Anti-citrullinated peptide antibodies and the progression of radiographic joint erosions in patients with early rheumatoid arthritis treated with the FIN-RACo combination and single disease-modifying antirheumatic drug strategies

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Abstract

Objectives

To evaluate the impact of antibodies to cyclic citrullinated peptide (ACPAs) on radiographic progression in patients with early rheumatoid arthritis (RA) initially treated either with a combination of 3 disease-modifying antirheumatic drugs (DMARDs) or with a single DMARD.

Methods

This study included 129 patients with early active RA initially randomised to treatment either with a combination of methotrexate, sulfasalazine, hydroxychloroquine, and prednisolone (FIN-RACo) (n=69) or with a single DMARD (initially sulfalasalazine) with or without prednisolone (SINGLE) (n=60). After 2 years, the use of DMARDs and prednisolone became unrestricted. Radiographic progression in hands and feet was assessed at baseline and at 1, 2, 3, 4 and 5 years. ACPAs at baseline were determined with enzyme immunoassay.

Results

ACPAs were positive in 92 (71%) patients. ACPA-positive vs. negative patients were more frequently rheumatoid factor (RF) positive (83% vs. 22%, p<0.001) and had an erosive disease (54% vs. 22%, p<0.001) at baseline. The presence of ACPA was associated with radiographic progression in FIN-RACo group even when the impact of RF was controlled; the radiographic progression was remarkably slower in ACPA-negative than in ACPA-positive cases (RF adjusted change over time between groups p=0.034). In the SINGLE group, the radiographic changes progressed parallel in ACPA-negative and positive patients.

Conclusion

Most ACPA-positive RA patients have joint erosions already at diagnosis. ACPA positivity in early RA was related to radiographic progression even in patients treated initially with the FIN-RACo regimen. The initial FIN-RACo therapy seems to slow down the progression of joint damage in ACPA-negative patients.

Key words

antibodies to cyclic citrullinated peptide (ACPAs), radiographic progression, FIN-RACo, DMARDs

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This work was supported by Competitive Research Funding of the Pirkanmaa Hospital District, Tampere University Hospital.

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Received on June 24, 2010; accepted in revised form on February 3, 2011.

Competing interests: none declared.

Introduction

Patients with rheumatoid arthritis (RA) have the potential to develop destructive symmetric polyarthritis. Despite active research, the causes and detailed pathogenesis of this autoimmune syndrome remain to be elucidated, but positivity for related HLA-DRB1 alleles, the so-called shared epitope (SE) (1), and for autoantibodies is known to be associated with susceptibility to and severity of RA. Cigarette smoking is the best established environmental risk factor for seropositive RA (2). However, probably none of the risk factors alone triggers clinical disease.

Until the discovery of antibodies directed to citrullinated protein antigens (ACPAs), the most important RA-associated autoantibodies have been rheumatoid factors (RFs). Several studies have already shown that positive ACPAs discern erosive RA patients from non-erosive cases early (3-6). In our previous large population-based cross-sectional study, high levels of ACPAs even predicted mortality in RA patients (7). Autoantibody formation against post-translationally citrullinated peptides is even thought to be involved in the disease pathogenesis. The presence of ACPAs strongly correlates with the presence of SE (4). The present knowledge suggests that immunisation against citrullinated antigens in SE+ persons triggered by environmental factors like cigarette smoke may initiate a special subtype of RA (8-10).

ACPA determination has rapidly become a widely used diagnostic tool for clinicians. According to EULAR recommendations for the management of early arthritis, ACPA positivity should be investigated in every patient presenting with early arthritis (11).

There is increasing evidence that effective antirheumatic therapy should be started early and targeted to remission. The treatment strategy including combinations of traditional DMARDs in early disease has induced significantly better responses including higher remission rates and retardation of radiographic joint damage progression as compared to the strategy applying single DMARDs as sequential monotherapy (12-15). However, it has also been speculated that "hit hard and early with a DMARD" strategy is superior to any combination therapies of DMARDs given that no good comparison of different combinations is available (16).

The aim of this study was to evaluate the impact of ACPAs on the radiographic outcome of patients with early RA initially treated either with a combination of methotrexate, sulfasalazine, hydroxychloroquine, and prednisolone (FIN-RACo) or with a single DMARD (initially sulfasalazine) with or without prednisolone (SINGLE).

Patients and methods

Patients and study design

From April 1993 to May 1995, a total of 199 DMARD-naive patients with recent-onset (duration of symptoms <2 years, median duration 6 months) clinically active RA (17), were admitted to this multicentre (n=18), investigator initiative, parallel-group, randomised study comparing the efficacy and tolerability of a therapy with FIN-RACo strategy with those treated by SINGLE strategy. Randomisation was organised with sequentially numbered envelopes consisting of blocks of 20, stratified with respect to RF (12). The FIN-RACo treatment was started with oral methotrexate 7.5 mg/week, sulfasalazine 500 mg twice daily, hydroxychloroquine 300 mg/day and prednisolone 5 mg/day, but the dosages could be adjusted to achieve remission. The highest dosages allowed were 15 mg/week for oral methotrexate, 2 g/day for sulfasalazine and 10 mg/day for prednisolone. If any of the components of the drug combination had to be discontinued for any reason, a combination of 3 DMARDs was restarted by replacing the discontinued DMARD with another as described in detail previously (12). The SINGLE strategy was performed according to the "sawtooth" principle by using sulfasalazine 2 g/day as the initial DMARD for all patients. The simultaneous use of up to 10 mg/day of oral prednisolone was allowed for patients with continuously active RA. The SINGLE strategy is also described in detail previously (12).

The treatment was targeted to remission by both the FIN-RACo and SINGLE

strategies. After two years the treatment strategy became unrestricted but the aim of treatment still was to achieve remission or to maintain it. In the original SINGLE group, a shift to combination treatment was recommended in the patients with active disease. The treatment strategy and the five-year follow-up results have been described in detail previously (14). In brief, regardless the original randomisation group, the patients with active RA could be treated liberally with increased doses of DMARDs (*e.g.* methotrexate up to 25 mg/week orally or parenterally, sulfasalazine up to 3 g/day) as well as with other DMARD combinations when clinically indicated and tolerated. In patients with long-term remission, low-dose prednisolone was the first drug to be tapered off. If prednisolone could be discontinued without losing remission, other DMARDs could be tapered down, one DMARD per year by reducing the dose gradually. Hydroxychloroquine was predetermined by the protocol to be the last DMARD to be used. If RA reactivated, the last drug regimen with which remission was maintained was reinstituted (14).

The DMARDs and predinisolone used and the median doses of methotrexate, sulfasalazine and prednisolone during the 5-year follow-up are shown in Table I. At the time of the study, methotrexate was used mostly per os, subcutaneous methotrexate was used for very few patients.

Ethical consideration

The study was performed according to the principles of the Declaration of Helsinki. The protocol was approved by the national health authorities and ethics committees in all 18 participating hospitals. All patients gave written informed consent.

Methods

The RA patients were assessed clinically at baseline and at regular intervals until five years or lost to follow-up. The ACR Core Data Set measures (18) were used to evaluate the clinical activity of RA as well as the response to treatment. DAS28 score was used to assess clinical disease activity (19).

Table I. The DMARDs and predinisolone used and the median doses of methotrexate, sulfasalazine and prednisolone during the five-year follow-up.

	FIN-RACo, n=69 (%)	SINGLE, n=60 (%)
Prednisolone	69 (100)	46 (77)
Sulfasalazine	69 (100)	60 (100)
Methotrexate	69 (100)	46 (77)
Hydroxychloroquine	69 (100)	35 (58)
Azathioprine	7 (10)	10 (17)
Cyclosporine	4 (6)	12 (20)
Auranophine	6 (9)	8 (13)
Intramuscular gold	5 (7)	10 (17)
Podophyllotoxine	3 (4)	3 (5)
D-penisillamine	0 (0)	1 (2)
Leflunomide	0 (0)	0 (0)
	Median dose	
	FIN-RACo	SINGLE
Methotrexate	7.5 mg/week	10 mg/week
Sulfasalazine	1 g/day	2 g/day
Prednisolone	5 mg/day	5 mg/day

Remission was defined according to the ACR criteria (20) with the modification that the fatigue and duration criteria were excluded, and all the 5 remaining criteria had to be fulfilled at appropriate visit before remission was confirmed (12, 14). Radiographs of the hands and feet were taken once a year. The radiographs were assessed by one experienced radiologist (L.L.) blinded with the clinical data, and scored by the method of Larsen *et al*. (21).

RF was determined at baseline at the laboratories of the centres participating in the FIN-RACo trial, based on the validated quantitative method and cut-off value at the particular laboratory. The HLA-DRB1-subtyping was performed by using sequence specific polymerase chain reaction (PCR) (22).

ACPAs were determined from a serum sample drawn at baseline and at 1, 2 and 5 years, and stored at -20°C until analysed. An enzyme immunoassay (Immunoscan RA, Euro-Diagnostica, Malmö, Sweden) was performed according to manufacturer's instructions with a cut-off value of 25 U/ml. Samples with results <25 U/ml were defined as negative. Samples giving optical density values over the highest standard (1600 U/ml) were diluted and reanalysed to get an approximate quantitative value in U/ml for the level of ACPA.

Statistical analysis

The descriptive values of the variables assessed were expressed as mean with standard deviation (SD), median with interquartile range (IQR) or as count with percentage. The most important outcomes are presented with 95 per cent confidence intervals. The comparison between groups was performed by *t*-test, permutation test or chi-square test. When there was a need to adjust confounding factors we used a bootstrap type ANCOVA. We used a random coefficient model for repeated measures to investigate the change over time between ANCA positive and negative patients. We calculated the area under the curve (AUC) with the trapezoidal method for DAS28. AUC was divided by the total time of study and results are depicted in time-weighted mean scores.

Results

The current study included 129 of the original 160 RA patients who completed the five-year follow-up study. Appropriate serum samples, clinical and radiographic data were available from all these patients for the whole 5-year follow-up period. Sixty-nine patients were initially randomised in FIN-RACo combination and 60 cases in SINGLE DMARD treatment strategy groups. The demographic and clinical data of the current study population of 129 patients did not differ from the original study population and neither did the demographic and clinical data of those 31 patients whose serum samples were not available for the current study.

The baseline demographic, clinical and radiographic characteristics of the study patients are shown in Table II and did not differ between treatment groups. ACPAs were detected in 92 (71%) of all patients at baseline. Forty-six (67%) patients in FIN-RACo group and 46 (77%) patients in SINGLE group were positive for ACPAs, respectively. Thus, the treatment groups did not differ from each other with respect to ACPA positivity (p=0.21). Neither did the quantitative ACPA levels differ significantly between the treatment groups at baseline. Median ACPA level in FIN-RACo group was 251 U/ml (95% CI 77.5–521 U/ml) and 393 U/ml (95% CI 181–694

Table II. Baseline demographic, clinical and radiographic characteristics of the patients.

Characteristic	Treatment strategy		*p*-value
	FIN-RACo (n=69)	SINGLE (n=60)	
Demographic			
Female, n (%)	42 (61)	39 (65)	0.63
Age (years), mean (SD)	46 (10)	48 (11)	0.39
Duration of disease (months), mean (SD)	7.3 (4.7)	8.2 (5.2)	0.31
Reumatoid factor present (%)	47 (68)	37 (62)	0.44
Measures of disease activity			
Erythrocyte sedimentation rate (mm/hr), mean (SD)	37 (24)	37 (21)	0.93
Number of swollen joints, mean (SD)	13 (6)	14 (7)	0.68
Number of tender joints, mean (SD)	19 (8)	20 (11)	0.48
Patient's global assessment (VAS), mean (SD)	46 (23)	48 (23)	0.60
Physician's global assessment (VAS), mean (SD)	44 (16)	46 (20)	0.63
Physical function (HAQ), mean (SD)	0.84 (0.54)	0.91 (0.63)	0.47
DAS28, mean (SD)	5.5 (1.0)	5.6 (1.1)	0.51
Radiographic			
Erosions in hand or foot radiographs (%)	29 (42)	29 (48)	0.47

VAS: visual analogue scale; HAQ: Health Assessment Questionnaire.

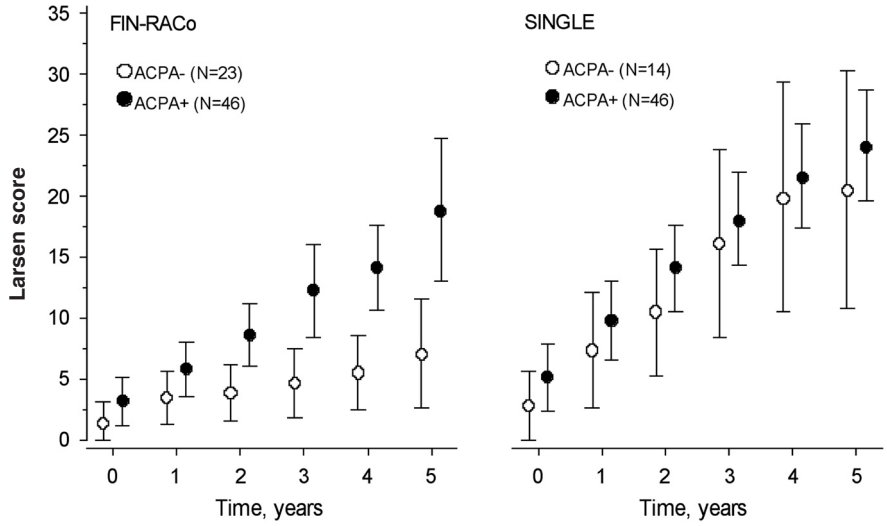

Fig. 1. Radiographic progression with regard to baseline ACPA findings adjusted for RF during the 5-year follow-up of RA patients treated with FIN-RACo and SINGLE treatment strategy.
FIN-RACo: RF adjusted change over time between groups p=0.034; SINGLE: RF adjusted change over time between groups p=0.95.

U/ml) in SINGLE group, respectively. The quantitative ACPA level was over 1600 U/ml in 22 patients at baseline (11 patients in each treatment group).

At baseline, the ACPA-positive RA patients were more frequently positive for RF (83% *vs*. 22%, $p<0.001$) and had more often an erosive disease (54% *vs*. 22%, $p<0.001$) than the ACPA-negative cases.

Data on SE was available in 125 out of the 129 patients. SE was found in 94 patients and 75 of them were ACPA-positive (79.8%) whereas SE was negative in 31 patients, 15 of whom were ACPA-positive (48.4%). The association between ACPA and SE was statistically significant, p=0.001.

During the 5-year follow-up period, the radiographic joint damage progression of the FIN-RACo group patients was slower in ACPA-negative than in ACPA-positive cases; RF adjusted change over time between ACPA-positive and negative patients was significant, p=0.034. In the SINGLE group cases, significant radiographic progression was observed in all cases irrespective of the ACPA status; RF adjusted change over time between ACPA-positive and negative patients was not significant, p=0.95 (Fig. 1).

Clinical remission rates at 1, 2 and 5 years in ACPA-positive and negative patients with respect to treatment group are shown in Table III. In both FIN-RACo and SINGLE groups, clinical remission rates were similar in ACPA-positive and negative cases. Thus, ACPA-positivity had no impact on the number of patients that achieved remission in either treatment group.

As the radiographic joint erosions progressed in ACPA-positive patients treated either with FIN-RACo or SINGLE but still the ACPA positivity did not have impact on clinical remission rates during the follow-up, we wanted to investigate the association between radiographic progression and clinical disease activity according ACPA groups. An AUC estimate of DAS28 score over time (0–5 yrs) was assessed. Radiographic progression was measured as a difference in Larsen score (Δ Larsen score) during the five-year follow-up. Among the patients with low clinical activity (DAS28 AUC score ≤3.2), the increase in Larsen score was significantly higher in ACPA-positive than in ACPA-negative patients (RF and Larsen baseline score adjusted difference (Δ) in Larsen score between ACPA-positive and negative patients; p=0.043) (Fig. 2.). There was no significant difference in Δ Larsen scores between ACPA-positive and negative patients with increased clinical disease activity (DAS AUC score >3.2).

To evaluate the influence of DMARDs on quantitative ACPA levels, all available serum samples drawn at 1, 2 and 5 years were tested for ACPAs. Only patients with complete dataset (serum samples) during the whole follow-up period (baseline, 1, 2 and 5 years) were used for analyses (48 FIN-RACo and 37 SINGLE patients). The quantitative level of ACPAs decreased significantly in both treatment groups during the 5-year follow-up but the difference between groups was not significant (Fig. 3). In addition, ACPA status was very constant for a single patient during the 5-year follow-up period, *i.e*. ACPA-

Table III. Remission rates (%) in ACPA positive and negative patients treated with FIN-RACo and SINGLE DMARD strategy at 1, 2 and 5 years.

Treatment strategy	ACPA+ n (%)	ACPA- n (%)	*p*-value
FIN-RACo			
1 yr	9 (20)	7 (32)	0.36
2 yrs	17 (39)	9 (45)	0.078
5 yrs	13 (30)	7 (35)	0.77
SINGLE			
1 yr	5 (11)	3 (23)	0.36
2 yrs	8 (18)	2 (16)	0.99
5 yrs	7 (16)	4 (33)	0.22

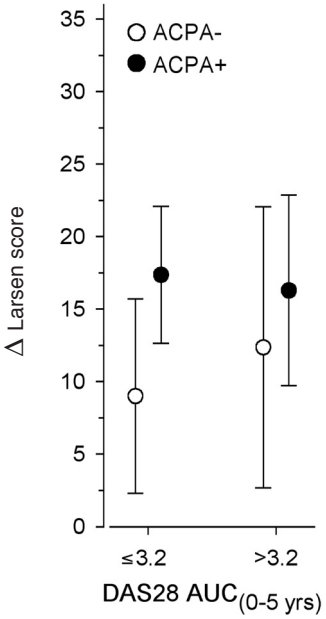

Fig. 2. The adjusted (RF and Larsen score at baseline) change in Larsen score (Δ) during the 5-year follow up in ACPA-positive and negative patients with regard to AUC (area under curve) estimate of DAS28 score.

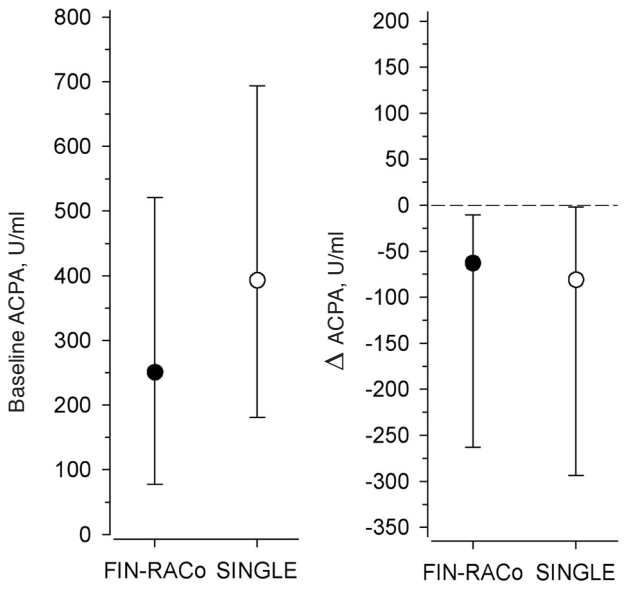

Fig. 3. The median of ACPA titers/levels at baseline (left panel) and median change during the 5-year follow-up in RA patients treated with FIN-RACo and SINGLE treatment strategy. Whiskers show 95% confidence intervals.

negative patients remained negative and ACPA-positive patients remained positive irrespective of the initial DMARD strategy (FIN-RACo *vs*. SINGLE).

Discussion

The main finding of the present study confirms that ACPA positivity associates with progressive radiographic joint destruction in patients with recent-onset RA. Our findings also imply that most RA patients genetically predisposed to become ACPA-positive, are already positive when the clinical disease emerges.

Second, ACPA positivity was associated with progressive radiographic joint erosions even in RA patients treated initially with the FIN-RACo strategy when the FIN-RACo treatment seemed to slow down the radiographic progression in patients negative for ACPA. The DMARD therapy regimens the patients received in this study were carefully defined and strictly followed by both patients and clinicians. Thus, the bias caused by the effects of varying drug therapies on immune system remained as a minor one. The finding that radiographic erosions progress in both treatment groups in ACPA-positive patients but the progression remarkably slows down in ACPA-negative patients treated with FIN-RACo DMARD strategy in early phase of the disease raises the question whether ACPA-positive and negative RA are separate diseases. Van der Helm-van Mil *et al*. (23) have analysed a cohort of 228 ACPA-positive and 226 ACPA-negative RA patients. At inclusion, the phenotype of RA was similar in ACPA-positive and ACPA-negative patients but during the follow-up, ACPA-positive RA patients had more swollen joints and more severe radiologic destruction despite similar distribution of affected joints.

Third, radiographic progression was observed to be greater in ACPA-positive than negative patients with low clinical disease activity measured by AUC estimate of DAS28. Disconnection between inflammation and joint destruction has been observed in recent studies with TNF blockade (24, 25). In the ATTRACT trial, treatment with infliximab plus methotrexate showed radiographic benefit even in RA patients with no clinical improvement (25). In the TEMPO trial, radiographic progression increased with an increasing serum CRP and DAS28 score in patients treated with methotrexate alone, but this classic relationship was uncoupled in patients treated with methotrexate plus etanercept showing no radiographic progression (25). It should be observed that during the conduction of the present study no biological agents were available.

The most important clinical challenge concerns the optimal therapeutic option for ACPA-positive RA patients. In the BeSt Trial (26), early RA patients were randomised to be treated either with sequential monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with methotrexate, sulfasalazine and prednisolone (group 3), or initial combination therapy with methotrexate and infliximab (group 4). In the two years' follow-up, the association of ACPA status with progression of joint damage was analysed according to different treatment strategies. ACPA positivity was predictive for progressive joint disease only in sequential monotherapy group (OR 12.6; 3.0-51.9) indicating that effective treatment can prevent radiographic progression, even in patients with risk fac-

tors for severe joint damage (26). Our 5-year follow-up results imply that the FIN-RACo combination is not alone an optimal option in preventing radiologic progression in ACPA-positive RA patients. Since the FIN-RACo combination is effective in inducing high rate of clinical remissions and biologicals in reducing radiographic progression, the question arises whether the FIN-RACo combination plus a biological agent like a TNF inhibitor would be an option in inducing both clinical and radiographic remission in ACPA-positive patients with early RA. Certainly, there is need for further studies evaluating the impact of biological agents on the radiological outcome of early RA with respect to ACPAs.

To conclude, most ACPA-positive RA patients had joint erosions already at diagnosis. Radiographic erosions progressed in ACPA-positive patients treated even with FIN-RACo strategy. We also found disconnection between radiological progression of joint damage and clinical disease activity in ACPA groups. In patients treated with SINGLE DMARD strategy during the first 2 years, erosions progressed also in ACPA-negative patients, while treatment with the FIN-RACo DMARD strategy remarkably slowed down the radiographic progression in patients negative for ACPAs. As ACPAs seem to be a constitutive predictor of joint destruction process, RA should be divided to ACPA-positive and negative subclasses requiring separate treatment strategies.

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