Arthritis Research & Therapy

RESEARCH

Open Access

The role of Anti-PAD4, Anti-CarP, and Anti-RA33 antibodies combined with RF and ACPA in predicting abatacept response in rheumatoid arthritis

Alberto Floris[1,2*†], Maria Maddalena Angioni[1,2†], Mattia Fadda[1,2], Micaela Rita Naitza[1,2], Mattia Congia[1,2], Elisabetta Chessa[1,2], Matteo Piga[1,2] and Alberto Cauli[1,2]

Abstract

Objectives To explore the role of newly emerging autoantibodies (AAbs) - peptidyl-arginine deiminase 4 (aPAD4), carbamylated proteins (aCarP), and anti-RA33 (aRA33) - alongside the traditionally assessed rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), in predicting the response to abatacept (ABT) and its retention rate in rheumatoid arthritis (RA) patients.

Methods Data from 121 consecutive ABT-treated RA patients were recorded. The RF and ACPA status were retrospectively assessed by reviewing the patients' clinical records. Positivity for aPAD4, aCarP and aRA33 were determined by Enzyme-Linked Immunosorbent Assay (ELISA). The achievement of a moderate or good EULAR response at 6 months and the 3-years retention were analyzed as treatment outcomes. Multiple logistic regression models and Cox regression hazard analysis models were built to identify the association between such outcomes and the different AAbs, after adjustment for different confounders. The AAbs were assessed both individually and in different combinations to identify the most robust predictive model.

Results In the studied cohort, RF, ACPA, aPAD4, aCarP and aRA33-Ab tested positive in 74.4%, 69.4%, 43.8%, 23.9%, 14.9% patients, respectively. A moderate or good EULAR response at 6 months was achieved by 64.5% of subjects and the cumulative 3-years retention rate was 56.6%. A higher EULAR response rate was recorded in patient with positivity for RF (67% in subjects tested positive vs. 58% in negative), ACPA (68% vs. 57%), aPAD4 (68% vs. 62%), and aCarP (72% vs. 62%), although statistical significance was not reached likely due to sample size limitations. Similarly, ACPA, aPAD4, aCarP were associated with higher 3-year retention rates, though not statistically significant individually. The combined analysis revealed that positivity for ACPA and/or aPAD4 predicted a significantly higher EULAR response rate at 6 months compared with double negativity (adjusted OR 2.7, p 0.026). Furthermore, positivity for at least one of

†Alberto Floris and Maria Maddalena Angioni contributed equally to this work.

*Correspondence:
Alberto Floris
alberto.floris@unica.it; albertofloris1@gmail.com

Full list of author information is available at the end of the article

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

ACPA, aPAD4, or aCarP predicted a significantly higher 3-year ABT retention rate compared to triple negativity (62.1% single or double positive vs. 33.5% triple negative, adjusted HR 0.48, p 0.022).

Conclusion This study highlights the potential benefits of using a combined assessment of ACPA aPAD4 and aCarP in predicting effectiveness of ABT in RA.

Keywords Rheumatoid arthritis, Abatacept, Biomarkers, Auto-antibodies

Introduction

The European League Against Rheumatism (EULAR) recommendations state that the treatment of rheumatoid arthritis (RA) should be aimed at reaching a sustained remission or, at the very least, low disease activity (LDA) into a treat to target strategy [1]. They also endorse that treatment should be adjusted if the target has not been reached at predetermined time points [1]. However, such approach may result in a loss of valuable time, increasing the risk of joint damage, exposure to ineffective treatment, loss of physical function and decreased quality of life. For this reason, substantial efforts have been directed towards identifying markers that could predict the response to various therapies and, therefore, enable physicians to prescribe the most suitable treatment for each patient, shifting from a "trial and error" approach towards a "personalized medicine" [2, 3].

Abatacept (ABT) is a selective T cell co-stimulation modulator that was approved for the treatment of adults with moderate-to-severe RA[4]. The efficacy and safety of ABT have been widely demonstrated in several RCTs and in observational studies [5]. In the view of a personalized medicine, many authors have attempted to identify predictors of response to ABT, with most evidence on the association between better treatment outcomes and seropositivity for rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA), that are the two best known serological markers of RA diagnosis and prognosis evaluation [6–11]. In the ACTION study, Alten R et al. showed a significantly higher rate of moderate or good EULAR response achievement in patient with single (83.4%) or double (87.7%) positivity for RF and ACPA, compared with patients tested negative for both the AAbs (75.6%, p 0.007) [6]. Concordantly, in the same study a higher 12 months drug survival was recorded in bio-naïve patients with double positivity for ACPA and RF (HR 0.056, 95%CI 0.38 to 0.82), and in biologic-failure patients with double or single positivity for such antibodies (HR 0.64, 95%CI 0.52 to 0.79; and HR 0.62, 95%CI 0.48 to 0 0.79, respectively).[6] Other studies confirmed the role of RF and ACPA in predicting the ABT treatment response analyzing different outcome measures and time points [7].

Despite the demonstration of antibodies as the more promising predictors of ABT response, poor data are currently available on the role of others AAbs that are emerging as potential biomarkers in RA, challenging the current classification of seropositive and seronegative disease. [8] Among these, there are the peptidyl-arginine deiminase 4, the carbamylated proteins and the anti-RA33 [9–12].

Anti-PAD4 (aPAD4) are antibodies that target the peptidyl-arginine deiminase 4, enzyme that play a critical role in generating autoantigens in RA by converting arginine residues into citrulline and then favoring the AAbs formation. Anti-PAD4 can precede the RA onset and are found in the pre-clinical phase in a subset of RA, suggesting a significant role in the disease pathogenesis, like ACPA and RF [13]. The precise effect of this AAbs on the enzyme functionality and activity is not completely understood [13], although some authors reported that aPAD4 may induce a decreased enzyme's requirement of calcium for activation and then increased catalytic efficiency [14]. aPAD4 are found in 30–40% of the RA patients, and in 2–18% of the RF and ACPA seronegative individuals [13]. Some studies demonstrated a significant association between aPAD4 positivity and a more erosive disease and greater radiographic damage accrual [13].

Anti-CarP antibodies (aCarP) recognize proteins post-translationally modified following carbamylation rather than citrullination. Despite a high degree of resemblance between citrulline and homocitrulline (a carbamylation-derived product), most ACPA and aCarP antibodies do not seem to be cross-reactive [8]. Their estimated prevalence in RA was around 42% (16% prevalence in subgroups of ACPA-negative patients). In addition to ACPA, RF and aPAD4, also aCarP appear positive many years before the diagnosis of RA [15] and have been demonstrated to be associated greater with joint damage accrual, both in ACPA positive and negative RA patients [16].

Anti-RA33 antibodies (aRA33) target a specific antigen known as heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP-A2/B1) having a molecular weight of 33 kD. These AAbs have been identified in approximately 30% of patients with RA [12]. Although their role in the pathogenesis of RA is not fully understood, several authors support their utility as diagnostic markers especially in early RA, particularly because their high specificity. Poor data are available on their prognostic value. [12].

This study aimed to analyze the role of aPAD4, aCarP, and aRA33 antibodies, alongside RF and ACPA, in

predicting the achievement and maintenance of ABT treatment response. These markers were evaluated both individually and in various combinations to identify the most robust predictive models.

Methods

Study design and population

From the RA cohort of the Rheumatology Unit of the University Clinic of Cagliari, all patients fulling the following inclusion criteria were recruited for this study: (a) age ≥ 18 years, (b) diagnosis of RA according to the 2010 ACR/EULAR classification criteria [17], (c) treatment with ABT with/without methotrexate (or other cs-DMARDs) with/without glucocorticoids, (d) moderate to high disease activity score, (e) follow-up duration of at least 6 months.

Demographic and clinical data were retrospectively collected from the patients' medical records, including routinely detected RF and ACPA status. For the experimental objective of this study, the same patients were also tested for aPAD4, aCarP, and aRA33.

To assess the primary outcomes, disease activity was measured at baseline and after six months by the Disease Activity Score on 28 joint with erythrocyte sedimentation rate (DAS28-ESR) [18]. Particularly, the primary outcome was set as the achievement of a six months good or moderate response, according to the EULAR criteria [18] (hereafter simply referred to as EULAR response). Indeed, the achievement of EULAR response was defined by a DAS28-ESR improvement from baseline > 1.2 or a DAS28 improvement ranging from > 0.6 to ≤ 1.2 with achievement of a target of a DAS28-ESR state ≤ 5.19. See the representation of the EULAR response criteria in the supplementary materials. The 3-years retention rate was analyzed as secondary outcome, to indirectly explore the long-term treatment effectiveness [18].

Assessment of autoantibodies

The RF and ACPA status were collected from the patients' medical records, since they were routinely detected and recorded in all RA patients followed at the Rheumatology Unit of the University Clinic of Cagliari. In particular, RF was assessed by latex-enhanced immunoturbidimetric assay (Atellica CH RF assay, any isotype, Siemens Healthcare Diagnostic Inc., USA, cut-off value 14 UI/ml), and ACPA by automated enzyme fluoroimmunoassay (anti citrullinated peptide, IgG, Phadia 250 EliA CCP, Sweden; cut-off value 10 UI/ml).

For the experimental objective of this study, aPAD4, aCarP, and aRA33 were purposefully measured in serum samples collected by the same physicians responsible for gathering clinical data and stored at -80 °C until analysis. These samples could be taken at varying time points during the patients' disease and treatment history, that may not correspond with the time at the ABT initiation. The enzyme-linked immunosorbent assay (ELISA) and absorbance reader were used to assesses both the aPAD4 (PAD4 Autoantibody assay, any isotype, CAYMAN Chemical, USA), aCarP (Carp-Ab assay, any isotype, MyBioSource, USA) and aRA33 antibodies (anti-RA33 assay, any isotype, MyBioSource, USA), according to manufacturer's instructions. In accordance with previous studies [9, 19, 20], since commercial In vitro diagnostic medical devices (IVDs) are not yet available and only research-use arrays exist, the reference values were estimated based on the results from a control group of 20 sex- and gender-matched healthy subjects and defined as values exceeding the 95th percentile (103105 U/mL for aPAD4, 751 ng/mL for aCarP, and 90 ng/mL for aRA33).

Statistical analysis

Summary statistics were calculated for demographics, clinical variables, and autoantibody status. The association between the EULAR response at 6 months from ABT initiation and seropositivity for RF, aCCP, aPAD4, aCarP, and aRA33 - both individually and in various combinations - was evaluated using logistic regression analysis (backward stepwise method) adjusted for sex, age, baseline DAS28, concomitant glucocorticoids and conventional synthetic (cs)-DMARDs usage, and biologic treatment line. In the primary analysis, the different AAbs combinations were studied as a dichotomous variable, classified as "positive" if at least one of the assessed antibodies was positive, and "negative" if all the analyzed antibodies were negative (e.g., RF and/or ACPA positive vs. both negative). The antibody combinations that were significantly associated with the EULAR response were also studied in the secondary analysis as a dichotomous variable, classified as "positive" if all the assessed antibodies were positive, and "negative" if all the analyzed antibodies were negative (e.g., RF and ACPA double positive vs. both negative). Finally, considering the entire antibodies panel, the association between the count of parallelly positive AAbs (as continuous variable) and the EULAR response achievement was evaluated. The results of the significant associations were expressed as adjusted odds ratios (adjOR) and 95% confidence intervals (95% CI).

The same approach was applied to assess the role of seropositivity for the various AAbs, both individually and in combinations, and the 3-year drug survival of abatacept. The cumulative drug retention rates were calculated and graphically represented using the Kaplan–Meier method. The impact of different antibody patterns on the risk of drug discontinuation was assessed by Cox proportional-hazard regression analysis (backward stepwise method), adjusted for the same covariates applied in the aforenoted analysis on the EULAR response achievement. Separate models were built to consider

Table 1 Baseline features of the enrolled RA cohort

	n **= 121**
Females, n (%)	92 (76.0%)
Diagnosis age, mean (SD) yrs	43.3 (16.3)
ABT initiation age, mean (SD) yrs	59.1 (13.3)
ABT initiation – AAbs assessment interval, mean (SD) yrs	0.6 (3.4)
Smokers, n (%)	55 (45.5)
BMI, n (%)	25.2 (3.8)
Disease duration, mean (SD) yrs	15.0 (11.2)
Tender joint count, mean (SD)	8.7 (5.6)
Swollen joint count, mean (SD)	5.1 (4.7)
ESR, mean (SD) mm/h	39.5 (26.6)
DAS 28, mean (SD) score	5.0 (1.0)
ABT treatment line	
Bio-naïve, n (%)	28 (23.1%)
1 previous b-DMARD, n (%)	26 (21.5%)
2 previous b-DMARD, n (%)	43 (35.5%)
≥ 3 previous b-DMARD, n (%)	24 (19.8%)
Concomitant cs-DMARD, n (%)	91 (75.2%)
Methotrexate, n (%)	61 (50.4%)
Leflunomide, n (%)	26 (21.5%)
Concomitant glucocorticoids	93 (76.9%)

RA, rheumatoid arthritis. ABT, abatacept. BMI, body mass index. ESR, erythrocyte sedimentation rate. CRP, C-reactive protein. DAS 28, disease activity score on 28 joints. b-DMARD, biologic disease modifying anti-rheumatic disease

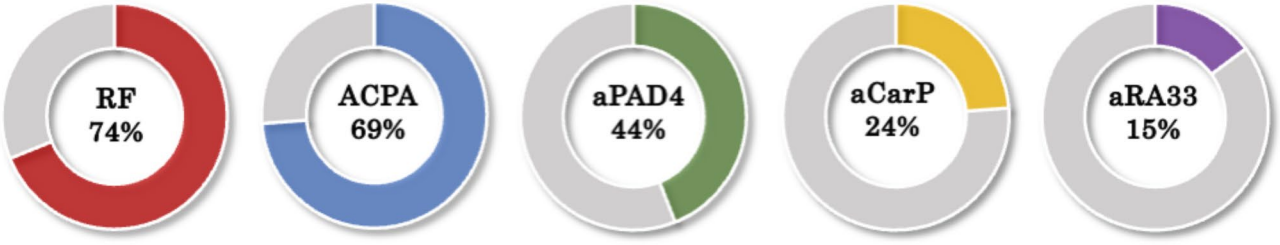

Fig. 1 Prevalence of the individual autoantibodies. RF, rheumatoid factor. ACPA, anti-citrullinated protein antibodies. aPAD4, anti-peptidyl-arginine deiminase 4. aCarP, anti-carbamylated proteins. aRA33, anti-RA33

discontinuation due to lack/loss of efficacy. No separate model was created to predict discontinuation due to adverse events because of the low number of withdrawals for safety issues.

The statistical significance was set for p-value < 0.05.

Results

Study population

In total, 121 adult patients with RA were enrolled in this study. The female-to-male ratio was 3:1. The mean (SD) age and disease duration at the initiation of ABT were 59.1 (13.3) years and 15.0 (11.2) years, respectively (Table 1). ABT was the first biologic therapy in 23% of patients, the second in 21.5%, the third in 35.5%, and the fourth or subsequent in the remaining 19.8%. In most subjects (75.2%) ABT was administered in combination with a cs-DMARD (methotrexate in 67.0% of cases). Detailed demographic and clinical characteristics of the cohort are provided in Table 1.

Autoantibodies prevalence and combinations

RF, ACPA, aPAD4, aCarP and aRA33 tested positive in, respectively, 90 (74.4%), 84 (69.4%), 53 (43.8%), 29 (23.9%), 18 (14.9%) patients (Fig. 1). The different combinations of AAbs positivity are represented in Table 2. When considering only the two commonly assessed serological biomarkers in RA - RF and ACPA – 94 (77.7%) patients would traditionally be classified as "seropositive". Out of these 94 patients, 80 (85.1%) were positive for both antibodies. Among the remaining 27 (22.3%) patients who were negative both for RF and ACPA, 6 were positive for aPAD4, 5 for aCarP, and 5 for aRA33. Therefore, when all five antibodies are considered, 106 (87.6%) subjects were classified as "seropositive" for at least one of these antibodies, while 15 (12.4%) were negative for all antibodies.

Table 2 Prevalence of different AABs combinations

RF	ACPA	aPAD4	aCarP	aRA33	
+	+	-	-	-	27 (22.3%)
+	+	+	-	-	25 (20.1%)
-	-	-	-	-	15 (12.7%)
+	+	+	+	-	11 (9.1%)
+	+	-	+	-	5 (4.1%)
+	-	-	-	-	5 (4.1%)
+	+	+	-	+	4 (3.3%)
-	-	+	-	-	4 (3.3%)
+	+	+	+	+	3 (2.5%)
+	+	-	+	+	3 (2.5%)
-	+	-	-	-	3 (2.5%)
-	-	+	-	-	3 (2.5%)
+	+	-	-	+	2 (1.7%)
+	-	+	-	-	2 (1.7%)
-	-	-	-	+	2 (1.7%)
+	-	+	+	-	1 (0.8%)
+	-	-	+	-	1 (0.8%)
+	-	-	-	+	1 (0.8%)
-	+	+	-	-	1 (0.8%)
-	-	+	-	+	1 (0.8%)
-	-	+	+	+	1 (0.8%)
-	-	-	+	+	1 (0.1%)

RF, rheumatoid factor. ACPA, anti-citrullinated protein antibodies. aPAD4, anti-peptidyl-arginine deiminase 4. aCarP, anti-carbamylated proteins. aRA33 anti-RA33

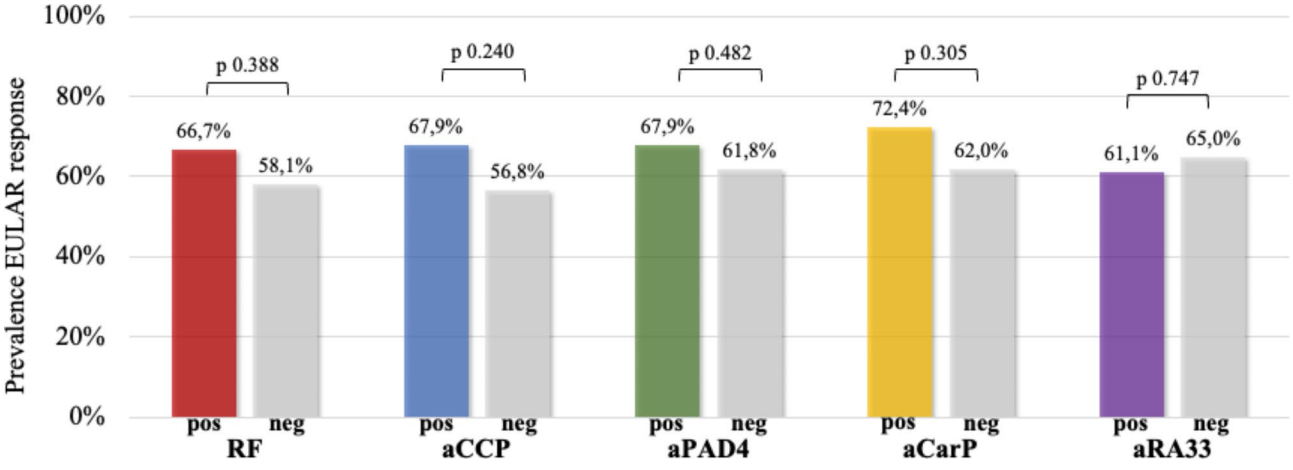

Fig. 2 Association between single antibodies positivity and achievement of EULAR response at 6 months. RF, rheumatoid factor. ACPA, anti-citrullinated protein antibodies. aPAD4, anti-peptidyl-arginine deiminase 4. aCarP, anti-carbamylated proteins. aRA33 anti-RA33

EULAR response and the association with the AAbs status

The EULAR response was achieved at 6 months by 78 (64.5%) patients.

When various AAbs were individually assessed, a higher EULAR response rate was recorded in patients tested positive for RF, ACPA, aPAD4, and aCarP, with a relative difference between positive and negative subjects ranging from 6.1 to 11.1%, that, however, was not statistically significant (Fig. 2).

The aRA33 positivity showed an inverse trend (Fig. 2). Given their overall prevalence (< 15.0%) and limited ability to discriminate between responders and non-responders (relative difference in terms of EULAR response rate < 4%), aRA33 were not included in subsequent models testing the performance of different AAbs combinations in predicting the ABT response. (Fig. 3)

When the various AAbs combinations were evaluated, the positivity of ACPA and/or aPAD4 was significantly associated with a higher rate of achieving a six-months EULAR response (adOR 2.7, 95% CI 1.1 to 6.4, $p = 0.026$) .(Fig. 3). For ACPA and/or PAD4 positivity the positive predictive value was 70% (95%CI 55–73%), the negative

Assessed AAbs combinations	N (%) of EULAR responders			Delta	Logistic regression		
	At least one AAb +		All AAbs -		OR (95%CI)	adjOR (95%CI)	p
RF and/or ACPA + vs double -	62 (66.0%)	vs	16 (59.3%)	6.7%	1.5 (0.6 to 3.9)		0.522
RF and/or aPAD4 + vs double -	43 (67.2%)	vs	12 (50.0%)	17.2%	2.8 (0.9 to 8.3)		0.138
RF and/or aCarP + vs double -	63 (66.3%)	vs	15 (57.7%)	8.6%	1.8 (0.7 to 4.6)		0.416
ACPA and/or PAD4 + vs double -	**65 (69.9%)**	**vs**	**13 (46.4%)**	23.5%	**3.5 (1.3 to 9.1)**	**2.7 (1.1 to 6.4)**	**0.026**
ACPA and/or CarP + vs double -	61 (67.0%)	vs	17 (56.7%)	10.3%	2.0 (0.8 to 4.5)		0.304
PAD4 and/or CarP + vs double -	43 (65.2%)	vs	35 (63.6%)	1.6%	1.2 (0.5 to 2.7)		0.862
RF and/or ACPA and/or aPAD4 + vs double -	68 (68.0%)	vs	10 (47.6%)	20.4%	2.3 (0.9 to 6.1)		0.081
RF and/or ACPA and/or aCarP + vs double -	65 (65.7%)	vs	13 (59.1%)	6.6%	1.6 (0.6 to 4.7)		0.561
RF and/or aPAD4 and/or aCarP + vs double -	68 (67.3%)	vs	10 (50.0%)	17.3%	2.8 (0.9 to 8.3)		0.139
ACPA and/or aPAD4 and/or aCarP + vs double -	67 (68.4%)	vs	11 (47.8%)	20.6%	2.4 (0.9 to 5.9)		0.068
RF and/or ACPA and/or aPAD4 and/or aCarP + vs double -	70 (67.3%)	vs	8 (47.1%)	20.2%	3.4 (1.0 to 10.8)		0.106

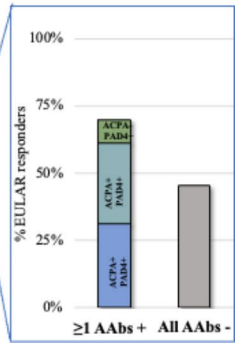

Fig. 3 Performance of assessment of different combinations of autoantibodies (AAbs) in predicting the achievement of EULAR response at six months from abatacept initiation. Positivity (+) of at least one AAbs into different combinations versus all AAbs negative (-) was evaluated. In the histogram on the left side the significant only combinations significantly associated with EULAR response is represented. RF, rheumatoid factor. ACPA, anti-citrullinated protein antibodies. aPAD4, anti-peptidyl-arginine deiminase 4. aCarP, anti-carbamylated proteins. EULAR, European alliance of associations against rheumatisms

predictive value 54% (95%CI 38–69%), and the number needed to treat (NNT) 4.26 (95%CI 2.3 36.1).

The enhanced predictive ability of the combined assessment of ACPA and aPAD4 (positivity of at least one the two AAbs) assessment in predicting the response to ABT is particularly evident from the following findings: (a) among the ACPA-negative group ($n = 37$), the patients who were PAD4-positive ($n = 9$) had a significantly higher likelihood of response compared to those who were PAD4-negative (88.9% vs. 46.4%, adjOR 12.5, 95% CI 1.3 to 119.6, $p = 0.028$); (b) conversely, in patients with double negativity for ACPA and aPAD4, the EULAR response rate dropped to 46.4%. No significant difference was observed in the EULAR response rate when comparing double-positive versus double-negative patients (63.9% vs. 46.4%, $p = 0.151$).

When the entire panel of all AAbs was evaluated, the count of positive AAbs (continuous variable) did not significantly associated with EULAR response. No significant association was found also after excluding aRA33.

Three-years drug survival and association with the AAbs status

At the end of the 3-year follow-up period, 48 (39.7%) patients discontinued ABT treatment. In particular, 41 stopped treatment due to inefficacy and 7 due to adverse effects (5 mild allergic skin reactions, 1 reduced compliance, 1 general malaise).

The cumulative retention rate, calculated using the Kaplan-Meier method, was 56.6% when considering any reason for withdrawal, 59.0% when considering only inefficacy, and 87.9% when considering only adverse effects.

When the different AAbs were individually assessed, a higher 3-years retention rate was recorded in patients with positivity for ACPA, aPAD4, and aCarP (supplementary materials). However, statistical significance was reached only for the association between ACPA-positivity and drug-survival including only withdrawals for inefficacy (supplementary materials).

Considering treatment withdrawals for any reason, the combined assessment of ACPA, aPAD4 and aCarP showed that the positivity of at least one of three antibodies was associated with a significantly higher retention rate (Kaplan-Meier estimated cumulative retention rate: 62.1% vs. 33.5%), and then a lower likelihood of treatment discontinuation (adjHR 0.492, 95% CI 0.268 to 0.901, p 0.022), when compared with triple negativity (Fig. 4). The combined assessment of solely ACPA and PAD4 was also associated with higher retention rate when compared with double negativity but was a slighting lower effect size (Fig. 4). These results were furtherly confirmed with more remarkable effect size in the sub-analysis considering only withdrawals for inefficacy, where the likelihood of treatment discontinuation was significantly lower in patients with at least one positivity among ACPA, aPAD4 and aCarP versus triple negativity (adjHR 0.48, 95% CI 0.218 to 0.800, p 0.022).

When comparing triple positivity versus triple negativity for the same AAbs, no significant difference in terms of drug survival was recorded (data not shown).

Discussion

Our study confirmed the effectiveness of ABT in the treatment of RA patients. With a 64% of six-months EULAR response rate and a 56% of 3-year retention rate, our results are consistent with previous real-world studies reporting the achievement of an EULAR response in approximately two-third of ABT-treated patients [21] and a retention rate of about 65%, with adverse effects accounting for less than 30% of discontinuations. [22]

However, the most interesting and original data from our study regard the role of newly emerging AAbs, particularly aPAD4 and aCarP, alongside traditionally assessed RF and ACPA, in predicting treatment response and drug

	Cox Regression hazard analysis		
	HR (95%CI)	adjHR (95%CI)	p
RF and/or ACPA + vs double -	0.95 (0.47 to 1.85)		0.730
RF and/or aPAD4 + vs double -	0.97 (0.47 to1.98)		0.715
RF and/or aCarP + vs double -	0.89 (0.45 to 1.75)		0.503
ACPA and/or PAD4 + vs double -	0.56 (0.30 to 1.05)	0.49 (0.27 to 0.90)	0.022
ACPA and/or CarP + vs double -	0.71 (0.38 to 1.33)		0.157
PAD4 and/or CarP + vs double -	0.64 (0.36 to 1.16)		0.101
RF and/or ACPA and/or aPAD4 + vs double -	0.81 (0.40 to 1.68)		0.506
RF and/or ACPA and/or aCarP + vs double -	0.82 (0.40 to 1.68)		0.506
RF and/or aPAD4 and/or aCarP + vs double -	0.83 (0.41 to 1.71)		0.370
ACPA and/or aPAD4 and/or aCarP + vs double -	0.55 (0.29 to 1.05)	**0.48 (0.22 to 0.80)**	**0.022**
RF and/or ACPA and/or aPAD4 and/or aCarP + vs double -	17.3 (0.42 to 720.7)		0.143

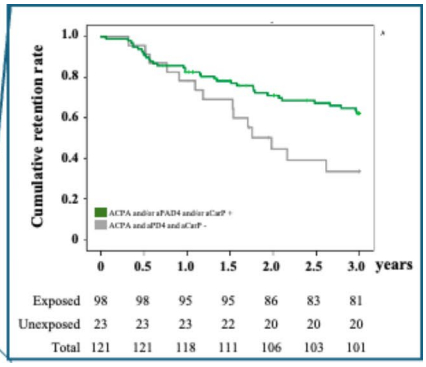

Fig. 4 Performance of assessment of different combinations of autoantibodies (AAbs) in predicting the abatacept drug survival. Positivity (+) of at least one AAbs into different combinations versus all AAbs negative (-) was evaluated. In the Kaplan-Meyer curves on the left side the cumulative retention rate of the most performing AAbs combination is represented. RF, rheumatoid factor. ACPA, anti-citrullinated protein antibodies. aPAD4, anti-peptidyl-arginine deiminase 4. aCarP, anti-carbamylated proteins. HR, hazard ration. adjHR, adjusted hazard ratio estimated by backwards stepwise Cox proportional regression analysis

survival in RA patients treated with ABT. Indeed, our findings offer valuable insights into the potential for these markers to enhance personalized treatment approaches in RA.

Few studies have so far examined the role of aPAD4, aCarP and aRA33 as predictors of treatment response in RA, particularly with ABT. Most research has focused on their use as diagnostic biomarkers or prognostic factors, consistently linking them to more severe and progressive disease [9, 11, 13, 23]. To our knowledge, specific data on the potential role of such AAbs as predictors of response to ABT are available only for the aCarP, and they are conflicting. Indeed, in a study of sixty RA patients, Kumar et al. observed a significantly greater reduction in DAS28-CRP during the first six months of ABT therapy in aCarP-positive patients compared to negative ones ($p = 0.003$). No significant results were found when stratifying the cohort by ACPA and/or RF positivity [24]. Similarly, Castellanos-Moreira et al. reported a significantly greater reduction in DAS28 at three months of follow-up in aCarP positive patients treated with ABT compared to aCarP negative subjects (-1.904 vs. -0.212; $p < 0.005$): moreover, they found that EULAR responders had higher baseline aCarP levels than non-responders (451.3 AU ± 675.4 AU vs. 152.6 ± 158.3; $p = 0.018$) [25]. In a subanalysis of a non-randomized multicenter prospective cohort on 43 RA patients treated with ABT, the authors found that baseline and changes of IgM-ACPA levels, but not aCarP (any isotype) associated with therapeutic response [26]. No data are specifically available regarding the potential role of aPAD4 in predicting the ABT treatment response. One study comparing the effectiveness of tofacitinib, adalimumab or methotrexate monotherapy showed that aPAD4 were associated with a slightly greater decline in DAS28-CRP levels post-treatment in early RA ($p = 0.049$), but no significant associations were found in established RA or other clinical outcomes such as ACR20, ACR50, and ACR70 [27].

Our study seems to suggest that patients who tested positive for RF, ACPA, aPAD4, and aCarP had a higher rate of EULAR response achievement at six months. However, statistical significance was not reached when these antibodies were individually assessed, likely due to the limited sample size. Overall, our data aligns with previous research demonstrating the role of ACPA in predicting the ABT-treatment response, where differences in response rates between ACPA-positive and ACPA-negative patients also averaged around 10%. This suggests that larger cohorts are needed to achieve statistical significance. Nevertheless, in the context of the main objective of this study, which was to evaluate the potential added value of integrating emerging AAbs in predicting the ABT-treatment response, we found that the positivity of at least one of ACPA and aPAD4 significantly increased the odds of achieving the EULAR response at six months compared with double negativity for these AAbs. This is due to the ability of aPAD4 to detect responsive patients among ACPA-negative subjects, and particularly to the remarkable reduction of the response rate in double-negative patients.

Similarly, when the 3-year drug survival was analyzed, patients who tested positive for ACPA, aPAD4, and aCarP, as individually assessed, had a higher drug retention rate. However, only ACPA reached statistical significance when evaluating withdrawals due to ABT inefficacy. The combined assessment of ACPA, aPAD4, and aCarP showed an enhanced ability to predict ABT drug survival, as the positivity for at least one of these three autoantibodies significantly associated with a higher retention rate compared to triple negativity.

Interestingly, the absolute number of parallelly positive AAbs did not correspond to a proportional increase in the likelihood of achieving the EULAR response or drug

survival. It seems to suggest that the number of positive antibodies is less relevant than whether the RA is "seronegative", particularly for both ACPA, PAD4, and CarP.

Regarding the aRA33 antibodies, they had the lowest prevalence (14.9%) and the lowest discriminative ability in our cohort, suggesting a lack of added value in their determination for predicting ABT treatment response.

Overall, our results seem to suggest the benefit of integrating the newer biomarkers— aPAD4 and aCarP — with traditional ones to improve accuracy in predicting the achievement and maintenance of response to ABT treatment. The enhanced predictive ability of the combined assessment of ACPA, aPAD4, and aCarP seems to derive from a redefinition of "seropositive" and "seronegative" RA, that, as suggested by other authors [28], should not be solely based on the evaluation of RF or ACPA. Indeed, the emerging AAbs may help to bridge the serological gap in RA by reducing the rate of "truly seronegative RA" and extending the group of "seropositive RA", that seem to be characterized by a distinctive pathogenic features, including a more prominent involvement of humoral immunity that may result in a variable responsiveness to different targeted therapies such as ABT. This hypothesis would align with the upstream mechanism of ABT, whereby co-stimulation blockade inhibits T-helper cell activation with a subsequent impact on B cells and autoantibody production [6]. Moreover, this hypothesis may be further supported by the results of the AMPLE study, where ABT showed superior efficacy to adalimumab at 24 weeks in RA patients with positivity for RF and ACPA, with further greater benefits in subjects with risk alleles encoding HLA-DRB1 shared epitope, that in turn is associated with humoral immune response.

Finally, an interesting speculation could stem from the results of our study combined with those of the APIPPRA trial [29], where T-cell co-stimulation modulation with abatacept for 12 months reduced progression to RA in at-risk patients identified based on ACPA and RF positivity and inflammatory arthralgia [29]. Indeed, based on these data and the demonstration that also CarP and PAD4 may precede the RA onset in many patients [13, 15], future studies may highlight the added value of incorporating also these emerging antibodies in identifying at high-risk patients for whom a preventive approach may be feasible and the co-stimulation blockade a rationale option.

This work has some limitations. The study's retrospective design and relatively small sample size may have potentially impacted the statistical power and generalizability of the findings, particularly regarding the survival analysis. Moreover, the study design and the sample size prevented to make a comparative analysis on the role of the different AAbs in predicting the response to other treatment beside ABT. Another limitation of our study regards the different time points in which the AAbs were tested, that may not correspond with the time at the ABT initiation. For this reason, we chose to assess the AAbs status (positive /negative) and not their titer, as it may result less affected by different ongoing treatment.

In conclusion, this study suggests the potential benefits of using a combined assessment of ACPA aPAD4 and aCarP in predicting treatment response and long-term outcomes in RA patients treated with ABT. Further and specifically designed prospective analyses on wider cohorts and diverse treatments are needed to validate our results and enhance predictive models. Additionally, exploring the mechanistic roles of these autoantibodies in RA pathogenesis could provide deeper insights into their utility as therapeutic targets.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-024-03470-y.

Supplementary Material 1

Acknowledgements
The authors would like to thank all the patients participating in this research.

Author contributions
Study conception: AF, MMA, AC. Study design: AF, MMA, AC. Experimental lab analyses: MMA, MN. Acquisition, analysis or interpretation of the data: AF, MMA, MF, MN, MC, EC, MP. Drafting the article or substantively revised it: AF, MMA, MF, MN, MC, EC, MP, AC. All authors read and approved the final manuscript.

Funding
This work was supported by Bristol Myers Squibb. The funder had no role in conceptualization, design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Local Ethical Committee (PROT. PG/2021/19418) of the Azienda Ospedaliero Universitaria of Cagliari and written informed consent was obtained from all participants.

Consent for publication
written informed consent for publication was obtained from all participants.

Competing interests
A.F.: consulting and/or speaking fees from Amgen, Eli Lilly, Janssen, Abbvie. M.C.: consulting and/or speaking fees from Amgen, Eli Lilly, Novartis. A.C.: consulting and/or speaking fees from Abbvie, Amgen, Biogen, BMS, Galapagos, Eli Lilly, Janssen, Novartis, Pfizer, UCB. The other authors declare no conflicts of interest.

Author details
[1]Department of Medical Science and Public Health, Rheumatology Unit, University of Cagliari, Azienda Ospedaliero Universitaria di Cagliari, SS 554 Monserrato (CA), Bivio Sestu, Monserrato 09042, Italy
[2]Rheumatology Unit, Azienda Ospedaliero Universitaria di Cagliari, Monserrato, Italy

Received: 17 September 2024 / Accepted: 28 December 2024
Published online: 15 January 2025

Refernces

1. Smolen JS, Landewé RBM, Bijlsma JWJ, Burmester GR, Dougados M, Kerschbaumer A, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis. 2020;79:685–99.
2. Roodenrijs NMT, van der Goes MC, Welsing PMJ, Tekstra J, van Laar JM, Lafeber FPJG, et al. Is prediction of clinical response to methotrexate in individual rheumatoid arthritis patients possible? A systematic literature review. Joint Bone Spine. 2020;87:13–23.
3. Narváez J, Magallares B, Díaz Torné C, Hernández MV, Reina D, Corominas H, et al. Predictive factors for induction of remission in patients with active rheumatoid arthritis treated with tocilizumab in clinical practice. Semin Arthritis Rheum. 2016;45:386–90.
4. Moreland L, Bate G, Kirkpatrick P. Abatacept. Nat Rev Drug Discov. 2006;5:185–6.
5. Blair HA, Deeks ED. Abatacept: A Review in Rheumatoid Arthritis. Drugs. 2017;77:1221–33.
6. Alten R, Mariette X, Lorenz H-M, Galeazzi M, Cantagrel A, Nüßlein HG, et al. Real-world predictors of 12–month intravenous abatacept retention in patients with rheumatoid arthritis in the ACTION observational study. RMD Open. 2017;3:e000538.
7. Sokolove J, Schiff M, Fleischmann R, Weinblatt ME, Connolly SE, Johnsen A, et al. Impact of baseline anti-cyclic citrullinated peptide-2 antibody concentration on efficacy outcomes following treatment with subcutaneous abatacept or adalimumab: 2-year results from the AMPLE trial. Ann Rheum Dis. 2016;75:709–14.
8. Ajeganova S, Huizinga TWJ. Seronegative and seropositive RA: alike but different? Nat Rev Rheumatol. 2015;11:8–9.
9. Kolarz B, Ciesla M, Rosenthal AK, Dryglewska M, Majdan M. The value of anti-CarP and anti-PAD4 as markers of rheumatoid arthritis in ACPA/RF negative rheumatoid arthritis patients. Ther Adv Musculoskelet Dis. 2021;13:1759720×21,989,868.
10. Boeters DM, Trouw LA, van der Helm-van Mil AHM, van Steenbergen HW. Does information on novel identified autoantibodies contribute to predicting the progression from undifferentiated arthritis to rheumatoid arthritis: a study on anti-CarP antibodies as an example. Arthritis Res Ther. 2018;20:94.
11. Pollmann S, Stensland M, Halvorsen EH, Sollid LM, Kvien TK, Fleckenstein B, et al. Anti-PAD4 autoantibodies in rheumatoid arthritis: levels in serum over time and impact on PAD4 activity as measured with a small synthetic substrate. Rheumatol Int. 2012;32:1271–6.
12. Yang X, Wang M, Zhang X, Li X, Cai G, Xia Q, et al. Diagnostic accuracy of anti-RA33 antibody for rheumatoid arthritis: systematic review and meta-analysis. Clin Exp Rheumatol. 2016;34:539–47.
13. Martinez-Prat L, Lucia D, Ibarra C, Mahler M, Dervieux T. Antibodies targeting protein-arginine deiminase 4 (PAD4) demonstrate diagnostic value in rheumatoid arthritis. Ann Rheum Dis. 2019;78:434–6.
14. Darrah E, Giles JT, Ols ML, Bull HG, Andrade F, Rosen A. Erosive Rheumatoid Arthritis Is Associated with Antibodies That Activate PAD4 by Increasing Calcium Sensitivity. Sci Transl Med. 2013 [cited 2024 Jul 15];5. Available from: https://www.science.orghttps://doi.org/10.1126/scitranslmed.3005370.
15. Shi J, Stadt LA van de, Levarht EWN, Huizinga TWJ, Hamann D, Schaardenburg D van, et al. Anti-carbamylated protein (anti-CarP) antibodies precede the onset of rheumatoid arthritis. Ann Rheum Dis. 2014;73:780–3.
16. van Delft MAM, Huizinga TWJ. An overview of autoantibodies in rheumatoid arthritis. J Autoimmun. 2020;102,392.
17. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62:2569–81.
18. van Riel PLCM, Renskers L. The Disease Activity Score (DAS) and the Disease Activity Score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. Clin Exp Rheumatol. 2016;34:S40–4.
19. Halvorsen EH, Pollmann S, Gilboe I-M, Heijde D van der, Landewé R, Ødegård S, et al. Serum IgG antibodies to peptidylarginine deiminase 4 in rheumatoid arthritis and associations with disease severity. Ann Rheum Dis. 2008;67:414–7.
20. Martinez-Prat L, Martinez-Taboada V, Santos C, Lopez-Hoyos M, Mahler M. Anti-Protein-Arginine Deiminase 4 IgG and IgA Delineate Severe Rheumatoid Arthritis. Diagn Basel Switz. 2022;12:2187.
21. Gottenberg JE, Ravaud P, Cantagrel A, Combe B, Flipo RM, Schaeverbeke T, et al. Positivity for anti-cyclic citrullinated peptide is associated with a better response to abatacept: data from the 'Orencia and Rheumatoid Arthritis' registry. Ann Rheum Dis. 2012;71:1815–9.
22. Manfredi A, Fornaro M, Bazzani C, Perniola S, Cauli A, Rai A, et al. Retention rate of biologic and targeted synthetic anti-rheumatic drugs in elderly rheumatoid arthritis patients: data from GISEA registry. Front Med. 2024 [cited 2024 May 24];11. Available from: https://www.frontiersin.org/articles/10.3389/fmed.2024.1349533.
23. Zhang G, Xu J, Du D, Liu Y, Dai L, Zhao Y. Diagnostic values, association with disease activity and possible risk factors of anti-PAD4 in rheumatoid arthritis: a meta-analysis. Rheumatology. 2024;63:914–24.
24. Kumar R, Piantoni S, Boldini M, Garrafa E, Bazzani C, Fredi M, et al. Anti-carbamylated protein antibodies as a clinical response predictor in rheumatoid arthritis patients treated with abatacept. Clin Exp Rheumatol. 2021;39:91–7.
25. Castellanos-Moreira R, Rodríguez-García SC, Gomara MJ, Ruiz-Esquide V, Cuervo A, Casafont-Solé I, et al. Anti-carbamylated proteins antibody repertoire in rheumatoid arthritis: evidence of a new autoantibody linked to interstitial lung disease. Ann Rheum Dis. 2020;79:587–94.
26. Kawashiri SY, Endo Y, Nishino A, Shimizu T, Ueki Y, Eiraku N, et al. Fri0098 Association Between the Serologic Status of Isotype-Specific Autoantibodies and Therapeutic Efficacy in Rheumatoid Arthritis Patients Treated with Abatacept: A Prospective Ultrasound Cohort Study in Japan. Ann Rheum Dis. 2020;79:628–628.
27. Cappelli LC, Hines D, Wang H, Bingham CO, Darrah E. Anti-Peptidylarginine Deiminase 4 Autoantibodies and Disease Duration as Predictors of Treatment Response in Rheumatoid Arthritis. ACR Open Rheumatol. 2024;6:81–90.
28. Pratt AG, Isaacs JD. Seronegative rheumatoid arthritis: Pathogenetic and therapeutic aspects. Best Pract Res Clin Rheumatol. 2014;28:651–9.
29. Cope AP, Jasenecova M, Vasconcelos JC, Filer A, Raza K, Qureshi S, et al. Abatacept in individuals at high risk of rheumatoid arthritis (APIPPRA): a randomised, double-blind, multicentre, parallel, placebo-controlled, phase 2b clinical trial. The Lancet. 2024;403:838–49.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.