Association between autoantibody level and disease activity in rheumatoid arthritis is dependent on baseline inflammation

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Abstract

Objective

It is still controversial whether autoantibody (AAb) serum levels have a value for response monitoring in rheumatoid arthritis (RA). Therefore, we retrospectively investigated a real-life outpatient RA cohort to determine which factors are associated with change in serum AAb levels and RA disease activity. The primary goal of the study was to determine predictors for changes in DAS28 and autoantibodies over time and identify traits of non-rituximab treated patients, which would define strong association of disease activity with changes in AAb-levels.

Methods

Seventy-eight patients with seropositive RA were monitored for DAS28, CRP, ESR, anti-cyclic citrullinated peptides (CCP), anti-mutated citrullinated vimentin (MCV), and rheumatoid factor (RF). Using linear mixed regression modelling, factors influencing DAS28 and serum AAb were determined. Patients showing above (good correlators) and below (bad correlators) average correlation of serum AAb with DAS28 were further characterised.

Results

In non-rituximab treated patients (88.5%), associations of changes in AAb and DAS28 were strengthened with more morning stiffness (p=0.002), DMARD use (p=0.02), tender joints (p=0.01), swollen joints (p<0.01), higher ESR (p<0.01) and VAS (p<0.001) at baseline. Decrease of anti-CCP was also predicted by longer disease duration (-4.4 U/ml per year disease duration, p=0.048) and/or no erosions (-2.0 U/ml/month, p<0.01) at baseline, whereas erosive disease predicted an increase (+1.4 U/ml/month, p=0.015) in anti-CCP. Conversely, patients with erosive disease showed a trend to decrease RF (-1.9 U/ml/month, p=0.06).

Conclusion

In non-rituximab treated RA patients, the association between disease activity and change in autoantibody levels is not static, but strengthens with increase in signs of inflammation (ESR, VAS, swollen joints, tender joints, morning stiffness) at baseline. Therefore, studies of changes in AAb need to consider baseline inflammation as confounder.

Key words

rheumatoid arthritis autoantibodies, rheumatoid factor, anti-citrullinated protein antibodies, biomarker, disease activity, DAS28, anti-CCP antibodies, anti-MCV antibodies

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Key message: In non-B cell depleted RA patients, association of autoantibody level and disease activity is not static, but dependent on baseline inflammatory state.

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Introduction

It is still controversial whether levels of autoantibodies are valid for monitoring disease activity in rheumatoid arthritis (RA). On the other hand, it is accepted that autoantibodies, particularly anti-citrullinated protein antibodies (ACPA) have prognostic value in terms of predicting radiologic progression (1) and development of RA from undifferentiated arthritis (2, 3), respectively. The predictive value of anti-CCP antibodies is especially high in RF negative individuals (4). However, combined measurement of serum IgM-RF or IgA-RF, respectively, and anti-CCP antibody levels substantially increases predictive value for radiological outcome (5-7) and long term prognosis at 10 year follow up was best predicted by a combined measure of anti-CCP and CRP at baseline (8, 9). ACPA and RF, respectively, seem to be independent predictors of radiologic outcome in RA (9-11), pointing to a different pathophysiological role of these autoantibody types.

However, there are also contrary opinions and one study states that baseline determination of RF and ACPA does not predict radiologic outcome, but presence of ACPA during the first three years of follow up is associated with outcome at five years or greater (12).

In terms of monitoring disease activity, one retrospective study of 17 selected patients suggested that fluctuations of anti-CCP antibodies over time were associated with changes in inflammatory parameters and thus concluded that anti-CCP might be a valid parameter to monitor disease activity (13). However, several other groups reported that the presence of ACPA at first diagnosis predicts worse outcome, but a change in ACPA levels does not reflect changes in disease activity (14-16). Another study found some association of ACPA and HAQ score values, however, again no association between DAS28 and ACPA levels (17). Furthermore, a decrease of serum IgM-RF and less so anti-CCP occurred in RA patients treated with anti-TNF, however, DAS28 after 24 weeks could again not be predicted by these changes in autoantibody levels (18).

Since anti-MCV antibody levels at baseline predict disease activity at 2 years, anti-MCV antibodies might be superior as compared to anti-CCP antibodies in terms of disease monitoring (19, 20). Indeed, a longitudinal follow-up of 42 RA patients with moderate to severe disease activity showed that serum levels of anti-MCV antibodies associate with changes in DAS28 (16). A longitudinal study specifically designed to investigate anti-MCV serum level as parameter to monitor disease activity, showed some, but not meaningful associations of changes in anti-MCV with changes in DAS28 (21).

In summary, ACPA and RF status at baseline determines prognosis of patients with RA. However, if changes in autoantibody levels help to monitor disease activity in RA is still controversial, with some reports showing such association, but most arguing against it. To clarify this issue, we conducted this retrospective, longitudinal study and tried to define determinants of autoantibody level and patient traits that determine the strength of association between ACPA and/or RF changes and disease activity.

Methods

Patient characteristics and study design

Seventy-eight patients diagnosed with RA according to the ACR classification criteria (22) and treated at the clinic for rheumatology and clinical immunology were included if they were positive for anti-CCP, anti-MCV, and/or RF, respectively. Triple seronegative patients (anti-MCV, anti-CCP and RF negative), pregnant patients, and patients under 18 years of age were excluded. The data from selected patients was retrospectively extracted from the patient's medical documentation starting from the first visit in the year 2011–2012 and at regular visits thereafter at 3, 6, 12, 18, and 24 month. The study was approved by the local ethics committee at the university hospital Regensburg, Germany (ref. no. 15-101-0029). The primary goal of the study was to determine predictors for changes in DAS28 and autoantibodies over time and identify traits of non-rituximab treated patients, which would define strong association of disease activity with changes in AAb-levels.

Measurement of autoantibodies

All blood parameters were determined by standard procedures in the central clinical laboratory of the Bad Abbach Medical Center. In brief, anti-MCV were determined by ELISA according to manufacturers' protocol (Orgentec Inc.). Values were regarded as normal if determined to be <20 U/ml. The upper limit of the test was 1000 U/ml. Anti-CCP antibodies were detected by Chemiluminescence using a COBAS™ e411 System (Roche Inc.) following standard procedures. Values <17 U/ml were regarded as normal, the upper limit of the test was 500 U/ml. RF was deteceted by turbidimetry using COBAS INTEGRA™ (Roche Inc.). Values <14 U/ml were regarded as normal.

Statistical analysis

Mixed linear regression modelling was used to investigate parameters influencing DAS28 and autoantibody levels over time. Time was treated as a linear variable in the model, and an interaction term (variable of interest (VARi) x time) was included. The general template of the model was as follows: DAS28 = b0 + b1 x VARi + b2 x time + b3 x time*VARi. The following variables were considered as covariates in the analysis: categorical: sex, erosive disease, morning stiffness, RF positive, NSAID use, DMARD use, continuous: age [yrs], duration [yrs], body weight [kg], leucocytes [/µl], predinsolon [mg/d], CRP [mg/l], ESR [mm/h], VAS [mm], swollen joints, tender joints. Adjustment for intra-individual effects was done by including a random intercept in the linear regression model. To determine patient traits best associated with a high association of autoantibody changes and DAS28, we divided patients in two groups (good correlators: 50% smallest residuals and bad correlators: 50% highest residuals) according to their individual residuals in the model. Patient traits of these two groups were compared by Fisher's exact test for count data or Welch's test for metric variables. *p*-values below 0.05 were regarded as significant. Calculations were performed using the statistical software R, 3.4.1 (The R Foundation for Statistical Computing).

Table I. Patients characteristics at study entry.

Patient characteristics at baseline	value
Age [yrs] mean (SD)	62.31 (10.4)
Sex % (n)	m: 44.9 (35)
	f: 55.1 (43)
Height [m] mean (SD)	1.7 (0.9)
Weight [kg] mean (IQR)	78.8 (66.0–89.8)
ACPA positivity %	98.7
RF positivity %	92.1
Erosive disease %	50
Disease duration [yrs] mean (IQR)	8.7 (1.1-12.3)
RF [U/ml] mean (IQR)	149 (26.4-149.0)
Anti-CCP [U/ml] % (n)	<100: 24.2 (16)
	101-500: 33.3 (22)
	>500: 42.2 (28)
	NA: 12
Anti-MCV [U/ml] % (n)	<40: 34.8 (23)
	40-250: 30.3 (20)
	>250: 34.8 (23)
	NA: 12
CRP mean (IQR)	25.5 (3.68-25.48)
Leucocytes [/µl] mean (SD)	9.2 (2.8)
ESR [mm/h] mean (IQR)	26.0 (7.0-42.3)
VAS [mm] mean (IQR)	52.7 (60-80)
Tender joints [n] mean (SD)	6.25 (6.75)
Swollen joints [n] mean (SD)	3.6 (6.7)
DAS28 mean (SD)	4.2 (1.7)
Follow-up radiographs available %(n)	43.6 (34)
NSAID use % (n)	43.6 (34)
DMARD use % (n)	52.6 (41)
Biologicals % (n)	33.3 (26)
- Rituximab % (n)	11.5 (9)
Prednisolone user % (n)	79.5 (62)
Prednisolone dose [mg/d] mean (IQR)	8.0 (2.1-10)
Prednisolone dose users [mg/d] mean (IQR)	10.1 (5.0-10.0)

CCP: cyclic citrullinated peptide; CRP: C-reactive protein; DAS28: disease activity score 28 joints; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; IQR: interquartile range; MCV: mutated citrullinated vimentin; NSAID: non-steroidal anti-inflammatory drug; RF: rheumatoid factor; SD: standard deviation; yrs: years; VAS: visual analogue scale.

Results

Baseline patient characteristics and general predictors of DAS28 reduction

For baseline patient characteristics see Table I. To further characterise our cohort, we determined parameters that associated with overall DAS28 changes. DAS28 decreased during the observation period (Fig. 1A) with an average reduction of DAS28 of -0.03 per month (p<0.00001), which did not differ between female and male patients. Furthermore, average DAS28 reduction was not associated with duration of disease at baseline (p=0.182), or age at baseline (p=0.076).

Patients with baseline bone erosions showed a statistical trend (p=0.068) towards less DAS28 reduction over time (-0.0219/month) as compared to non-erosive disease (-0.0456/month). This finding remained after adjusting for age, duration of disease, sex, and body weight. Irrespective of ACPA status (anti-MCV and/or anti-CCP positive), DAS28 reduction was almost not evident in RF negative individuals (-0.009/month) as compared to RF positive individuals, who on average showed a DAS28 reduction of -0.034/month (p<0.00001). Anti-CCP levels at baseline were associated with 2.13 times greater reduction of DAS28 per month as compared to average DAS28 reduction achieved over time (estimate contribution of anti-CCP: -0.01/month *vs.* time: -0.0047/month, p<0.01). Levels of anti-MCV were also directly associated with greater reduction of DAS28 over time, however, the contribution of this effect as compared to anti-CCP was less pronounced (p=0.04).

Predictors of anti-CCP levels

Overall, anti-CCP levels showed no change over time (p=0.833, Fig. 1B).

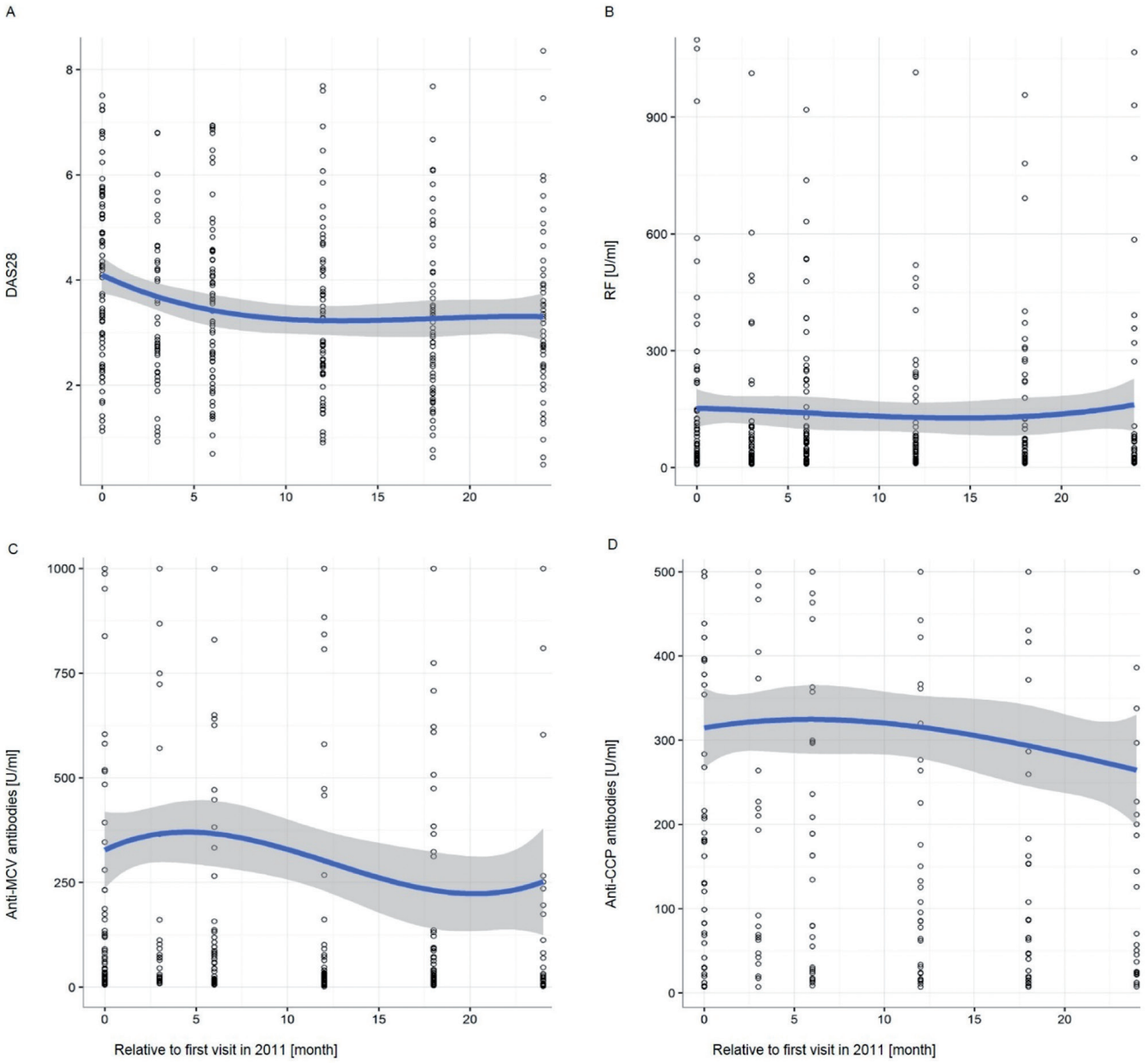

Fig. 1. Levels of DAS28 and autoantibodies over time. In each panel the mean level over time is shown (blue line) with 95% confidence interval (shaded grey). Each dot represents one measurement for one individual patient. Time in month was calculated relative to first visit in 2011. **A**) DAS28, **B**) anti-CCP (U/ml), **C**) anti-MCV (U/ml), **D**) rheumatoid factor (RF, U/ml).

Also, age at baseline (p=0.7) or sex (f: p=0.18; m: p=0.21) showed no association with changes in anti-CCP levels. However, patients with longer disease duration at baseline showed greater reduction of anti-CCP over time (-4.4 U/ml per year disease duration, p=0.048). Interestingly, erosions at baseline predicted an increase in anti-CCP (+1.4 U/ml/month, p=0.015), whereas no erosions at baseline related to a decrease (-1.9 U/ml/month, p=0.003). After adjusting for age, sex, body weight, and duration of disease, this influence remained stable (with initial erosion: +1.6 U/ml/month, p=0.008; no initial erosion: -2.0 U/ml/month, p=0.0017). Looking for certain treatments potentially influencing anti-CCP, we found that use of rituximab was clearly associated with a decrease in anti-CCP (-2.15 U/ml/month, p=0.0026). In contrast, rituximab non-users show a statistical trend towards an increase of anti-CCP over time (+0.84 U/ml/month, p=0.097). Anti-MCV positivity predicts a slightly higher initial anti-CCP value, in average 8.28 U/ml (p=0.003). However, the decrease in anti-CCP over time was not dependent on anti-MCV or RF status.

Predictors of anti-MCV levels

Similar to anti-CCP, anti-MCV levels did not change over time (p=0.816, Fig. 1C) in overall analysis. Age at baseline (p=0.89) and sex (m: p=0.69, f: p=0.52) did also not influence anti-MCV levels. In contrast to anti-CCP, disease duration at baseline had no effect on anti-MCV over time (p=0.55). Like for anti-CCP, anti-MCV levels numerically de-

Table II. Patient traits determining association of changes in DAS28 with fluctuations in anti-MCV serum titre.

T0 (first visit)	mean good correlator (n=24)	mean bad correlator (n=26)	OR	5%	95%	*p*-value
Sex			1.17	0.33	4.14	0.78
Erosive disease			0.72	0.20	2.53	0.77
Morning stiffness			0.11	0.01	0.53	**0.002**
RF positive			0.91	0.06	13.67	1
NSAID use			0.71	0.20	2.50	0.58
DMARD use			4.37	1.18	17.88	**0.02**
			t-value	5%	95%	*p*-value
Age [yrs]	63.9	58.5	1.91	-0.2	11.1	0.06
Duration [yrs]	6.8	9.1	-0.83	-7.9	3.2	0.40
Body weight [kg]	79.2	77.3	0.39	-7.7	11.4	0.69
Leucocytes [/µl]	8.3	8.7	-0.38	-2.0	1.3	0.70
Predinsolon [mg/d]	5.6	5.8	-0.10	-3.6	3.3	0.91
CRP [mg/l]	20.4	10.0	1.5	-3.0	23.7	0.12
ESR [mm/h]	28.1	13.4	2.48	2.7	26.5	**0.01**
VAS [mm]	61.2	32.6	3.92	13.8	43.4	**0.0003**
Swollen joints [n]	5.2	1.8	2.56	0.7	6.0	**0.01**
Tender joints [n]	6.4	3.2	2.18	0.2	6.2	**0.03**

CRP: C-reactive protein; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; MCV: mutated citrullinated vimentin; NSAID: non-steroidal anti-inflammatory drug; RF: rheumatoid factor; VAS: visual analogue scale.

Table III. Patient traits determining association of changes in DAS28 with fluctuations in anti-CCP serum titre.

T0 (first visit)	mean good correlator (n=24)	mean bad correlator (n=26)	OR	5%	95%	*p*-value
Sex			0.85	0.24	2.99	1
Erosive disease			1	0.28	3.48	1
morning stiffness			0.11	0.01	0.53	**0.002**
RF positive			0.91	0.06	13.67	1
NSAID use			0.71	0.20	2.50	0.58
DMARD use			4.37	1.18	17.88	**0.02**
			t-value	5%	95%	*p*-value
Age [yrs]	63.5	59.0	1.54	-1.3	10.2	0.12
Duration [yrs]	6.3	9.6	-1.18	-8.8	2.2	0.24
Body weight [kg]	77.9	78.8	-0.19	-10.5	8.7	0.84
Leucocytes [/µl]	8.6	8.4	-0.38	-1.4	1.9	0.77
Predinsolon [mg/d]	6.2	5.2	0.58	-2.4	4.4	0.56
CRP [mg/l]	20.6	9.8	1.6	-2.6	24.1	0.11
ESR [mm/h]	28.9	12.5	2.82	4.7	28.1	**0.007**
VAS [mm]	62.0	31.7	4.25	15.9	44.7	**0.0001**
Swollen joints [n]	5.2	1.8	2.56	0.7	6.0	**0.01**
Tender joints [n]	7.1	2.5	3.34	1.8	7.4	**0.002**

CCP: cyclic citrullinated peptide; CRP: C-reactive protein; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; NSAID: non-steroidal anti-inflammatory drug; RF: rheumatoid factor; VAS: visual analogue scale.

crease in non-erosive patients (-2.15 U/ml/month, p=0.64) and increase (1.38 U/ml/month, p=0.33) in patients with baseline erosions. However, this observation did not reach significance. RF status also did not show a statistically significant contribution to fluctuations of anti-MCV over time. Also similar to anti-CCP, use of rituximab predicted a decrease in anti-MCV (-3.5 U/ml/month, p=0.038), whereas non-rituximab treatment showed no influence on anti-MCV levels over time (+1.25 U/ml/month, p=0.30).

Predictors of RF levels during treatment

Similar to anti-CCP and anti-MCV, RF did not change over time during standard treatment (p=0.71, Fig. 1D), and showed no significant association to age at baseline (p=0.70), sex (m: p=0.46, f: p=0.92) or duration of disease (p=0.44). However, in contrast to anti-CCP and anti-MCV, erosions at baseline showed a statistical trend to predict a decrease in RF levels over time (-1.9 U/ml/month, p=0.066), whereas no erosions at baseline had no significant influence on RF levels (+1.5 U/ml/month, p=0.17). Even after adjusting for age, sex, duration of disease, and body weight, the effect of erosion status at baseline on RF titres over time remained stable. Like anti-CCP and anti-MCV, rituximab use was associated with a decrease in RF over time (-2.9 U/ml/month, p=0.02), whereas non-rituximab use did not change RF levels (p=0.35).

Determinants for the association of autoantibody levels with disease activity

To determine patient traits that are associated with a better correlation of autoantibody fluctuation with DAS28, we excluded rituximab users from analysis, since rituximab has strongest influence on autoantibody levels in itself, as shown by the analysis above. The rest of the patients were divided into two groups, good correlators and bad correlators, by ranking patients according to residuals in the model. The two groups were than compared subsequently. As shown in Tables I-III the pattern of traits which were different between good correlators and bad correlators are similar between autoantibody classes. Fluctuations in autoantibodies associate best with DAS28 in patients that had morning stiffness, were on DMARDs, and had higher ESR and VAS as well as more tender joints and swollen joints at the first visit (Tables I-III). Overall, it seems that autoantibody fluctuations in patients with high disease activity at baseline correlate best with changes in DAS28.

Discussion

We conducted this retrospective RA cohort study to determine predictors for changes in DAS28 and autoantibodies

Table IV. patient traits determining association of changes in DAS28 with fluctuations in RF serum titre.

T0 (first visit)	mean good correlator (n=24)	mean bad correlator (n=26)	OR	5%	95%	*p*-value
Sex			0.85	0.24	2.99	1
Erosive disease			0.72	0.20	2.53	0.77
Morning stiffness			0.11	0.01	0.53	**0.002**
RF positive			0.91	0.06	13.67	1
NSAID use			0.98	0.28	3.45	1
DMARD use			6.38	1.65	28.19	**0.003**
			t-value	5%	95%	*p*-value
Age [yrs]	63.7	58.8	1.71	-0.8	10.6	0.09
Duration [yrs]	6.7	9.2	-0.88	-8.0	3.1	0.38
Body weight [kg]	78.0	78.6	-0.12	-10.2	9.0	0.9
Leucocytes [/µl]	8.4	8.6	-0.27	-1.9	1.4	0.78
Predinsolon [mg/d]	4.5	7.0	-1.4	-6.1	0.9	0.14
CRP [mg/l]	20.1	9.9	1.5	-3.0	23.5	0.12
ESR [mm/h]	27.7	13.1	2.48	2.7	26.4	**0.01**
VAS [mm]	58.7	35.2	3.06	8.0	39.0	**0.003**
Swollen joints [n]	5.2	1.8	2.49	0.6	6.0	**0.01**
Tender joints [n]	6.4	3.2	2.18	0.2	6.2	**0.03**

CRP: C-reactive protein; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; NSAID: non-steroidal anti-inflammatory drug; RF: rheumatoid factor; VAS: visual analogue scale.

over time and to identify traits that characterise patients in whom autoantibody levels can be used to monitor treatment response in a real life setting.

Changes of autoantibody levels were primarily associated with rituximab treatment. This finding confirms known literature (23) and is highly expected, since autoantibodies are produced by the B cell compartment, which is targeted by rituximab. However, rituximab does not target long-living plasma cells in the bone marrow, since these cells do not express CD20 anymore (24). Therefore, this result indicates that not all autoantibody producing cells are long-living plasma cells in RA patients. After all, the effect of rituximab on autoantibody production might be primarily mediated by "blocking the road" towards newly generated antibody secreting cells, as suggested before (24). Although the reduction in autoantibodies is markedly pronounced in rituximab treated patients, such decrease is also observed with other biologic agents, like tocilizumab and anti-TNF (25). However, together with our findings, results of such studies might also be interpreted as resulting from selection bias, since patients switched to biologics are usually patients with higher disease activity who, according to our results, show the greatest drop in autoantibody levels irrespective of used treatment regimen.

Patients with erosive disease show an increase in ACPA and a tendency to decrease RF over time. One hypothesis from this observation would be that erosions lead to a microenvironment pushing ACPA production. It is known that ACPA directly contribute to formation of erosions by interacting with osteoclasts (26), however, it has not been observed that erosions lead to increased ACPA formation. Erosions are presumably areas of high inflammatory activity with activated fibroblasts producing proinflammatory cytokines contributing to a microenvironment suitable for activation of B cells and even germinal center formation (27), further contributing to autoantibody production (28-30). In addition, peptidyl arginine deiminase (PAD), regulating the citrullination of proteins, are more expressed and active in these areas, since they are upregulated and activated by local inflammatory processes (31). Therefore, erosions might be indeed a reactor for ACPA, however, this is a hypothesis to be tested in further studies.

RFs role in forming erosions or *vice versa* is less clear. Our results indicate that patients who had erosions at baseline showed a trend to decrease RF over time. This might reflect that contrary to ACPA production, production of RF is unrelated to local processes. Indeed, it has been suggested, that IgM-RF is produced by freshly activated B cells in the periphery (32) and that the contribution of IgM-RF to pathophysiology is primarily mediated by forming immune complexes locally in the joint, binding to, *e.g.* IgG-ACPA that are bound to a local target, followed by activation of the complement system (32, 33).

The primary goal of our study was to determine traits that would identify patients that show high association of disease activity and change in autoantibody levels. Overall, we found, that fluctuation of autoantibody levels associates better with disease activity in patients that show higher grades of morning stiffness, are on DMARDs, and have higher ESR and VAS as well as more tender and swollen joints at the first visit. Again, one could argue that these traits reflect higher disease activity and as shown and discussed above, these patients also show greater reduction of autoantibody levels and better treatment response, although prognosis is worse (1, 4-9, 34). Therefore, it is not surprising, that traits of patients reflecting high inflammatory load identify patients with the best association of changes in disease activity and changes in autoantibody levels.

As additional findings, we observed, that non-erosive patients and patients with higher autoantibody levels have the greatest reduction in DAS28 over time under real life treatment conditions and it is known, that patients with higher autoantibody levels have more active disease (3, 35). Taken together, this suggests that treatment in general is more effective in patients with higher autoantibody levels. Indeed, it has been discussed that ACPA positivity is a good marker for response to rituximab treatment (36, 37). However, it has also been realised that other biologic agents, *e.g.* anti-IL-6 strategies also seem to work better in patients with positive autoantibody status and higher disease activity (38). Therefore, it seems to be

a general principle that signs of high inflammatory activity, including high levels of autoantibodies, predict better response to anti-inflammatory therapy. This may be simply due to the fact that response characteristics are not linear and greatest reduction can be conveyed in a highly active system as opposed to a system that is almost at its steady state. On the other hand, non-erosive patients tend to have milder disease overall (39) and therefore might respond better to standard care overall.

A limitation of our study is the retrospective nature of the analysis and the heterogeneity of our study population, however, the latter best reflects a realistic outpatient setting. Another limitation is that this was only a monocentric study and therefore limits generalisability. Smoking habit was not monitored systematically in the study population, which might be a limitation of the study, since smoking is discussed at least as a modulator of initial autoantibody formation (40, 41). Although, possible changes of autoantibody levels over time, which might be induced by cigarette smoking have not been studied systematically to our knowledge.

We show that changes of autoantibody levels, regardless of class and treatment, are associated with disease activity in highly active patients, whereas patients with low inflammatory activity do not show this association. Therefore, part of the controversies regarding changes of autoantibodies over time might result from mixing patients with low and high disease activity in the analysis (14-18, 21). In conclusion, studies investigating changes of autoantibody levels in RA should always consider markers of inflammatory activity, like tender and swollen joints, ESR, pain VAS and morning stiffness as confounders when interpreting results.

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