year 3	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available RF (IU/ml) after year three radiograph	0.000	0.000	0.424	1	0.515	1.000
Constant	0.079	0.104	0.576	1	0.448	1.082

Tab. 6: Effect of RF in radiographic progression at baseline and after 3 years.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors.

After adjusting for disease activity (SDAI at baseline), the effect of RF on radiographic progression lost significance (baseline: p=0.099; year 3: p=0.562), whereas disease activity showed a strong association at both baseline (p=0.021) and after 3 years (0.010), indicating that disease activity is an indicator for radiographic progression from a prospective and

retrospective point and thus more related to damage progression than RF (Tab. 7); also that a lot of the association of RF with progression is likely mediated through its effects on disease activity, as has been recently reported.[5] Although disease activity was associated with RF levels at both time points (p<0.001), the association of RF on progression decreased from baseline (Wald score 2.72) to three years (Wald score 0.34).

Baseline	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available RF (IU/ml) at first radiograph	0.000	0.000	2.719	1	0.099	1.000
SDAI	0.021	0.009	5.315	1	0.021	1.021
Constant	-0.255	0.161	2.519	1	0.113	0.775

Year 3	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available RF (IU/ml) after year three radiograph	0.000	0.000	0.336	1	0.562	1.000
SDAI	0.024	0.009	6.700	1	0.010	1.024
Constant	-0.227	0.164	1.913	1	0.167	0.797

Tab. 7: Effect of RF in radiographic progression at baseline and after 3 years adjusted for disease activity.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors; SDAI, Simplified Disease Activity Index.

The situation was similar after adjusting for the cumulative disease activity over the three-year observational period, expressed as SDAI (AUC): the association of RF on progression is still borderline significant at baseline (p=0.052), but not at all apparent after three years (p=0.513), whereas disease activity was confirmed as indicator for damage progression at both time points (p=0.001). (Tab. 8)

Baseline	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available RF (IU/ml) at first radiograph	0.001	0.000	3.760	1	0.052	1.001
SDAI (AUC)	0.000	0.000	10.868	1	0.001	1.000
Constant	-0.425	0.163	6.822	1	0.009	0.654

Year 3	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available RF (IU/ml) after year three radiograph	0.000	0.000	0.427	1	0.513	1.000
SDAI (AUC)	0.000	0.000	11.662	1	0.001	1.000
Constant	-0.364	0.162	5.042	1	0.025	0.695

Tab. 8: Effect of RF in radiographic progression at baseline and after 3 years adjusted for cumulative disease activity.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors; SDAI, Simplified Disease Activity Index; AUC, Area under the curve.

7.2.2 Effect of ACPA on radiographic progression

In an analogous analysis, the probability of future progression significantly increases with higher ACPA levels, at baseline (p=0.001), as well as when looking back after 3 years (p<0.001). There was essentially no difference in the strength of this association (Wald scores: 11.3 vs. 13.0, respectively). (Tab. 9) This is likely due to the fact that there are almost no converters to negative ACPA and also the changes to very low ACPA are rare: mean (standard deviation) ACPA levels at baseline and after three years were 187.2 (232.6) IU/ml and 145.3 (169.5) IU/ml, respectively.

Baseline	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available ACPA (IU/ml) at first radiograph	0.001	0.000	11.333	1	0.001	1.001
Constant	-0.154	0.122	1.599	1	0.206	0.858

Year 3	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available ACPA (IU/ml) after year three radiograph	0.002	0.001	12.979	1	0.000	1.002
Constant	-0.193	0.125	2.377	1	0.123	0.824

Tab. 9: Effect of ACPA in radiographic progression at baseline and after 3 years.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors.

When we adjusted for disease activity (SDAI at baseline), in contrast to RF, the effect of ACPA on radiographic progression kept its significance from baseline to the three years time point (baseline p<0.001; year 3 p<0.001), as well as in comparison with the unadjusted model (see Wald scores, Tab. 9). The latter was the case, even although the association of disease activity was similarly strong as in the RF model (see respective Wald scores, Tab. 7). This implies that radiographic progression is independently related to ACPA and to disease activity. (Tab. 10)

Baseline	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available ACPA (IU/ml) at first radiograph	0.002	0.000	12.698	1	0.000	1.002
SDAI	0.024	0.009	6.603	1	0.010	1.024
Constant	-0.488	0.179	7.454	1	0.006	0.614

Year 3	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available ACPA (IU/ml) after year three radiograph	0.002	0.001	13.099	1	0.000	1.002
SDAI	0.024	0.009	6.762	1	0.009	1.024
Constant	-0.519	0.182	8.104	1	0.004	0.595

Tab. 10: Effect of ACPA in radiographic progression at baseline and after 3 years adjusted for disease activity.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors; SDAI, Simplified Disease Activity Index.

After adjusting for the cumulative disease activity over the three-year observational period, expressed as SDAI (AUC), the independent effect of ACPA on radiographic progression remained at both time points (p=0.001). (Tab. 11)

Baseline	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available ACPA (IU/ml) at first radiograph	0.001	0.000	10.604	1	0.001	1.001
SDAI (AUC)	0.000	0.000	11.200	1	0.001	1.000
Constant	-0.586	0.176	11.102	1	0.001	0.557

Year 3	B	S.E.	Wald	df	Sig.	Exp(B)
Nearest available ACPA (IU/ml) after year three radiograph	0.002	0.001	12.010	1	0.001	1.002
SDAI (AUC)	0.000	0.000	11.117	1	0.001	1.000
Constant	-0.623	0.179	12.114	1	0.001	0.536

Tab. 11: Effect of ACPA in radiographic progression at baseline and after 3 years adjusted for cumulative disease activity.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors; SDAI, Simplified Disease Activity Index; AUC; Area under the curve.

7.3 Seroconversion and radiographic progression (*objective 2*)

7.3.1 RF seroconversion and radiographic progression

The next step was to evaluate the association of seroconversion, as the extreme end of serological improvement, and radiographic progression. Therefore we first divided our population (n=450) according to their course of RF: 54.2% (n=244) of patients were seropositive for RF at baseline as well as after three years; 7.8% (n=35) were positive for RF at baseline, but seroconverted to negativity during the observational period; 33.3% (n=150) of patients were negative for RF at both time points; and 4.7% (n=21) were negative at baseline and positive at three years. (Fig. 9)

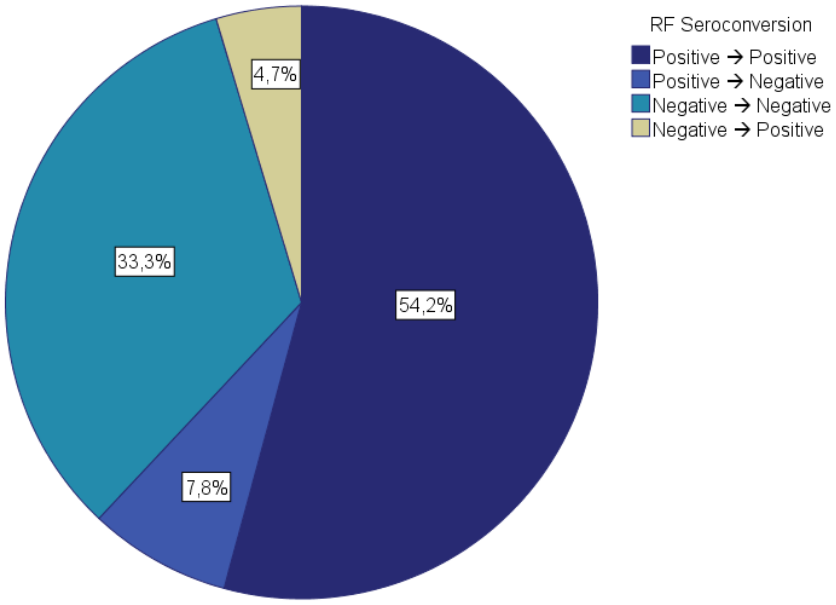

Fig. 9: Patients according to their RF course using a cut-off of >12 IU/ml to define seropositivity.

We studied to what extent radiographic progression was different in these groups: overall 52.7% (n=237) of the patients experienced of radiographic damage over the three-year observational period. The highest percentage of progression was observed in patients seroconverting from positive to negative (60%). There was virtually no difference in the proportion of patients progressing between the RF non-converters and converters (57.4% vs. 60.0%). Only the RF negative patients, who remained seronegative, showed clearly less progression than all other groups (42.2%) (p=0.028 by Chi^2 test). (Tab. 12) Interestingly enough, the progression rate among the 35 patients converting to negativity was almost

identical with those 21 converting to positivity, but the overall progression was even higher in the former (12.5 increase for conversion to negativity vs. 6.1 increase for conversion to positivity).

RF Seroconversion status (n)	Progression rate	Mean (SD) progression in SvH
Positive → Positive (244)	57.4%	8.7 (12.3)
Positive → Negative (35)	60.0%	12.5 (16.0)
Negative → Negative (150)	42.7%	6.1 (7.6)
Negative → Positive (21)	57.1%	6.1 (5.5)
Overall (450)	52.7%	8.0 (11.2)
p-value	0.028 (by Chi^2 test)	0.009 (by ANOVA)

Tab. 12: RF Seroconversion in radiographic progression.
Abbreviations: SD, Standard deviation; SvH; Sharp/van der Heijde Score.

7.3.2 ACPA Seroconversion and radiographic progression

Also for seroconversion of ACPA we divided our population (n=450) in four groups as above. Whereas, we could observe at least a few seroconversions in the case of RF, there were virtually none for ACPA (1.6% in either way); 60.2% (n=271) of the patients were positive for ACPA at baseline as well as after three years, and 36.7% (n=165) were ACPA negative at both time points (Fig. 10). This obviously also explains the sustained statistical prediction of ACPA at baseline and at three years as identified in section 7.2.2.

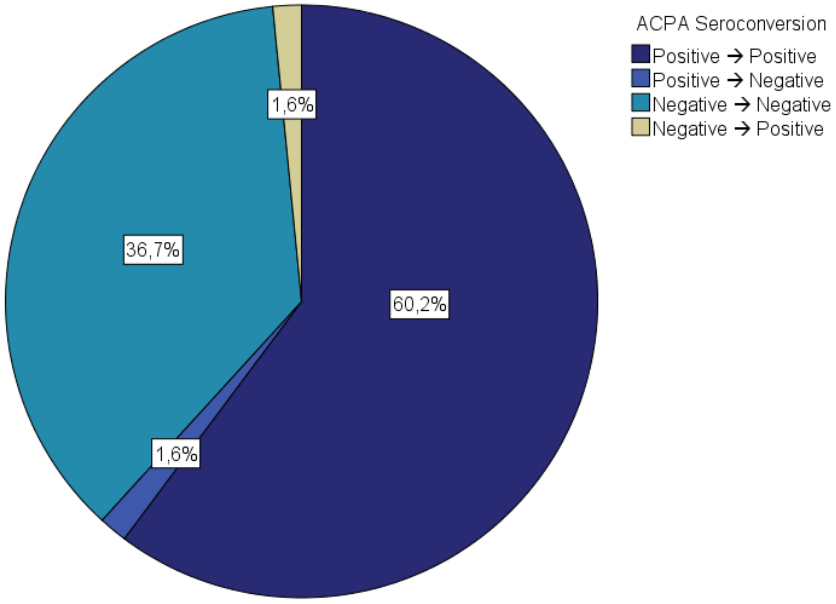

Fig. 10: Patients according to their ACPA course using a cut-off of >10 IU/ml to define seropositivity.

Although we could not observe enough seroconversions for making a distinct statement about the effect of ACPA seroconversion on radiographic progression, we examined in a further step the association between the course of ACPA and progression of damage (Tab. 13): overall 52.7% (n=237) patients developed radiographic damage during the three-year observational period. In the group of ACPA positive-positive patients the rate of progressors was higher than in the cohort of all-time negative patients (60.1% vs. 41.8%), highlighting ACPA positivity as predictor for radiographic progression ($p<0.001$ by Chi^2 test).

ACPA Seroconversion status (n)	Progression rate	Mean (SD) progression in SvH
Positive → Positive (271)	60.1%	9.5 (12.9)
Positive → Negative (7)	57.1%	7.3 (6.3)
Negative → Negative (165)	41.8%	5.9 (7.6)
Negative → Positive (7)	14.3%	4.1 (7.1)
Overall (450)	52.7%	8.0 (10.8)
p-value	<0.001 (by Chi^2 test)	0.009 (by ANOVA)

Tab. 13: ACPA Seroconversion in radiographic progression.
Abbreviations: SD, Standard deviation; SvH; Sharp/van der Heijde Score.

Due to the fact, that we observed virtually no ACPA seroconversions, we did not further pursue our investigations on that topic at this point.

7.4 RF seroconversion: detailed analysis comparing converting with non-converting RF positive patients (*objective 2*)

7.4.1 Crude analysis

Here, we compared RF seroconverting patients to non-seroconverting RF positive patients to evaluate seroconversion as a predicting marker for radiographic progression. There were 244 patients in the group of patients, who were positive for RF at baseline as well as at endpoint, and 35 patients, who seroconverted during the three-year observational period to negative. We compared these two groups of patients on the basis of autoantibody levels and disease activity at baseline as well as their changes, radiographic damage at baseline and damage progression after three years (Tab. 14).

	Mean (Standard deviation)		**p-value by Mann-Whitney-U test**
	Positive → Positive (n=244)	Positive → Negative (n=35)	
Baseline RF (IU/ml)	319.2 (661.1)	78.2 (202.2)	p<0.001
Δ RF (IU/ml)	126.1 (545.4)	66.2 (202.2)	p=0.908
Baseline ACPA (IU/ml)	271.2 (237.5)	244.2 (253.7)	p=0.256
Δ ACPA (IU/ml)	57.7 (137.1)	94.2 (151.4)	p=0.234
Baseline SDAI	13.8 (11.4)	19.4 (10.9)	p=0.003
Δ SDAI	5.7 (11.7)	5.5 (11.1)	p=0.468
SDAI (AUC)	14032.4 (11862.3)	20337.6 (14871.6)	p=0.007
Baseline damage (SvH)	42.3 (61.0)	46.6 (43.4)	p=0.095
Year 3 progression (SvH)	8.7 (12.3)	12.5 (16.0)	p=0.334

Tab. 14: Characteristics of RF positive patients, who seroconvert, or not.
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

The levels of RF at baseline were more than four times higher in the group of patients remaining seropositive (319.2 vs. 78.2 IU/ml), but seroconverting patients seemed to suffer from more active RA at the first visit (SDAI 13.8 vs. 19.4) and over the three-year observational period (p=0.007). We observed a greater, but non-significant change in RF for patients who remained seropositive compared to the converting patients (126.1 vs. 66.2 IU/ml), while the decrease of disease activity was about the same (SDAI: 5.7 vs. 5.5), i.e.

endpoint SDAI was still much higher in seroconverting patients. Also, baseline damage was about equal in both groups (42.3 vs. 46.6 in the SvH score).

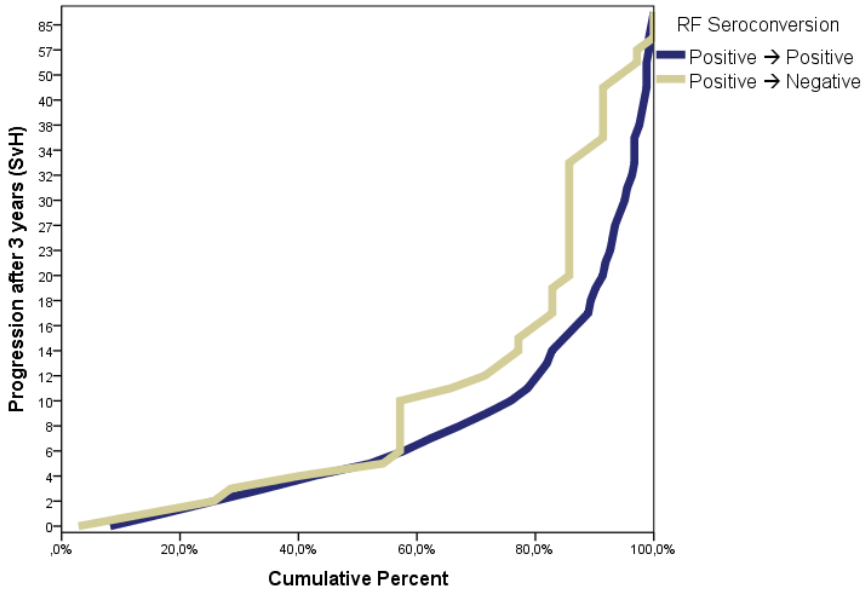

Fig. 11: Radiographic progression in RF positive patients, who seroconvert, or not.

Radiographic progression after the three-year observational period was considerably, but not significantly higher in the group of seroconverting patients (8.7 vs. 12.5 in the SvH score). The extent of damage progression is illustrated in Fig. 11 by using probability plots, in which the degree of progression is ordered by size, and shown as cumulative proportions. There was no difference between RF converters and non-converters in ACPA levels at baseline (p=0.256) or in their change after three years (p=0.234), indicating an effect of RF seroconversion on radiographic progression independent of ACPA.

7.4.2 Matched for disease activity and baseline damage

In a next step we matched these two groups of patients for their initial disease activity (SDAI at baseline) as well as for the initial radiographic damage, considering these two as major predictors for radiographic progression. We found 29 matched pairs (Tab. 15).

	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Positive → Positive (n=29)	Positive → Negative (n=29)	
Baseline RF (IU/ml)	267.9 (517.5)	84.7 (222.1)	p<0.001
Δ RF (IU/ml)	77.9 (318.7)	72.7 (222.1)	p=0.250
Baseline SDAI	19.3 (11.2)	19.4 (10.9)	p=0.913
Baseline ACPA (IU/ml)	335.5 (237.2)	224.6 (256.2)	p=0.066
Δ ACPA (IU/ml)	81.3 (131.4)	79.5 (130.5)	p=0.775
Δ SDAI	10.5 (11.8)	5.5 (11.1)	p=0.409
SDAI (AUC)	16923.8 (12440.7)	21914.5 (15700.1)	p=0.199
Baseline damage (SvH)	50.5 (45.8)	49.7 (46.3)	p=0.913
Year 3 progression (SvH)	11.2 (12.5)	12.6 (16.9)	p=0.845

Tab. 15: Characteristics of RF positive patients, who seroconvert, or not matched for SDAI and radiographic damage at baseline.
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

Although levels of RF at baseline are reduced by about 60 IU/ml due to the process of matching in the group of patients with sustained seropositivity, they were still more than three times higher than in the group of seroconverting patients (267.9 vs. 84.7 IU/ml). Similarly, in the matched analysis, ACPA levels at baseline tended to be higher in the group of non-converters (335.5 vs. 224.6 IU/ml); their change over the three-year observational period was about equal in both groups (81.3 vs. 79.5 IU/ml). Baseline disease activity by SDAI was comparable (approximately 19) in both groups, and so was radiographic damage (approximately 50 points in the SvH score). No significant difference was seen in decrease of RF (77.9 vs. 72.7 IU/ml), in decrease of disease activity (SDAI 10.5 vs. 5.5), or in cumulative disease activity over the three-year observational period (p=0.199).

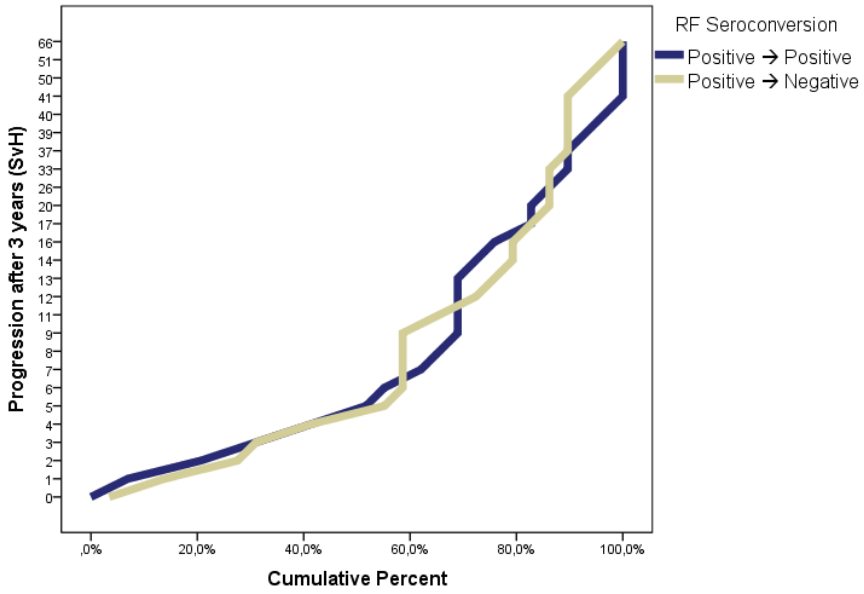

Fig. 12: Radiographic progression in RF positive patients, who seroconvert, or not matched for SDAI and radiographic damage at baseline.

As a result of the matching process, i.e. given similar baseline damage and disease activity, progression of radiographic damage after 3 years was similar in both groups (p=0.845). (Fig. 12)

7.4.3 Matched for RF levels and baseline damage

After matching for disease activity at baseline and baseline damage, the change in RF levels became similar in between the groups of RF converters and non-converters. Therefore, in an additional analysis we matched for baseline RF levels and baseline damage to maintain the signal of change between the converters and non-converters.

	Mean (Standard deviation)		**p-value by Mann-Whitney-U test**
	Positive → Positive (n=33)	Positive → Negative (n=33)	
Baseline RF (IU/ml)	82.0 (159.3)	79.2 (208.2)	p=0.142
Δ RF (IU/ml)	13.3 (52.8)	67.2 (208.2)	p=0.072
Baseline ACPA (IU/ml)	244.3 (219.3)	239.9 (250.9)	p=0.497
Δ ACPA (IU/ml)	31.1 (105.6)	91.2 (151.4)	p=0.137
Baseline SDAI	11.3 (9.4)	19.4 (11.2)	p=0.003
Δ SDAI	3.1 (10.2)	6.3 (9.8)	p=0.059
SDAI (AUC)	13755.0 (11642.1)	20309.1 (15328.6)	p=0.046
Baseline damage (SvH)	43.6 (41.2)	44.1 (41.7)	p=0.985
Year 3 progression (SvH)	9.6 (12.8)	11.4 (15.1)	p=0.979

Tab. 16: Characteristics of RF positive patients, who seroconvert, or not matched for RF levels and radiographic damage at baseline.
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

By matching for RF levels at baseline, RF levels were now equal in both groups (82.0 vs. 79.2 IU/ml). As a consequence, the difference of changes in RF levels in between the two groups became apparent again (13.3 vs. 67.2 IU/ml); again, ACPA levels, as well as their change, reacted in the same way as RF levels do as a result of the matching progress. Seroconverting patients suffered from significantly more active disease at baseline (p=0.003) and over the three-year observational period (p=0.046). Due to the process of matching already formed damage at baseline was comparable in both groups (43.6 vs. 44.1 in the SvH score). (Tab. 16)

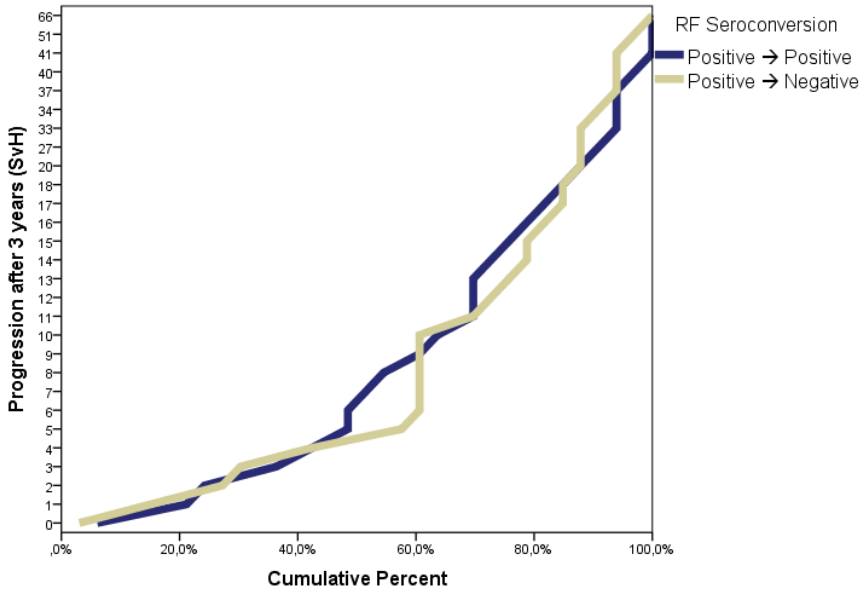

Fig. 13: Radiographic progression in RF positive patients, who seroconvert, or not matched for RF levels and radiographic damage at baseline.

As a result of the matching process, i.e. given similar RF levels and structural damage at baseline, progression of radiographic damage after 3 years was again very similar in both groups (p=0.979). (Fig. 13)

7.5 Changes in RF levels and radiographic progression: tertile approach (*objective 3*)

As an additional step, we evaluated the decrease of RF during the disease process as predicting marker for radiographic progression. From the initial 279 seropositive patients, we excluded 27 patients, whose RF was worsening over the three years period, resulting in a total population of 252 patients. Then we divided the population of patients according to their level of RF: we calculated the tertiles of RF level at baseline and then divided our population into three groups of seropositive patients according to their levels: RF high positive patients with a RF level > 250.6 IU/ml), RF moderate positive patients with a RF level ≤ 250.6 IU/ml, but > 67.3 IU/ml and a group of RF low positive patients with a RF level ≤ 67.3 IU/ml, but > 12 IU/ml.

For these groups, we determined the patients who converted to the respective other groups (Fig. 14):

- high positive remaining high positive (16.3%, n=41);
- high positive turning moderate positive (11.9%, n=30);
- high positive turning low positive (5.2%, n=13);
- high positive patients with complete seroconversion (0.4%, n=1);
- moderate positive remaining moderate positive (15.5%, n=39);
- moderate positive turning low positive (14.3%, n=36);
- moderate positive patients with complete seroconversion (3.2%, n=8);
- low positive remaining low positive (23.0%, n=58);
- low positive patients with complete seroconversion (10.3%, n=26).

We then studied the structural progression over three years in these groups (Tab. 17).

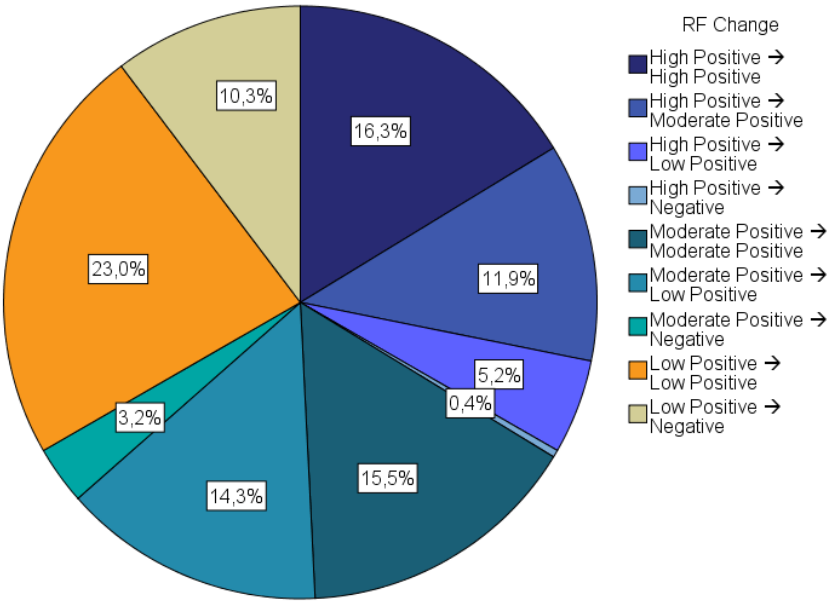

Fig. 14: Changes in RF levels.

Changes in RF levels (n)	Progression rate	p-value
High Positive → High Positive (41)	58.5%	0,345 (by Chi^2 test)
High Positive → Moderate Positive (30)	66.7%	
High Positive → Low Positive (13)	76.9%	
High Positive → Negative (1)	0	
Moderate Positive → Moderate Positive (39)	41.0%	0,064 (by Chi^2 test)
Moderate Positive → Low Positive (36)	63.9%	
Moderate Positive → Negative (8)	75.0%	
Low Positive → Low Positive (58)	58.6%	0,936 (by Chi^2 test)
Low Positive → Negative (26)	57.7%	
Overall (252)	58.7%	0,244 (by Chi^2 test)

Tab. 17: Changes in RF levels and radiographic progression.

We observed the greatest percentage of progressing patients in the groups with greater changes of RF, which are the groups who switch from high to low positive (76.9%), and those becoming RF-negative from an initial moderate positivity (75.0%). In general, it may be stated, that there is a greater amount of progression with greater decline, which can be seen best in the comparison of the initial RF moderate positive groups of patients (moderate positive → moderate positive: 41.0% progression; moderate positive → low positive: 63.9% progression; moderate positive → negative: 75.0% progression). This effect could also be observed in the groups of the initial RF high positive patients ($p<0.244$ by Chi^2 test).

7.6 RF positive patients: comparing improvers with non-improvers (tertile approach) (*objective 3*)

In addition to evaluating complete seroconversion as predictive marker for radiographic progression, we also studied the possible effect of decreasing RF levels on damage progression, using the same tertiles as described in section 7.5 (high positive: RF level > 250.6 IU/ml; moderate positive: RF level ≤ 250.6 IU/ml, > 67.3 IU/ml; low positive: RF level ≤ 67.3 IU/ml, > 12 IU/ml).

We analysed the differences of initial RF positive patients regarding their variable courses of RF and radiographic progression. Because of their small patient number, we did not include seroconverting patients, who initially had high positive RF levels (n=1) and seroconverting patients, who initially had moderate positive RF levels (n=8). Therefore, the following comparisons resulted from that:

- high positive patients remaining high positive (n=41) vs. high positive patients turning moderate positive (n=30) (Tab. 18, Fig. 15);
- high positive patients remaining high positive (n=41) vs. high positive patients turning low positive (n=13) (Tab. 19, Fig. 16);
- moderate positive patients remaining moderate positive (n=39) vs. moderate positive patients turning low positive (n=36) (Tab. 20, Fig. 17);
- low positive patients remaining low positive (n=58) vs. seroconverting patients, who initially had low positive RF levels (n=26) (Tab. 21, Fig. 18).

We then compared these groups of patients on the basis of RF at baseline, change of RF, initial disease activity, change of disease activity, cumulative disease activity over the three-year observational period, radiographic damage at baseline, and damage progression over three years.

7.6.1 Matched for disease activity and baseline damage

In the next step we matched the groups for their SDAI at baseline as well as for their initial radiographic damage, concerning disease activity and already formed damage as the two major predictors for further radiographic progression. This should eliminate the influence of these two factors on radiographic progression and provide information about the (disease activity) independent effect of decreasing RF levels. The following number of matched pairs resulted from that:

- high positive patients remaining high positive vs. high positive patients turning moderate positive (n=21 matched pairs) (Tab. 18, Fig. 15);
- high positive patients remaining high positive vs. high positive patients turning low positive (n=11 matched pairs) (Tab. 19, Fig. 16);
- moderate positive patients remaining moderate positive vs. moderate positive patients turning low positive (n=17 matched pairs) (Tab. 20, Fig. 17);
- low positive patients remaining low positive vs. seroconverting patients, who initially had low positive RF levels (n=17 matched pairs) (Tab. 21, Fig. 18).

a) Patients with high positive RF levels remaining high positive vs. patients changing from high to moderate positivity

Crude	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	High → High (n=41)	High → Moderate (n=30)	
Baseline RF (IU/ml)	1056.4 (1356.6)	470.7 (286.6)	p=0.003
Δ RF (IU/ml)	348.1 (1242.1)	330.5 (282.5)	p=0.015
Baseline SDAI	15.2 (11.4)	16.3 (10.9)	p=0.631
Δ SDAI	5.4 (13.4)	10.2 (11.5)	p=0.192
SDAI (AUC)	15795.6 (12042.3)	12438.5 (8621.8)	p=0.295
Baseline damage (SvH)	41.2 (58.4)	53.1 (61.4)	p=0.105
Year 3 progression (SvH)	8.3 (8.7)	8.7 (8.5)	p=0.726

Matched	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	High → High (n=21)	High → Moderate (n=21)	
Baseline RF (IU/ml)	937.3 (783.4)	395.1 (175.9)	p=0.001
Δ RF (IU/ml)	230.6 (711.5)	251.0 (172.2)	p=0.080
Baseline SDAI	14.8 (11.2)	15.1 (10.3)	p=0.870
Δ SDAI	4.3 (14.1)	10.2 (11.1)	p=0.216
SDAI (AUC)	15297.2 (11667.6)	12086.2 (8883.9)	p=0.428
Baseline damage (SvH)	43.3 (65.0)	44.4 (60.4)	p=0.801
Year 3 progression (SvH)	8.4 (8.6)	9.8 (9.4)	p=0.570

Tab. 18: RF high positive remaining patients with from high to moderate positive changing patients: crude (above) and matched for SDAI and radiographic damage at baseline (bottom).
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

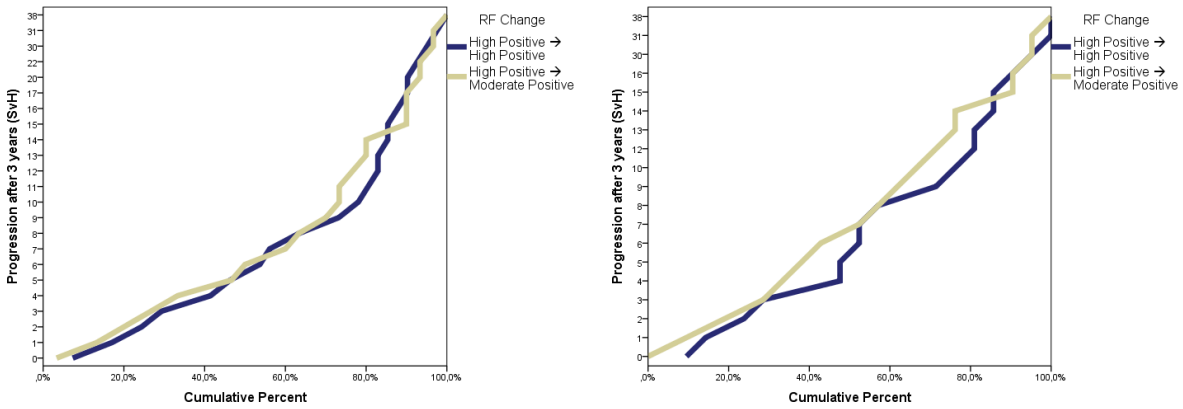

Fig. 15: Radiographic progression in RF high positive remaining patients and patients with from high to moderate positive changing RF levels: crude (left) and matched for SDAI and radiographic damage at baseline (right).

b) Patients with high positive RF levels remaining high positive vs. patients changing from high to low positivity

Crude	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	High → High (n=41)	High → Low (n=13)	
Baseline RF (IU/ml)	1056.4 (1356.6)	477.4 (190.5)	p=0.085
Δ RF (IU/ml)	348.1 (1242.1)	441.4 (180.7)	p=0.016
Baseline SDAI	15.2 (11.4)	23.9 (18.2)	p=0.158
Δ SDAI	5.4 (13.4)	11.2 (10.5)	p=0.251
SDAI (AUC)	15795.6 (12042.3)	26153.8 (26812.7)	p=0.390
Baseline damage (SvH)	41.2 (58.4)	35.8 (36.0)	p=0.378
Year 3 progression (SvH)	8.3 (8.7)	24.3 (36.6)	p=0.287

Matched	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	High → High (n=11)	High → Low (n=11)	
Baseline RF (IU/ml)	708.1 (552.8)	480.1 (208.0)	p=0.365
Δ RF (IU/ml)	490.9 (1377.9)	443.5 (197.7)	p=0.007
Baseline SDAI	19.3 (14.3)	20.5 (14.6)	p=0.797
Δ SDAI	9.4 (13.0)	12.1 (10.7)	p=0.710
SDAI (AUC)	16064.4 (12922.8)	16815.1 (13628.1)	p=0.949
Baseline damage (SvH)	30.6 (34.8)	37.3 (39.2)	p=0.606
Year 3 progression (SvH)	5.0 (4.5)	20.3 (34.5)	p=0.217

Tab. 19: RF high positive remaining patients with from high to low positive changing patients: crude (above) and matched for SDAI and radiographic damage at baseline (bottom).
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

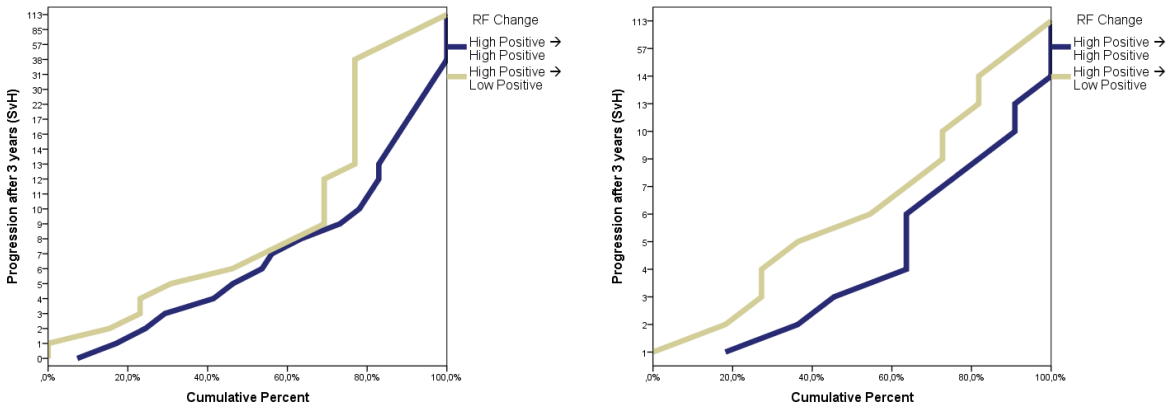

Fig. 16: Radiographic progression in RF high positive remaining patients and patients with from high to low positive changing RF levels: crude (left) and matched for SDAI and radiographic damage at baseline (right).

c) Patients with moderate positive RF levels remaining moderate positive vs. patients changing from moderate to low positivity

Crude	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Moderate → Moderate (n=39)	Moderate → Low (n=36)	
Baseline RF (IU/ml)	152.2 (53.5)	108.9 (44.4)	p<0.001
Δ RF (IU/ml)	36.2 (57.3)	67.3 (46.8)	p=0.016
Baseline SDAI	13.0 (11.6)	14.1 (10.3)	p=0.493
Δ SDAI	4.3 (13.6)	7.0 (10.9)	p=0.837
SDAI (AUC)	11544.4 (7152.5)	13352.6 (9065.0)	p=0.294
Baseline damage (SvH)	28.1 (57.0)	39.1 (43.1)	p=0.091
Year 3 progression (SvH)	5.6 (7.5)	7.3 (10.7)	p=0.150

Matched	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Moderate → Moderate (n=17)	Moderate → Low (n=17)	
Baseline RF (IU/ml)	141.5 (56.5)	109.8 (47.9)	p=0.092
Δ RF (IU/ml)	34.9 (61.9)	68.2 (53.4)	p=0.079
Baseline SDAI	12.9 (11.7)	12.8 (10.4)	p=0.892
Δ SDAI	3.2 (13.7)	6.5 (11.5)	p=0.823
SDAI (AUC)	11396.8 (6067.9)	11674.7 (4668.1)	p=0.610
Baseline damage (SvH)	27.7 (32.8)	27.4 (32.9)	p=0.946
Year 3 progression (SvH)	5.8 (9.0)	6.0 (5.0)	p=0.259

Tab. 20: RF moderate positive remaining patients with from moderate to low positive changing patients: crude (above) and matched for SDAI and radiographic damage at baseline (bottom).
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

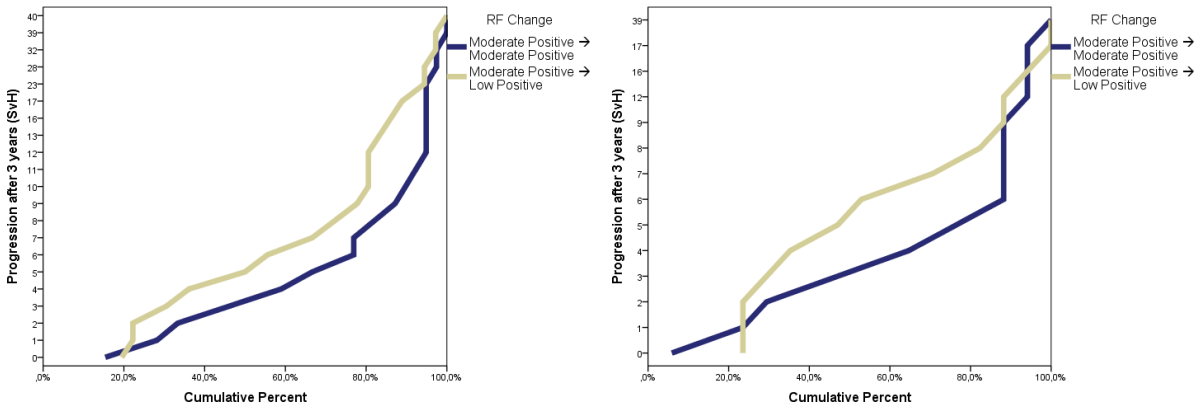

Fig. 17: Radiographic progression in RF moderate positive remaining patients and patients with from moderate to low positive changing RF levels: crude (left) and matched for SDAI and radiographic damage at baseline (right).

d) Patients with low positive RF levels remaining low positive vs. seroconverting patients with low positive RF levels at baseline

Crude	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Low → Low (n=58)	Low → Negative (n=26)	
Baseline RF (IU/ml)	37.6 (15.6)	28.1 (14.8)	p=0.007
Δ RF (IU/ml)	3.8 (20.2)	16.1 (14.8)	p=0.005
Baseline SDAI	11.2 (10.1)	18.3 (12.2)	p=0.007
Δ SDAI	4.1 (10.1)	4.3 (9.3)	p=0.383
SDAI (AUC)	13330.2 (10865.5)	19746.3 (14099.3)	p=0.026
Baseline damage (SvH)	53.2 (77.3)	43.5 (42.2)	p=0.611
Year 3 progression (SvH)	8.8 (7.8)	11.6 (15.9)	p=0.942

Matched	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Low → Low (n=17)	Low → Negative (n=17)	
Baseline RF (IU/ml)	37.7 (16.1)	26.2 (13.6)	p=0.016
Δ RF (IU/ml)	7.9 (15.5)	14.2 (13.6)	p=0.357
Baseline SDAI	16.7 (12.3)	17.7 (13.1)	p=0.919
Δ SDAI	7.6 (14.4)	2.9 (9.8)	p=0.531
SDAI (AUC)	14466.2 (8908.7)	20542.2 (15652.1)	p=0.339
Baseline damage (SvH)	44.5 (44.1)	44.4 (43.7)	p=1.000
Year 3 progression (SvH)	9.5 (7.8)	12.8 (18.4)	p=0.760

Tab. 21: RF low positive remaining patients and seroconverting patients with low positive RF levels at baseline: crude (above) and matched for SDAI and radiographic damage at baseline (bottom).
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

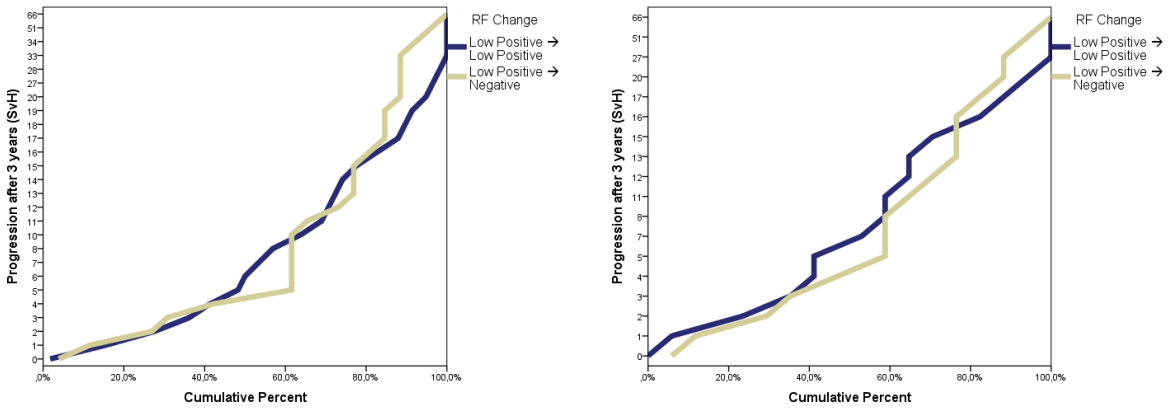

Fig. 18: Radiographic progression in RF low positive remaining patients and seroconverting patients with low positive RF levels at baseline: crude (left) and matched for SDAI and radiographic damage at baseline (right).

Within all the crude analyses, we could mostly observe the higher amount of radiographic progression after three years in patients with greater RF level changes (SvH 8.3 vs. 8.7 in the group of high positive patients remaining high positive vs. high positive patients turning moderate positive; 8.3 vs. 24.3 in the group of high positive patients remaining high positive vs. high positive patients turning low positive; 5.6 vs. 7.3 in the group of moderate positive patients remaining moderate positive vs. moderate positive patients turning low positive; and 8.8 vs. 11.6 in the group of low positive patients remaining low positive vs. seroconverting patients, who initially had low positive RF levels). This was in most analyses accompanied by a higher disease activity at baseline and over the three-year observational period, but also a bigger decline in disease activity, and higher amounts of already existing structural damage in the groups of converters.

After matching the groups for their SDAI at baseline and their initial radiographic damage in order to eliminate the influence of these two factors on radiographic progression, damage progression was about equal between the groups of converters and non-converters, although damage progression still tend to be higher in the groups of changing RF levels: SvH 8.4 vs. 9.8 in the group of high positive patients remaining high positive vs. high positive patients turning moderate positive; 5.0 vs. 20.3 in the group of high positive patients remaining high positive vs. high positive patients turning low positive; 5.8 vs. 6.0 in the group of moderate positive patients remaining moderate positive vs. moderate positive patients turning low positive; and 9.5 vs. 12.8 in the group of low positive patients remaining low positive vs. seroconverting patients, who initially had low positive RF levels. Importantly, the matching did also lead to a more or less similar absolute change in RF levels in patients in the different groups. The overall progression was lower after selecting the matching patients.

7.6.2 Matched for RF levels and baseline damage

It is apparent that after matching for disease activity at baseline and baseline damage the change in RF levels became similar in the RF converters and non-converters. Therefore, in an additional analysis we matched for baseline RF levels and baseline damage to maintain the signal of change between the converters and non-converters. The following number of matched pairs resulted from that:

- high positive patients remaining high positive vs. high positive patients turning moderate positive (n=15 matched pairs) (Tab. 22, Fig. 19);
- high positive patients remaining high positive vs. high positive patients turning low positive (n=12 matched pairs) (Tab. 23, Fig. 20);
- moderate positive patients remaining moderate positive vs. moderate positive patients turning low positive (n=15 matched pairs) (Tab. 24, Fig. 21);
- low positive patients remaining low positive vs. seroconverting patients, who initially had low positive RF levels (n=21 matched pairs) (Tab. 25, Fig. 22).

a) Patients with high positive RF levels remaining high positive vs. patients changing from high to moderate positivity

Crude	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	High → High (n=41)	High → Moderate (n=30)	
Baseline RF (IU/ml)	1056.4 (1356.6)	470.7 (286.6)	p=0.003
Δ RF (IU/ml)	348.1 (1242.1)	330.5 (282.5)	p=0.015
Baseline SDAI	15.2 (11.4)	16.3 (10.9)	p=0.631
Δ SDAI	5.4 (13.4)	10.2 (11.5)	p=0.192
SDAI (AUC)	15795.6 (12042.3)	12438.5 (8621.8)	p=0.295
Baseline damage (SvH)	41.2 (58.4)	53.1 (61.4)	p=0.105
Year 3 progression (SvH)	8.3 (8.7)	8.7 (8.5)	p=0.726

Matched	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	High → High (n=15)	High → Moderate (n=15)	
Baseline RF (IU/ml)	606.9 (418.9)	505.1 (314.2)	p=0.436
Δ RF (IU/ml)	-24.9 (647.6)	378.9 (319.0)	p=0.002
Baseline SDAI	13.14 (11.9)	16.1 (13.2)	p=0.621
Δ SDAI	4.5 (14.1)	8.5 (13.3)	p=0.618
SDAI (AUC)	13552.0 (9686.6)	10334.1 (7034.9)	p=0.325
Baseline damage (SvH)	48.0 (73.7)	47.6 (67.4)	p=0.775
Year 3 progression (SvH)	4.6 (3.9)	7.7 (6.3)	p=0.233

Tab. 22: RF high positive remaining patients with from high to moderate positive changing patients: crude (above) and matched for RF levels and radiographic damage at baseline (bottom).
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

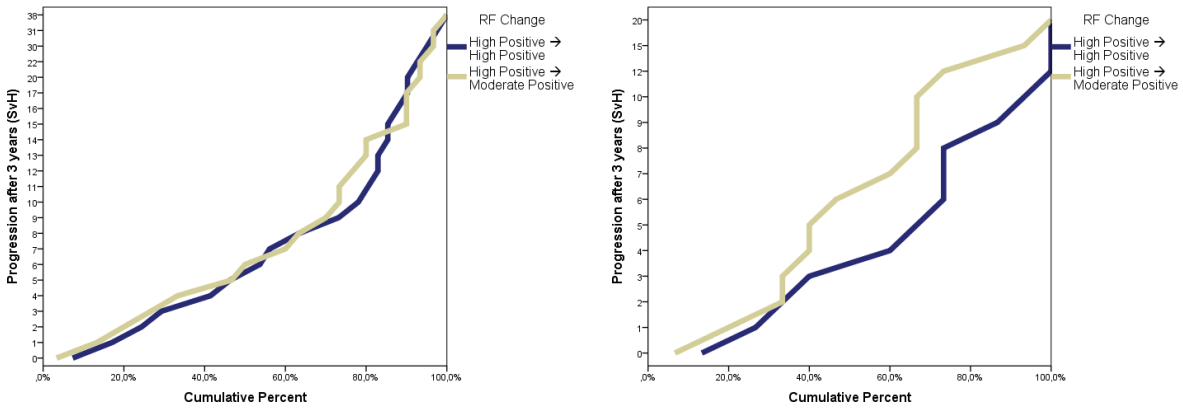

Fig. 19: Radiographic progression in RF high positive remaining patients and patients with from high to moderate positive changing RF levels: crude (left) and matched for RF levels and radiographic damage at baseline (right).

b) Patients with high positive RF levels remaining high positive vs. patients changing from high to low positivity

Crude	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	High → High (n=41)	High → Low (n=13)	
Baseline RF (IU/ml)	1056.4 (1356.6)	477.4 (190.5)	p=0.085
Δ RF (IU/ml)	348.1 (1242.1)	441.4 (180.7)	p=0.016
Baseline SDAI	15.2 (11.4)	23.9 (18.2)	p=0.158
Δ SDAI	5.4 (13.4)	11.2 (10.5)	p=0.251
SDAI (AUC)	15795.6 (12042.3)	26153.8 (26812.7)	p=0.390
Baseline damage (SvH)	41.2 (58.4)	35.8 (36.0)	p=0.378
Year 3 progression (SvH)	8.3 (8.7)	24.3 (36.6)	p=0.287

Matched	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	High → High (n=12)	High → Low (n=12)	
Baseline RF (IU/ml)	480.8 (243.9)	481.8 (198.3)	p=0.799
Δ RF (IU/ml)	-121.0 (273.8)	447.6 (187.3)	p<0.001
Baseline SDAI	11.3 (10.0)	23.7 (19.1)	p=0.118
Δ SDAI	1.5 (11.0)	9.6 (9.8)	p=0.095
SDAI (AUC)	10455.7 (6480.1)	27969.7 (27157.2)	p=0.089
Baseline damage (SvH)	32.1 (32.1)	36.5 (37.5)	p=0.843
Year 3 progression (SvH)	7.6 (5.6)	26.2 (37.5)	p=0.410

Tab. 23: RF high positive remaining patients with from high to low positive changing patients: crude (above) and matched for RF levels and radiographic damage at baseline (bottom).
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

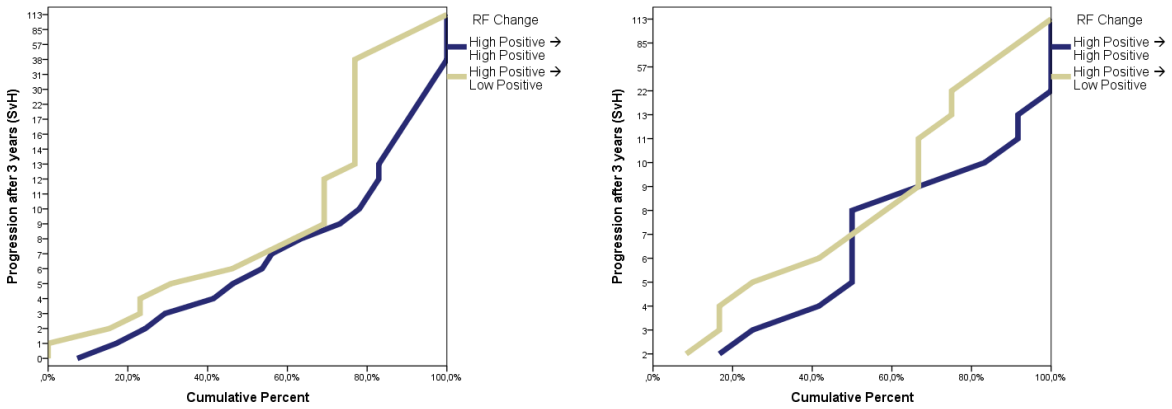

Fig. 20: Radiographic progression in RF high positive remaining patients and patients with from high to low positive changing RF levels: crude (left) and matched for RF levels and radiographic damage at baseline (right).

c) Patients with moderate positive RF levels remaining moderate positive vs. patients changing from moderate to low positivity

Crude	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Moderate → Moderate (n=39)	Moderate → Low (n=36)	
Baseline RF (IU/ml)	152.2 (53.5)	108.9 (44.4)	p<0.001
Δ RF (IU/ml)	36.2 (57.3)	67.3 (46.8)	p=0.016
Baseline SDAI	13.0 (11.6)	14.1 (10.3)	p=0.493
Δ SDAI	4.3 (13.6)	7.0 (10.9)	p=0.837
SDAI (AUC)	11544.4 (7152.5)	13352.6 (9065.0)	p=0.294
Baseline damage (SvH)	28.1 (57.0)	39.1 (43.1)	p=0.091
Year 3 progression (SvH)	5.6 (7.5)	7.3 (10.7)	p=0.150

Matched	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Moderate → Moderate (n=15)	Moderate → Low (n=15)	
Baseline RF (IU/ml)	124.1 (50.8)	124.7 (53.3)	p=0.902
Δ RF (IU/ml)	15.0 (48.9)	88.7 (54.6)	p<0.001
Baseline SDAI	8.4 (4.9)	18.0 (11.6)	p=0.061
Δ SDAI	2.6 (9.3)	10.5 (12.0)	p=0.167
SDAI (AUC)	10437.5 (4116.2)	13791.2 (8165.5)	p=0.270
Baseline damage (SvH)	18.1 (19.2)	19.8 (18.9)	p=0.775
Year 3 progression (SvH)	6.7 (7.0)	5.5 (4.7)	p=0.653

Tab. 24: RF moderate positive remaining patients with from moderate to low positive changing patients: crude (above) and matched for RF levels and radiographic damage at baseline (bottom).
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

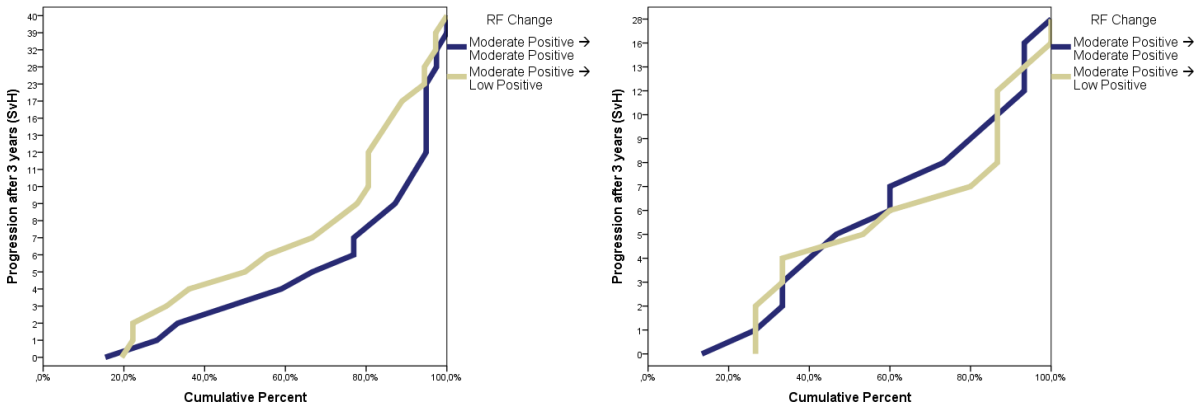

Fig. 21: Radiographic progression in RF moderate positive remaining patients and patients with from moderate to low positive changing RF levels: crude (left) and matched for RF levels and radiographic damage at baseline (right).

d) Patients with low positive RF levels remaining low positive vs. seroconverting patients with low positive RF levels at baseline

Crude	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Low → Low (n=58)	Low → Negative (n=26)	
Baseline RF (IU/ml)	37.6 (15.6)	28.1 (14.8)	p=0.007
Δ RF (IU/ml)	3.8 (20.2)	16.1 (14.8)	p=0.005
Baseline SDAI	11.2 (10.1)	18.3 (12.2)	p=0.007
Δ SDAI	4.1 (10.1)	4.3 (9.3)	p=0.383
SDAI (AUC)	13330.2 (10865.5)	19746.3 (14099.3)	p=0.026
Baseline damage (SvH)	53.2 (77.3)	43.5 (42.2)	p=0.611
Year 3 progression (SvH)	8.8 (7.8)	11.6 (15.9)	p=0.942

Matched	Mean (Standard deviation)		p-value by Mann-Whitney-U test
	Low → Low (n=21)	Low → Negative (n=21)	
Baseline RF (IU/ml)	30.7 (14.7)	30.7 (15.1)	p=0.860
Δ RF (IU/ml)	1.7 (19.7)	18.7 (15.1)	p=0.001
Baseline SDAI	8.1 (6.2)	18.9 (13.1)	p=0.006
Δ SDAI	0.8 (6.9)	3.8 (10.7)	p=0.280
SDAI (AUC)	12677.2 (10643.5)	19939.6 (14380.3)	p=0.061
Baseline damage (SvH)	35.3 (40.2)	35.8 (38.2)	p=0.734
Year 3 progression (SvH)	7.3 (6.2)	12.0 (17.6)	p=0.970

Tab. 25: RF low positive remaining patients and seroconverting patients with low positive RF levels at baseline: crude (above) and matched for RF levels and radiographic damage at baseline (bottom).
Abbreviations: SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score; AUC, Area under the curve.

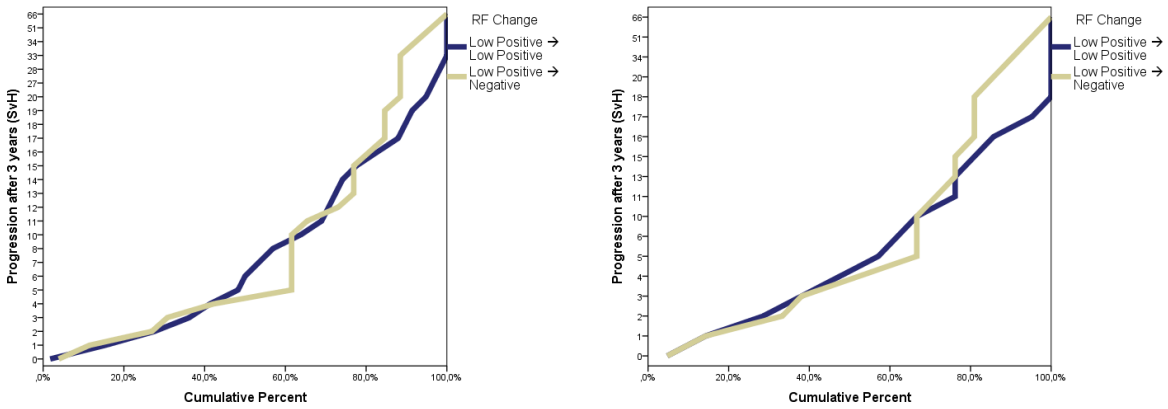

Fig. 22: Radiographic progression in RF low positive remaining patients and seroconverting patients with low positive RF levels at baseline: crude (left) and matched for RF levels and radiographic damage at baseline (right).

Similar to the analyses in 7.6.1, even after matching for RF levels and structural damage at baseline, we could generally observe a higher amount of radiographic progression in the group of RF reducers or converters: SvH 4.6 vs. 7.7 in the group of high positive patients remaining high positive vs. high positive patients turning moderate positive; 7.5 vs. 26.2 in the group of high positive patients remaining high positive vs. high positive patients turning low positive; 6.7 vs. 5.5 in the group of moderate positive patients remaining moderate positive vs. moderate positive patients turning low positive; and 7.3 vs. 12.0 in the group of low positive patients remaining low positive vs. seroconverting patients, who initially had low positive RF levels.

The matching analyses did not only confirm previously reported findings on risk factors for radiographic progression, but also demonstrated the two main differences between RF converters and non-converters: first, patients with decreasing RF levels seem to suffer from more active disease at baseline and over the three-year observational period; and second, more structural damage has already occurred in these patients. This might reflect an inflammatory potential, which makes changing RF levels possible in the first place.

7.7 Decrease of RF levels as potential risk factor for radiographic progression (*objective 3*)

Within the matching analyses presented above, we observed that patients with greater changes in RF levels show a higher amount of radiographic progression, and that after matching for disease activity levels at baseline and already formed structural damage this difference becomes less apparent. (Tab. 26)

Year 3 progression (SvH)	Crude Mean (SD)	Matched for disease activity and baseline damage Mean (SD)	Matched for RF levels and baseline damage Mean (SD)
Positive → Positive	8.7 (12.3)	11.2 (12.5)	9.6 (12.8)
Positive → Negative	12.5 (16.0)	12.6 (16.9)	11.4 (15.1)
p-value by Mann-Whitney-U test	p=0.334	p=0.845	p=0.979
High positive → High positive	8.3 (8.7)	8.4 (8.6)	4.6 (3.9)
High positive → Moderate positive	8.7 (8.5)	9.8 (9.4)	7.7 (6.3)
p-value by Mann-Whitney-U test	p=0.726	p=0.570	p=0.233
High positive → High positive	8.3 (8.7)	5.0 (4.5)	7.6 (5.6)
High positive → Low positive	24.3 (36.6)	20.3 (34.5)	26.2 (37.5)
p-value by Mann-Whitney-U test	p=0.287	p=0.217	p=0.410
Moderate positive → Moderate positive	5.6 (7.5)	5.8 (9.0)	6.7 (7.0)
Moderate positive → Low positive	7.3 (10.7)	6.0 (5.0)	5.5 (4.7)
p-value by Mann-Whitney-U test	p=0.150	p=0.259	p=0.653
Low positive → Low positive	8.8 (7.8)	9.5 (7.8)	7.3 (6.2)
Low positive → Negative	11.6 (15.9)	12.8 (18.4)	12.0 (17.6)
p-value by Mann-Whitney-U test	p=0.942	p=0.760	p=0.970

Tab. 26: Summary of the matching analyses presented in sections 7.4 and 7.6.
Abbreviations: SvH; Sharp/van der Heijde Score; SD, Standard deviation.

This may supports the hypothesis that reversible RF levels are associated with the active disease process, but that there are also irreversible RF levels that confer a fixed risk. The latter might be low, given that there was no trend in the amount of progression between patients staying high positive, moderate positive, or low positive with their RF. At the same time, conclusions have to be made with caution, since the absolute changes in RF levels ended up being relatively similar between the different matched patient groups.

This hypothesis was confirmed when using the absolute change scores of RF crude (p=0.024), as well as adjusted for the absolute change levels of SDAI (p=0.025) and for the cumulative disease activity over the three-year observational period (p=0.037), in a logistic regression analysis. The change in SDAI on the other hand did not diminish the association of RF change with progression, indicating that it is simply the higher disease activity thread at baseline and over time that confers the dominant risk. (Tab. 27)

	B	S.E.	Wald	df	Sig.	Exp(B)
Δ RF (IU/ml)	0.001	0.000	5.081	1	0.024	1.001
Constant	0.051	0.097	0.271	1	0.602	1.052

	B	S.E.	Wald	df	Sig.	Exp(B)
Δ RF (IU/ml)	0.001	0.000	3.101	1	0.078	1.001
Baseline SDAI	0.020	0.009	4.670	1	0.031	1.020
Constant	-0.197	0.158	1.563	1	0.211	0.821

	B	S.E.	Wald	df	Sig.	Exp(B)
Δ RF (IU/ml)	0.001	0.000	5.022	1	0.025	1.001
Δ SDAI	0.002	0.003	0.490	1	0.484	1.002
Constant	-0.031	0.109	0.082	1	0.775	0.969

	B	S.E.	Wald	df	Sig.	Exp(B)
Δ RF (IU/ml)	0.001	0.000	4.341	1	0.037	1.001
SDAI (AUC)	0.000	0.000	10.594	1	0.001	1.000
Constant	-0.368	0.157	5.456	1	0.020	0.692

Tab. 27: Effect of change in RF levels and disease activity in radiographic progression.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors; SDAI, Simplified Disease Activity Index; AUC, Area under the curve.

7.8 Matrix risk model for the prediction of radiographic progression (*objective 3*)

7.8.1 Pooled patients (ACPA positive and negative)

The previous analyses suggested the development of a matrix predicting radiographic progression including major risk factors of disease activity at baseline and already existing radiographic damage, as well as RF levels at baseline and the decrease of RF over the three-year observational period. From our population we could identify 197 patients, for which all of these considered risk factors were available, and whose RF levels decrease over the three-year observational period.

We performed logistic regression analysis to examine the effect of selected risk factors on radiographic progression and to predict probability of structural damage. The risk factors were included as dichotomous variables based on clinical utility and the ability of identifying subgroups of relevant size: disease activity at baseline (SDAI ≤15 Vs. SDAI >15) and already existing radiographic damage (SvH score ≤20 Vs. SvH score >20), as well as RF levels at baseline (RF ≤120 IU/ml Vs. RF >120 IU/ml) and the decrease of RF over the three-year observational period (RF ≤60 IU/ml Vs. RF >60 IU/ml). Progression was again defined as a threshold change in SvH score ≥5 over the past three years. (Tab. 28)

Predictors of progression	B	S.E.	Wald	df	Sig.	Exp(B)
Baseline RF	-0.742	0.504	2.165	1	0.141	0.476
Decrease of RF	0.739	0.506	2.136	1	0.144	2.095
Baseline SDAI	0.465	0.310	2.258	1	0.133	1.592
Baseline damage	1.080	0.305	12.567	1	0.000	2.943
Constant	-0.456	0.300	2.311	1	0.128	0.634

Tab. 28: Effect of RF at baseline, decrease of RF over three years, disease activity at baseline and structural damage at baseline on radiographic progression.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors; SDAI, Simplified Disease Activity Index.

A colour scheme ranging from blue (low risk) to red (high risk) was used to enhance visual readability. The numbers in each cell of the matrix represent the percentage (95% confidence interval, CI) of patients who have the baseline characteristics and the corresponding change In RF levels. For example, a patient with RA and a SDAI >15, RF levels at baseline >120 IU/ml and already formed radiographic damage >20 points in the SvH Score and whose RF levels decrease by more than 60 IU/ml would have a 75% (95% CI 62%, 84%) predicted probability of radiographic progression. (Fig. 23)

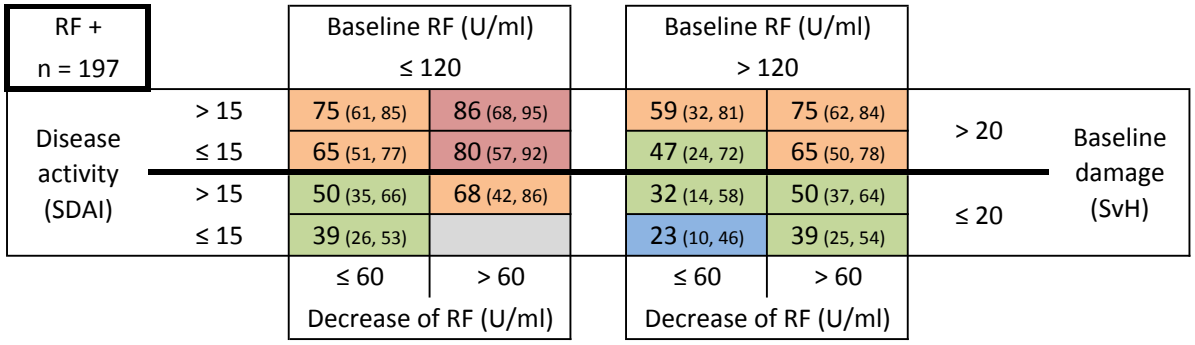

Fig. 23: Matrix risk model for the prediction of radiographic progression.
The numbers in each cell represent the percentage (95% CI) of patients who had radiographic progression out of all patients, who have the baseline characteristics. Colour scheme: blue: 0-25%; green: 26-50%; orange: 51-75%; red: 76-100% predicted probability of radiographic progression. Grey boxes represent a combination of risk factors which none of our patients could fit in. SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score.

Here again, greater changes of RF levels were associated with greater degree of radiographic progression. Interestingly, patients with lower RF levels at baseline seem to progress more likely than those with higher levels.

7.8.2 ACPA positive patients

Furthermore patients were pooled for their ACPA state (positive vs. negative), considering ACPA as one major predictor for radiographic progression too. From our population we could identify 164 ACPA positive patients, who were also positive for RF at baseline. In the group of ACPA positive patients, the selected risk factors showed stronger association than in the non-pooled population. (Tab. 29)

Predictors of progression	B	S.E.	Wald	df	Sig.	Exp(B)
Baseline RF	-1.265	0.594	4.533	1	0.033	0.282
Decrease of RF	0.981	0.590	2.767	1	0.096	2.666
Baseline SDAI	0.590	0.352	2.810	1	0.094	1.803
Baseline damage	0.998	0.341	8.586	1	0.003	2.712
Constant	-0.132	0.339	0.151	1	0.698	0.876

Tab. 29: Effect of RF at baseline, decrease of RF over three years, disease activity at baseline and structural damage at baseline on radiographic progression in ACPA positive patients.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors; SDAI, Simplified Disease Activity Index.

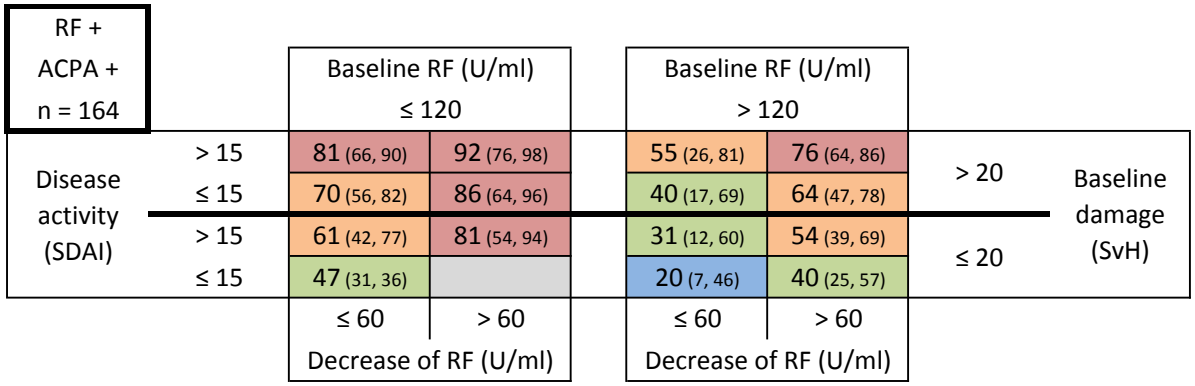

Fig. 24: Matrix risk model for the prediction of radiographic progression in ACPA positive patients.
The numbers in each cell represent the percentage (95% CI) of patients who had radiographic progression out of all patients, who have the baseline characteristics. Colour scheme: blue: 0-25%; green: 26-50%; orange: 51-75%; red: 76-100% predicted probability of radiographic progression. Grey boxes represent a combination of risk factors which none of our patients could fit in. SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score.

As anticipated, the group of ACPA positive patients showed higher predicted probabilities for radiographic damage. (Fig. 24)

7.8.3 ACPA negative patients

In the group of 33 ACPA negative patients, we could observe much lower predicted probability of radiographic progression, indirectly confirming ACPA as one major predictor of radiographic progression. (Fig. 25) Interestingly the impact of the selected risk factors on radiographic progression was much lower in the group of ACPA negative patients. (Tab. 30)

Predictors of progression	B	S.E.	Wald	df	Sig.	Exp(B)
Baseline RF	1.297	1.251	1.074	1	0.300	3.657
Decrease of RF	-0.742	1.205	0.379	1	0.538	0.476
Baseline SDAI	0.473	0.843	0.314	1	0.575	1.604
Baseline damage	1.795	0.885	4.111	1	0.043	6.018
Constant	-1.887	0.837	5.077	1	0.024	0.152

Tab. 30: Effect of RF at baseline, decrease of RF over three years, disease activity at baseline and structural damage at baseline on radiographic progression in ACPA negative patients.
Abbreviations: B, value for the logistic regression equation for predicting the dependent variable from the independent variable; S.E., standard errors associated with the coefficients; Wald, Wald chi-square value; df, degrees of freedom for each of the tests of the coefficients; Sig., significance; Exp(B), odds ratio for the predictors; SDAI, Simplified Disease Activity Index.

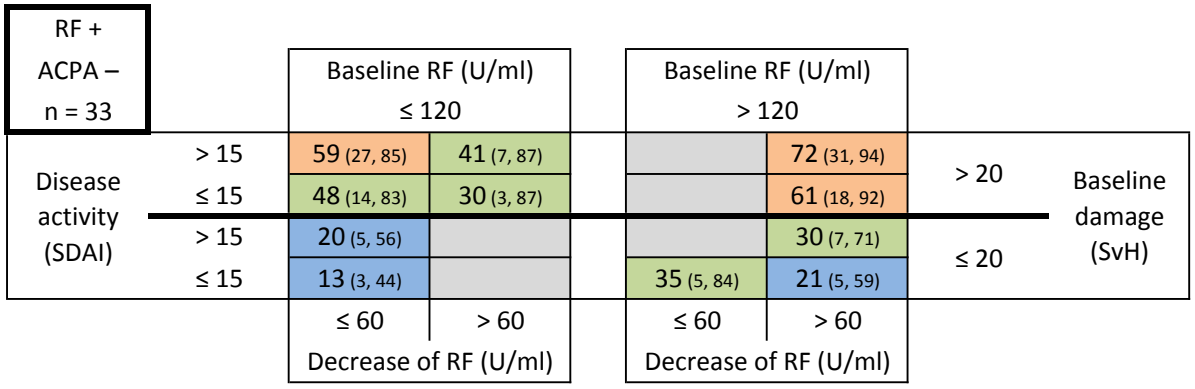

Fig. 25: Matrix risk model for the prediction of radiographic progression in ACPA negative patients.
The numbers in each cell represent the percentage (95% CI) of patients who had radiographic progression out of all patients, who have the baseline characteristics. Colour scheme: blue: 0-25%; green: 26-50%; orange: 51-75%; red: 76-100% predicted probability of radiographic progression. Grey boxes represent a combination of risk factors which none of our patients could fit in. SDAI, Simplified Disease Activity Index; SvH; Sharp/van der Heijde Score.

Concomitantly with the much lower impact of the selected risk factors on radiographic progression in the group of ACPA negative patients, this risk model could not support our previous assumption that greater changes in RF levels are associated with a higher amount of radiographic progression.

8 Discussion

Rheumatoid factors and antibodies against citrullinated peptides are well-established markers of RA. They serve diagnostic purposes on the one hand, which is also clear from their prominent role in contemporary classification criteria of RA, but also are clearly in the focus of prognostic considerations in RA. 'Seropositive' patients are considered to have more aggressive disease and a higher risk of structural progression. Now, as these antibodies may not persist, particularly RF, which has been shown repeatedly to change in the course of treated RA, the concept of 'seropositive' and 'seronegative' RA is challenged.

The aim of the current study was to investigate whether changes of RF and ACPA are just serological phenomena, or whether they also reflect improvement in disease prognosis as well. Since previous findings presented a tight link between higher levels of AAB and damage progression, as well as an association between decreasing AAB levels and a reduction of disease activity, one could expect that a decrease of AAB levels might be associated with a better outcome.[5-7] In fact, in our analyses we found the opposite, namely that with greater decline in RF levels comes greater amount of progression. This at first glance intriguing finding is put back in context by the fact that greater changeability in RF levels was also closely linked to a higher disease activity. Due to the fact that ACPA seems to be much less reactive than RF, we were not able to perform the same analyses on both AAB.

Our initial objective was to investigate the association of AAB levels and structure. A logistic regression model showed an increasing probability of radiographic progression with higher levels of RF at baseline ($p=0.039$), whereas this effect almost vanishes after three years ($p=0.515$). This shows that the effect of RF on damage progression is greater at an early stage of disease indicating some sort of change in this role for RF over time. After adjusting these results for disease activity, the effect of RF on radiographic progression clearly lost its significance ($p=0.099$ at baseline, respectively $p=0.562$ after three years), while disease activity showed a significant association at both measuring points.

One inference from the results of our study is that the disease activity related RF is clearly associated with structural progression, while the putative constant ('unchangeable' or

'fixed') RF is not or much less associated. If both were of similar structural significance, one would expect that patients with constantly high positive levels have higher progression rates than patients who remain constantly moderate or constantly low with their RF levels during the given observation period. This was, at least in our analysis here, not observable.

In contrast to RF, the effect of ACPA on radiographic progression also showed significance after adjusting for disease activity ($p<0.001$ at baseline and after three years), indicating that radiographic damage in ACPA positive patients is related to independent effects of ACPA as well as of higher disease activity. ACPA shows even more strength of this association than baseline SDAI (Wald scores: 12.7 vs. 6.6 at baseline, respectively 13.1 vs. 6.8 after three years).

At this point, one may thus conclude that (a) disease activity explains parts of the association between the RF change and structural consequences, that (b) this association is (statistically) stronger for disease activity than for change in RF levels, (c) ACPA shows less plasticity in the course of RA disease activity, and (d) detectable ACPA have strong structural implications. This supports the following concepts: (1) measureable RF levels comprise reversible (disease activity related) and irreversible (disease defining, and constant) components; and (2) serological links to RA disease activity and to RA progression have to be viewed differentially.

How can some of these epidemiological findings be explained? First, why is RF partly reversible[7, 66, 67] and associated with disease activity[5, 7, 68, 69] and progression[5, 7, 68, 69], while ACPA is only poorly responsive to treatment[66, 70, 71], less associated with disease activity[72], while still associated with progression?[6, 60]

The relationship of RF with disease activity, as well as its absence for ACPA, may be related to the primarily observed isotypes, where RF is of IgM and ACPA are mostly of IgG isotype. Firstly, IgM may activate complement to a greater extent than IgG.[73] This can boost the inflammatory response via inflammatory complement breakdown products and/or complement-receptor mediated macrophage activation.[74-76] ACPA on the other hand might preferentially activate inhibitory Fcγ-receptors mitigating the inflammatory response.[77]

Aside from the isotype, another aspect may be the cellular origin of these antibodies. ACPA likely represent products of long-lived memory plasma cells[78], which are less tightly linked

to the inflammatory disease process[79]. RF on the other hand may be at least partly produced by a subset of B lymphocytes, B-1 cells[80-82], which has been proposed already long ago.[83] These cells have a clearly higher plasticity than plasma cells, and therefore more directly link to the inflammatory response and clinical disease activity.

Nevertheless, here we primarily studied to what extent radiographic progression was different between patients changing, and not changing their RF and ACPA status (seroconverters and non-seroconverters). Since no useable signal on seroconversion was detectable for ACPA as opposed to RF, our respective analyses mainly relate to the latter. We could observe higher percentage of progression rate in seroconverting than in patients remaining positive (60.0% vs. 57.4%, p=0.028). Also mean progression was greater in the group of seroconverters (12.5 points in the SvH score in the group of seroconverters vs. 8.7 points in the group of non-seroconverters, p=0.334). In a next step we made comparisons between these two groups of patients concerning radiographic progression on basis of RF levels and disease activity at baseline, changes of RF levels and disease activity over the three-year observational period, as well as already existing structural damage at baseline. Levels of RF at baseline were more than four times higher in the group of patients remaining seropositive (p<0.001), indicating that actual seroconversion is much more likely to occur in patients with lower RF levels. However, at the end there was not that much difference in decline of RF levels over time (p=0.908). Seroconverting patients suffered from more active disease at baseline (p=0.003) and over the three-year observational period (p=0.007), but decrease was once again similar in between the two groups (p=0.468). Also, radiographic damage at baseline was about equal in both groups (p=0.095).

To eliminate the two major predictors of radiographic progression – disease activity and existing damage – we matched seroconverters and non-converters for these two risk factors. As a result of the matching process, we could observe that progression of radiographic damage after three years was similar in both groups now (12.6 points in the SvH score in the group of seroconverters vs. 11.2 points in the group of non-seroconverters, p=0.845). Since this matching analysis led to same changes in RF levels between converters and non-converters, to maintain the signal of this change, we also matched for RF levels at baseline and again, baseline damage. As a result, despite apparent difference in RF changes (p=0.072), damage progression was still equal in both groups (p=0.979).

When we compared in our analyses the various groups based on tertiles of initial RF levels, disease activity and structural damage at baseline, as well as their changes over time; here too, radiographic progression tended to be higher in the groups of patients whose RF levels change more over time. We consistently found higher disease activity levels at baseline and over the three-year observational period, as well as greater change of it, in the groups of RF changers. When we then again matched the groups for disease activity and structural damage at baseline, as well as for RF levels and structural damage at baseline, progression of damage was about equal between the groups. It seems that the only thing that distinguishes seroconverters from non-seroconverters is the higher disease activity.

This all is therefore again providing clues to the complex triangle between reversibility of serological findings, their association with disease activity, and their association with structural progression. We finally created a matrix risk model predicting probability of radiographic progression including major risk factors of disease activity at baseline and already existing radiographic damage, as well as RF levels at baseline and the decrease of RF over the three-year observational period. In addition, we stratified patients by their ACPA status. The matrix confirmed our previous findings and once again verified changes of RF as risk factor for radiographic progression. This effect seems to play a role in particular in the group of ACPA positive patients, whereas ACPA negative patients do not tend to be that much affected from changing in RF levels.

As a final thought, many of the discussed topics may come together and explain findings during the pathogenesis of RA and in preclinical and early clinical disease. It is apparent from epidemiological studies that serological abnormalities, such as particularly RF, appear long before the clinical onset of disease[41, 84]. Although the events triggering these detectable immune responses remain to a great part enigmatic, there seems – at least on the group level – to be an increase of RF levels over time. Furthermore, it has been postulated that RA progression is accelerated in early disease[85] and, at the same time, shown that RF levels clearly improve more upon treatment in RA with shorter duration than in more longstanding disease[7, 66, 67]. Again, this reversibility is pointing towards a larger disease activity related component in the beginning of RA, and potentially linking this to a greater risk of progression for patients with early RA as compared to established RA.

One limitation in our study assessments was the fact that we did not include therapy in our analyses, since it is well documented that biological treatments dramatically reduce radiographic progression.[86, 87] Furthermore, the patient numbers within the matching analyses in 7.4 and 7.6 were small. In addition, we cross-sectionally selected our patients with no regard to their disease duration and putative AAB changes that had already taken place at that time.

In conclusion, we were able to show that changes of RF levels indeed reflect a change in prognosis of RA, since greater changes are associated with a higher probability of radiographic progression. Greater changeability of RF levels is closely linked to higher disease activity. This might support the link of a particular type of variable RF to disease activity, which confers the higher inflammatory potential of patients improving their RF levels. In fact, this disease activity associated RF component, potentially allows total RF levels to change with a change in disease activity, which ultimately may lead to our observation of RF levels changing with therapy. Nevertheless, RF also confers a risk of progression independent of disease activity. This exciting and complex web of the RA disease process and outcomes shall continue to challenge us and give thoughts for future clinical and translational experiments.

9 Supplement

9.1 List of abbreviations

ACPA	Anti-citrullinated protein antibodies
ACR	American College of Rheumatology
ADAMTS	A disintegrin-like and metalloproteinase with trombospondin
bDMARD	Biological disease modifying anti-rheumatic drug
CCP2	Second-generation CCP assay
CRP	C-reactive protein
csDMARD	Conventional synthetic disease modifying anti-rheumatic drug
DIP	Distal interphalangeal
DMARD	Disease modifying anti-rheumatic drug
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
HC gp39	Human cartilage derived glycoprotein 39
HLA	Human leukocyte antigen
IL	Interleukin
JAK	Janus kinase
JSN	Joint space narrowing
MCP	Metacarpophalangeal
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinases
MTP	Metatarsophalangeal
NSAID	Non-steroidal anti-inflammatory drug
PIP	Proximal interphalangeal
RA	Rheumatoid arthritis
RANKL	Receptor Activator of NF-κB Ligand
RF	Rheumatoid factor
SDAI	Simplified Disease Activity Index
SE	Shared epitope
SLE	Systemic lupus erythematosus
SvH	Sharp/van der Heijde
TNF-α	Tumor necrosis factor alpha
tsDMARD	Targeted synthetic disease modifying anti-rheumatic drug

9.2 List of figures

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10 References

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