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Carotid Atherosclerosis, Disease Measures, Oxidized Low-density Lipoproteins, and Atheroprotective Natural Antibodies for Cardiovascular Disease in Early Rheumatoid Arthritis –– An Inception Cohort Study

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Carotid Atherosclerosis, Disease Measures, Oxidized Low-density Lipoproteins, and Atheroprotective Natural Antibodies for Cardiovascular Disease in Early Rheumatoid Arthritis — An Inception Cohort Study

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ABSTRACT. ***Objective.*** Although an enhanced risk of cardiovascular disease (CVD) in persons with rheumatoid arthritis (RA) is well established, the mechanisms behind it remain unclear. We studied whether carotid atherosclerosis, RA disease measures, or potential cardiovascular biomarkers influenced the incidence of CVD in an RA inception cohort.

Methods. RA disease measures and CVD biomarkers were assessed at 0, 3, 12, 24, and 60 months after disease onset, and carotid ultrasonography after 5 years. The study outcome was incident CVD events — acute myocardial infarction, angina pectoris, congestive heart failure, or ischemic cerebrovascular event. Survival analysis and Cox and longitudinal regressions were used for statistical analyses.

Results. A total of 105 patients, without CVD events prior to RA onset, experienced 17 CVD events, an incidence rate of 1.35 events per 100 person-years (95% CI 0.71–2.0). The rate of CVD events did not differ with regard to measures of carotid intima-media thickness, but it was higher for patients with bilateral carotid plaques than for those without (p = 0.012). Improvement in Disease Activity Score for 28 joints, visual analog scale for pain, and Stanford Health Assessment Questionnaire score over the first year, as well as usage of methotrexate (MTX), was associated, independent of age, with reduction of risk of CVD event [hazard ratios 0.68 (95% CI 0.5–0.97), 0.97 (95% CI 0.95–0.99), 0.35 (95% CI 0.15–0.82), and 0.34 (95% CI 0.12–0.91), respectively]. In longitudinal analyses, increasing oxidized low-density lipoprotein (oxLDL) and probability for low antiphosphorylcholine antibodies (anti-PC) were observed in those who experienced a subsequent CVD event.

Conclusion. Bilateral carotid plaques were associated with poor CVD-free survival. Early reductions of inflammation, pain, and disability as well as MTX usage were associated with better CVD outcome. Elevated oxLDL and low IgM anti-PC levels may link chronic inflammation in RA to enhanced risk of CVD events. (First Release May 15 2012; J Rheumatol 2012;39:1146–54; doi:10.3899/jrheum.111334)

Key Indexing Terms:
RHEUMATOID ARTHRITIS CARDIOVASCULAR DISEASE CAROTID ARTERY PLAQUE
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Recently, it has been recognized that cardiovascular disease (CVD) is a major contributor to mortality in rheumatoid arthritis (RA), leading to a reduction in lifespan by an average of 5 to 15 years in both men and women[1]. The risk of CVD in patients with RA is 1.5 to 2-fold that in the general population[2]. In spite of the clear association between RA and CVD, the explanation of the link between these conditions is challenging. The excess of CVD and cardiovascular mortality in RA is mostly observed after 10 years of disease duration[3,4], but it is unclear when the risk for CVD starts. Thus, there is a need for prospective inception cohort studies from disease onset with long followup, in which independent effects of clinically relevant markers of future CVD events can be elucidated.

In RA, the presence of subclinical atherosclerosis, measured by increased carotid intima-media thickness (cIMT) and occurrence of plaque, has been found frequently[5,6], and also early in the disease process[7]. However, there are few studies addressing predictors of subclinical atherosclerosis in RA, and

still fewer have addressed the influence of subclinical atherosclerosis on cardiovascular events[8,9]. To date, it is difficult to draw conclusions to what extent findings of carotid atherosclerosis have clinical significance and whether a greater burden of atherosclerosis translates to cardiovascular outcomes in RA.

Recently, we reported the association of the apolipoproteins and the novel risk marker IgM antibodies against phosphorylcholine (anti-PC) with subclinical atherosclerosis in patients with early RA[10]. Here, in the same prospective RA inception cohort study with an observation period > 10 years, we analyzed whether carotid atherosclerosis, longitudinal RA disease measures, and potential cardiovascular biomarkers could be associated with incident CVD events.

MATERIALS AND METHODS

Patients. We investigated the inception cohort of 114 patients of the BARFOT (Better Anti-Rheumatic FarmacOTherapy) observational study[10], who at study inclusion had an RA diagnosis according to the American College of Rheumatology criteria[11], a disease duration < 1 year, who were < 70 years of age, and who had started with disease-modifying antirheumatic drugs (DMARD) in accord with the recommended treatment strategy in Sweden. That strategy implied initial monotherapy and early use of low-dose oral glucocorticoids, and "step-up" combination therapy reserved for more severe disease, with the goal to achieve remission. Between June 2000 and March 2004, when these patients had been followed for 5 years since diagnosis, they had undergone high-resolution B-mode ultrasonography of the carotids as reported[10].

All study participants provided written informed consent. The study was approved by the local Ethics committee and was performed in accord with the Declaration of Helsinki.

Assessment of CVD outcomes. The study outcome was the first-ever CVD event occurring during the followup. Information on incident CVD event was obtained retrospectively and validated through a structured review of the medical records. CVD events were predefined as any incident acute myocardial infarction [AMI; a diagnosis based on history, electrocardiogram (ECG), and/or echocardiography together with typical enzymatic pattern and/or angiography], angina pectoris (registered as history of typical chest pain with compatible ECG or myocardial scintigraphy, stress ECG, or stress echocardiography and/or angiography), congestive heart failure (CHF; a recorded diagnosis based on history of at least 1 episode of symptomatic heart failure with continued dyspnea, New York Heart Association class 2–4, together with typical radiography and/or echocardiography) or ischemic cerebrovascular event (both ischemic stroke and transient ischemic attack diagnosed by typical clinical picture with neurological deficits and/or verified with computerized tomography and/or magnetic resonance imaging).

CVD risk factor assessments. Traditional cardiovascular risk factors. Information on smoking was obtained at study inclusion and at the 5-year assessment. Smoking status was characterized as daily ever-smoking (current or past) or never smoking. Hypertension was considered if at least 3 consecutive measurements of elevated blood pressure (systolic blood pressure ≥ 140 or diastolic ≥ 90 mm Hg) were documented and/or prescription of antihypertensive drug during followup. Diabetes mellitus was defined as history of diabetes and/or prescription of antidiabetic medication during followup. At the 5-year assessment body mass index (BMI; kg/m^2), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were determined. Hyperlipidemia was defined as TC ≥ 5.0 mmol/l or LDL ≥ 3.0 mmol/l and/or prescription of lipid-lowering drugs.

RA disease measures. Medical history and physical and routine laboratory examinations were assessed at inclusion and at 3, 6, 12, 24, and 60 month visits; and later, annually. Disease activity was measured with the Disease Activity Score (DAS28)[12] composite index. Functional assessment was carried out using the Swedish version of the Stanford Health Assessment Questionnaire (HAQ; range 0 to 3)[13]. Pain was measured by a visual analog scale (VAS pain; range 0 to 100 mm). At each followup visit information on medication was updated and regular use of drugs was considered [methotrexate (MTX), antimalarials, any other DMARD, biologics, glucocorticoids, and nonsteroidal antiinflammatory drugs (NSAID)] during at least 6 months throughout the followup.

C-reactive protein (CRP) was measured by a non-high sensitivity assay and low levels were reported as < 10 mg/l. Rheumatoid factor (RF) was measured by the agglutination test, where a positive titer was > 1/20.

Potential novel risk factors. At each visit up to 5 years of followup, supplementary nonfasting blood samples were drawn and aliquots of plasma were stored at –70°C until analysis for apolipoproteins, oxidized LDL (oxLDL), and IgM anti-PC. Apolipoproteins A1 (apoA1) and B (apoB) were determined by immunoturbidimetry (Synchron LX; Beckman Coulter, Brea, CA, USA).

oxLDL and IgM anti-PC were determined by ELISA (Mercodia AB, Uppsala, Sweden; and Athera CVDefine kit, Athera Biotechnologies AB) as described[14,15], and were expressed as arbitrary units. IgM anti-PC measurement range was from 6.25 to 100 U/ml; samples giving absorbance above 100 U/ml were diluted as appropriate and reassayed. Intra- and inter-assay coefficients of variations for IgM anti-PC were 5.2% and 1.1%, and for oxLDL 6.2% and 4.0%, respectively.

Carotid intima-media measurements. Right and left carotid arteries were examined with a duplex scanner (Aspen Acuson; Acuson Corp., Mountain View, CA, USA) using a 7 MHz linear array transducer. The far wall of the common carotid artery (CCA), 0.5 to 1.0 cm proximal to the beginning of the carotid bulb, was used for measurements of cIMT, defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo, and was measured in the region free of atherosclerotic plaques. For right and left CCA mean values of the cIMT within the 10-mm section were estimated, and the mean cIMT was calculated as follows: [(right + left)/2]. Carotid plaque, defined as a localized intima-media thickening > 1 mm and at least a 100% increase in thickness compared with adjacent wall segments, was screened for in the common, internal, and external carotids[16]. Plaque occurrence was classified as the absence of plaque (none), the presence of unilateral plaque, and the presence of bilateral plaques. Carotid measurements were carried out by 2 certified ultrasonographers. The intrareader coefficient of variation for cIMT was 3.2%.

Statistical analysis. In descriptive statistics, all 114 patients were surveyed. Continuous variables were summarized as mean (SD) or median (interquartile range; IQR), as appropriate, and categorical variables as frequencies (percentages). Chi-square, Fisher exact test, or Mann-Whitney U test was used for comparisons, as appropriate. cIMT and anti-PC levels were dichotomized by tertiles or determined as continuous variables as indicated.

In survival analyses, the 105 patients without CVD event prior to RA were included. One of these, with a CVD event during the first 5 years of disease, was excluded from analyses when required for correct interpretation of the statistical results. CVD incidence rates (with 95% confidence interval for a Poisson count) were presented as events per 100 person-years at risk, performed separately for the whole followup period and for the observation period after carotid ultrasonography examination. The Kaplan-Meier method was used to describe CVD event-free survival for the groups stratified by cIMT in tertiles or occurrence of carotid plaque, and Mantel-Cox log-rank analysis was applied to determine differences between survival functions obtained from the groups. Cox proportional hazards regression analysis was used to estimate the effect of predictors on the risk of first-ever CVD event, and age adjustment was applied for variables showing an effect with $p < 0.10$ in univariate Cox analyses. A 95% CI for hazard ratio (HR) was used to estimate the likely range of effect for the population coefficients.

In longitudinal analyses, the 105 patients with no CVD event before onset of RA were included. The variables measured repeatedly over the first 5 years after RA diagnosis were analyzed with mixed linear modeling for continuous

variables or generalized estimating equations (GEE) for dichotomized variables, with 2 between-group factors (occurrence of CVD event or non-CVD event) and 1 within-group factor, "time" (0, 3, 12, 24, 60 months, with exception of 1 case, as noted, whose factor "time" was limited up to 12 months). To allow for the means of the response variables to differ between CVD and non-CVD groups as time progressed, an interaction term with factor "time" was included in the models. The best-fit model was chosen according to the covariance structure and the smallest value of the Akaike Information Criterion (AICC) and Bayesian Information Criterion (BIC).

Log transformation was performed if required. Level of significance was $\alpha < 0.05$. Analyses were performed with the Statistica package, release 9 (StatSoft Scandinavia AB, Tulsa, OK, USA), and SPSS, version 18 (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 114 patients, 31.6% were men. At inclusion, mean age of the patients was 50.6 (SD 11.2) years, mean DAS28 was 5.37 (SD 1.12), and 67 patients (58.8%) were RF-positive. At the 5-year assessment, 80 (70.2%) patients were classified as having hyperlipidemia; however, only 17 (14.9%) patients received treatment with statins during followup. Then the mean cIMT was 0.67 mm (IQR 0.6–0.77) and bilateral carotid plaques were detected in 46 (40.4%) participants. During followup the prevalence of smoking history and hypertension was high: 80 (70.2%) subjects were ever-smokers and 67 (58.8%) were identified with hypertension.

The observation period started between January 1, 1995, and October 30, 1999, i.e., when the patients were included in the BARFOT study. The followup period for survival analyses lasted until an incident CVD event, death, loss to followup, or January 1, 2011, whichever came first. Fifteen (13.2%) patients were censored before the end of the study, of whom 8 subjects died of malignancies, 3 of infections, and 1 of Alzheimer disease, and 1 committed suicide; then 2 patients were lost to followup approximately 6 and 8 years after inclusion. Of the whole cohort of 114 patients, 9 had a history of CVD event before RA onset, 6 had AMI, 2 angina pectoris, and 1 ischemic cerebrovascular event.

Patients' characteristics according to whether they experienced an incident CVD event before or after RA onset are presented in Table 1. Compared with those who had no CVD events during the study period, patients who experienced CVD events after RA onset were older, were more frequently classified with hyperlipidemia, had lower DAS28 scores, had increasing oxLDL levels between time of inclusion and the 5-year assessment, had worse disease control measured by reduction (Δ) in DAS28, HAQ and VAS pain between inclusion and the 1-year assessment, were less often treated with MTX, and were more frequently found to have bilateral carotid plaques. These characteristics differed also from those who had their CVD event before RA diagnosis, where, additionally, cIMT was greater than but disease characteristics were similar to those without events.

Rates of incident CVD events and CVD event-free survival. The 105 patients with no history of CVD events prior to RA onset comprised 1259 person-years of observation by the censoring date January 1, 2011, with mean followup of 12 (SD 2.9) years. These patients experienced 17 incident CVD events, i.e., 16.2% of the patients, corresponding to an incidence rate of 1.35 events per 100 person-years (95% CI 0.71–2.0). Of these, 1 had AMI, 3 angina pectoris, 5 ischemic cerebrovascular events, and 8 CHF. The cases of CHF were not attributed to diabetes, obesity, alcohol abuse, valvular heart disease, or arrhythmia.

In the 104 participants (excluding the case of stroke occurring before carotid ultrasonography), the time to CVD event did not differ between patients separated into the 2 lower tertiles of cIMT versus the upper tertile (≥ 0.73 mm) at the 5-year assessment, and the means for CVD event-free survival times since inclusion into the study were 15.0 (95% CI 14.4–15.6) and 14.4 years (95% CI 13.2–15.6), respectively ($p = 0.45$, log-rank test; Figure 1A). Additional analysis restricted to those with strictly atherosclerosis-related CVD outcomes (AMI, angina pectoris, or ischemic cerebrovascular event) did not change that result. However, there was a significant difference in time to CVD event between the patients when they were separated according to occurrence or absence of bilateral carotid plaques (Figure 1B), with mean times for CVD event-free survival since study inclusion of 13.9 (95% CI 12.8–15.0) and 15.2 years (95% CI 14.7–15.8), respectively ($p = 0.012$, log-rank test); Table 2 presents the numbers of events, observation periods, and rates of CVD events over time, according to tertiles of cIMT and occurrence or absence of carotid plaque.

Factors associated with CVD events. Univariate Cox regressions revealed the variables associated with the hazard of incident CVD event (Table 3). Among traditional factors, only age, but not gender, ever-smoking, hypertension, diabetes mellitus, BMI, was associated with increased risk of CVD event. The baseline levels of apolipoproteins, oxLDL, IgM anti-PC, or inflammatory variables failed to show any association with CVD events. In contrast, a low decrease in DAS28, HAQ score, and VAS for pain over the first year, increase in oxLDL over 5 years, and HAQ at the 5-year assessment were associated with shorter CVD-free survival (Table 3).

In age-adjusted models (Table 3), it was found that early reduction of inflammation (risk decreased by 32% per additional level of DAS28 improvement at the 1-year assessment), pain relief (30% risk reduction per 10 units of VAS decrease at the 1-year assessment), and improvement of functional status (risk decreased by 65% per each additional level of HAQ reduction 1 year after RA onset) were associated with a better CVD outcome; whereas higher functional disability was associated with a poorer CVD outcome (risk increased by 154% per each additional level of HAQ scale at the 5-year assessment). In addition, use of MTX showed a protective role, with age-adjusted HR = 0.34 (95% CI 0.12–0.91); hence, there was a risk reduction of CVD event by 66% in those taking MTX, with a doubled risk in those not treated with MTX. Neither regular use of antimalarials, biologics, glucocorticoids, or

Table 1. Characteristics of the 114 patients with rheumatoid arthritis (RA), according to incident cardiovascular disease (CVD) events. Values expressed as mean (SD) or median (IQR).

Variables	Incident CVD Event		
	Never (referent group)	After RA Onset	Before RA Onset
No. patients	88	17	9
Age at RA onset, yrs	48.6 (10.9)	55.3 (7.2)*	63.9 (7)***
Men, n (%)	25 (28.4)	4 (23.5)	7 (77.8)**
CVD risk factors during the study			
Ever-smoking, n (%)	60 (68.2)	13 (76.5)	7 (77.8)
Hypertension, n (%)	48 (54.5)	12 (70.6)	7 (77.8)
Diabetes mellitus, n (%)	5 (5.7)	2 (11.8)	1 (11.1)
Body mass index at 5 yrs, kg/m^2	26 (4.8)	24.9 (4.3)	23.9 (2.9)
Hyperlipidemia, n (%)	57 (64.8)	15 (88.2)*	8 (88.9)
Novel markers at baseline			
oxLDL, U/I	53 (44.8–64.9)	55.8 (48.9–64.6)	41.4 (28.9–64.4)
IgM anti-PC, U/ml	70.6 (33.6–148.6)	76.7 (38–141.8)	57.3 (47.6–59)
ΔoxLDL 0–60 mo	–9 (–17)	–21 (–18.5)*	–9.6 (–20)
Δanti-PC 0–60 mo	6.7 (–3.4; 29.5)	13.9 (–10; 50.5)	3.3 (–0.9; 18.1)
RA manifestations at baseline			
ESR, mm/h	39 (27–56)	36 (17–52)	35 (29–47)
CRP, mg/l	24 (11–48)	22 (6–43)	26 (11–42)
DAS28	5.5 (1)	4.6 (1.3)*	5.5 (1)
HAQ	1.2 (0.5)	1 (0.8)	1 (0.3)
VAS pain	48.7 (20.6)	38.2 (29.3)	51.6 (16.4)
RF positivity, n (%)	52 (59.1)	12 (70.6)	3 (33.3)
ΔESR 0–12 mo	26.6 (22)	23.5 (18.8)	23.8 (18.2)
ΔCRP 0–12 mo	13 (0–34)	12 (–2; 31)	17 (2–25)
ΔDAS28 0–12 mo	2.4 (1.5)	1.5 (1.2)**	2.4 (1.2)
ΔHAQ 0–12 mo	0.7 (0.6)	0.3 (0.5)*	0.5 (0.6)
ΔVAS pain 0–12 mo	25.5 (26.4)	5.4 (16.8)*	24.9 (37.8)
HAQ at 5 yrs	0.6 (0.5)	1.0 (0.8)*	0.5 (0.5)
RA treatment during the study			
Methotrexate, n (%)	76 (86.4)	11 (64.7)*	4 (44.4)**
Antimalarials, n (%)	34 (38.6)	9 (52.9)	2 (22.2)
Biologics, n (%)	34 (38.6)	8 (47.1)	2 (22.2)
Glucocorticoids, n (%)	43 (48.9)	10 (58.8)	6 (66.7)
NSAID, n (%)	56 (63.6)	11 (64.7)	5 (55.6)
Carotid ultrasound measurements			
Mean cIMT, mm	0.66 (0.59–0.75)	0.66 (0.6–0.77)	0.77 (0.73–0.99)**
cIMT, upper tertile†, n (%)	26 (29.5)	6 (35.3)	6 (66.7)*
Carotid plaque			
None, n (%)	30 (34.1)	4 (23.5)	0
Unilateral, n (%)	31 (35.2)	2 (11.8)*	1 (11.1)**
Bilateral, n (%)	27 (30.7)	11 (64.7)**	8 (88.9)*

* $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. † cIMT upper tertile ≥ 0.73 mm. CVD: cardiovascular disease; TC: total cholesterol; LDL: low-density lipoprotein; oxLDL: oxidized LDL; anti-PC: anti-phosphorylcholine antibodies; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: Disease Activity Score in 28 joints; HAQ: Health Assessment Questionnaire; VAS: visual analog scale; RF: rheumatoid factor; Δ: reduction between assessments at times as indicated; NSAID: nonsteroidal antiinflammatory drug; cIMT: carotid intima-media thickness.

NSAID nor the carotid measures proved to have any statistically significant association with CVD outcomes. Still, the presence of bilateral carotid plaques, but not cIMT, was associated with strictly atherosclerosis-related CVD outcomes (CHF excluded), with age-adjusted HR = 6.31 (95% CI 1.27–31.4; p = 0.025).

In longitudinal analyses, log oxLDL, low IgM anti-PC, log CRP, HAQ, and VAS pain showed different development as time progressed when the patients were stratified by occurrence or absence of incident CVD events (Figure 2A-2D; log CRP not shown). Thus, log oxLDL, HAQ, and probability of low anti-PC increased over time, and log CRP and VAS pain were higher in individuals who experienced a subsequent CVD event. The statistically significant difference in the progression of values over time did not change after further adjustments for age, sex, and MTX use, with the exception of

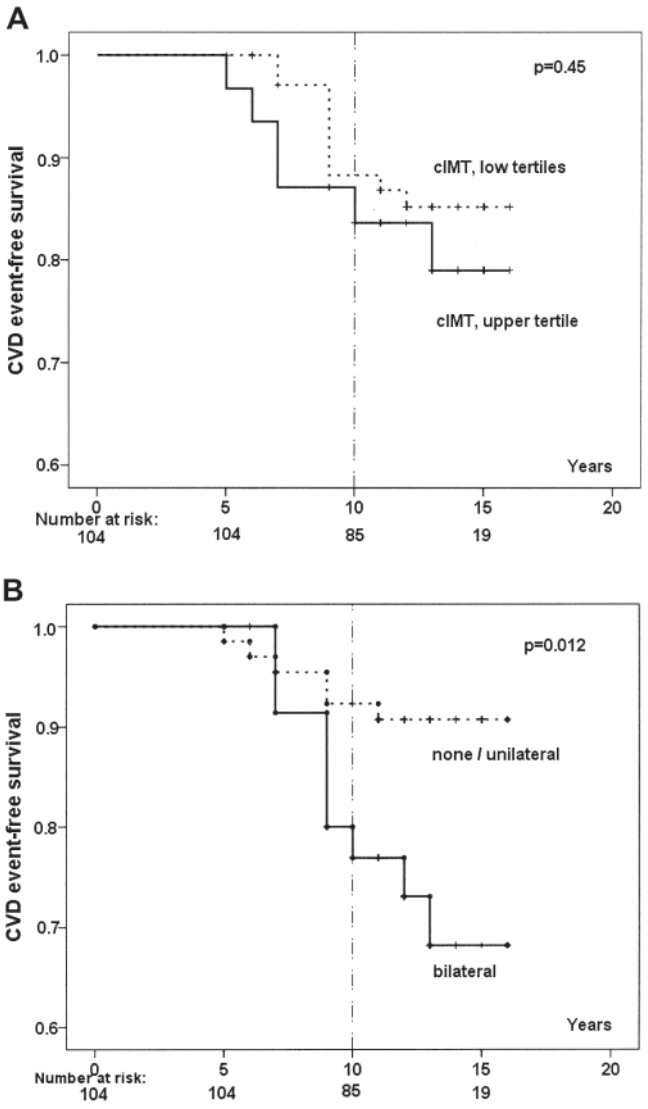

Figure 1. Kaplan-Meier curves show the CVD event-free survival in 104 RA patients with no history of CVD events, stratified by carotid intima-media thickness (cIMT; upper tertile, ≥ 0.73 mm) in tertiles (A); and by occurrence of bilateral carotid plaque (B). Vertical lines mark survival rates 10 years after onset of RA disease. P values compare between-group difference by log-rank test.

log CRP over time (trend for statistically significant between-group difference after correction for age, p = 0.069). Measures of apolipoproteins, ESR, and DAS28 over the first 5 years of RA disease failed to show significant associations with the study outcome.

DISCUSSION

This observational prospective study in an inception cohort of patients with early RA demonstrated that CVD event-free survival was significantly different between those who had bilateral carotid plaques after 5 years of RA disease compared with those without plaque or with unilateral plaque. Further, the risk of subsequent incident CVD events was inversely associated with change in DAS28, HAQ, and VAS pain between study inclusion and the 1-year assessment. Also, regular use of MTX was strongly associated with better CVD outcome. Contrarily, increasing oxLDL levels and low levels of atheroprotective IgM anti-PC, a vague decrease of levels of CRP, HAQ, and VAS pain over the first 5 years of disease were associated with occurrence of CVD events throughout the study.

The observed frequency of incident CVD events in our clinic-based study, 16.2% of patients, is somewhat higher, whereas incidence of AMI is lower, than the findings in other RA cohorts. Thus, van Halm, *et al* found CVD events in 9% of RA patients[17], and Evans, *et al* reported incident acute coronary syndrome in 10.4% of patients[9], while Holmqvist, *et al* revealed the incidence rate of AMI of 0.5 per 100 person-years[18] compared to approximately 0.1 per 100 person-years in our study. These discrepancies may be related to differences in study design, the study populations, and the method chosen to ascertain diagnosis of CVD. Also, in our study the definition of cardiovascular outcomes was broader, encompassing both strictly atherosclerosis-related outcomes and outcomes with other possible nonatherosclerotic underlying mechanisms, such as congestive heart failure. Currently, the relationship between heart failure and RA is not completely understood. Some investigators ascribe heart failure mostly to ischemic heart disease, but other pathways than atherosclerosis, for example inflammatory pathways, may also contribute[19]. In patients in our cohort we were able to exclude nonischemic causes of the heart failure, such as diabetes, obesity, alcohol abuse, valvular heart disease, and arrhythmia. However, we should be cautious in extrapolating our results to "pure" atherosclerotic disease. Ideally, the question of strictly atherosclerotic disease and heart failure in RA should be addressed separately in future investigations.

Although it is currently acknowledged that the cardiovascular risk in RA is substantially elevated, it is debated when the increased risk starts during the course of RA disease[20]. In established RA, disease duration is independently associated with both atherosclerosis and CVD[21,22]. However, in RA inception cohorts no increase in mortality and CVD has been found during the first 10 years of followup[4]. The explanations for this delay may include the lead time for CVD to be manifest, direct or indirect effects of cumulative systemic inflammation, or accumulated functional disability and sedentary lifestyle[23,24].

The importance of disease-related risk factors for CVD in RA is apparent in the patients in our study when they are divided into those with CVD events before and those with events after diagnosis of RA. Thus, the presence of traditional risk factors and carotid atherosclerosis (both presence of plaque and cIMT) was pronounced in those with CVD events before RA onset, while presence of plaque and inflammation-dependent factors were prominent in those with occurrence of CVD events after RA onset. It seems possible that a

Table 2. Rates of incident cardiovascular disease (CVD) events (per 100 person-years) in the 104 patients with RA, according to cIMT in tertiles and carotid plaque occurrence on carotid ultrasound at the 5-year assessment.

Followup Periods and Variables	Incident CVD Events, n (95% CI)	Person-yrs	Rate per 100 Person-yrs (95% CI)
Whole study period			
cIMT, low tertiles	10 (3.8–16.2)	883	1.13 (0.43–1.83)
cIMT, upper tertile	6 (1.2–10.8)	375	1.6 (0.32–2.88)
Plaque none/unilateral	6 (1.2–10.8)	840	0.71 (0.14–1.29)
Plaques bilateral	10 (3.8–16.2)	418	2.39 (0.91–3.88)
After ultrasound examination of carotids			
cIMT, low tertiles	10 (3.8–16.2)	504	1.98 (0.75–3.21)
cIMT, upper tertile	6 (1.2–10.8)	214.5	2.8 (0.56–5.03)
Plaque none/unilateral	6 (1.2–10.8)	493.5	1.22 (0.24–2.2)
Plaques bilateral	10 (3.8–16.2)	225	4.44 (1.69–7.2)

cIMT: carotid intima-media thickness.

Table 3. Hazard ratios (HR) for incident cardiovascular disease in the 105 patients with RA (1 case excluded when indicated), univariate and age-adjusted Cox regression analyses.

Variables	HR (95% CI)	p	Age-adjusted HR (95% CI)	p
Age at RA onset	1.06 (1.01–1.11)	0.016	—	
ΔoxLDL (60–0 mo)†	1.03 (1.01–1.06)	0.008	1.03 (1.0–1.06)	0.035
ΔDAS28 (0–12 mo)	0.69 (0.49–0.98)	0.041	0.68 (0.5–0.97)	0.035
ΔHAQ (0–12 mo)	0.36 (0.15–0.86)	0.020	0.35 (0.15–0.82)	0.016
ΔHAQ (0–60 mo)†	0.29 (0.15–0.58)	0.000	0.26 (0.13–0.55)	0.000
ΔVAS pain (0–12 mo)	0.97 (0.95–0.99)	0.004	0.97 (0.95–0.99)	0.005
HAQ at 5 yrs†	2.53 (1.31–4.87)	0.006	2.54 (1.31–4.92)	0.006
MTX use	0.32 (0.12–0.87)	0.025	0.34 (0.12–0.91)	0.033
Bilateral carotid plaque†	3.34 (1.21–9.22)	0.020	2.16 (0.66–7.05)	0.20

† Analyses where 1 case was excluded due to CVD occurrence about 2 years after disease onset. oxLDL: oxidized low-density lipoprotein; DAS28: Disease Activity Score in 28 joints; HAQ: Health Assessment Questionnaire; VAS: visual analog scale; MTX: methotrexate; cIMT: carotid intima-media thickness.

different set of risk factors contribute to CVD and that the cardiovascular outcomes may be explained by different mechanisms of atherogenesis, plaque formation, plaque rupture, and atherothrombosis. Accordingly, in an autopsy study the patients with RA had less histological evidence of atherosclerosis but greater evidence of inflammation, as well as more vulnerable and inflamed high-risk plaques, compared to controls[25].

The clinical significance of subclinical atherosclerosis for incident CVD events after onset of RA was shown in this study by findings of an approximately 4-fold increased rate of incident CVD events in patients identified with bilateral carotid plaques as compared to those without plaque or with unilateral plaque. However, our study did not demonstrate the clinical importance of cIMT in survival analyses.

The significance of cumulative inflammation for incident CVD events was shown here by the association of lower CRP levels over 5 years and reduction in DAS28 during the first year of RA disease with better CVD outcome. The lower DAS28 reduction in patients with CVD events should, however, be interpreted with caution as the mean level was already lower at baseline. Further, the evidence for acceleration of atherogenesis in a chronic inflammatory milieu measured by CRP or ESR and the significance of nonspecific inflammatory markers for CVD have not been consistent across studies[26]. Few investigators have reported relationships between DAS measures and CVD outcomes, subclinical atherosclerosis, and biomarkers of endothelial dysfunction[7,27,28]. The questions of whether lack of associations depended on study designs, lack of accurate measurements of cumulative effects, short followup, or low power, or reflected a complex relationship between 2 multifaceted diseases should be addressed in larger trials in RA inception cohorts.

Also, an insufficient improvement in HAQ during the first year of RA disease and a higher HAQ level 5 years after disease onset heightened the risk of CVD events. This finding expands an earlier report concerning the predictive value of HAQ at 1 year for subsequent all-cause and CVD mortalities[29]. Suboptimal disease control in early stages of RA disease, measured by change in DAS28, HAQ, and VAS pain over the first year, may reflect a greater degree of functional decline in later stages of disease with effects on the cardiovascular prognosis and outcomes.

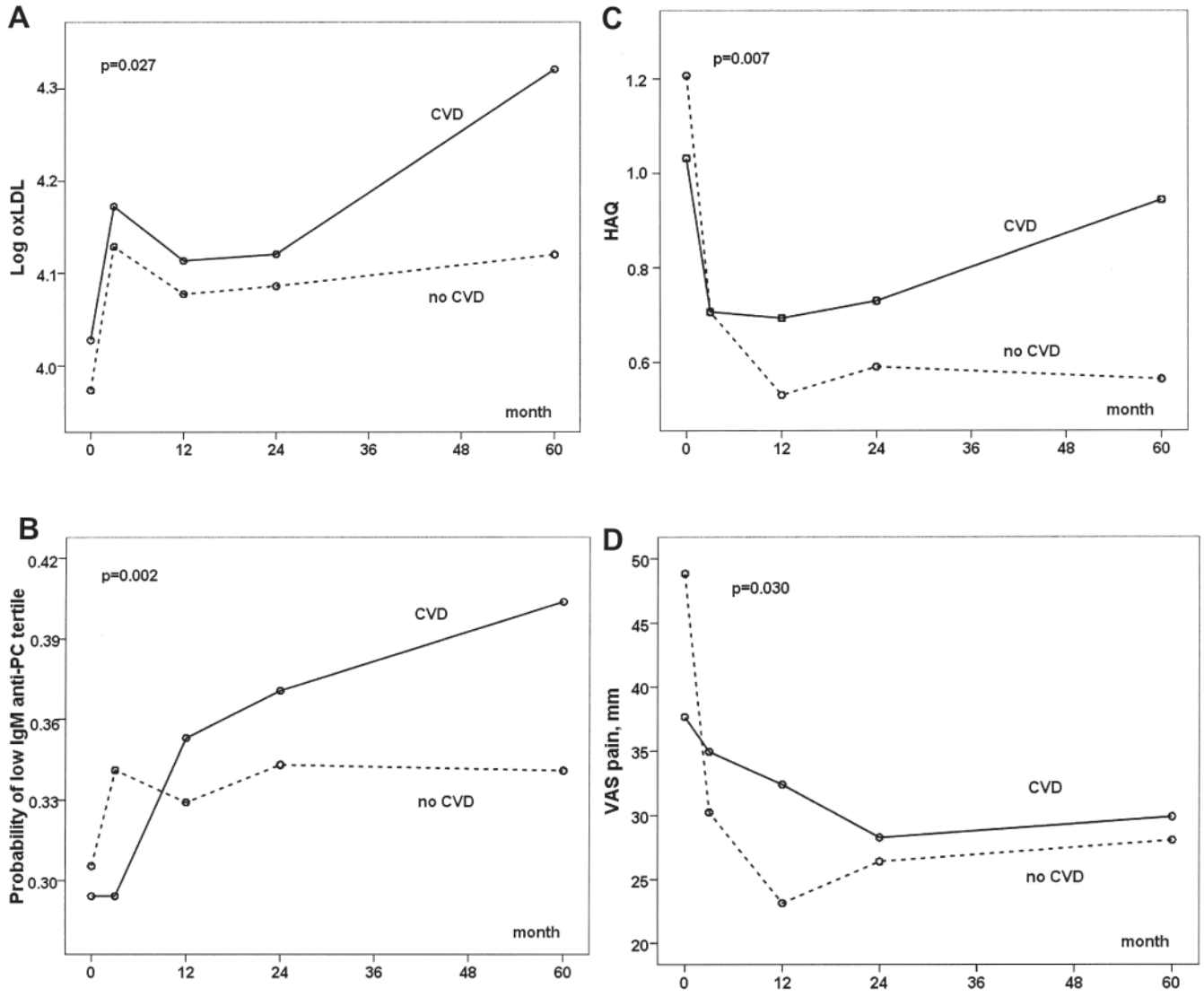

Figure 2. Predicted means of variables plotted against time by incident CVD events throughout the study in the 105 patients with RA (1 case contributed only up to 12 months of followup before occurrence of a CVD event), analyzed by mixed linear models. A: log oxidized low-density lipoprotein (oxLDL); B: probability of the low tertile of IgM antiphosphorylcholine antibodies (anti-PC); C: Stanford Health Assessment Questionnaire (HAQ); D: VAS for pain. P values compare interaction with time factor.

The significance of chronic pain in morbidity has not been sufficiently studied. Yet chronic pain and pain-related psychiatric illness have been shown to contribute to increased cardiovascular-related mortality in RA[30,31]. Our study suggests that a reduction in pain during the first year after RA diagnosis may be associated with an independent protective cardiovascular effect. Therefore, along with physical examination and laboratory tests, simple qualitative measures of patient self-reported pain need to be included in future research and in the followup of patients with RA.

We have largely confirmed previous studies about beneficial effects of MTX on cardiovascular outcomes[32,33]. It seems likely that the decrease of inflammation following use of MTX may improve CVD outcomes, but other MTX-specific effects can also exist[32]. Acknowledging the atheroprotective role of MTX, the current European League Against Rheumatism guidelines recommend it as part of cardiovascular risk management in patients with RA[34].

The mechanisms for the assumed accelerated atherosclerosis in autoimmune rheumatic disease include classical risk factors, the chronic inflammatory process, immune dysregulation, effects of treatments, and genetic determinants[35]. Recent data support the assumption that atherosclerosis is an inflammatory autoimmune disease, and that all parts of the immune system take part in atherosclerosis formation[36].

In our study, oxLDL levels increased over the first 5 years of RA disease in individuals who experienced a subsequent CVD event. In the general population increasing plasma

oxLDL levels are associated with increased risk for CVD[37] and predictive for CVD, independently of traditional lipid and cardiovascular risk factors[38], as well as for rupture-prone atherosclerotic plaques[39]. Additionally, serum oxLDL levels are elevated in RA and correlate with disease activity independently of other inflammatory markers, suggesting the importance of oxLDL in a chronic inflammatory condition *per se*[40,41]. We previously reported increasing oxLDL levels during the first 5 years of followup in this RA cohort, despite amelioration of inflammation, and also a trend to positive association between oxLDL levels and carotid measures[10]. Taking these observations together, we propose that oxLDL may have significance for cardiovascular events, probably due to plaque instability, but further studies are needed.

As well, low levels of natural antibodies such as IgM anti-PC during the first 5 years of RA disease characterized patients with subsequent CVD events, which extends the evidence of their atheroprotective role in RA[10]. Anti-PC of the IgM subclass, representing a natural immune response, may inhibit proatherogenic and proinflammatory effects in infection, autoimmunity, and atherogenesis by decreased uptake of oxLDL and inhibition of the inflammatory effects of oxidized phospholipids[42]. Higher levels of IgM anti-PC might also have a protective role against progression of early atherosclerosis as demonstrated by repetitive measurement of cIMT[43], while low levels of IgM anti-PC might be an independent risk marker causally related to major CVD outcomes[15,44,45].

Although not significantly linked to the cardiovascular outcomes, traditional risk factors such as history of ever smoking, hypertension, and hyperlipidemia were prevalent in our patients. Traditional risk factors may behave differently in RA and in the general population. Thus, in the large AMORIS study, the predictive value of lipids for AMI and ischemic stroke were not consistent in the patients with RA[46]. However, the lack of associations do not exclude a potential influence of traditional risk factors for underlying proatherogenic mechanisms and future CVD events.

The strengths of our study are a long observation period, repetitive measurements of disease characteristics and novel biomarkers at the same timepoints throughout the followup, and reliable data sources for cardiovascular events and traditional risk factors. We recognize the limitations, such as a relatively low number of patients, few participants followed for more than 10 years, and lack of carotid ultrasound evaluation at inclusion. The observational design limits conclusions of causality, but the results provide the important hypothesis-generating observation.

In summary, bilateral carotid plaques but not cIMT measurements were associated with poor CVD outcome. Patients with functional disability seemed to belong to a high-risk group for future incident CVD events, and they might be targeted for intensive reduction of risk factors. The results support that reduction in DAS28, HAQ, and VAS pain over the first year after diagnosis may independently contribute to improve CVD prognosis. When it comes to reduction of risk for cardiovascular disease, control of inflammation should still be the goal for treatment. Further, pain evaluation should not be neglected in clinical care of patients with RA. Elevated oxLDL and low levels of atheroprotective IgM anti-PC might link atherogenesis and inflammation, and could be of particular use for CVD risk prediction in RA.

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