Novel visualization techniques towards identification of atherosclerotic patients at risk

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CHAPTER 3

Does reduction of disease activity improve early markers of cardiovascular disease in newly diagnosed Rheumatoid Arthritis patients?

Lodewijk de Groot, Nynke A. Jager, Johanna Westra, Andries J. Smit, Cees G.M. Kallenberg, Marcel D. Posthumus, Marc Bijl

Rheumatology (accepted)
Abstract

OBJECTIVES The incidence of cardiovascular disease (CVD) is increased in rheumatoid arthritis (RA). The current study was designed to evaluate whether reduction of disease activity will influence early markers of cardiovascular disease.

METHODS In a prospective longitudinal study 58 newly diagnosed RA patients and 58 age and sex matched healthy controls (HC) were included. Endothelial dysfunction was measured by small artery elasticity (SAE), endothelial cell activation was assessed by measuring soluble vascular cellular activation molecule-1 (sVCAM-1) and von Willebrand Factor (vWF). Advanced glycation endproducts (AGEs) were quantified by skin autofluorescence. After one year measurements were repeated in all RA patients.

RESULTS At entry, SAE was decreased in RA (median 3.4 ml/mmHg100, range 1.2-9.0) vs. HC (6.1, 5.0-15.3), P<0.0001 and sVCAM-1 and vWF were increased: 391 ng/ml (256-680) vs. 341 (223-691), P=0.0015 and 120 ng/ml (26.5-342) vs. 99 (22-298), P=0.02, respectively. SAE was inversely correlated with DAS-28 (r=-0.31, P=0.016). AGEs were increased 2.55 a.u. (1.29-4.65) vs. 2.12 (1.32-3.82) in HC P=0.003. In multivariate analysis, presence of RA, age and systolic blood pressure were independently and inversely related to SAE. After one year SAE had significantly improved in RA from 3.4 (1.2-9.0) to 3.8 (1.5-10.3), P=0.03.

CONCLUSION Endothelial dysfunction is present in newly diagnosed RA patients, independently of traditional risk factors and is inversely correlated with disease activity. By reducing disease activity endothelial dysfunction improves, though not to normal values. Besides reduction in disease activity targeting traditional risk factors remains important in preventing CVD in RA.

Keywords: Rheumatoid arthritis, endothelial cell activation, endothelial dysfunction, small artery elasticity, intima media thickness, advanced glycation end products, atherosclerosis
Introduction

Morbidity, and mortality due to cardiovascular diseases (CVD) in RA is increased (1,2). CVD is the result of atherosclerosis and formation of atherogenic plaques (3). Three stages are identified in the formation of atherogenic plaques: Endothelial cell (EC) activation, endothelial cell dysfunction and finally preclinical atherosclerosis (4). In addition to known traditional risk factors such as smoking, hypertension, overweight and dyslipidemia, other non-traditional-risk factors are supposed to play an important role in increased cardiovascular mortality and morbidity in RA. Among these, CRP, auto-antibodies, oxidized LDL and Advanced Glycation Endproducts (AGEs) are recognized (5). AGEs accumulate in tissues with slow turnover such as the vessel wall, cartilage and skin. AGEs can be quantified in the skin, urine and serum and are increased in longstanding RA (6). AGEs can ligate to the receptor of AGE (RAGE), which, as it is present on endothelial cells, results in EC activation and finally formation of sVCAM-1 (7). In this way the AGE-RAGE interaction becomes a self maintaining and re-enforcing process. We hypothesize that systemic inflammation is a major non-traditional risk factor that contributes significantly to the development of premature atherosclerosis in RA. We hypothesize that even in newly diagnosed RA AGE accumulation and early stages of atherosclerosis, i.e. endothelial activation and dysfunction, will be present and that these changes can be reversed by reducing disease activity.

Methods

Patients and controls
The study was designed as a prospective longitudinal study with RA patients compared with healthy controls at inclusion (t=0). After one year patients served as their own controls. Consecutive patients with age 18-80 yrs, fulfilling the American College of Rheumatology (ACR) criteria for RA (8) and having symptoms of RA since maximum one year, were asked to participate. The patients had to be mentally able to understand the study information and had to subscribe the informed consent. Patients with diabetes mellitus (fasting blood glucose level> 7 mmol/L, or use of antidiabetic drugs), pregnancy, renal impairment (serum creatinin> 140μmol/L) or surgery or myocardial infarction in the past three months were excluded. In total 58 patients and 58 healthy controls (HC) were included. Patients and HC were recruited between august 2008 and september 2010. Information concerning traditional risk factors for CVD was obtained from all study participants, as described earlier (9). Patients were treated according to the DREAM protocol, a DAS-guided scheme, aiming at remission (DAS-28<2.6) (10). Treatment for hypertension or dyslipidemia was not pre-specified in the protocol and at the discretion of the treating physician. The study was approved by the local medical ethics committee of the University Medical Center of Groningen and informed consent was obtained from all study participants.
Blood sampling and analysis
After an overnight fast blood was sampled and creatinine, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and glucose were measured using routine procedures. Serum was stored at -20°C for analysis of endothelial cell activation markers.

Measurement of disease activity, joint damage and cumulative CRP
For assessment of disease activity the Disease Activity Score for 28 joints was used (DAS-28) (11,12). Cumulative CRP was calculated as described (13).

Measurement of Endothelial cell activation markers
Serum sVCAM-1 levels were measured by ELISA according to manufacturers guidelines (R&D Systems, Abingdon, UK). vWF was measured by ELISA as previously described (14).

Measurement of Small Artery Elasticity (SAE), intima-media thickness (IMT), and AGEs
SAE was measured as described before (15). Coefficient of variation for SAE was 11.2% (16). IMT was measured in a standardized way by the same experienced technicians. Coefficient of variation of IMT measurement is approximately 5% (17,18). AGEs were measured using the auto-fluorescence reader (AGE-reader, DiagnOptics Technologies BV Groningen, The Netherlands). Coefficient of variance is 5.8% (19).

Statistical methods
Data are expressed as median (range), unless stated otherwise. The sample size of 60 patients was calculated on the assumption that 50% of RA patients would achieve remission, and SAE in these patients would normalize (from 4.5 to 7.7 ml/mmHg100; with SD of 3.7 found in an earlier study (20) Expecting 5% loss to follow-up 60 patients were invited. Two-sample t-tests or Mann-Whitney-U tests were used as appropriate to make comparisons between patients and controls for continuous variables. For categorical variables the chi-square method and for very small expected frequencies the Fisher’s exact test were used. Gaussian distribution of the data was analyzed with D’Agostino and Pearson omnibus normality test. Correlation analysis was performed by Pearson correlation when variables were normally distributed, otherwise the Spearman correlation was used. To control for unbalanced cardiovascular risk factors between patients and HC multivariate regression analysis was used. Multivariate linear regression analysis for predictors of SAE at t=0 was performed by using forward inclusion of variables with P values ≤0.10 in univariate analysis. The probability of F for entry was 0.05, implicating that variables are included in the model until the statistical significance does not improve anymore. Longitudinal data were analyzed using Wilcoxon matched-pairs signed rank test. Analyses were performed using GraphPad Prism version 4.03 2005 and SPSS version 14.0. Two-sided P-values <0.05
were considered significant.

Results

Clinical characteristics of patients and controls
RA patients had an unfavorable cardiovascular risk profile. In comparison to HC, RA patients were more often smokers, had more frequently hypertension, had lower HDL-levels, and higher triglyceride levels. Most patients were on NSAIDs. Use of antihypertensive medication as well as statin use remained constant. After one year a second measurement could be obtained in all but 5 patients.

Endothelial dysfunction, endothelial activation, intima media thickness and AGES
At inclusion, endothelial dysfunction, as reflected by SAE was present in RA patients. SAE was 3.4 ml/mmHg100 (1.2-9.0) vs. 6.1 (2.0-15.3) in HC (P<0.0001). Endothelial activation was present in patients as well. sVCAM-1 in RA patients was 391 ng/ml (256-680) vs. 341 (223-691) in HC (P=0.0015) and vWF was 120 ng/ml (26.5-342) vs. 99 (22-298) P=0.017. IMT was not different between RA and HC on t=0, being 0.73mm (0.45-1.64) in RA vs. 0.72 (0.39-1.46) in HC. AGEs were increased in RA (2.55 a.u. (1.29-4.65) vs. 2.12 (1.32-3.82) in HC P=0.003. In multivariate analysis with forward inclusion of variables that had a P value <0.10 in univariate analysis, endothelial dysfunction was independently associated with the presence of RA, age and systolic blood pressure. The adjusted R² of this model was 0.454 (table 1). In RA patients, SAE was inversely correlated with DAS-28, (r=-0.30, P=0.03), AGE's (r=-0.33, P=0.016), systolic blood pressure (r=-0.56, P<0.0001) and IMT (r=-0.26, P=0.05). AGEs correlated with DAS-28 (r=0.29, P= 0.03), systolic RR (r=0.37, P=0.005), glucose levels (r=0.35, P=0.01) and IMT (r=0.55, P<0.0001)
Longitudinal data

DAS-28 improved from 4.64 (range 2.1-7.8) to 2.15 (0.17-5.53) P< 0.0001. After 1 year (t=1) SAE improved in RA patients, although it did not normalize to levels found in HC. SAE increased from 3.4, (1.2-9.0) to 3.8, (1.5-10.3), P=0.03. No significant change was found in sVCAM-1, vWF, IMT or AGEs (data not shown). Systolic blood pressure did not change significantly, whereas diastolic blood pressure decreased slightly from median 80 mmHg (56-108) to 79 (58-112), P=0.03. Total cholesterol increased from 5.0 mmol/L (2.9-9.1) to 5.5 (3.7-10.1), HDL increased from 1.2 mmol/L (0.8-2.7) to 1.5 (0.8-3.0), and LDL increased from 3.2 mmol/L (1.4-6.5) to 3.7 (1.3-6.7). Only the rise in HDL reached significance (P=0.0001). Change in DAS-28 did not correlate with the change in SAE, also no relation was found between change in SAE and cumulative CRP. To further substantiate the relation between disease activity, endothelial activation and dysfunction RA patients were divided in those reaching remission (DAS < 2.6) after one year versus those not reaching remission. Patients in remission (n=36) showed improvement in SAE (from 3.2 (1.2-9.0) to 3.9 (1.5-10.3), P=0.02)
whereas those not reaching remission (n=15) had a deterioration in SAE from 4.0 (1.3-8.0) to 3.8 (1.5-8.4) P=0.6. No difference was found in endothelial activation markers and AGEs regarding patients in remission vs. no remission (table 2).

**Table 2.** Comparison of patients in remission vs patients not in remission on $t=1$

<table>
<thead>
<tr>
<th></th>
<th>Remission (n=36)</th>
<th></th>
<th>No remission (n=15)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t=0$</td>
<td>$t=1$</td>
<td>P-value</td>
<td>$t=0$</td>
</tr>
<tr>
<td>SAE</td>
<td>3.2 (1.2-9.0)</td>
<td>3.9 (1.5-10.3)</td>
<td>0.02</td>
<td>4.0 (1.3-8.0)</td>
</tr>
<tr>
<td>VCAM</td>
<td>377 (256-680)</td>
<td>338 (224-637)</td>
<td>0.13</td>
<td>410 (297-620)</td>
</tr>
<tr>
<td>vWF</td>
<td>115 (35-328)</td>
<td>120 (38-321)</td>
<td>0.28</td>
<td>173 (27-342)</td>
</tr>
<tr>
<td>AGEs</td>
<td>2.53 (1.29-3.45)</td>
<td>2.60 (1.52-4.29)</td>
<td>0.67</td>
<td>2.62 (1.44-4.65)</td>
</tr>
<tr>
<td>IMT</td>
<td>0.73 (0.49-1.16)</td>
<td>0.74 (0.48-1.21)</td>
<td>0.61</td>
<td>0.79 (0.45-1.62)</td>
</tr>
<tr>
<td>DAS-28</td>
<td>4.5 (2.1-7.8)</td>
<td>1.9 (0.17-2.58)</td>
<td>$&lt;0.0001$</td>
<td>5.1 (2.9-7.8)</td>
</tr>
</tbody>
</table>

SAE; Small Artery Elasticity, VCAM; Vascular Cellular Adhesion Molecule, vWF; von Willebrand Factor, AGEs; Advanced Glycation Endproducts, IMT; Intima media Thickness, DAS-28; Disease Activity Score of 28 joints

**Discussion**

Our study shows that already very early in the course of RA endothelial activation and dysfunction are present as well as increased accumulation of AGEs. It remains to be established whether these changes occur in the very short period (<1 year) of overt clinical disease, or reflect a much longer period of subclinical inflammation. It is known from previous studies that already years before the actual diagnosis of RA levels of inflammatory cytokines like IL-6, TNF-α and IFN-γ are increased, indicating presence of subclinical inflammation (21). The unaltered IMT, despite increased presence of several traditional cardiovascular risk factors, in our patients at inclusion, argues against longstanding pro-atherosclerotic events before disease onset. This suggests that disease activity in itself indeed contributes significantly to the development of atherosclerosis, which is supported by the inverse relation we found between SAE and DAS-28. Furthermore, in multivariate analysis presence of RA proved to be an independent risk factor for decreased SAE at baseline.

Another, cross-sectional, study performed in patient with early RA not only showed a decreased SAE in RA patients, but also a significant increase in IMT (22). The fact that this study as well as other studies (23-25) in contrast to our study did show an increase of IMT even at baseline in early RA might be due to differences in methods or patient characteristics. For example in our study median DAS-28 was 4.64, in comparison to 5.6 in the study of Adhikari et al (22).

In contrast to the unaltered IMT, we found that even in early RA at time of diagnosis AGEs
in the skin were increased, suggesting that a subclinical (inflammatory) process proceeds the occurrence of clinical RA. So far no studies have been performed in early RA showing an increase in AGEs in the skin. AGEs in the skin indicate that the body has been exposed to oxidative stress or higher glucose levels. In diabetes increase of AGEs has been shown to be a predictor of macro- and microvascular events (26,27). It may be that also in RA increase of AGEs in the skin indicates risk for future cardiovascular events, in particular because, in contrast to SAE, AGEs persisted at higher levels despite lowering disease activity.

Endothelial function improved after one year of treatment, but this improvement was relatively low and SAE by far did not reach normal values. The fact that systolic blood pressure did not decrease in RA may contribute in the failure to reach normal SAE values. Patients who received remission though showed an improvement in SAE, whereas patients who did not reach remission did not change. Together, these findings support the recommendation that reduction of disease activity might reduce progression of CVD in RA. The GO-BEFORE trial in early RA showed superiority of golimumab+MTX over MTX monotherapy in reducing markers of endothelial activation (28). Several other studies have been performed that showed a better improvement in endothelial dysfunction in established RA, especially when treated with TNF blocking agents (29-32). However these studies were performed in established RA with anti-TNF as DMARD therapy. Also disease activity was much higher in these studies. One study also showed improvement in SAE in an early RA cohort (33).

It might be that the period of one year of treatment is too short to show an improvement of the endothelial function to normal levels. Alternatively, and supported by our data, even after reaching remission endothelial activation still remains present, making normalization of SAE, as part of the same process, unlikely. The fact that AGEs are increased at baseline might be of importance in this concept. Formation of AGE’s is a self reinforcing process and as such might drive the atherosclerotic process independent of clinically active disease. Our data suggest that even earlier intervention or longer follow-up is needed to address this issue.

Conclusions

Our study shows that endothelial dysfunction is present in early RA and can only very partially be improved by reducing disease activity in the first year of treatment. This indicates that reducing traditional risk factors remains important in preventing CVD in RA.
ENDOTHELIAL DYSFUNCTION AND DISEASE ACTIVITY IN EARLY RA

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