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Associations between an expanded autoantibody profile and treatment responses to biologic therapies in patients with rheumatoid arthritis

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ABSTRACT

Background: Although biologics represent a major advance in rheumatoid arthritis (RA), many patients fail to achieve adequate responses to these agents. We examined whether combined positivity to three well-characterized autoantibodies predicts treatment response among RA patients initiating biologics.

Methods: The study included biologic-naïve patients initiating anti-TNF treatment, biologic-exposed patients switching to rituximab or tocilizumab, and patients (biologic naïve or exposed) initiating abatacept. Rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP) antibody, and IgG antibodies to malondialdehyde-acetaldehyde (MAA) were measured using banked enrollment serum. The relationship between the number of autoantibodies positive (0–3) and treatment response (absolute improvement in 28-joint Disease Activity Score [DAS28-CRP] or improvement > 1.2) at 6 months was examined using multivariable linear and logistic regression.

Results: Of 1,229 patients initiating biologics, 79% were women; 89% were Caucasian. The number of baseline RA-related autoantibodies positive was associated with improved treatment response in a dose-dependent fashion. Compared to patients seronegative for all autoantibodies, adjusting for covariates, those positive for all three were more than twice (OR 2.35; 95% CI 1.57–3.51) as likely to achieve DAS28 improvement > 1.2 units. Associations of autoantibody positivity with biologic treatment response were strongest for anti-CCP antibody, persisted in analyses limited to biologic naïve patients, and did not appear to differ markedly among different agents examined.

Conclusion: An expanded autoantibody profile appears to significantly predict RA treatment response to biologic treatment in a dose-dependent fashion. Incorporating these serologic profiles with additional biomarkers or other informative patient characteristics could provide an opportunity to personalize RA management.

Rheumatoid arthritis (RA) is characterized by polyarticular inflammation, which can lead to progressive joint destruction with resulting disability and an increased risk of mortality [1]. There are no pathognomonic lab tests or clinical findings for RA. However, serum autoantibodies are routinely measured in the clinical setting to aid in the diagnosis or classification of RA. Such disease-related autoantibodies include rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA). Recently, work led by our group has demonstrated that circulating autoantibodies targeting malondialdehyde-acetaldehyde (MAA; adducts generated during oxidative stress) are increased in RA relative to controls, enriched in RA synovium compared to that of osteoarthritis patients, are associated with but not completely overlapping with the

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presence of both RF and ACPA, and are often accompanied by extra-articular disease manifestations [2–4].

In addition to their role in disease classification, both RF and ACPA portend a more aggressive natural course in RA [5,6]. Likewise, we have shown that autoantibodies to MAA are associated with higher disease activity in RA [2] and these autoantibodies appear to decrease over time (although remain detectable) in the context of effective RA management [7]. An emerging body of evidence suggests that RA-related autoantibodies may be informative as part of a precision medicine approach, predicting RA treatment response [8–13]. The availability of predictive biomarkers has the potential to limit exposure and risk related to agents that are unlikely to be efficacious while reducing the time interval between diagnosis and attaining adequate disease control. For example, prior studies have demonstrated that individual autoantibody status may selectively predict biologic treatment response in RA. Specifically, both RF and ACPA positivity are each associated with improved treatment responses to rituximab, with ACPA positivity portending an approximate 3-fold odds of good response compared to ACPA negativity [11]. More recent studies have suggested that ACPA and/or RF positivity are also associated with greater clinical response to abatacept, with antibody status being comparably less predictive with the use of anti-tumor necrosis factor (TNF) inhibitors [8,9]. To date, there have been no studies evaluating whether anti-MAA antibody status predicts future biologic treatment response in RA.

Therefore, the aim of the current study was to evaluate whether combined positivity to three RA-associated autoantibodies (RF, ACPA, and anti-MAA antibody) predicts treatment response in patients with RA initiating biologic therapy. In this study, we used a well-characterized study population of RA patients initiating biologics to examine whether an expanded autoantibody profile would selectively predict treatment response to TNF inhibitors or non-TNF biologics including abatacept, tocilizumab, and rituximab, and whether that response was differential according to the biologic DMARDs mechanism of action. We hypothesized that clinical responses with biologic therapies would be positively associated with the number of pre-treatment autoantibodies detected in a dose-dependent fashion.

1. Methods and materials

1.1. Study participants

The study included participants from the Comparative Effectiveness Registry to study Therapies for Arthritis and Inflammatory Conditions (CERTAIN), a prospective, non-randomized cohort study of patients with RA that was initiated in 2010 [14]. Participants were assessed at three-month intervals for up to one year with banked serum available from enrollment and over follow-up. Patients enrolled in CERTAIN (between 2010 and 2014) fulfilled the 1987 American College of Rheumatology (ACR) classification criteria for RA [15], had at least moderate disease activity defined by a clinical disease activity index (CDAI) score > 10 at the time of biologic initiation, and were either starting or switching biologic agents (abatacept, adalimumab, certolizumab, etanercept, golimumab, infliximab, rituximab or tocilizumab). This study included a sub-cohort of CERTAIN: 1) biologic naïve patients initiating anti-TNF; 2) all patients initiating abatacept, irrespective of prior biologic exposure; and 3) patients initiating non-TNF/non-abatacept biologics, all with prior biologic exposure. The study was limited to all patients for whom banked serum samples and complete disease activity assessments were available at enrollment and at 6 months of follow-up to minimize missing data and to avoid outcome misclassification with earlier treatment withdrawal (Supplemental Figure 1). Reflecting CERTAIN's pragmatic study design, patients were treated at the discretion of the treating provider throughout observation. All CERTAIN participants provided informed consent prior to enrollment and the study was approved by both a central Institutional Review Board (IRB) [the New England IRB] and local and university-based IRBs if required at individual sites [15].

1.2. Disease activity assessments

For this sub-study, disease activity was assessed using the 28-joint Disease Activity Score (DAS28-CRP) with a primary outcome of experiencing at least a 1.2-unit improvement at 6 months. A change of 1.2 units or more has been demonstrated to reflect the minimal clinically important improvement for the DAS28-CRP [16]. We opted to examine a change in disease activity rather than achievement of an absolute disease activity threshold such as a DAS28-CRP < 2.6 (corresponding to clinical remission in RA [17]) as the former would be less dependent on baseline values. Moreover, sustained remission in RA is uncommon in clinical practice [18] and using such low threshold would limit our ability to detect meaningful associations given the low frequency of outcomes likely observed. The absolute change in DAS28-CRP over the first 6 months of observation was examined as a secondary outcome. Recognizing that baseline values influence the subsequent responses, baseline DAS28-CRP was accounted for as a covariate in all models shown.

1.3. Autoantibody measurement

RF and ACPA were measured using banked serum from enrollment in a central laboratory (ICON Central Laboratories, Farmingdale, NY). IgG anti-MAA antibody was measured using ELISA in the Experimental Immunology Laboratory at the University of Nebraska Medical Center as previously described [2]. We limited our analyses to the IgG anti-MAA antibody isotype as IgA and IgM antibody isotypes yielded similar results (data not shown) and we have previously shown that changes in IgG anti-MAA (vs. IgA and IgM) are most strongly correlated with treatment response in patients with RA [7]. RF was measured using particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Rotkruez, Switzerland; positivity > 14 U/ml) while ACPA was measured using a commercially available third-generation anti-cyclic citrullinated peptide (CCP) antibody ELISA (INOVA Diagnostics, San Diego, CA; positivity > 20 U/ml). As anti-MAA antibody is non-specific, detectable in most individuals at varying concentrations and no firm threshold has been established to define its seropositivity [3], IgG anti-MAA antibody positivity was defined as concentrations falling in the highest two tertiles among study participants, rendering a frequency of positivity that approximates the frequency of positivity for RF and ACPA. Participants were classified based on the number of baseline autoantibodies positive (range 0–3).

1.4. Statistical analysis

Participant characteristics were compared across 4 biologic treatment groups (anti-TNF, abatacept, tocilizumab, rituximab) using chi square tests for categorical variables or one-way ANOVAs for continuous variables. Associations between the number of baseline autoantibodies positive (1, 2, or 3 positive vs. 0) and DAS28-CRP improvement of 1.2 units or more at 6 months were examined using multivariable logistic regression. Covariates for multivariate models were identified a priori and included age, sex, race (Caucasian, African American, other), smoking status (never, former, current), body mass index (kg/m^2), concomitant use of non-steroidal anti-inflammatory drugs (NSAIDs), concomitant prednisone or methotrexate use, prior biologic exposure (yes/no), and baseline DAS28-CRP. Data for all covariates and disease activity measures were replete with the exception of NSAID use. For this variable, a missing response was imputed to mean "no current NSAID exposure." In secondary analyses, associations between the number of baseline autoantibodies with absolute change in DAS28-CRP were examined using multivariable linear regression, accounting for the same covariates noted above. In sensitivity analyses, these analyses were repeated among biologic naïve anti-TNF and abatacept-treated patients

excluding patients with previous biologic exposure. In additional analyses, we examined these associations in stratified analyses among patients receiving individual biologic agents, grouping anti-TNF use as a single exposure.

To examine the incremental predictive value of autoantibody status, beyond that attributed to other covariates, receiver operator curves (ROCs) were generated and area-under-the-curves (AUCs) were calculated. Specifically, we generated two ROCs, one including all of the variables included in our final multivariable model examining a DAS28-CRP response of 1.2 units or more and the other including the same variables but excluding the number of autoantibodies positive. To more closely examine the incremental predictive capacity of the different autoantibodies, we compared the fit of nested models using likelihood ratio tests. We specifically examined whether a multivariable model including only a single autoantibody status (ACPA, RF, or IgG anti-MAA alone) was significantly improved when values for the other two autoantibodies were added to the model. All analyses were completed using Stata version 16.1 (Stata Corp, College Station, TX).

2. Results

2.1. Patient characteristics

Among the 1,229 biologic courses examined in 1175 unique patients, the different biologic agents initiated included anti-TNF agents (n = 435; 35%), abatacept (n = 380; 31%), tocilizumab (n = 297; 24%), and rituximab (n = 117; 14%) (Table 1). Fifty-two patients contributed 2 separate biologic courses and 1 patient had three. The study population was comprised predominantly of women (79%), had a mean age of 57 years, and a majority (59%) had been exposed to prior biologic therapy. At enrollment, patients had active arthritis, reflected in a mean baseline DAS28-CRP score of 4.8. Patient characteristics were similar across biologic treatment groups with few exceptions. Patients initiating non-TNF biologics were older while those receiving rituximab were more likely to report current or former smoking status. Patients initiating anti-TNF agents were more likely to be receiving concomitant methotrexate and, along with those receiving rituximab, were more likely to be seropositive for RF.

2.2. Autoantibody status

A majority of the patients in this study were seropositive for ACPA (55%) or RF (60%). Using serum concentrations falling in the upper two tertiles to define positivity for IgG anti-MAA, 37% of patients were seropositive for all three autoantibodies; 22% were positive for two of three; 28% were positive for just one; and only 14% were seronegative for all 3. The various frequencies of seropositivity based on combinations of the three different autoantibodies are shown in Fig. 1. Twenty-one percent of patients were positive for IgG anti-MAA but negative for ACPA and RF.

2.3. Associations of autoantibody status with change in DAS28

In univariate analyses, both ACPA (60.2% vs. 46.4%; OR 1.74; 95% CI 1.38, 2.21) and RF (58.2% vs. 47.9%; OR 1.51; 95% CI 1.19, 1.92) positivity were individually associated with a greater likelihood of achieving at least a 1.2-unit DAS28-CRP improvement following the initiation of biologic therapy (Table 2). Likewise, in univariate linear regression, both ACPA (β −0.31; 95% CI −0.46, −0.16) and RF (β −0.29; 95% CI −0.45, −0.14) positivity were individually associated with greater absolute reduction (improvement) in DAS28-CRP over the first 6 months of biologic treatment. IgG MAA antibody positivity was not significantly associated with a DAS28-CRP decrease of 1.2 units (55.2% vs. 52.1%; OR 1.13; 95% CI 0.89, 1.45) but was associated with a small decrease in DAS28-CRP (β −0.16; 95% CI −0.33, −0.001).

In univariate analyses, the number of autoantibodies positive demonstrated a linear dose-dependent relationship with DAS28-CRP improvement, examined either as an improvement of 1.2 units or more or as an absolute change (p for trend < 0.001 for both). Associations between the number of autoantibodies positive and treatment

Table 1
Baseline patient characteristics at the time of enrollment (N = 1229).

	Total (n = 1229)	Anti-TNF (n = 435)	Abatacept (n = 380)	Tocilizumab (n = 297)	Rituximab (n = 117)	P value
Demographics and Health Behaviors						
Age, years	57 (13)	55 (13)	59 (13)	58 (13)	58 (12)	0.001
Female	971 (79)	333 (77)	299 (79)	248 (84)	91 (78)	0.16
Race						
White	1091 (89)	377 (88)	338 (90)	271 (92)	105 (90)	0.82
African American	75 (6)	31 (7)	22 (6)	15 (5)	7 (6)	
Other	54 (4)	22 (5)	17 (5)	10 (3)	5 (4)	
Smoking status						
Never	577 (48)	194 (46)	184 (49)	153 (52)	46 (39)	<0.001
Former	410 (34)	123 (29)	128 (34)	106 (36)	53 (45)	
Current	221 (18)	104 (25)	63 (17)	36 (12)	18 (15)	
Body mass index, kg/m^2	30 (7)	30 (7)	30 (7)	30 (7)	31 (8)	0.64
RA-Related Factors						
Anti-CCP positive	662 (55)	246 (57)	207 (56)	143 (50)	66 (59)	0.22
RF positive	726 (60)	287 (66)	212 (57)	151 (52)	76 (66)	<0.001
Anti-MAA positive	816 (67)	288 (67)	254 (67)	193 (65)	81 (69)	0.86
Number of positive autoantibodies						
0	162 (14)	48 (11)	56 (15)	44 (16)	14 (13)	0.14
1	331 (28)	113 (26)	99 (27)	93 (33)	26 (24)	
2	255 (22)	104 (24)	70 (19)	58 (20)	23 (21)	
3	437 (37)	163 (38)	139 (38)	88 (31)	47 (43)	
NSAID use	297 (24)	100 (23)	89 (23)	83 (28)	25 (21)	0.35
Prednisone use	400 (33)	138 (32)	133 (35)	89 (30)	40 (34)	0.53
Methotrexate use	740 (60)	319 (73)	219 (58)	143 (48)	59 (50)	<0.001
Prior biologic therapy	722 (59)	0 (0)	308 (81)	297 (100)	117 (100)	–
DAS28-CRP	4.8 (1.1)	4.9 (1.1)	4.7 (1.1)	5.0 (1.1)	4.9 (1.0)	0.02

Anti-TNF, anti-tumor necrosis factor; RA, rheumatoid arthritis; Anti-CCP, antibody to cyclic citrullinated peptides; RF, rheumatoid factor; anti-MAA, antibody to malondialdehyde-acetaldehyde adducts; NSAID, non-steroidal anti-inflammatory drug; DAS28-CRP, disease activity score for 28 joints – calculated using C-Reactive Protein measures. Missing values: Age – 1, Female – 1, Race – 9, Smoking Status – 21, Body mass index – 1, Anti-CCP – 32, RF – 15, Anti-MAA – 5, NSAID – 0, Prednisone – 0, Methotrexate – 0, Prior biologic therapy – 0, DAS28-CRP – 32.

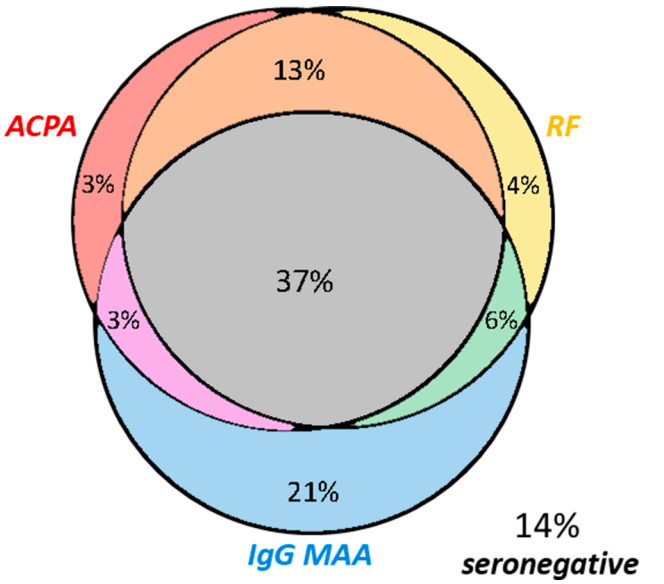

Fig. 1. Frequency of different combinations of autoantibody positivity patients with RA.

response to biologic therapies remained following multivariable adjustment (Table 2). After accounting for covariates, compared to seronegative individuals, patients with one positive antibody had 1.58-times greater odds (95% CI 1.04–2.40), those with two positive antibodies had 2.05-times greater odds (95% CI 1.33–3.18), and those with three positive antibodies had 2.35-times greater odds (95% CI 1.57–3.51) to have an improvement > 1.2 DAS28-CRP units after 6 months of biologic treatment (p for trend < 0.001). These odds ratios corresponded to response frequencies of 40.8%, 51.0%, 56.8%, and 59.7% for those with 0, 1, 2, or 3 autoantibodies positive, respectively. Compared to patients seronegative for all three autoantibodies, those with one positive antibody had an average improvement in disease activity that was 0.25 DAS28-CRP units greater (95% CI 0.02–0.48), those with two positive antibodies had an improvement of 0.36 units greater (95% CI 0.12–0.60), and those with three positive antibodies had an improvement of 0.48 units greater (95% CI 0.26–0.70) (p for trend < 0.001). Associations between other covariates examined and treatment response are shown in Table 2.

In subsequent sensitivity analyses limited to 507 biologic naïve patients, the associations between the number of autoantibodies positive and treatment response were similar (Table 3). In separate analyses examining the associations in patients receiving individual biologics, we observed similar associations between the number of autoantibodies positive and treatment response with anti-TNF agents and abatacept as in the overall analysis without significant differences between the different biologic classes (Supplemental Table 1). Associations between autoantibody status and treatment response with tocilizumab and rituximab (all with a history of prior biologic exposure) were less consistent (Supplemental Tables 2–3).

2.4. Incremental model performance with inclusion of autoantibody status

ROCs corresponding to multivariable logistic regression models predicting a DAS28-CRP improvement of at least 1.2 units, with and without considering the number of autoantibodies positive, are shown in Fig. 2. Model discrimination was only slightly better for the model including the number of positive autoantibodies (AUC 0.687) compared to the model without (AUC 0.672) (Fig. 2). Likelihood ratio (LR) tests were then used in order to examine the relative contribution of the three different autoantibodies to the multivariable linear and logistic regression models (Table 4). Adding RF and IgG anti-MAA positivity to a model already including ACPA status yielded no significant improvement in model fit when modeling either change in DAS28-CRP (p = 0.245) or a DAS28-CRP change of 1.2 units or more (p = 0.863). In contrast, sequential models including ACPA positivity (regardless of the other autoantibodies added) universally resulted in significant improvements in model fit.

Table 2
Unadjusted and adjusted associations of covariates with treatment response 6 months after initiating biologic treatment, represented by DAS28 change (N = 1229).

	DAS28 improvement ≥ 1.2 Odds Ratio (95%CI)		Change in DAS28 Beta Coefficient (95% CI)	
	Unadjusted	Adjusted	Unadjusted	Adjusted
IgG MAA, RF, CCP Positivity	***Referent***	***Referent***	***Referent***	***Referent***
0	1.59 (1.07, 2.34)	1.58 (1.04, 2.40)	−0.27 (−0.52, −0.02)	−0.25 (−0.48, −0.02)
1	2.03 (1.35, 3.05)	2.05 (1.33, 3.18)	−0.41 (−0.67, −0.15)	−0.36 (−0.60, −0.12)
2	2.23 (1.53, 3.24)	2.35 (1.57, 3.51)	−0.52 (−0.75, −0.28)	−0.48 (−0.70, −0.26)
3	P trend < 0.001	P trend < 0.001	P trend < 0.001	P trend < 0.001
Demographics and Health Behaviors				
Age, per 10 years	1.01 (0.93, 1.11)	–	0.01 (-0.05, 0.07)	–
Female	0.82 (0.61, 1.08)	0.86 (0.63, 1.19)	0.17 (-0.01, 0.36)	0.11 (-0.06, 0.29)
Race	***Referent***	***Referent***	***Referent***	***Referent***
White	0.49 (0.30, 0.81)	0.43 (0.25, 0.75)	0.37 (0.05, 0.69)	0.35 (0.05, 0.64)
African American	0.62 (0.36, 1.09)	0.51 (0.27, 0.94)	0.21 (-0.15, 0.58)	0.37 (0.04, 0.71)
Other	***Referent***	***Referent***	***Referent***	***Referent***
Smoking status	1.17 (0.90, 1.52)	1.13 (0.84, 1.50)	−0.06 (-0.23, 0.11)	−0.04 (-0.20, 0.12)
Never	1.22 (0.89, 1.69)	0.89 (0.62, 1.27)	−0.20 (-0.41, 0.01)	0.04 (-0.16, 0.24)
Former	0.97 (0.95, 0.98)	0.96 (0.94, 0.98)	0.02 (0.01, 0.04)	0.03 (0.02, 0.04)
Current	***RA-Related Factors***			
Body mass index, kg/m2	0.66 (0.51, 0.87)	0.77 (0.57, 1.03)	0.30 (0.13, 0.48)	0.16 (-0.00, 0.32)
RA-Related Factors	0.83 (0.65, 1.06)	–	0.08 (-0.08, 0.24)	–
NSAID use	0.84 (0.66, 1.06)	–	0.11 (-0.05, 0.26)	–
Prednisone use	0.75 (0.59, 0.94)	0.76 (0.44, 1.29)	0.22 (0.07, 0.38)	0.01 (-0.29, 0.31)
Methotrexate use	1.61 (1.43, 1.81)	1.73 (1.52, 1.97)	−0.47 (-0.54, −0.40)	−0.48 (-0.54, −0.41)
Prior biologic therapy	***Treatment Assignment***			
Baseline DAS28-CRP	***Referent***	***Referent***	***Referent***	***Referent***
Treatment Assignment	0.75 (0.59, 0.95)	0.96 (0.55, 1.67)	0.28 (0.12, 0.43)	0.23 (-0.08, 0.54)
Anti-TNF				
Non-TNF biologic				

IgG MAA, immunoglobulin G for malondialdehyde-acetaldehyde adducts; RF, rheumatoid factor; CCP, cyclic citrullinated peptides; DAS28-CRP, disease activity score for 28 joints – calculated using C-Reactive Protein measures; NSAID, non-steroidal anti-inflammatory drug; TNF, tumor necrosis factor; Among those seronegative for all 3 autoantibodies, the unadjusted mean (SD) improvement in DAS28 at 6 months was 1.03 (1.29) units.

3. Discussion

RA is a progressive disease that can lead to irreversible joint destruction and debilitation. Currently available conventional synthetic (cs)-DMARDs can effectively treat RA and halt the progression of the disease, although this is true only for a limited proportion of patients. In patients experiencing suboptimal treatment responses to csDMARDs, the

Table 3
Multivariable associations of patient factors with treatment response represented by DAS28 change, limited to biologic naïve anti-TNF and abatacept patients (n = 507).

	DAS28 Decrease ≥ 1.2Odds Ratio (95% CI)	Absolute DAS28 Change Beta Coefficient (95% CI)
IgG MAA, RF, CCP Positivity	***Referent***	***Referent***
0	2.12 (1.06, 4.23)	−0.40 (−0.79, −0.01)
1	3.30 (1.61, 6.80)	−0.61 (−1.01, −0.20)
2	3.58 (1.80, 7.11)	−0.76 (−1.14, −0.38)
3	P trend < 0.001	P trend < 0.001
Demographics and Health Behaviors		
Female (vs male)	0.66 (0.40, 1.08)	0.35 (0.07, 0.62)
Race	***Referent***	***Referent***
White	0.55 (0.25, 1.21)	0.34 (−0.11, 0.79)
African American	0.70 (0.28, 1.74)	0.03 (−0.48, 0.53)
Other		
Smoking status	***Referent***	***Referent***
Never	1.06 (0.67, 1.68)	0.05 (−0.21, 0.31)
Former	0.68 (0.40, 1.16)	0.24 (−0.06, 0.54)
Current		
Body mass index, kg/m2	0.98 (0.95, 1.01)	0.02 (0.00, 0.04)
RA-Related Factors		
NSAID use at baseline	0.79 (0.50, 1.24)	0.16 (−0.10, 0.42)
Baseline DAS28-CRP	1.66 (1.35, 2.04)	−0.43 (−0.54, −0.32)
Treatment Assignment		
Anti-TNF	***Referent***	***Referent***
Abatacept	0.94 (0.54, 1.65)	0.24 (−0.08, 0.56)

IgG MAA, immunoglobulin G for malondialdehyde-acetaldehyde adducts; RF, rheumatoid factor; CCP, cyclic citrullinated peptides; DAS28-CRP, disease activity score for 28 joints – calculated using C-Reactive Protein measures; NSAID, non-steroidal anti-inflammatory drug; TNF, tumor necrosis factor

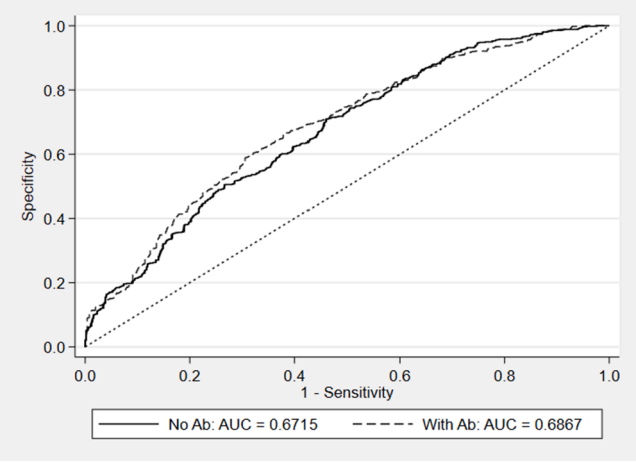

Fig. 2. Receiver Operator Curves (ROC) for multivariable logistic regression models predicting DAS28 improvements of at least 1.2 units 6 months after initiating biologic treatment. Area under the curve (AUC) shown for each model. Solid line: ROC generated using all variables shown in Table 1 excluding age and number of positive auto-antibodies. Dashed line: ROC generated using all variables above in addition to number of positive autoantibodies.

initiation of biologic therapy has been shown to be a highly effective management strategy in a number of randomized controlled trials [17,18]. Unfortunately, there is currently no way of identifying the patients most likely to benefit from biologic therapy or, when faced with several viable biologic modalities, the agent most likely to prove effective for an individual patient. Results from our study suggest that an expanded autoantibody profile that includes antibody to MAA in addition to the more conventional ACPA and RF measures could provide

Table 4
Nested model comparisons from multivariable models using likelihood ratio (LR) tests to determine if the additional variables make a significant contribution to the model.

Outcome	Predictors	LR p-value
Change in DAS28, Baseline to 6 Months	CCP alone vs. CCP, RF, IgG anti-MAA	0.245
	RF alone vs. RF, CCP, IgG anti-MAA	0.008
	IgG anti-MAA alone vs. IgG anti-MAA, CCP, RF	0.001
	CCP/RF alone vs. IgG anti-MAA, CCP, RF	0.115
Improvement in DAS28 of 1.2 or greater, Baseline to 6 months	CCP alone vs. CCP, RF, IgG anti-MAA	0.863
	RF alone vs. RF, CCP, IgG anti-MAA	0.002
	IgG anti-MAA alone vs. IgG anti-MAA, CCP, RF	<0.001
	CCP/RF alone vs. IgG anti-MAA, CCP, RF	0.596

*All models contain sex, race, smoking status, BMI, NSAID use indicator, prior biologic indicator, DAS28 at baseline, and TNF/Non-TNF indicator.

significant, albeit modest, predictive capacity among patients with RA initiating biologic treatment. Compared to those who were seronegative for all three autoantibodies examined, patients with at least one positive autoantibody achieved significantly greater improvements with biologic therapy. Moreover, we observed a dose-dependent relationship between the number of positive autoantibodies detected and the magnitude of clinical improvement achieved following biologic initiation. While the addition of autoantibody status assessment to models containing readily available clinical information yielded statistically significant associations, it is important to recognize that this addition led to only incremental improvements in prediction. Even so, of the three autoantibodies examined, ACPA clearly yielded the greatest predictive characteristics.

These findings are consistent with data from other studies that have evaluated autoantibody positivity and clinical responses to biologic treatments. It has been previously shown, for instance, that ACPA-positive RA patients who received either abatacept or an anti-TNF therapy experienced substantially more improvement in clinical disease activity after 6 months of treatment compared to seronegative patients [9]. The same study also showed similar associations between autoantibody status and treatment response for the different agents examined [9], in agreement with our results that also did not demonstrate significant differences when comparing different biologic mechanisms of action. Additional studies have shown that ACPA positivity predicts greater biologic treatment response with rituximab [11] as well as abatacept in biologic naïve patients [10]. By simultaneously examining multiple biologic therapies, our results suggest that these previously reported associations of autoantibody status with treatment response may not be agent specific.

Explanations for why autoantibody positivity might predict treatment responsiveness in this way are not currently known. One possibility is that these findings indicate a broader issue of disease misclassification. Individuals who are seropositive for these autoantibodies are more likely to represent “true” RA whereas other individuals may have conditions that mimic RA that, while manifesting similarly, are characterized by a distinct disease pathway that render these alternative conditions inherently less responsive to treatment with biologics. Similarly, autoantibody production correlates with disease activity and

severity on both a molecular and immune level, and as such, may serve as a non-specific surrogate for those most likely to benefit from anti-inflammatory interventions. Were this to be the case, these patients would perhaps be more responsive to therapies besides biologics. Simultaneous assessments of treatment responses in both seropositive and seronegative patients to other therapies, such as csDMARDs or glucocorticoids, may shed further light on the matter.

Further, the biologic therapies all target or modulate molecules or cells mediating adaptive autoimmune responses – anti-TNF inhibits adaptive autoimmune produced TNFα; tocilizumab inhibits IL-6 which regulates B and T cell responses; rituximab depletes B cells which can serve as professional antigen presenting cells, produce autoantibodies, and produce cytokines; and abatacept disrupts T cell co-stimulation. Both B and T cell phenotypes have been demonstrated to predict treatment responses in RA. Prior studies have shown, for instance, that different B cell phenotypes infiltrating diseased synovium may play protective or aggravating roles in RA treatment [19]. Likewise, prior reports have highlighted correlations between peripheral blood T cell repertoire, autoantibody expression and RA disease activity in DMARD-treated patients [20–22]. Given seropositivity reflects an adaptive autoimmune response, our results suggest that by preselecting RA patients with a clear autoimmune etiology (based on seropositivity) the clinical response rate to biologics which target the adaptive immune response is increased.

A potential limitation of this study is that the requirement for the patients initiated on biologics to have at least 6 months of follow-up may have inadvertently excluded patients who switched drug courses earlier than 6 months after starting the biologic. If this occurred, the results would likely underestimate the associations observed between the number of autoantibodies positive and treatment responses. Additionally, the biologic agent initiated for individual study participants was not randomly assigned, but rather chosen at the discretion of investigators whose patients were enrolled in CERTAIN, raising the possibility of selection bias. While we examined only a limited number of currently available therapies, the biologic agents included (anti-TNF inhibitors, abatacept, tocilizumab, and rituximab) are frequently used in clinical practice and integral to the treatment pathway for a large and growing number of patients with RA. Even so, other biologic agents and targeted small molecule agents are frequently used for the treatment of RA and were not examined based upon the time period of the study. Additional therapies for RA now include other interleukin (IL)-6 receptor inhibitors (i.e., sarilumab) and the Janus kinase (JAK) inhibitors (e.g. tofacitinib, baricitinib, and upadacitinib). Likewise, this study focused on a limited number of autoantibodies that have been identified in RA. In addition to RF, ACPA, and IgG anti-MAA examined herein, other autoantibodies such as those to peptidyl-arginine deiminase 4 (PAD4), antikeratin antibodies (AKA), antiperinuclear factor antibody (APF), antibodies against carbamylated proteins (anti-CarP) [23–26], and others could be informative in future studies. Autoantibody to PAD4, for example, has recently been shown to be associated with improved treatment responses as part of a post-hoc investigation using banked samples from a large randomized clinical trial comparing the addition of etanercept to triple combination csDMARDs (addition of hydroxychloquine and sulfasalazine) in patients failing methotrexate monotherapy [27].

In conclusion, the results of this study, which utilized an expanded autoantibody profile that consisted of ACPA, RF, and IgG anti-MAA antibodies, showed that the number of positive autoantibodies predicted treatment response in patients with RA following the initiation of a biologic therapy in a dose-dependent fashion. Of the three autoantibodies analyzed, ACPA positivity yielded the greatest predictive capacity and this association did not appear to differ substantially based on which biologic agent was used. Although the autoantibody profile examined yielded statistically significant associations with treatment response, the predictive capacity (above and beyond that observed with the use of readily available clinical factors) appears to be modest and likely insufficient at present for routine adoption in clinical practice. Additional study, incorporating other informative clinical factors or other novel biomarkers, will be needed before these autoantibodies could be leveraged as part of day-to-day practice for the prediction of treatment response in RA.

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CRediT authorship contribution statement

Alison D. Petro: Writing - original draft, Writing - review & editing, Visualization, Project administration. **Joseph Dougherty:** Writing - original draft, Writing - review & editing, Visualization. **Bryant R. England:** Data curation, Methodology, Writing - review & editing. **Harlan Sayles:** Data curation, Formal analysis, Visualization, Writing - review & editing. **Michael J. Duryee:** Validation, Data curation, Writing - review & editing. **Carlos D. Hunter:** Validation, Data curation, Writing - review & editing. **Joel M. Kremer:** Investigation, Resources, Data curation, Writing - review & editing, Funding acquisition. **Dimitrios A. Pappas:** Investigation, Resources, Data curation, Writing - review & editing. **William H. Robinson:** Writing - review & editing. **Jeffrey R. Curtis:** Investigation, Methodology, Resources, Data curation, Writing - review & editing, Funding acquisition. **Geoffrey M. Thiele:** Conceptualization, Validation, Resources, Data curation, Writing - review & editing, Supervision. **Ted R. Mikuls:** Conceptualization, Methodology, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Appendix A. Supplementary material

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