Published in final edited form as:

Arthritis Rheumatol. 2019 May ; 71(5): 696–702. doi:10.1002/art.40791.

Association of baseline peptidylarginine deiminase 4 autoantibodies with favorable response to treatment escalation in rheumatoid arthritis

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Abstract

Objective: To determine if the baseline presence of autoantibodies to peptidylarginine deiminase 4 (PAD4) predicts therapeutic response to biologic and conventional disease-modifying antirheumatic drugs (DMARDs) in patients with rheumatoid arthritis (RA) who failed methotrexate (MTX) monotherapy.

Methods: Baseline serum from 282 RA patients who failed MTX monotherapy were screened for the presence of anti-PAD4 antibodies by immunoprecipitation. Clinical response to either triple DMARD (MTX, sulfasalazine, and hydroxychloroquine) or MTX-etanercept combination therapy was determined at 24- and 48-weeks post-treatment initiation. Disease activity was measured using the disease activity score for 28-joint counts (DAS28) and erosive disease was quantified using the Sharp van der Heijde scoring method. Generalized estimating equations (GEE) were used to model the clinical responses to treatment between patients with or without baseline anti-PAD4 antibodies.

Results: Anti-PAD4 antibodies were associated with male sex, a history of never (vs. ever) smoking, and anti-citrullinated protein antibodies. At baseline, patients with anti-PAD4 antibodies had a longer disease duration and significantly more radiographic joint damage than anti-PAD4 negative individuals but did not differ in their DAS28. In unadjusted analyses and multivariable

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Author Contributions

All authors contributed to the preparation of this manuscript and approved the final version for publication. ED and TRM guided data collection, interpretation, and analysis. ED, AR, and TRM designed the study. JRO and TRM provided clinical samples and data from patients in the RACAT study. FY performed statistical analysis and LCC aided in data interpretation.

Financial interests: ED received a research grant from Pfizer; ED and AR are authors on licensed Patent No. 8,975,033 entitled "Human Autoantibodies Specific for PAD3 which are Cross-reactive with PAD4 and their Use in the Diagnosis and Treatment of Rheumatoid Arthritis and Related Diseases"; AR received support for participating in the Siemens Healthineers conference; TRM received research funding from Bristol Myers Squibb and is a consultant for Pfizer; and LCC has research funding from Bristol Myers Squibb and is a consultant for Regeneron/Sanofi. The other authors have no disclosures.

GEE models, patients with anti-PAD4 antibodies exhibited greater improvements in DAS28 and less radiographic progression than anti-PAD antibody negative patients at 24- and 48-weeks, independent of treatment received.

Conclusion: Although anti-PAD4 antibodies were associated with worse baseline radiographic joint damage, suggesting a history of active or undiagnosed disease, treatment escalation therapy was more effective in reducing disease activity and slowing the progression of joint damage in this patient subset.

Disease-modifying antirheumatic drugs (DMARDs) have greatly improved clinical outcomes for patients with rheumatoid arthritis (RA). Among these, methotrexate (MTX) is the most commonly prescribed first-line DMARD but is often insufficient for long-term disease control when used as oral monotherapy (1). Following sub-optimal responses to MTX monotherapy therapy, addition of other DMARDs including tumor necrosis factor α (TNF α) inhibitors, has been shown to be an effective therapeutic strategy and is currently the standard of care (2, 3). While these treatment algorithms have greatly improved disease prognosis, this step-wise approach and variable treatment response between individuals suggests the need to personalize the early selection of effective therapeutics. Initiation of effective therapy early in the disease course can prevent the accumulation of joint damage and disability in RA (4). Interestingly, differential treatment responses among patients with the RA-associated autoantibodies, rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs), have been reported in some studies, suggesting that baseline autoantibody status may associate with clinical responses to specific DMARDs (5).

Antibodies to peptidylarginine deiminase 4 (PAD4), a key enzyme in RA pathogenesis, have been identified in the serum of 23–45% of patients with established disease and have been associated with severe erosive joint disease in several RA cohorts (6–8). Radiographic joint damage was greatest in a subset of RA patients who harbored anti-PAD4 antibodies that cross-reacted with the related enzyme PAD3 (9, 10). Antibodies that recognize PAD3 alone have not been described. In a small open-label TNF α -inhibitor study, the baseline presence anti-PAD4 antibodies was associated with persistent disease activity and progression of erosive disease despite 12 months of treatment (11). In an observational study of patients with long-standing RA, anti-PAD3/4 cross-reactive antibodies were associated with increased progression of erosive joint disease (9), but this trend was not observed in a similar study of patients with early-RA (10).

The prevalence and association of anti-PAD4 antibodies with severe erosive joint disease in patients with RA suggests that they may be informative predictors of poor clinical response to DMARD therapy, however, data from a large study of patients receiving standardized treatment with rigorous pre- and post-treatment clinical assessments are lacking. To address this, the association of baseline anti-PAD4 antibodies with the clinical response to triple DMARD (MTX, sulfasalazine, and hydroxychloroquine) or MTX-etanercept combination therapy in RA patients who had active disease despite MTX monotherapy was explored.

PATIENTS AND METHODS

Study subjects

Sera from 282 patients in the Veterans Affairs Cooperative Study Program (CSP) 551 RA: Comparison of Active Therapies (RACAT) study were retrospectively screened for anti-PAD4 and anti-PAD3/4 antibodies (3). Study participants provided written informed consent. RACAT was a double-blind non-inferiority trial comprised of patients with RA who had active disease despite MTX monotherapy and were randomized to receive either triple DMARD (MTX, sulfasalazine, and hydroxychloroquine) or combination therapy (MTX and etanercept). Baseline demographic, clinical, and serological variables were collected. Anti-CCP antibody (Diastat, Axis-Shield Diagnostics, Dundee, UK) and rheumatoid factor by nephelometry (RF; Siemens, Munich, Germany) were measured using banked serum from baseline. The disease activity score for 28-joint counts (DAS28) calculated using the erythrocyte sedimentation rate (ESR) and radiographic burden of erosive disease, as calculated by the Sharp van der Heijde scoring method (12), were determined at baseline, as well as at 24 and 48 weeks post-treatment initiation. At 24 weeks, patients whose DAS28 did not improve by at least 1.2 points were switched to the other treatment arm in a blinded fashion.

PAD autoantibody detection

Anti-PAD4 and PAD3/4 cross-reactive antibodies were detected in patient serum by immunoprecipitation of S³⁵-labeled *in vitro* transcribed and translated (Promega) PAD4 or PAD3 protein as described (6, 9). Immunoprecipitated proteins were separated by SDS-PAGE, visualized by radiography, and quantified by densitometry. A densitometry value >0.02 was considered positive. Baseline sera from patients in the RACAT cohort were first screened for autoantibodies to PAD4, then anti-PAD4 positive sera were further screened for cross-reactivity to PAD3. Patients positive for both PAD4 and PAD3 reactivity were designated as having “anti-PAD3/4 cross-reactive antibodies (XR)” based on published work (9, 10). Individuals with reactivity to PAD4 but not to PAD3 were determined to have “anti-PAD4 mono-specific antibodies (P4)”, and patients who were negative were designated as “anti-PAD antibody negative (P0)”.

Statistical analyses

Patients were grouped by their baseline anti-PAD antibody status and demographic, clinical, and serologic variables were compared using SAS 9.4 (SAS Inc, Cary, NC). Baseline treatment assignments, the number of patients whose treatment was switched, and treatment after switching was also compared between groups. The total anti-PAD4 antibody positive group (P4 and XR patients combined) was also compared to P0 individuals for these parameters. Normally distributed variables were compared using Student's t-tests, non-normally distributed variables were compared using the Kruskal-Wallis test and categorical variables were compared using Chi-squared or two-sided Fisher's exact tests, as appropriate. Generalized estimating equations (GEE) were used to model the clinical responses between patients with or without anti-PAD4 antibodies with regard to DAS28 and Sharp score. P4 and XR patients were combined in these analyses given similar changes observed in DAS28 and Sharp scores in these groups over follow-up. A GEE model with identify link and

compound symmetry correlation among repeated measures was used to model the DAS28 scores over time, while a GEE log link model for count data and compound symmetry correlation among repeated measures was used to model the Sharp scores over time. Both GEE models included baseline anti-PAD4 antibody status, follow up time, and interaction between anti-PAD4 and follow up time to assess the association of anti-PAD4 with the change in the DAS28 or Sharp score. The GEE models also included other covariates such as age, sex, race, body mass index, disease duration, anti-CCP status, treatment arm, treatment switching and smoking status. The changes in the DAS28 score and relative changes in Sharp score were estimated and compared between treatment groups at each follow up visit. Similar models were created using baseline anti-CCP status, and its interaction with follow up or the anti-PAD4 status and the corresponding interaction as the primary covariates. The Quasi- Akaike Information Criterion (QIC) was used to identify the optimal model when modeling the DAS28 or Sharp score over time, as is appropriate for GEE, a non-likelihood-based model (13). To ensure a fair comparison using the same dataset, the models under comparison were restricted to patients with non-missing values for both anti-PAD4 and anti-CCP status at baseline. The QIC score between the anti-CCP and anti-PAD4 models were compared and the model with the lower QIC score was determined to be the model with the better fit.

RESULTS

Anti-PAD4 antibodies are associated with radiographic joint disease at baseline

Anti-PAD4 antibodies were present in 26% (74/282) of patients at baseline. Demographic, clinical and serological characteristics of patients grouped by anti-PAD4 antibody status are shown in Table 1. Compared to anti-PAD4 negative individuals, patients with anti-PAD4 antibodies were more often male and less likely to have ever smoked. They also had a longer RA duration and were more likely to be anti-CCP positive, with a 98% higher median anti-CCP titer, than anti-PAD antibody negative individuals. While there were no significant differences in baseline measures of disease activity or inflammation between the groups, including DAS28, ESR, and number of tender or swollen joints, patients with anti-PAD4 antibodies had more radiographic joint damage, as reflected by a 190% higher median baseline Sharp score compared to anti-PAD4 negative individuals.

Among the anti-PAD4 antibody positive individuals, 58% had anti-PAD4 mono-specific antibodies (P4) and 42% had anti-PAD3/4 cross-reactive antibodies (XR). Subanalysis of anti-PAD4 positive individuals according to P4 or XR status did not reveal any significant demographic or clinical differences, other than a slightly higher mean swollen joint count in P4 individuals (Table 1). Despite similar randomization to triple DMARD or MTX-etanercept combination therapy between P0, P4, and XR patients, XR patients were 2.9-times less likely to be switched to the alternative treatment arm at 24 weeks, compared to P0 individuals. Since treatment switching was blinded and driven by a lack of clinical response to treatment at 24 weeks, this suggested a more favorable early treatment response in XR positive individuals.

Anti-PAD4 positive patients respond more favorably to treatment

To determine if the clinical response to treatment escalation differed based on the anti-PAD4 autoantibody subtype present at baseline, the mean change in DAS28 or Sharp score in P0, P4, and XR patients at 24- and 48-weeks post-treatment was calculated (Table S1). In unadjusted analyses, patients in all antibody groups exhibited a decrease in DAS28 at 24- and 48-weeks post-treatment, indicating a reduction in disease activity, but progressed in their erosive joint disease, as measured by an increase in Sharp score. The magnitude of DAS28 improvement was greater in P4 and XR (−2.36 and −2.56 DAS28 units, respectively) patients than P0 individuals (−2.08 DAS28 units) at 48 weeks, with a similar improvement observed in P4 and XR patients. Similarly, less progression of radiographic joint damage was observed in P4 and XR individuals (33% and 29% higher Sharp score in 48 weeks post treatment vs. baseline, respectively) compared to patients with no anti-PAD antibodies (69% higher Sharp score in 48 weeks post treatment vs. baseline). Similar trends in DAS28 and Sharp score according to anti-PAD antibody subtype were observed in patients irrespective of treatment with triple DMARD or MTX-etanercept combination therapy (Table S2).

Multivariable GEE models were then created to estimate the mean change in DAS28 or relative change in Sharp scores by anti-PAD antibody status following treatment. Due to the similar baseline characteristics and clinical outcomes observed in P4 and XR individuals in both treatment arms (Tables S1 and S2), clinical responses of anti-PAD4 antibody positive patients (combined P4 and XR groups) were compared to anti-PAD4 negative individuals. Although MTX non-responders with anti-PAD4 antibodies had significantly higher Sharp scores at baseline compared to anti-PAD4 negative individuals (Table 1), they had more favorable clinical outcomes in response to treatment, even after adjusting for age, sex, race, body mass index, disease duration, anti-CCP status, treatment switching and smoking status (Tables 2 and 3). While both patient groups had a reduction in disease activity following treatment, patients with anti-PAD4 antibodies had a 22–23% greater decrease in DAS28 compared to antibody negative individuals (Table 2). This equated to a 2.27 vs. 1.84-point drop in DAS28 score at 24 weeks ($p=0.02$) and a 2.55 vs. 2.09-point drop at 48 weeks ($p=0.008$) in patients with anti-PAD4 antibodies compared to those without. Despite the observed improvement in disease activity with treatment, patients in both groups demonstrated modest radiographic disease progression, as indicated by slightly increasing Sharp scores at 24 and 48 weeks (Table 3). However, anti-PAD4 positive patients demonstrated less relative progression of their joint disease compared to antibody negative individuals [8% vs. 24% increase in Sharp score at 24 weeks ($p=0.01$), and a 7% vs. 27% increase at 48 weeks ($p=0.002$)].

To compare how models containing either anti-CCP or anti-PAD4 as a co-variate perform in relation to the change in DAS28 or Sharp score, the QIC was calculated for each model. When modeling DAS28, models including anti-PAD4 or anti-CCP antibodies performed similarly with a QIC of 824.4 and 823.2, respectively. In contrast, when modeling Sharp score, the multivariable model including anti-CCP performed better than the model including anti-PAD4 antibodies (QIC of −46535.1 versus −44522.4, respectively). However, in contrast to anti-PAD4 antibodies, baseline anti-CCP antibodies were associated with more radiographic disease progression compared to antibody negative individuals [23% vs. 7%

increase in Sharp score at 24 weeks ($p=0.02$), and a 26% vs. 7% increase at 48 weeks ($p=0.006$).

DISCUSSION

This study explored the utility of baseline anti-PAD4 antibodies in predicting future treatment response in RA patients who had active disease despite MTX monotherapy. Baseline clinical characteristics associated with anti-PAD4 antibodies in the RACAT cohort were consistent with previous studies including a longer disease duration, less frequent smoking history, association with anti-CCP antibodies, and higher Sharp scores (6–8, 14). Although patients with anti-PAD4 antibodies had the most radiographic joint damage at baseline, importantly, they had better clinical outcomes following triple DMARD or MTX-etanercept combination therapy than anti-PAD4 antibody negative individuals. This more favorable response was indicated by a greater decrease in disease activity by DAS28 and less progression of radiographic joint damage as measured by Sharp score. These data suggest that although patients with anti-PAD4 antibodies who fail MTX monotherapy have a higher burden of erosive disease at baseline, they respond favorably to treatment escalation therapy, as measured by traditional markers of disease activity.

These results are both surprising and intriguing given the significant radiographic joint disease burden found in anti-PAD4 positive patients at baseline in this and other cohorts (6–8). The only published treatment study exploring the predictive value of anti-PAD4 antibodies concluded that patients with baseline anti-PAD4 antibodies had persistent disease activity and progressive erosive disease despite 12 months of treatment with TNF α -inhibitor therapy (11). Important differences in study design may have contributed to the differing conclusions between this and our current study. The study by Halvorsen et al was a small open-label analysis of 40 patients who started TNF α -inhibitor therapy (i.e. adalimumab, infliximab, or etanercept) as part of routine clinical care. Information on inclusion criteria as well as past and concomitant DMARD use was not provided, and baseline erosive disease or DAS28 was not controlled for in the statistical modeling due to the small sample size (11). Our larger cohort study provided increased statistical power, focused exclusively on patients who had suboptimal responses to MTX monotherapy, and contained two treatment escalation arms, both of which included concomitant MTX therapy, providing a well-characterized patient population for studying the effect of baseline anti-PAD4 antibodies on treatment response. In addition, our use of GEE models for longitudinal data analysis allowed for the estimated change in radiographic joint damage and DAS28 following treatment to be determined at the individual patient level, as a function of baseline anti-PAD4 antibody status.

We have previously explored the association of anti-PAD3/4 cross-reactive antibodies with progression of radiographic joint disease in two observational cohorts of patients with RA. In a study of patients with established disease (9), patients with anti-PAD3/4 antibodies were significantly more likely to experience radiographic progression compared to anti-PAD negative individuals over a mean follow-up period of 39 months (9). In a smaller study of early RA patients (disease duration <2 years), baseline Sharp score, rather than anti-PAD3/4 antibodies, was associated with the progression of erosive disease over a 36-month period

(10). There are crucial differences between these observational studies and our current RACAT sub-study that are worth noting. In contrast to the RACAT cohort, patients in these two previous studies were receiving treatment with diverse therapeutics as part of routine clinical care and had a much longer time between radiographs (36 and 39 months vs. 6 and 12 months in RACAT). This highlights important unknowns about the durability of the favorable treatment response observed in anti-PAD4 positive RACAT patients past the 12-month time point as well as the persistence of subclinical disease that may drive progression over the longer term. In addition, our focus on patients whose disease was not adequately controlled by MTX monotherapy precludes us from determining the effect of baseline anti-PAD4 antibodies on the clinical response to MTX alone. Additional double-blind treatment studies and those with a longer follow-up period are therefore necessary to determine the generalizability of these findings to other RA patient populations.

Although disease activity improved and radiographic joint damage slowed more significantly in patients with anti-PAD4 antibodies once effective therapy was initiated, the extensive joint damage observed in these patients at baseline suggests a history of inadequate disease control. This could reflect the accumulation of joint damage early in disease prior to diagnosis, a longer disease duration before diagnosis, suboptimal disease management prior to study enrollment, or an accumulation of occult joint damage in the absence of classic disease activity markers, as has been described in the general RA population (4). The reported prevalence of anti-PAD4 antibodies in 18% of patients pre-clinically and in 21% of patients with early RA suggests that future treatment studies of early RA cohorts are needed to identify the therapeutic window for this unique patient subset and expedite disease diagnosis (10, 15). It may be that clinical indicators, other than traditional disease activity measures, are needed to detect and prevent the accumulation of joint damage in patients with anti-PAD4 antibodies. Our finding that patients with anti-PAD4 antibodies have a high burden of joint damage at baseline but respond favorably to treatment escalation therapy once initiated, highlights the importance of understanding the early events that drive joint damage in this unique patient subset.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank David Hines from the Rheumatic Disease Research Core Center at Johns Hopkins for providing technical support.

Grant support: This work was supported by the Jerome L. Greene Foundation (ED and LCC), VA Merit (CX000896, TRM), VA Cooperative Study Program (JRO), National Institute of General Medical Sciences (U54GM115458, TRM) and National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) (grant numbers P30 AR053503 and P30 AR070254, ED and LCC) at the National Institutes of Health (NIH). The content of this paper is solely the responsibility of the author and does not represent the official views the NIH.

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Table 1:

Baseline characteristics of RACAT patients by anti-PAD antibody status

	Group 1: Anti-PAD4 negative	Group 2: Anti-PAD4 positive		Group 1 vs. 2 (P)	P0 vs. P4 (P)	P0 vs. XR (P)	P4 vs. XR (P)
	P0 (n = 208)	P4 (n = 43)	XR (n = 31)				
Age, years	58.2 ± 12.0	57.7 ± 13.1	56.9 ± 14.2	0.61	0.79	0.59	0.80
Male gender, n (%)	113 (54)	29 (67)	21 (68)	0.048	0.11	0.16	0.98
Caucasian, n (%) **	184 (88)	35 (81)	30 (97)	0.89	0.21	0.22	0.071
Ever smoking, n (%)	152 (73)	25 (58)	20 (65)	0.048	0.051	0.32	0.58
RA duration, years *	1 (1-3)	1 (1-6)	5 (1-10)	0.010	0.23	0.004	0.12
RF Positivity, n (%)	140 (67.3)	34 (79.1)	21 (67.7)	0.26	0.13	0.96	0.27
Anti-CCP positivity, n (%) **	133 (63.9)	34 (79.1)	27 (87.1)	0.004	0.075	0.013	0.54
Anti-CCP titer *	54.3 (1.5-209.2)	130.4 (13.7-225.5)	91.4 (24.5-245.2)	0.027	0.10	0.082	0.84
Sharp Score *	5 (2-12)	13 (5-25)	15 (2-29)	0.0002	0.002	0.012	0.72
DAS-28 *	5.8 (5.2-6.4)	5.6 (5.2-6.7)	5.7 (5.1-6.6)	0.91	0.94	0.93	0.99
Tender Joint Count *	13 (9-18)	13 (9-18)	13 (9-16)	1.00	0.95	0.94	0.88
Swollen Joint Count *	11 (8-14.5)	11 (9-15)	9 (6-13)	0.89	0.19	0.17	0.047
ESR *	23.5 (11.5-40)	20 (10-42)	18 (11-31)	0.45	0.55	0.57	0.87
Treatment at baseline:							
Triple DMARD, n (%)	110 (53)	19 (44)	13 (42)	0.15	0.30	0.26	0.85
MTX-etanercept, n (%)	98 (47)	24 (56)	18 (58)				
Treatment Switched at 24 weeks, n (%) **	61 (29)	12 (28)	3 (10)	0.13	0.85	0.028	0.079
Treatment after switching:							
Triple DMARD, n (%)	103 (50)	23 (53)	16 (52)	0.64	0.64	0.83	0.87
MTX-etanercept, n (%)	105 (50)	20 (47)	15 (48)				

P0= anti-PAD antibody negative; P4= anti-PAD4 mono-specific antibodies; XR= anti-PAD3/4 cross-reactive antibodies; Mean ± standard deviation is given unless otherwise indicated;

* The median (Q1-Q3) is given to describe the data. Nonparametric Wilcoxon rank sum test was used for comparison;

** Fisher exact tests were used for comparison between the XR group vs the other treatment group.

Table 2:

Estimated change in DAS28 with treatment according to anti-PAD4 antibody status

GEE model	Weeks Post-Treatment	Estimated Change in DAS28 (95% CI)		p-value
		Anti-PAD4 Negative	Anti-PAD4 Positive	
Unadjusted	24	-1.83 (-2.02 to -1.65)	-2.25 (-2.58 to -1.92)	0.03
	48	-2.09 (-2.26 to -1.92)	-2.50 (-2.81 to -2.20)	0.02
Adjusted *	24	-1.84 (-2.02 to -1.65)	-2.27 (-2.60 to -1.94)	0.02
	48	-2.09 (-2.26 to -1.92)	-2.55 (-2.84 to -2.26)	0.008

* GEE model adjusted for age, sex, race, body mass index, disease duration, anti-CCP status, treatment arm, treatment switching and smoking status, p value was based on testing the interaction between time and Anti-PAD4 status, or equivalently comparing the difference in the DAS28 changes between the Anti-PAD4 negative and Anti-PAD4 positive groups.

Table 3:

Estimated change in Sharp score with treatment according to anti-PAD4 antibody status

GEE model	Weeks Post-Treatment	Estimated Relative Change in Sharp score (95% CI)		p-value
		Anti-PAD4 Negative	Anti-PAD4 Positive	
Unadjusted	24	0.21 (0.12 to 0.31)	0.03 (−0.05 to 0.12)	0.006
	48	0.24 (0.15 to 0.34)	0.03 (−0.06 to 0.12)	0.001
Adjusted *	24	0.24 (0.15 to 0.33)	0.08 (0.00 to 0.16)	0.01
	48	0.27 (0.18 to 0.37)	0.07 (−0.02 to 0.16)	0.002

* GEE model adjusted for age, sex, race, body mass index, disease duration, anti-CCP status, treatment arm, treatment switching and smoking status. The estimated relative changes in share score quantifies the relative changes in the share score from baseline. For example, in the unadjusted GEE model, we anticipated a 21% increase in the sharp score in 24 months from baseline for the anti-pad4 negative group. The p values were based on testing the interaction between time and Anti-PAD4 status, or equivalently comparing the difference in the relative sharp score changes from baseline between the Anti-PAD4 negative and Anti-PAD4 positive groups.