

## No significant change in arterial stiffness in RA after 6 months and 1 year of rituximab treatment

Sylvain Mathieu<sup>1,2</sup>, Bruno Pereira<sup>2,3</sup>, Jean-Jacques Dubost<sup>1,2</sup>, Jean-René Lussan<sup>2,4</sup> and Martin Soubrier<sup>1,2</sup>

### Abstract

**Objective.** The excess cardiac risk, found in RA has been attributed to biological inflammation. Effective control of inflammation may be of benefit in reducing cardiovascular risk in RA patients. The aim of this study is to investigate the effects of 24 and 52 weeks of rituximab treatment on arterial stiffness and cardiovascular risk factors.

**Methods.** Arterial stiffness was measured by augmentation index (AIx) and pulse wave velocity (PWV), and other cardiovascular risk factors (lipid profile, blood pressure) were collected in active RA patients.

**Results.** Thirty-three patients, of whom 29 were females, with a mean age of 60.9 (12.0) years were included. Thirty patients had positive RFs, 27 had positive anti-CCP antibody and 93.9% ( $n=31$ ) were erosive. Nineteen patients were non-responders to anti-TNF- $\alpha$  treatments. After rituximab treatment, no change was observed in arterial stiffness, neither after 6 nor after 12 months [PWV 8.1 (3.1)m/s at baseline, 8.1 (2.8) at 6 months, 8.0 (2.7) at 1 year,  $P=0.924$ ; and AIx 30.4 (8.2)% at baseline, 28.6 (7.6) at 6 months, 29.4 (6.7) at 1 year,  $P=0.216$ ]. Total and low-density lipoprotein cholesterol levels increased significantly but high-density lipoprotein (HDL) and triglyceride levels were unchanged. The atherogenic index (total cholesterol/HDL cholesterol) was increased, but not to a level of significance. No change was found in other cardiovascular risk factors. DAS-28 according to levels of ESR and CRP and biologic inflammation were significantly improved.

**Conclusion.** Arterial stiffness did not improve after 6 and 12 months of rituximab therapy. The treatment had a beneficial effect on biologic inflammation and disease activity, but caused a pro-atherogenic lipid profile.

**Key words:** rheumatoid arthritis, arterial stiffness, cardiovascular risk markers, rituximab.

### Introduction

There is substantial evidence of excess cardiovascular morbidity and mortality in patients with RA [1–2]. The reasons for accelerated atherosclerosis in RA are likely to be multifactorial. Studies of RA populations did not find that traditional cardiovascular risk factors by themselves were

sufficient to explain the increased cardiovascular events seen in these patients [3, 4].

Atherosclerosis is an inflammatory disease, and systemic inflammation plays a pivotal role in the increased incidence of cardiovascular disease in RA [4]. Systemic inflammation has been recently reported to be correlated with arterial stiffness [5] and was known to affect endothelial function by different mechanisms, including reduction in nitric oxide bioavailability, degradation of elastin on vascular wall and attenuation of the survival of endothelial progenitor cells [6].

Arterial stiffness, a marker of the cardiovascular risk that can directly accelerate the atherosclerotic process and can predict the occurrence of cardiovascular events [7], is increased in RA patients [8] and correlated to the importance of biological inflammation and structural damage [9, 10]. However, TNF- $\alpha$  inhibitors, which are

<sup>1</sup>Service de Rhumatologie, Gabriel Montpied Teaching Hospital, <sup>2</sup>Fac Médecine, Univ Clermont 1, <sup>3</sup>DRCI and <sup>4</sup>Service de Cardiologie, Gabriel Montpied Teaching Hospital, Clermont-Ferrand, France.

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Correspondence to: Sylvain Mathieu, Rheumatology Department, Gabriel Montpied Teaching Hospital, Place H. Dunant, Clermont-Ferrand 63000, France.  
E-mail: smathieu@chu-clermontferrand.fr

known to reduce biologic inflammation after therapy, had conflicting effects on arterial stiffness in RA [8, 11].

Rituximab, a chimeric anti-CD20 mAb, improves disease activity in RA patients who experience inadequate response or intolerance to TNF- $\alpha$  inhibitor treatment [12]. B cells have been reported to have a role in atherosclerosis with pro-atherogenic properties, and recently, in mice, B-cell depletion caused a significant reduction in atherosclerosis [13]. The present study aimed to assess the effects of 24 and 52 weeks of rituximab therapy in RA on arterial stiffness, a marker of subclinical atherosclerosis and lipid profile.

## Patients and methods

RA patients who fulfilled the ACR 1987 revised criteria were eligible for enrolment. Those enrolled had more than two unsuccessful TNF- $\alpha$  blockade treatments or had contraindications to these treatments and persistent active RA [DASs according to levels of ESR (DAS-28<sub>ESR</sub>) and CRP (DAS-28<sub>CRP</sub>) were >3.2]. Patients were treated with rituximab according to medical practitioners' opinion. They received two i.v. infusions of rituximab (1000 mg each) and methylprednisolone (100 mg each) 2 weeks apart. At baseline and after 24 and then 52 weeks of treatment, clinical assessment, laboratory examinations and arterial stiffness were recorded. The study was approved by the local Research Ethics Committee of Gabriel Montpied Teaching Hospital and all subjects gave written informed consent.

### Clinical assessment

Disease duration and medications were recorded. The number of tender joints (TJs) and swollen joints (SJs) was recorded for each patient. The global assessment score of disease activity and the level of pain were recorded by the patient using a 100-mm horizontal visual analogue scale (VAS). DAS-28<sub>ESR</sub> and DAS-28<sub>CRP</sub> were calculated at baseline, 6 and 12 months post-treatment.

### Non-invasive vascular assessment

#### Augmentation index

The Sphygmocor apparatus (Atcor Medical, Sydney, Australia) was used to perform pulse wave analysis. Blood pressure was recorded in the supine position. Radial artery waveforms were recorded from the wrist of the dominant arm with a tonometer. The augmentation index (AIx), a measure of systemic arterial stiffness, was calculated by the integral software as the difference between the second and first systolic peaks, expressed as a percentage of the pulse pressure.

#### Pulse wave velocity

Pulse wave velocity (PWV) was measured along the descending thoracoabdominal aorta using the foot-to-foot velocity method. Briefly, waveforms were obtained transcutaneously over the common carotid artery and the right

femoral artery; the time delay ( $t$ ) was measured between the feet of the two waveforms. The distance ( $D$ ) covered by the waves was assimilated to the distance measured between the two recording sites. PWV was calculated as  $PWV = D$  (metres)/ $t$  (seconds).

### Laboratory examinations

In the morning, after an overnight fast, venous blood was sampled for the measurement of serum concentrations of CRP, ESR and lipid profile [total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides]. The atherogenic index (total cholesterol/HDL cholesterol) was then calculated.

### Statistical analysis

Mean (s.d.) or median and interquartile range (IQR) for quantitative variables and number of patients (percentages within parentheses) for categorical variables were calculated. To compare the time course evolution (baseline, 6 months and 12 months) of different parameters, a repeated-measures analysis of variance (ANOVA) test was used followed by a Tukey–Kramer test to compare differences between and within groups. This analysis was completed by linear mixed models that allow consideration of random subject effects: random intercept and slope. When the normality of a parameter was not achieved using the Shapiro–Wilk test (ESR and CRP, for example), two options were considered: (i) log transformation was performed and previous models have been used or (ii) generalized linear mixed models were implemented for parameters that are not Gaussian distribution and not log-transformation normal distribution. The residual normality was checked for all models presented in this article. Power analyses have been conducted to see whether the sample size is sufficient to detect changes over time. A  $P < 0.05$  (two-tailed) was considered statistically significant. Statistical analysis was performed using Stata software (version 10; Stata-Corp LP, College Station, TX, USA).

## Results

Table 1 shows the baseline comorbidities, cardiovascular risk factors, demographic characteristics and concurrent medications of RA patients. Thirty-three patients, of whom 29 (87.9%) were females, with a mean age of 60.9 (12.0) years and long-standing disease of 17.6 (8.6) years were included (Table 1). Thirty (90.9%) RA patients had positive RFs, 27 (81.8%) had positive anti-CCP antibody and 93.9% ( $n = 31$ ) were erosive. Nineteen patients (57.6%) were non-responders to anti-TNF- $\alpha$  treatments and 13 had contraindications to these treatments.

After rituximab treatment, no change was observed in PWV and AIx, neither after 6 nor after 12 months [PWV 8.1 (3.1) m/s at baseline, 8.1 (2.8) at 6 months, 8.0 (2.7) at 1 year,  $P = 0.924$ ; and AIx 30.4 (8.2)% at baseline, 28.6 (7.6) at 6 months, 29.4 (6.7) at 1 year,

**TABLE 1** Baseline characteristics of RA patients treated with rituximab

Characteristics	Rituximab group (n = 33)
Age, mean (s.d.), years	60.9 (12.0)
Male/female, n	4/29
Ever/never smoking, n	7/26
Disease duration, mean (s.d.), years	17.6 (8.6)
Systolic BP, mean (s.d.), mmHg	129.8 (21.1)
Diastolic BP, mean (s.d.), mmHg	71.0 (8.8)
BMI, mean (s.d.), kg/m <sup>2</sup>	25.0 (5.6)
Diabetes, n (%)	5 (15.2)
RF positive, n (%)	30 (90.9)
Anti-CCP positive, n (%)	27 (81.8)
Erosive RA, n (%)	31 (93.9)
SJ count, mean (s.d.)	8.9 (4.8)
ESR, mean (s.d.), mm/h	36.8 (25.9)
CRP, mean (s.d.), mg/l	23.4 (24.7)
DAS-28 <sub>ESR</sub> , mean (s.d.)	5.7 (1.1)
DAS-28 <sub>CRP</sub> , mean (s.d.)	5.4 (0.9)
DMARDs, n (%)	
MTX alone	20 (60.6)
LEF alone	1 (3.0)
HCQ alone	3 (9.1)
SSZ alone	0 (0.0)
Combination	2 (6.1)
None	7 (21.1)
CSs, mg/day	
n (%)	23 (75.8)
Mean (s.d.)	7.6 (5.3)
NSAIDs, n (%)	9 (27.3)
Statins, n (%)	3 (9.1)
Anti-hypertensive therapy, n (%)	13 (39.4)

BP: blood pressure.

$P=0.216$ ] (Table 2). A significant increase in concentrations of total cholesterol [4.9 (1.0) mmol/l at baseline, 5.6 (0.8) at 6 months, 5.5 (1.3) at 1 year,  $P < 0.001$ ] and LDL cholesterol [2.7 (0.6) mmol/l at baseline, 3.0 (0.7) at 6 months, 3.1 (1.0) at 1 year,  $P=0.029$ ] were observed. We found an increase in the atherogenic index (total cholesterol/HDL cholesterol), but not to a level of significance [3.06 (0.79) at baseline, 3.29 (0.96) at 6 months, 3.37 (1.03) at 1 year,  $P=0.517$ ], but HDL and triglyceride levels were unchanged (Table 2). No change was found in levels of blood pressure or BMI. DAS-28<sub>ESR</sub> [5.7 (1.1) at baseline, 3.7 (1.6) at 6 months, 3.9 (1.1) at 1 year,  $P < 0.001$ ] and DAS-28<sub>CRP</sub> [5.4 (0.9) at baseline, 3.4 (1.3) at 6 months, 3.6 (1.2) at 1 year,  $P < 0.001$ ] were significantly improved. After 6 months of rituximab treatment, 11 RA patients achieved both DAS-28 remission and a good European League Against Rheumatism (EULAR) response (11/33, 33.3%). Four more patients obtained a good EULAR response without DAS-28 remission. At 1 year of treatment, seven RA patients had DAS remission and a good EULAR response (7/27, 25.9%) and four more patients had only a good EULAR response. We found a significant decrease in parameters of biologic inflammation [ESR 36.8 (25.9) mm/h at baseline, 25.8 (16.8) at 6 months, 25.0 (15.6) at 1 year,  $P < 0.001$ ; and CRP 23.4 (24.7) mg/l at baseline, 10.6 (11.9) at 6 months, 12.0 (12.3) at 1 year,  $P < 0.001$ ] (Table 2).

No change was obtained in Alx or PWV after rituximab in RA patients achieving remission at 6 or 12 months (data not shown). No significant modification in lipid profile was found after rituximab treatment in subgroup analysis, i.e. in RA patients with DAS remission or not, those with good EULAR response or not and those with biologic inflammation or not (data not shown).

**TABLE 2** Differences in parameters after 6 or 12 months of rituximab treatment

Characteristics	Rituximab therapy (n = 33)			P-value <sup>a</sup>
	Baseline	6 months	12 months	
Alx, %	30.4 (8.2)	28.6 (7.6)	29.4 (6.7)	0.216
PWV, m/s	8.1 (3.1)	8.1 (2.8)	8.0 (2.7)	0.924
Total cholesterol, mmol/l	4.9 (1.0)	5.6 (0.8)	5.5 (1.3)	<0.001
HDL cholesterol, mmol/l	1.7 (0.5)	1.7 (0.4)	1.7 (0.5)	0.060
LDL cholesterol, mmol/l	2.7 (0.6)	3.0 (0.7)	3.1 (1.0)	0.029
Triglycerides, mmol/l	1.2 (0.6)	1.3 (0.7)	1.1 (0.6)	0.144
Atherogenic index	3.1 (0.8)	3.3 (1.0)	3.4 (1.0)	0.517
TJC	9.2 (5.3)	3.3 (3.5)	3.6 (3.0)	<0.001
SJC	8.9 (4.8)	4.1 (4.5)	4.0 (3.2)	<0.001
Patient Activity VAS	60.8 (19.5)	31.4 (24.9)	35.3 (27.1)	<0.001
ESR, mm/h	36.8 (25.9)	25.8 (16.8)	25.0 (15.6)	<0.001
CRP, mg/l	23.4 (24.7)	10.6 (11.9)	12.0 (12.3)	0.003
DAS-28 <sub>ESR</sub>	5.7 (1.1)	3.7 (1.6)	3.9 (1.1)	<0.001
DAS-28 <sub>CRP</sub>	5.4 (0.9)	3.4 (1.3)	3.6 (1.2)	<0.001
Steroids dose, mg/day	7.6 (5.3)	5.4 (3.9)	4.1 (3.4)	<0.001

Values are mean (s.d.). Bold values are significant. <sup>a</sup>ANOVA test. TJC: TJ count; SJC: SJ count.

## Discussion

In this study we found that 6 and 12 months of rituximab treatment did not improve arterial stiffness. PWV, considered as the gold standard to measure arterial stiffness [7], was not improved, nor Aix, a composite measurement of arterial stiffness and wave reflection that depends on the velocity of the pressure wave [7].

Studies assessing the effects of rituximab on subclinical atherosclerosis in RA are scarce. Endothelial function plays a key role in early atherosclerosis and contributes to the development of clinical features in the later stages of the vascular disease. In the study of Gonzalez-Juanatey *et al.* [14], six consecutive RA patients (five women; age range 55–79 years) with active disease refractory to TNF- $\alpha$  inhibitor therapy were treated by two infusions of rituximab 1000 mg each separated by 2 weeks. Kerekes *et al.* [15] assessed the effects of rituximab on flow-mediated dilation (FMD), a marker of endothelial dysfunction, in five female RA patients who received two infusions of 1000 mg rituximab. In the both studies, rituximab treatment resulted in a rapid improvement after 2 weeks of treatment, and the role of methylprednisolone administered with rituximab had been questioned in FMD [15]. However, a continuing improvement at 16 weeks in the five patients studied by Kerekes *et al.* and at 24 weeks in 5/6 patients studied by Gonzalez-Juanatey *et al.* should be considered as solely attributable to rituximab therapy [14–16].

The lack of improvement we observed in arterial stiffness in 33 RA patients after 6 months of rituximab treatment is inconsistent with the improvement in endothelial function reported in these two previous studies that involved a smaller number of patients. The absence of significant improvement in arterial stiffness after 6 months of rituximab could be explained by the longer time needed for the treatment to be effective. Rituximab is not an anti-cytokine therapy and onset of its action is slower than that of anti-TNF treatment. Indeed, B-cell depletion during rituximab therapy is not always fully complete and requires about 12 weeks [17]. This could explain why, after 6 months of treatment, anti-TNF therapy, but not rituximab, could bring about an improvement in arterial stiffness [8]. However, after 1 year of treatment, there was still no change in arterial stiffness.

The lack of improvement in arterial stiffness does not necessarily mean that rituximab has no beneficial effect on cardiovascular risk. TNF- $\alpha$  blockade treatment was not consistently observed to improve arterial stiffness in all studies. However, it was recently reported to decrease cardiovascular mortality in RA patients [18].

The effect of rituximab on lipid profile in the present study is pro-atherogenic. This deleterious effect has already been reported after TNF- $\alpha$  inhibitors in RA, but results are conflicting.

A possible limitation of our study was that we did not measure the lipoproteins and apolipoproteins, which provide a better assessment of cardiovascular risk [19]. However, guideline groups previously recommended a cholesterol-based approach and excluded

apolipoproteins from routine clinical use. The number of included patients in our study may seem insufficient, but power analyses conducted to see whether the sample size was sufficient to detect changes over time showed that considering the observed decrease in ESR after 12 months of treatment and an intra-class correlation coefficient (ICC) from 0.58, the power of our study was >95%. For CRP, the power was near 85% (ICC = 0.19). Another limitation is the lack of a control group, which makes the evaluation of the time needed to assess any change in stiffness status more difficult. However, according to the medical literature, 6 months of follow-up are enough to demonstrate a relevant change of arterial stiffness [8].

In this study, arterial stiffness was not improved after 6 and 12 months of rituximab therapy. The treatment had a beneficial effect on biologic inflammation and disease activity, but caused a pro-atherogenic lipid profile. The management of dyslipidaemia is necessary in RA, as recommended by the EULAR. In patients treated by rituximab with dyslipidaemia, the treatment of choice should be dietary advice followed by fibrates rather than statins that could decrease the efficacy of rituximab in RA [20].

### Rheumatology key messages

- Rituximab does not improve arterial stiffness after 6 or 12 months of treatment.
- Rituximab decreases systemic inflammation and disease activity, but causes a pro-atherogenic lipid profile.

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## References

- 1 Meune C, Touzé E, Trinquart L *et al.* Trends in cardiovascular mortality in patients with rheumatoid arthritis over 50 years: a systematic review and meta-analysis of cohort studies. *Rheumatology* 2009;48:1309–13.
- 2 Aviña-Zubieta JA, Choi HK, Sadatsafavi M *et al.* Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Arthritis Rheum* 2008;59:1690–7.
- 3 Solomon DH, Kremer J, Curtis JR *et al.* Explaining the cardiovascular risk associated with rheumatoid arthritis: traditional risk factors versus markers of rheumatoid arthritis severity. *Ann Rheum Dis* 2010;69:1920–5.
- 4 Sattar N, McCarey DW, Capell H *et al.* Explaining how ‘high-grade’ systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation* 2003;108:2957–63.
- 5 van Bussel BC, Schouten F, Henry RM *et al.* Endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness over a 6-year period. *Hypertension* 2011;58:588–95.
- 6 Verma S, Kuliszewski MA, Li SH *et al.* C-reactive protein attenuates endothelial progenitor cell survival,

- differentiation, and function: further evidence of a mechanistic link between C-reactive protein and cardiovascular disease. *Circulation* 2004;109:2058–67.
- 7 Mäki-Petäjä KM, Wilkinson IB. Anti-inflammatory drugs and statins for arterial stiffness reduction. *Curr Pharm Des* 2009;15:290–303.
  - 8 Mäki-Petäjä KM, Hall FC, Booth AD *et al.* Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor- $\alpha$  therapy. *Circulation* 2006;114:1185–92.
  - 9 Van Doormun S, McColl G, Jenkins A *et al.* Screening for atherosclerosis in patients with rheumatoid arthritis. Comparison of two in vivo tests of vascular function. *Arthritis Rheum* 2003;28:72–80.
  - 10 Wong M, Toh L, Wilson A *et al.* Reduced arterial elasticity in rheumatoid arthritis and the relationship to vascular disease factors and inflammation. *Arthritis Rheum* 2003;48:81–9.
  - 11 Van Doornum S, McColl G, Wicks IP. Tumour necrosis factor antagonists improve disease activity but not arterial stiffness in rheumatoid arthritis. *Rheumatology* 2005;44:1428–32.
  - 12 Cohen SB, Emery P, Greenwald MW *et al.* Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum* 2006;54:2793–806.
  - 13 Ait-Oufella H, Herbin O, Bouaziz JD *et al.* B cell depletion reduces the development of atherosclerosis in mice. *J Exp Med* 2010;207:1579–87.
  - 14 Gonzalez-Juanatey C, Llorca J, Vazquez-Rodriguez TR *et al.* Short-term improvement of endothelial function in rituximab-treated rheumatoid arthritis patients refractory to tumor necrosis factor  $\alpha$  blocker therapy. *Arthritis Rheum* 2008;59:1821–4.
  - 15 Kerekes G, Soltész P, Dér H *et al.* Effects of rituximab treatment on endothelial dysfunction, carotid atherosclerosis, and lipid profile in rheumatoid arthritis. *Clin Rheumatol* 2009;28:705–10.
  - 16 Sandoo A, Toms T, Zanten JV *et al.* Short-term effects of rituximab on flow-mediated dilatation may be mediated by intravenous glucocorticoids: comment on the article by Gonzalez-Juanatey *et al.* *Arthritis Rheum* 2009;61:854–5.
  - 17 Dass S, Rawstron AC, Vital EM *et al.* Highly sensitive B cell analysis predicts response to rituximab therapy in rheumatoid arthritis. *Arthritis Rheum* 2008;58:2993–9.
  - 18 Barnabe CCM, Martin BJ, Ghali WA. Systematic review and meta-analysis: anti-tumor necrosis factor alpha therapy and cardiovascular events in rheumatoid arthritis. *Arthritis Care Res* 2011;63:522–9.
  - 19 Walldius G, Jungner I, Aastveit AH *et al.* The apoB/apoA-I ratio is better than the cholesterol ratios to estimate the balance between plasma proatherogenic and antiatherogenic lipoproteins and to predict coronary risk. *Clin Chem Lab Med* 2004;42:1355–63.
  - 20 Arts E, Jansen TL, Den Broeder A *et al.* Statins Inhibit the anti-rheumatic effects of rituximab in rheumatoid arthritis—results from the Dutch Rheumatoid Arthritis Monitoring (DREAM) Registry. Abstract supplement. *Arthritis Rheum* 2010;62:S766.