The course of clinically suspect arthralgia and early rheumatoid arthritis : clinical features, imaging and genetics

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Summary and discussion

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In this thesis, studies were performed on the very early and early phases of rheumatoid arthritis (RA). In **Part I**, the phase of Clinically Suspect Arthralgia (CSA) was investigated. Since it became clear that early aggressive treatment of RA has much more effect in terms of preventing joint damage progression and achieving remission, a challenge in the rheumatologic field is now to identify and treat RA as soon as possible. The earliest moment to clinically recognise patients who may develop RA is the phase of CSA. In Part II and III, studies were performed within early RA. In **Part II**, genetic risk factors for a more severe disease course, mainly joint damage progression, were studied. These studies contributed to our understanding of processes that are fundamental to disease progression. **Part III** focussed on other outcomes in RA, among which patient-reported outcomes.

PART I: THE PHASE OF CLINICALLY SUSPECT ARTHRALGIA

This thesis started (**Chapter 2**) with a review on literature on the preclinical phases of RA [1]. This revealed that there is convincing evidence that autoantibody development and maturation occurs before clinically detectable arthritis develops and suggestive evidence that systemic and local inflammation are already present in this phase. RA development can thus be considered a multiple hit process in which RA-related processes can be active already years before RA is diagnosed. The studies reviewed were mainly performed in autoantibody-positive populations and we observed that studies on the preclinical and very early phases of autoantibody-negative RA were scarce. This review ended with a research agenda for studies on the very early phases of RA.

The phase of symptoms without clinically apparent arthritis is the first moment that imminent RA can be clinically recognised. Since the symptoms that are characteristic for this phase are not yet known [1], we studied this phase by investigating patients with Clinically Suspect Arthralgia (CSA). CSA was defined as arthralgia without clinically detectable arthritis that was considered by the rheumatologist clinically suspect to progress to RA and thus, the decision on whether a patient had CSA depended on the clinical expertise of the rheumatologist. In addition, the decision on the presence of CSA was made at the first visit before additional tests were performed and thus did not depend on the autoantibody-status of the patient. This concept differed from that used in other studies focusing on this symptomatic phase that studied autoantibody-positive patients with unspecified arthralgia or non-specific musculoskeletal symptoms [2–4]. The advantage of the CSA approach is that it is in line with the clinical practice where patients present with certain symptoms and the decision to perform additional investigations is based on the clinical presentation. Furthermore, it allows identification of both autoantibody-negative and autoantibody-positive RA in the early symptomatic phase.

The set-up of the rheumatology outpatient clinic of the Leiden University Medical Centre is uniquely suited to identify patients in very early disease phases. General practitioners have been encouraged for several years to refer any patient with a suspicion of arthritis. The

start of an Early Arthritis *Recognition* Clinic (EA*R*C) in 2010, initially aimed to improve early detection of clinical arthritis, also provided an excellent opportunity to identify patients with CSA [5]. Since 2012 these patients have been included in an observational cohort and these patients were studied in this thesis.

In **Chapter 3**, we investigated the characteristics of patients with CSA at inclusion in the cohort. Subclinical inflammation of hand and foot as measured by MRI was present in 44% of the CSA patients. Subclinical MRI-inflammation was here defined as a RAMRIS MRI-inflammation score of ≥3. This cut-off was quite arbitrarily but based on MRI-findings of 19 symptom-free persons in which a score of ≥3 was rare. Furthermore, 28% of the CSA patients were positive for RA-related autoantibodies. We observed that CSA patients with MRI-inflammation were older and more frequently ACPA-positive than patients without MRI-inflammation. However, a combination of clinical and serological characteristics incompletely differentiated patients with and without MRI-inflammation. These data suggested that the information provided by MRI cannot be easily replaced by commonly used clinical and serological markers and that MRI-detected inflammation may have some diagnostic value. This was later on, in Chapter 5, further explored.

Studies on the preclinical and very early phases of RA that were performed by other groups thus far were mainly done in patients carrying autoantibodies and the very early phase of autoantibody negative RA was relatively unexplored. Therefore, in **Chapter 4**, we studied subclinical MRI-inflammation in ACPA-negative CSA patients and observed that RAMRIS MRI-inflammation scores of ACPA-negative CSA patients were significantly higher than those of 19 age-matched symptom-free controls. This suggested that ACPA-negative RA has, similar as ACPA-positive RA [6] an early phase of symptoms without clinical arthritis in which subclinical MRI-inflammation is present.

In **Chapter 5**, we studied patients with CSA and the presence of subclinical MRI-inflammation in these patients for the first time longitudinally. However, a relevant issue of the use of MRI in the early phases of RA is that MRI is a very sensitive imaging technique and it is unknown which scores should be considered as normal and which reflect pathology. Data of several MRI-studies on small numbers of symptom-free persons (including the MRI-data in symptom-free persons used in Chapter 3 and 4) showed 'MRI-abnormalities' to some extent, but were difficult to compare because different MRIs, scanning protocols and scoring methods were used [7]. Therefore, we recently performed a large-scale MRI-study in 193 symptom-free persons recruited from the general population [8]. MRI-detected inflammation was prevalent in these persons without joint symptoms as 72% had a RAMRIS MRI-inflammation score ≥1; synovitis and BME were more prevalent than tenosynovitis. MRI-inflammation was especially prevalent at higher age and at preferential locations (MCP2, MCP3, wrist and MTP1 joints). These findings suggested an influence of aging, which was observed both in these symptom-free persons as in the CSA patients studied in Chapter 3, and potentially of mechanical strains because some of the preferential locations

for MRI-inflammation in the symptom-free persons are also known as preferential location for arthritis and destruction in RA. Based on these data of MRI-findings in symptom-free persons reference values for a normal MRI were suggested. These values were specified for age, MRI-feature and anatomic location. To prevent false-positive MRIs in our CSA patients, we applied these reference values to define the presence of subclinical MRI-inflammation for our study within CSA patients.

Within our longitudinal study in patients with CSA (**Chapter 5**) we observed that 17% of all patients progressed from CSA to clinical arthritis within the first year after inclusion. Patients with subclinical MRI-inflammation had an increased risk to develop clinical arthritis as 31% of the patients with a positive MRI developed arthritis within one year. The majority did so within the first 4-5 months after inclusion, indicating that the period of CSA and subclinical inflammation is relatively short. When subclinical MRI-inflammation was absent, progression to clinical arthritis was rare (6%). In addition, we observed that tenosynovitis was more predictive than synovitis and BME as it associated independent of the other MRI-features with arthritis development. Tenosynovitis is uncommon in the general population [8], has been reported to be frequently present in early RA [9] and has been demonstrated to be present in mice in the preclinical phase before synovitis developed [10]. Together, these data may suggest that tenosynovitis may be a very early and potentially the initiating feature in arthritis development. Repeated MRI with short-time intervals during the process of arthritis development would give more insight in the timing of pathologic events occurring inside the joints. Within CSA, also ACPA-positivity associated with progression from CSA to arthritis. Both the presence of subclinical MRI-inflammation and ACPA-positivity were independently predictive for arthritis development. We used the presence and absence of MRI-inflammation and ACPA to stratify CSA patients in groups with different risks on arthritis development. Although the absolute value of MRI might be higher in ACPA-negative than in ACPA-positive CSA patients because ACPA-positive patients had already a higher prior risk of arthritis development, present data suggested that MRI is diagnostically relevant in the phase of CSA. This role is probably located in both identifying patients with an increased risk of arthritis development and ruling out imminent arthritis. The latter because the prior chance of arthritis decreased from 9% to 3% in the ACPA-negative CSA patients when the MRI was negative and from 63% to 40% in the ACPA-positive patients.

The decision on whether a patient had CSA was based on the clinical expertise of the rheumatologist as the symptoms that are characteristic for the early symptomatic phase of RA are not well-characterised. In **Chapter 6**, we studied the value of clinical expertise as selection criterion for CSA. Clinical expertise is a valuable tool in the medical and also specifically for the rheumatologic diagnostic process. For example, the clinical expertise was used in the process to develop the 2010 ACR/EULAR criteria for RA [11] and for the set-up of the French ESPOIR cohort [12]. We observed that clinical expertise is also useful for selecting arthralgia patients at risk of RA because arthralgia patients that were considered by their

rheumatologist to have CSA had an odds ratio of 55 to develop RA compared to arthralgia patients not considered to have CSA.

In **Chapter 7**, we aimed to define the clinical characteristics of patients with arthralgia who are considered at risk of RA. It can be assumed that interventions in the symptomatic phase preceding the onset of clinical arthritis may be more effective in terms of reducing the risk of disease persisting and preventing joint damage. However, studies to address this require the inclusion of homogeneous sets of patients. Therefore, with a EULAR taskforce comprising 18 rheumatologists, a methodologist, 3 health professionals, 2 patients and a research fellow we defined a set of clinical features that best characterise patients with arthralgia that are according to the clinical expert-opinion at risk of RA development. A three-phase process was used consisting of 1) identifying relevant items using a Delphi approach, 2) deriving candidate criteria by evaluating patients that were presented on paper and 3) by validating the criteria with newly referred arthralgia patients. The following set of parameters that describe arthralgia at risk of RA was defined: joint symptoms of recent-onset (duration <1 year), symptoms located in MCP joints, duration of morning stiffness 60 minutes, most severe symptoms present in the early morning, presence of a first-degree relative with RA, difficulty with making a fist and positive squeeze-test of MCP joints. In the validation phase, this combination of parameters was accurate in identifying arthralgia patients that were considered at risk of RA development as the AUC was 0.92. Test characteristics belonging to the number of positive parameters were presented and depending on the study a more sensitive or specific definition can be used.

Further perspectives on studies within CSA

In short, based on this thesis we learned that:

- The clinical expertise is useful to identify arthralgia patients who may develop RA, because patients with CSA are at increased risk of developing arthritis.
- Both ACPA-positive and ACPA-negative RA have a phase with CSA and MRI-detected subclinical inflammation.
- MRI-detected subclinical inflammation might have a diagnostic value in patients with CSA. This is true for both ACPA-positive and ACPA-negative patients but the absolute value might be higher in ACPA-negative patients.
- The developed definition of arthralgia at risk for RA which represents the consensus-based expert opinion of rheumatologist can serve as basis for future studies and trials in the CSA phase.

Our approach to study the symptomatic phase of RA without clinical arthritis is in line with the care at Dutch rheumatologic outpatient clinics. Therefore this might allow implementations of the results obtained in this thesis in Dutch rheumatologic care. However, first, replication of our findings in independent CSA populations, which do not yet exist, is needed.

To address the question whether intervention in the symptomatic phase preceding arthritis development is beneficial it is needed that patients with arthralgia with an increased risk on RA are identified. To this end, the risk factors for progression from CSA to clinical arthritis that we identified in our CSA cohort are helpful as they will contribute to accurate risk stratification within CSA. Subclinical MRI inflammation and ACPA were the most important risk factors and we performed risk stratification based on these two factors. However, our CSA population was too small to develop a full prediction model which may provide most accurate risk stratification. Such a prediction model, including all potential predictors (such as patient characteristics, symptoms, findings at physical examination, serological inflammation markers, different autoantibodies and MRI-inflammation), will reveal which factors are independently predictive for the development of arthritis and may allow to stratify the risk of arthritis/RA development more accurately. To this end, a large CSA population is needed of which part can be used for identification and the other part for validation. In addition, to perform trials in the symptomatic phase it is important to include a homogeneous group of patients. For this, the consensus expert-opinion based definition of CSA consisting of 7 clinical items is helpful. However, it is unknown how good this definition is in identifying patients who will later on progress to RA (thus the predictive accuracy of the definition). Therefore, a subsequent prospective study is needed in which patients with CSA according to the definition will be longitudinally followed on the development of RA. The diagnostic accuracy of the clinical definition alone is most likely not highly accurate because it is only based on clinical features. Presumably, combining the clinical definition with findings of additional investigations, such as results of serological tests (f.e. ACPA, RF and/or CRP) or imaging (f.e. MRI or US) will improve the diagnostic accuracy and will results in criteria for 'imminent RA'. This process is similar as the process that first led to the definition of inflammatory back pain which was subsequently integrated in the ASAS classification criteria [13,14]. Future research in which our own CSA cohort might be part of a large international prospective study with multiple cohorts will hopefully result in accurate risk stratification and can be the basis of dedicated trials.

We observed that MRI-detected subclinical inflammation might have a diagnostic value in patients with CSA and that MRI-detected tenosynovitis was most predictive for progression from CSA to clinical arthritis. This thesis comprised the first MRI-studies in patients with CSA. Further studies are needed to determine if the diagnostic value of MRI can be improved by assessing for example MRI-inflammation at specific locations or combinations of MRI-features. In addition, the value of other imaging techniques such as ultrasonography (US) in the CSA phase is not yet determined. Although MRI and US can both depict tenosynovitis, MRI has several advantages compared to US, such as that MRI is a minimal operator-dependent procedure and the presence of a validated scanning and scoring protocol for MRI [15]. If validated scanning and scoring protocol for US will become available, further studies are needed to assess the value of US in CSA.

Next to studies on predicting which CSA patients will progress to clinical arthritis, longitudinal studies within CSA patients may also provide more insight in mechanisms underlying RA development at both systemic and local levels. Biomarkers, such as gene expression profiles and autoantibody profiles can be assessed and related to MRI-detected inflammation and arthritis development. Longitudinal MRIs in individual patients will shed light on what happens inside the joints. Comparing MRIs performed in CSA and in the early clinical arthritis phase will reveal whether the extent and localisation of MRI-inflammation change during conversion from CSA to clinical arthritis.

Furthermore, additional work is needed for early identification of RA (at risk) within the primary care. General practitioners (GPs) have, as gatekeeper for access to rheumatologic care, an important role in early identification of patients with (an increased risk of) RA. GPs work in populations with different background risks and the symptoms that are characteristic for RA at risk in the GP population are unknown. Their guideline recommends that patients suspected for RA should be referred on short-term, but no specific recommendations on the symptoms and signs that should be assessed before referral are included [16]. The set-up of an EARC has improved the identification of early arthritis substantially [5], but whether knowledge on symptoms that are predictive for RA (at risk) in the GP population and the development of a referral tool would improve early identification further should be subject of subsequent studies.

Summary of research agenda:

- Replication of the findings done in our CSA cohort in independent CSA cohorts.
- To identify with high accuracy a homogenous group of CSA patients who will progress to RA. Additional risk factors for progression to RA should be identified that can contribute to dedicated risk stratification and might finally result in criteria for 'imminent RA'. The EULAR definition for CSA and European collaboration of the taskforce may be helpful to this end.
- If we can identify arthralgia patients at risk accurately, trials will reveal whether intervention can prevent onset of clinical arthritis, disease chronicity, functional disability or quality of life or whether it will reverse subclinical MRI-inflammation.
- Evaluating whether the diagnostic accuracy of MRI-detected inflammation in the CSA phase can be improved by evaluating f.e. specific locations and/or combinations of MRI-features.
- Evaluating of the value of imaging modalities such as ultrasonography and PET-CT in the CSA phase.
- Examining the sequence of pathologic events that occur in the period between onset of CSA and arthritis development both on systemic and local level in longitudinal studies.
    - Systemic level: gene expression profiles, serological inflammatory markers and epitope spreading of (different) autoantibodies.

- Local/joint level: repeated MRIs and determining the timing of the inflammatory features that are visualised (synovitis, tenosynovitis, BME, erosions).
- Development of referral tool for first line care in order to further decrease the time of referral to second line care of patients at risk of or with RA.

PART II: GENETIC FACTORS AND DISEASE OUTCOME IN RHEUMATOID ARTHRITIS

In this part, genetic risk factors for a more severe course of RA were investigated. Studying genetic variations in relation to disease outcome can increase our comprehension of disease progression, may convey novel targets for focused therapy and may improve personalised medicine. The main studied outcome was joint damage progression, one of the hallmarks of RA which can be measured objectively by scoring radiographs of hands and feet using the Sharp-van der Heijde scoring method [17]. The other studied outcome was arthritis persistence which is the other hallmark of RA and can be investigated by studying its opposite, the achievement of DMARD-free sustained remission [18].

For present studies, we selected patients fulfilling the 1987 ACR criteria for RA. In our view, these criteria for patient selection were most appropriate to perform basic research as the use of the 2010 ACR/EULAR criteria would have resulted in a more heterogeneous study population [11].

Prediction of joint damage severity on the level of the individual patients is not yet accurate, hampering individualised treatment. Matrices developed to predict rapid radiographic progression correctly classified only approximately half of all patients [19–21] and are not used in clinical practice. At the start of this thesis, several genetic risk factors had been found to be associated with joint destruction in previous studies [22–32]. When adding these genetic factors to a prediction model for radiographic progression that already included traditional factors, we observed that the predictive accuracy improved from 56% correct classifications to 62% (**Chapter 8**). In addition, genetic risk factors together explained 12-18% of the variance in radiographic progression. However, still 38% of patients were incorrectly classified by the full model and we considered the predictive performance of the derived model including genetic factors insufficient for use in clinical practice.

Replication of findings is crucial in the field of genetics. Therefore, in addition to the Leiden Early Arthritis Clinic (EAC) cohort [33], several other cohorts were used in the studies on genetic risk factors, including the Swedish Umeå [34], Spanish HCSC-RA [35], North-American Wichita [36], NDB [37] and NARAC [38], and French ESPOIR [12] cohorts. These cohorts were all smaller than the EAC cohort and comprised less radiographs over time, though could be used to replicate and substantiate observed associations. In **Chapter 9**, the initially published finding that a variant in *FOXO3A* was associated with joint damage progression in two cohorts of the UK [39] was not replicated in five other cohorts suggesting that *FOXO3A* is not a major factor regulating the severity of the course of RA.

Using candidate-gene approaches, we identified two genetic variants that were associated with joint damage progression within the ACPA-negative RA population. This is relevant because the large majority of risk variants for progression have been identified in ACPA-positive or pooled populations. First, rs9138 in *SPP1*, initially identified as susceptibility variant for RA [40] and encoding osteopontin which has a function in bone formation and remodeling was observed to associate with radiographic progression within ACPA-negative RA (**Chapter 10**). Second, in **Chapter 11**, variants that have been described to associate with radiographic progression but for which the results of different studies were incongruent were studied in six cohorts. Rs2900180 in *C5-TRAF1* significantly associated with radiographic progression; the association was confined to the ACPA-negative subgroup. The region of rs2900180 in *C5-TRAF1* was fine-mapped and another variant had a stronger association, but we could not statistically distinguish which variant was most important. The studied variants in *IL-6*, *IL-10*, and *FRCL3* were not associated and the initial findings on these variants done in studies with lower patient numbers and radiographs could be considered false-positive, underlining the relevance of replication of findings. For both rs9138 in *SPP1* and for rs2900180 in *C5-TRAF1* there was data available that the (region of the) variant is related to expression on RNA or protein level [40–42]. These studies, done on the level of genetics and expression suggested that the identified regions are relevant in pathways mediating disease progression.

The *HLA-DRB1* region is the most important genetic risk factor for both RA development and progression that is identified thus far. In particular the SE alleles, sharing a similar amino sequence at position 70-74 in the peptide-binding groove, and acting via ACPAs on disease development and outcome, are relevant [22,43,44]. However, the underlying biological pathway is not yet unravelled. Recently, a further refinement of the association of HLA and RA was proposed. Using advanced statistical methods, the strongest association with RA development was reported for HLA-DRB1 position 11 (or 13 which are in high linkage disequilibrium); this association was independent of the SE alleles [45,46]. Studying four cohorts, we observed that the amino acids Valine or Leucine at position 11 were associated with joint damage progression (**Chapter 12**). This association was independent of the presence of the SE alleles but not independent of ACPA. Future studies will reveal whether taking position 11 and 13 into account will be helpful in identifying the pathogenic antigens that result in immune activation and autoantibody production, thereby stimulating disease development and progression.

In **Chapter 13**, a candidate-gene study on arthritis persistence (the absence of achieving DMARD-free sustained remission) was performed. Genetic risk factors for joint damage progression were studied in relation to persistence and it was observed that besides the *HLA-DRB1* SE alleles, rs2104286 in *IL2RA* was associated with arthritis persistence in two cohorts. In addition, lower soluble IL2Rα (CD25) levels associated with a higher chance of remission. Intriguingly, *IL2RA* and SE are the only variants identified thus far that are

associated with RA development, joint damage progression and persistent inflammation. This underlines the relevance of these variants, but also suggested that the mechanisms driving joint damage progression and disease persistence are partially different.

In **Chapter 14**, serum level osteoprotegerin (OPG) was studied in relation to arthritis persistence. Besides the well-known role of OPG in bone metabolism, OPG also has pro-inflammatory effects and it was reported that the serum level was associated with achieving Disease Activity Score (DAS)-remission the next year [47]. Here, we replicated this latter finding. In addition, OPG level also associated with DMARD-free sustained remission. Together these data suggested that OPG levels are reflective of a process influencing the severity of inflammation both on the short and long-term.

Further perspectives on studies on risk factors for disease outcome

Including the genetic risk factors identified in this thesis, thus far, fourteen genetic variants have been identified and were replicated to associate with radiographic progression: *HLA-DRB1, CD40, IL15, DKK1, IL2RA, GRZB, IL4R, SPAG16, C5orf30, MMP9*, intergenic downstream of *ZFP36L1* and *C14orf181, OPG, SPP1* and *C5-TRAF1*. We observed that these variants together explained approximately 20% of the variance in radiographic progression. This cannot be directly compared to the estimation that 45-58% of the severity of joint damage is heritable which was estimated in Icelandic RA patients [48], but it suggests that part of the heritability is still missing. In line with this, we observed that radiographic progression could not be accurately predicted using all known, both traditional and genetic, risk factors. This 'missing heritability' might be explained by not yet identified common genetic variants that associate with joint damage, rare variants with large effects on joint damage or by gene-gene or gene-environment interactions. To this end, radiographic data of several cohorts should be combined to enable large studies.

The other long-term outcome that was studied was arthritis persistence (the absence of achieving DMARD-free sustained remission). It is likely that achieving DMARD-free sustained remission will become a preferred treatment goal in the future, but only few risk factors for arthritis persistence are known thus far. We performed a candidate-gene study and hypothesised that genetic risk factors for joint damage might also be risk factors for arthritis persistence. This approach sounds reasonable as both outcomes are a reflection of the long-term disease course. However, in fact there is no clear evidence that underlying processes of joint damage and arthritis persistence are overlapping. In addition, it is unclear whether the patients with severe joint damage are similar to the patients with persistent arthritis. Ideally, we had performed a hypothesis-free genome-wide association study (GWAS) or had analysed the whole Immunochip [49] in relation to arthritis persistence. Unfortunately, this was hampered by the low frequency of DMARD-free sustained remission and the absence of multiple cohorts with data on this disease outcome. Collection of data on this outcome in multiple cohorts would allow such large genetic studies.

Thus far, multiple genetic risk factors have been identified for RA development or disease progression within RA. Previously, it was observed that the genetic variants that are associated with susceptibility to RA and joint damage progression of RA are largely different [30]. In this thesis, we studied the genetic risk factors for joint damage, which are mainly located in genes involved in inflammation, immunity or bone/cartilage metabolism, in relation to arthritis persistence and observed that also these were largely non-overlapping. The *HLA-DRB1* alleles and a variant in *IL2RA*, both located in genes involved in immunity/inflammation, were the only variants that associated with both joint damage progression and arthritis persistence. Variants in genes involved in bone/cartilage metabolism associated with joint damage, but not with arthritis persistence. Overall, these data suggest that the processes driving the development of RA, progression of joint damage within RA and persistence of arthritis within RA are largely different. However, further studies are needed to unravel these pathways and to give more insight in whether the identified variants are causal and how these variants are involved in disease development and disease progression. For this, large fine-mapping studies and functional studies are needed, respectively to identify all variants that are linked to the variants with the strongest association and to determine the potential functional consequences of these variants. Recent advances in technology and bioinformatics may be helpful to this end [50].

In addition, we found 2 serological biomarkers that were associated with arthritis persistence, i.e. high soluble IL2Rα and OPG levels. These findings might give additional clues for targeted intervention. Interestingly, lowering soluble IL2Rα (CD25) levels with anti-CD25 (daclizumab) have been shown to be effective in multiple sclerosis [51]. In addition, upregulation of regulatory T-cells with low-dose IL2 was beneficial in type 1 diabetes [52]. Further research on longitudinal measured biomarker levels would reveal the relevance of specific serological biomarkers for arthritis persistence and their potential role in guiding treatment decisions.

PART III: OTHER OUTCOMES IN RHEUMATOID ARTHRITIS

Due to improved treatment strategies, severe joint damage is less prevalent nowadays and therefore, other outcome measures will become more important. A good candidate would be arthritis persistence and its opposite achieving DMARD-free sustained remission, which is the closest proxy of cure of RA and can be assessed rather objectively [18]. This outcome was used in Chapter 13 and 14. In **Chapter 15**, the occurrence and relevance for patients of achieving DMARD-free sustained remission was studied. We observed that with nowadays treatment strategies the chance to achieve this favourable outcome is increased. It was observed that also from patient perspective achieving DMARD-free sustained remission is an outcome to pursue as this status reflected resolution of symptoms and disability. This underlines the relevance of this outcome.

Patient-reported outcomes such as fatigue, functional ability and work ability are

also important [53] outcome measures in RA. Fatigue is a frequently reported symptom in RA, associated with functional disability and considered one of the most important outcomes by patients. The causation of fatigue in RA is thought to be multidimensional [54] and the contribution of inflammation in unclear. We studied the long-term course of fatigue (**Chapter 16**) and observed that fatigue is a persistent problem in RA. In addition, the extent of inflammation over time significantly associated with the severity of fatigue though the effect sizes were small, indicating that non-inflammatory pathways should be considered important as well. Interestingly, improved treatment strategies that have resulted in less inflammation and improved objective outcomes of RA have not resulted in less severe fatigue. Therefore, fatigue in RA remains an 'unmet need'.

FINAL CONCLUSIONS

The field of RA is moving into identification of patients as early as possible and the ultimate aim is to prevent RA becoming a chronic disease. To this end, the studies on the phase of Clinically Suspect Arthralgia (CSA), described in Part I of this thesis provided relevant insights. Patients with arthralgia that were considered by the rheumatologist to have an increased risk to progress to RA (CSA) had indeed an increased risk of RA. In addition, subclinical MRI-inflammation preceded clinical arthritis with a few months. Future research will shed more light on processes underlying progression from CSA to RA and effectiveness of treatment initiation in the CSA phase.

The severity of the course of RA is variable between patients and this cannot be yet accurately predicted. The studies in Part II and III contributed to the understanding of these differences in severity. Three genetic risk factors for more severe joint damage progression (two non-HLA and one HLA variation) and one for arthritis persistence were identified. Further research on functional implications of the identified variants and whether they might be useful as biomarkers to guide treatment decisions is needed.

DMARD-free sustained remission, the opposite of arthritis persistence, will probably become an increasingly important outcome in RA as it approximates cure of RA, is relevant from patient perspective and is increasingly achievable nowadays although the majority of patients is not yet able to achieve this outcome. Future studies will reveal whether this beneficial outcome can be achieved more frequently when treatment is initiated in the phase of CSA.

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