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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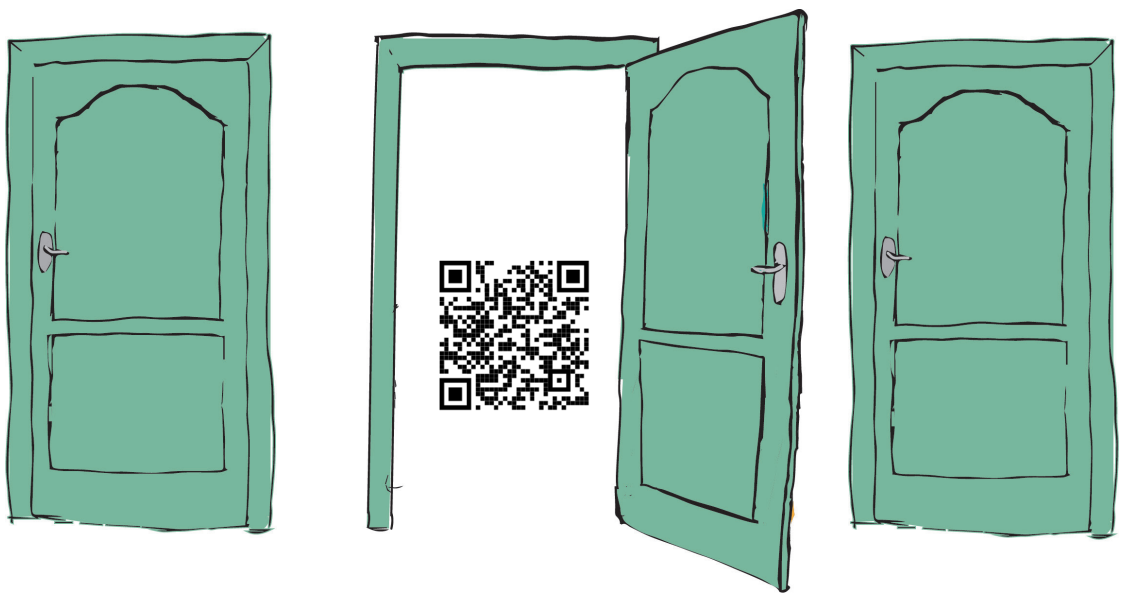
LONG-TERM OUTCOMES



CHAPTER 7

Enhanced treatment strategies and distinct disease outcomes among autoantibody-positive and -negative rheumatoid arthritis patients over 25 years: a longitudinal cohort study in the Netherlands

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ABSTRACT

Background

Based on different genetic and environmental risk factors and histology, it has been proposed that rheumatoid arthritis (RA) consists of two types: autoantibody-positive and autoantibody-negative RA. However, until now, this remained hypothetical. To assess this hypothesis, we studied whether the long-term outcomes differed for these two groups of RA-patients.

Methods and Findings

In the Leiden Early Arthritis Cohort, 1285 consecutive RA-patients were included between 1993-2016 and followed yearly. Treatment protocols in routine care improved over time, disregarding autoantibody-status, 5 inclusion periods were used as instrumental variables: 1993-1996 delayed mild disease modifying anti-rheumatic drug (DMARD) initiation (reference period); 1997-2000 early mild DMARDs; 2001-2005 early methotrexate; 2006-2010 early methotrexate followed by treat-to-target adjustments; 2011-2016 similar to 2006-2010 plus additional efforts for very early referral.

Three long-term outcomes were studied: SFDR (persistent absence of clinical synovitis after DMARD-cessation), mortality and functional disability measured by yearly health assessment questionnaires (HAQ). Treatment response on the short-term (disease activity) was measured by DAS28-ESR. Linear mixed models and Cox regression were used, stratified for autoantibody-positivity, defined as IgG anti-CCP2 and/or IgM rheumatoid factor-positivity.

823 patients had autoantibody-positive RA (mean age 55, 67% female); 462 patients autoantibody-negative RA (age 60, 64% female). Age, gender and percentage of autoantibody-positive patients were constant throughout the inclusion periods.

Disease activity significantly decreased over time within both groups. SDFR-rates increased since introduction of treat-to-target (HR 2006-2010: 3.35 [1.46 to 7.72; p=0.004] & HR 2011-2016: 4.57 [1.80 to 11.6; p=0.001]) in autoantibody-positive RA, but not in autoantibody-negative RA. In autoantibody-positive RA, mortality decreased significantly since treat-to-target treatment-adjustments (HR 2006-2010: 0.56 [0.34 to 0.92; p=0.023] & HR 2011-2016: 0.33 [0.14 to 0.77; p=0.010]), but not in autoantibody-negative RA (HR 2006-2010: 0.79 [0.40 to 1.56; p=0.50] & HR 2011-2016: 0.36 [0.10 to 1.34; p=0.13]). Similarly, functional disability improved in autoantibody-positive RA since 2001-2005 (range -0.16 [-0.29 to -0.03; p=0.043] to -0.32 [-0.44 to -0.20; p<0.001]) units improvement), but not in autoantibody-negative RA (range 0.10 [-0.12 to 0.31; p=0.38] to -0.13 [-0.34 to 0.07; p=0.20]) units improvement). Limitations to note were that treatment was not randomized but protocolized and instrumental variable analysis was used to obtain comparable groups, and that a limited spread of ethnicities was included.

Conclusions

Although the disease activity has improved in both autoantibody-positive and autoantibody-negative RA in recent decades, the response in long-term outcomes differed. We propose that it is time to subdivide RA in autoantibody-positive RA (type 1) and autoantibody-negative RA (type 2), in the hope that this leads to stratified treatment in RA.

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AUTHOR SUMMARY

Why Was This Study Done?

- Patients with rheumatoid arthritis (RA) have different risk factors and histology (microscopic anatomy) depending on the presence or absence of autoantibodies (anti-citrullinated protein antibodies and rheumatoid factor).
- Because it is suspected that RA with and without autoantibodies are two distinct diseases with a different pathophysiology, we hypothesized that these two types of RA react differently to improvements in treatment strategies that have taken place over the last decades.

What Did the Researchers Do and Find?

- Since its start in 1993, the inclusion criteria of the Leiden early arthritis cohort have not changed and included RA patients remained similar, apart from earlier diagnosis, therefore RA patients from different years were comparable. Treatment protocols enhanced over time, but were similar for patients with and without autoantibodies.
- We studied the changes in disease activity and three long term outcomes of RA patients with and without autoantibodies over time (inclusion period was a proxy for treatment strategy).
- We found that while disease activity improved in both patient groups, the long term outcomes (the possibility to permanently stop medication, mortality and functional disability) only improved in RA patients with autoantibodies.

What Do These Findings Mean?

- The disconnection between improvement in disease activity and subsequent improvement in longterm outcomes in RA without autoantibodies suggest that the underlying pathogenesis of RA with and without autoantibodies is different.
- We propose that it is time to formally subdivide RA into type 1 (with autoantibodies) and type 2 (without autoantibodies).

INTRODUCTION

Careful clinical observations over time have led to the description of diseases. In addition, subdividing of diseases has also been based on clinical observations, whilst differences in pathogenetic aetiology were identified subsequently. For instance subdividing diabetes in type 1 and type 2 was based on differences in clinical presentation (young versus older and obese patients); this distinction was confirmed by treatment response to insulin, and subsequently fuelled targeted etiological studies [1].

Rheumatoid arthritis (RA) is considered a syndrome. During the last decade it was observed that there are differences in RA-patients with and without autoantibodies (such as Rheumatoid factor (RF) and anti–citrullinated protein antibodies (ACPA)). Autoantibody-positive RA has a different genetic background [2], different environmental risk factors [3,4], slight differences in the preclinical symptomatic phase and first clinical presentation [5-7], differences in histology [8], differences in the synovial fluid cytokine profile [9] and, when left untreated, more severe joint destruction [5]. Nonetheless, the aetiology and pathophysiology of RA is still incompletely understood. It is unclear if there is one pathophysiological genesis, in which the presence of autoantibodies is promoted by certain genetic factors and where autoantibodies act as a 'severity' factor. Or, alternatively, that there are two different mechanisms of disease development. When distinct disease-mechanisms exist, treatment response may differ. Whether autoantibody-positive and autoantibody-negative RA have different mechanisms can therefore be addressed by clinical evaluation of long-term results in response to changes in treatment strategy.

Slight differences in effect of some drugs have been described between autoantibody-positive and autoantibody-negative RA-patients based on trial-data [10-13], but these are based on selected groups of RA-patients with a limited follow-up duration. We will take advantage of a large longitudinal cohort including incident RA-patients without selection from a region during the last 25 years; to our knowledge this is currently the largest observational cohort of RA. Treatment of RA has changed over time and improvements in strategies (e.g. early start, treat-to-target treatment adjustments) were not different for autoantibody-positive and autoantibody-negative patients. To evaluate whether autoantibody-positive RA and autoantibody-negative RA are two disease types, we studied the associations between changing treatment-strategies and disease activity in the short-term as well as three long-term outcomes.

METHODS

Longitudinal cohort

The Leiden Early Arthritis Clinic is a population based inception cohort including all consecutive patients newly presenting with recent-onset arthritis, that was started in 1993 and has been described in [14]. Inclusion criteria were presence of synovitis determined at physical examination by rheumatologists and symptom duration of <2 years. The department of rheumatology in the Leiden University Medical Center is the only centre for rheumatic diseases in a semi-rural area with >400,000 inhabitants. Since the start of the cohort general practitioners (GPs) were informed on the relevance of early referral and patients referred with suspicion on early arthritis were seen with priority, generally <2 weeks. Of note, in line with Dutch GP-guidelines, autoantibodies were rarely determined in primary care [15]. Written informed consent was obtained from all participants. The study was approved by the local medical ethics committee ('Commissie Medische Ethiek' of the Leiden University Medical Centre; B19.008).

For this study we selected the patients with RA (clinical diagnosis plus fulfilment of 1987-ACR-criteria). The use of the 1987-criteria (instead of the 2010-criteria) excluded influences of temporary changes in views on diagnosing RA and of the inverse relationship between presence of autoantibodies and degree of inflammation on the classification [16,17]. Between 2/24/1993 and 31/12/2016, 1377 patients enrolled in the cohort were classified with RA.

At the first visit, rheumatologists and patients completed questionnaires (among which the health assessment questionnaire disability index (HAQ)), swollen and tender joint counts (SJC, TJC) were performed, and blood samples taken for routine diagnostic laboratory screening (including erythrocyte sedimentation rate (ESR), immunoglobulin M– rheumatoid factor (positive if ≥3.5 IU/ml). From 2006, ACPA (anti-CCP2, Eurodiagnostica, positive if ≥25 U/ml; from 2009 EliA CCP, Phadia, positive if ≥7U/ ml) was measured. In patients included before 2006, ACPA-status was assessed retrospectively on stored baseline serum samples using the Eurodiagnostica assay. Since seroconversion is rare, repeated ACPA and/or RF measurements during follow-up were not studied [18]. In six patients autoantibody-status was not available, consequently they were excluded from the analyses (S1 Fig).

Protocolized follow-up visits were performed twice in the first year and yearly thereafter, as long as patients were treated at the outpatient clinic. Follow-up ended in case of death, release from care due to sustained DMARD-free remission (SDFR), moving to another area or withdrawal of informed consent while remaining treated. As data were collected at regular rheumatologist visits withdrawal of informed consent was rare. Data from the Statistics Netherlands from our region showed that moving away from the Leiden area was also infrequent (<3% annually) [19]. Inherent to the design, follow-up was shorter in the more recent inclusion periods. The majority of missing follow-up visits (not due to inclusion date) was due to mortality or SDFR.

Definition autoantibody-positive and autoantibody-negative

Patients with ACPA and/or RF were categorized as autoantibody-positive; double negative patients as autoantibody-negative. For practical reasons the distinction in type 1 and type 2 respectively is based on the autoantibodies that are currently used in the clinic. It could be that if more factors were included, eg other autoantibodies or other factors such as obtained from histology, a better division into groups would have been obtained [20-23]. Our primary goal, however, was to investigate the main distinction into autoantibody-positive and autoantibody-negative RA as it is used in clinical practice.

Treatment

Patients were treated in routine care according to protocols. 86 of 1377 RA-patients were treated within randomized clinical trials that were not in line with the treatment guidelines at that time and excluded, leaving 1285 RA-patients for analyses (S1 Fig). Temporal changes in treatment strategies concerned the initial start as well as treatment adjustments over time; both improvements in strategies are reflected by inclusion period as proxy. Patients included between 2/24/1993-31/12/1996 (n=168) received initial NSAIDs and started mild DMARDs with delay. Patients included between 1/1/1997-31/12/2000 (n=185) were treated early but not with methotrexate (e.g. hydroxychloroquine and sulfasalazine) [24]. Patients included between 1/1/2001-31/12/2005 (n=207) started early with methotrexate [25]. From 2006 onwards early methotrexate was followed by treat-to-target treatment adjustments, indicating treatment adjustments in case of increase disease activity scores (DAS) (1/1/2006-31/12/2010, n=335) [26]. Furthermore, because the value of very early treatment became even more apparent in 2010, and as GP-delay contributed most to the total delay in our region [27], from 2011 onwards on top of the existing regimen additional efforts were undertaken to further reduce referral delay by instituting an early arthritis recognition clinic, which is a screening clinic for the presence of inflammatory arthritis (1/1/2011-31/12/2016, n=390) [27-29].

In line with absence of guidelines that initial treatment should be adapted to autoantibody status [30,31], initial treatment choices were not directed by autoantibodies. Subsequent treatment decision were targeted at DAS; this was independent of patient characteristics. Thus protocols were similar for type 1 and 2.

Anti-TNF was the first biologic that became available in the early 2000s for RA-patients that failed on ≥2 conventional DMARDs [32]. Over time other biologics were registered, though the indication remained similar in the Netherlands. S1 Table provides information about the use of biologics at different follow-up durations, for type 1 and 2 separately. The usage was slightly higher in type 1, especially after introduction of treat-to-target.

Outcomes

Disease activity reflected the direct results of treatment; measured with the DAS28-ESR [33]. Since 2006 treatment is aimed at this short-term target to eventually improve long-term outcomes. Three long-term outcomes were studied: SDFR, mortality and functional disability. SDFR was defined as the sustained absence of synovitis (by physical examination) after discontinuation of DMARD therapy (including biologics, systemic or intra-articular corticosteroids) for the entire follow-up after DMARD-withdrawal, and this follow-up had to be at least one year after DMARD-stop [34]. This stringent and innovative definition of long-term remission is the opposite of disease persistence and became increasingly achievable [35]. After achievement of SDFR, patients were followed for median 5.5 years, to verify its sustainability. Patients that achieved DMARD-free remission but developed a late flare during this follow-up (n=23) were not considered as being in SDFR. All medical files of patients with ≥1 year follow-up were retrospectively explored on SDFR until April 2017. Mortality status was obtained from the civic registries on June 1, 2018. Functional disability, is one of the most important outcome from patients' perspective [36], and was measured yearly with the HAQ ranging from 0-3 (no-severe disability) [37,38].

Statistical Analyses

Main analyses were done for type 1 and 2 RA separately. Inclusion period was used as instrumental variable for treatment strategy. Within each type, improvements over time were compared to the reference period (inclusion 1993-1996).

Next, improvements over time compared to the reference period were compared between the two types by including an interaction term in the models to quantify the difference in improvent over time between the two types.

Time to SDFR was analysed with Cox regression. SDFR-status was censored at the date of revision of the medical files or at an earlier date when they were lost to follow-up or had died.

Mortality was analysed with Cox regression; follow-up was censored at the date of data extraction. Mortality was not compared to the general population because determination of excess mortality in RA relative to the population requires >10 years of follow-up to become apparent [39,40]; this follow-up duration was absent for the recent inclusion periods.

Missing data on DAS (complete DAS missing, 0% baseline and 3% follow-up) and HAQ (13% baseline, 22% annual follow-up) of attended visits were imputed using multivariate multiple imputation with predictive mean matching (100 cycles, 30 datasets). DAS and HAQ were analysed with linear mixed models. Because both outcomes rapidly decreased within the first year, the first year was analyzed separately from the remaining follow-up [41-43]. Slope of decrease in the first year was analysed with a random intercept and an identity covariance matrix. The course after the first year was analysed with a random intercept, random slope and continuous auto-regressive covariance matrix of order 1. Estimated marginal means were calculated. Percentages of DAS28-ESR remission (<2.6) at 1 and 3 years were tested with chi-square tests [44].

To minimize the influence of the association of the studied exposure and follow-up duration, analyses were truncated at 15 years follow-up and follow-up duration was not included as covariate in any of the analyses. All analyses were corrected for age and gender to improve model fit. As none of the measured baseline covariates are true confounders on the relationship between treatment strategy and outcomes, because they are not associated with the exposure or regarded to be the causal path (see S1 Text and S2 Fig for explanation), no other corrections were made.

No formal prospective analysis plan was written down and submitted prior to performing the analyses. Widths of the intervals have not been adjusted for multiplicity and p-values <0.05 were considered significant. R 3.6.1 with packages described in Text S2 were used. This study is reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline (See S1 Checklist).

Sensitivity Analyses

In a sensitivity analysis RA was defined according to the 2010-criteria.

In response to requests during peer review, to assess whether the difference in age at onset between the disease types might influence the results, patients aged <65 years at diagnosis were analysed in a sensitivity analysis.

For SDFR and mortality a sensitivity analysis was done, as due to differences in symptom duration at baseline, patients could not have presented themselves to the EAC because the studied event (SDFR, death) had already happened. To assess the influence of this possible left-truncation, correction for left-truncation was applied.

Finally, data for both disease types were plotted per inclusion periods for all outcomes; this was done for illustration.

RESULTS

Baseline characteristics

823 patients had type 1 RA; the mean age at first presentation was 55, 67% was female (Table 1). 462 patients had type 2; their mean age was 60, 64% was female. Age, gender and percentage of RA types were constant throughout the inclusion periods (p=0.59, p=0.28 and p=0.42, respectively), showing that similar RA-patients were included over time. Within both RA types, patients presented with shorter symptom duration, lower numbers of swollen and tender joints and lower acute phase reactants in more recent inclusion periods, reflecting that earlier presentation was paralleled with less severe disease (Table 1).

Disease activity

In type 1 RA, DAS improved in the first year and during subsequent follow-up (Fig 1; Table 2). Percentage of patients achieving DAS28-ESR remission (<2.6) significantly increased, e.g. from 13% in the oldest inclusion period, to 50% at year 1 and 61% at year 3 in the most recent period (S3 Fig).

In the type 2 RA, DAS also improved, especially in the first year (Fig 2; Table 3). DAS28-ESR remission percentages increased from 32% in the oldest inclusion period, to 54% at year 1 and 71% at year 3 in the most recent period (S3 Fig).

Sustained DMARD-free remission

In type 1 RA, SDFR significantly increased over time, especially since the start of treat-to-target (Fig 1; Table 2). In type 2 RA, there was no significant increase in SDFR (Fig 2; Table 3).

Mortality

Compared to the reference period, mortality decreased significantly in type 1 RA since the start of treat-to-target (Fig 1; Table 2). No significant association was found in type 2 RA (Fig 2; Table 3), although hazard ratios were in the same direction as in type 1 RA.

Functional Disability

In type 1 RA, functional disability improved over time since the start of early methotrexate, both in the first year and the subsequent years (Fig 1; Table 2). In type 2 in contrast, improvement was absent (Fig 2; Table 3).

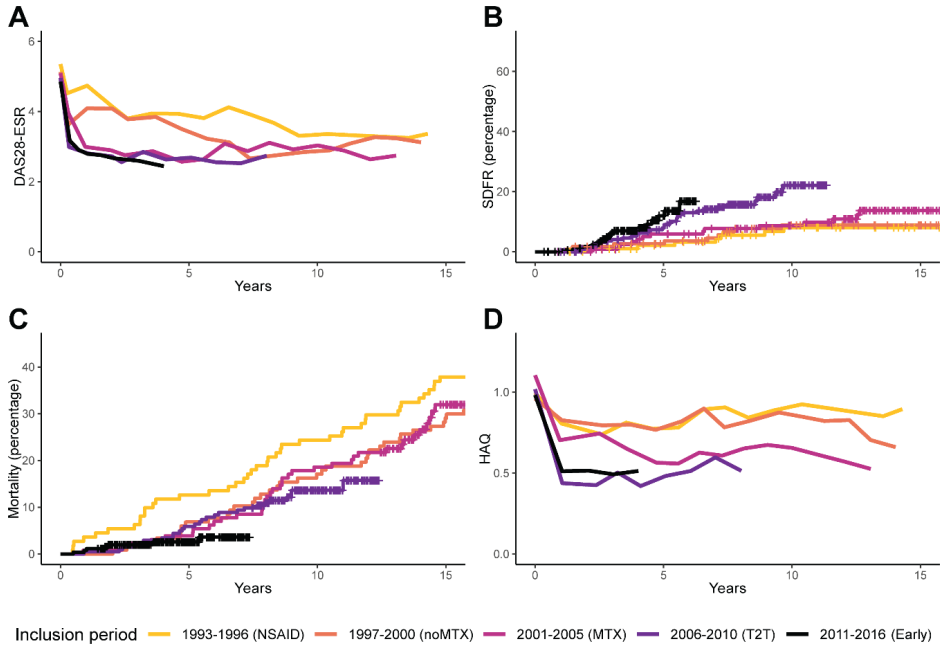
Table 1: Characteristics of patients with type 1 (autoantibody-positive; A) and type 2 RA (autoantibody-negative; B) at first presentation to the early arthritis clinic.

	1993-1996 (n = 112, 67%)	1997-2000 (n=118, 64%)	2001-2005 (n = 129, 62%)	2006-2010 (n = 203, 61%)	2011-2016 (n = 261, 67%)	p-value
Women, n (%)	77 (69)	82 (70)	91 (71)	136 (67)	167 (64)	0.70
Age in years, mean (SD)	56 (16)	55 (16)	55 (15)	54 (15)	56 (15)	0.63
Symptom duration, days median (IQR)	153 (84-306)	156 (84-304)	147 (72-264)	146 (61-270)	103 (53-227)	0.006
Current smoker, n (%)	35 (33)	35 (33)	29 (27)	40 (22)	74 (30)	0.21
28-SJC, median (IQR)	6 (3-10)	7 (4-12)	4 (2-7)	4 (2-7)	4 (2-7)	<0.001
28-TJC, median (IQR)	7 (3-13)	7 (3-14)	7 (3-12)	6 (3-11)	5 (2-9)	<0.001
ESR, median (IQR)	46 (26-70)	32 (20-54)	30 (18-55)	29 (14-42)	29 (14-41)	<0.001
VAS general health, median (IQR)	43 (17-70)	44 (26-66)	53 (34-72)	56 (29-72)	70 (50-80)	<0.001
DAS28-ESR, median (IQR)	5.5 (4.2-6.5)	5.2 (4.2-6.1)	5.2 (4.3-6.0)	4.9 (4.2-6.0)	4.8 (4.1-5.7)	0.02
HAQ, median (IQR)	1.0 (0.6-1.4)	0.8 (0.4-1.6)	1.0 (0.6-1.6)	1.0 (0.5-1.5)	1.0 (0.5-1.5)	0.12

B.	1993-1996 (n = 56, 33%)	1997-2000 (n = 67, 36%)	2001-2005 (n = 78, 38%)	2006-2010 (n = 132, 39%)	2011-2016 (n = 129, 33%)	p-value
Women, n (%)	38 (68)	41 (61)	57 (73)	80 (61)	79 (61)	0.34
Age in years, mean (SD)	56 (15)	59 (19)	60 (14)	61 (16)	62 (14)	0.16
Symptom duration, days median (IQR)	126 (61-220)	92 (62-219)	120 (74-234)	109 (59-176)	85 (45-189)	0.06
Current smoker, n (%)	17 (30)	11 (18)	14 (20)	24 (21)	28 (22)	0.52
28-SJC, median (IQR)	9 (4-14)	12 (7-19)	6 (3-10)	6 (3-10)	6 (3-10)	<0.001
28-TJC, median (IQR)	9 (3-19)	13 (6-20)	11 (5-19)	9 (4-13)	7 (3-11)	<0.001
ESR, median (IQR)	40 (22-56)	28 (16-47)	27 (16-47)	31 (9-46)	25 (11-41)	0.008
VAS general health, median (IQR)	46 (25-63)	50 (26-62)	56 (36-75)	64 (44-79)	70 (60-80)	<0.001
DAS28-ESR, median (IQR)	5.6 (4.5-6.3)	5.8 (4.8-6.5)	5.6 (4.4-6.7)	5.3 (4.4-6.3)	5.2 (4.4-6.0)	0.19
HAQ, median (IQR)	1.1 (0.8-1.6)	0.9 (0.5-1.4)	1.1 (0.8-1.8)	1.1 (0.8-1.5)	1.0 (0.6-1.5)	0.15

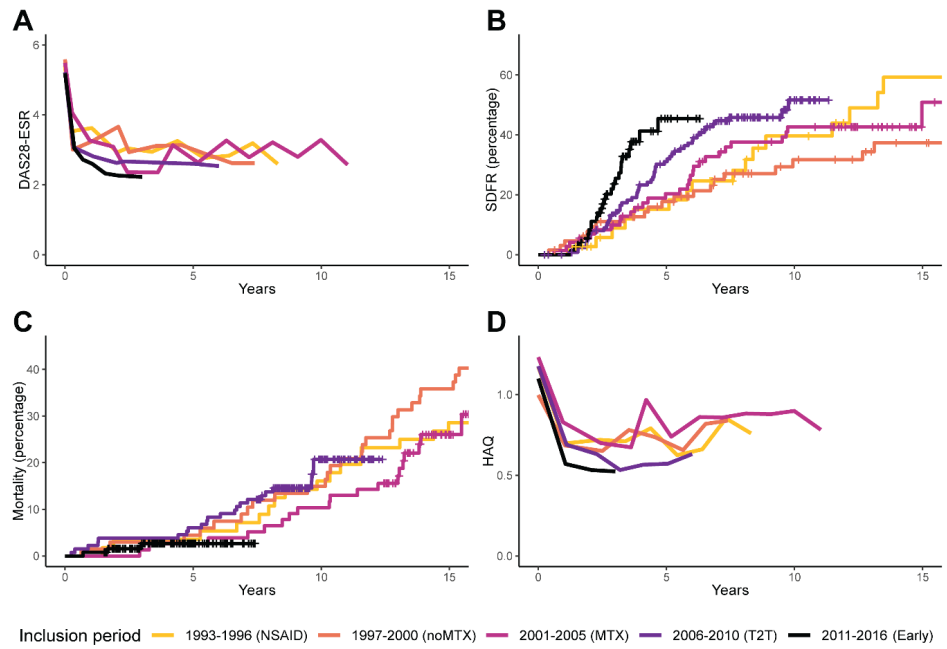
Legend: N, number of patients; SD, standard deviation; IQR, inter quartile range; SJC, swollen joint count; TJC, tender joint count; ESR, erythrocyte sedimentation rate; VAS, visual analogue scale; DAS, disease activity score; HAQ, health assessment questionnaire. p-value; results of Kruskal-Wallis H-test (Fisher's exact test for proportions and ANOVA for normally distributed variables).
The percentage of patients with type 1 or 2 RA for the different inclusion periods was stable over time (p=0.42).
The 28-SJC and 28-TJC counts are the number of swollen and tender joints, respectively, out of 28 joints assessed.
The VAS general health is a self-reported assessment, ranging from 0 to 100.
The DAS28-ESR ranges 2-9.4, with higher scores indicating more disease activity.
The HAQ (HAQ-DI) ranges 0-3, with higher scores indicating more disability.

Figure 1: Disease activity over time (A) and the long-term outcomes sustained DMARD-free remission (B), mortality (C) and functional disability (D) in type 1 (autoantibody-positive) RA.



Legend: For DAS28-ESR and HAQ, mean values of imputed data from visits that were attended are shown; when <20% of patients attended the visit, lines were truncated.
DAS, disease activity score; ESR, erythrocyte sedimentation rate; SDFR, sustained DMARD-free remission; HAQ, health assessment questionnaire; NSAID, nonsteroidal anti-inflammatory drugs; MTX, methotrexate; T2T, treat-to-target; Early, early treatment;
The DAS28-ESR ranges 2-9.4, with higher scores indicating more disease activity. Remission is defined as a score <2.6 and a change of >1.2 is considered a clinically relevant change [44].
The HAQ ranges 0-3, with higher scores indicating more disability. The minimally important difference is 0.22 [38].
For SDFR, at 5 years, 85%, 87%, 89%, 82% and 32% of patients from inclusion period 1993-1996 to 2011-2016, respectively, were still in at risk. At 10 years, this was 79%, 71%, 70%, 15%, 0% and at 15 years 56%, 59%, 12%, 0%, 0%.
For mortality, at 5 years, 87%, 93%, 96%, 94% and 42% of patients from inclusion period 1993-1996 to 2011-2016, respectively, were still in at risk. At 10 years, this was 76%, 83%, 81%, 38%, 0% and at 15 years 62%, 71%, 35%, 0%, 0%.

Figure 2: Disease activity over time (A) and the long-term outcomes: sustained DMARD-free remission (B), mortality (C) and functional disability (D) in type 2 (autoantibody-negative) RA.



Legend: For DAS28-ESR and HAQ, mean values of imputed data from visits that were attended are shown; when <20% of patients attended the visit, lines were truncated.
DAS, disease activity score; ESR, erythrocyte sedimentation rate; SDFR, sustained DMARD-free remission; HAQ, health assessment questionnaire; NSAID, nonsteroidal anti-inflammatory drugs; MTX, methotrexate; T2T, treat-to-target; Early, early treatment.
The DAS28-ESR ranges 2-9.4, with higher scores indicating more disease activity. Remission is defined as a score <2.6 and a chnge of >1.2 is considered a clinically relevant change [44].
The HAQ ranges 0-3, with higher scores indicating more disability. The minimally important difference is 0.22 [38].
For SDFR, at 5 years, 73%, 74%, 72%, 62% and 14% of patients from inclusion period 1993-1996 to 2011-2016, respectively, were still in at risk. At 10 years, this was 41%, 45%, 47%, 9%, 0% and at 15 years 22%, 31%, 8%, 0%, 0%.
For mortality, at 5 years, 96%, 96%, 97%, 94% and 27% of patients from inclusion period 1993-1996 to 2011-2016, respectively, were still in at risk. At 10 years, this was 84%, 85%, 90%, 34%, 0% and at 15 years 71%, 64%, 26%, 0%, 0%.

Table 2: Disease activity during the first year and subsequent follow-up and long-term outcomes: sustained DMARD-free remission, mortality and functional disability per inclusion period compared to the reference period for type 1 (autoantibody-positive) RA.

	DAS28-ESR, slope in first year		**DAS28-ESR over time after first year**		**Sustained DMARD free remission**		**Mortality**		**HAQ, slope in first year**		**HAQ over time, after first year**	
	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val	Hazard ratio[c]	p-val	Hazard ratio[c]	p-val	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val
Inclusion period												
1993-1996	Ref[d]		Ref[d]		Ref		Ref		Ref[d]		Ref[d]	
1997-2000	-0.38 (-0.87;0.10)	0.12	**-0.41 (-0.66;-0.16)**	**0.002**	1.14 (0.42;3.05)	0.80	0.74 (0.47;1.15)	0.18	0.01 (-0.19;0.21)	0.89	-0.02 (-0.15;0.11)	0.58
2001-2005	**-1.70 (-2.21;-1.20)**	**<0.001**	**-0.86 (-1.12;-0.61)**	**<0.001**	1.66 (0.67;4.12)	0.27	0.71 (0.46;1.11)	0.13	**-0.28 (-0.49;-0.07)**	**0.009**	**-0.16 (-0.29;-0.03)**	**0.043**
2006-2010	**-1.62 (-2.08;-1.17)**	**<0.001**	**-1.04 (-1.28;-0.80)**	**<0.001**	**3.35 (1.46;7.72)**	**0.004**	**0.56 (0.34;0.92)**	**0.023**	**-0.33 (-0.51;-0.14)**	**0.001**	**-0.32 (-0.44;-0.20)**	**<0.001**
2011-2016	**-1.54 (-1.96;-1.12)**	**<0.001**	**-1.07 (-1.32;-0.83)**	**<0.001**	**4.57 (1.80;11.6)**	**0.001**	**0.33 (0.14;0.77)**	**0.010**	**-0.29 (-0.46;-0.12)**	**0.001**	**-0.26 (-0.38;-0.14)**	**0.008**

Bold numbers indicate p-values < 0.05.
[a] Difference in slope in the first year compared to the slope in 1993-1993; analyzed with linear mixed models corrected for age and gender. A negative number indicates a steeper slope.
[b] Difference in mean over time compared the mean over time in 1993-1996; analyzed with linear mixed models corrected for age and gender.
[c] Hazard ratios compared to 1993-1996; analyzed with Cox regression corrected for age and gender.
[d] The estimated marginal mean, adjusted for age and gender, in type 1 RA for inclusion period 1993-1996 was -0.34 (-0.70 to 0.03) for the slope in DAS28-ESR in the first year, 3.58 (3.39 to 3.76) for DAS28-ESR over time after the first year, -0.15 (-0.29 to 0.00) for slope in HAQ in the first year and 0.78 (0.68 to 0.88) for HAQ over time after the first year.
DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; p-val, p-value.

Table 3: Disease activity during the first year and subsequent follow-up and the long-term outcomes: sustained DMARD-free remission, mortality and functional disability per inclusion period compared to the reference period for type 2 (autoantibody-negative) RA.

	DAS28-ESR, slope in first year		**DAS28-ESR over time after first year**		**Sustained DMARD free remission**		**Mortality**		**HAQ, slope in first year**		**HAQ over time, after first year**	
	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val	Hazard ratio[c]	p-val	Hazard ratio[c]	p-val	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val
Inclusion period												
1993-1996	Ref[d]		Ref[d]		Ref		Ref		Ref[d]		Ref[d]	
1997-2000	-0.53 (-1.30;0.24)	0.18	0.08 (-0.32;0.49)	0.69	0.61 (0.32;1.18)	0.14	0.67 (0.35;1.30)	0.24	0.16 (-0.13;0.44)	0.29	0.03 (-0.19;0.24)	0.81
2001-2005	**-0.88 (-1.66;-0.11)**	**0.025**	-0.03 (-0.43;0.37)	0.89	0.80 (0.43;1.48)	0.48	0.57 (0.28;1.13)	0.11	0.05 (-0.25;0.35)	0.75	0.10 (-0.12;0.31)	0.38
2006-2010	**-0.78 (-1.48;-0.08)**	**0.029**	-0.26 (-0.63;0.11)	0.17	1.11 (0.63;1.97)	0.71	0.79 (0.40;1.56)	0.50	0.02 (-0.24;0.28)	0.87	-0.09 (-0.28;0.10)	0.34
2011-2016	**-1.08 (-1.75;-0.41)**	**0.002**	**-0.44 (-0.84;-0.04)**	**0.030**	1.89 (0.97;3.67)	0.060	0.36 (0.10;1.34)	0.13	-0.02 (-0.27;0.23)	0.89	-0.13 (-0.34;0.07)	0.20

Bold numbers indicate p-values < 0.05.
[a] Difference in slope in the first year compared to the slope in 1993-1993; analyzed with linear mixed models corrected for age and gender. A negative number indicates a steeper slope.
[b] Difference in mean over time compared the mean over time in 1993-1996; analyzed with linear mixed models corrected for age and gender.
[c] Hazard ratios compared to 1993-1996; analysed with Cox regression and corrected for age and gender.
[d] The estimated marginal mean, adjusted for age and gender, in type 2 RA for inclusion period 1993-1996 was -1.27 (-1.81 to -0.72) for the slope in DAS28-ESR in the first year, 2.70 (2.40 to 3.01) for DAS28-ESR over time after the first year, -0.46 (-0.67 to -0.25) for slope in HAQ in the first year and 0.62 (0.47 to 0.78) for HAQ over time after the first year. DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; p-val, p-value

Comparison of improvement of type 1 and type 2

To assess whether more improvement was indeed observed in type 1 RA compared to type 2 RA, change with respect to the reference period was compared between the two disease types by adding an interaction term to the models. More improvement for the outcomes DAS over time, SDFR and functional disability was observed in type 1 RA (Table 4). This was statistically significant for these outcomes in the inclusion period 2006-2010 (early methotrexate followed by treat-to-target treatment adjustments).

Sensitivity analyses

According to the 2010-criteria, 1421 patients had RA, 957 type 1 and 474 type 2 (S4 Fig). Due to the composition of these criteria, type 2 RA required ≥11 involved joints for classification [16,17]. Indeed this group had high joint counts, especially high tender joints in the latest periods when acute phase reactants and swollen joint counts at diagnosis decreased (S2 Table). This possibly resulted in incomparability in disease activity between the periods within type 2 RA. Results for type 1 were similar when RA was defined according to the 1987-criteria. For type 2 little improvement in DAS was present and effect sizes of long-term outcomes were in line with the main results (S3,4 Table).

Analyses were repeated in patients aged <65 years at diagnosis; similar results were obtained except for a non-significant improvement in mortality in type 1 RA, possibly caused by a lower number of events (S5,6 Table).

Effect sizes for the outcomes SFDR and mortality after correction for left truncation were similar (S7 Table).

For illustration, head-to-head comparisons between type 1 and type 2 RA within the inclusion periods are shown in S5-8 Fig.

Table 4: Differences in improvement of disease outcomes between type 1 (autoantibody-positive) and type 2 (autoantibody-negative) rheumatoid arthritis with enhanced treatment strategies over 25 years

	DAS28-ESR, slope in first year		**DAS28-ESR over time after first year**		**Sustained DMARD free remission**		**Mortality**		**HAQ, slope in first year**		**HAQ over time, after first year**	
	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val	Hazard ratio[c]	p-val	Hazard ratio[c]	p-val	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val
Inclusion period												
1993-1996	Ref[d]		Ref[d]		Ref		Ref		Ref[d]		Ref[d]	
1997-2000	0.14 (-0.75;1.04)	0.75	-0.46 (-0.94;0.03)	0.068	1.80 (0.55;5.92)	0.33	1.02 (0.47;2.23)	0.96	-0.14 (-0.49;0.21)	0.42	-0.06 (-0.30;0.19)	0.65
2001-2005	-0.82 (-1.73;0.08)	0.073	**-0.70 (-1.18;-0.22)**	**0.004**	2.10 (0.70;6.28)	0.18	1.22 (0.54;2.73)	0.64	-0.33 (-0.69;0.03)	0.069	-0.21 (-0.46;0.04)	0.095
2006-2010	-0.82 (-1.64;0.00)	0.050	**-0.70 (-1.14;-0.25)**	**0.002**	**2.93 (1.08;7.90)**	**0.034**	0.82 (0.37;1.83)	0.63	**-0.35 (-0.66;-0.05)**	**0.024**	**-0.22 (-0.44;0.00)**	**0.046**
2011-2016	-0.47 (-1.23;0.29)	0.22	**-0.55 (-1.04;-0.05)**	**0.030**	2.10 (0.71;6.22)	0.18	1.11 (0.26;4.85)	0.89	-0.27 (-0.56;0.02)	0.064	-0.11 (-0.35;0.13)	0.37

Bold numbers indicate p-values < 0.05.
The overall p-value of the interaction term in the models (e.g. the p-value for difference in improvement between the two subtypes over all inclusion periods) was 0.072 for DAS28-ESR slope in first year, <0.001 for DAS28-ESR over time after first year, 0.28 for Sustained DMARD free remission, 0.91 for mortality, 0.016 for HAQ slope in first year and 0.10 for HAQ over time after first year.
[a] Additional improvement in type 1 with respect to type 2. A negative number corresponds to additional change downward in type 1 with respect to the reference period (e.g. more decrease in the first year with respect to the reference period). Since a lower DAS/HAQ is better, a negative number indicates more improvement in type 1.
[b] Additional improvement in type 1 with respect to type 2. A negative number corresponds to additional change downward of the mean after the first year in type 1 with respect to the reference period. Since a lower DAS/HAQ is better, a negative number indicates more improvement in type 1.
[c] Additional improvement in type 1 with respect to type 2. A number above 1 corresponds to additional SDFR in type 1 with respect to the reference period. Since more SDFR is better, a number above 1 indicates more improvement in type 1.
[d]Additional improvement in type 1 with respect to type 2. A number below 1 corresponds to less mortality in type 1 with respect to the reference period. Since a lower mortality is better, a number below 1 indicates more improvement in type 1.
DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire p-val, p-value.

DISCUSSION

Summary of findings

During the last 25 years, the treatment of RA has changed in several aspects. We studied outcomes of RA and observed that improved treatment strategies were paralleled by reduced disease activity in autoantibody-positive and autoantibody-negative RA, but resulting significant improvements in the long-term outcomes, SDFR, mortality and functional disability, were only present in autoantibody-positive RA and not in autoantibody-negative RA. In line with these findings, DAS, SDFR and functionality had greater improvements over the last 25 years within autoantibody-positive than within autoantibody-negative RA. Especially the introduction of treat-to-target treatment adjustments associated with significantly greater improvements in autoantibody-positive RA than in autoantibody-negative RA. The disconnection between improvements in disease activity and in several longterm outcomes suggest that the underlying pathogenesis of autoantibody-positive and autoantibody-negative RA is different. We therefore propose that the time has come to subdivide RA in type 1 and type 2.

Comparisons with other studies

Subdivisions of disease are ideally underpinned with identified differences in etiopathology. However clinical observations have frequently been the basis of subdivisions of diseases and preceded the identification of pathophysiological mechanisms. Both types of RA have a different genetic background. Whereas >100 genetic risk factors are identified for type 1, few genetic factors have been related to type 2 RA [45]. Known environmental risk factors are associated with predominantly one of the two types [3,4]. These data, together with observed differences in histology [8], may also point towards different underlying mechanisms.

Etiopathogenetic research in the last decade has focused most on autoantibody-positive RA, but a causal relationship for the autoantibodies has not been proven. Further pathogenic research is needed for both type 1 and type 2 RA.

Strengths and limitations of this study

We have studied the autoantibodies that are daily used in clinical practice (ACPA, RF). Several new autoantibodies have recently been identified; most co-occur in patients that also harbor ACPA or RF [20-23]. Few percent of ACPA- and RF-negative patients were found positive for novel autoantibodies, leaving the so-called 'serological gap' largely unchanged. There was insufficient power to assess which autoantibodies are optimal for the characterization of type 1 RA. It is a subject for further research to determine whether the division can be optimized by incorporation of recently identified autoantibodies or other markers (e.g. obtained from histology) [46].

Autoantibody-positivity was determined with the cut-offs that are also used in daily clinical practice in our hospital. Some patients might have values just around the cut-off at baseline and therefore might change in autoantibody-positivity over time. Previous research in the EAC cohort has shown that sero-conversion towards autoantibody-negativity is rare, even when SDFR is achieved, and that seroconversion was mostly caused by fluctuations of levels around the cut-off [18]. Similarly, data from our cohort show that seroconversion from autoantibody-negativity to autoantibody-positivity is also infrequent (2% after 1-year follow-up; Fig S9). Thus autoantibody status is quite stable after diagnosis.

Type 2 patients had a clinical diagnosis of RA, fulfilled classification criteria, and lacked ACPA and RF. It has been suggested that autoantibody-negative RA is heterogeneous in nature. We find it important to formally consider autoantibody-negative RA as a separate entity, but we cannot exclude that type 2 RA consists of different subtypes. This was beyond the scope and power of this study.

To assess the response to improved treatment strategies without exposing patients to outdated and less effective treatments, historical data was used and inclusion period as instrumental variable for treatment strategy. As an alternative to randomisation, instrumental variable analysis uses a proxy (inclusion period) to create groups with comparable patients that receive different treatment strategies. Between these groups, treatment strategies can be compared without confounding by indication, under the assumption that allocation to the groups is random. Since inclusion criteria of the Leiden EAC have not changed over time, year of RA diagnosis was assumed random. Importantly, initial treatment protocols and treat-to-target protocols were similar for patients with and without autoantibodies, making the instrument similar for both patient groups.

Treatment was targeted at DAS-remission since 2006, and was never targeted at autoantibodies (notable, ACPA results became available for rheumatologists in this study from 2006 onwards). While type 2 RA had a slightly higher baseline DAS and in type 1 mean DAS over time decreased more, mean DAS and remission rates were similar or better in type 2 RA in all periods. Observed differences in long-term outcomes are therefore unlikely the result of better adherence to treat-to-target in autoantibody-positive patients. Also the finding that patients with autoantibodies more often required biologics to achieve DAS-remission (S1 Table) merely underlines the difference between both types.

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Progression of joint destruction was not studied as outcome, because the natural course of type 2 RA involves little structural damage and a lack of improvement can also be explained by the inability to measure this [5]. The long-term outcomes studied here, on the other hand, had the potential for improvement, also in patients with type 2 RA.

Mortality was studied without adjusting for mortality in the general population because excess mortality in RA is heavily dependent on follow-up duration, which differs between the inclusion cohorts [40]. Although a significant improvement in mortality was observed in type 1 RA and not in type 2 RA, effect sizes were in the same direction. Analyses of longer follow-up in larger cohorts, that also adjust for mortality in the general population are needed to determine if excess mortality reduced differently between the two groups.

In current treatment strategies SDFR is not targeted. Although innovative, this is an interesting outcome from an immunological perspective, that resembles 'cure'. Prolonged follow-up duration is required to determine the sustainability of DMARD-free remission after DMARD-cessation. An advantage of our data is that we had median 5.5 years of follow-up after DMARD-stop.

RA was defined according to the 1987-criteria (not the 2010-criteria) to exclude influences of temporal changes in rheumatologists views on diagnosing RA. Furthermore, autoantibodies load heavily in the 2010-criteria. It is known that much inflammation is needed in the absence of autoantibodies to fulfill the 2010-criteria [16,17]. Even more, in our data higher tender joint counts were needed to classify RA in recent periods, possibly resulting in incomparability in DAS within the current set of autoantibody-negative 2010-RA patients. Nonetheless, similar results in long term outcomes were found.

Future implications

Possible implications of formal subdivision of RA are execution of more focused pathogenetic studies, development of treatment protocols adapted to disease type, and performance of trials per disease type. Ultimately a better distinction leads to improved personalized care.

Conclusion

In sum, to our knowledge this is the first long-term study in a large cohort of RA-patients with data of 25 years of follow-up. Based on the demonstrated differences in long-term outcomes, and supported by previous findings on risk factors, we propose to subgroup RA in type 1 and type 2, in the hope that this leads to stratified treatment in RA.

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