Differences and similarities of autoantibody-positive and autoantibody-negative rheumatoid arthritis during the disease course: on our way to personalized medicine

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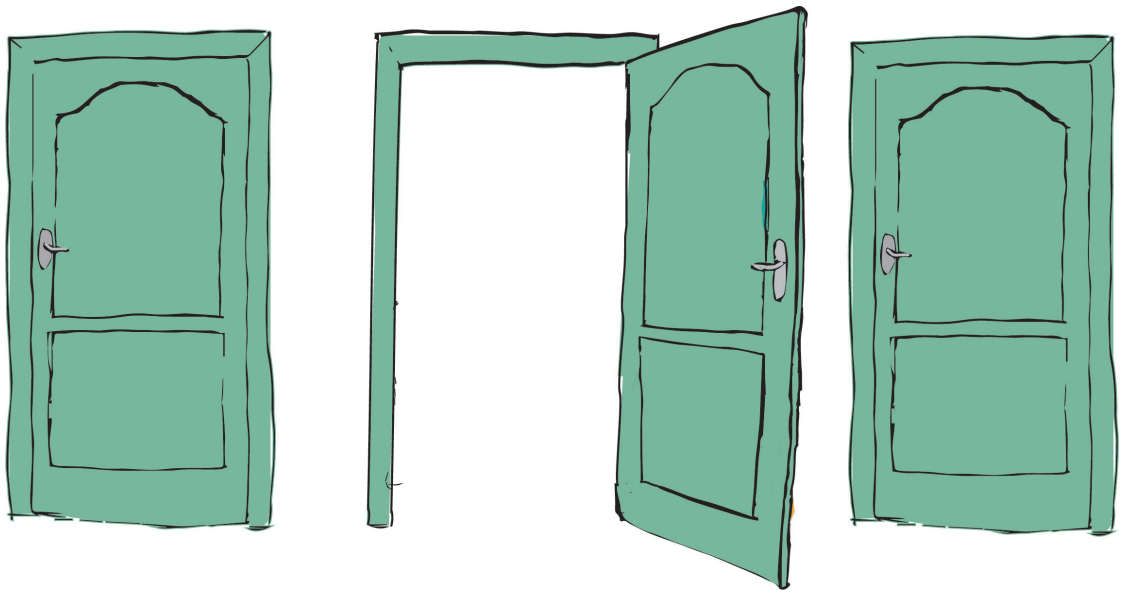
CHAPTER 9

Summary and discussion



In this thesis we aimed to assess the differences and similarities between autoantibody-positive and autoantibody-negative RA from the start of the of complaints to the end of the disease. We studied the symptomatic pre-arthritis phase, the early arthritis phase and long-term outcomes of rheumatoid arthritis patients. These phases were studied on the joint level with MRI, on the patient level with disease activity and patient reported outcomes (PROs) and on the society level using data from all rheumatoid arthritis patients from the Leiden region that presented to the LUMC since 1993.

SUMMARY OF FINDINGS

Pre-arthritis

In **Chapter 2**, we analysed which combinations of MRI-features at onset were predictive for RA-development in symptomatic patients without arthritis, to increase our comprehension of locations of RA-onset and to improve the predictive accuracy of MRI based on a unique cohort of clinically suspect arthralgia (CSA) patients. We identified that MCP extensor peritendinitis is among the tissues affected by RA already in the CSA phase. Furthermore, we improved prediction making. Based on the predictors "presence of MCP extensor peritendinitis" and "number of locations with subclinical inflammation" five risk categories were defined, of which the PPVs were up to 67% in the highest category. Thereafter these findings were validated in an independent set of patients, with PPVs up to 63%. The next step is to integrate these MRI data with other relevant biomarkers. Nonetheless, this enhanced the use of MRI in prediction of arthritis development in CSA patients.

Early arthritis

In **Chapter 3**, we hypothesized that if MRI-detectable tenosynovitis is a true RA-feature, the sensitivity for RA is high at diagnosis, in both autoantibody-positive and autoantibody-negative RA, and lower in other diseases. We showed that the large majority (>80%) of early RA patients have tenosynovitis at small hand and foot joints. This high sensitivity was present in both autoantibody-positive and autoantibody-negative RA, and was much lower in other arthritides. Furthermore, the sensitivity of tenosynovitis for RA was comparable to synovitis. These data imply that tenosynovitis, next to synovitis, is a true RA feature. This comprehension may fuel future research into the role of juxta-articular synovial inflammation in the pathogenesis of both autoantibody-positive and autoantibody-negative RA.

In **Chapter 4**, we determined trends in incidence of autoantibody-positive and autoantibody-negative RA over two decades in the Leiden region. We hypothesized that part of the incidence increase of autoantibody-negative RA is explained by aging of the population and this might lead to an increase of autoantibody-negative RA in the future. Using data from the Leiden EAC and population data from the Leiden area, we found an increasing incidence of autoantibody-negative RA that was absent in autoantibody-positive RA. Moreover, we show that the increase in autoantibody-negative RA is indeed in part explained by aging of the population. This will make autoantibody-negative RA more prevalent the coming years (estimated increase of ~11% in 20 years) and promotes the need for research in this subset of RA.

In **Chapter 5**, we studied the relationship between MRI detected inflammation and fatigue and found that MRI inflammation was not associated with simultaneous fatigue at diagnosis and during disease course in both autoantibody-positive and autoantibody-negative patients. Studying time orders, we observed that a decrease in MRI inflammation in the first year was associated with decrease in fatigue in the second year, however the standardized effect size was similar to clinical disease activity as measured by the DAS. Therefore, overall MRI inflammation did not aid in explaining fatigue not explained by the DAS. This suggests there is a ceiling effect for explaining fatigue by inflammation and supports the concept that fatigue in patients with classified RA is in part disconnected from inflammation. Consequently, the results imply that aiming at imaging remission instead of clinical remission does not lower fatigue in autoantibody-positive and autoantibody-negative RA.

In **Chapter 6**, we studied patterns of MRI inflammation decrease in 216 consecutive RA and UA patients who received early DMARD-treatment. We used cross-lagged models to evaluate the influence of two time-patterns: a simultaneous pattern ("change in one inflammatory feature associated with change in another feature") and a subsequent pattern ("change in one inflammatory feature preceded change in another feature"), in three time-periods (0-4 months, 4-12 months, 12-24 months). We observed a simultaneous decrease of synovitis, tenosynovitis and osteitis. In addition, synovitis decrease preceded tenosynovitis decrease. In autoantibody-positive but not in autoantibody-negative patients, synovitis decrease preceded osteitis decrease. Therefore patters of subsequent change were partly different in the autoantibody-positive and autoantibody-negative disease. This suggests that different inflammatory pathways underlie MRI-inflammation in autoantibody-positive and autoantibody-negative RA.

Long-term outcomes

In **Chapter 7**, we studied the response of long-term outcomes of autoantibody-positive and autoantibody-negative RA patients to treatment strategies that have changed over the last 25 years. We observed that included RA patients had remained similar, apart from earlier diagnosis; therefore, RA patients from different years were

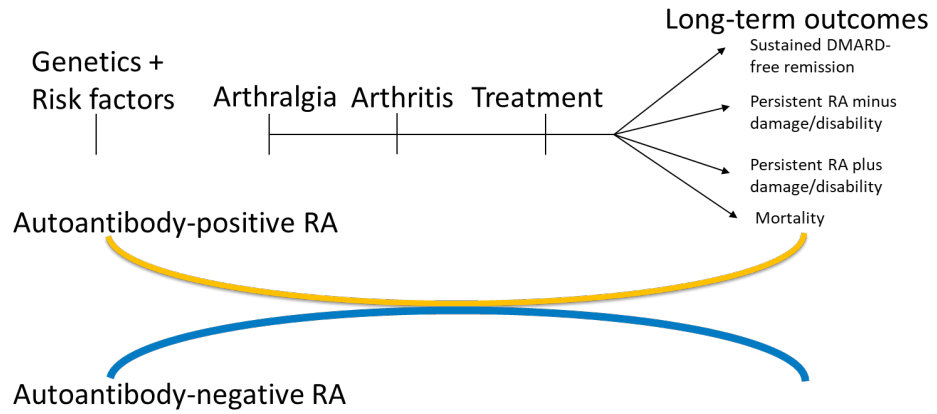
comparable. We found that while disease activity improved in both autoantibody-positive and autoantibody-negative RA patients, the long-term outcomes (the possibility to permanently stop medication, mortality, and functional disability) only improved in autoantibody-positive RA patients. The disconnection between improvement in disease activity and subsequent improvement in long-term outcomes in RA without autoantibodies suggests that the underlying pathogenesis of RA with and without autoantibodies is different. Based on our data, we think it is time to make a differentiation in RA and accordingly divide it into autoantibody-positive (type 1) and autoantibody-negative (type 2) subsets. This differentiation will stimulate focused etiopathologic studies as well as stratified clinical trials.

In **Chapter 8**, we aimed to answer the question whether mortality in rheumatoid arthritis (RA) has normalized, as contradicting results had been published. In many of the studies on mortality two important factors are not sufficiently taken into account: follow-up duration and disease subtypes (such as autoantibody-positivity). To assess the true impact of early intensive treatment on mortality we performed a large study (>1200 RA-patients) with up to 25 years of follow-up and sufficient power to stratify for follow-up duration and autoantibody status. We showed that excess mortality has resolved since the introduction of early intensive treatment in autoantibody-negative RA, but excess mortality remains an issue in autoantibody-positive RA.

COMPARISONS WITH OTHER STUDIES

As summarized above, we studied differences and similarities of autoantibody-positive and autoantibody-negative RA from start of complaints to the end of disease. We found that these RA subtypes have many differences as well as similarities. Altogether, the amount of similarity between the two RA types seems to depend on the phase of the disease that is studied. As visualized in Figure 1, the differences between autoantibody-positive and autoantibody-negative RA are most prominent before the start of complaints and in the long-term outcomes after treatment. Conversely, the two types are more similar in the phase from the start of complaints until the initial response to treatment. In total, this implicates that autoantibody-positive and autoantibody-negative RA are two distinct diseases with different pathophysiology. Next, we will further elaborate on the course of (dis-)similarity of autoantibody-positive and autoantibody-positive RA and the implications of these (dis-)similarities.

Figure 1. Summary of differences and similarities of autoantibody-positive and autoantibody-negative RA



Pre-arthritis

Pre-arthralgia

The pre-arthritis phase generally consists of an asymptomatic and a symptomatic phase. In this thesis, the pre-symptomatic phase was not studied. However, previous research showed that autoantibody-positive and autoantibody-negative RA have major differences in this phase: They have different genetic risk factors [1-3], different environmental risk factors [4,5] and per definition autoantibodies are not detected in autoantibody-negative RA while these are often present before complaints in autoantibody-positive RA.[6]

Prediction of arthritis development in arthralgia

In the phase of symptomatic pre-arthritis (Phase (D) according to the EULAR study group for risk factors for RA), previous research is predominantly aimed at predicting arthritis development in either autoantibody-positive arthralgia patients or relatives of autoantibody-positive arthralgia patients.[7-9] In these autoantibody-positive arthralgia patients, morning stiffness, C-reactive protein (CRP), the shared epitope, tenderness of the joints and imaging detected inflammation have been identified as predictors for arthritis development in multiple studies.[10-12] Particularly, inflammation around the tendons as detected by imaging was shown to be predictive in this group.[8,13]

In this thesis, we studied the Leiden clinically suspect arthralgia (CSA) cohort. To our knowledge, this is the only arthralgia cohort that also includes a significant amount of autoantibody-negative patients. Previously, it was shown that MRI-detected subclinical inflammation has a positive predictive value of ~30% in CSA patients, with a negative predictive value of ~95%.[14] In **Chapter 2**, we showed that we could improve the

positive predictive value of MRI up to 75% while keeping the high negative predictive value. This was done by also incorporating the number of locations with subclinical inflammation and the presence of inflammation around the MCP tendons. More recently, we have shown that this predictive value is independent of autoantibodies.. [15] Altogether, imaging detected inflammation, particularly in the tendon sheaths, is predictive for arthritis development in both autoantibody-positive and autoantibody-negative RA.

Regarding other predictors, in concurrence with autoantibody-positive patients, CRP, shared epitope and morning stiffness are also (borderline) associated with arthritis development in CSA.[14,16] Overall, predictors for arthritis development are rather similar for autoantibody-positive and autoantibody-negative arthralgia patients.

Disease course between arthralgia and arthritis

Differences between autoantibody-positive and autoantibody-negative patients in the disease course between arthralgia and arthritis have been scarcely studied. Burgers et al. showed that autoantibody-positive and autoantibody-negative CSA patients that eventually convert to arthritis have many similarities at symptom onset and presentation with arthralgia. The differences were a higher tender joint count and more difficulties in making a fist in autoantibody-negative patients and a longer symptom duration at presentation and shorter time to arthritis in autoantibody-positive patients. [17] Ten Brinck et al. suggested that the course of MRI inflammation was similar for autoantibody-positive and autoantibody-negative patients, but autoantibody-positive patients had more osteitis when they presented with CSA.[18]

Combining these studies, it can be concluded that while some small differences can be observed at presentation with arthralgia, the predictors of arthritis development and the disease course from CSA presentation to arthritis are rather similar, except for a shorter time to arthritis development in autoantibody-positive patients. Altogether, autoantibody-positive and autoantibody-negative patients are rather similar in this phase.

Early arthritis

At presentation with arthritis, previous research showed that autoantibody-positive and autoantibody-negative RA patients are rather similar clinically: they have similar joint distribution, similar disease activity, similar disability, similar morning stiffness and similar age and gender distribution. [19-21] Conflicting results have been reported about initial treatment response: during initial treatment DAS has been reported both to be lower and higher in autoantibody-positive and autoantibody-negative patients under randomized and protocolized treatment and therefore results are inconclusive.

[22,23] In this thesis we also identified a difference in the early arthritis phase: we showed that incidence of autoantibody-negative RA was higher in the elderly (**Chapter 4**). However, altogether autoantibody-positive and autoantibody-negative RA are rather similar clinically in the early arthritis phase.

In this thesis we also studied MRI in the phase of early arthritis and initial treatment response and also found many similarities: synovitis and tenosynovitis are equally as often present at first presentation (**Chapter 3**); MRI inflammation does not help in explaining fatigue in both autoantibody-positive and autoantibody-negative RA (**Chapter 5**); All inflammatory features decrease simultaneously after initial treatment and synovitis decrease precedes tenosynovitis decrease (**Chapter 6**). We also identified one difference: in autoantibody-positive but not in autoantibody-negative patients, synovitis decrease preceded osteitis decrease in the second year (**Chapter 6**). Altogether, we can conclude that autoantibody-positive and autoantibody-negative RA are also rather alike in the early arthritis phase when studied with MRI.

Long-term outcomes

Previous research into long-term outcomes in autoantibody-positive and autoantibody-negative RA revealed that autoantibody-positive patients have more damage progression, more swollen joints during follow-up and have a lower chance of achieving sustained DMARD free remission (SDFR).[19,24] Conversely, the pattern of joint involvement was similar and comparable PROs were described under treat-to-target treatment regimes.[19,25] However, the effect of treatment on long-term outcomes in autoantibody-positive and autoantibody-negative RA were scarcely studied.

To study effect of treatment on long term outcomes of autoantibody-positive and autoantibody-negative RA, logically, long term follow up is needed. Very long-term follow-up (>10y) is rare in randomized clinical trials (RCTs), as these are very costly. In the rare case that RCTs extend their follow-up to this time, treatment is often less strictly protocolized and more similar between arms, thereby making a RCT more comparable to a cohort study.[26] In this thesis, we took advantage of 25 years of follow-up of the Leiden EAC. To our knowledge, this is currently the largest observational cohort of RA.[27]

In **Chapter 7,** we found that disease activity improved in both patient groups. This was to be expected as the treat-to-target strategy, that is aimed at lowering DAS below a certain threshold, has been implemented around 2006. In contrast to the DAS, the other long-term outcomes (sustained DMARD-free remission, mortality, and functional disability) only improved in autoantibody-positive RA. This disconnection

between DAS and other long-term outcomes in autoantibody-negative patients is in stark contrast with the aim of treat-to-target strategies as the aim is to "lower the DAS on the short-term to enhance other outcomes on the long-term". Moreover, this disconnection implicates a different disease mechanism in autoantibody-negative RA. Also supporting the hypothesis of differences in disease mechanism, we observed that sustained DMARD-free remission and functional disability improved more in autoantibody-positive patients than in autoantibody-negative patients. While (changes in) treatment strategies were similar for autoantibody-positive and autoantibody-negative RA, improvement in long-term outcomes differed, again implying differences in different disease mechanisms.

In reaction to this study, one might argue that autoantibody-positive patients might have been treated more intensely before 2006, when treatment was less strictly aimed at a DAS target. If this would have been the case, one would expect less improvement in long-term outcomes with stricter treatment strategies after 2006 in autoantibody-positive RA. We observed the opposite, making it implausible that more intense treatment of autoantibody-positive patient before 2006 caused our results.

Therefore, we conclude that although disease activity has improved in both autoantibody-positive and autoantibody-negative RA, the response in long-term outcomes in recent decades with enhanced treatment strategies differed. Altogether, autoantibody-positive and autoantibody-negative RA seem rather different with respect to long-term outcomes and effect of treatment on long-term outcomes.

Mortality

In this thesis, we studied mortality in autoantibody-positive and autoantibody-negative RA patients in two different ways and found different results; In **Chapter 7,** we found that mortality significantly improved in autoantibody-positive RA whereas no significant improvement was found in autoantibody-negative RA. However, effect sizes were in the same direction and we observed no significant difference in mortality improvement between the two RA subtypes. Correction for age and gender was performed in these analyses but no adjustment for mortality in the general population was performed because excess mortality in RA is heavily dependent on follow-up duration and these follow-up durations differ between the cohorts studied.

In **Chapter 8,** we studied mortality corrected for the general population and follow-up duration. We found that mortality is normalized in ACPA-negative RA but not in ACPA-positive RA. Because standardized mortality rates cannot be compared between groups with a different age, gender and diagnosis-year distribution, comparisons between groups were not performed.[28]

Intuitively, these results might seem contradictory. However, the two chapters answer different questions: "Has mortality improved with enhanced treatment?" and "Is excess mortality still present with enhanced treatment?". An open question is whether excess mortality has improved since the introduction of enhanced treatment. However, to investigate this, two comparable large group of patients with similar age, gender and diagnosis-year distribution should be treated with either old or enhanced treatment strategies for >15 years. Unfortunately, this study is unfeasible and might also be unethical.

In conclusion, whether excess mortality has improved with enhanced treatment in autoantibody-negative RA is still to be debated. However, research into this subject might not be prioritized because excess mortality is less prominent in this group. In contrast, in autoantibody-positive RA, while mortality seems to have improved with enhanced treatment strategies, after longer follow-up excess mortality is still present. Therefore, research into treatment for excess mortality in autoantibody-positive RA is warranted. Still, with respect to the aim of this thesis, both studies show remarkable differences between autoantibody-positive and autoantibody-negative RA regarding to the long term outcome mortality.

IMPLICATIONS OF FINDINGS

Time to subdivide RA into type 1 and type 2

The aim of this thesis was to systemically study the differences between autoantibody-positive and autoantibody-negative RA from start of complaints to the end of disease. Previous research had already shown large differences between autoantibody-positive and autoantibody-negative RA before the start of complaints. We found that these disease types were rather similar in the phase from start of complaints to initial treatment response. In stark contrast, long-term outcomes and influence of treatment on long-term outcomes was very dissimilar. A graphical representation of this is presented in Figure 1. Altogether, we conclude that the differences between autoantibody-positive and autoantibody-negative RA before complaints and in long-term outcomes imply a (partly) different disease mechanism. Therefore, we propose that it is time to subdivide RA into autoantibody-positive RA (type 1) and autoantibody-negative RA (type 2).

Implications of subdividing RA

If the hypothesis that type 1 and type 2 RA have (partly) different disease mechanisms is accepted, all previous research in RA should be revaluated and future research should be redirected. This is because risk factors and effect of treatment on outcomes might differ between the two types. And while correction for ACPA and/or RF has become

increasingly popular in research articles, stratification for autoantibody status in the only way to identify these differences.

In particular, as radiological damage and SDFR are more present in type 1 and type 2 respectively, studies with these outcomes might be primarily driven by one of the two disease types and cannot be generalised to the other type without further thought. Therefore, studies that used these outcomes and did not stratify for disease type should be revaluated. While doing this, it should be kept in mind that results of these studies might only apply to one disease type.

Finally, the 2010 classification criteria heavily load on the presence of autoantibodies. This is the result of the aim to early identify patients with persistent and/or erosive disease. Indeed, these criteria facilitated more early classification in type 1 patients.[29] However, the additional value of these criteria in type 2 patients is still to be elucidated and the need >10 affected joints (tender/swollen) to fulfill the 2010 criteria might have promoted classification of autoantibody-negative patients with more pain rather than patients with persistent and/or erosive disease. In the future, research could be aimed at identifying risk factors for persistent and/or erosive disease in autoantibody-negative early arthritis patients with a clinical diagnosis of UA. This with the ultimate aim to optimize early classification of type 2 RA.

Importance of type 2 RA

While RA research several decades ago predominantly focused on damage as an outcome, type 2 RA was originally seen as the mild subtype of RA and received less attention. As clinical relevant damage has become rare, PROs have become increasingly important.[30] Previous research has shown that with respect to PROs, type 2 RA is not a "mild" subtype. Also, in **Chapter 7,** we showed that with respect to long-term outcomes such as DAS, HAQ, mortality and SDFR, type 1 and type 2 are becoming increasingly similar. Therefore, type 2 RA has become less "mild" and research into type 2 is becoming increasingly important.

Another reason type 2 is becoming increasingly important is the rising prevalence of this RA subtype; In **Chapter 4**, we showed that the incidence of type 2 is rising, partly due to aging of the population. Also, we showed that disease duration has not shortened: In **Chapter 7,** we showed that SDFR rates did not rise in this type and in **Chapter 8**, we showed that excess mortality is no longer present in this RA type. Altogether, a rising incidence and a similar disease duration will result in a rising prevalence of type 2 RA. In contrast, type 1 RA will have a less prominent rising incidence due to aging. Type 1 also has improved mortality and improved SDFR and therefore will probably become less prevalent in comparison to type 2.

While type 2 is becoming less "mild" and more prevalent in comparison to type 1, less is known about this RA type and newer treatment strategies might be less effective in this RA type. With regard to treatment, in **Chapter 7** we showed that treatment has been intensified in this type but that this did not result in improvement of long-term outcomes. Therefore, when applying enhanced treatment strategies, doctors might be overtreating their type 2 patients.[31] Further research is needed to elucidate which treatment strategies do improve outcomes of type 2 patients.

With regard to pathophysiology, also less is known about type 2. While it is still debated whether autoantibodies play an active role in type 1 RA or are "innocent bystanders", [32] autoantibodies provide an anchor for pathophysiologic research in RA and therefore this research primarily focuses on type 1 RA, leaving a gap in knowledge about the pathophysiology of type 2 RA. Finally, with regard to diagnosis, the 2010 criteria are heavily dependent on autoantibodies and therefore the consequence of applying these criteria in type 2 patients has been insufficiently studied.

In conclusion, we want to emphasize that while type 1 RA is seen as the more severe type, type 2 RA is becoming increasingly prevalent and relatively more severe. Since less is known about type 2 in terms of optimal diagnosis, treatment strategies and pathophysiology, we want to advocate for more research into the optimal diagnosis, treatment and pathophysiology of type 2 RA.

Optimal division of type 1 and type 2

In this thesis, we promote the subdivision of RA into type 1 and type 2. However, how this division should exactly be performed should be based on future research. The division between autoantibody-positive and autoantibody-negative RA is most often based on RF, ACPA or both. Because these autoantibodies often cooccur, the resulting divisions are quite similar: in this thesis they were used interchangeably. RF+/ACPA- patients are generally older at onset compared to with RF+/ACPA+ patients, show similar incidence trends as RF-/ACPA- patients and have relatively milder damage progression.[33-37] In addition, RF is more prevalent in the general population.[38,39] Therefore, it might be more appropriate to make the subdivision between type 1 and type 2 strictly on ACPA.

Future research might result in even further subdivision of RA, especially of type 2 RA, since this type is suggested to be more heterogenous. Research into further subdivision might help to elucidate whether autoantibody level, number of autoantibodies or presence of other autoantibodies aid the optimal subdivision.[40-43] It is possible that other markers reflecting the underlying pathophysiology such as histology or metabolomic / lipidomic markers might help in making the best distinction. Ideally, the

division is made based on differences in pathophysiological mechanisms, but as long as these are unknown, epidemiological studies can be used. As the difference between type 1 and type 2 RA is most prominent pre-arthralgia and in long-term outcomes, these disease phases should be studied to elucidate what the optimal subdivision should be.

Tenosynovitis in type 1 and type 2 RA

Many studies described in this thesis show that tenosynovitis plays a prominent role in both type 1 and type 2 early RA: tenosynovitis predicts arthritis development in arthralgia patients, tenosynovitis is present in >80% of early RA patients and dissolving of tenosynovitis is associated with previous synovitis decrease. These results are interesting because tenosynovitis is a form of juxta-articular synovial inflammation and not intra-articular inflammation. Because RA is seen as a disease of the joints, intra-articular inflammation is historically associated with RA. However, also other forms of juxta-articular inflammation have been shown to play a role in early RA. Intermetatarsal bursitis is associated with early RA compared to other diagnoses.[44] The pathophysiology and the interaction of these juxta-articular and intra-articular forms of synovial inflammation remain to be elucidated in both RA types.

FINAL CONCLUSIONS

In short, based on this thesis, we learned that:

1. It is time to subdivide RA in autoantibody-positive RA (type 1) and autoantibody-negative RA (type 2) to enable stratified diagnosis, treatment and research in RA.
2. The prevalence of type 2 RA will rise due to increasing incidence, similar sustained DMARD-free remission rates and absence of excess mortality.
3. The goal to improve long-term outcomes by achieving remission on the short term has not been achieved in type 2 RA.
4. MRI-detected tenosynovitis is an early disease feature with high sensitivity and specificity for both type 1 and type 2 RA.

9

SUMMARY OF RESEARCH AGENDA

Type 1 and type 2 RA

- To systemically review RA studies that are stratified for autoantibody status to elucidate what is known about type 1 and type 2 RA, separately.
- To elucidate whether type 2 RA is indeed more heterogeneous and whether this type should be further subdivided.
- To develop a prediction model for persistence of autoantibody-negative early arthritis with the aim to reevaluate and maybe amend classification criteria in type 2 RA.
- To search for treatment strategies in type 2 RA that do not only decrease DAS but also improve long term outcomes.
- To optimize the distinction between type 1 and type 2 RA based on epidemiology pre-arthritis and in long-term outcomes, but also on other markers reflecting the underlying pathophysiology such as histology, metabolomics, lipidomics and autoantibody characteristics.
- To elucidate pathophysiological differences between type 1 and type 2 RA.

Tenosynovitis

- To examine the morphologic, histologic and molecular characteristics of tenosynovitis in early RA.
- To elucidate the etiology, interaction and timing of juxta-articular and intra-articular synovial inflammation in early RA.
- To further homogenize and validate scoring methods for tenosynovitis on MRI an ultrasound.
- To elucidate whether a tendon sheath is present around the extensor tendons at the MCP level and whether peritendinitis on MRI is in fact tenosynovitis.
- To further develop shorter and less costly MRI protocols to visualize tenosynovitis.

9

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