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ORIGINAL RESEARCH

# Autoantibody biomarkers and first-line therapy response in RA: findings from the CAP48 cohort

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## ABSTRACT

**Objective** A panel of antibodies against three antigens, University Hasselt (UH)-rheumatoid arthritis (RA).305, 318 and 329, has been associated with lack of response to first-line therapy in the Care in early RA trial. This study aimed to determine the association of this antibody panel with first-line therapy response in an independent cohort.

**Methods** Anti-UH-RA.305/318/329 antibody reactivity was determined using ELISA in 165 baseline samples of the CAP48 cohort, an observational cohort of patients with early and naïve RA treated mainly with methotrexate monotherapy. Multivariable analyses assessed associations between baseline antibody reactivity and failure to reach remission or low disease activity (LDA) at 3, 6, 9 and 24 months according to the Disease Activity Score 28-joint C-reactive protein (DAS28CRP) and clinical/simplified disease activity index (SDAI).

**Results** In the total RA cohort, baseline anti-UH-RA.305/318/329 antibody reactivity was significantly higher in patients not achieving LDA versus those achieving LDA at 9 months (31.6% vs 11.3% for DAS28CRP/SDAI; OR 3.64, 95% CI 1.34 to 9.91, p=0.045). In patients with seronegative RA, a significant association between antibody reactivity and not achieving LDA based on DAS28CRP (42.1% vs 12.5%; OR 5.09, 95% CI 1.2 to 27.0, p=0.05) was already observed at 6 months. After 24 months, baseline antibody positivity remained significantly associated with not achieving LDA based on SDAI (OR 29.9, 95% CI 2.5 to 109.2, p=0.01) in patients with seronegative status.

**Discussion** In the CAP48 cohort, the anti-UH-RA antibody panel was associated with a lack of response to first-line therapy for certain clinical measures, observed at 9 months in the total RA cohort and at 6 and 24 months in patients with seronegative status. The antibody panel should be further validated for its use in early personalised RA treatment.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Rheumatoid arthritis (RA) treatment is still largely guided by a trial-and-error approach, and over one-third of patients with RA fail to respond adequately to these standard treatments. In a recent study, we identified a novel panel of antibodies (University Hasselt (UH)-RA.305, 318, 329) that correlated with a lack of first-line treatment response in RA.

### WHAT THIS STUDY ADDS

⇒ This study assessed the association between baseline anti-UH-RA.305/318/329 antibody reactivity and treatment outcome in the CAP48 RA cohort, with associations with failure to achieve low disease activity observed at 9 months in the total cohort and at 6 and 24 months in the seronegative subgroup.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ In the long run, the integration of this antibody panel into clinical practice may facilitate earlier identification of patients who are less likely to benefit from conventional first-line therapies, allowing for more timely and individualised treatment interventions. Such a shift could contribute to improved long-term disease outcomes by enhancing the precision of care and selecting the best treatment trajectory.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterised by persistent inflammation that primarily affects the joints.[1] Due to its progressive nature and potential for irreversible damage, early and effective treatment is crucial to limit disease progression. Current guidelines from the European Alliance of Associations for Rheumatology (EULAR) recommend a combination of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), preferably methotrexate (MTX) and short-term glucocorticoids (GC) as the first-line treatment strategy to achieve remission or low disease activity (LDA).[2] Approximately one-third of patients fail to respond adequately to this first-line therapy and require second-line therapies, such as biologics DMARDs (bDMARDs) or targeted DMARDs (tsDMARDs).[3–5] Although achieving a good response within the first 3 months is strongly associated with improved

long-term outcomes, treatment escalation in routine practice is typically considered only after an insufficient response is observed, which may delay optimal disease control and contribute to adverse clinical and psychosocial outcomes.[6–10]

Currently, there are no reliable biomarkers to accurately predict a patient's response to first-line RA treatment, resulting in a trial-and-error approach to therapy. Some studies have identified potential predictors of MTX response, such as low CD39 expression on regulatory T cells, which impairs their suppressive function and correlates with poor outcomes of treatment with MTX.[11] Also, synovial biomarkers such as fibrinoid necrosis and CD68 could identify patients with early RA with higher disease activity and predict a better MTX treatment response at 3 months.[12] Other factors, such as pro-inflammatory cytokines (eg, interleukin (IL)-1β, IL-6), acute-phase reactants (erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)), clinical variables (disease activity and duration) and demographic factors (age and sex), have been linked to MTX non-response.[13–17] However, the lack of consistency and clinical validation of these markers has hindered their clinical utility. Moreover, autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), are well-established RA markers but have yielded inconsistent results in predicting therapy response.[18–20]

We recently identified antibodies against a panel of three antigens, University Hasselt (UH)-RA.305, 318 and 329, which were associated with lack of response to first-line therapy in the global RA population and patients with seronegative RA, based on early (week 8) and sustained (week 8–52) remission and LDA outcomes.[21] These antibodies were identified in the Care in Early RA (CareRA) trial, which involved 13 rheumatology practices in Flanders and studied different doses of GC with csDMARDs, including MTX, in early RA. Antigen discovery was performed using serological antigen selection (SAS), in which human RA synovial complementary (c)DNA phage display libraries were screened with baseline serum from patients who did not achieve remission by week 16 based on the Disease Activity Score 28-joint C-reactive protein (DAS28CRP) (n=20). This yielded 41 reactivity patterns, of which six antigens with high reactivity in non-remission pools and low reactivity in remission pools were further assessed in 136 individual CareRA baseline samples using phage ELISA. Antibodies against UH-RA.305, 318 and 329 demonstrated the strongest predictive value, identifying 36% of week-8 non-responders compared with 13% of responders (positive likelihood ratio ($LR^+$)=2.7, p=0.003).[21] In the current study, we aimed to investigate the potential of the anti-UH-RA.305/318/329 antibody panel as a biomarker for first-line therapy response in the CAP48 cohort, a prospective observational study of patients with early RA managed in routine clinical practice, where treatment decisions were guided by physician discretion rather than standardised protocols.[22]

## MATERIALS AND METHODS

### Patient samples

The CAP48 cohort is a Belgian multicentric, observational study comprising more than 600 patients under 50 years of age with early RA, juvenile idiopathic arthritis (JIA), systemic lupus erythematosus (SLE) or diffuse systemic sclerosis (DSS). At the time of analysis, the overall cohort included 373 patients with RA, 185 patients with JIA, 73 patients with SLE and 23 patients with DSS. CAP48 inclusion criteria for patients with RA were age <50 years, symptom onset within the past 12 months, meeting the 2010 American College of Rheumatology/EULAR classification criteria for RA and not having received prior DMARD therapy at baseline.[22] Patients were monitored quarterly during the first year and annually thereafter for up to 5 years. Peripheral blood samples, along with demographic, clinical and treatment data, were collected at baseline and at each follow-up visit.[22]

For the present study, a subset of patients with RA from the CAP48 cohort was selected at random from all eligible patients with available baseline serum samples and clinical data. This subset included 165 patients with RA with a mean age of 36 years; 82% were female and the median baseline DAS28CRP was 4.3. First-line therapy was initiated at baseline and was predominantly MTX, with a small proportion of the patients (n=31, 20.3%) receiving concomitant GC. Table 1 summarises the demographic and clinical characteristics of the included patients with RA.

Remission (rem) and LDA were assessed using the DAS28CRP, Simplified Disease Activity Index (SDAI) and Clinical Disease Activity Index (CDAI) at baseline and follow-up (3, 6, 9 and 24 months) visits. Remission and LDA were defined using standard cut-offs: DAS28CRP rem as <2.6, LDA as ≤3.2; CDAI rem as ≤2.8, LDA as ≤10; and SDAI rem as ≤3.3, LDA as ≤11. Sustained remission (sust rem) or sustained LDA (sust LDA) during the first year was defined as meeting the respective criteria across all follow-up assessments within that year. Patients who did not meet rem or LDA criteria at any of these visits during that year were classified as non-sust rem or non-sust LDA, respectively. The distribution of patients who did/did not reach rem/LDA at various time points, or sust rem/LDA, is shown in table 2.

### Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

### Phage ELISA

Antibody reactivity in serum was assessed against antigens UH-RA.305/318/329 displayed on phage surfaces using phage ELISA, as previously outlined in a study by Quaden *et al*.[23] In brief, 96-well high-binding microplates (Greiner, Belgium) were coated overnight at 4°C with 3.5 µg/mL anti-M13 mouse monoclonal antibody (clone MM05T, Sino Biological, China), diluted in a

**Table 1** Demographic and clinical features of patients with RA from the CAP48 cohort (n=165)

| Characteristic | | | | | |
|---|---|---|---|---|---|
| Age (years), mean (SD) | | | 36.1 (9.2)* | | |
| Gender (female), n (%) | | | 133 (82)* | | |
| Disease duration at BL (weeks), median (IQR) | | | 2.79 (0–11.1)† | | |
| GC use, n (%) | | | 31 (20.3)‡ | | |
| RF-positive, n (%) | | | 100 (63)§ | | |
| ACPA-positive, n (%) | | | 98 (61)§ | | |
| RF and ACPA-seronegative (%) | | | 46 (29)§ | | |
| **Clinical characteristic** | **Baseline** | **3M** | **6M** | **9M** | **24M** |
| CRP (mg/mL), median (IQR) | 0.7 (0.2–1.7) | 0.25 (0.1–0.94)¶ | 0.2 (0.1–6.4)‡ | 0.29 (0.1–0.62)** | 0.2 (0.1–0.52)** |
| DAS28CRP, median (IQR) | 4.3 (3.4–5.4) | 3.1 (1.8–4.1)¶ | 2.7 (1.2–3.6)‡ | 2.48 (1.6–3.4)** | 2.0 (1.5–3.38)¶ |
| Delta DAS28CRP, median (IQR) | – | 1.3 (0.68–2.3)¶ | 1.5 (0.71–2.4)‡ | 1.9 (0.8–2.8)** | 1.9 (0.9–3.0)¶ |
| SDAI, median (IQR) | 20.2 (13–31.8) | 9.8 (3.7–18.8)¶ | 6.7 (2.5–14.4)‡ | 5.8 (2.0–12.5)** | 5.2 (1.6–12.5)** |
| CDAI, median (IQR) | 18.5 (12–29.3)* | 10 (4.3–17.7)¶ | 7 (2.7–14.6)¶ | 6.8 (2.0–12.5)** | 4.3 (1.6–12.6)** |

*Missing values for 2 patients.
†Missing values for 1 patient.
‡Missing values for 11–20 patients.
§Missing values for 4–10 patients,
¶Missing values for 20–40 patients.
**Missing values for 41–55 patients.
ACPA, anti-citrullinated protein antibodies; BL, baseline; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS28CRP, Disease Activity Score in 28 joints with C-reactive protein; GC, glucocorticoids; M, months; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index.

**Table 2** Distribution of remission or LDA in patients with RA used for validation of antibody reactivity in individual samples

| | (Sust) rem n/N (%)* | Non-(sust) rem n/N (%)* | (Sust) LDA n/N (%)* | Non-(sust) LDA n/N (%)* |
|---|---|---|---|---|
| rem/LDA | | | | |
| 3M DAS28CRP | 58/142 (41) | 84/142 (59) | 75/142 (53) | 67/142 (47) |
| 3M SDAI | 33/142 (23) | 109/142 (77) | 76/142 (53) | 66/142 (47) |
| 3M CDAI | 20/129 (16) | 109/129 (84) | 66/129 (51) | 63/129 (49) |
| 6M DAS28CRP | 73/150 (49) | 77/150 (51) | 98/150 (65) | 52/150 (35) |
| 6M SDAI | 51/149 (34) | 98/149 (66) | 102/149 (68) | 47/149 (32) |
| 6M CDAI | 37/139 (27) | 102/139 (73) | 91/139 (65) | 48/139 (35) |
| 9M DAS28CRP | 63/118 (53) | 55/118 (47) | 80/118 (68) | 38/118 (32) |
| 9M SDAI | 43/118 (36) | 75/118 (64) | 80/118 (68) | 38/118 (32) |
| 9M CDAI | 34/112 (30) | 78/112 (70) | 73/112 (65) | 39/112 (35) |
| 24M DAS28CRP | 81/128 (63) | 47/128 (37) | 93/128 (73) | 35/128 (27) |
| 24M SDAI | 49/116 (42) | 67/116 (58) | 81/116 (70) | 35/116 (30) |
| 24M CDAI | 49/124 (40) | 75/124 (60) | 85/124 (69) | 39/124 (31) |
| Sust remission/LDA | | | | |
| 3M–12M DAS28CRP | 16/96 (17) | 80/96 (83) | 30/96 (32) | 66/96 (68) |
| 3M–12M SDAI | 11/96 (11) | 85/96 (89) | 33/96 (34) | 63/96 (66) |
| 3M–12M CDAI | 4/79 (5) | 75/79 (95) | 19/79 (24) | 60/79 (76) |

*Number and percentage of patients with RA who did (rem/LDA) or did not (non-rem/non-LDA) reach remission/LDA at 3, 6, 9 and 24 months or sust rem/sust LDA.
CDAI, Clinical Disease Activity Index; DAS28CRP, Disease Activity Score in 28 joints with C-reactive protein; LDA, patient reaching low disease activity; M, months; non-LDA, patient not reaching low disease activity; non-rem, patient not reaching disease remission; RA, rheumatoid arthritis; rem, patient reaching disease remission; SDAI, Simplified Disease Activity Index; sust, sustained.

coating buffer (0.1 M sodium carbonate-bicarbonate buffer, pH 9.6). After washing three times with phosphatase buffered saline (PBS; pH 7.4) containing 0.1% Tween-20 and one time with PBS (shaking at room temperature (RT)) and blocking with PBS supplemented with 5% skimmed milk powder (2 hours at 37°C), antigen-expressing phage (7.0×1011 cfu/mL in PBS with 5% (w/v) skimmed milk powder (Marvel PBS (MPBS)) were added and incubated at 37°C for 1 hour followed by 30 min at RT. This was followed by washing after which the diluted serum samples (1:100 in 5% MPBS) were incubated for 1 hour at 37°C and 30 min at RT. Detection was performed using horseradish peroxidase-conjugated goat anti-human IgG-Fc antibody (1 mg/mL, Bethyl, USA) for 1 hour at RT (1/10 000 in 5% MPBS). Following another wash, signal development was performed using 3,3′,5,5′-tetramethylbenzidine substrate for 10 min, and absorbance was read at 450 nm after stopping the reaction with 1.8 M sulfuric acid. Samples were tested in duplicate and repeated independently at least twice. Non-specific binding was controlled by testing against empty phage (without antigen display), excluding samples with optical density above 0.5 for empty phage. Results were expressed as the ratio of antigen-expressing phage optical density to empty phage optical density. The coefficient of variation for duplicate measurements and experimental repeats was accepted when it was below 20%.

### Statistical analysis

The antibody positivity cut-off was determined using the Pruned Exact Linear Time algorithm analysis in R Studio (V.2023.12.1+402, Posit PBC, USA) and was set at five times the standard deviation (SD) above the mean ratio (average (AVG)+5 SD) of all non-reactive samples (represented by the lowest subgroup from the changepoint analysis). A sample was classified as positive if it demonstrated antibody reactivity to at least one antigen in the UH-RA.305/318/329 panel.

Statistical analyses were conducted using SAS JMP Pro V.17.2 (SAS, USA), with significance defined as $p \leq 0.05$. Antibody positivity against individual UH-RA antigens was compared between the non-rem group and rem group by applying Fisher's exact test. A one-tailed test was applied to assess whether antibody positivity was significantly more frequent in the non-rem group. Associations between antibody panel reactivity and failure to achieve remission or LDA were evaluated using nominal logistic regression. The disease activity indices DAS28CRP, SDAI and CDAI were analysed as binary dependent variables. Univariable models included antibody reactivity against the panel as the sole predictor, whereas multivariable models additionally adjusted for age, sex and seronegativity (negative for both ACPA and RF). Predictor selection in multivariable analyses was performed using stepwise-backward elimination, retaining variables with $p \leq 0.05$ in the final model. Samples lacking follow-up measurements for the relevant disease activity index were excluded from the corresponding comparison; no imputation of missing data was performed.

Receiver operating characteristic (ROC) curves were generated to assess the discriminatory performance of anti-UH-RA.305/318/329 reactivity for predicting failure to achieve remission or LDA across the three disease activity indices.

To account for multiplicity (three disease activity indices across four time points; 12 hypotheses), adjusted p values were computed using the adaptive step-down Bonferroni procedure of Hochberg and Benjamini. This method provides asymptotic control of the family-wise error rate while incorporating an estimate of the number of true null hypotheses, allowing a less conservative adjustment than traditional Bonferroni-based procedures. The procedure is valid under general dependence structures.[24] Multiplicity corrections were applied separately for univariable and multivariable models and independently for analyses in the full RA cohort and the seronegative subgroup.

## RESULTS

### Baseline antibody reactivity against UH-RA antigens in total patients and patients with seronegative status

At baseline, a subset of patients with RA demonstrated antibody reactivity to the UH-RA antigen panel. Specifically, reactivity was observed in 2.4% (4/165) of patients with RA for UH-RA.305, 4.8% (8/165) for UH-RA.318 and 12.7% (21/165) for UH-RA.329. When considered as a panel (UH-RA.305/318/329), 17.6% (29/165) of patients with RA exhibited reactivity to at least one of the antigens. Among patients with seronegative RA, 4.3% (2/46) were antibody positive for UH-RA.305, 13.0% (6/46) for UH-RA.318 and 15.2% (7/46) for UH-RA.329. Overall, 26.1% (12/46) of patients with seronegative status showed reactivity to at least one of the UH-RA antigens.

### Baseline antibody reactivity against UH-RA. antigens was associated with non-LDA in the total RA cohort and patients with seronegative RA

Previously, we showed that baseline antibody reactivity against the UH-RA.305/318/329 panel was associated with non-rem and non-LDA in the CareRA trial.[21] To confirm these findings in an independent cohort, we assessed whether baseline antibody reactivity to this panel correlated with lack of therapy response at various time points in the CAP48 cohort. Baseline anti-UH-RA.305/318/329 antibody reactivity was significantly associated with failure to achieve LDA at 9 months after correction for multiple testing in the total RA cohort. Specifically, antibody positivity was observed in 31.6% of patients not achieving LDA versus 11.3% of patients with LDA using DAS28CRP and SDAI 9 months after baseline (table 3) with associations statistically significant in both univariable and multivariable analyses (OR (95% CI): DAS28CRP and SDAI: 3.64 (1.34 to 9.91), p=0.045 (table 3)). For the CDAI measure

**Table 3** Baseline anti-UH-RA.305/318/329 antibody reactivity according to disease LDA at different time points in the total RA cohort

| Disease activity measure | BL panel reactivity non-LDA* | BL panel reactivity LDA* | Univariable model† | | | Multivariable model‡ | | |
|---|---|---|---|---|---|---|---|---|
| | | | OR (95% CI) | P value | P value corrected | OR (95% CI) | P value | P value corrected |
| All RA (n=112–150)§ | | | | | | | | |
| 3M DAS28CRP | 11/67 (16.4) | 12/75 (16.0) | 1.04 (0.42 to 2.53) | 0.95 | 1.0 | 1.11 (0.43 to 2.84) | 0.82 | 1.0 |
| 3M SDAI | 11/66 (16.7) | 12/76 (15.8) | 1.07 (0.43 to 2.62) | 0.89 | 1.0 | 1.14 (0.45 to 2.88) | 0.76 | 1.0 |
| 3M CDAI | 11/63 (17.5) | 8/66 (12.1) | 1.53 (0.58 to 4.24) | 0.39 | 1.0 | 1.87 (0.67 to 5.54) | 0.235 | 1.0 |
| 6M DAS28CRP | 12/52 (23.1) | 12/98 (12.2) | 2.15 (0.88 to 5.25) | 0.091 | 0.46 | 2.15 (0.88 to 5.2) | 0.09 | 0.45 |
| 6M SDAI | 10/47 (21.3) | 14/102 (13.7) | 1.7 (0.68 to 4.15) | 0.253 | 1.0 | 1.55 (0.59 to 3.95) | 0.37 | 1.0 |
| 6M CDAI | 11/48 (22.9) | 13/91 (14.3) | 1.78 (0.72 to 4.37) | 0.21 | 1.0 | 1.71 (0.66 to 4.35) | 0.26 | 1.0 |
| 9M DAS28CRP | 12/38 (31.6) | 9/80 (11.3) | **3.64 (1.34 to 9.91)** | **0.009** | **0.045** | **3.64 (1.34 to 9.91)** | **0.009** | **0.045** |
| 9M SDAI | 12/38 (31.6) | 9/80 (11.3) | **3.64 (1.34 to 9.91)** | **0.009** | **0.045** | **3.64 (1.34 to 9.91)** | **0.009** | **0.045** |
| 9M CDAI | 12/39 (30.8) | 9/73 (12.3) | **3.16 (1.2 to 8.6)** | **0.02** | 0.1 | **3.16 (1.2 to 8.6)** | **0.02** | 0.1 |
| 24M DAS28CRP | 7/35 (20.0) | 11/93 (11.8) | 1.86 (0.63 to 5.21) | 0.25 | 1.0 | 1.6 (0.51 to 4.74) | 0.41 | 1.0 |
| 24M SDAI | 7/35 (20.0) | 9/81 (11.1) | 1.99 (0.66 to 5.89) | 0.22 | 1.0 | 1.68 (0.51 to 5.34) | 0.39 | 1.0 |
| 24M CDAI | 8/39 (20.5) | 8/85 (9.4) | 2.48 (0.84 to 7.33) | 0.097 | 0.49 | 2.48 (0.84 to 7.33) | 0.097 | 0.49 |

Bold values represent significant results with p values ≤0.05.
*Number and percentage of anti-UH-RA.305/318/329 antibody positive baseline samples from total patients with RA who did (LDA) or did not (non-LDA) reach LDA at different time points according to different disease activity measures.
†Univariable model for prediction of non-LDA using baseline anti-UH-RA.305/318/329 antibody reactivity. A p value ≤0.5 was considered to be statistically significant, and the 95% CI was analysed.
‡Multivariable model for prediction of non-LDA using baseline anti-UH-RA.305/318/329 antibody reactivity, corrected for age, gender and RF/ACPA status.
§Analysis based on all tested individual baseline total RA samples with available clinical data on LDA. P values were corrected for multiple testing in both univariable and multivariable models based on the adaptive step-down Bonferroni procedure of Hochberg and Benjamini.
ACPA, anti-citrullinated protein antibodies; BL, baseline; CDAI, Clinical Disease Activity Index; DAS28CRP, Disease Activity Score in 28 joints with C-reactive protein; LDA, patient reaching low disease activity; M, months; non-LDA, patient not reaching low disease activity; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; UH, University Hasselt.

for LDA at 9 months, following correction, the effect observed at this time point did not reach significance at the conventional 5% threshold, although it remained statistically significant at the 10% level (table 3). Antibody reactivity to each antigen in the panel was further evaluated with respect to DAS28CRP LDA status at 9 months. For UH-RA.329, 23.6% of patients without LDA were antibody-positive compared with 7.5% of patients with LDA (p=0.017; $LR^+$=3.2). Although similar trends were noted for UH-RA.305 and UH-RA.318, these differences were not statistically significant (table 4). In the seronegative RA subgroup, although comprising a small number of patients, baseline antibody positivity was significantly more frequent in patients not achieving LDA (42.1%) compared with LDA (12.5%) based on DAS28CRP at 6 months (table 5), in both univariable (OR (95% CI): 5.09 (1.2 to 27.0), p=0.026) and multivariable (OR (95% CI): 5.09 (1.2 to 27.0), p=0.05) models.

**Table 4** Baseline anti-UH-RA antibody reactivity according to DAS28CRP LDA at 9 months

| UH-RA. antigen | BL antibody reactivity 9M non-LDA | BL antibody reactivity 9M LDA | $LR^+$ | P value |
|---|---|---|---|---|
| UH-RA.305 | 3/38 (7.9) | 1/80 (1.3) | 6.1 | 0.098 |
| UH-RA.318 | 3/38 (7.9) | 3/80 (3.8) | 2.1 | 0.29 |
| UH-RA.329 | 9/38 (23.6) | 6/80 (7.5) | 3.2 | **0.017** |
| Panel of UH-RA.305/318/329 | 12/38 (31.6) | 9/80 (11.3) | 2.8 | **0.009** |

Antibody positivity against individual UH-RA antigens was compared between groups by applying Fisher's exact test. To test the probability that anti-UH-RA antibody positivity was greater in the non-LDA than in the LDA group, the one-tailed test was used. The number and percentage of anti-UH-RA positive baseline samples from patients who did (LDA) or did not (non-LDA) reach DAS28CRP remission at 9 months are shown. This analysis represents a comparison at a single time point and for a single outcome measure; therefore, no correction for multiple testing was applied. The panel of three antibodies indicates antibody reactivity against at least one of the antigens, namely, UH-RA.305, UH-RA.318 or UH-RA.329. Bold values represent significant results with p values ≤0.05.
BL, baseline; DAS28CRP, Disease Activity Score in 28 joints with C-reactive protein; LDA, low disease activity; $LR^+$, positive likelihood ratio; M, months; RA, rheumatoid arthritis; UH, University Hasselt.

**Table 5** Baseline anti-UH-RA.305/318/329 antibody reactivity according to LDA at different time points in patients with seronegative RA

| Disease activity measure | BL panel reactivity non-LDA* | BL panel reactivity LDA* | Univariable model† | | | Multivariable model‡ | | |
|---|---|---|---|---|---|---|---|---|
| | | | OR (95% CI) | P value | P value corrected | OR (95% CI) | P value | P value corrected |
| Seronegative RA (n=26–43)§ | | | | | | | | |
| 3M DAS28CRP | 5/16 (31.3) | 5/24 (20.8) | 1.72 (0.4 to 7.57) | 0.46 | 0.46 | 2.13 (0.47 to 9.87) | 0.32 | 0.64 |
| 3M SDAI | 5/16 (31.3) | 5/24 (20.8) | 1.72 (0.4 to 7.57) | 0.46 | 0.46 | 1.76 (0.36 to 8.58) | 0.48 | 0.96 |
| 3M CDAI | 5/14 (35.7) | 5/24 (20.8) | 2.11 (0.48 to 9.54) | 0.32 | 0.32 | 1.66 (0.3 to 9.07) | 0.56 | 0.96 |
| 6M DAS28CRP | 8/19 (42.1) | 3/24 (12.5) | **5.09 (1.2 to 27.0)** | **0.026** | **0.026** | **5.09 (1.2 to 27.0)** | **0.026** | **0.05** |
| 6M SDAI | 7/18 (38.9) | 4/25 (16.0) | 3.34 (0.83 to 15.22) | 0.091 | 0.091 | 3.54 (0.69 to 19.4) | 0.13 | 0.26 |
| 6M CDAI | 7/17 (41.2) | 4/23 (17.4) | 3.33 (0.81 to 15.42) | 0.096 | 0.096 | 2.45 (0.49 to 13.25) | 0.27 | 0.54 |
| 9M DAS28CRP | 6/12 (50.0) | 4/17 (23.5) | 3.25 (0.68 to 17.35) | 0.14 | 0.14 | 3.25 (0.68 to 17.35) | 0.14 | 0.28 |
| 9M SDAI | 6/12 (50.0) | 4/17 (23.5) | 3.25 (0.68 to 17.35) | 0.14 | 0.14 | 3.25 (0.68 to 17.35) | 0.14 | 0.28 |
| 9M CDAI | 6/12 (50.0) | 4/14 (28.6) | 2.5 (0.51 to 13.6) | 0.26 | 0.26 | 2.5 (0.51 to 13.6) | 0.26 | 0.52 |
| 24M DAS28CRP | 5/11 (45.5) | 3/20 (15.0) | 4.72 (0.89 to 29.5) | 0.068 | 0.068 | 3.37 (0.52 to 23.1) | 0.2 | 0.4 |
| 24M SDAI | 6/11 (56.6) | 1/19 (5.3) | **21.5 (2.83 to 462.4)** | **0.002** | **0.002** | **29.85 (2.5 to 109.2)** | **0.005** | **0.01** |
| 24M CDAI | 6/12 (50.0) | 2/19 (10.5) | **8.5 (1.51 to 70.2)** | **0.015** | **0.015** | **6.54 (1 to 64.43)** | **0.05** | 0.1 |

Bold values represent significant results with p values ≤0.05.
*Number and percentage of anti-UH-RA.305/318/329 antibody positive baseline samples from patients with seronegative RA who did (LDA) or did not (non-LDA) reach LDA at different time points according to different disease activity measures.
†Univariable model for prediction of non-LDA using baseline anti-UH-RA.305/318/329 antibody reactivity. A p value ≤0.5 was considered to be statistically significant, and the 95% CI was analysed.
‡Multivariable model for prediction of non-LDA using baseline anti-UH-RA.305/318/329 antibody reactivity, corrected for age, gender and RF/ACPA status.
§Analysis based on all tested individual baseline seronegative RA samples with available clinical data on LDA. P values were corrected for multiple testing in both univariable and multivariable models based on the adaptive step-down Bonferroni procedure of Hochberg and Benjamini.
ACPA, anti-citrullinated protein antibodies; BL, baseline; CDAI, Clinical Disease Activity Index; DAS28CRP, Disease Activity Score in 28 joints with C-reactive protein; LDA, patient reaching low disease activity; M, months; non-LDA, patient not reaching low disease activity; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; UH, University Hasselt.

At 24 months, while associations in the total RA group did not reach statistical significance, they were significant for non-LDA in patients with seronegative RA when measured using SDAI (OR (95% CI): 21.5 (2.83 to 462.4), p=0.002 for the univariable analysis and 29.9 (2.5 to 109.2), p=0.01 for the multivariable analysis) and CDAI (OR (95% CI): 8.5 (1.51 to 70.2), p=0.015 for the univariable analysis) (table 5). This association was reflected in the marked difference in baseline antibody positivity between LDA and non-LDA patients with seronegative RA at 24 months: 56.6% of patients without LDA versus 5.3% of patients with LDA based on SDAI, and 50% versus 10.5% based on CDAI. Notably, the wide CIs reflect the small number of patients in this subgroup, which limits the robustness of these estimates. Graphical representation of the ROC curves of the significant results (based on the uncorrected p values) of the multivariable analysis is shown in online supplemental figure 1.

Finally, the association between sustained non-LDA and antibody reactivity against our panel at baseline was investigated. In the total RA cohort, antibody reactivity was more frequently observed in patients with non-sust-LDA compared with those with sust-LDA, but this difference was not statistically significant. Similarly, in patients with seronegative status, there was a trend toward higher antibody reactivity in patients with non-sust-LDA based on DAS28CRP and SDAI, though these associations were not statistically supported (online supplemental table 1).

Collectively, these results confirm the association between anti-UH-RA. antibody reactivity and failure to achieve LDA in the CAP48 cohort, with associations emerging at 6 months for patients with seronegative RA and at 9 months for total patients with RA.

### Baseline antibody reactivity against UH-RA. antigens was associated with non-remission in the total RA cohort and patients with seronegative RA

Remission was also evaluated as an outcome, representing a more stringent benchmark of therapy response compared with LDA.[1] In the total RA population, the largest differences in baseline antibody positivity between non-remission and remission patients, and correspondingly the highest OR, were observed at 9 months, with the non-remission SDAI therapy response approaching significance (22.7% non-rem vs 9.3% rem; OR (95% CI): 2.86 (0.97 to 10.5), p=0.057 for multivariable analysis) (table 6). Because none of the remission outcomes reached statistical significance, no correction for multiple testing was applied. Similarly, in patients with seronegative status, baseline antibody reactivity showed a trend at 9 months toward association with SDAI non-remission (OR (95% CI): 4.44 (0.84 to 34.95), p=0.081

**Table 6** Baseline anti-UH-RA.305/318/329 antibody reactivity according to disease remission at different time points in the total RA cohort

| Disease activity measure | BL panel reactivity non-rem* | BL panel reactivity rem* | Univariable model† | | Multivariable model‡ | |
|---|---|---|---|---|---|---|
| | | | OR (95% CI) | P value | OR (95% CI) | P value |
| All RA (n=112–150)§ | | | | | | |
| 3M DAS28CRP | 12/84 (14.3) | 11/58 (19.0) | 0.71 (0.3 to 1.77) | 0.459 | 0.71 (0.3 to 1.77) | 0.452 |
| 3M SDAI | 16/109 (14.7) | 7/33 (21.2) | 0.64 (0.24 to 1.81) | 0.384 | 0.64 (0.24 to 1.81) | 0.384 |
| 3M CDAI | 15/109 (13.7) | 4/20 (20.0) | 0.64 (0.2 to 2.45) | 0.485 | 0.64 (0.2 to 2.45) | 0.487 |
| 6M DAS28CRP | 14/77 (18.2) | 10/73 (13.7) | 1.4 (0.58 to 3.47) | 0.45 | 1.35 (0.54 to 3.49) | 0.52 |
| 6M SDAI | 15/98 (15.3) | 9/51 (17.6) | 0.84 (0.35 to 2.16) | 0.713 | 0.8 (0.31 to 2.13) | 0.65 |
| 6M CDAI | 18/102 (17.6) | 6/37 (16.2) | 1.1 (0.42 to 3.28) | 0.843 | 1.06 (0.39 to 3.22) | 0.913 |
| 9M DAS28CRP | 13/55 (23.6) | 8/63 (12.7) | 2.13 (0.82 to 5.82) | 0.121 | 2.13 (0.82 to 5.82) | 0.121 |
| 9M SDAI | 17/75 (22.7) | 4/43 (9.3) | 2.86 (0.97 to 10.5) | 0.057 | 2.86 (0.97 to 10.5) | 0.057 |
| 9M CDAI | 17/78 (21.8) | 4/34 (11.7) | 2.09 (0.7 to 7.75) | 0.195 | 1.96 (0.64 to 7.32) | 0.247 |
| 24M DAS28CRP | 9/47 (19.2) | 9/81 (11.1) | 1.89 (0.69 to 5.24) | 0.21 | 1.7 (0.59 to 4.9) | 0.32 |
| 24M SDAI | 12/67 (17.9) | 4/49 (8.2) | 2.45 (0.79 to 9.52) | 0.12 | 2.45 (0.79 to 9.52) | 0.12 |
| 24M CDAI | 12/75 (16.0) | 4/49 (8.2) | 2.14 (0.7 to 8.05) | 0.19 | 2.2 (0.7 to 8.44) | 0.18 |

*Number and percentage of anti-UH-RA.305/318/329 antibody positive baseline samples from patients with total RA who did (rem) or did not (non-rem) reach remission at different time points to different disease activity measures.
†Univariable model for prediction of non-rem using baseline anti-UH-RA.305/318/329 antibody reactivity. A p value ≤0.5 was considered to be statistically significant, and the 95% CI was analysed.
‡Multivariable model for prediction of non-rem using baseline anti-UH-RA.305/318/329 antibody reactivity, corrected for age, gender and RF/ACPA status.
§Analysis based on all tested individual baseline total RA samples with available clinical data on remission. No correction for multiple testing was carried out as all of the p values were not statistically significant.
ACPA, anti-citrullinated protein antibodies; BL, baseline; CDAI, Clinical Disease Activity Index; DAS28CRP, Disease Activity Score in 28 joints with C-reactive protein; M, months; non-rem, patient not reaching disease remission; RA, rheumatoid arthritis; rem, patient reaching disease remission; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; UH, University Hasselt.

for multivariable analysis) (table 7). At 24 months, baseline antibody reactivity showed a trend towards association with SDAI non-remission (OR (95% CI): 6.54 (0.91 to 13.4), p=0.06) (table 7).

For both the total RA cohort and the subgroup of patients with seronegative RA, no statistically significant differences in baseline antibody reactivity were observed between non-remission and remission across all remission indices (DAS28CRP, SDAI and CDAI; tables 6 and 7) at 3 and 6 months. Furthermore, baseline antibody reactivity against the UH-RA. antigens was not associated with sustained non-remission across any disease activity index in the total RA population (online supplemental table 2).

## DISCUSSION

Results of this study indicate that antibody reactivity to the UH-RA.305/318/329 panel was more frequent among patients with RA who did not achieve LDA compared with those who did achieve LDA according to the DAS28CRP at 9 months (OR 3.64) after the start of first-line treatment. Although most of the patients received csDMARD therapy, a small proportion (9.5%) did receive bDMARDs during the study period, and this rate was stable across 3, 6 and 9 months, suggesting that these treatments are unlikely to explain the differences in predictive associations observed at month 9. Moreover, in patients with seronegative RA, the antibody panel was significantly associated with the lack of LDA at both 6 (OR 5.09) and 24 months (OR 29.9) after the start of treatment. Earlier results from the CareRA trial showed that baseline antibody reactivity to this panel was associated with failure to achieve remission as early as 8 weeks but with comparable effect size (OR 3.63 for DAS28CRP) with particularly strong predictive value in patients with seronegative status (OR 17.3 for week-8 non-rem).[21] Notably, the proportion of antibody-positive patients among non-responders versus responders in CAP48 at 9 months (DAS28CRP non-LDA: 31.6% vs 11.3%) was similar to that in CareRA at 8 weeks (DAS28CRP non-rem: 36% vs 13%). Thus, the association between the anti-UH-RA antibody panel and lack of therapy response is of similar magnitude in both studies, but the timing differs, occurring later in CAP48 than in CareRA.

The differing treatment protocols between these cohorts provide important context for interpreting the timing of observed associations between antibody reactivity and disease activity. The CAP48 cohort is a prospective observational study of younger adults (<50 years) that reflects routine clinical practice, with treatment, most often MTX monotherapy and limited GC use, left to the

**Table 7** Baseline anti-UH-RA.305/318/329 antibody reactivity according to disease remission at different time points in patients with seronegative RA

| Disease activity measure | BL panel reactivity non-rem* | BL panel reactivity rem* | Univariable model† | | Multivariable model‡ | |
|---|---|---|---|---|---|---|
| | | | OR (95% CI) | P value | OR (95% CI) | P value |
| Seronegative RA (n=26–43)§ | | | | | | |
| 3M DAS28CRP | 6/24 (25.0) | 4/16 (25.0) | 1 (0.23 to 4.6) | 1 | 1.1 (0.24 to 5.4) | 0.89 |
| 3M SDAI | 8/31 (25.8) | 2/9 (22.2) | 1.21 (0.23 to 9.31) | 0.825 | 1.56 (0.28 to 13.1) | 0.64 |
| 3M CDAI | 7/31 (22.6) | 3/7 (42.9) | 0.39 (0.06 to 2.35) | 0.28 | 0.39 (0.07 to 2.35) | 0.29 |
| 6M DAS28CRP | 8/24 (33.3) | 3/19 (15.8) | 2.67 (0.64 to 13.9) | 0.183 | 3.12 (0.55 to 21.6) | 0.2 |
| 6M SDAI | 8/29 (27.6) | 3/14 (21.4) | 1.4 (0.32 to 7.57) | 0.66 | 1.47 (0.28 to 8.9) | 0.65 |
| 6M CDAI | 8/30 (26.7) | 3/10 (30.0) | 0.84 (0.18 to 4.66) | 0.839 | 0.82 (0.13 to 5.24) | 0.82 |
| 9M DAS28CRP | 6/14 (42.9) | 4/15 (26.7) | 2.06 (0.44 to 10.53) | 0.358 | 2.06 (0.44 to 10.53) | 0.358 |
| 9M SDAI | 8/17 (47.1) | 2/12 (16.7) | 4.44 (0.84 to 34.95) | 0.081 | 4.44 (0.84 to 34.95) | 0.081 |
| 9M CDAI | 8/17 (47.1) | 2/9 (22.2) | 3.11 (0.55 to 25.2) | 0.21 | 3.11 (0.55 to 25.2) | 0.21 |
| 24M DAS28CRP | 6/15 (40.0) | 2/16 (12.5) | 4.67 (0.86 to 36.9) | 0.075 | 3.42 (0.52 to 29.9) | 0.2 |
| 24M SDAI | 6/17 (35.3) | 1/13 (7.7) | 6.54 (0.91 to 134.1) | 0.06 | 6.54 (0.91 to 134.1) | 0.06 |
| 24M CDAI | 6/17 (35.3) | 2/14 (14.3) | 3.27 (0.6 to 25.7) | 0.174 | 2.5 (0.4 to 20.69) | 0.33 |

*Number and percentage of anti-UH-RA.305/318/329 antibody positive baseline samples from patients with seronegative RA who did (rem) or did not (non-rem) reach remission at different time points according to different disease activity measures.
†Univariable model for prediction of non-rem using baseline anti-UH-RA.305/318/329 antibody reactivity. A p value ≤0.5 was considered to be statistically significant, and the 95% CI was analysed.
‡Multivariable model for prediction of non-rem using baseline anti-UH-RA.305/318/329 antibody reactivity, corrected for age, gender and RF/ACPA status.
§Analysis based on all tested individual baseline RA seronegative samples with available clinical data on remission. No correction for multiple testing was carried out as all of the p values were not statistically significant.
ACPA, anti-citrullinated protein antibodies; BL, baseline; CDAI, Clinical Disease Activity Index; DAS28CRP, Disease Activity Score in 28 joints with C-reactive protein; M, months; non-rem, patient not reaching disease remission; RA, rheumatoid arthritis; rem, patient reaching disease remission; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; UH, University Hasselt.

rheumatologist's discretion.[22] In contrast, the CareRA trial was a randomised controlled trial, which applied a predefined treat-to-target strategy in which patients were stratified by prognostic profile and treated with predefined MTX-based strategies that included GC bridging, leading to rapid disease suppression and enabling baseline antibody reactivity to predict non-remission as early as week 8. This rapid onset mirrors earlier studies showing that GC bridging enhances early treatment efficacy, with higher remission rates (65.1% vs 46.8%) and quicker disease activity reductions (DAS28CRP Area under the curve (AUC): 11.18 vs 13.84) compared with MTX monotherapy.[7 25] The delayed associations in the current study using the CAP48 cohort may reflect the time-dependent action of MTX, where it requires weeks to months to reach therapeutic levels and achieve clinical effectiveness, as demonstrated in pharmacokinetic studies where steady-state levels were achieved after approximately 22 weeks.[26] In this study, the therapeutic response was slower than that of the CareRA trial, with only 41% of CAP48 patients achieving DAS28CRP remission at 3 months (table 2), compared with 67% patients by week 8 in the CareRA trial.[21] As a result, the association between antibody reactivity and clinical outcomes only emerged at later time points after sufficient exposure to effective therapy. Alternatively, the lack of associations at early time points in the current study could be due to insufficient statistical power because of a smaller proportion of non-responders at early time points as well as a relatively low rate of antibody reactivity. Taken together, these observations indicate that the timing and detectability of associations may depend on both therapeutic context and cohort characteristics.

Furthermore, the absence of a significant association between baseline antibody reactivity and stringent remission endpoints in this study may reflect the challenging nature of achieving remission in routine clinical settings. Remission represents a more rigorous target when compared with LDA, typically attained by only 30–60% of patients with RA, whereas LDA is achieved more frequently (50–80%) depending on the specific disease activity criteria used.[27–30] This discrepancy may explain why stronger associations were observed with LDA rather than remission outcomes, especially in an observational cohort where treatment intensity and adherence may vary. In addition, the lower remission rates observed in the CAP48 cohort may have limited statistical power, further contributing to the lack of significant associations.

While our study provides valuable insights into the association between baseline antibody reactivity against

the UH-RA. antigens and treatment outcomes in patients with RA, several limitations should be considered. The relatively small sample sizes, especially within patients with seronegative status, which is a clinically important group, may limit the statistical power to detect significant associations. As the seronegative subgroup comprised fewer patients, effect estimates should be interpreted in this context, although the overall association patterns were consistent with those seen in the total cohort. One limitation of our approach is that analyses of disease activity focused on the achievement of remission or LDA based on established cut-offs, rather than on the magnitude of improvement from baseline. However, attainment of remission or LDA early in the treatment course has been shown to be more clinically informative than relative change in disease activity.[6] Another limitation is that although the anti-UH-RA. antibody panel showed predictive potential, antibody testing was limited to baseline only. Thus, dynamic changes in antibody levels over time were not assessed, which could provide additional insight into their clinical relevance. Nonetheless, baseline remains the most clinically relevant time point for a marker, as treatment decisions are made before initiation of therapy. Finally, the observational design of the CAP48 cohort, while reflective of real-world clinical practice, introduces potential confounding due to variability in treatment decisions, timing of evaluations and follow-up intensity across centres. Despite modest sensitivity, the UH-RA.305/318/329 panel could still help to bridge a key clinical gap by identifying otherwise unrecognised early non-responders, and its performance could be enhanced in the future through combination with additional biomarkers as part of a multimarker precision-medicine approach. However, before clinical implementation can be considered, the added value of the UH-RA.305/318/329 antibody panel over standard treat-to-target strategies must be formally evaluated in a randomised controlled trial in the broad RA population, with subgroup analyses in patients with seronegative status. Such a study would compare antibody-guided treatment strategies with standard clinical judgement and assess whether this approach improves outcomes such as achievement of remission or LDA and treatment escalation. In conclusion, baseline antibody reactivity against UH-RA. antigens is associated with lack of therapy response (non-LDA), in the global RA population at 9 months (DAS28CRP/SDAI) and patients with seronegative status at 6 and 24 months (DAS28CRP and SDAI, respectively) in the CAP48 cohort. These findings are partly concordant with earlier observations and suggest that the anti-UH-RA antibody panel may capture biologically relevant differences in treatment responsiveness. Nevertheless, further validation in larger, prospective and interventional studies is needed to determine the biomarker potential of this antibody panel.

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**Competing interests** VS and PV have a patent application filed on the biomarkers described in this report. All other authors have no competing interests to declare.

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**Ethics approval** This study was conducted in accordance with the Helsinki Declaration and approved by local ethics committees of Hasselt University (CME2022/056) and Comité d'Ethique Hospitalo-Facultaire-Saint-Luc-UCLouvain (2013/24MAI/293). Written informed consent was obtained from all participants.

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