Changes in anti-cyclic citrullinated peptide antibodies and rheumatoid factor isotype serum levels in patients with rheumatoid arthritis following treatment with different biological drugs

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Abstract

Objective

Anti-cyclic citrullinated peptide antibodies (anti-CCP) are a serological marker of rheumatoid arthritis (RA), and also have a prognostic value for more aggressive disease. Whether anti-CCP levels may change during treatment according to clinical response is matter of debate. Likewise, it is unknown whether different biological drugs have peculiar effects on anti-CCP levels. This study aimed to investigate changes in anti-CCP serum levels in RA patients on biological drugs with different mechanism of action.

Methods

We studied 71 patients with active RA tested positive for anti-CCP who started a first biological drug (54 anti-TNF-α drug, 9 rituximab, 8 tocilizumab). In 14 patients stopping anti-TNF-α treatment for ineffectiveness, rituximab was started. Anti-CCP and rheumatoid factor (RF) isotypes (IgM, IgA, IgG) levels were measured at entry, 12 months and again at 12 months after swapping to rituximab.

Results

After 1 year of therapy of the first biological drug, patients taking anti-TNF-α drugs showed a significant reduction of the anti-CCP levels (p=0.002), and all RF isotypes (p=0.003). Also patients treated with rituximab or tolicizumab had a significant decrease in anti-CCP (p=0.01) and RF isotype levels (p=0.01). Anti-CCP levels did not correlated with DAS28 over time. In patients switching to rituximab after failure of TNF-α blockers, anti-CCP levels did not change at 12 months (p=0.06), despite of the reduction of DAS28 (p=0.02) and RFs levels (p=0.02).

Conclusion

Our study showed that anti-CCP levels may change during RA course, regardless of the biological drug used and the clinical response.

Key words

anti-CCP, rheumatoid factor IgA, rheumatoid factor IgG, rheumatoid factor IgM, rituximab, DAS28

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Introduction

Anti-cyclic citrullinated peptide antibodies (anti-CCP) are considered highly specific for rheumatoid arthritis (RA) and are associated to more aggressive RA (1-6). It is conceivable that anti-CCP synthesis may vary depending on the disease activity and changes in anti-CCP serum levels may reflect the clinical response to the treatment. Furthermore, being biological drugs targeting a specific step of RA pathogenic pathways, it may presume that the impact on antibody synthesis might be different depending on the mechanism of action of the blocking agent.

The availability of a biomarker, meant as biological measure with the sensitivity to change according to the disease activity, could aid the physicians in assessing the clinical response and making their therapeutic decisions, and anti-CCP antibodies might be a promising candidate. Some studies have explored this hypothesis but the results are not univocal, as changes in anti-CCP levels have been found or not correlating with the clinical response to anti-TNF-α drugs or rituximab (7). The aim of our study was to investigate the changes in anti-CCP serum levels in RA patients treated with rituximab after failure of a first anti-TNF-α drug, and the correlation of anti-CCP with clinical response.

Patients and methods

Patients

Patients with RA were retrospectively selected from a local longitudinal database. The local ethics committee (Azienda Policlinico, Bari) approved the register and prior written informed consent to take part was obtained from all patients in compliance with the Helsinki Declaration. RA was diagnosed according to the 1987 ACR revised criteria (8) and patients having active disease began a treatment with a biological drug after inadequate response to one or more disease modifying drugs (DMARDs).

To the purpose of this analysis, we enrolled only RA patients who tested positive anti-CCP, having available serum samples and starting a first ever TNF-α inhibitor (adalimumab, certolizumab, etanercept, infliximab) or a non-TNF-α inhibitor (rituximab, tocilizumab) prescibed by the rheumatologist on its own decision. Patients switching to rituximab, after failure of the first anti-TNF-α drug, were also gathered. All drugs were given at the doses recommended in the marketing registrations and rituximab was administrated every 6 months. As clinical outcome the achievement of a "good" EULAR (European League against Rheumatisms) clinical response, defined as a DAS28 change from baseline ≥1.2, was considered.

Anti-cyclic citrullinated peptide antibodies and rheumatoid factor assay

Serum samples from RA patients were frozen at -30°C and anti-CCP and RF assays were run at the same time.

• *Autoantibody measurement*

RF isotypes were measured by the random access fully automated ImmunoCAP 250 instrument (Phadia, Italy), using newly developed fluorescence enzyme immunoassays (EliA RF IgM, RF IgA, RF IgG; Pharmacia Diagnostics, Freiburg, Germany). Anti-CCP autoantibodies were determined by a fully automated CLIA processing system Zenith RA (Menarini, Italy), using a commercial anti-CCP chemiluminescence immunoassay (Zenith RA CCP IgG; Technogenetics, Lodi, Italy).

Statistical analysis

Differences in means for normally distributed continuous variables were compared by the analysis of variance (ANOVA), and the Kruskal-Wallis test was used to compare not normally-distributed continuous variables. Differences in the distribution of frequencies were assessed by chi-squared test. Within-group comparisons between baseline and each follow-up time intervals were evaluated by *t*-test. Correlations were performed by Pearson's analysis for continuous variables. Multiple regression analysis by stepwise forward selection was performed to evaluate baseline predictors of "good" EULAR response. All statistical analysis was made using the SPSS (v. 22) statistical software, and a p-value <0.05 was considered statistically significant.

Results

Baseline characteristics

We selected 71 patients with RA tested positive for anti-CCP who commenced a first biological drug. Fifty-four patients started an anti-TNF-α drug, while 9 patients began RTX and 8 TCZ. Patients were subdivided into two groups according to the type of biological drug, anti-TNF-α drug or non-anti-TNF-α drug (Table I). No significant difference was found between the two groups in terms of age, duration of disease, gender, DAS28, and autoantibody levels.

Changes in antibody levels after the first biological drug

After 1 year of therapy of the first biological drug, the anti-CCP levels dropped from 240 309 at baseline to 122±110 at 12 months (p=0.0001), the IgA-RF levels from 60±60 to 45±49 (p=0.01), the IgM-RF levels from 68±72 to 43±56 (p=0.0001), and the IgG-RF levels from 36±40 to 26±32 (p=0.001). Table II shows anti-CCP and RF levels in patients taking anti-TNF-α or non-anti-TNF-α drugs. A significant decrease in their levels occurred after 1 year of treatment.

Clinical response and correlations with autoantibodies

DAS28 was significantly reduced following 1 year of treatment (baseline 4.4±1.3, 1 year 3.4±1.6, p=0.001) with the first biological drug. At baseline, the anti-CCP levels did not correlate with DAS28, nor did with RF isotypes. Pooling baseline and 1 year values together, DAS28 strongly correlated with IgM-RF or IgG-RF, but did not with the anti-CCP levels (Fig. 1). Crude multivariate logistic regression analysis showed that IgA-RF and duration of disease were associated with the "good" EULAR response (Table III). However, after adjustment, baseline IgA-RF was the only predictor of "good" EULAR response".

Changes in antibody levels and DAS28 after the second biological drug

In 14 patients, not responding to TNF-α blockers and starting RTX, DAS28 was 4.19±1.2 at onset, then increased to 4.9±1.5 (p=0.2) while treated with the first anti-TNF-α drug, and then significantly decreased to 3.4±1.0 (p=0.02, *vs*. 1 year) after swapping to RTX. However, autoantibody levels did not follow changes in DAS28. Anti-CCP levels were 166.3±172 at baseline, 83.6±91 (p=0.05) after 1 year, then rose to 108.3±98 (p=0.06 *vs*. 1 year) during RTX treatment. RF-IgM levels were 109.6±93 at onset, decreased to 76±77 (p=0.1) at 1 year, then significantly declined to 13.7±24 (p=0.008 *vs*. 1 year) after RTX course. RF-IgA lev-

Table I. Baseline demographics.

	Patients on anti-TNF-α drugs (n=54)	Patients on non-anti-TNF-α drugs (n=17)
Age (years)	56.1 ± 14	55.4 ± 12
Female (%)	79	53
Duration of disease (years)	9.0 ± 9.2	7.8 ± 7.2
HAQ-DI	1.4 ± 0.7	1.1 ± 0.8
ESR-DAS28	4.3 ± 1.4	4.6 ± 1.1
Biological drug	Adalimumab (n. 3) Certolizumab (n. 1) Etanercept (n. 16) Infliximab (n. 34)	Rituximab (n. 9) Tocilizumab (n. 8)
Anti-CCP serum levels	243 ± 320	238 ± 192
Anti-CCP (%)	100	100
RF-IgM (%)	82	88
RF-IgA (%)	56	88
RF-IgG (%)	98	100
Glucocorticoids (%)	69	76
DMARDs (%)	81	88

HAQ-DI: Health Assessment Questionnaire-Disability Index; ESR-DAS28: Erythrocyte Sedimentation Rate-Disease Activity Score 28 joints; anti-CCP, anti-cyclic citrullinated peptide antibodies; RF: rheumatoid factor; DMARDs: disease-modifying anti-rheumatic drugs.

Table II. Changes in serum anti-CCP and RF isotype levels during the first year of therapy.

	Patients on anti-TNF-α drugs (n=54) (mean ±SD)			Patients on non-anti-TNF-α drugs (n=17) (mean ±SD)		
	Baseline	1 year	p-value	Baseline	1 year	p-value
Anti-CCP	243 ± 320	116 ± 106	0.002	238 ± 192	141 ± 121	0.01
RF-IgM	59 ± 67	40 ± 55	0.003	97 ± 82	53 ± 62	0.01
RF-IgA	52 ± 56	42 ± 47	0.1	83 ± 68	55 ± 56	0.03
RF-IgG	34 ± 39	26 ± 33	0.007	43 ± 43	25 ± 30	0.007
ESR-DAS28	4.3 ± 1.4	3.6 ± 1.7	0.04	4.6 ± 1.1	3.1 ± 1.1	0.003

anti-CCP: anti-cyclic citrullinated peptide antibodies; RF: rheumatoid factor; ESR-DAS28: Erythrocyte Sedimentation Rate-Disease Activity Score 28 joints.

Table III. Baseline covariates predictors of "good" EULAR response at 24 months in all RA patients following the first biological drug evaluated by multivariate logistic regression analysis.

	Unadjusted analysis			Model after forward selection		
Baseline covariates	OR	95% CI	p value	OR	95% CI	p value
Anti-CCP	0.99	0.99–1.00	0.32			
RF-IgM	0.96	0.98-1.01	0.60			
RF-IgA	1.02	1.00-1.05	0.01	1.01	1.00-1.02	0.03
RF-IgG	0.99	0.98-1.01	0.92			
DMARDs (yes/no)	0.20	0.19-2.29	0.20			
Prednisone (yes/no)	3.56	0.58-21.8	0.16			
Disease duration	0.98	0.97-0.99	0.02			
Age	1.04	0.97-1.10	0.20			
Sex (male/female)	6.04	0.85-42.6	0.71			

Anti-CCP: anti-cyclic citrullinated peptide antibodies; RF: rheumatoid factor; DMARDs: disease-modifying anti-rheumatic drugs; OR: odds ratio; CI: confidence intervals.

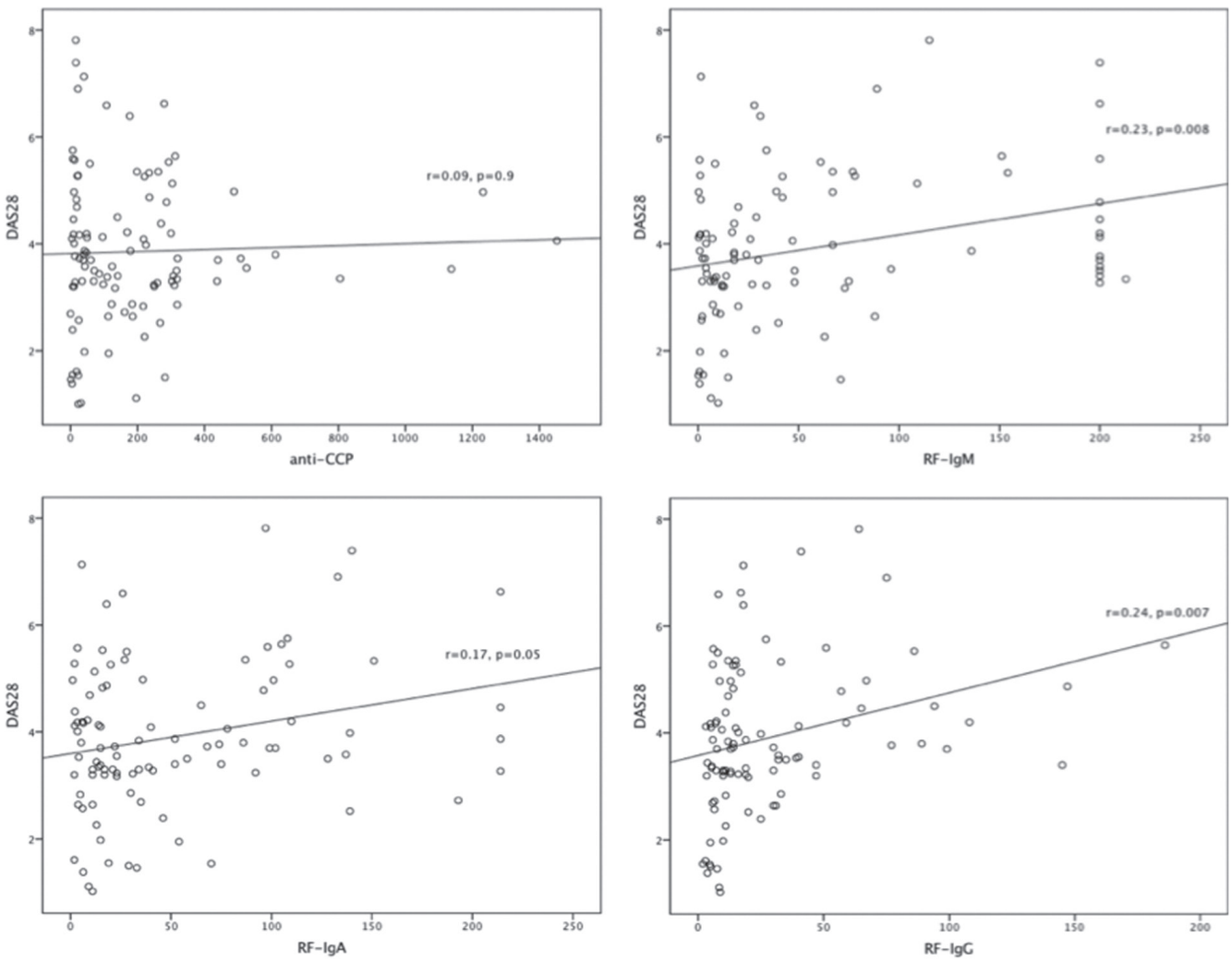

Fig. 1. Correlations between antibody levels, anti-CCP, RF-IgM, RF-IgA, RF-IgG, and DAS28 in the whole RA cohort while treated with the first biological drug. Baseline and 1 year observations were pooled together. DAS28, Disease Activity Score 28 joints. Pearson's correlation analysis.

els were 51.3±35 at onset, decreased to 42.3±34 (p=0.4) at 1 year, then reduced to 16.7±28 (p=0.4) after RTX. RF-IgG levels were 60±43 at onset, decreased at 36.2±26 (p=0.02) at 1 year, then further declined to 11.1±8 (p=0.05 *vs*. 1 year) after RTX therapy (Fig. 2).

Discussion

Whether anti-CCP antibodies may be considered a true biomarker of RA is matter of debate and clinical studies have yielded conflicting results, but a tight correlation between the anti-CCP levels and activity of RA disease over time has never been clearly demonstrated (9). It is conceivable that drugs targeting B cells may have higher impact on anti-CCP levels than those blocking TNF-α. On the same basis it is thought that B cells depleting treatment such rituximab is greatly effective in RA patients with high anti-CCP antibody titres (10). Previous studies have shown a significant decrease in anti-CCP and RF levels following RTX therapy in RA patients (7). Instead, anti-CCP levels remained unchanged despite the clinical response in 6 out of 7 RA patients on RTX therapy (11).

Aim of our study was to evaluate changes in anti-CCP levels in RA patients across 1 year of therapy with TNF-α inhibitors, RTX or TCZ, and in those patients who, after failing anti-TNF-α drugs, initiated RTX. We speculated that assessing changes in the anti-CCP levels in patients sequentially treated with B cell depleting therapy after failure of a first anti-TNF-α drug could give further insights into the question whether anti-CCP may be regarded as biomarker of clinical response in RA patients. Furthermore, the RF isotypes (IgM, IgA, and IgG) levels were investigated. We found a significant decrease in anti-CCP levels in the whole RA cohort at 1 year, regardless of the biological drug. Anti-CCP levels did not correlate with DAS28 nor did with 1-year "good" EULAR response. Instead, RF-IgA levels were a weak predictor of clinical response. A skewed behaviour of anti-CCP and RF has been already reported in RA, anti-CCP levels being not affected by treatment, but RF-IgM or RF-IgA correlating with disease activity or clinical response to biological drugs (12, 13). Whether this discrepancy might reflect a different role played by anti-CCP and RF in RA pathogenesis should be worthy to be addressed.

We focused on the antibody profile in 14

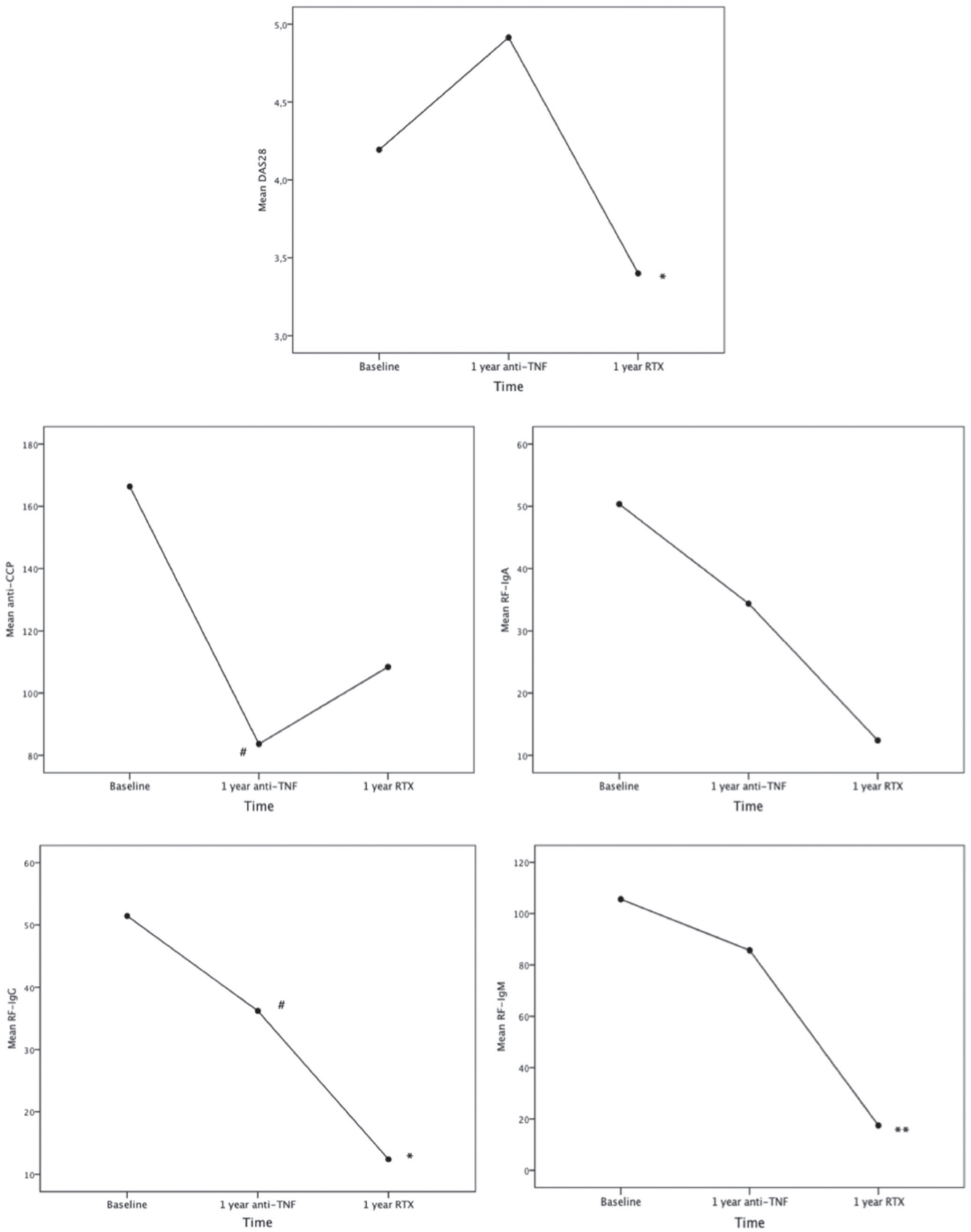

Fig. 2. DAS28 values and sequential antibody levels, anti-CCP, RF-IgM, RF-IgA, RF-IgG, in 14 RA patients failing the first anti-TNF and starting rituximab. Time points were: baseline of the study, 1 year of treatment with anti-TNF, and 1 year of treatment after swapping to rituximab. Comparisons by paired *t* test analysis. DAS28, Disease Activity Score 28 joints. $^{*}p<0.05$ *vs*. 1 year anti-TNF, $^{**}p<0.01$ *vs*. 1 year anti-TNF, $^{\#}p<0.05$ *vs*. baseline.

RA patients with active disease, changing the treatment into RTX because of failure of the first TNF-α blocker. Despite of the effectiveness of B cell depleting therapy, the anti-CCP levels tended to increase after 1 year, instead a further decrease in all RF isotype levels was noticed. These findings suggest that disease activity, assessed by DAS28, and anti-CCP titre changes are uncoupled and this is quite surprising given the key role ascribed to the anti-CCP in the pathogenesis of RA. It may be speculated that anti-CCP is a genetically determined marker of RA, but it is not a biomarker of disease course.

However, some weaknesses of our study must be taken in account. The number of patients was low, nevertheless the lack of association of DAS28 with anti-CCP levels over time was quite clear-cut. The wide range of disease duration may be a confounding factor as it has demonstrated that the antibodies levels tend to fall in the first year of the disease and become stable in the next five years (14). Finally, the design of the study might be a bias against the correlation of anti-CCP levels and disease activity, as we retrospectively measured the anti-CCP and RF levels in selected RA patients tested positive for anti-CCP. In conclusions, the question whether anti-CCP level has sensitivity to change and may be regarded as biomarker of disease activity in RA remains unanswered.

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