Comparison of lipid and lipid-associated cardiovascular risk marker changes after treatment with tocilizumab or adalimumab in patients with rheumatoid arthritis

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ABSTRACT

Objective Compare changes in lipids and lipid-associated cardiovascular (CV) risk markers in patients with rheumatoid arthritis (RA) treated with tocilizumab or adalimumab. Methods Post-hoc analysis was performed in patients with RA who received tocilizumab intravenously every 4 weeks or adalimumab subcutaneously every 2 weeks for 24 weeks in the ADACTA trial. Lipid and lipid-associated CV risk biomarkers, including high-density lipoprotein-associated serum amyloid A (HDLSAA), secretory phospholipase A2 IIA (sPLA2 IIA) and lipoprotein (a) (Lp(a)) were measured at baseline and at week 8. Results The study included 162 patients treated with tocilizumab and 162 patients treated with adalimumab; HDL-SAA and sPLA2 IIA were measured in a subpopulation of 87 and 97 patients, respectively. Greater increases in mean low-density lipoprotein cholesterol (LDL-C) (0.46 mmol/L (95% CI 0.30 to 0.62)), high-density lipoprotein cholesterol (HDL-C) (0.07 mmol/L (0.001 to 0.14)), total cholesterol (TC) (0.67 mmol/L (0.47 to 0.86)), triglycerides (0.24 mmol/L (0.10 to 0.38)) and TC:HDL ratio (0.27 (0.12 to 0.42)) occurred with tocilizumab from baseline to week 8. HDL-SAA, sPLA2 IIA and Lp(a) decreased more with tocilizumab than adalimumab. Moderate early elevations in LDL-C, HDL-C and triglyceride levels were reported in Phase II and Phase III clinical trials of patients with RA treated with the interleukin-6 (IL-6) receptor inhibitor tocilizumab (TCZ); the TC:HDL-C ratio either decreased or remained unchanged. In contrast, a decline in Lp(a) with TCZ treatment and a change in HDL protein composition occurred. Lipid changes have also been reported in patients with RA treated with tumour necrosis factor (TNF)-α inhibitors. Patients with RA treated with adalimumab had increased HDL-C and apolipoprotein A1 levels, with no change in LDL-C or triglyceride levels, and improvement in atherogenic ratios. Data on the effect of TNF-α blockers on Lp(a) are mixed, though most did suggest a reduction. Described here is a post-hoc analysis of data from a clinical trial that compared IL-6 and TNF-α signalling inhibition to assess the impact of these therapeutic strategies on lipid-associated CV risk biomarkers and their relationship to treatment response. The dearth of such comparator trials despite an urgent need for better understanding of any differential effects of these agents on CV risk parameters makes this analysis important.

INTRODUCTION

Patients with rheumatoid arthritis (RA) are at increased risk of cardiovascular disease (CVD) compared with the general population.1 2 Traditional risk factors for CVD do not appear to fully explain this increased risk,3 and additional factors, including inflammation, may contribute to CVD risk in RA.4 5 The impact of inflammation on lipid levels is complex and may manifest as changes in total cholesterol (TC) levels and in lipid particle-associated proteins, such as serum amyloid A (SAA) and secretory phospholipase A2 IIA (sPLA2 IIA); both are identified biomarkers of increased cardiovascular (CV) risk.5–8 Patients with severe, untreated RA may have very low lipid levels, which is paradoxical when considering their increased risk of CVD. In contrast, treatment of active disease can lead to elevated levels of TC, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in conjunction with reduced levels of inflammation.9 Lipoprotein(a) (Lp(a)) levels are increased in patients with RA.10 The association of Lp(a) with CVD in the general population has been assessed through genetic and Mendelian randomisation studies.11–13 These studies strongly point to Lp(a) as a causal agent in the process of atherogenesis.14–17 Moderate early elevations in LDL-C, HDL-C and triglyceride levels were reported in Phase II and Phase III clinical trials of patients with RA treated with the interleukin-6 (IL-6) receptor inhibitor tocilizumab (TCZ); the TC:HDL-C ratio either decreased or remained unchanged. In contrast, a decline in Lp(a) with TCZ treatment and a change in HDL protein composition occurred.16 Lipid changes have also been reported in patients with RA treated with tumour necrosis factor (TNF)-α inhibitors.17 Patients with RA treated with adalimumab had increased HDL-C and apolipoprotein A1 levels, with no change in LDL-C or triglyceride levels, and improvement in atherogenic ratios.18 19 Data on the effect of TNF-α blockers on Lp(a) are mixed, though most did suggest a reduction.20–23 Described here is a post-hoc analysis of data from a clinical trial that compared IL-6 and TNF-α signalling inhibition to assess the impact of these therapeutic strategies on lipid-associated CV risk biomarkers and their relationship to treatment response. The dearth of such comparator trials despite an urgent need for better understanding of any differential effects of these agents on CV risk parameters makes this analysis important.
**PATIENTS AND METHODS**

**Patients**

This post-hoc study included patients from the ADACTA trial (ClinicalTrials.gov number NCT01119859). ADACTA was a Phase IV study that assessed the efficacy of TCZ as monotherapy compared with adalimumab as monotherapy in adults who had RA for ≥6 months and who were intolerant of or not good candidates for continued use of methotrexate (MTX).24 A total of 326 patients were randomly assigned 1:1 to receive either TCZ 8 mg/kg monotherapy intravenously every 4 weeks plus subcutaneous placebo every 2 weeks or adalimumab 40 mg monotherapy subcutaneously every 2 weeks plus intravenous placebo every 4 weeks for 24 weeks. Patients had to discontinue all synthetic disease-modifying antirheumatic drugs (DMARDs) within an appropriate washout period before baseline; any patient requiring treatment with a synthetic or biological DMARD was withdrawn from the study.25 Analyses of core lipids and Lp(a) were performed in the ADACTA safety population, which included all patients who received at least one dose of study medication and had at least one post-dose safety assessment. Additional analyses of lipid-associated CV risk biomarkers were performed in 184 patients (97 adalimumab, 87 TCZ) who consented to donate serum bio-repository samples for further exploratory analysis and who provided both baseline and week 8 samples. Week 8 samples provided a larger sample size than do later time points, and previous studies have shown that lipid changes observed after 6 weeks remain stable with continued TCZ treatment.23

**Assessments**

Core lipid panel (LDL-C, HDL-C, TC and triglycerides) and lipid-associated CV risk biomarker levels (Lp(a), HDL-SAA and sPLA2 II A) were analysed in serum samples obtained at baseline and at week 8. Week 8 was selected because of the limited availability of bio-repository samples later in the study although data for Lp(a) and core lipids were available for later time points. With the exception of LDL, which was calculated using the formula TC−HDL-C−(triglycerides/5), the core lipid panel was measured using the β-quantification method. The core lipid panel and Lp(a) was analysed by Covance Central Laboratory Services (Indianapolis, Indiana, USA). Assays for HDL-SAA and sPLA2 II A levels were performed at Pacific Biometrics (Seattle, Washington, USA).

sPLA2 II A was determined with the use of an enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, Michigan, USA) by coupling a monoclonal capture antibody with detection by acetylcholinesterase/Fab conjugate and 5′,5′-dithiobis 2-nitrobenzoic acid (DTNB). EDTA serum Lp(a) (Denka Seiken, Tokyo, Japan) was quantified using immunoturbidimetric assay kits. These assays were performed with a Roche Modular P autoanalyzer (Roche Diagnostics, Indianapolis, Indiana, USA).

For determination of HDL-SAA, serum HDL particles were isolated by polyethylene glycol 8000 (PEG-8000; Promega, Madison, Wisconsin, USA) precipitation, as described by Chiba et al.26 Briefly, equal volumes of 13.0% PEG (P-4463; Sigma-Aldrich, St Louis, Missouri, USA) were mixed to precipitate non-HDL proteins and lipoproteins. After centrifugation for 5 min at 18 000g, supernate SAA was determined by EIA (Abayzme; Needham, Massachusetts, USA). An anti-SAA monoclonal capture and a horseradish peroxidase-conjugated polyclonal antibody were used with 3,3′,5,5′-tetramethylbenzidine (TMB) detection.

**Statistical analyses**

Differences between TCZ and adalimumab in change from baseline to week 8 in LDL-C, HDL-C, TC, triglycerides, TC:HDL ratio and Lp(a) were assessed using a post-hoc analysis of covariance model adjusted for baseline laboratory parameters.

Differences in change from baseline to week 8 in HDL-SAA and sPLA2 II A were assessed using the Kruskal–Wallis test. Log transformation of the data was investigated because of the skewed distribution of these two parameters. The transformation did not allow the parameters to be normally distributed; therefore, the non-parametric statistical method of the Kruskal–Wallis test was used to account for the skewed distribution of the data. Change from baseline to week 8 was summarised and was also split by efficacy responders. Week 24 American College of Rheumatology (ACR) 20, ACR50 and European League against Rheumatism (EULAR) good/moderate responders and non-responders were used to analyse HDL-SAA, sPLA2 II A and Lp(a) levels by efficacy. Change in Lp(a) from >50 mg/dL at baseline to ≤50 mg/dL at week 8 was summarised based on recommendations that suggest Lp(a) levels should ideally be <50 mg/dL in patients at intermediate or high risk of CVD/coronary heart disease (CHD).27 All statistical analyses were post-hoc; therefore, no adjustments were made for multiple testing, and no statistical significance can be claimed.

**RESULTS**

The ADACTA safety population consisted of 324 patients who were intolerant of or for whom MTX was inappropriate. The bio-repository population consisted of 184 patients from the ADACTA safety population. Baseline demographics and disease characteristics were similar between the two treatment arms and between the safety and bio-repository populations (see online supplementary table S1). Core lipid parameters, including LDL-C, HDL-C, TC, triglyceride levels and TC:HDL ratio, increased from baseline to week 8 with TCZ monotherapy; numerically smaller changes were observed for these lipids with adalimumab monotherapy.

Differences in mean (95% CI) change from baseline between arms were as follows: 0.46 mmol/L (0.30 to 0.62; p<0.0001) for LDL-C; 0.07 mmol/L (0.001 to 0.14; p=0.0453) for HDL-C; 0.67 mmol/L (0.47 to 0.86; p<0.0001) for TC; 0.24 mmol/L (0.10 to 0.38; p=0.0008) for triglycerides and 0.27 (0.12 to 0.42; p=0.0005) for TC:HDL ratio (table 1).

Reductions in HDL-SAA levels were observed from baseline to week 8 in patients treated with TCZ monotherapy and in those treated with adalimumab monotherapy. A greater treatment effect was observed in the TCZ group; median (IQR) change from baseline to week 8 was –3.2 mg/L (–11.1, –1.0) and –1.1 mg/L (–7.1, 0.6) for TCZ and adalimumab, respectively (p=0.0077) (table 2 and figure 1A). Reductions in sPLA2 II A levels were also observed with both TCZ and adalimumab monotherapy, with greater reductions seen in the TCZ group; median (IQR) change from baseline to week 8 was –4.1 mg/L (–7.8, –1.1) and –1.3 mg/L (–2.9, 0.8) for TCZ and adalimumab, respectively (p<0.0001) (table 2 and figure 1B). Greater reductions in Lp(a) were observed in patients treated with TCZ monotherapy than with adalimumab monotherapy; the difference in adjusted means (95% CI) was –7.12 mg/dL (–9.9 to –4.4) (p<0.0001) (table 3 and figure 1C). Higher proportions of patients who had baseline Lp(a) levels >50 mg/dL improved (defined as achieving Lp(a) levels ≤50 mg/dL) by week 8 in the TCZ monotherapy group (11/21 patients [52.4%]) than in the adalimumab monotherapy group (6/24 patients [25.0%]).

**Changes in HDL-SAA, sPLA2 II A and Lp(a) levels by efficacy response**

Changes from baseline to week 8 in Lp(a), sPLA2 II A and HDL-SAA were also evaluated relative to efficacy response.
Greater reductions were seen in all three lipid-associated CV risk biomarkers at week 8 in both efficacy responders and non-responders who received TCZ monotherapy than in those who received adalimumab monotherapy. In ACR20 responders and non-responders, median IQR changes from baseline to week 8 in HDL-SAA were $–3.3 \text{ mg/L} (–10.0, –1.0)$ and $–3.1 \text{ mg/L} (–12.95, –1.15)$, respectively, with TCZ and $–1.0 \text{ mg/L} (–6.8, 0.55)$ and $–1.1 \text{ mg/L} (–8.7, 1.2)$, respectively, with adalimumab. Similar trends were observed for ACR50 and EULAR good/moderate responders and non-responders for HDL-SAA and for sPLA2 IIA (table 4) and Lp(a) (table 5).

**DISCUSSION**

It was previously demonstrated that though LDL-C levels increase in patients treated with TCZ, Lp(a) levels may decline, and favourable changes in HDL-C level and HDL composition may occur.16 Published data regarding the effect of TNF-α blockers on Lp(a) are conflicting.19–22 Data collected in the
ADACTA study allowed, for the first time, a direct comparison of the effect of anti-TNF (aTNF) and anti–IL-6 therapies on lipids and lipid-associated CV risk biomarkers in the context of a single study in which other trial parameters were balanced between groups by strict randomisation and blinding. Post-hoc analyses showed a greater increase in LDL-C and greater reductions in Lp(a), HDL-SAA and sPLA2 IIA levels over the first 8 weeks of treatment in patients with RA who received TCZ monotherapy compared with those who received adalimumab monotherapy. Reductions in HDL-SAA, sPLA2 IIA and Lp(a) were observed in efficacy responders and non-responders, suggesting that the cytokines themselves—as opposed to, or at least in addition to, other disease activity-related pathways—mediate some of the effects observed. Although reductions for both responders and non-responders treated with TCZ monotherapy appear to be larger than for those treated with adalimumab monotherapy, the small sample size for each group precludes statistical comparison.

Increased inflammation in patients with autoimmune disorders such as RA is associated with quantitative and qualitative modifications of circulating lipids. Recent data suggest that decreased LDL-C and HDL-C levels in patients with active RA are associated with increased cholesterol catabolism rather than decreased synthesis, with the reverse true following treatment of inflammation.28 These data are somewhat reassuring.

Nevertheless, it should be noted that parallel lipid kinetic data do not exist for TCZ or for aTNF therapies. In addition to

<table>
<thead>
<tr>
<th>Table 2 Change from baseline to week 8* in HDL-SAA and sPLA2 IIA (bio-repository population)</th>
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<tbody>
<tr>
<td>ADA 40 mg SC q2w, n=97</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>HDL-SAA (mg/L)</td>
</tr>
<tr>
<td>Baseline Median (IQR)</td>
</tr>
<tr>
<td>Mean (SD)</td>
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<td>n=64 n=58</td>
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</tr>
<tr>
<td>Mean (SD)</td>
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<tr>
<td>n=62 n=55</td>
</tr>
<tr>
<td>sPLA2 IIA (ng/mL)</td>
</tr>
<tr>
<td>Baseline Median (IQR)</td>
</tr>
<tr>
<td>Mean (SD)</td>
</tr>
<tr>
<td>n=90 n=77</td>
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<tr>
<td>Change from baseline to week 8 Median (IQR)</td>
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<tr>
<td>Mean (SD)</td>
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<td>n=86 n=73</td>
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</table>

n represents number of patients contributing to summary statistics.
*Includes only patients with both baseline and week 8 values.
†Comparison between medians (ADA vs TCZ) using the Kruskal–Wallis test. ADA, adalimumab; HDL-SAA, high-density lipoprotein-associated serum amyloid A; IV, intravenous; q2w, every 2 weeks; q4w, every 4 weeks; SC, subcutaneous; sPLA2 IIA, secretory phospholipase A2 II; TCZ, tocilizumab.

Figure 1 Change from baseline to week 8 in HDL-SAA (A, bio-repository population), sPLA2 IIA (B, bio-repository population) and Lp(a) (C, safety population)—patient-level data. ADA, adalimumab; HDL-SAA, high-density lipoprotein-associated serum amyloid A; IV, intravenous; Lp(a), lipoprotein (a); sPLA2 IIA, secretory phospholipase A2 II; q2w, every 2 weeks; q4w, every 4 weeks; SC, subcutaneous; TCZ, tocilizumab.
quantitative changes, inflammation may be accompanied by changes in lipid particle size and protein composition that may result in a potentially pro-atherogenic lipid profile. In particular, SAA, sPLA$_2$, IIA and cholesteryl ester transfer protein can remodel the lipid and protein composition of HDL particles. The Association of HDL with SAA and sPLA$_2$ IIA impairs antioxidative and atheroprotective capacity of HDL. Displacement of HDL cargo proteins that have antioxidant activity, such as apoA1 and paraoxonase 1, with these acute-phase reactants may displace in HDL cargo proteins that have antioxidant activity, such as apoA1 and paraoxonase 1, with these acute-phase reactants with TCZ may be, at least partially, independent of the RA treatment response. Lp(a) occurs in non-responders suggests that the reduction in SAA, sPLA$_2$ IIA for week 24 ACR20, ACR50 and EULAR good/moderate responders and non-responders (bio-repository population)

Table 4 Change from baseline to week 8* in HDL-SAA and sPLA$_2$ IIA for week 24 ACR20, ACR50 and EULAR good/moderate responders and non-responders (bio-repository population)

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Statistic</th>
<th>ADA 40 mg SC q2w, n=162</th>
<th>TCZ 8 mg/kg IV q4w, n=162</th>
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<tbody>
<tr>
<td>Lp(a) (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>162</td>
<td>162</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>157</td>
<td>156</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>–1.1 (15.1)</td>
<td>–7.6 (12.0)</td>
</tr>
<tr>
<td>min, max</td>
<td></td>
<td>–102.5, 86.4</td>
<td>–60, 21.9</td>
</tr>
<tr>
<td>Difference in adjusted means (95% CI)†</td>
<td></td>
<td>–7.12 (–9.9 to –4.4)</td>
<td>p Value‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.0001</td>
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</tbody>
</table>

*Includes only patients with both baseline and week 8 values.
†Difference and 95% CI based on adjusted least square means (TCZ-ADA).
‡ANCOVA model was adjusted for baseline Lp(a). p Values were unadjusted for multiple testing; therefore, no statistical significance can be claimed.
ADA, adalimumab; ANCOVA, analysis of covariance; IV, intravenous; Lp(a), lipoprotein (a); q2w, every 2 weeks; q4w, every 4 weeks; SC, subcutaneous; TCZ, tocilizumab.

Elevated Lp(a) levels can contribute to atherogenesis by depositing LDL-C in the intima of the vascular wall by the recruitment of inflammatory cells or the binding of proinflammatory oxidised phospholipids. Lp(a) shares structural homology with plasminogen and plasmin and has potentially brinolytic properties, which could be beneficial in promoting clot stabilisation and thrombosis. Therefore, the results of the current analysis may be explained by an effect of IL-6 inhibition that is independent of its anti-arthritis effect, similar to its effect on C reactive protein production. Decreases in HDL-SAA, sPLA$_2$ IIA and Lp(a) levels also occurred in adalimumab non-responders; although they appeared to have occurred to a lesser extent than in TCZ.
In conclusion, despite the overall increased LDL-C levels observed with TCZ compared with adalimumab, TCZ may potentially exert beneficial actions on HDL-SAA, sPLA2 IIA and Lp(a), suggesting that IL-6 blockade may work in different ways to influence CVD risk.

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Contributors CG, KT and MK were involved in the conception and design of the study. CG, IBM, AK, KT, MK, JP and NS were involved in the analysis and interpretation of the data. CG, KT and JP wrote the initial draft of the manuscript. All authors drafted the article or revised it critically for important intellectual content; all authors approved the final version to be submitted for publication and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Competing interests CG has received fees for speaking and/or consulting from AbbVie, BMS, Roche, Pfizer, Celgene, MSD, Janssen and AB2 Bio, and he has received research funding from Roche, AbbVie, MSD and AB2 Bio. IBM has received grants and personal fees from Roche (during the conduct of the study), Pfizer, UCB, BMS, AbbVie and Amgen (outside the submitted work). AK has received grants from Roche (outside the submitted work). KT is an employee of Roche Products and owns share options in Roche. MK is an employee of Genentech and owns share options in Roche. JP is an employee of Roche Products and owns share options in Roche. NS has received fees for consulting from Roche, UCB and Sanofi/Regeneron and for speaking from Janssen.

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