Early phase clinical and biological markers associated with subclinical atherosclerosis measured at 7 years of evolution in an early inflammatory arthritis cohort

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Abstract

Objective

Accelerated atherosclerosis has emerged as a critical issue in rheumatoid arthritis (RA). There is a need to better understand the link between RA and atherosclerosis. Our aim was to identify parameters associated with the development of subclinical atheroma in a very early arthritis (VErA) cohort

Methods

VErA-cohort patients were prospectively recruited from 1998 to 2002. Arthritis treatment was standardised from onset. The clinical, biological and radiological parameters of all patients were collected from inclusion. Carotid intima-media thickness (cIMT) was measured 7 years after their first symptoms.

Results

Among 105 patients included, 82 developed RA (mean age at onset: 51.7±12.8 years). Mean carotid artery IMT at year 7 was 0.67±0.12 mm. Larger thickness defined by values above the median (0.66) was associated with inclusion age ($p<10^{-6}$), swollen joint count (p=0.01), DAS44 (p=0.048) and hypertension (p=0.006). In contrast, anti-CCP positivity (>50 UA/ml) was associated with thinner cIMT (p=0.03). Baseline as well as cumulated values of markers reflecting systemic inflammation, lymphocyte activation, endothelial dysfunction and oxidative stress were not correlated with carotid subclinical atherosclerosis. Major independent atheroma risk factors retained by multivariate analyses were hypertension (OR 4.33 [1.59–11.73]; p=0.004) and swollen joint count at inclusion (OR 3.87 [1.54–9.72]; p=0.004), while methotrexate use was a protective marker (OR 0.27 [0.11–0.71]; p=0.007).

Conclusion

This study conducted from the VErA vascular cohort of community-cases of RA confirm that cIMT is under the influence of classical CV risk (hypertension), disease marker (SJC) and methotrexate intake

Key words

undifferentiated arthritis, rheumatoid arthritis, early arthritis, atherosclerosis, cardiovascular disease

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Introduction

Rheumatoid arthritis (RA) patients have a higher risk of cardiovascular (CV) events than the general population. The meta-analysis by Meune *et al*. (1) confirmed a 60% increase in risk of CV death in RA patients compared with general population [standardised mortality ratio (SMR) 1.6; 95% CI: 1.5–1.8]. Surprisingly, this study did not show a decrease in SMR over the five past decades, despite changes in RA course. These results underlined the necessity to target specifically a reduction in CV mortality in RA patients. Previous studies also showed increased risk of several cardiovascular morbidities during the course of RA: myocardial infarction [incidence rate ratio (IRR) 2.10; 95% CI: 1.52–2.89], stroke [IRR 1.91; 95% CI 1.73–2.12] and congestive heart failure [hazard ratio 1.87, 95% CI::1.47–2.39] (2, 3). It is important to highlight that myocardial infarction risk increases rapidly following RA diagnosis as observed in a large inception cohort of RA diagnosed between 1995 and 2006 (4). This finding can be explained by the fact that inflammatory changes often precede diagnosis and changes in risk factors such as lipid levels can occur in the pre-clinical phase, which may impact on the vasculature very early. Currently, RA is considered as an independent CV risk factor, as important as diabetes mellitus (5, 6). Recommendations (7, 8) emphasised the importance of vascular risk management for RA patients.

Many studies concerned the identification of markers predictive of CV complications in established RA. To assess subclinical atherosclerosis, many of them performed measurement of carotid artery intima-media thickness (cIMT) which is a well-established non invasive technique correlated with traditional CV risk factors and with 10-year risk of CV events (9). In this regard, the ACCF/AHA guidelines emphasised that carotid US including measurement of cIMT is a useful technique for cardiovascular risk assessment in asymptomatic adults (10). The utility of cIMT measurements in observational studies from RA patients has recently been proven in the meta-analysis by van Sijl *et al*. (11). Even though some studies have shown no increased cIMT in RA patients, most of them confirmed more severe atherosclerosis lesions, with an overall cIMT difference of 0.09 mm (95%CI: 0.07–0.11 mm) between RA patients and control (11). As occurred for carotid plaques, cIMT yielded a high predictive power for the development of cardiovascular events over a 5-year follow-up period in RA patients (12). This subclinical atherosclerosis was mainly correlated with age, duration of RA and systemic inflammation. Indeed, in long-standing RA patients, the magnitude and chronicity of the inflammatory response measured by C-reactive protein (CRP) correlates directly with the presence of subclinical atherosclerosis; those exhibiting the highest mean CRP levels had greater cIMT values (13). Nevertheless, no consensual model predictive of CV events in RA has yet been accepted. Moreover, these studies provide no data about atherosclerosis in early inflammatory arthritis.

Endothelial dysfunction, another relevant sign of very early atherosclerosis, can be assessed non-invasively by other techniques such as flow-mediated dilatation (FMD) or pulse wave analysis. In general, early endothelial dysfunction precedes subclinical atherosclerosis. Indeed, decreased FMD may provide earlier information on subclinical atherosclerosis than cIMT since both surrogate markers of atherosclerosis are correlated only in patients with long disease duration (14).

Atherogenesis is a complex process associating the oxidative stress, the endothelial dysfunction, the activation of T lymphocytes and monocytes/macrophages, the formation and accumulation of foam cells (15). Interestingly, many pro-inflammatory cytokines and proteins such as TNF-α, IL-1β, IL-6, CRP, play key roles both in RA pathophysiology and in the early stages of atherogenesis (16, 17). Many other pathways are also common to these two diseases: oxidative stress, angiogenesis, auto-immunity. In fact, previous studies showed overexpression of crucial molecules or cells in RA patients and in patients suffering from vascular complications (intercellular-cell-adhesion molecule [ICAM-1], vascular cell-adhesion

molecule-1 [VCAM-1], oxidised low-density lipoproteins [oxLDL], activated T lymphocytes, vascular endothelial growth factor [VEGF]) (18-20).
The objectives of our study were:
i. to assess the presence of subclinical atherosclerosis, measured by cIMT, at 7 years of arthritis evolution, in the VErA cohort of patients prospectively followed for early inflammatory arthritis (RA or undifferentiated arthritis [UA]);
ii. to identify surrogate markers associated with the degree of atheromatous disease, based on the knowledge of both RA and atherosclerosis pathogenesis.

Patients and methods

Patients

A total of 310 patients with early arthritis were prospectively recruited between October 1998 and January 2002 in 2 French regions, *i.e.* the entire Upper Normandy province (1,800,000 inhabitants) and metropolitan Amiens (300,000 inhabitants). The methodology of the VErA cohort was previously described (21). Briefly, inclusion criteria were: man or woman, age ≥18 years, ≥2 swollen joints confirmed by a rheumatologist, swelling persisting >4 weeks, symptoms lasting <6 months; no previous glucocorticoid nor DMARDs. Exclusion criteria were: associated inflammatory back pain, pregnancy or breastfeeding, foreseen move during the next 10 years. This study was approved by the Upper Normandy Ethics Committee (French law 88-1138; 20 December 1988; file 95/138/HP). All patients gave their informed written consent at the time of inclusion.

Therapeutic recommendations

For the first 2 years, treating rheumatologists were given recommendations so that the included patients would be treated homogeneously. The guidelines had been devised before early intensive DMARDs administration became the internationally accepted strategy and prior to biotherapy availability in France. Schematically, it was recommended not to use systemic glucocorticoids unless necessitated by very active disease, and then briefly at the lowest possible dose. For DMARDs, it was recommended to start with hydroxychloroquine (6 mg/kg/day), to be replaced or combined with oral methotrexate, starting at 7.5 mg/week.

Followup evaluations

All of the patients were followed for at least 2 years, except for those with definitively (non-RA) diagnosed arthritis, who were withdrawn from the study as soon as the entity was recognised. All other patients who subsequently fulfilled the ACR RA criteria (22) or had undifferentiated arthritis (UA) were followed, even if their disease was in spontaneously or posttreatment-induced remission. Follow-up visits were conducted 3 and 6 months after inclusion and then every 6 months.

Clinical markers

At baseline and then at every follow-up consultation, the following clinical parameters were recorded: age, sex, Ritchie articular index, number of swollen joints of a total of 44 and the Health Assessment Questionnaire (HAQ) score. To evaluate disease activity, we calculated the Disease Activity Score 44 (DAS44) comprising 3 variables (Ritchie articular index, number of swollen joints of a total of 44 and erythrocyte sedimentation rate/1st hour [ESR])(23).

Classical cardiovascular risk factors

A retrospective inquiry concerning classical cardiovascular risk factors of the patients at the onset of their disease was conducted during the consultation at 7 years of follow-up across medical chart review and patient survey. The following cardiovascular risk factors were collected: hypertension (defined as a repeatedly elevated blood pressure exceeding 140 over 90 mmHg and/or intake of antihypertensive medication), hypercholesterolaemia (LDL-cholesterol >1.6 g/l or 4.1 mmol/l), hypertriglyceridaemia (>1.5 g/l or 1.7 mmol/l), diabetes (fasting blood glucose: 1.26 g/l or 7 mmol/l), prior or ongoing tobacco smoking, personal and/or family history of cardiovascular events. At this time, patients' weight and height were measured; body mass index (BMI) was calculated and the waist/hip ratio (WHR) was determined to evaluate android obesity (BMI >30 kg/m^2 and/or WHR >1.00 for men or >0.85 for women) (24).

Biological markers of RA

Systemic inflammation was assessed at each visit by measuring ESR and the C-reactive protein (CRP) concentration. Serum rheumatoid factor (RF) levels were determined with the latex-fixation test (Rapitest® RF, Behring Diagnostics, Westwood, MA), with the positivity threshold set at ≥20 international units (IU)/ml. Serum anti-cyclic citrullinated peptide (CCP) antibodies were detected with an enzyme-linked immunosorbent assay (ELISA), using second-generation kits (EuroImmun, GMBH, GroB Grönau, Germany), with the positivity threshold set at ≥10 AU/ml.

Biological parameters of CV risks

At inclusion (prior to DMARDs and/or corticosteroids initiation), 12 and 24 months of arthritis progression, the following parameters were measured: soluble CD40L, a marker of lymphocyte and platelet activation, with an ELISA kit (Quantikine®, R&D Systems, Minneapolis, MN); light-density lipoprotein oxidation (oxLDL) and advanced oxidation protein products (AOPP) to evaluate oxidative stress, respectively, with an ELISA (Mercodia® AB, Uppsala, Sweden) and spectrophotometry; vascular cell-adhesion molecule-1 (VCAM-1) and E-selectin, markers of endothelial dysfunction, with ELISA kits (Quantikine®); soluble vascular endothelial growth factor receptor-1 (sVEGFR1), an anti-angiogenesis marker, assessed with an ELISA (Quantikine®). VEGF and angiopoietin-1 were also evaluated to study angiogenesis with specific ELISA only at inclusion and 12 months.

Genetic markers

In order to assess the contribution of genetic background in the development of atherosclerosis in the context of early arthritis, we analysed 3 genetic susceptibility factors: HLA-DRB1-SE, PTPN22 620W and TNFRII 196R alleles. The different laboratory protocols used to identify these 3 genetic markers were previously described (25).

Radiological assessment

Standardised hand-and-wrist and foot radiographs were obtained at inclusion, 6 months of follow-up and then annually. The films were read independently by 2 rheumatologists [PF and OM], with consensus reached by discussion in the case of disagreement (21). This reading enabled calculation of van der Heijde modified Sharp erosion, joint-narrowing and total scores (26).

Technique for cIMT assessment of atherosclerosis

The cIMT was measured according to standardised modalities (27): an HDI 5000 Philips ultrasonograph (2001) with a linear L7-4 probe at a frequency of 10 MHz obtained 5 images in B-mode of each carotid artery's IMT in a longitudinal section centered on the common carotid (defined as the segment 15 mm proximal to carotid dilation). The Philips QLAB v.2 2002 programme was used to analyse the images. After elimination of the extreme values, the mean of the 3 remaining cIMT values was calculated for each carotid. The average of the cIMT of the 2 carotid segments was used in the analyses. The presence of carotid atheromatous plaques was also evaluated. Plaques were defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding cIMT value or demonstrates a thickness of 1.5 mm.

Statistical analyses

The inclusion characteristics of RA and UA groups were compared with Student's *t*-tests (for quantitative variables) and with Fisher's exact test (for qualitative variables). Univariate analyses compared groups of patients with cIMT thinner than *versus* thicker or equal to the cohort median (0.66 mm) using a Mann-Whitney U-test for medians of continuous variables and a Fisher's exact test for qualitative variables. Variables with *p*-values less than 0.10 were entered into the logistic-regression multivariate analysis (SPSS software) to identify independent parameters associated with thicker carotid IMT.

Table I. Characteristics of the vascular VErA cohort at inclusion.

Characteristic*	Total (n=105)	RA (n=82)	UA (n=23)	*P*
Age (yr)	51.7 ± 12.8	51.9 ± 13.2	50.8 ± 11.5	NS
Female sex	69 (66)	54 (66)	15 (65)	NS
Ritchie index	8.9 ± 9.1	10.0 ± 9.8	5.1 ± 4.8	0.02
No. of swollen joints	10.4 ± 8.3	12.0 ± 8.3	4.7 ± 5.7	0.0001
DAS44	3.23 ± 1.25	3.48 ± 1.21	2.43 ± 1.02	0.003
HAQ	1.0 ± 0.7	1.05 ± 0.65	0.69 ± 0.68	0.02
ESR (mm/1st h)	25.9 ± 23.6	27.6 ± 24.4	20.3 ± 20.5	NS
CRP (mg/l)	22.0 ± 32.2	22.9 ± 30.4	18.7 ± 38.4	NS
RF titer (IU/ml)	105.7 ± 314.5	135.0 ± 350.8	1.2 ± 5.8	NS
RF positivity	41 (39.0)	40 (49)	1 (4)	0.0002
Anti-CCP antibodies titer (AU/ml)	30.2 ± 42.6	37.4 ± 45.6	4.4 ± 20.8	0.001
Anti-CCP antibodies positivity	38 (36.2)	37 (45)	1 (4)	0.0001
Total modified Sharp score	1.0 ± 2.6	1.1 ± 2.9	0.4 ± 1.2	NS
HLA-DRB1 shared epitope	47/89 (53)	43/71 (60)	4/18 (22)	0.007
PTPN22 1858T	21/98 (21)	15/75 (20)	6/23 (26)	NS
TNFRII 196R	42/98 (43)	37/75 (49)	5/23 (22)†	0.03
Hypertension	33 (31)	29 (36)	4 (17)	NS
Hypercholesterolaemia	19 (18)	16 (20)	3 (13)	NS
Hypertriglyceridaemia	7 (7)	5 (6)	2 (9)	NS
Diabetes mellitus	9 (8)	6 (7)	3 (13)	NS
Tobacco smoker	19 (18)	16 (20)	3 (13)	NS
Vascular history				
Familial	19 (18)	14 (18)	5 (23)	NS
Personal	1 (1)	1 (1)	0	NS
BMI (kg/m²)	26.3 ± 5.8	26.8 ± 5.3	25.5 ± 5.0	NS
Android obesity	53 (50)	42 (52)	11 (48)	NS
No. of classical CV risk factors	1.55 ± 1.09	1.61 ± 1.11	1.32 ± 0.99	NS

*Values are expressed as means ± SD or n (%).
RA: rheumatoid arthritis; UA: undifferentiated arthritis; DAS: disease activity score; HAQ: Health Assessment Questionnaire; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; CV: cardiovascular; BMI: body mass index.

Table II. Treatments received by the VErA vascular cohort patients during the 7 years of follow-up.

Treatment*	Total (n=105)	RA (n=82)	UA (n=23)
Nonsteroidal antiinflammatory drugs	91 (87)	74 (90)	17 (74)
Corticosteroids	49 (47)	43 (52)	6 (26)
Hydroxychloroquine	33 (31)	27 (33)	6 (26)
Methotrexate	58 (55)	55 (67)	3 (13)†
Anti-tumor necrosis factor-α	12 (11)	12 (15)	0
Statins	8 (8)	8 (10)	0

*Values are expressed as n (%).
†$p<10^{-5}$ between rheumatoid arthritis (RA) and undifferentiated arthritis (UA).

Results

Characteristics of the VErA population

Out of the 310 patients included, 105 patients (82 RA and 23 UA) still followed, after 7 years, in the VErA cohort were included in this study. Their demographic, clinical, biological, radiological and genetic characteristics at inclusion are given in Table I. All RA patients fulfilled the revised ACR criteria (28). At baseline, these patients (69 women and 36 men) presented active diseases defined by a mean DAS44 of 3.23 and elevated values of inflammatory parameters (mean ESR: 25.9±23.6 mm/1st h; mean CRP 22.0±32.2 mg/l). Among them, 39.0% were RF-positive and 36.2% had anti-CCP antibodies. Radiological evaluation yielded van der Heijde modified Sharp erosion, joint-narrowing and total scores of 0.3±1.4, 0.6±1.8 and 1.0±2.6, respectively. RA and UA patients were comparable for age and sex. In contrast, at inclusion, RA patients had significantly more active disease with higher Ritchie index, swollen joints counts, DAS44 and HAQ scores. Their anti-CCP antibody

Table III. Correlations between carotid intima-media thickness defined with a cut-off (median value of 0.66) and the different clinical, radiological and biological markers evaluated at inclusion and their areas under the time-concentration curves.

Marker	At inclusion			Area under the curve (over 2-year follow-up)		
	cIMC < 0.66 (n = 51)	cIMC ≥ 0.66 (n = 54)	*p*	cIMC < 0.66 (n = 51)	cIMC ≥ 0.66 (n = 54)	*p*
Sex (M/F)	15/36	21/33	0.41	-	-	
Classical CV risk factor						
Age	45 (19-75)	56.5 (35-76)	10-6	-	-	-
High blood pressure, n (%)	9 (17.6)	24 (44.4)	0.006	-	-	-
Hypercholesterolaemia, n (%)	8 (15.7)	11 (20.4)	0.62	-	-	-
Tobacco exposure, n (%)	12 (23.5)	7 (13)	0.20	-	-	-
Diabetes , n (%)	4 (7)	5 (8)	1	-	-	-
BMI (kg/m²)	25.6 (15.1 – 44.4)	26.6 (17.5 – 41.1)	0.42	-	-	-
Waist-to-hip ratio°	0.88 (0.68 – 1.11)	0.91 (0.72-1.09)	0.17	-	-	-
No. of factors	1 (0-5)	2 (0-4)	0.20	-	-	-
Classical RA						
Ritchie articular index	6 (0-29)	8 (0_48)	0.20	4.14 (0 – 20.86)	3.15 (0 – 17.25)	0.56
No. of swollen joints	6 (0-32)	9 (0-34)	0.01	4.14 (0 – 21.71)	5.93 (0 – 18.29)	0.06
DAS44	2.82 (0.91-5.55)	3.28 (0.98-7.45)	0.05	2.11 (0.79 -5.26)	2.25 (0.96-4.53)	0.80
HAQ	0.87 (0-3.37)	0.94 (0-2.62)	0.78	0.63 (0.02-2.14)	0.45 (0 – 2.04)	0.44
Modified Sharp erosion score	0 (0-9)	0 (0-8)	0.43	-	-	-
Modified total Sharp score	0 (0-10)	0 (0-12)	0.24	-	-	-
HLADRB1 shared epitope*, n (%)	25 (49)	22 (40.7)	0.67	-	-	-
PTPN22 1858T allele*, n (%)	10 (19.6)	11 (20.4)	1	-	-	-
Inflammation						
ESR mm1sh	17 (4-100)	16 (4-110)	0.78	13 (3.7 – 46.7)	14.7 (3.9 – 79)	0.25
CRP mg/l	9 (5-106)	9 (5-185)	0.88	7 (5 – 37)	8.36 (4.71–62.14)	0.24
Autoimmunity						
RF (IU/ml)	0 (0-2560)	0 (0-1400)	0.19	5.60 (0 – 1275)	0 (0 – 670)	0.19
Anti-CCP antibodies positivity (≥ 50 AU/ml) n (%))	20 (39.2)	11 (20.4)	0.03	-	-	-
CD40L	225 (14 – 2924)	484 (5 – 5166)	0.16	1737 (216-5557)	2079 (324-7738)	0.36
Angiogenesis						
VEGF	357 (106-1058)	387 (147-1220)	0.97	697 (181-2716)	531 (144-4000)	0.37
sVEGFR1-β	205 (0-96)	164 (0-96)	0.18	135 (0-159)	126 (12-202)	0.65
Angiopoietin-1	23 (0-200)	25 (3.35-200)	0.77	40 (1-197)	35 (6-200)	0.40
Endothelial dysfunction						
VCAM-1	847 (452 - 2070)	869 (422 - 2124)	0.34	766 (471-1325)	760 (385-1485)	0.31
E-Selectin	40 (14 – 129)	48 (15 – 266)	0.25	42 (14-107)	50 (18-223)	0.12
Oxidative stress						
Oxidised LDL	46 (19 – 122)	48 (11 – 142)	0.66	45 (27.3-122.3)	46.3 (10.8-94.5)	0.56
AOPP	73 (17 – 194)	83 (34 – 228)	0.37	73 (28 - 194)	88 (42 – 176)	0.17

Results are expressed as median (lower and upper limits) unless indicated otherwise. °data collected at 7-year follow-up. *at least one at-risk allele.
CV: cardiovascular; BMI: body mass index; RA: rheumatoid arthritis; DAS: disease activity score; HAQ: Health Assessment Questionnaire; RF: rheumatoid factor; CCP: cyclic citrullinated peptide; ESR: erythrocyte sedimentation rate (mm/1sth); CRP: C-reactive protein (mg/l); VEGF: vascular endothelial growth factor; sVEGFR1: soluble vascular endothelial growth factor receptor-1; VCAM-1: vascular cell adhesion molecule-1; LDL: light-density lipoprotein; AOPP: advanced oxidation protein products.

mean levels were also significantly higher; they were more frequently RF- and/or anti-CCP2 antibody-positive and carriers of the *HLA-DRB1* shared epitope and of the *TNFRII 196R* allele. Structural damage at inclusion was comparable for the 2 patient groups.

Classical cardiovascular risk factors are also reported in Table I. The mean number of classical risk factors per patient was 1.55±1.09, with comparable frequencies of each factor in the 2 groups. Carotid IMT, measured at 7-year follow-up was 0.67±0.12 mm (median: 0.66), with no significant difference according to the type of inflammatory disease. Eleven patients (6 RA and 5 UA) had atheromatous plaques in their carotid arteries.

All the treating rheumatologists adhered to the DMARDs guidelines.

During the 7 years of follow-up preceding cIMT measurement, 87% of the patients had taken NSAIDs, 47% received corticosteroids, 55% took methotrexate, 11% were prescribed anti-tumour necrosis factor-alpha (TNF-α) biologics and 8% were on statins (Table II). Corticosteroid doses were <10 mg/day. RA patients had received methotrexate significantly more frequently than UA patients, and none of the latter patients were treated with anti-TNF-α agents.

Relationships between carotid cIMT and the different markers

Since our objective was to identify specific precocious markers of subclinical atherosclerosis during the course of early arthritis, correlation tests were conducted on the entire population, independently of RA or UA diagnosis (Table III). Univariate analyses, considering the median cIMT value (0.66)

as a cut-off value, revealed significant associations between higher cIMT and age at inclusion ($p=10^{-6}$) or hypertension ($p=0.006$). The number of swollen joints and the DAS44 score at inclusion were associated with the importance of atheromatous lesions ($p=0.01$ and 0.048, respectively). In contrast, surprisingly, anti-CCP–antibody positivity was weakly associated with a lower cIMT value ($p=0.03$). Furthermore, all biomarkers reflecting systemic inflammation, angiogenesis, endothelial dysfunction and oxidative stress were not correlated with cIMT. Moreover, no relationship was found between the evolution of these markers during the first 2 years of follow-up and higher cIMT except a trend for the number of swollen joints ($p=0.06$). Finally, patient's genetic markers and treatments were not correlated with subclinical atheroma. However, a trend was found between low cIMT and methotrexate exposure ($p=0.08$)

All the markers associated with increased cIMT in our univariate analysis were subjected to multivariate logistic-regression analysis, after adjustment for age. This multivariate analysis retained two risk factors (hypertension and number of swollen joints) and one protective marker (methotrexate exposure) (Table IV). A model was built using these independent variables, leading to a proportion of correctly classified patients of 72%. Area under the ROC curve was 0.70

Table IV. Independent baseline factors associated with cIMT (as a dichotomous variable) using multiple logistic regression analysis from selected data according to univariate analysis.

Variable	Standard regression coefficient	Standard error	Odds ratio with 95% confidence interval	*p*
Number of swollen joints	1.35	0.47	3.86 (1.53-0.71)	0.004
Hypertension	1.46	0.51	4.32 (1.6-11.7)	0.004
MTX intake	- 1.30	0.48	0.27 (0.10-0.70)	0.007

Discussion

The first aim of the VErA vascular study was to assess the presence of subclinical atherosclerosis in patients suffering from early inflammatory arthritis. Even though functional measures of the vasculature are particularly relevant, our study was only focused on morphological vascular abnormalities

Since carotid IMT is increasingly used as a surrogate marker for atherosclerosis and as a predictor of future cardiovascular events in healthy subjects and in RA (29), carotid ultrasound was performed in our cohort at 7 years of follow-up. It has been recently demonstrated that carotid assessment by sonography is more sensitive than coronary calcification score to detect subclinical atherosclerosis in a population of 100 patients with established RA (30). Moreover, this tool has also been proved to be useful to establish the actual cardiovascular risk of RA as many patients included in the category of moderate risk according to well-established risk charts like the Systematic Coronary Risk Evaluation (SCORE) have carotid plaques (31).

Our cohort is somewhat unique in that the patients were included very early, less than 6 months after the onset of their disease, and that they were conservatively treated during the first 2 years. Previous studies by Chung *et al*. (32) and Hafström *et al*. (33) concerning "recent RA", have included established RA patients, satisfying ACR criteria (22) and progressing for <5 years and <1 year. Thus, our population is one of the largest for a prospective study on subclinical atherosclerosis during inflammatory arthritis, after the ÓRALE cohort (34, 35) and that of Kumeda *et al*. (36). The frequencies of classical cardiovascular risk factors varied widely according to the RA cohort but our findings are comparable, with the exception of the American study by del Rincón *et al*. (34) and the Israeli study by Abu-Shakra *et al*. (37). These differences may be due to discrepancies in the characteristics of general population in each country. Interestingly, although 53 (50%) of our patients had android obesity, only 22 patients had a BMI >30 kg/m^2 and the others merely had elevated waist-to-hip ratios. These observations suggest that BMI calculation underestimates the android distribution of fat that might play a role in the enhanced vascular risk of RA patients. Characteristics of our patients (age, sex ratio) were comparable to those of other studies but their clinical markers of disease activity, RF concentrations and radiological scores were lower. These differences can be explained by the recent onset of our patients' disease and by the criteria of our population-based recruitment. Nevertheless, the mean cIMT in the VerA vascular cohort (0.67±0.12 mm), that has been measured 7 years after the onset of symptoms, was comparable to those of previous studies conducted by Hafström *et al*. (0.67±0.12 mm) or Dessein *et al*. (0.65±0.22 mm), concerning established RA patients (33, 38) and more recently, in the 114 patients with recent RA (<12 months) from the BARFOT study population in which mean cIMT was also 0.67 (0.6-0.77) after 5-years of RA disease (39), but we did not have any matched group to compare. Even though comparison of cIMT values is difficult among studies because of huge variations of mean cIMT values in RA and in control groups and of the potential impact of conventional and biologic DMARDS that have the ability to reduce cIMT in responders (11, 40, 41), data are generally similar as shown previously. Moreover, several works such as that conducted by Corrales *et al*. in 370 RA patients with a mean disease duration of 10 years, without history of cardiovascular events, suggest that patients with a mean cIMT <0.60 mm and no plaque can be considered as being free of atherosclerosis (30). Such profile has been observed in a small population de 35 early (<12 months) RA patients since mean cIMT value was 0.50±0.16 mm, which was however significantly higher than that in healthy controls (0.44±0.09) (42). Thus, taken together, those results suggest that the degree of subclinical atherosclerosis is higher in established RA than in early inflammatory arthritis. But the important limitations of the present study are the lack of a matched control group and

the fact that carotid assessment was not performed at the study inclusion.

Our second objective was to identify surrogate markers associated with subclinical atherosclerosis during the course of early inflammatory arthritis. The specificity of our study was to focus on biochemical markers reflecting the different phases of the pathophysiological processes involved in atherosclerosis and not only on the usual markers of arthritis and classical cardiovascular risk factors. Since cIMT may be dependent on the course of different parameters, we carried out serial measurements of these biological markers in order to evaluate both baseline and cumulated data. With this regard, characterisation of cumulative inflammation seems to be a better indication of the overall inflammatory burden and subsequent risk of developing atherosclerosis (43).

As expected, age at inclusion was the main marker associated with the development of atheromatous lesions. Hypertension was also strongly correlated with high cIMT values: indeed, hypertension had the strongest link with the presence of atheromatous lesions in multivariate analysis (OR 4.33, 95% CI [1.59–11.73]; p=0.004). This result is in accordance with previous data, notably those obtained from the Dudley Rheumatoid Arthritis Comorbidity Cohort (DRACCO) in which hypertension and insulin resistance were found to be associated with increased IMT (44). In our study, the other CV risk factors were not related to subclinical atherosclerosis. In a recent report, higher levels of pro-atherogenic apolipoproteins apoB and apoB/apoA1 ratio were independently associated with enhanced detection of bilateral carotid plaques in a cohort of 114 patients with recent RA (39). However, we have not assessed these lipoproteins and, in addition, only a few patients had carotid plaques in our cohort.

During the first 2 years of follow-up, all of the patients were subsequently treated homogeneously in a nonaggressive manner, in accordance with protocol recommendations and the therapeutic approach practiced in France at the time the protocol was written. Consequently, the impact of therapeutic on the markers studied at the onset of the disease was limited. Most of previous studies examining atherosclerosis in RA reported only treatment at inclusion and not those prescribed previously. A few teams analysed the effect of cumulative treatment (32, 45, 46), particularly del Rincón *et al*. (46), who emphasised the deleterious impact of the cumulative corticosteroid dose on atheroma. In our cohort, no relationship was found between cIMT values and NSAIDs or corticosteroid treatments. Nevertheless, the protective role of methotrexate on CV events in RA patients was confirmed (47). Univariate analyses also identified that the number of swollen joints and the DAS44 score at inclusion, reflecting arthritis activity, were the clinical markers significantly associated with carotid atherosclerosis. The role of systemic inflammation, highlighted in several reports (48-53), was not confirmed in our study, both at baseline and during the first 2 years of follow-up. In this respect, association between systemic inflammation and subclinical atherosclerosis is not clearly evident. It was mainly observed in cross-sectional studies while findings between cumulative inflammation and atherosclerosis are contrasting (39, 42, 44, 54). Nevertheless, a link between mean CRP and ESR values and increased risk of cardiovascular events was observed in a cohort study of RA (55).

These discrepancies are probably related to the degree of disease activity in the different cohorts. In the present study, RA patients had moderate systemic inflammation that could explain why cumulative inflammation was not high enough to lead to vascular abnormalities. Among the most specific biological markers, those reflecting lymphocyte activation (CD40L), angiogenesis (VEGF, sVEGFR1, angiopoïetin), endothelial dysfunction (VCAM1, E-selectin) and oxidative stress (oxidised LDL, AOPP) were not significantly associated with carotid atheroma. This is in accordance with another population-based study that did not disclose a significant association between cIMT/carotid plaques and well-documented markers of endothelial cell activation such as sICAM-3, sICAM-1, sVCAM-1, sP-selectin and sE-selectin in 29 patients undergoing anti-TNF therapy (56). In contrast, anti-CCP–antibody positivity at inclusion was associated with lower cIMT values.

The 'protective' role of anti-CCP antibodies against atherosclerosis development might suggest that the more 'inflammatory' forms of early arthritis (with elevated ESR and CRP values) are at higher risk of vascular complications, while the more 'autoimmune' forms (with elevated RF and anti-CCP antibodies) have more structural damage (57). In contrast, the study by López-Longo *et al*. (58) showed that the presence of anti-CCP antibodies was independently associated with ischemic heart disease (OR 2.8, 95% CI 1.19–6.56; p=0.009), but not with traditional CV risk factors, in a cohort of 937 RA patients. The discrepancy with our results may be due to huge differences in the population studied: mean disease duration (10.5±8.3 years), presence of rheumatoid nodules (20.2%), associated renal disease (14.3%) or pulmonary disease (9.8%) representing more evolved and severe forms of RA. Since anti-CCP positive patients are considered as having a more severe disease, we can postulate that they have been treated more aggressively, which might have reduced the influence that chronic inflammation might have had on the development of atherosclerotic disease. But we can not exclude that anti-CCP might have a more pathogenic atherosclerotic effect in long-standing RA patients than in those with early arthritis. In this respect, our findings are in accordance with those obtained from the Nijmegen early RA cohort in which there was no relationship between anti-CCP positivity and/or titers and the occurrence of non-fatal or fatal cardiovascular events within a 10-years follow-up (59). Thus, the precise contribution of anti-CCP antibodies in cardiovascular risk during early inflammatory arthritis requires further evaluation in larger cohorts.

Other genetic and biological markers such as HLA-DRB1*04 alleles carry-

ing shared epitope, osteoprotegerin and angiopoïetin-2 have been suggested to be associated to cardiovascular disease in RA patients (60-63). Those markers have not been investigated in the present study.

Our earlier systematic analysis of the literature (64) showed that markers, other than classical risk factors, associated with subclinical atherosclerosis in RA patients were: the duration of RA progression, the number of deformed joints, age at the time of diagnosis, CRP, ESR, the soluble intracellular adhesion molecule-1 (sICAM-1) level and treatment with corticosteroids and anti-TNF-α. The multiparameter study we have conducted from the VErA vascular cohort has not led to the identification of new biomarkers associated with carotid subclinical atherosclerosis Multivariate analysis emphasised the role of 2 risk factors (hypertension and number of swollen joints at inclusion) and a protective element (methotrexate use). These findings confirmed the multifactorial origin of atherosclerosis, associating classical cardiovascular risk factors and markers of arthritis activity. The protective role of methotrexate supported reported results showing fewer cardiovascular deaths in RA patients taking this drug (47). However, there is a controversy about the impact of DMARDs on the progression on atherosclerosis since some studies have shown either progression or reduction of cIMT in patients undergoing anti-TNF therapy (3, 65). A decreased risk of having unstable plaques mediated by the anti-inflammatory effect of methotrexate or biologic agents may account for the decreased risk of cardiovascular events, regardless of cIMT progression due to age, traditional cardiovascular risk factors and inflammation (65, 66).

There are several limitations in the present study. Markers of vascular dysfunction such as arterial stiffness indices (PWV) or FMD have not been assessed. Because we, like many other authors, did not measure the cIMT at inclusion, we cannot affirm that the value increased or not over time. The markers analysed were thus correlated with atheromatous lesions at 7 years, but are not truly predictive of atheroma aggravation. Only the study by Nagata-Sakurai *et al*. (48) evaluated markers associated with increased carotid IMT, between inclusion and 18–36 months later, in 62 RA patients and 63 controls: their analysis indicated a correlation between cIMT and markers of inflammation (ESR and CRP). Many models "predicting" subclinical atherosclerosis were developed in several studies (34, 36, 45, 48, 49, 66, 67). The point common to these different models is that they systematically integrated classical cardiovascular risk factors, markers of RA activity and, sometimes, more specific markers (neutrophil counts, hypothyroidism, von Willebrand factor). In our study, biochemical markers analysed were defined based on the pathophysiological characteristics of RA and atherosclerosis (oxidative stress, endothelial dysfunction, autoimmunity, angiogenesis) and had not been evaluated in earlier investigations (other than sICAM-1). Nevertheless, none of the soluble markers reflecting lymphocyte activation and endothelial dysfunction can be considered nowadays as robust markers of these pathophysiological disorders

Using the very specific population of VErA cohort, that represent community cases of very early RA with a moderate degree of activity and severity, having an accurate follow-up since the early signs of arthritis, we have observed that both the inflammatory disease, reflected by the joint count, and the classical CV risk factor hypertension, were associated with cIMT thickness. This confirms that both inflammation and classical CV risk factor must be scrutinised and targeted for the aim of modifying the CV outcomes of RA. This is strengthened by our data suggesting a protective role of methotrexate.

Key messages

- Subclinical atheroma in a cohort of early inflammatory arthritis is multifactorial.
- Those factors include traditional risk factor and articular disease activity.
- Hypertension and swollen joint count are the most significant parameters.
- Methotrexate intake has a protective effect.

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