Sustained changes in lipid profile and macrophage migration inhibitory factor levels after anti-tumour necrosis factor therapy in rheumatoid arthritis


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ABSTRACT

Background: Macrophage migration inhibitory factor (MIF) has recently emerged as an important cytokine possibly linking rheumatoid arthritis (RA) and atherogenesis. Because atherogenesis is accelerated in RA this was conducted to investigate whether anti-tumour necrosis factor (TNF) therapy could lead to sustained downregulation of systemic MIF levels and improvement in lipid profiles.

Methods: Fifty RA patients with active disease (disease activity score in 28 joints (DAS28) ≥ 3.2), who started adalimumab therapy at 40 mg every other week, were included. At baseline, weeks 16 and 52 serum levels of MIF and lipids were assessed. In addition, the DAS28 and serum C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR) were determined.

Results: After 16 weeks of adalimumab therapy, both DAS28 and MIF levels were significantly decreased (p < 0.001 and p = 0.020, respectively). This was sustained up to week 52 (p < 0.001 and p = 0.012, respectively). CRP levels and ESR were significantly reduced after 16 and 52 weeks of adalimumab therapy (p < 0.001). High-density lipoprotein cholesterol levels increased at week 16 (p < 0.001), but remained at baseline at week 52. Apolipoprotein (apo) A-I levels increased at week 16 (p < 0.001) and remained stable (p = 0.005). This resulted in an improved apo B/A-I ratio.

Conclusions: The results underline the sustained down-regulation of MIF as a potential new mechanism by which anti-TNF therapy might reduce vascular inflammation, and as such perhaps cardiovascular morbidity in RA patients. This hypothesis is supported by an improved apo B/A-I ratio as well as reduced CRP levels in these patients.
To explore the relationship between inflammation and factors involved in atherogenesis, we investigated the early and long-term effects of anti-TNF therapy on serum MIF levels and known risk factors such as C-reactive protein (CRP) levels and the lipid profile in RA patients.

PATIENTS AND METHODS

Patients
Fifty RA patients with active disease (disease activity score in 28 joints (DAS28) ≥3.2) were included in the study. All patients received adalimumab 40 mg subcutaneously every other week in combination with methotrexate in a stable dose for at least 8 weeks. The concomitant use of prednisone (<10 mg/day) and non-steroidal anti-inflammatory drugs was allowed if stable for at least one month. Approval for this study was obtained from the institutional ethics review committee at the Academic Medical Center/University of Amsterdam. All participants gave written informed consent.

Clinical assessments
RA disease activity was assessed at baseline and weeks 16 and 52 after the start of adalimumab treatment using the DAS28. Clinical response was evaluated by the EULAR response criteria. For comparison of data between responders (good and moderate) and non-responders we used response measured at week 16. In addition, the presence of extra-articular manifestations (such as vasculitis, nodules and pleuritis) was noted before entry into the study.

Cardiovascular risk factor profiles
In the assessment of cardiovascular risk factors the following data were recorded: medical history including cardiovascular events, smoking (current smoker, ever smoker) and current medication, hypertension, dyslipidaemia, diabetes and body mass index (BMI; kg/m²).

Lipid profiles
Serum total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, triglyceride and lipoprotein (a) (Lp(a)) levels were assessed by standard laboratory techniques. Apolipoprotein (apo) A-I and apo B levels were measured by an automated nephelometric assay using an array protein system nephelometer (Beckman, Mijdrecht, The Netherlands). In addition, erythrocyte sedimentation rate (ESR; mm/h) and CRP levels (mg/l) were determined. Blood was drawn from patients while fasting at baseline and at 16 and 52 weeks after initiation of adalimumab therapy. All values were determined by the GLP certified routine clinical chemistry laboratory at the Academic Medical Center in Amsterdam.

MIF ELISA
Natural serum MIF levels (pg/ml) were determined with a commercial quantitative sandwich-enzyme immunoassay (human MIF, DY289, R&D Systems Inc, Minneapolis, Minnesota, USA). The assay was performed according to the manufacturer’s instructions. Fasting serum samples were stored at −80°C and analysed all at once.

Statistical analysis
A paired t test or the Wilcoxon signed ranks test, whichever was appropriate, was used to determine significant changes from baseline. Probability values less than 0.05 were considered statistically significant in a two-tailed test. This exploratory

Table 1 Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>All n = 50</th>
<th>Responders n = 39</th>
<th>Non-responders n = 11</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>51 (13)</td>
<td>51 (12)</td>
<td>47 (17)</td>
<td>0.331</td>
</tr>
<tr>
<td>Female (%)</td>
<td>38 (76)</td>
<td>28 (72)</td>
<td>10 (91)</td>
<td>0.190</td>
</tr>
<tr>
<td>Disease duration, months (IQR)</td>
<td>59 (33–145)</td>
<td>61 (29–149)</td>
<td>54 (34–142)</td>
<td>0.935</td>
</tr>
<tr>
<td>Erosive disease (%)</td>
<td>33 (66)</td>
<td>26 (67)</td>
<td>7 (64)</td>
<td>0.851</td>
</tr>
<tr>
<td>Rheumatoid factor positive (%)</td>
<td>36 (72)</td>
<td>29 (74)</td>
<td>7 (64)</td>
<td>0.484</td>
</tr>
<tr>
<td>Anti-CCP positive (%)</td>
<td>35 (70)</td>
<td>28 (72)</td>
<td>7 (64)</td>
<td>0.602</td>
</tr>
<tr>
<td>Extra articular manifestations (IQR)</td>
<td>14 (28)</td>
<td>12 (31)</td>
<td>2 (18)</td>
<td>0.412</td>
</tr>
<tr>
<td>DAS28 (SD)</td>
<td>5.6 (1.1)</td>
<td>5.7 (1.1)</td>
<td>5.3 (0.8)</td>
<td>0.225</td>
</tr>
<tr>
<td>BMI, kg/m² (SD)</td>
<td>27 (6.3)</td>
<td>26 (6.7)</td>
<td>26 (6.7)</td>
<td>0.357</td>
</tr>
<tr>
<td>Smokers, current (%)</td>
<td>12 (24)</td>
<td>10 (26)</td>
<td>2 (18)</td>
<td>0.509</td>
</tr>
<tr>
<td>Smokers, ever (%)</td>
<td>31 (62)</td>
<td>27 (69)</td>
<td>4 (36)</td>
<td>0.047*</td>
</tr>
<tr>
<td>SBP, mm Hg (SD)</td>
<td>132 (15)</td>
<td>133 (16)</td>
<td>129 (12)</td>
<td>0.506</td>
</tr>
<tr>
<td>DBP, mm Hg (SD)</td>
<td>80 (9)</td>
<td>80 (9)</td>
<td>83 (8)</td>
<td>0.241</td>
</tr>
<tr>
<td>ESR, mm/h (IQR)</td>
<td>20 (11–35)</td>
<td>20 (11–35)</td>
<td>20 (15–36)</td>
<td>0.824</td>
</tr>
<tr>
<td>CRP, mg/l (IQR)</td>
<td>17 (5–20)</td>
<td>10 (4–22)</td>
<td>8 (5–16)</td>
<td>0.779</td>
</tr>
<tr>
<td>Diabetes mellitus type 2 (%)</td>
<td>4 (8)</td>
<td>4 (10)</td>
<td>0 (0)</td>
<td>0.268</td>
</tr>
<tr>
<td>Previous cardiovascular event (%)</td>
<td>4 (8)</td>
<td>3 (6)</td>
<td>1 (9)</td>
<td>0.687</td>
</tr>
<tr>
<td>Statin use (%)</td>
<td>6 (12)</td>
<td>5 (13)</td>
<td>1 (9)</td>
<td>0.717</td>
</tr>
<tr>
<td>Antihypertensive drug use (%)</td>
<td>16 (32)</td>
<td>13 (41)</td>
<td>3 (27)</td>
<td>0.704</td>
</tr>
<tr>
<td>Methotrexate, mg/week (SD)</td>
<td>18.4 (7.6)</td>
<td>18.0 (7.7)</td>
<td>19.5 (7.5)</td>
<td>0.500</td>
</tr>
<tr>
<td>Use of corticosteroids (%)</td>
<td>16 (32)</td>
<td>11 (28)</td>
<td>5 (45)</td>
<td>0.279</td>
</tr>
<tr>
<td>Prednisone dose, mg/day (SD)</td>
<td>7.8 (2.4)</td>
<td>7.8 (2.4)</td>
<td>8.0 (2.7)</td>
<td>0.268</td>
</tr>
<tr>
<td>Use of NSAID (%)</td>
<td>36 (72)</td>
<td>28 (78)</td>
<td>8 (73)</td>
<td>0.951</td>
</tr>
</tbody>
</table>

Mean values (SD), median and interquartile range (IQR) or percentages are shown.
*p Values <0.05 (two-sided) are significant.
BMI, body mass index; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; NSAID, non-steroidal anti-inflammatory drug; SBP, systolic blood pressure.
study was not powered to correct for multiple comparisons by Bonferroni correction. Independent samples t tests were used for subanalysis to detect differences in baseline values or changes after treatment between groups. Correlations were assessed with the Pearson product-moment or Spearman rank-order correlation coefficients, whichever was appropriate. Stepwise backward multivariable linear regression analysis was used to identify possible baseline predictors of change in MIF levels at weeks 16 and 52. Because delta MIF levels had a skewed distribution, values were rank-transformed before linear regression analysis. Baseline variables included in the analysis were sex, BMI, MIF, CRP, triglyceride, total cholesterol and HDL levels. Values are expressed as the mean (SD) or median and interquartile range (IQR), whichever was appropriate. SPSS 12.0.2 for Windows was used.

RESULTS

Patients and clinical response

The baseline patient characteristics of 50 patients are shown in table 1. The DAS28 score decreased significantly after 16 weeks (DAS28 5.7, SD 1.2) and 52 weeks (5.4, SD 1.4) of adalimumab therapy compared with baseline (5.6, SD 1.2; both p<0.001). At week 16 all patients were evaluable for clinical response: 11 (22%) patients were EULAR non-responders, 25 (50%) were moderate responders and 14 (28%) were good responders. At week 52 there were 44 patients with an evaluable response, of whom six (14%) were non-responders, 18 (41%) were moderate responders and 20 (45%) were good responders. Six patients dropped out of the study between 16 and 52 weeks follow-up due to lack of efficacy in five patients and a serious adverse event in one patient.

Pretreatment serum MIF levels

A large variability in MIF levels was observed between patients varying from the lowest detectable concentration of 60 pg/ml up to 6571 pg/ml. There was no significant relationship with the use of low-dose corticosteroids or the dosage of methotrexate, nor with clinical measures of disease activity at baseline (data not shown).

Pretreatment apo A-I and HDL levels are inversely correlated with systemic inflammation

There was a negative correlation between the pretreatment apo A-I and CRP levels (r = −0.338, p = 0.017) as well as ESR (r = −0.347, p = 0.014). Similarly, pretreatment HDL-cholesterol correlated inversely with CRP levels and ESR (r = −0.290, p = 0.041 and r = −0.340, p = 0.016, respectively).

Interestingly, higher pretreatment HDL levels correlated with lower MIF levels before the initiation of adalimumab therapy (r = −0.294, p = 0.040). As expected, baseline LDL-cholesterol was significantly lower in the six patients who used statins compared with the other 44 who did not use a statin (p = 0.007); other lipoproteins did not differ between these groups. All patients who used statins were known to have a history of hypercholesterolaemia. Four of the six patients had had a previous cardiovascular event, and all six were on concomitant antihypertensive drugs. One patient also had type 1 diabetes.

Sustained downregulation of MIF and inflammatory parameters, but not of HDL-cholesterol after adalimumab therapy

Serum MIF levels were significantly decreased 16 weeks after the initiation of adalimumab therapy (median 171 pg/ml, IQR 60–444) compared with baseline (median 333 pg/ml, IQR 93–1544, p = 0.020). This effect was sustained up to week 52 (median 145, IQR 60–335, p = 0.012; fig 1). CRP and ESR levels decreased significantly after 16 and 52 weeks of adalimumab therapy (both p<0.001, table 2).

The mean HDL-cholesterol levels increased at week 16 compared with baseline (p<0.001). However, HDL levels returned to nearly baseline at week 52 (table 2). LDL-cholesterol levels did not change after adalimumab treatment. Furthermore, Lp(a) levels decreased significantly at week 16 up to week 52 after treatment (both p = 0.001, table 2).

Improvement of the atherogenic index after adalimumab therapy

The mean apo A-I levels (high levels are thought to be cardioprotective) were significantly increased to 1.56 mmol/l (22%) at week 52 (p = 0.050). The total cholesterol/HDL ratio showed a temporary improvement (p = 0.054), due to the increase in HDL cholesterol, nor with clinical measures of disease activity at baseline (data not shown).

Extended report

![Figure 1](image-url)
levels in combination with gender predicted 45% of the variance in change of the MIF concentration at week 16 (adjusted R² = 0.453). The MIF concentration at baseline alone predicted 38% of the variance in change in the MIF concentration at week 52 (adjusted R² = 0.383). A significantly larger decrease in the MIF concentration was seen in female compared with male patients at week 16 (p = 0.011), but no gender difference was observed at week 52. We observed no association between baseline levels of inflammation or lipid profile and changes in MIF levels after treatment.

**Baseline MIF concentrations and gender predict changes in MIF after adalimumab treatment**

With stepwise backward multivariable linear regression analysis we identified baseline predictors for change in MIF levels after treatment. Included in the analysis were the following baseline variables: sex, BMI, MIF, CRP, triglyceride, total cholesterol and HDL levels. No baseline predictors for change in MIF concentration over time were identified other than patient gender and pretreatment MIF concentrations. Baseline MIF concentration in combination with gender predicted 45% of the variance in change of the MIF concentration at week 16 (adjusted R² = 0.453). The MIF concentration at baseline alone predicted 38% of the variance in change in the MIF concentration at week 52 (adjusted R² = 0.383). A significantly larger decrease in the MIF concentration was seen in female compared with male patients at week 16 (p = 0.011), but no gender difference was observed at week 52. We observed no association between baseline levels of inflammation or lipid profile and changes in MIF levels after treatment.

**Changes in MIF levels and lipid profile in relation to clinical response**

We analysed whether changes in MIF concentration differ between EULAR responders compared with non-responders. We found no relationship between clinical response and changes in MIF concentration at week 16 nor at week 52. However, the decrease in the Lp(a) concentration was greater in responders than in non-responders at week 16 (p = 0.018), with a similar trend at week 52 (p = 0.087). Furthermore, in accordance with previous data, an increase in HDL levels at week 16 was associated with a decrease in the DAS28 score at the same time.

**Table 2 Changes in MIF levels and lipid profile over time**

<table>
<thead>
<tr>
<th></th>
<th>Week 0 (n = 50)</th>
<th>Week 16 (n = 50)</th>
<th>p Value</th>
<th>Week 52 (n = 44)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIF, pg/ml (IQR)</td>
<td>333 (90–1544)</td>
<td>171 (60–444)</td>
<td>0.020*</td>
<td>145 (60–335)</td>
<td>0.012*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l (SD)</td>
<td>4.86 (1.07)</td>
<td>5.06 (1.16)</td>
<td>0.053</td>
<td>4.98 (1.13)</td>
<td>0.301</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/l (SD)</td>
<td>1.52 (0.38)</td>
<td>1.66 (0.38)</td>
<td>&lt;0.001*</td>
<td>1.62 (0.39)</td>
<td>0.061</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/l (SD)</td>
<td>2.88 (0.95)</td>
<td>2.96 (1.01)</td>
<td>0.392</td>
<td>2.93 (1.02)</td>
<td>0.577</td>
</tr>
<tr>
<td>Triglycerides, mmol/l (SD)</td>
<td>1.01 (0.51)</td>
<td>0.99 (0.54)</td>
<td>0.513</td>
<td>0.94 (0.43)</td>
<td>0.350</td>
</tr>
<tr>
<td>Apo A-1, mmol/l (SD)</td>
<td>1.46 (0.25)</td>
<td>1.56 (0.22)</td>
<td>&lt;0.001*</td>
<td>1.56 (0.21)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Apo B, mmol/l (SD)</td>
<td>0.99 (0.25)</td>
<td>1.00 (0.27)</td>
<td>0.465</td>
<td>0.99 (0.28)</td>
<td>0.816</td>
</tr>
<tr>
<td>Lp(a), mmol/l (IQR)</td>
<td>198 (65–356)</td>
<td>175 (85–377)</td>
<td>&lt;0.001*</td>
<td>171 (47–375)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Total cholesterol/HDL (SD)</td>
<td>3.33 (0.93)</td>
<td>3.15 (0.65)</td>
<td>0.034*</td>
<td>3.19 (0.84)</td>
<td>0.272</td>
</tr>
<tr>
<td>Apo B/Apo A-I (SD)</td>
<td>0.70 (0.21)</td>
<td>0.65 (0.20)</td>
<td>0.014*</td>
<td>0.65 (0.20)</td>
<td>0.050</td>
</tr>
<tr>
<td>Glucose, mmol/l (SD)</td>
<td>5.13 (1.97)</td>
<td>4.95 (1.14)</td>
<td>0.847</td>
<td>5.04 (1.34)</td>
<td>0.930</td>
</tr>
<tr>
<td>CRP, mg/l (IQR)</td>
<td>8.0 (4.6–19.6)</td>
<td>3.8 (1.8–9.1)</td>
<td>&lt;0.001*</td>
<td>2.7 (1.1–5.6)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Values are represented as mean (SD) or median and interquartile range (IQR). *p Values <0.05 (two-sided) are significant.

Apo, apolipoprotein; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein (a); MIF, macrophage migration inhibitory factor.
point (r = -0.308, p = 0.050). Similarly, HDL levels increased more in EULAR responders than in non-responders, although this difference did not reach statistical significance (p = 0.068). The increase in apo A-I was no different between response groups.

**DISCUSSION**

Both RA and atherosclerosis are related to chronic inflammation. There is increasing evidence that TNF and MIF are involved in these conditions and that the role of these cytokines is linked. In both RA and atherosclerosis enhanced MIF levels have been observed at the site of inflammation and MIF was shown to mediate leucocyte recruitment into the inflamed joint and vessel wall. Furthermore, MIF can mediate integrin activation and induce the expression of other inflammatory cytokines, such as IL-6, TNF and matrix metalloproteinases, associated with joint damage in RA and plaque instability in atherosclerosis. The role of TNF is supported by the observation that anti-TNF therapy may reduce the increased cardiovascular risk associated with RA by decreasing systemic inflammation. Previous work has shown that TNF blockade may influence lipid levels, insulin resistance, vascular adhesion molecule expression and endothelial function. We performed the present study to provide more insight into the mechanisms that could be involved in the effects of anti-TNF therapy on cardiovascular risk. The results confirm our hypothesis that adalimumab treatment leads to the downregulation of MIF, with potential beneficial consequences for vascular inflammation. Moreover, we show for the first time that long-term TNF blocking therapy with adalimumab has a favourable influence on the lipid profile of RA patients.

It has previously been suggested that chronic systemic inflammation in RA and subsequent atherogenesis are partly the result of chronic cytokine overflow from the inflamed joints into the circulation. Anti-TNF therapy has been shown to diminish local inflammation in the joints by decreasing synovial cell infiltration and the expression of adhesion molecules, chemokines and cytokines, which coincides with a reduction in acute phase reactants. A decrease in CRP levels was previously shown to be accompanied by a reduction in synovial MIF and TNF expression in the same patient when disease activity was reduced by conventional DMARD therapy. In light of these data we hypothesised that pro-inflammatory cytokine release from the inflamed joint could be diminished after adalimumab treatment, resulting in a decrease in systemic levels of cytokines, including MIF (fig 2). Consistent with this notion we found serum MIF levels to be significantly down-regulated within 16 weeks after adalimumab therapy, an effect that was sustained up to one year after the initiation of treatment. Whether anti-TNF therapy reduces MIF in the atherosclerotic lesion in patients is as yet unknown, but beneficial effects of TNF inhibition on atherosclerotic lesions have been demonstrated in animal atherosclerosis models. A decrease in MIF expression could lead to reduced monocyte and T-cell influx into the inflamed vessel wall, hence arresting plaque formation.

HDL levels increased temporarily after treatment, resulting in an improved atherogenic index (total cholesterol/HDL-cholesterol) at week 16, which was no longer present after one year of adalimumab treatment. A brief rise in HDL-cholesterol, also known as an inverse phase reaction, is to be expected after reversing the inflammatory state and was previously reported in other studies with infliximab. In addition, we observed a sustained increase in apo A-I levels and thus an improvement of the apo B/A-I ratio. Based on these findings one can speculate that the apo B/A-I ratio may better reflect the cardiovascular risk profile after TNF blocking therapy than the traditional atherogenic index (total cholesterol/HDL), as differential effects of adalimumab therapy can be observed for apo A-I and HDL levels. Of interest, serum apo A-I has previously been shown to inhibit T-cell contact-induced monocyte activation. As a result cytokine (TNFα and IL-1β) production by monocytes was inhibited while monocyte proliferation remained unaltered. These data indicate a novel anti-inflammatory mechanism of this apolipoprotein. Conceivably, the sustained increase of apo A-I levels in our study might lead to the inhibition of T-cell-induced monocyte activation, both in the inflamed synovium and in the vessel wall.

Lp(a) has been demonstrated to have a spectrum of pathogenic activities among which are increased vascular adhesion molecule expression, chemotaxis of monocytes, foam cell formation, smooth muscle cell proliferation and increased platelet aggregation. Different clinical studies have shown Lp(a) levels to be an independent risk factor for developing CHD. The significant decrease in levels of Lp(a) both 16 and 52 weeks after adalimumab therapy could thus contribute to a decrease in the pro-atherogenic state. This could be a direct effect of anti-TNF therapy but may also be an indirect effect of the overall diminishment in inflammation.

The open-label rather than placebo-controlled design is obviously a limitation of this study, as we cannot conclude with complete certainty that the decrease in MIF levels resulting from decreased inflammation was a direct effect of TNF blockade or merely a result of regression to the mean. However, the patients had persistent disease activity in spite of at least two conventional DMARD before inclusion in the study, suggesting that the reduction in inflammation was the result of TNF blockade. The data presented in this exploratory study thus provide the rationale for future studies with a controlled design to confirm the effects on lipid profiles and MIF levels in relation to cardiovascular endpoints.

In conclusion, TNF blocking therapy reduced systemic MIF levels, possibly reflecting a reduction in the atherogenic state. Apart from reduced systemic inflammation, as shown by reduced CRP and ESR levels, the sustained decrease in the apo B/apo A-I ratio suggests a favourable effect of adalimumab treatment on markers associated with atherogenesis.

**Acknowledgements:** The investigators would like to thank the research nurses Margot Colomijn and Mariane Anson for performing the clinical assessments and Laura Splint for performing the MIF ELISA.

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**Competing interests:** PPT is a member of the advisory board of Abbott and has received honoraria for lectures. The study sponsors had no involvement in the study design, the collection, analysis and interpretation of the data, writing the report, or the decision to submit the paper for publication.

**Ethics approval:** Approval for this study was obtained from the institutional ethics committee.

**Patient consent:** Obtained.

**REFERENCES**


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