# Tolerance and immune regulation in rheumatoid arthritis

Dekkers, J.S.

# PART II

## AUTOIMMUNITY IN EARLY DISEASE

*Hans Holbein der Jügere (1497-1543) Erasmus writing (detail), 1523, Le Louvre Paris, France*

# Chapter 6

## Autoantibody Status is not Associated with Early Treatment Response to first-line Methotrexate in Patients with Early Rheumatoid Arthritis

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Jacqueline S. Dekkers[1], Sytske Anne Bergstra[1], Arvind Chopra[2], Mohammed Tikly[3], João Eurico Fonseca[4], Karen Salomon-Escoto[5], Tom W.J. Huizinga[1], Diane van der Woude[1]

1. Department Of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
2. Centre For Rheumatic Diseases, Pune, India
3. Division Of Rheumatology, University of Witwatersrand, Johannesburg, South Africa.
4. Rheumatology Research Unit, Lisbon Academic Medical Centre, Portugal
5. Department Of Rheumatology, UMass Memorial Medical Center, Massachusetts, USA

**ABSTRACT**

**Objectives**

In rheumatoid arthritis (RA), the relationship between autoantibody status and treatment response to methotrexate remains unclear. We investigated the association between autoantibody status and early remission in newly diagnosed RA-patients treated with methotrexate using real-world data.

**Methods**

RA-patients initially treated with methotrexate were selected from an international observational database (METEOR). Patients were stratified into autoantibody-positive (rheumatoid factor (RF)- and/or anti-citrullinated-protein antibodies (ACPA)-positive) or autoantibody negative (RF- and ACPA- negative). The effect of autoantibody status on the chance of achieving remission within 3 to 6 months was analysed using Cox-proportional hazards regression.

**Results**

Data from 1826 RA-patients were available for analysis. DAS remission was achieved in 17% (318/1,826). This was similar in autoantibody-positive (17% (282/1629)) and -negative patients (18% (36/197)). Hence, autoantibody positivity was not associated with remission (HR0.89, 95%CI 0.57;1.38). Similar findings were found when stratified for methotrexate monotherapy (HR0.75, 95%CI 0.41;1.37) or combination treatment (HR0.76, 95%CI 0.37;1.54). Good physical function (HAQ<0.5) was achieved in 33% (530/1590) of all patients. Autoantibody-positivity was also not associated with HAQ<0.5 (HR1.05, 95%CI 0.71;1.57).

**Conclusions**

Autoantibody status is not associated with early remission in newly diagnosed RA-patients receiving methotrexate. This indicates that methotrexate is effective as initial treatment strategy regardless of autoantibody status.

**Introduction**

Rheumatoid arthritis (RA) is generally considered to consist of two separate entities: autoantibody-positive and -negative disease, each with distinct genetic and environmental risk factors and disease outcomes. Autoantibody-positive patients have worse long-term outcomes with less functional ability, worse disease activity and more radiographic joint damage(1-3). The presence of autoantibodies is associated with a better treatment response to rituximab(4) and abatacept(5), but whether an association exists with other (more commonly used) drugs, most notably methotrexate, remains unknown.

The presence of rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA), especially at high levels, is mentioned as poor prognostic factor for treatment response to methotrexate in international treatment recommendations(6) even though the relationship between autoantibody status and treatment response to methotrexate is unclear. Some studies have suggested that autoantibody-positive patients might respond better to methotrexate, while other studies do not support this conclusion(7-10). In a previous cohort study disease-modifying antirheumatic drug (DMARD) treatment was equally effective in autoantibody-positive and -negative patients, but treatment differed and not all patients received methotrexate(11, 12). As methotrexate is the most widely used anti-rheumatic drug in clinical practice(6), it would be important to know whether the presence of autoantibodies is associated with better treatment response, since patients may benefit from treatment tailored to "autoantibody status". We therefore investigated the relationship between autoantibody status and remission in newly diagnosed RA-patients treated with first-line methotrexate.

## Methods

### Study population

Data were obtained from the METEOR register(13). This is an international, observational database of patients with a diagnosis of RA according to the rheumatologist, attending daily clinical practice. Data of RA-patients with symptom duration <5 years, baseline Disease Activity Score (DAS) >1.6 and follow-up visits after 3-6 months were selected. At least one of the following measures had to be available: DAS, DAS28, *Simplified Disease Activity Index* (*SDAI*), Clinical Disease Activity Index (CDAI), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) or health assessment questionnaire (HAQ). Data regarding autoantibody status (ACPA and/or RF, which were measured locally at the participating centers) had to be available and methotrexate had to be part of initial treatment. Data were gathered between 1995-2017 and contained irregular time intervals and different numbers of follow-up visits per patient due to the observational design of the database. All follow-up visits within 3 to 6 months after treatment initiation were selected.

### Statistical analysis

Patients were stratified into autoantibody-positive (RF- and/or ACPA-positive) and -negative patients (RF- and ACPA-negative) and summary statistics were generated for baseline characteristics. Missing data were imputed using multivariate normal multiple imputation (30 imputations)(14). The variables patient-reported pain, ACPA and DAS contained most missing values (17 to 28% of values missing). Variables included for imputation were: year of birth, sex, RF, ACPA, CRP, ESR, weight, height, Ritchie articular index, SDAI, CDAI, erosions, HAQ, swollen joint count, DAS, DAS28, doctor's and patient's global assessment, and patient-reported pain. The effect of autoantibody status on the chance of achieving remission (DAS<1.6) or good physical function (HAQ<0.5) was analysed using Cox-proportional hazards regression with DAS or HAQ as time-dependent covariates(15) and correcting for confounders. Sub-analyses of methotrexate treatment strategies were performed. Patients were stratified into four groups according to initial medication strategy and we tested whether there was effect modification of these medication groups. In addition, we evaluated the presence of effect modification by testing for statistical interaction between autoantibody status and country. If no interactions were found (p>0.10), data of all countries were combined. Moreover, patients were stratified based symptom duration and assessed whether symptom duration was an effect modifier. Analyses were performed using StataSE14 (StataCorp LP).

**Sensitivity analysis**

Complete case analysis was performed as a sensitivity analysis. Linear mixed model (LMM) analyses were performed as sensitivity analyses to assess the effect of autoantibody status on DAS, HAQ and ESR as continuous outcomes during treatment with methotrexate. To account for irregular time intervals, random intercept and slope were added to each model. Interaction between time in follow-up and autoantibody status was added and adjusted for potential confounders, to assess whether the effect of autoantibody status on treatment outcome differed over time. Sensitivity analysis based on patients of European centers was performed to count for the dissimilarity between the frequency of seronegative patients between countries.

**Results**

Of the individuals registered in the METEOR database, 1,826 patients fulfilled the selection criteria. A flow-chart of patient selection is depicted in Figure 1. Patients originated from 20 different countries, with 93% of data originating from India, South-Africa, Portugal, the Netherlands, the United States, Mexico and Great Britain. 1,629 (89%) patients were autoantibody-positive (RF positive n=1,554, ACPA positive n=849) and 197 patients (11%) were autoantibody-negative. Baseline characteristics are depicted in Table 1. At baseline, autoantibody-positive patients were younger, had longer disease duration, higher baseline DAS and increased levels of acute-phase reactants ($p< 0.001$). Autoantibody-negative patients more often received methotrexate monotherapy and less frequently combination therapy with other conventional synthetic DMARDs compared to autoantibody-positive patients ($p< 0.001$).

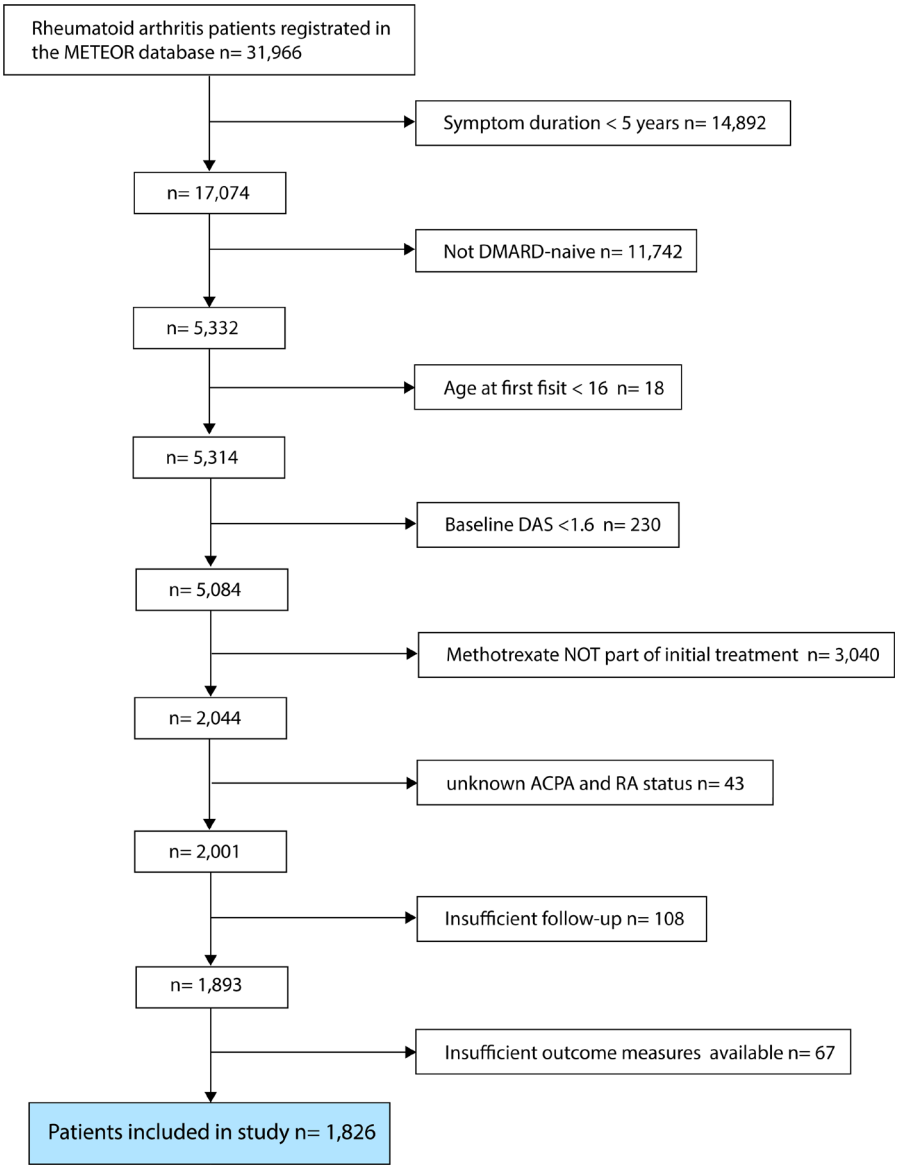


**Figure 1. Flow-chart of patient selection.** No effect modification of country on autoantibody status was present (p=0.62), therefore data of all countries were combined. In a period of 6 months, DAS remission was achieved by a similar percentage of autoantibody-positive (17%, 282/1629) and -negative (18%, 36/197) patients. The probability of achieving DAS remission over time is depicted for autoantibody-positive and negative RA-patients in Figure 2. Accounting for potential confounders, autoantibody positivity did not associate with remission (HR 0.89, 95%CI 0.57;1.38) (Table 2). Similar findings were found when stratified for methotrexate monotherapy (HR 0.75, 95%CI 0.41;1.37) or combination treatment (HR 0.76, 95%CI 0.37;1.54).

**Table 1. Complete case analysis**

| **DAS Remission** | **Hazard ratio** | **95% CI for HR** | | **p Value** |
|---|---|---|---|---|
| | | **Lower** | **Upper** | |
| *A: Adjusted Model** | 1.01 | 0.66 | 1.55 | 0.97 |
| *B: Adjusted Model** | 0.84 | 0.46 | 1.54 | 0.57 |
| *C: Adjusted Model** | 0.78 | 0.40 | 1.53 | 0.47 |
| **HAQ <0.5** | **Hazard ratio** | **95% CI for HR** | | **p Value** |
| | | **Lower** | **Upper** | |
| E: Adjusted Model* | 1.13 | 0.78 | 1.63 | 0.51 |
| F: Adjusted Model* | 0.95 | 0.54 | 1.67 | 0.86 |
| G: Adjusted Model* | 1.38 | 0.82 | 2.34 | 0.22 |

**Complete case analysis based on cox-proportional hazard regression analysis. Selection of covariates for adjustment was based on potential confouding variables with uneven distribution and change-in-estimation (e.g. significant change in OR).** Potential counfouding variables tested: sex, age, symptom duration at diagnosis, follow-up duration, DAS at baseline, HAQ at baseline, country, smoking and medication.

**A/E:** association between autoantibody positivity and DAS remission/HAQ<0.5

**B/F:** methotrexate monotherapy stratum

**C/G:** combinationtherapy stratum

*Adjusted for sex, symptom duration at diagnosis, country, smoking, methotrexate treatment strategies and DAS/HAQ at baseline

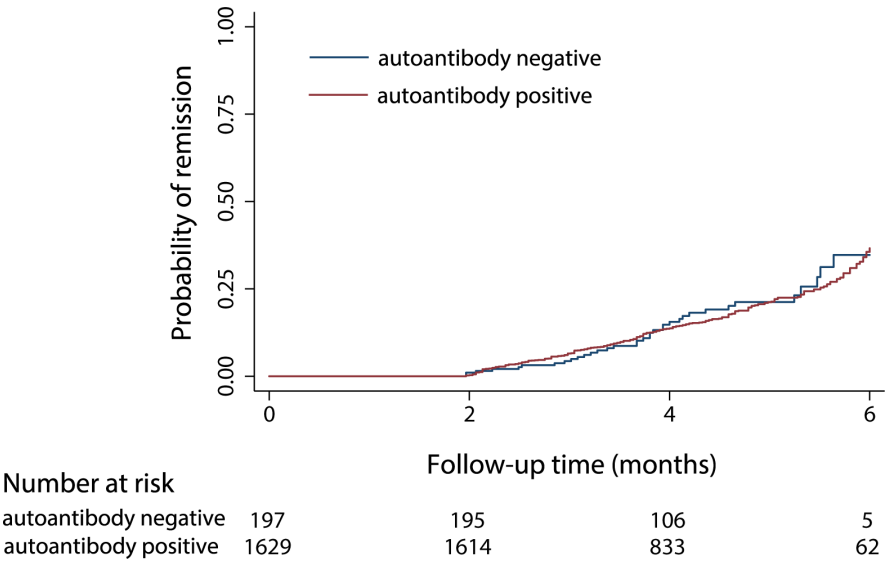


**Figure 2. Probability of achieving remission over time.** Newly diagnosed RA-patients were analysed for probability of achieving remission (DAS < 1.6). Month 0 corresponds to the time of starting initial treatment with methotrexate mono- or combination therapy. Autoantibody-positive patients (ACPA and/or RF) (red line, n=1,629) and autoantibody-negative patients (ACPA and RF negative) (blue line, n=197) are depicted. The depicted figure is based on non-imputed data. RA, rheumatoid arthritis; DAS, disease activity score; ACPA, anti-citrullinated protein antibodies; RF, rheumatoid factor.

Regarding good physical function (HAQ < 0.5), 236 patients had good physical function at baseline and an additional 33% (530/1590) achieved this outcome within 6 months. Percentages were comparable for autoantibody-positive (35%, 491/1413) and -negative (33%, 39/117) patients. Thus, autoantibody positivity did not associate with HAQ<0.5 (HR1.05, 95%CI 0.71;1.57). Complete case sensitivity analysis showed very similar results as the analysis based on imputed data (Table 3). Additional analyses based on LMM revealed no differences between autoantibody-positive and -negative patients regarding DAS (p=0.71), HAQ (p=0.59) or ESR (p=0.27). Sub-analyses of methotrexate treatment strategies revealed no presence of effect modification by medication strategy (p>0.10). In addition, we stratified based on symptom duration and assessed whether symptom duration was an effect modifier, but also did not find such an effect (p=0.22).

Although ACPA and RF often co-occur, RF testing has been used longer and is in some countries still determined more often than ACPA. We therefore performed a sensitivity analysis to evaluate the association between RF-positivity and short-term remission. In line with our previous findings, RF-positivity alone did not associate with short-term DAS remission nor the ability to regain function (Table 4). Additional sensitivity analysis based on patients of European centers only, revealed similar results (Table 5).

**Table 2. Patient characteristics at baseline**

| | ***All RA-patients n=1,826*** | | ***Autoantibody-positive n=1,629*** | | ***Autoantibody-negative n=197*** | | |
|---|---|---|---|---|---|---|---|
| ***Characteristic*** | Values available | Summary statistics | Values available | Summary statistics | Values available | Summary statistics | p-value |
| *ACPA (positivity), n (%)* | 1,121 | 849 (75) | 924 | 849 (92) | 197 | 0 (0) | <0.001 |
| *RF (positivity), n (%)* | 1,810 | 1,554 (85) | 1,613 | 1,554 (96) | 197 | 0 (0) | <0.001 |
| *Gender (female), n (%)* | 1,814 | 1,429 (79) | 1,620 | 1,288 (80) | 194 | 141 (73) | 0.03 |
| *Age at diagnosis (years), mean ±SD* | 1,815 | 48±13 | 1,619 | 47±13 | 197 | 54±16 | <0.001 |
| *Symptom duration (months), median (IQR)* | 1,826 | 15 (6;36) | 1,629 | 18 (7;36) | 197 | 7 (3;17) | <0.001 |
| *Visit count, mean ±SD* | 4,265 | 3.14±1.05 | 3,782 | 3.13±1.02 | 483 | 3.27±1.24 | 0.13 |
| *Follow-up duration (months), mean ±SD* | 1,826 | 4.2±1.2 | 1,629 | 4.2±1.2 | 197 | 4.2±1.1 | 0.98 |
| *Cigarette smoking, n (%)* | 1,602 | | 1,461 | | 141 | | <0.001 |
| *Never* | | 1,353 (85) | | 1,250 (86) | | 103 (73) | |
| *Current* | | 158 (10) | | 140 (10) | | 18 (13) | |
| *Past* | | 91 (6) | | 71 (5) | | 20 (14) | |
| *ESR (mm/hr), median (IQR)* | 1,588 | 51 (29;85) | 1,413 | 55 (31;85) | 175 | 30 (15;48) | <0.001 |
| *CRP (mg/dl), median (IQR)* | 1,498 | 23 (9;49) | 1,324 | 24 (11;52) | 154 | 10 (3;24) | <0.001 |
| *VAS, median (IQR)* | 1,357 | 50 (50;75) | 1,212 | 50 (50;75) | 145 | 50 (35;75) | 0.59 |
| *SJC in 44 joints, median (IQR)* | 1,664 | 5 (2;10) | 1,492 | 5 (2;10) | 172 | 6 (3;11) | 0.12 |
| *RAI, median (IQR)* | 1,661 | 9 (5;16) | 1,489 | 10 (5;16) | 172 | 6 (4;9.5) | 0.13 |
| *DAS, mean ±SD* | 1,078 | 3.8±1.1 | 979 | 3.9±1.0 | 117 | 3.4±1.1 | 0.35 |
| *HAQ, median (IQR)* | 1,505 | 1.0 (0.6;1.6) | 1,384 | 1.0 (0.6;1.6) | 121 | 1.1 (0.5;1.6) | 0.32 |
| ***First-line treatment strategy:*** | | | | | | | |
| *MTX monotherapy, n (%)* | 1,826 | 653 (36) | 1,629 | 549 (34) | 197 | 104 (53) | |
| *MTX & csDMARD & glucocorticoid , n (%)* | 1,826 | 806 (44) | 1,629 | 728 (45) | 197 | 78 (40) | |
| *MTX & synthetic DMARD, n (%)* | 1,826 | 351 (19) | 1,629 | 338 (21) | 197 | 13 (7) | |
| *MTX & biological DMARD, n (%)* | 1,826 | 16 (<1) | 1,629 | 14 (<1) | 197 | 2 (<1) | |

All data are mean and SD or median and interquartile range (IRQ, 25$^{th}$ ; 75$^{th}$ percentile). ACPA, anti-citrullinated protein antibody; RF, rheumatoid factor, ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, visual analogue scale general health patient; SJC, swollen joint count on a 44-joint count; RAI, Ritchie articular index on a 53 joint count; DAS, disease activity scores (ESR) on a 44-joint count; HAQ, Health Assessment Questionnaire; SDAI, Simplified Disease Activity Index; MTX, methotrexate; DMARD, disease modifying anti-rheumatic drugs.

**Table 3. Cox-proportional hazard regression analysis**

| | | 95% CI for HR | | |
|---|---|---|---|---|
| ***DAS Remission*** | **Hazard ratio** | **Lower** | **Upper** | **p Value** |
| *A: Adjusted Model** | 0.89 | 0.57 | 1.38 | 0.61 |
| *B: Crude Model* | 0.96 | 0.67 | 1.35 | 0.79 |
| *C: Adjusted Model*** | 0.75 | 0.41 | 1.37 | 0.35 |
| *D: Crude Model* | 0.88 | 0.56 | 1.38 | 0.57 |
| *E: Adjusted Model*** | 0.76 | 0.37 | 1.54 | 0.45 |
| *F: Crude Model* | 1.26 | 0.72 | 2.21 | 0.42 |

| | | 95% CI for HR | | |
|---|---|---|---|---|
| ***HAQ <0.5*** | **Hazard ratio** | **Lower** | **Upper** | **p Value** |
| *A: Adjusted Model** | 1.05 | 0.71 | 1.57 | 0.80 |
| *B: Crude Model* | 1.65 | 1.19 | 2.28 | <0.01 |
| *C: Adjusted Model*** | 0.79 | 0.44 | 1.42 | 0.43 |
| *D: Crude Model* | 2.02 | 1.26 | 3.25 | <0.01 |
| *E: Adjusted Model*** | 1.28 | 0.74 | 2.22 | 0.38 |
| *F: Crude Model* | 1.65 | 1.03 | 2.66 | 0.04 |

**Selection of covariates for adjustment was based on potential confouding variables with uneven distribution and change-in-estimation (e.g. significant change in OR).** Potential confouding variables tested: sex, age, symptom duration at diagnosis, follow-up duration, DAS at baseline, HAQ at baseline, country, smoking and medication.

**A/B:** association between autoantibody positivity and DAS remission/HAQ<0.5.

**C/D:** methotrexate monotherapy stratum.

**E/F:** combination therapy stratum

*Adjusted for sex, symptom duration at diagnosis, country, smoking, methotrexate treatment strategies and DAS/HAQ at baseline

**Adjusted for sex, symptom duration at diagnosis, country, smoking and DAS/HAQ at baseline

**Table 4. Cox-proportional hazard regression analysis for rheumatoid factor**

| | | 95% CI for HR | | |
|---|---|---|---|---|
| ***DAS Remission*** | **Hazard ratio** | **Lower** | **Upper** | **p Value** |
| *A: Adjusted Model** | 1.05 | 0.70 | 1.58 | 0.81 |
| *B: Crude Model* | 0.96 | 0.70 | 1.32 | 0.82 |
| *C: Adjusted Model*** | 1.05 | 0.55 | 2.00 | 0.89 |
| *D: Crude Model* | 0.83 | 0.55 | 1.25 | 0.37 |
| *E: Adjusted Model*** | 0.95 | 0.50 | 1.79 | 0.87 |
| *F: Crude Model* | 1.31 | 0.79 | 2.15 | 0.29 |

| | | 95% CI for HR | | |
|---|---|---|---|---|
| ***HAQ <0.5*** | **Hazard ratio** | **Lower** | **Upper** | **p Value** |
| *C: Adjusted Model** | 1.14 | 0.82 | 1.60 | 0.44 |
| *D: Crude Model* | 1.39 | 1.06 | 1.83 | 0.02 |
| *C: Adjusted Model*** | 0.86 | 0.50 | 1.48 | 0.59 |
| *D: Crude Model* | 1.90 | 1.25 | 2.90 | <0.01 |
| *E: Adjusted Model*** | 1.31 | 0.85 | 2.02 | 0.22 |
| *F: Crude Model* | 1.26 | 0.87 | 1.82 | 0.23 |

**Selection of covariates for adjustment was based on potential confouding variables with uneven distribution and change-in-estimation (e.g. significant change in OR).** Potential counfouding variables tested: sex, age, symptom duration at diagnosis, follow-up duration, DAS at baseline, HAQ at baseline, country, smoking and medication.

**A/B:** association between rheumatoid factor positivity and DAS remission/HAQ<0.5.

**C/D:** methotrexate monotherapy stratum.

**E/F:** combinationt herapy stratum

*Adjusted for sex, symptom duration at diagnosis, country, smoking, methotrexate treatment strategies and DAS/HAQ at baseline

**Adjusted for sex, symptom duration at diagnosis, country, smoking and DAS/HAQ at baseline

**Table 5. Cox-proportional hazard regression analysis: sensitivity analysis based on European countries**

| | | 95% CI for HR | | |
|---|---|---|---|---|
| **DAS Remission** | **Hazard ratio** | **Lower** | **Upper** | **p Value** |
| A: Adjusted Model* | 1.09 | 0.53 | 2.25 | 0.81 |

| | | 95% CI for HR | | |
|---|---|---|---|---|
| **HAQ <0.5** | **Hazard ratio** | **Lower** | **Upper** | **p Value** |
| B: Adjusted Model* | 1.44 | 0.63 | 3.26 | 0.38 |

**A/B:** association between autoantibody positivity and DAS remission/HAQ<0.5

*Adjusted for sex, symptom duration at diagnosis, country, smoking, methotrexate treatment strategies and DAS/HAQ at baseline

**Discussion**

This study reveals that autoantibody status does not associate with short-term DAS remission and good physical function in newly diagnosed RA-patients receiving methotrexate in a real-world setting. Autoantibody-positive RA-patients present with more severe disease (higher baseline disease activity and increased levels of acute-phase reactants) compared to autoantibody-negative patients. We found that 17% of newly diagnosed RA-patients achieved DAS remission, independent of autoantibody status. In accordance, the percentage of patients achieving a good physical function was independent of the autoantibody status. Together, these findings indicate that methotrexate is effective as initial treatment strategy regardless of autoantibody status.

Previous intervention studies suggested that the presence of ACPA in early RA-patients may be associated with a better response to methotrexate, with higher levels indicating an improved response (10, 16, 17). Moreover, ACPA-positive undifferentiated arthritis patients receiving methotrexate were found to be less likely to progress to RA[10]. A possible explanation for the discrepancies between these previous findings and our data may be related to differences in study populations, with the previous randomized controlled trials including only a selection of RA patients with high baseline disease activity. A strength of our study is that it is based on a large international real-world cohort that best mimics routine clinical practice. Our findings are in line with the results of a previous cohort study reporting equal treatment responses independent of autoantibody status in patients receiving various different initial DMARDs (11).

This study has several limitations. As the METEOR database is an observational database gathered during daily clinical practice most variables contain missing values. Data regarding ACPA status were more often missing compared to RF status, which may be explained by the time period of inclusion. To account for missing data we applied multiple imputations (14). Reciprocal analysis based on non-imputed data revealed no differences between autoantibody-positive and -negative patients. Another concern with real-world data is the variation in clinical scoring and a higher noise to signal ratio. It is to be expected that differences in DAS or other outcome measurements exist between different centres. However, it is less likely that measurements within one centre differ between autoantibody-positive and -negative individuals. Due to the observational nature of the database, we had to take several precautions to limit the influence of potential bias - multiple imputation, testing for effect modification and adjusting for potential confounders – but it is always possible that residual confounding remains.

A striking feature of our dataset was the high percentage of autoantibody-positive RA-patients (up to 89%), which is higher than in European cohorts but consistent with other international cohorts (11, 18). Some countries, particularly those with limited financial resources, included only a very limited number of autoantibody-negative patients, perhaps

because these patients are less frequently referred to rheumatologists in those settings. Never smokers were more prevalent among autoantibody positive RA patients. This is remarkable, since smoking is associated with ACPA-positive RA(19).This can be explained by the large proportion of Indian patients included in the analyses. The majority of these patients were autoantibody positive. After exclusion of Indian patients, 54% (350/651) of autoantibody positive patients were never smokers compared to 86% (1,250/1,461) found in the whole dataset. It has been previously published that especially the number of women smoking in India is low (approximately 3%)(20). Furthermore, the use of smokeless tobacco products is relatively high in India, which was not captured in this database (21).

In our dataset, information regarding autoantibody status was limited to ACPA and RF serology. We cannot exclude that some of the autoantibody-negative patients express other RA-associated serological markers such as anti-carbamylated protein antibodies or anti-acetylated protein antibodies (22). However, these novel autoantibodies are expected to be present in only a limited proportion of the ACPA- and RF-negative population.

In conclusion, we found that autoantibody status was not associated with early remission in newly diagnosed RA-patients receiving methotrexate in real-world clinical practice. The results from our study therefore do not support the hypothesis that treatment should be tailored to “autoantibody status” when it comes to initiating methotrexate therapy as first-line anti-rheumatic treatment. Rather, our results indicate that that methotrexate is effective as initial treatment strategy regardless of autoantibody status.

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