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Disease Response in Rheumatoid Arthritis Across Four Biologic Therapies Associates with Improvement in Paraoxonase-1 Activity

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A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Clinical Research

By

Amir Ali Razmjou

2023

ABSTRACT OF THE THESIS

Disease Response in Rheumatoid Arthritis Across Four Biologic Therapies Associates with Improvement in Paraoxonase-1 Activity

by

Amir Ali Razmjou

Master of Science in Clinical Research

University of California, Los Angeles, 2023

Professor Marc Suchard, Chair

Background/Purpose: Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme, which has been implicated as a biomarker of cardiovascular (CV) risk in patients with rheumatoid arthritis (RA).

Methods: 1213 adult RA patients in the CERTAIN registry with moderate or high disease activity (CDAI >10) who initiated a biologic agent that had not been previously used for their treatment (tocilizumab (TCZ), n=296; abatacept (ABA), n= 374; TNFα inhibitors (TNFi) n= 427; rituximab (RTX), n=116) had serum specimens analyzed for PON1 activity by arylesterase (ARYL), lactonase (LAC) and paraoxonase (PON) assays at baseline and after 6 months of new biologic

therapy. A targeted panel of oxylipins was evaluated in a subset of patients with the lowest and highest 6-month DAS28 responses in each treatment group.

Results: PON1 activity increased in the entire cohort after 6 months of new biologic therapy, showing the greatest, most consistent increases in the TCZ group. Increases in the LAC and ARYL activities of PON1 after 6 months of therapy associated with treatment response to new biologic therapies measured by DAS28, CDAI, and ACR 20, 50, or 70 responses (OR (CI 95%) of 3.33 (1.52, 7.27), and 3.37 (1.45, 7.83), $p<0.005$ for ACR responses (LAC/ARYL) respectively), after controlling for other RA disease characteristics. DAS28-CRP high responders had significantly greater decreases in 12-HETE ($p=0.0005$) than low responders.

Conclusion: Improvement in disease activity across 4 classes of biologic therapies with different mechanisms of action associates with improvement in the activity of PON1, a protein associated with reduction in systemic oxidative stress and CV risk.

The thesis of Amir Ali Razmjou is approved.

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2023

TABLE OF CONTENTS (PAGES)

Introduction:

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

The Corevitas (formerly Corrona) registry was initiated in 2001 and is the largest independent database in North America collecting standardized outcome metrics from both rheumatologists and patients[8]. CERTAIN (Comparative Effectiveness Registry to study Therapies for Arthritis and Inflammatory CoNditions) is a prospective cohort study of adult patients with RA recruited from the Corevitas RA Registry network that had at least moderate disease activity and had either started therapy with or switched to a TNF inhibitor (TNFi) or non-TNFi biologic DMARD. Biorepository samples and clinical data including disease activity measures were collected at baseline and longitudinally in this patient cohort following the start of a new therapy[9].

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. Investigation of these associations is expected to further our understanding of mechanisms by which reduction in RA

disease activity, irrespective of specific treatment, associates with reduction in cardiovascular risk, an observation noted in several observational studies[4].

Methods:

Study design:

This study was conducted as a historical cohort study of patients enrolled in the Comparative Effectiveness Registry to Study Therapies for Arthritis and Inflammatory Conditions (CERTAIN)[9]. Of note, the CERTAIN study was a prospective cohort sub-study of the former Consortium of Rheumatology Researchers of North America (CORRONA) registry, now known as CorEvitas. Patients provided informed consent, and the study was approved by the appropriate institutional review boards. Patients were recruited into the study if their treating rheumatologist deemed it appropriate to start or switch to a new biologic agent in usual clinical practice. Inclusion criteria for CERTAIN were as follows: 1) adult RA patients fulfilling the 1987 ACR RA classification criteria, 2) moderate disease activity as defined by CDAI >10, 3) patients starting or switching to a new biologic, which included biologic naïve patients. Clinical and laboratory assessments were conducted at baseline (prior to initiation of a new biologic), and again at 6 months (after initiation of a new biologic). Patients were divided into the following biologic treatment groups: 1) tumor-necrosis factor inhibitors (TNFi), 2) rituximab (RTX), 3) abatacept (ABA), 4) tocilizumab (TCZ). Baseline demographic information, RA disease activity (CDAI, DAS28-CRP), and RA disease features were collected for all patients. PON1 activities and oxylipin values were measured in stored serum samples collected at 0 and 6 months as described below.

Outcomes:

The primary outcome was the changes in PON activity from baseline to 6 months across the 4 treatment groups. Secondary outcomes included the associations between changes in PON activity and oxylipins with RA disease response (ACR 20/50/70 responses, DAS28-CRP, and CDAI).

PON1 activity and oxylipins:

PON1 activity was measured in all patient serum samples by paraoxonase (PON), lactonase (LAC), and arylesterase (ARYL) assays as described previously[10]. Mass spectrographic analysis was performed for a targeted lipidomics panel of oxylipins in 50 patients with the best DAS28 treatment response and 50 patients with the worst DAS28 treatment response in each therapeutic group. A SCIEX 5500 QTrap was run in negative ion mode as described previously[11] and controlled by Analyst 1.6.2 software, for a lipidomics panel including: 20-hydroxy leukotriene B4 (20-OH LTB4), thromboxane B2 (TBX2), prostaglandin E2 (PGE2), 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, 14-s hydroxydocosahexaenoic acid (14sHDHA), and 5-oxo-HETE[10].

Statistical analysis:

Data were analyzed using JMP Pro 14.0 (SAS Institute Inc., Cary, NC, USA). Prior to data analysis, $Log_{10}(x)$ or $Log_{10}(x+1)$ transformation was performed so that the data followed a normal distribution, which included PON1 activities (PON, LAC, ARYL), and the oxylipins. Initial analysis

included descriptive statistics for baseline demographics of patients divided by their treatment groups, and between group differences were determined by one-way ANOVA for continuous demographic variables, and Chi-squared testing for categorical demographics variables. Analysis for the change from baseline to 6 months in traditional lipids, PON1 activity, and oxylipins was done by comparing the mean change to a prespecified change of zero, using a paired t-test. Multivariate ordinal logistic regressions were used to understand the relationship between change in PON activity, and ACR 20/50/70 responses. For this ordinal outcome, non-responders were coded as "0", ACR 20 responders as "1", ACR 50 responders as "2", and ACR 70 responders as "3". Finally, multivariate linear regressions were performed to evaluate the relationship of changes in PON activity, with changes in RA disease activity (DAS28-CRP, CDAI). Covariates for the models were selected by backwards stepwise regression with a predetermined set of covariates (age, sex, BMI, seropositivity, RA disease duration). Odds ratios (OR) were calculated for logistic regressions, and beta-coefficients (rates of change) were presented for linear models. Significance level was pre-specified at $p<0.05$.

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, gender was 79% female, race was 89% white, average BMI was 30.2±7.1 kg/m^2, seropositivity (RF or CCP) was 70%, 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 to the other treatment groups including younger age (55.3±12.8 years) and lower disease duration (5.0±7.0 years), as expected for patients starting their first TNFi. Baseline levels of traditional lipids (total cholesterol, LDL, HDL) and PON activity (PON, LAC, ARYL) were similar across groups, with the exception of higher triglycerides in the TCZ group. Baseline disease activity measures were similar across groups, although there were small numerical differences noted in DAS28-CRP and HAQ (Table 1).

Changes in Lipid Measures Following 6 Months of New Biologic Therapy

Across the entire cohort, significant increases in PON, LAC, and ARYL activities of PON1 were observed (+8.3 (3.6) U/L, +15.7 (4.7) U/L, and +14.1 (4.4) U/L respectively, all $Log_{10}(x)*10^3$ transformed (n= 1091); mean (SEM), all $p<0.05$). Patients treated with TCZ had the greatest, most consistent increases in all three domains of PON activity, including PON, LAC, and ARYL as well as significant decreases in 8 of 15 measured oxylipins (Table 2). In addition, as expected, TCZ treated patients showed statistically significant increases in all traditional lipids, including a rise in total cholesterol of 8.4 (2.0) mg/dL, LDL of 6.8 (1.8) mg/dL, HDL of 1.6 (0.8) mg/dL, and triglycerides of 14.7 (5.6) mg/dL (mean (SEM)). No other treatment group demonstrated similar consistent, statistically significant changes in all lipid assessments including traditional lipids, PON1 activities, and oxylipins. Patients treated with ABA showed significant rises in 7 of 15 measured oxylipins (Table 2).

Increases in PON1 Activity Associate with ACR Treatment Responses

The relationship of changes in PON activity from 0 to 6 months with ACR 20/50/70 responses was evaluated using an ordinal logistic regression multivariate model (Table 3). When controlling for BMI, seropositivity, and RA disease duration, greater increases in LAC and ARYL activities of PON1 were significantly associated with greater odds of achieving ACR 20, 50, or 70 responses for the total cohort with OR (CI 95%) of 3.33 (1.52, 7.27), and 3.37 (1.45, 7.83) respectively (both $p<0.005$). The paraoxonase activity of PON1 also had a positive relationship with an OR of 2.63 (0.96, 7.19), although did not reach statistical significance ($p=0.06$). As expected, RA clinical disease characteristics also associated with likelihood of ACR 20/50/70 responses in these models. Higher baseline BMI, longer disease duration, and seropositivity associated with decreased odds of achieving ACR 20, 50, or 70 responses at 6 months. When utilizing the same models and stratifying by treatment group, there were positive associations of PON1 activities with ACR responses, which reached statistical significance in LAC and ARYL activities of the TNFi and ABA groups. Individual logistic regression analyses for ACR 20/50/70 responses were also performed and demonstrated similar results (Supplemental Table 11).

Associations of PON1 Activity with DAS28 and CDAI

The relationship of changes in PON activity with changes in DAS28-CRP and CDAI from 0 to 6 months was investigated with multivariate linear regression analyses. When controlling for BMI and seropositivity, there were negative associations noted in all PON activities with DAS28, with statistical significance in PON, LAC and ARYL, with a coefficient (SE) of -1.14 (0.34), -0.91 (0.26), and -0.97 (0.28), respectively (all $p<0.05$). Greater increases in PON1 activity following 6 months

of biologic therapy were associated with greater decreases in DAS28-CRP. When stratified by treatment group there were negative correlations seen again within each group, with statistical significance in the TNFi and ABA groups (2/3 PON activities), and in the RTX group (1/3 PON activities) (Table 4). When CDAI was used as the disease activity outcome instead of DAS28, PON1 activities again showed significant and inverse correlations in multivariate analyses with greater increases in the activity of PON1 associating with greater decreases in CDAI after 6 months of biologic therapy (Supplemental Table 2). Significant associations between PON1 activities and DAS28 and CDAI responses were noted only in the individual non-TCZ treatment groups.

Association of Oxylipins with RA Treatment Response

Oxylipins were measured for a subgroup of patients, including of the patients with the 50 highest and 50 patients with the lowest DAS28-CRP response (most negative change in DAS28-CRP constituted high response, least negative change in DAS28-CRP constituted low response). Across all treatment groups, we found that DAS28-CRP high responders had significantly greater decreases in 12-HETE ($p=0.0005$) than low responders (Table 5). When the analysis was stratified by treatment group, there were significant decreases of oxylipins noted in the DAS28-CRP high responders of TNFi (14S-HDHA(14S-HDoHE)), in RTX (TXB2, 12-HETE), and TCZ (14S-HDHA(14S-HDoHE)) (all $p<0.05$) (Supplemental Table 3).

Associations of changes in oxylipins with changes in DAS2-CRP were also evaluated in linear regression models and a positive, statistically significant correlation was noted in the univariate model with TXB2 (0.9 (0.4)), and 12HETE (0.9 (0.2)) coefficient (SE) (Supplemental

Table 4). Greater decreases in these oxylipins associated with greater decreases in disease activity. In additional analyses controlling for RA disease features, which may affect DAS28 response including BMI and seropositivity, the association of DAS28 with 12HETE (0.8 (0.2)) remained statistically significant (Supplemental Table 4). Of note for all oxylipin analysis, there was a lower sample size, and limited to those "best" and "worst" DAS28-CRP responders, and thus was exploratory in nature.

Relationship 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 PGFα, and 14S-HDHA(14S-HDoHE) with PON, and TXB2 with ARYL (Supplemental Table 5). Greater increases in PON1 activity associated with greater decreases in these oxylipin levels. 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 several PON1 activities, including 6trans12epi LTB4+ LTB4 and 5-oxoETE demonstrating significant relationships with PON, ARYL, and LAC, and LXA4 demonstrating significant relationships with PON and ARYL (Supplemental Table 5). Finally, in patients treated with ABA there was a significant negative relationship of PON and 14S-HDHA (14S-HDoHE), and significant positive relationships of ARYL and TXB2 and LXA4 (Supplemental Table 5).

Discussion:

RA patients 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[12]. Both traditional CV risk factors as well as ongoing RA disease activity contribute to CV risk in RA patients. Growing evidence suggests a differential CV risk dependent on the stage of RA disease activity, with patients in acute flare or high disease activity having the greatest risk, and patients in remission having a comparable risk of CVD to 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 is a result of 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, although significant epidemiologic work suggests a beneficial effect of TNFi therapy in reducing CV risk, a recent randomized trial showed no difference in the effect of TNFi versus triple therapy on vascular wall inflammation measured by FDG PET CT as a CV risk measure[13]. More work in this area for better understanding of mechanisms driving CV risk in active RA appears needed.

The present study utilized a unique design and robust dataset to compare different biologics and their effects on PON1 activity and oxylipins in RA patients starting four new classes of biologic therapy. Increases in PON1 activity were noted across the entire cohort of patients treated with different biologic therapies, although were only statistically significant and consistent across PON1 activities in the tocilizumab group. Consistent with prior observations, we also found that patients treated with tocilizumab had significant increases in

traditional lipid markers (TC, LDL, HDL, TG), which were not demonstrated with other biologic therapies.

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 anti-oxidant, and anti-inflammatory properties through its role in metabolizing pro-inflammatory oxidized lipid mediators and preventing the formation of oxidized-LDL, which drives inflammation in the artery wall[1]. PON1 function has been linked to the development of CV disease and atherosclerosis in both the general population, as well as RA patients[1,14]. Our prior investigations have shown that higher PON1 activity is associated with reduced risk of subclinical carotid atherosclerosis in RA patients, as well as reduced CV and malignancy events in RA patients treated with tofacitinib[3,5].

Because PON1 is an HDL-associated enzyme, increases in PON1 activity with tocilizumab may be due in part to the increases in HDL cholesterol (surrogate marker for HDL particle numbers), which warrants further study. In addition, tocilizumab acts as a competitive inhibitor of the IL-6 receptor, causing compensatory increases in IL-6 levels by competitively inhibiting IL-6 receptor-mediated clearance[15]. IL-6 directly upregulates production of PON1 by the liver, thereby increasing PON1 activity[16]. In contrast, abatacept 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[17]. 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 not only increasing traditional lipid

markers and PON1 activity, but also simultaneously decreasing levels of several oxylipins warrants further investigation for biologic and clinical relevance.

Across treatment groups, we noted associations of increases in PON1 with decreases in disease activity measured by ACR 20,50, and 70 treatment responses as well as CDAI, and DAS28, which were significant in the non-TCZ groups. We found that in the total cohort, the odds of achieving ACR 20/50/70 responses was significantly higher with greater increases in PON1 activity. 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 due to relatively smaller numbers. Furthermore, we query whether the associations were not statistically significant in the TCZ group due to the marked increases in PON1 activity with TCZ treatment alone, perhaps linked to drug mechanism as discussed above, limiting the ability to note significant disease activity associations.

Work by our group has previously 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 histologic damage[10]. In the current analysis, we saw significant decreases in several oxylipins in RA patients treated with TCZ, but significant rises in several oxylipins from baseline to 6 months in patients treated with ABA. This data warrants further confirmation in additional cohorts and the physiologic and clinical significance of these changes warrants further investigation. Additionally, we show that some oxylipins did correlate with RA disease activity, including increases in TXB2 and 12-HETE

correlating positively with DAS28-CRP, and DAS28-CRP high responders having significant decreases in 12-HETE compared to 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, the predominant body of this research comes from placebo-controlled studies of one therapeutic, rather than studies comparing multiple treatments. There are well known effects of both tocilizumab and tofacitinib significantly increasing the values of traditional lipid profiles in RA, with subsequent data suggesting possible increases in major adverse CV events in patients with established CVD treated with tofacitinib as compared to TNFi[18-20]. Additionally, individual clinical trial biorepository assessments of infliximab, tofacitinib, and tocilizumab have shown significant increases in PON1 activity and decreases in pro-inflammatory lipid mediators compared to placebo[21-23]. However, to our knowledge, no study has previously evaluated PON1 and systemic oxidative stress measured by a panel of oxylipins across four biologic therapies.

In summary, this data may present clues to novel mechanisms and biomarkers for CV disease risk in RA, which is of particular importance given the difficulty of risk stratification in RA patients, as traditional lipid profiles underestimate CV disease risk in such patients[14]. Given this underestimation of CV disease risk, in the European League Against Rheumatism (EULAR) guidelines of CV risk and rheumatic disease, several multipliers have been proposed for application to traditional risk stratification models, including a 1.5x multiplier for RA[24]. However, only one CV risk calculator to date, the ERS RA risk calculator, considers the patient's RA disease control in CV risk assessment[25]. This current study adds to the growing body of literature in this area, exploring the role of PON1 and oxylipins in CV risk and disease activity of

RA patients. While further investigation into the clinical implications of these potential markers is needed, they may represent a potential novel mechanism by which RA CV disease risk can be stratified and monitored[26].

There are several limitations to this study. Given it is an observational cohort, we could not ideally control for the likely biases of the different treatment groups representing distinct patient populations, although we did perform multivariate analysis to control for some disease features. The cohort studied was predominantly Caucasian. Additionally, we did not have an arm for JAK-inhibitors, which are increasingly used targeted-synthetic DMARDs associated with lipid increases. We did not have a placebo group to compare changes to which poses limitations in analysis. The paraoxonase 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 was limited to a subset of the lowest and highest DAS28-CRP responders within each treatment group, which introduces issues with generalizability of those findings to the whole cohort, and limited power in detecting significant differences and correlations.

Conclusions:

Improvement in disease activity across 4 classes of biologic therapies with different mechanisms of action associates with improvement in the activity of PON1, a protein associated with CV risk reduction. Increases in PON1 activity were most marked in the TCZ group, which also had the greatest increases in total, LDL, and HDL cholesterol levels. Across treatment groups, improvement in PON1 activity associated with greater odds of treatment

response. These findings suggest a potential mechanism by which improvement in RA disease activity by multiple therapies reduces CV risk through improvement in the activity of PON1.

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

	TNFI (n=427)	RTX (n=116)	ABA (n=374)	TCZ (n=296)	Between group 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 naive	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 ANOVA for continuous variables, and Chi-squared testing for categorical variables.
Significance testing on PON/LAC/ARYL performed on Log_{10} transformed data.
*p<0.05
HTN= hypertension, RF= rheumatoid factor, CCP= anti-cyclic citrullinated peptide antibody, TC= total cholesterol, LDL= low density lipoprotein, HDL= high density lipoprotein, TG= triglycerides, PON= paraoxonase activity, ARYL=arylesterase activity, LAC= lactonase activity

Table 2. Changes of traditional lipids, PON activity, and bioactive lipid mediators from baseline to six months.

	TNFi	RTX	ABA	TCZ
	N=420	**N=112**	**N=359**	**N=284**
TC^ (mg/dL)	1.3(1.4)	0.04(2.8)	0.5(1.5)	8.4(2.0)*
LDL^ (mg/dL)	0.9(1.3)	0.3(2.4)	-0.6(1.3)	6.8(1.8)*
HDL^ (mg/dL)	0.8(0.6)	0.5(1.1)	0.2(0.5)	1.6(0.8)*
TG^ (mg/dL)	2.1(3.8)	5.0(7.6)	5.4(3.4)	14.7(5.6)*
	N=379	**N=111**	**N=330**	**N=272**
PON#	1.2(5.7)	-7.5(10.2)	3.7(6.5)	30.3(8.3)*
LAC#	15.0(8.4)	1.5(11.7)	-0.2(8.2)	41.9(9.5)*
ARYL#	10.9(7.6)	16.9(12.1)	3.9(8.8)	29.8(8.2)*
	N=83	**N=92**	**N=82**	**N=92**
6k PGF1α%#	0.5(1.5)	-2.4(1.4)	-2.4(1.6)	-2.4(1.4)
TXB2%#	79.7(35.7)*	39.7(29.2)	2.5(33.3)	-14.2(29.1)
PGE2%#	13.4(11.3)	-5.4(6.6)	25.5(14.7)	-4.4(2.7)
LXA4%#	8.9(9.1)	-2.2(7.3)	15.9(12.1)	-8.9(3.8)*
RvD1%	1.9(1.1)	0.3(0.8)	1.8(1.6)	-1.0(0.7)
13,14-dihydro-15k PGF2α%	1.1(1.0)	-0.07(0.9)	2.0(2.9)	1.2(0.9)
6trans12epi LTB4 + LTB4%#	-13.3(8.3)	-5.8(17.9)	75.6(36.3)*	-16.8(8.2)*
13HODE%	20.0(26.5)	9.4(20.6)	111.4(49.5)*	-61.1(21.1)*
9HODE%	22.0(27.3)	9.8(21.7)	121.3(50.4)*	-77.7(21.1)*
15HETE%	26.4(22.7)	5.8(17.2)	96.6(42.8)*	-36.0(14.7)*
14S-HDHA% (14S-HDoHE)	23.9(24.8)	3.7(16.1)	51.1(36.2)	-15.5(12.5)

11HETE[%]	27.6(23.4)	8.0(17.9)	103.8(43.9)*	-44.9(15.9)*
12HETE[%]	14.0(52.0)	2.4(41.1)	-1.4(53.8)	-64.1(42.6)
5HETE[%]	32.0(42.0)	11.8(31.5)	165.9(62.5)*	-101.4(36.4)*
5-oxoETE[%]	13.5(14.7)	-2.1(13.2)	68.2(29.5)*	-26.8(8.3)*

Values are mean (SEM)
Values represent differences from baseline to 6 months (6 month minus baseline (change))
^N=1175
[#]PON values transformed by $Log_{10}(x)$ for significance testing; values reported as $Log_{10}(x)*10^3$ (N=1090-1091). Units are Log(U/mL)
[%]oxylipin values transformed by $Log_{10}(x+1)$ for significance testing; values reported as $Log_{10}(x+1)*10^3$ (N=204-350 across each oxylipin). Units are Log(ng/mL).
[#]Additional missingness of data in these oxylipins with lower sample sizes compared to non-marked oxylipins.
*Mean change is significant different from zero by paired t-test, $p<0.05$

Table 3A. Multivariate ordinal logistic regression of associations of PON1 with ACR 20/50/70 responses (entire cohort).

	ACR 20/50/70 OR (CI 95%)	**p-value**
ARYL[#]	3.37 (1.45, 7.83)	0.005
BMI	0.97 (0.95, 0.99)	0.001
Seropositivity	0.77 (0.67, 0.89)	0.0003
Disease Duration	0.98 (0.97, 0.99)	0.004
LAC[#]	3.33 (1.52, 7.27)	0.003
BMI	0.97 (0.95, 0.99)	0.001
Seropositivity	0.78 (0.68, 0.90)	0.0005
Disease Duration	0.98 (0.97, 0.99)	0.004
PON[#]	2.63 (0.96, 7.19)	0.06
BMI	0.97 (0.95, 0.99)	0.001
Seropositivity	0.78 (0.68, 0.90)	0.001
Disease Duration	0.98 (0.97, 0.99)	0.004

Ordinal logistic regression adjusted for BMI, seropositivity, and disease duration.
[#]X (X=PON, LAC, ARYL) values are difference in $log_{10}X$ from 0 to 6 months. Units are Log(U/mL).
PON=paraoxonase; LAC=lactonase; ARYL=arylesterase; BMI=body mass index

Table 3B. Multivariate ordinal logistic regression for associations of PON1 with ACR 20/50/70 response (individual treatment groups).

	TNFi (N=359)	RTX (N=102)	ABA (N=313)	TCZ (N=255)
PON[#]	4.98 (0.81, 30.72)	0.42 (0.01, 17.42)	3.37 (0.51, 22.31)	1.91 (0.31, 11.66)
LAC[#]	4.81 (1.45, 15.93)*	0.53 (0.02, 13.51)	5.50 (1.19, 25.48)*	1.76 (0.34, 8.95)
ARYL[#]	4.43 (1.13, 17.35)*	0.12 (0.00, 4.12)	6.90 (1.57, 30.22)*	2.39 (0.37, 15.63)

*p<0.05

Ordinal logistic regression adjusted for BMI, seropositivity, and disease duration.

Values are OR (95% CI).

[#]X (X=PON, LAC, ARYL) values are difference in $\log_{10}$X from 0 to 6 months. Units are Log(U/mL).

PON=paraoxonase; LAC=lactonase; ARYL=arylesterase; BMI=body mass index.

Table 4. Multivariate linear regression analyses of associations of change in PON1 with DAS28CRP after 6 months of new biologic therapy.

	Total Cohort (N=1033)	TNFi (N=361)	RTX (N=104)	ABA (N=313)	TCZ (N=255)
PON[#]	-1.14 (0.34)*	-1.57 (0.62)*	-2.68 (1.13)*	-1.02 (0.62)	-0.37 (0.63)
BMI	0.02 (0.01)*	0.02 (0.01)	0.04 (0.02)*	0.03 (0.01)*	0.02 (0.01)
Seropositive	0.11 (0.05)*	0.15 (0.08)	-0.04 (0.15)	0.21 (0.08)*	-0.01 (0.08)
LAC[#]	-0.91 (0.26)*	-0.75 (0.42)	0.13 (1.00)	-1.36 (0.48)*	-0.70 (0.55)
BMI	0.02 (0.01)*	0.02 (0.01)*	0.04 (0.02)*	0.03 (0.01)*	0.02 (0.01)
Seropositive	0.12 (0.04)*	0.17 (0.08)*	0.03 (0.15)	0.21 (0.08)*	-0.02 (0.09)
ARYL[#]	-0.97 (0.28)*	-0.99 (0.47)*	-1.19 (0.97)	-1.03 (0.46)*	-0.54 (0.63)
BMI	0.02 (0.01)*	0.02 (0.01)	0.04 (0.02)*	0.03 (0.01)*	0.02 (0.01)
Seropositive	0.12 (0.05)*	0.17 (0.08)*	0.03 (0.15)	0.21 (0.08)*	-0.01 (0.08)

Values are estimate (SE).

[#]X (X=PON, LAC, ARYL) values are difference in $\log_{10}$X from 0 to 6 months. Units are Log(U/mL).

*p<0.05

PON=paraoxonase; LAC=lactonase; ARYL=arylesterase; BMI=body mass index (kg/m^2); seropositive=+RF or CCP; TNFi=tumor necrosis factor inhibitor; RTX=rituximab; ABA=abatacept; TCZ=tocilizumab

Supplemental Table 1. Multivariate logistic regression analyses of PON activity and divided by ACR 20/50/70 treatment responses.

Total Cohort N=1029

	ACR20	ACR50	ACR70
PON#	4.32 (0.71, 27.55)	4.06 (1.18, 14.33)*	3.43 (0.62, 17.53)
LAC#	3.36 (1.47, 7.87)*	3.07 (1.18, 8.12)*	3.87 (1.07, 13.70)*
ARYL#	3.40 (1.40, 8.45)*	2.28 (0.80, 6.47)	6.57 (1.59, 26.41)*

TNFi Cohort N=359

	ACR20	ACR50	ACR70
PON#	5.28 (0.77, 39.96)	13.04 (1.50, 129.75)*	6.23 (0.36, 118.11)
LAC#	7.16 (1.89, 30.20)*	4.27 (1.08, 18.23)*	3.59 (0.59, 20.95)
ARYL#	4.84 (1.12, 22.82)*	4.03 (0.84, 20.86)	9.32 (1.11, 80.65)*

RTX Cohort N=102

	ACR20	ACR50	ACR70
PON#	0.41 (0.01, 22.68)	0.32 (0.00, 68.13)	1.52 (0.00, 4835.17)
LAC#	0.27 (0.01, 7.84)	2.58 (0.03, 284.72)	49.05 (0.06, 83252.96)
ARYL#	0.13 (0.00, 4.45)	0.02 (0.00, 3.35)	0.87 (0.00, 1425.95)

ABA Cohort N=313

	ACR20	ACR50	ACR70
PON#	2.67 (0.37, 20.61)	2.37 (0.21, 24.96)	7.71 (0.33, 147.56)
LAC#	5.39 (1.08, 31.00)*	1.57 (0.25, 10.44)	1.87 (0.16, 20.79)
ARYL#	7.72 (1.59, 44.61)*	1.80 (0.27, 11.36)	3.44 (0.27, 38.47)

TCZ Cohort N=255

	ACR20	ACR50	ACR70
PON#	1.60 (0.23, 11.36)	3.41 (0.35, 32.98)	0.65 (0.01, 16.78)
LAC#	1.41 (0.25, 7.90)	3.12 (0.37, 27.15)	5.91 (0.25, 113.15)
ARYL#	1.89 (0.26, 13.55)	3.40 (0.29, 40.19)	16.71 (0.46, 553.59)

*$p<0.05$

Values are OR (95% CI).

Covariates of multivariate models also include BMI, seropositivity, RA disease duration (all significant).

#X (X=PON, LAC, ARYL) values are difference in $\log_{10}$X from 0 to 6 months. Units are Log(U/mL).

PON=paraoxonase; LAC=lactonase; ARYL=arylesterase; TNFi=tumor necrosis factor inhibitor; RTX=rituximab; ABA=abatacept; TCZ=tocilizumab

Supplemental Table 2: Multivariate linear regression analysis of associations of change in PON1 with CDAI after 6 months of biologic therapy.

	Total Cohort	TNFi	RTX	ABA	TCZ
PON[#]	-7.39 (3.43)*	-13.68 (6.46)*	-24.43 (11.76)*	-8.96 (6.11)	0.92 (6.13)
BMI	0.23 (0.06)*	0.25 (0.10)*	0.41 (0.17)*	0.17 (0.11)	0.18 (0.11)
Seropositive	0.60 (0.45)	-0.34 (0.82)	-0.90 (1.54)	1.96 (0.79)*	0.24 (0.88)
LAC[#]	-6.66 (2.66)*	-4.96 (4.39)	-6.03 (10.33)	-12.90 (4.82)*	-2.66 (5.38)
BMI	0.24 (0.06)*	0.25 (0.10)*	0.37 (0.17)*	0.16 (0.11)	0.19 (0.11)
Seropositive	0.65 (0.45)	-0.18 (0.83)	-0.45 (1.55)	1.93 (0.78)*	0.29 (0.88)
ARYL[#]	-6.39 (2.81)*	-6.03 (4.91)	-15.91 (9.90)	-9.86 (4.49)*	2.69 (6.20)
BMI	0.24 (0.06)*	0.24 (0.10)*	0.37 (0.17)*	0.18 (0.11)	0.19 (0.11)
Seropositive	0.67 (0.45)	-0.21 (0.83)	-0.33 (1.53)	1.92 (0.78)*	0.33 (0.88)

Values are estimate (SE).

[#]X (X=PON, LAC, ARYL) values are difference in $\log_{10}$X from 0 to 6 months. Units are Log(U/mL).

*p<0.05

PON=paraoxonase; LAC=lactonase; ARYL=arylesterase; BMI=body mass index (kg/m^2); seropositive=+RF or CCP; TNFi=tumor necrosis factor inhibitor; RTX=rituximab; ABA=abatacept; TCZ=tocilizumab

Supplemental Table 3. Change in oxylipins by DAS28-CRP response stratified by treatment group.

a. TNFi Cohort (N=83)

	DAS28 Response				
	N (Low)	**Low**	**N (High)**	**High**	**p-value**
6k PGF1α[%]	26	0.9(2.3)	23	-0.01(2.0)	0.76
TXB2[%]	35	92.3(46.5)	38	68.2(54.1)	0.74
PGE2[%]	29	22.5(21.4)	28	3.9(6.1)	0.41
LXA4[%]	28	18.7(18.7)	30	-0.2(2.3)	0.33
RvD1[%] (N=82-93)	40	2.0(2.1)	43	1.7(1.0)	0.89
13,14-dihydro-15k PGF2α[%]	40	1.2(1.8)	43	1.0(1.1)	0.93
6trans12epi LTB4 + LTB4[%]	33	-18.3(16.5)	33	-8.4(2.4)	0.56
13HODE[%]	40	55.1(50.0)	43	-13.2(21.8)	0.21
9HODE[%]	40	57.3(50.0)	43	-10.9(24.5)	0.23
15HETE[%]	40	52.1(44.5)	43	2.4(14.4)	0.29

14S-HDHA% (14S-HDoHE)	40	53.5(46.4)	42	-4.2(19.7)	0.26
11HETE%	40	56.3(45.0)	43	0.8(16.6)	0.25
12HETE%	40	92.2(79.5)	43	-57.7(66.8)	0.15
5HETE%	40	89.5 (74.0)	43	-21.4(42.1)	0.20
5-oxoETE%	40	44.2(27.9)	43	-15.1(10.1)	0.051

b. RTX Cohort (N=93)

	DAS28 Response				
	N (Low)	**Low**	**N (High)**	**High**	**p-value**
6k PGF1α%	28	-0.5(2.6)	25	-4.9(2.6)	0.15
TXB2%	41	98.9(38.0)	44	-16.2(43.5)	0.05*
PGE2%	32	-11.4(13.6)	34	0.2(2.0)	0.41
LXA4%	29	-11.1(14.6)	31	2.8(2.8)	0.35
RvD1% (N=82-93)	46	0.1(1.1)	46	0.2(1.1)	0.93
13,14-dihydro-15k PGF2α%	46	1.0(1.4)	46	-1.1(1.1)	0.24
6trans12epi LTB4 + LTB4%	34	-24.9(36.8)	36	8.9(5.3)	0.37
13HODE%	46	-5.2(34.9)	46	15.4(21.1)	0.61
9HODE%	46	-13.0(37.7)	46	23.1(20.3)	0.40
15HETE%	46	-5.8(27.8)	46	4.0(16.3)	0.76
14S-HDHA% (14S-HDoHE)	45	11.5(21.5)	45	-13.5(22.7)	0.42
11HETE%	46	-6.6(30.0)	46	8.7(15.0)	0.65
12HETE%	46	106.3(51.2)	46	-114.2(60.4)	0.007*
5HETE%	46	-26.9(45.6)	46	29.3(38.8)	0.35
5-oxoETE%	46	-17.9(24.3)	46	14.6(10.7)	0.23

c. ABA Cohort (N=82)

	DAS28 Response				
	N (Low)	**Low**	**N (High)**	**High**	**p-value**
6k PGF1α%	22	-5.4(2.5)	31	-0.3(2.0)	0.11
TXB2%	33	0.2(61.3)	39	4.4(33.7)	0.95
PGE2%	27	-1.7(4.3)	32	48.4(26.5)	0.07
LXA4%	22	1.7(3.8)	28	27.0(21.3)	0.25

RvD1[%] (N=82-93)	38	1.6(2.6)	44	1.9(2.1)	0.93
13,14-dihydro-15k PGF2α[%]	38	-2.6(1.5)	44	5.9(5.2)	0.12
6trans12epi LTB4 + LTB4[%]	28	47.5(49.6)	37	96.8(51.8)	0.49
13HODE[%]	38	75.5(57.8)	44	142.3(78.0)	0.49
9HODE[%]	38	78.4(59.9)	44	158.3(78.6)	0.42
15HETE[%]	38	70.1(57.2)	44	119.4(63.1)	0.56
14S-HDHA[%] (14S-HDoHE)	38	57.6(53.3)	44	45.5(50.0)	0.87
11HETE[%]	38	74.2(58.3)	44	129.4(64.9)	0.53
12HETE[%]	38	55.1(82.7)	44	-50.2(70.5)	0.34
5HETE[%]	38	126.8(79.4)	44	199.7(94.7)	0.56
5-oxoETE[%]	38	57.5(44.8)	44	77.5(39.4)	0.74

TCZ Cohort (N=92)

	DAS28 Response				
	N (Low)	**Low**	**N (High)**	**High**	**p-value**
6k PGF1α[%]	26	-1.9(1.9)	22	-3.1(2.1)	0.67
TXB2[%]	39	30.4(53.6)	45	-52.8(27.4)	0.17
PGE2[%]	30	0.0(3.3)	27	-9.3(4.1)	0.08
LXA4[%]	28	-5.4(2.7)	27	-12.5(7.2)	0.36
RvD1[%] (N=82-93)	43	-0.7(1.0)	49	-1.2(1.0)	0.71
13,14-dihydro-15k PGF2α[%]	43	2.1(1.4)	49	0.4(1.2)	0.38
6trans12epi LTB4 + LTB4[%]	34	-7.1(7.5)	34	-26.5(14.5)	0.24
13HODE[%]	43	-70.6(25.9)	49	-52.8(32.7)	0.67
9HODE[%]	43	-92.3(26.2)	49	-64.8(32.3)	0.51
15HETE[%]	43	-14.1(15.8)	49	-55.3(23.6)	0.15
14S-HDHA[%] (14S-HDoHE)	42	11.6(16.0)	48	-39.2(18.3)	0.04*
11HETE[%]	43	-23.6(19.1)	49	-63.5(24.6)	0.20
12HETE[%]	43	22.6(56.8)	49	-140.1(61.0)	0.054

5HETE%	43	-68.6(49.1)	49	-130.2(53.3)	0.40
5-oxoETE%	43	-18.6(11.0)	49	-34.0(12.2)	0.35

DAS28-CRP response defined as the 50 samples with the greatest negative change (high responders), versus the least negative change (low responders).
Oxylipin values are changes from baseline to 6 months (6 month minus baseline)
*p<0.05 between means
Values are Mean(SEM)
%Values represented as $Log_{10}(x+1)*10^3$
Additional missingness of data in some oxylipins (6k PGF1α, TXB2, PGE2, LXA4, 6trans12epi LTB4 + LTB4) with lower sample sizes compared others.

Supplemental Table 4: Univariate and multivariate linear regression analyses of change in oxylipins and associations with change in DAS28CRP after six months of biologic therapy.

Univariate Linear Regression	
	Total Cohort
6k PGF1α% (N=48-54)	-8.4 (12.9)
TXB2% (N=72-86)	0.9 (0.4)*
PGE2% (N=57-66)	-1.7 (1.6)
LXA4% (N=50-61)	-1.0 (2.1)
RvD1% (N=82-93)	2.4 (10.2)
13,14-dihydro-15k PGF2α% (N=82-93)	-6.7 (7.0)
6trans12epi LTB4 + LTB4% (N=65-71)	-0.3 (0.7)
13HODE% (N=82-93)	0.0 (0.3)
9HODE% (N=82-93)	-0.1 (0.3)
15HETE% (N=82-93)	0.3 (0.4)
14S-HDHA% (14S-	0.9 (0.5)

HDoHE) (N=82-91)	
11HETE% (N=82-93)	0.3 (0.4)
12HETE% (N=82-93)	0.9 (0.2)*
5HETE% (N=82-93)	0.1 (0.2)
5-oxoETE% (N=82-93)	0.4 (0.6)
Multivariate Linear Regression	
	Total Cohort
TXB2%	0.05 (0.04)
BMI	0.05 (0.01)*
Seropositive	-0.0006 (0.12)
12HETE%	0.82 (0.22)*
BMI	0.05 (0.01)*
Seropositive	0.05 (0.11)

*p<0.05

Values are estimate (SE)

%Values represented as $Log_{10}(x+1)*10^3$, and represent change over 6 months.

Supplemental Table 5: Univariate linear regression analysis of change in PON1 activities as associations with change in oxylipin levels.

a. Total cohort

	PON	**LAC**	**ARYL**
6k PGF1α% (N=48-54)	1.59 (0.71)*	0.81 (0.93)	0.09 (0.91)
TXB2% (N=72-86)	0.01 (0.02)	0.04 (0.03)	0.07 (0.03)*
PGE2% (N=57-66)	-0.09 (0.09)	-0.10 (0.14)	0.01 (0.13)
LXA4% (N=50-61)	-0.06 (0.12)	0.08 (0.16)	0.12 (0.16)
RvD1% (N=82-93)	-0.65 (0.57)	-0.89 (0.79)	-0.76 (0.76)
13,14-dihydro-15k PGF2α% (N=82-93)	0.16 (0.39)	0.63 (0.54)	0.68 (0.51)

6trans12epi LTB4 + LTB4[%] (N=65-71)	-0.05 (0.04)	-0.06 (0.06)	-0.04 (0.05)
13HODE[%] (N=82-93)	-0.01 (0.02)	-0.01 (0.03)	-0.00 (0.03)
9HODE[%] (N=82-93)	-0.01 (0.02)	-0.03 (0.03)	-0.01 (0.03)
15HETE[%] (N=82-93)	-0.03 (0.02)	-0.03 (0.03)	-0.01 (0.03)
14S-HDHA[%] (14S-HDoHE) (N=82-91)	-0.06 (0.03)*	-0.05 (0.04)	-0.02 (0.04)
11HETE[%] (N=82-93)	-0.03 (0.02)	-0.04 (0.03)	-0.01 (0.03)
12HETE[%] (N=82-93)	-0.02 (0.01)	-0.02 (0.02)	0.00 (0.02)
5HETE[%] (N=82-93)	-0.01 (0.01)	-0.01 (0.02)	-0.01 (0.02)
5-oxoETE[%] (N=82-93)	-0.06 (0.03)	-0.08 (0.05)	-0.08 (0.05)

b. TCZ Cohort

	PON	**LAC**	**ARYL**
6k PGF1α[%] (N=48-54)	2.32 (1.39)	-1.49 (2.10)	-2.32 (2.00)
TXB2[%] (N=72-86)	-0.01 (0.04)	0.05 (0.05)	0.06 (0.05)
PGE2[%] (N=57-66)	0.52 (0.62)	-0.20 (0.77)	-0.30 (0.81)
LXA4[%] (N=50-61)	-1.79 (0.39)*	-0.44 (0.57)	-1.22 (0.60)*
RvD1[%] (N=82-93)	-1.07 (1.51)	-0.27 (1.87)	-2.81 (1.92)
13,14-dihydro-15k PGF2α[%] (N=82-93)	0.55 (1.11)	-1.16 (1.36)	-1.78 (1.41)
6trans12epi LTB4 +	-0.40 (0.18)*	-0.45 (0.21)*	-0.53 (0.23)*

LTB4% (N=65-71)			
13HODE% (N=82-93)	-0.00 (0.05)	-0.06 (0.06)	-0.03 (0.06)
9HODE% (N=82-93)	-0.04 (0.05)	-0.09 (0.06)	-0.06 (0.06)
15HETE% (N=82-93)	-0.09 (0.07)	-0.14 (0.09)	-0.17 (0.09)
14S-HDHA% (14S-HDoHE) (N=82-91)	-0.06 (0.08)	-0.12 (0.10)	-0.12 (0.11)
11HETE% (N=82-93)	-0.10 (0.06)	-0.12 (0.08)	-0.14 (0.08)
12HETE% (N=82-93)	0.01 (0.02)	-0.00 (0.03)	0.01 (0.03)
5HETE% (N=82-93)	-0.05 (0.03)	-0.04 (0.03)	-0.07 (0.04)
5-oxoETE% (N=82-93)	-0.26 (0.12)*	-0.31 (0.15)*	-0.37 (0.15)*

c. ABA Cohort

	PON	**LAC**	**ARYL**
6k PGF1α% (N=48-54)	1.57 (1.28)	-0.42 (1.78)	1.83 (1.76)
TXB2% (N=72-86)	0.06 (0.05)	0.13 (0.08)	0.17 (0.07)*
PGE2% (N=57-66)	-0.22 (0.15)	-0.12 (0.26)	0.03 (0.21)
LXA4% (N=50-61)	0.01 (0.19)	0.56 (0.31)	0.58 (0.27)*
RvD1% (N=82-93)	-1.45 (0.88)	-0.50 (1.47)	0.06 (1.26)
13,14-dihydro-15k PGF2α% (N=82-93)	-0.12 (0.50)	0.87 (0.82)	0.96 (0.70)
6trans12epi LTB4 + LTB4% (N=65-71)	-0.08 (0.05)	-0.02 (0.09)	-0.01 (0.08)

13HODE[%] (N=82-93)	-0.02 (0.03)	0.02 (0.05)	0.02 (0.04)
9HODE[%] (N=82-93)	-0.02 (0.03)	0.01 (0.05)	0.01 (0.04)
15HETE[%] (N=82-93)	-0.05 (0.03)	-0.01 (0.06)	0.01 (0.05)
14S-HDHA[%] (14S-HDoHE) (N=82-91)	-0.10 (0.04)*	-0.06 (0.07)	-0.02 (0.06)
11HETE[%] (N=82-93)	-0.04 (0.03)	-0.00 (0.05)	0.01 (0.05)
12HETE[%] (N=82-93)	-0.04 (0.03)	-0.03 (0.04)	0.02 (0.04)
5HETE[%] (N=82-93)	-0.00 (0.02)	0.03 (0.04)	0.04 (0.03)
5-oxoETE[%] (N=82-93)	-0.07 (0.05)	-0.01 (0.08)	-0.01 (0.07)

Subgroup analysis done on TCZ/ABA groups based on seeing significant changes in those groups in oxylipins over 6 months (Table 2).
Values are estimate (SE)
%Values represented as $Log_{10}(x+1)*10^3$, and represent change over 6 months.
X=BLM, Y=PON
*p<0.05

Appendix:

Outline: The analysis of this paper was conducted in multiple stages, from the initial analysis of the study, preparation for submission to the American College of Rheumatology (ACR) national scientific conference, oral presentation of the study at the ACR meeting, to final analysis in preparation for the current thesis and manuscript submission. The statistical methods and decision making will be presented in detail in the following pages. All analysis was done by Amir Razmjou with mentoring, and using the JMP statistical software package.

1. ACR Meeting Submission: This data was first analyzed for abstract submission to the 2022 ACR meeting, which took place in Philadelphia on November, 2022. The submitted abstract was selected for oral presentation. This initial analysis was performed with mentoring by Dr. Christina Charles-Schoeman (CCS), with some consultation with Dr. David Elashoff (DE).
    a. Demographics: Initially, descriptive analysis was done on the entire patient cohort (patient characteristics, RA disease features, baseline PON values, etc). This was followed by the same descriptive data (mean(SD), or n(%)) but stratified by the four biologic treatment groups.
    b. Change in traditional lipids/PON from baseline to 6 months: This was largely the primary outcome of the study, and at this stage was done by comparing the mean values at baseline versus the mean values at 6 months for the various traditional lipid markers/PON activities, and this was further stratified by treatment group. This was done by a 2-sided t-test. The methodology for this analysis was changed in the subsequent analysis for the manuscript.

c. Changes in PON1 activity from baseline to 6 months by ACR 20/50/70 response: The goal of this somewhat complex analysis was to see if patients who had varying degrees of disease response demonstrated different changes in their PON activity compared to those who did not. This analysis was done by comparing mean changes in PON for those who did have disease response versus those who did not, and then compared by a 2-sided t-test for significance. For example, those who reached ACR 20 response had their mean change in PON activity compared to the mean change in PON activity in those who did not achieve ACR 20 response. This was done for each type of PON activity, each of the three ACR response categories, and for the whole cohort, as well as divided by treatment group.

2. Manuscript and Thesis: After the oral presentation at the ACR, and with further discussion with CCS, further data was obtained on the cohort looking at values of a panel of oxylipins in a select group of patients. These patients were those with the fifty best, and worst RA disease responses (by DAS28) in each of the four biologic treatment groups. In addition to this new analysis, further statistical consultation was done with Dr. Jeffrey Gornbein (UCLA Biomath) which led to many changes in analytic technique. Only the significant differences and new methodology from prior will be described below.

    a. Data Transformation: In preparation of this analysis, given the highly skewed nature of PON and oxylipin values (somewhat typical of cytokines and immunologic biomarkers), $Log_{10}(x)$ or $Log_{10}(x+1)$ (if there were values between

0.0-1.0) transformation was performed on the data for better analysis with the statistical techniques used for the study.

b. Demographics: In addition to including data on baseline oxylipins, the main difference here was the inclusion of ANOVA, and Chi-squared testing to evaluate if there were significant differences in these various factors between the treatment groups. This is relevant information given the observational nature of this study and the biases these differences may introduce.

c. Change in PON1 and oxylipins from baseline to 6 months: Unlike the prior analysis, a more accurate significance testing was performed where rather than comparing the means from baseline to those at 6 months, the mean change was compared against a null hypothesis of "zero" via a t-test.

d. Changes in PON1 activity from baseline to 6 months and ACR 20/50/70 response: A major change was the utilization of a multivariate ordinal logistic model to test this association. This approach was better able to take into account the ordinal nature of the ACR 20/50/70 response, and consider all responses rather than simply testing ACR 20, 50, and 70 responses separately. Additionally, a multivariate approach was taken using a manual backwards stepwise technique, starting with known RA disease response predictors (age, sex, BMI, seropositivity, RA disease duration). This method allowed us to better estimate the individual effects of our predictors of interest while controlling for the aforementioned factors.

e. Changes in PON1 from baseline to 6 months and DAS28-CRP response: This analysis was done with a multivariate linear regression with the previously mentioned (2d) backwards stepwise approach.

f. Several other analyses were done using multivariate linear regressions to evaluate for PON1 activity, and oxylipins and their relationships with CDAI (other RA disease response measure), as well as with each other (PON1 activity and oxylipins). Many of the same analyses done for PON1 and ACR 20/50/70, and DAS28 response was also done using oxylipins and included in the supplementary tables. This analysis was not as robust given the smaller sample sizes (oxylipins were only collected from the 50 best/worst DAS28 responders), and not as generalizable since we had self-selected for specific RA responders.

References:

1. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol*. Apr 2001;21(4):473-80. doi:10.1161/01.atv.21.4.473

2. Bhattacharyya T, Nicholls SJ, Topol EJ, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA*. Mar 2008;299(11):1265-76. doi:10.1001/jama.299.11.1265

3. Charles-Schoeman C, Buch MH, Dougados M, et al. Risk of major adverse cardiovascular events with tofacitinib versus tumour necrosis factor inhibitors in patients with rheumatoid arthritis with or without a history of atherosclerotic cardiovascular disease: a post hoc analysis from ORAL Surveillance. *Annals of the Rheumatic Diseases*. 2023;82(1):119-129. doi:10.1136/ard-2022-222259

4. Rho YH, Chung CP, Oeser A, et al. Interaction between oxidative stress and high-density lipoprotein cholesterol is associated with severity of coronary artery calcification in rheumatoid arthritis. *Arthritis Care Res (Hoboken)*. Oct 2010;62(10):1473-80. doi:10.1002/acr.20237

5. Charles-Schoeman C, Lee YY, Shahbazian A, et al. Association of paraoxonase 1 gene polymorphism and enzyme activity with carotid plaque in rheumatoid arthritis. *Arthritis Rheum*. Nov 2013;65(11):2765-72. doi:10.1002/art.38118

6. Arts EE, Fransen J, Den Broeder AA, van Riel PLCM, Popa CD. Low disease activity (DAS28≤3.2) reduces the risk of first cardiovascular event in rheumatoid arthritis: a time-dependent Cox regression analysis in a large cohort study. *Ann Rheum Dis*. Oct 2017;76(10):1693-1699. doi:10.1136/annrheumdis-2016-210997

7. Myasoedova E, Chandran A, Ilhan B, et al. The role of rheumatoid arthritis (RA) flare and cumulative burden of RA severity in the risk of cardiovascular disease. *Ann Rheum Dis*. Mar 2016;75(3):560-5. doi:10.1136/annrheumdis-2014-206411

8. Baker JF, Reed G, Poudel DR, Harrold LR, Kremer JM. Obesity and Response to Advanced Therapies in Rheumatoid Arthritis. *Arthritis Care Res (Hoboken)*. Nov 2022;74(11):1909-1916. doi:10.1002/acr.24867

9. Pappas DA, Kremer JM, Reed G, Greenberg JD, Curtis JR. "Design characteristics of the CORRONA CERTAIN study: a comparative effectiveness study of biologic agents for rheumatoid arthritis patients". *BMC Musculoskelet Disord*. Apr 01 2014;15:113. doi:10.1186/1471-2474-15-113

10. Charles-Schoeman C, Wang J, Shahbazian A, et al. Suppression of inflammatory arthritis in human serum paraoxonase 1 transgenic mice. *Sci Rep*. 10 2020;10(1):16848. doi:10.1038/s41598-020-74016-w

11. Razmjou AA, Wang JM, Shahbazian A, Reddy S, Charles-Schoeman C. Suppressed paraoxonase-1 activity associates with elevated oxylipins and the presence of small airways disease in patients with rheumatoid arthritis. *Clin Rheumatol*. Jan 2023;42(1):75-82. doi:10.1007/s10067-022-06375-w

12. Løgstrup BB, Ellingsen T, Pedersen AB, et al. Cardiovascular risk and mortality in rheumatoid arthritis compared with diabetes mellitus and the general population. *Rheumatology (Oxford)*. Mar 02 2021;60(3):1400-1409. doi:10.1093/rheumatology/keaa374

13. Solomon DH, Giles JT, Liao KP, et al. Reducing cardiovascular risk with immunomodulators: a randomised active comparator trial among patients with rheumatoid arthritis. *Ann Rheum Dis*. Mar 2023;82(3):324-330. doi:10.1136/ard-2022-223302

14. Charles-Schoeman C. Cardiovascular disease and rheumatoid arthritis: an update. *Curr Rheumatol Rep*. Oct 2012;14(5):455-62. doi:10.1007/s11926-012-0271-5

15. Shimamoto K, Ito T, Ozaki Y, et al. Serum interleukin 6 before and after therapy with tocilizumab is a principal biomarker in patients with rheumatoid arthritis. *J Rheumatol*. Jul 2013;40(7):1074-81. doi:10.3899/jrheum.121389

16. Cheng CC, Hsueh CM, Chen CY, Chen TH, Hsu SL. Interleukin-6 upregulates paraoxonase 1 gene expression via an AKT/NF-κB-dependent pathway. *Biochem Biophys Res Commun*. Jul 19 2013;437(1):55-61. doi:10.1016/j.bbrc.2013.06.034

17. Bozec A, Luo Y, Engdahl C, Figueiredo C, Bang H, Schett G. Abatacept blocks anti-citrullinated protein antibody and rheumatoid factor mediated cytokine production in human macrophages in IDO-dependent manner. *Arthritis Res Ther*. Feb 07 2018;20(1):24. doi:10.1186/s13075-018-1527-x

18. Maini RN, Taylor PC, Szechinski J, et al. Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. *Arthritis Rheum*. Sep 2006;54(9):2817-29. doi:10.1002/art.22033

19. Lee EB, Fleischmann R, Hall S, et al. Tofacitinib versus methotrexate in rheumatoid arthritis. *N Engl J Med*. Jun 19 2014;370(25):2377-86. doi:10.1056/NEJMoa1310476

20. Ytterberg SR, Bhatt DL, Mikuls TR, et al. Cardiovascular and Cancer Risk with Tofacitinib in Rheumatoid Arthritis. *N Engl J Med*. Jan 27 2022;386(4):316-326. doi:10.1056/NEJMoa2109927

21. Popa C, van Tits LJ, Barrera P, et al. Anti-inflammatory therapy with tumour necrosis factor alpha inhibitors improves high-density lipoprotein cholesterol antioxidative capacity in rheumatoid arthritis patients. *Ann Rheum Dis*. Jun 2009;68(6):868-72. doi:10.1136/ard.2008.092171

22. McInnes IB, Thompson L, Giles JT, et al. Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: MEASURE, a randomised, placebo-controlled study. *Ann Rheum Dis*. Apr 2015;74(4):694-702. doi:10.1136/annrheumdis-2013-204345

23. IB M, I K, M B, et al. Effects of tofacitinib on lipid biomarkers in patients with active rheumatoid arthritis. Annals of the Rheumatic Diseases 2013. p. A416.

24. Agca R, Heslinga SC, Rollefstad S, et al. EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update. *Ann Rheum Dis*. Jan 2017;76(1):17-28. doi:10.1136/annrheumdis-2016-209775

25. Ahmed O, Krishnamurthy V, Kaba RA, Tahir H. The management of cardiovascular disease risk in patients with rheumatoid arthritis. *Expert Opin Pharmacother*. Jun 2022;23(8):947-958. doi:10.1080/14656566.2022.2076594

26. Ferreira HB, Melo T, Paiva A, Domingues MDR. Insights in the Role of Lipids, Oxidative Stress and Inflammation in Rheumatoid Arthritis Unveiled by New Trends in Lipidomic Investigations. *Antioxidants (Basel)*. Jan 02 2021;10(1)doi:10.3390/antiox10010045