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ORIGINAL RESEARCH

Disease response in rheumatoid arthritis across four biologic therapies associates with improvement in paraoxonase-1 activity and oxylipins

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ABSTRACT

Objective Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme, that has been implicated as a biomarker of cardiovascular risk in patients with rheumatoid arthritis (RA). We aimed to investigate how different biologic therapies affect levels of PON1 and oxylipins.

Methods 1213 adult patients with RA in the Comparative Effectiveness Registry to study Therapies for Arthritis and Inflammatory CoNditions cohort study with moderate-to-high disease activity (Clinical Disease Activity Index (CDAI) >10) who initiated a new biologic (tocilizumab (TCZ), n=296; abatacept, n=374; tumour necrosis factor inhibitors, n=427; rituximab, n=116) were followed prospectively with serum specimens analysed for PON1 activity by arylesterase (ARYL), lactonase (LAC) and PON assays at baseline and after 6 months of biologic therapy. A targeted panel of oxylipins was evaluated by liquid chromatography-mass spectrometry/mass spectrometry in a subset of patients with the lowest and highest 6-month Disease Activity Score 28 (DAS28)-C reactive protein (CRP) responses in each treatment group.

Results PON1 activity generally increased in the entire cohort after 6 months of new biologic therapy, showing the greatest, most consistent increases in the TCZ group. Increases in all three PON1 domains associated with significant decreases in disease activity in DAS28-CRP/CDAI ($p<0.05$), and increases in LAC/ARYL were significantly associated with the American College of Rheumatology 20/50/70 responses (OR (95% CI) of 1.12 (1.04, 1.22) and 1.13 (1.04, 1.23), $p<0.01$, respectively), after controlling for other RA disease characteristics. Some oxylipins, including 12-hydroxyeicosatetraenoic acid correlated with RA disease activity measures.

Conclusion Improvement in disease activity across four classes of biologics is associated with enhanced PON1 activity, which has significant implications for cardiovascular safety.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Suppression of paraoxonase-1 (PON1) activity has been associated with increased risk of cardiovascular events in patients with rheumatoid arthritis (RA).

WHAT THIS STUDY ADDS

⇒ Patients with RA starting a new biologic (tumour necrosis factor inhibitor, rituximab, tocilizumab or abatacept) show increases in their PON1 activity, with the most marked increases noted in patients treated with tocilizumab.

⇒ Disease activity improvements in RA are associated with increases in PON1 activity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study provides a potential mechanism by which improved RA disease activity leads to decreased cardiovascular risk through improvements in PON1 activity.

INTRODUCTION

High-density lipoprotein (HDL) and its associated antioxidant enzyme, paraoxonase-1 (PON1), play major roles in the pathogenesis of atherosclerosis through metabolism of pro-inflammatory, oxidised lipids and regulation of systemic oxidative stress.[1] Lower PON1 activity is associated with increased risk of cardiovascular (CV) events in the general population,[2] and it predicts risk of CV and malignancy events after controlling for traditional risk factors in patients with RA treated with the Janus kinase (JAK) inhibitor tofacitinib.[3] PON1 activity is suppressed by active disease,[4 5] and growing data also suggest that moderate-to-high RA disease activity is associated with increased CV risk in RA, while low disease activity or remission is associated with decreased CV risk.[6 7]

Some of PON1's major anti-oxidant roles are to inactivate low-density lipoprotein (LDL)-derived oxidised phospholipids, and to

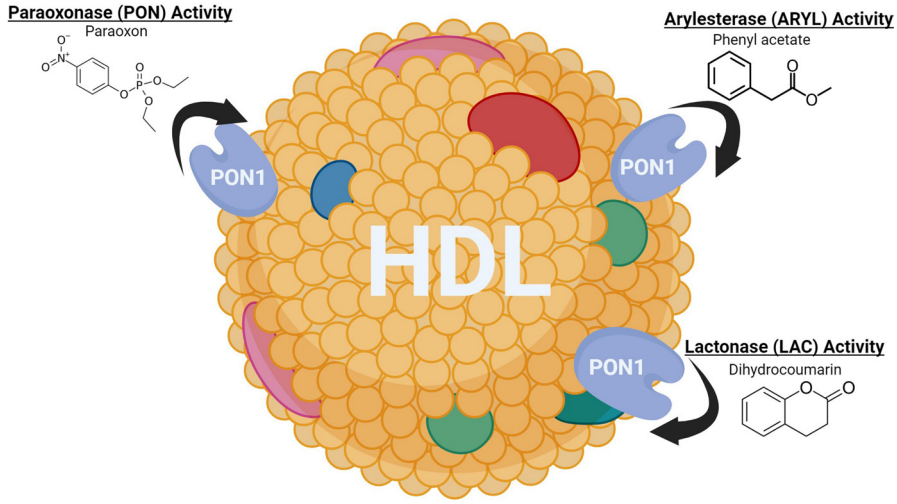

Figure 1 PON1 activities represented on an HDL particle. This figure depicts PON1 as an HDL-associated enzyme. Additionally, it shows the various in vitro activities of PON1 (PON, ARYL and LAC activities) and examples of their substrates which are used to measure PON1 activity. ARYL, arylesterase; LAC, lactonase; HDL, high-density lipoprotein; PON1, paraoxonase 1.

slow the oxidation of phospholipids in HDL.[8] PON1 has paraoxonase (PON), lactonase (LAC) and arylesterase (ARYL) enzyme activities, as in vitro HDL-associated PON1 hydrolyses substrates representing each of the three enzyme activities[8] (figure 1). However, the in vivo physiological substrates of PON1 remain unclear. Our recent work in a mouse model of RA demonstrated that overexpression of the human PON1 transgene, which increases circulating PON1 activity twofold to threefold, reduced arthritis scores and histological joint damage.[9] These improvements in inflammatory arthritis are associated with decreases in pro-inflammatory oxylipins compared with controls without changes of cytokine/chemokine levels. Oxylipins are oxidised metabolites of polyunsaturated fatty acids that serve as important paracrine modulators of innate immune responses including inflammatory responses and tissue repair.[10]

The CorEvitas (formerly Corrona) RA registry was initiated in 2001 and is the largest independent database in North America collecting standardised outcome metrics from both rheumatologists and patients.[11] The Comparative Effectiveness Registry to study Therapies for Arthritis and Inflammatory CoNditions (CERTAIN) study is a prospective cohort nested within the CorEvitas registry. Adult patients with RA were eligible to participate in CERTAIN if they had at least moderate disease activity at the time of an initiation of a biologic disease-modifying antirheumatic drug (bDMARD). Biorepository samples and clinical data including disease activity measures were collected at baseline and longitudinally, along with a large array of centrally processed laboratory values in this patient cohort following the initiation of a new biologic therapy.[12]

The aim of the current work was to determine the association of response to RA treatment across four different biologic classes of therapy with changes in PON1 activity and a targeted panel of oxylipins previously linked to systemic oxidative stress.[2] Investigation of these associations is expected to further our understanding of mechanisms by which reduction in RA disease activity, irrespective of specific treatment, is associated with reduction in CV risk, an observation noted in several studies.[4]

PATIENTS AND METHODS

Study design

This study was conducted as a prospective cohort study of patients enrolled in the CERTAIN study.[12] Of note, the CERTAIN study was a prospective cohort substudy of the former Consortium of Rheumatology Researchers of North America (formerly known as Corrona, now named CorEvitas). Patients were recruited into the study if their treating rheumatologist deemed it appropriate to start a new biologic agent. Inclusion criteria for CERTAIN were as follows: (1) adult patients with RA fulfilling the 1987 American College of Rheumatology (ACR) RA classification criteria; (2) moderate or high disease activity as defined by Clinical Disease Activity Index (CDAI) >10 at the time of a new biologic start; (3) initiating or switching to a new bDMARD. Clinical and laboratory assessments were conducted at baseline (on the day of and prior to initiation of a new biologic agent), and then every 3 months for a year. Patients who stopped the biologic agent prior to 12 months postinitiation exited CERTAIN. For the present analysis, a subset of patients were selected, baseline and 6-month data were used. The choice of bDMARD was that of the treating physician and was not randomised. The subset of the CERTAIN study analysed here included biologic-naïve patients initiating tumour necrosis factor (TNF) therapy, as well as biologic-experienced patients initiating rituximab (RTX) or tocilizumab (TCZ), and those starting abatacept (ABA) regardless of previous exposure. Patients were divided into the following biologic treatment groups: (1) TNF

inhibitors (TNFi); (2) RTX; (3) ABA; (4) TCZ. All TNFi patients were biologic-naïve (n=427), followed by 25% of ABA patients (n=93/374), with very few biologic-naïve patients in the RTX (n=2/116) and TCZ groups (n=2/296). This reflects real-life clinical practice, as TNFi are usually first-line biologics in RA, RTX/TCZ often second-line or third-line, and ABA either first-line or later. Baseline demographic information, RA disease activity (CDAI, Disease Activity Score 28 (DAS28)-C reactive protein (CRP)) and standardised RA clinical metrics were collected for all patients at all visits. PON1 activities and oxylipin values were measured from serum samples stored at −70°C collected at 0 and 6 months as described below.

Outcomes

The primary outcomes were the changes in PON1 activity from baseline to 6 months across the four treatment groups in relation to standardised disease activity metrics (ACR 20/50/70, DAS28-CRP and CDAI). Secondary outcomes included the associations between changes in oxylipins with RA disease responses.

PON1 activity, oxylipins, traditional lipids

PON1 activity was measured in all patient serum samples by PON, LAC and ARYL assays as described previously.[9] Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis was performed on an Agilent 1290/SCIEX 5500 QTRap LC-MS/MS system for a targeted lipidomics panel of over 40 oxylipins in 50 patient subsets of the larger cohort with the greatest DAS28 treatment response and 50 patients with the least DAS28 treatment response in each therapeutic group. Sample preparation and LC-MS/MS settings are as previously described[13] for a lipidomics panel including: 20-hydroxy leukotriene B4, thromboxane B2, prostaglandin E2, 6-trans 12-epi leukotriene B4 (6t12eLTB4), leukotriene B4 (LTB4), 13-hydroxyoctadecadienoic acid (13-HODE), 12-HODE, 9-HODE, 15-hydroxyeicosatetraenoic acid (15-HETE), 12-HETE, 11-HETE, 5-HETE, 14S-hydroxydocosahexaenoic acid (14S-HDHA) and 5-oxo-HETE.[9] HDL and LDL were measured in a central lab in all patients; LDL was measured directly, and not calculated. Rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) were also measured centrally.

Statistical analysis

Data analysis was performed using SAS V.9.4 (SAS Institute, Cary, North Carolina, USA). Descriptive statistics of baseline demographics, PON1 activity and lipids by treatment groups were reported, and comparison between groups were determined by one-way analysis of variance for continuous variables, and χ^2 tests for categorical variables. Prior to data analysis, PON1 activities (PON, LAC, ARYL), and oxylipins were $\log_{10}$ transformed to approximate a normal distribution. Mixed models were fitted to examine the effect of time, treatment and their interaction on PON1 activity, traditional lipids and oxylipins. A multivariate ordinal logistic regression model termed 'ACR-Max' was used to explore the association between change in PON activity and ACR 20/50/70 responses. ACR non-responders were coded as '0', ACR 20 responders as '1', ACR 50 responders as '2' and ACR 70 responders as '3'. Of note, patients who were included in a higher ACR response group were not included in the lower response categories, that is, the responders were mutually exclusive (ACR 70 responder was not included in ACR 50 or ACR 20 groups). In addition, separate logistic regression models were fitted using ACR 20, ACR 50 or ACR 70 responses as dependent variables. Finally, multivariable linear regressions were performed to evaluate the relationship of changes in PON1 activity, with changes in RA disease activity (DAS28-CRP, CDAI). Covariates for these models were selected by backwards stepwise regression with a predetermined set of covariates including age, sex, body mass index (BMI), seropositivity and RA disease duration, with a p value of 0.05 as the selection criteria. As a sensitivity analysis, a similar analysis was performed to control for the per cent change in HDL in addition to the aforementioned covariates because PON1 is an HDL-associated protein. ORs were presented for logistic and ordinal regressions, and beta-coefficients for linear models. Significance level was prespecified at $p<0.05$.

Patient and public involvement

The patient and public were not involved in the design, conduct, or reporting of this research project.

RESULTS

Patient clinical and laboratory characteristics

A total of 1213 patients were included in the study, with 427 (35%) in the TNFi group, 116 (10%) in the RTX group, 375 (31%) in the ABA group and 296 (24%) in the TCZ group. The average age of the cohort was 57.2±12.8 years, 79% were female, 89% were white, average BMI was 30.2±7.1 kg/m^2, 70% were seropositive (RF or CCP) and mean disease duration of RA was 8.8±9.1 years. Of note, there were some significant differences in the TNFi group, which included only biologic-naïve patients, compared with the other treatment groups with younger age (55.3±12.8 years) and lower disease duration (5.0±7.0 years) (table 1). Baseline levels of both traditional lipids (total cholesterol (TC), LDL, HDL) and PON activity (PON, LAC, ARYL) were assessed in all patients in a central laboratory and were similar across groups, except for higher triglycerides (TG) in the TCZ group. Baseline disease activity measures were similar across groups, although there were small numerical differences noted in DAS28-CRP and Health Assessment Questionnaire (table 1). Distribution of bDMARD experienced versus naïve is noted in table 1.

Changes in PON1 following 6 months of new biological therapy

Across the entire cohort, significant increases in PON, LAC and ARYL activities of PON1 were observed (+8.3

Table 1 Baseline cohort demographics, disease features, traditional lipids and PON1 activity by treatment group

	TNFi (n=427)	RTX (n=116)	ABA (n=374)	TCZ (n=296)	P value
Age (years)	55.3 (12.8)	58.0 (12.1)	58.7 (12.6)	57.6 (12.9)	0.001*
Female	326 (76.5)	90 (77.6)	295 (78.9)	247 (83.4)	0.14
Caucasian %	340 (79.6)	96 (82.8)	305 (81.6)	258 (87.2)	0.4
BMI (kg/m^2)	29.9 (7.1)	30.7 (7.5)	30.3 (6.8)	30.5 (7.5)	0.6
Disease duration (years)	5.0 (7.0)	12.2 (8.4)	9.7 (9.6)	11.7 (9.4)	0.0001*
Seropositive (RF or CCP)	317 (74.4)	89 (76.7)	255 (68.2)	188 (63.5)	0.01*
Swollen joint count	8.1 (5.7)	8.2 (4.8)	7.6 (5.3)	7.7 (5.6)	0.47
Tender joint count	11.3 (7.3)	11.9 (7.6)	10.9 (7.0)	11.9 (7.7)	0.28
C reactive protein (mg/L)	11.6 (20.7)	7.9 (13.5)	8.1 (12.5)	11.7 (17.8)	0.005*
CDAI	29.7 (13.5)	31.0 (12.0)	28.5 (12.1)	30.4 (12.9)	0.14
DAS28-CRP	4.9 (1.1)	4.9 (1.0)	4.7 (1.1)	5.0 (1.1)	0.01*
HAQ	1.0 (0.7)	1.1 (0.6)	1.0 (0.7)	1.1 (0.7)	0.01*
Current smokers	101 (24.5)	18 (15.5)	61 (16.5)	36 (12.2)	0.0003*
History of HTN	100 (23.4)	35 (30.1)	131 (35.0)	86 (29.0)	0.004*
Diabetes mellitus	41 (9.6)	7 (6.0)	35 (9.3)	38 (12.8)	0.17
Cholesterol-lowering agents	96 (22.5)	33 (28.4)	107 (28.6)	80 (27.0)	0.2
Biologic-naïve	427 (100.0)	2 (1.7)	93 (24.9)	2 (0.7)	0.0001*
Prednisone use	135 (31.6)	39 (33.6)	130 (34.8)	89 (30.1)	0.6
TC (mg/dL)†	189.9 (40.0)	191.7 (42.3)	193.5 (41.1)	196.8 (39.0)	0.2
LDL (mg/dL)†	111.6 (35.5)	110.9 (35.9)	113.8 (36.3)	113.6 (34.9)	0.8
HDL (mg/dL)†	60.9 (18.8)	63.3 (21.5)	61.0 (17.9)	62.8 (18.7)	0.4
TG (mg/dL)†	141.0 (84.3)	142.1 (92.7)	151.8 (86.3)	161.3 (99.2)	0.02*
PON (U/mL)	679.6 (497.9)	648.0 (428.8)	639.7 (497.1)	660.4 (473.9)	0.6
LAC (U/mL)	23.9 (8.5)	24.6 (7.8)	23.6 (8.3)	24.3 (7.8)	0.4
ARYL (U/mL)	253.9 (71.4)	250.1 (70.5)	249.4 (74.6)	254.6 (67.4)	0.5

Values are mean (SD), n (%) and represent those of the baseline visit.
Significance testing by one-way analysis of variance for continuous variables, and χ^2 test for categorical variables.
Significance testing on PON/LAC/ARYL performed on $\log_{10}$ transformed data.
LDL was measured and not calculated.
*P<0.05.
†All lipids were measured prospectively in a central laboratory.
ARYL, arylesterase activity; BMI, body mass index; CCP, anticyclic citrullinated peptide antibody; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28, Disease Activity Score 28; HAQ, Health Assessment Questionnaire; HDL, high-density lipoprotein; HTN, hypertension; LAC, lactonase activity; LDL, low-density lipoprotein; PON, paraoxonase activity; RF, rheumatoid factor; TC, total cholesterol; TG, triglycerides; TNFi, tumour necrosis factor inhibitors.

(3.6) U/L, +15.7 (4.7) U/L and+14.1 (4.4) U/L, respectively, all $\log_{10}(x)\times10^3$ transformed (n=1091); mean (SEM), all p<0.05). Patients treated with TCZ had the greatest, and most significant (all p<0.001) increases in all three domains of PON activity from 0 to 6 months (figure 2), as well as significant decreases in 8 of 15 measured oxylipins (online supplemental table 1). In a general linear mixed model with random subject effect (controlling for drug groups and time), there were significant differences noted between the drug groups for PON/LAC (overall p=0.005, p=0.003, respectively), with the TCZ group driving the differences. In addition, as expected, TCZ-treated patients showed statistically significant increases in all traditional lipids, including a mean (SEM) rise in TC of 8.4 (2.0) mg/dL, LDL of 6.8 (1.8) mg/dL, HDL of 1.6 (0.8) mg/dL and TG of 14.7 (5.6) mg/dL (online supplemental table 1). No other treatment groups demonstrated significant changes in PON activity or traditional lipid measures, except that patients treated with ABA showed significant rises in 7 of 15 measured oxylipins (online supplemental table 1).

Associations of ACR 20/50/70 treatment responses with PON1 activity

The relationship of ACR 20/50/70 responses to changes in PON activity from 0 to 6 months was evaluated using a novel multivariate ordinal logistic regression model, which we termed 'ACR-Max' (figure 3). After controlling

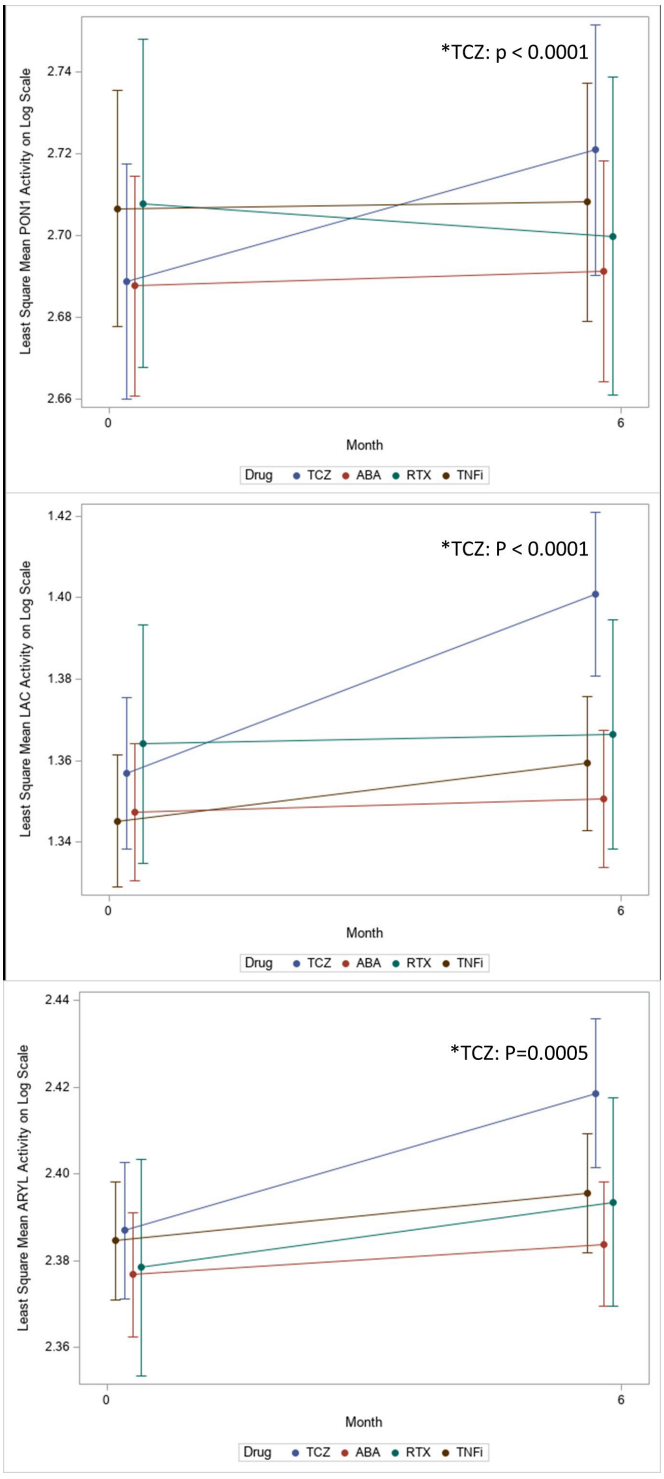

Figure 2 Change in PON activity from baseline to 6 months (least square mean). This figure depicts model-based least square mean PON activities (PON1—panel A, LAC—panel B, ARYL—panel C) from baseline to 6 months after initiation of a new biologic. TCZ led to significant increases in all three PON1 activity domains. ABA, abatacept; ARYL, arylesterase; LAC, lactonase; PON, paraoxonase; RTX, rituximab; TCZ, tocilizumab; TNFi, tumour necrosis factor inhibitor.

for BMI, seropositivity and RA disease duration, ACR-Max responses (likelihood of achieving a higher ACR response) were significantly associated with greater increases in the LAC and ARYL activities of PON1 over 6 months for the total cohort with OR (95% CI) of 1.12 (1.04, 1.22) and 1.13 (1.04, 1.23), respectively (both p<0.01). The PON activity of PON1 also had a positive relationship with the ACR-Max response with an OR (95% CI) of 1.10 (1.00, 1.22), although only reached borderline statistical significance (p=0.057). When these models were modified to additionally control for per cent change of HDL, change of LAC and ARYL activities remained significant associated with higher ACR-Max, with OR (95% CI) of 1.10 (1.02, 1.19) and 1.11 (1.02, 1.21), respectively (both p<0.05)(online supplemental table 2).

For subgroup analysis stratified by treatment group, there were similar associations of change in PON1 activity with ACR-Max responses, which reached statistical significance in the LAC and ARYL activities of the TNFi and ABA groups (online supplemental figure 1). Traditional binary logistic regression analyses for individual ACR 20/50/70 responses were also performed and demonstrated similar results to the ACR-Max; greater increases in PON1 activity over 6-month follow-up were associated with greater likelihood of achieving either ACR 20, 50 or 70 responses (online supplemental table 3 and online supplemental figure 2).

Associations of DAS28-CRP and CDAI reponses with PON1 activity

The relationship of changes in DAS28-CRP and CDAI with changes in PON1 activity from 0 to 6 months was investigated with multivariate linear regression analyses controlling for BMI, seropositivity and disease duration. In the total cohort, there were negative associations between changes of DAS28-CRP and CDAI, and changes of all PON1 activities (all p<0.05); greater decreases in RA disease activity measures were associated with greater increases in PON1 activity (table 2). When stratified by treatment group, there were largely negative correlations within each group, with statistical significance in TNFi with PON activity, and in ABA with ARYL/LAC activity in both DAS28-CRP and CDAI (table 2). As a sensitivity analysis, we additionally included per cent change in HDL in the above models. There were negative associations between change of PON1 activity and changes of DAS28-CRP and CDAI, with significance noted in all three PON1 domains with change in DAS28-CRP, as well as in LAC/ARYL with change in DAS28-CRP in ABA patients (online supplemental table 4).

Associations of RA treatment response with oxylipins

Oxylipins were measured for a subgroup of patients with the 50 highest and 50 lowest DAS28-CRP responses (the biggest decrease in DAS28-CRP constituted the high responders, the least decrease in DAS28-CRP constituted the low responders). Across all treatment groups, we found that DAS28-CRP high responders had significantly greater decreases in 12-HETE than low responders (p=0.0005). When the analysis was stratified by treatment

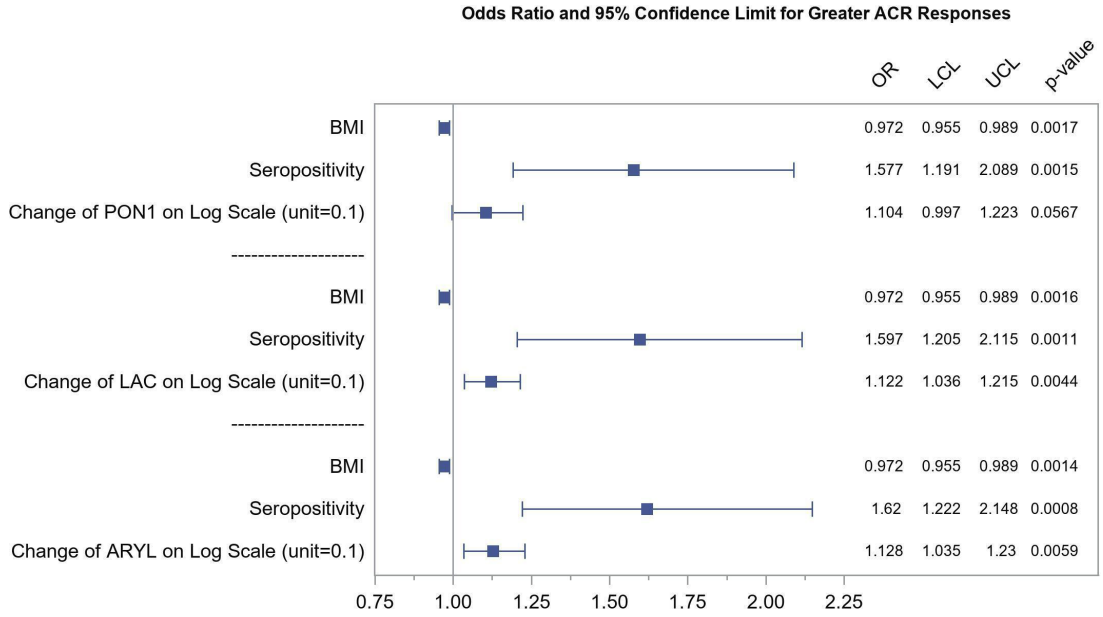

Figure 3 Multivariable ordinal logistic regression analysis of associations of change in PON1 with ACR-Max response. Model is an ordinal logistic regression for the effect of change in PON1 activity on a composite ACR 20/50/70 response (0=non-responder, 1=ACR 20 responder, 2=ACR 50 responder, 3=ACR 70 responder) adjusted for BMI, seropositivity, disease duration (not shown). PON1 values are difference in $\log_{10}(x)$ from 0 to 6 months. Units are log (U/mL). ACR, American College of Rheumatology; ARYL, arylesterase; BMI, body mass index; LAC, lactonase; LCL, lower confidence interval (5%); PON1, paraoxonase; UCL, upper confidence interval (95%).

group, there were significant decreases of oxylipins noted in the DAS28-CRP high responders of TNFi (5-oxo-HETE), of RTX (TXB2, 12-HETE) and of TCZ (14S-HDHA (14S-HDoHE)) (all p<0.05) (online supplemental tables 5, 6, 7 and 8).

Associations of changes in DAS28-CRP with changes in oxylipins were also evaluated in linear regression models and a positive, statistically significant correlation was noted in the univariate model with TXB2, and 12-HETE (0.9 (0.4), 0.9 (0.2) respectively, coefficient (SE), online supplemental table 9). Greater decreases in disease activity were associated with greater decreases in these oxylipins. In additional analyses controlling for RA disease features, including BMI and seropositivity—which may affect DAS28 response—the association of DAS28 with 12-HETE (0.8 (0.2)) remained statistically significant (online supplemental table 9). Of note, oxylipin analyses had smaller samples sizes and were limited to the 'best' and 'worst' DAS28-CRP responders. These analyses were thus exploratory in nature.

Table 2 Multivariable linear regression analysis to assess the associations between changes in PON1 activity and changes in DAS28-CRP and CDAI after 6 months of biologic therapy

	Total cohort	TNFi (n=361)	RTX (n=104)	ABA (n=313)	TCZ (n=255)
Change in DAS28-CRP					
PON†	−1.10 (0.34)*	−1.56 (0.62)*	−2.50 (1.13)*	−1.03 (0.63)	−0.36 (0.62)
LAC†	−0.81 (0.26)*	−0.75 (0.42)	0.08 (0.99)	−1.30 (0.49)*	−0.72 (0.55)
ARYL†	−0.94 (0.28)*	−0.99 (0.47)*	−1.0 (0.97)	−0.98 (0.04)*	−0.69 (0.64)
Change in CDAI					
PON†	−7.93 (3.44)*	−13.53 (6.45)*	−22.77 (11.75)	−9.00 (6.12)	0.95 (6.14)
LAC†	−6.51 (2.67)*	−4.95 (4.39)	−6.43 (10.24)	−12.39 (4.86)*	−2.87 (5.41)
ARYL†	−6.38 (2.82)*	−6.02 (4.90)	−15.27 (9.83)	−9.48 (4.52)*	2.04 (6.27)

Values are model coefficient estimate (SE).
General linear models with differences of DAS28-CRP and CDAI from 0 to 6 months as the dependent variable, controlling for seropositivity, RA disease duration and BMI (not shown).
*P<0.05.
†PON1 values are difference in $\log_{10}(x)$ from 0 to 6 months. Units are log (U/mL).
ABA, abatacept; ARYL, arylesterase; BMI, body mass index (kg/m^2); CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28, Disease Activity Score 28; LAC, lactonase; PON, paraoxonase; RTX, rituximab; TCZ, tocilizumab; TNFi, tumour necrosis factor inhibitor.

Associations of PON1 activity with oxylipins

The relationships of oxylipins and PON activities were explored with linear regression and showed modest, negative relationships throughout the CERTAIN cohort, with statistical significance noted for 6k prostaglandin F α, and 14S-HDHA (14S-HDoHE) with PON, and TXB2 with ARYL (all p<0.05). Greater increases in PON1 activity were associated with greater decreases in these oxylipin concentrations. Interestingly, when this relationship was explored specifically within patients treated with TCZ, which associated with the greatest effects on several oxylipins, there were more consistent, significant associations with PON1 activities. 6t12eLTB4+LTB4 and 5-oxo-HETE demonstrated significant relationships with PON, ARYL and LAC, while LXA4 showed significant relationships with PON and ARYL (all p<0.05). Finally, in patients treated with ABA, there was a significant negative relationship between PON and 14S-HDHA (14S-HDoHE), as well as significant positive relationships between ARYL and TXB2 and LXA4 (all p<0.05).

DISCUSSION

Patients with RA have increased risk of CV disease (CVD), including an over 50% increased risk of myocardial infarction and heart failure as well as an increased risk of stroke.[14] Both traditional CV risk factors as well as ongoing RA disease activity contribute to CV risk in patients with RA. Growing evidence suggests that CV risk varies depending on the stage of RA disease activity, with patients experiencing acute flares or high disease activity facing the greatest risk. In contrast, patients in remission have CVD risk comparable to that of patients without RA.[6 7]

While the association of RA disease activity with CV risk is well appreciated, the mechanisms responsible for the accelerated CVD in the setting of active RA are not well understood. In particular, we do not know if the reduced CV risk in low RA disease states results from modulation in specific immune or lipid pathways, driven by a specific drug target, or rather is a result of a shared final common pathway across RA therapies. For example, while significant epidemiological studies suggest a beneficial effect of TNFi therapy in reducing CV risk, a recent randomised trial found no difference in the effects of TNFi compared with triple therapy on vascular wall inflammation, as measured by FDG PET CT, which serves as a CV risk measure.[15] It is apparent that there is a need for greater insight regarding the biological mechanisms responsible for the differential CV risk associated with disease activity status.

The present study used a unique design and a uniquely robust dataset to compare different biologics and their effects on PON1 activity and oxylipins in patients with RA initiating four different classes of biologic therapy. Increases in PON1 activity were noted across the entire cohort of patients treated with different biologic therapies, although they were only statistically significant and consistent across PON1 activities in the TCZ group. Consistent with prior observations, we also found that patients treated with TCZ had significant increases in centrally processed traditional lipid markers (TC, LDL, HDL, TG), which were not demonstrated with other biologic therapies. It should also be noted that statistical significance and biologic relevance of changes in PON1 should not be conflated.

HDL plays a central role in the prevention of vascular inflammation and atherosclerotic plaque formation. Importantly, PON1 is the major HDL-associated enzyme responsible for much of its antioxidant and anti-inflammatory properties through its role in metabolising pro-inflammatory oxidised lipid mediators and preventing the formation of oxidised LDL, which drives inflammation in the arterial wall.[1] PON1 is synthesised primarily in the liver and appears mainly in serum, where it is associated with HDL. PON1 is considered a 'promiscuous enzyme', with the ability to hydrolyse many substrates and can be evaluated by its different activities, such as its PON activity (when paraoxon is used as substrate), its ARYL activity (when a non-phosphorous arylester such as phenyl acetate is used as substrate) or by its LAC activity (when lactones such as dihydrocoumarin are used as substrate) as in the current work.[8] PON1 activity as measured by these spectrophotometric assays has been inversely linked to the development of CVD and atherosclerosis in both the general population, as well as in patients with RA.[1 16] Our prior investigations have shown that higher PON1 activity is associated with reduced risk of subclinical carotid atherosclerosis in patients with RA, as well as reduced CV and malignancy events in patients with RA treated with tofacitinib.[3 5] The current study was not designed to evaluate effects on surrogate markers of CVD or CV events.

Because PON1 is an HDL-associated enzyme, we hypothesised that increases in PON1 activity with TCZ may be partly due to increases in HDL cholesterol (HDL-C), which serves as a surrogate marker for HDL particle numbers. However, in analyses adjusted for HDL-C levels, associations of TCZ with increases in PON1 activity remained, suggesting that increases in HDL-C do not account entirely for the observed increases in PON1 activity with TCZ treatment. TCZ also acts as a competitive inhibitor of the interleukin (IL)-6 receptor, causing compensatory increases in IL-6 levels by competitively inhibiting IL-6 receptor-mediated clearance.[17] IL-6 directly upregulates production of PON1 by the liver, thereby increasing PON1 activity.[18] This pathway may offer an alternative mechanism for the robust increase in PON1 activity with TCZ treatment seen in the current work. In contrast, ABA therapy has been associated with reduction of IL-6 levels, and in the current work showed some of the smallest changes in PON1 activity across all three assays.[19]

A limitation of the current study is the lack of an arm for targeted-synthetic DMARDs, such as tofacitinib, which has also been shown to increase traditional cholesterol

levels and PON1 activity in phase II and phase III clinical trials.[20] Additional work comparing the effects of tofacitinib and TCZ on lipid metabolism in patients with RA including other lipid mediators such as lipoprotein(a) as well as other effects on the HDL particle function and structure including increases in associated serum amyloid A levels with systemic inflammation may be of value.[21]

We also noted significant decreases in several pro-inflammatory oxylipins from baseline to 6 months in the TCZ group, which correlated with the increases in PON1 activity. This unique property of TCZ in increasing traditional lipid markers and PON1 activity, and simultaneously decreasing levels of several oxylipins warrants further investigation for biologic and clinical relevance. As noted, oxylipins are metabolites of polyunsaturated fatty acids, which regulate inflammatory responses,[10 22] and in previous work, we have identified higher circulating levels of multiple oxylipins in patients with RA compared with healthy controls, with trends observed for the highest levels in patients with RA with active disease and carotid atherosclerosis.[9] In these studies, lower circulating PON1 activity was also associated with higher levels of several oxylipins, including eicosanoid metabolites, which have long been implicated in atherosclerosis, platelet aggregation, vascular constriction and cardiac dysfunction.[23] Ultimately, it is interesting to consider that the known hyperlipidemic effects of TCZ treatment may not in fact be deleterious and increase CV risk, but potentially anti-inflammatory in nature. This is supported by prior research, including a randomised controlled trial that did not show increased CV events in patients with RA treated with TCZ compared with etanercept.[24]

Across treatment groups, we noted associations of disease activity responses measured by ACR 20, 50 and 70, as well as CDAI and DAS28, with increases in PON1 that were significant in the non-TCZ groups. We found that in the total cohort, greater increases in PON1 activity were associated with greater odds of achieving a higher ACR-Max response, as well as greater individual ACR 20/50/70 responses. Furthermore, greater increases in PON1 activity correlated significantly with decreases in both DAS28-CRP and CDAI. These associations were most marked in the ABA and TNFi groups, perhaps limited in power in the RTX group due to relatively smaller numbers. Furthermore, we can speculate that the lack of statistically significant associations in the TCZ group may be due to the marked increases in PON1 activity associated with TCZ treatment alone, potentially linked to the drug's mechanism of action as discussed above, limiting the ability to achieve statistical significance.

Previous work by our group has shown that overexpression of the human PON1 transgene in mice, which increases circulating PON1 activity, prevents increases in inflammatory oxylipins and reduces arthritis activity and histological damage.[9] In the current analysis, we saw significant decreases in several oxylipins in patients with RA treated with TCZ, but significant rises in several oxylipins from baseline to 6 months in patients treated with ABA. These data warrant further confirmation in additional cohorts, and the biological and clinical significance of these unique observations requires further investigation. Additionally, we found that some oxylipins correlated with RA disease activity; specifically, increases in TXB2 and 12-HETE showed a positive correlation with DAS28-CRP. High responders exhibited significant decreases in 12-HETE compared with low responders.

The effects of treatment with different biologic agents on both traditional and non-traditional CV risk markers have been studied previously in RA. However, majority of this research comes from placebo-controlled studies of single agents, rather than studies comparing multiple treatments. Additionally, individual clinical trial biorepository assessments of infliximab, tofacitinib and TCZ have shown significant increases in PON1 activity and decreases in pro-inflammatory lipid mediators compared with placebo.[25–27] However, to our knowledge, no study has previously evaluated both PON1 activity and systemic oxidative stress measured by a panel of oxylipins across four biologic therapies along with prospectively assessed validated clinical metrics. In addition, it is no longer considered ethical to place patients with RA on a placebo for the 6-month duration reported in our investigation.

In summary, we believe that our unique data present clues to novel mechanisms and biomarkers for CVD risk in RA. CV risk stratifications in patients with RA using traditional lipid profiles is known to underestimate actual disease risk.[16] The EULAR guidelines of CV risk and rheumatic disease have proposed a 1.5× multiplier for RA.[28] However, to date, only one CV risk calculator, the Expanded Risk Score (ERS) RA risk calculator, takes into account the patient's RA disease control in CV risk assessment.[29] The current study provides a unique perspective from four different prospective bDMARD cohorts, exploring the role of PON1 and oxylipins in CV risk and disease activity in patients with RA. While further long-term investigation into the clinical implications of these potential markers is needed, our observations introduce novel mechanisms that enhance our understanding of the complex molecular interactions that occur during successful clinical responses to various bDMARDs.[30]

There are several limitations to this study. While the study was prospective, patients were not randomised at baseline, which we believe is unlikely to affect the findings. Although the choice of biologic agent was based on the judgement of the treating physician, we believe that the consistent changes observed across interventions, along with the robust sample sizes, provide a high degree of biologic representativeness within the groups. It is therefore unlikely that selection or channelling bias has affected the results we report. The cohort studied was predominantly Caucasian. We did not include a placebo group to compare changes, as the CERTAIN study was designed as a drug intervention study; however, it is important to note that having a placebo group for 6 months is no longer considered ethical. Given the observational nature of the study, there were major variations in

the number of biologic-experienced versus biologic-naïve patients between the different groups, which is typical of real-world clinical practice. All of the drugs included were approved after regulatory mandates for placebo-controlled trials had been completed. The PON activity of PON1 is highly associated with the PON1Q192R polymorphism, which was not evaluated in the current work. Finally, our sample size for oxylipin analysis (total n=350 across all treatment groups) was intentionally limited to a subset of the lowest and highest DAS28-CRP responders within each treatment group to maximise the potential for finding biologically meaningful correlations with responses. Nevertheless, it is possible that these smaller numbers could have limited our ability to detect differences compared with the larger numbers in the entire cohort. However, the dramatic clinical response differences between the best and worst responders were intentionally selected to maximise the potential for finding biologically meaningful correlations with the responses.

CONCLUSIONS

Improvement in disease activity across four classes of biologic therapies with different mechanisms of action is associated with an increase in the activity of PON1, a protein linked to CV risk reduction. Increases in PON1 activity were most pronounced in the TCZ group, which also exhibited the greatest increases in TC, LDL cholesterol and HDL-C levels. Across treatment groups, improvement in PON1 activity was associated with greater odds of treatment response. These findings suggest a potential mechanism by which improvement in RA disease activity across multiple therapies reduces CV risk through enhanced PON1 activity.

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