Subclinical Atherosclerosis in Patients with Rheumatoid Arthritis and Low Cardiovascular Risk: The Role of von Willebrand Factor Activity

Gorica G. Ristić1*, Vesna Subota2, Toplica Lepić3, Dejana Stanisavljević4, Branislava Glišić1, Arsen D. Ristić5, Milan Petronijević1, Dušan Z. Stefanović1

1 Department of Rheumatology and Clinical Immunology, Military Medical Academy and Medical Faculty of the Belgrade Defence University, Belgrade, Serbia, 2 Institute of Medical Biochemistry, Military Medical Academy, Belgrade, Serbia, 3 Department of Neurology, Military Medical Academy and Medical Faculty of the Belgrade Defence University, Belgrade, Serbia, 4 Institute of Medical Statistics, Belgrade University School of Medicine, Belgrade, Serbia, 5 Department of Cardiology, Clinical Centre of Serbia and Belgrade University School of Medicine, Belgrade, Serbia

* goricaris@eunet.rs

Abstract

Background
To evaluate association between von Willebrand factor (vWF) activity, inflammation markers, disease activity, and subclinical atherosclerosis in patients with rheumatoid arthritis (RA) and low cardiovascular risk.

Methods
Above mentioned parameters were determined in blood samples of 74 non-diabetic, normotensive, female subjects, with no dyslipidemia (42 patients, 32 matched healthy controls, age 45.3±10.0 vs. 45.2±9.8 years). Intima-media thickness (IMT) was measured bilaterally, at common carotid, bifurcation, and internal carotid arteries. Subclinical atherosclerosis was defined as IMT > IMTmean+2SD in controls at each carotid level and atherosclerotic plaque as IMT > 1.5 mm. Majority of RA patients were on methotrexate (83.3%), none on steroids >10 mg/day or biologic drugs. All findings were analysed in the entire study population and in RA group separately.

Results
RA patients with subclinical atherosclerosis had higher vWF activity than those without (133.5±69.3% vs. 95.3±36.8%, p<0.05). Predictive value of vWF activity for subclinical atherosclerosis was confirmed by logistic regression. vWF activity correlated significantly with erythrocyte sedimentation rate, fibrinogen, modified disease activity scores (mDAS28–ESR, mDAS28–CRP), modified Health Assessment Questionnaire (p<0.01 for all), duration of smoking, number of cigarettes/day, rheumatoid factor concentration (p<0.05 for all), and...
anti-CCP antibodies (p<0.01). In the entire study population, vWF activity was higher in participants with subclinical atherosclerosis (130±68% vs. 97±38%, p<0.05) or atherosclerotic plaques (123±57% vs. 99±45%, p<0.05) than in those without. Duration of smoking was significantly associated with vWF activity (β 0.026, p = 0.039).

**Conclusions**

We demonstrated association of vWF activity and subclinical atherosclerosis in low-risk RA patients as well as its correlation with inflammation markers, all parameters of disease activity, and seropositivity. Therefore, vWF might be a valuable marker of early atherosclerosis in RA patients.

**Introduction**

The incidence of cardiovascular diseases (CVD) is higher in patients with rheumatoid arthritis (RA) than in general population [1] and increased carotid intima-media thickness (IMT) has been recommended for the cardiovascular risk stratification in these patients [2, 3]. Chronic inflammation, the basic feature of rheumatoid arthritis (RA), plays a major role in accelerated atherosclerosis in patients with RA through its influence on insulin resistance, lipid status, and atherothrombogenic factors, such as fibrinogen, D-dimer, von Willebrand factor (vWF), and plasminogen activator inhibitor (PAI) [4]. The vWF is considered a reliable marker of endothelial dysfunction/damage, which is an initial step in atherosclerosis [5, 6].

Independent association of vWF with the increased carotid intima-media thickness (IMT) was shown in asymptomatic subjects [7], but limited data are available regarding its relation with subclinical atherosclerosis in RA [8–10], and only two investigations analysed patients without atherosclerotic risk factors [11, 12]. Therefore, the aim of our study was to evaluate association between vWF activity, inflammation markers, disease activity, and carotid IMT in young, non-diabetic, normotensive, female RA patients, with no dyslipidemia.

**Methods**

The investigation was designed as a cross-sectional, single-centre study. All participants have signed two copies of a written informed consent to participate in this study (one given to the participant, one kept in the study files). The study protocol and the consent procedure were approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia.

**Patients and Controls**

The study population included 74 female subjects: 42 RA consecutive patients and 32 healthy controls. Patients fulfilled the American College of Rheumatology revised criteria for RA. Mean disease duration was 7.1±5.4 years. Extra-articular manifestations were present in 11.9%, rheumatoid factor (RF) in 69%, anti-cyclic citrullinated peptide (anti CCP) antibodies in 59.5% of patients. Mean modified disease activity score 28 (mDAS28-ESR) was 3.55±1.36, mDAS28-CRP 3.1±1.27, while mean modified Health Assessment Questionnaire (mHAQ) was 0.45±0.49. Treatment included: low-dose prednisolone in 73.8% (mean 4.0±4.9 years), methotrexate dose of 10.4±2.0 mg/week in 83.3% (mean 3.3±2.8 years), chloroquine in 76.2% (mean 3.7±2.7 years), combined methotrexate/chloroquine therapy in 62% (mean 2.6±1.8 years), and sulfasalazine in 23.8% (mean 2.5±1.8 years) of patients.
Control subjects were matched with RA group regarding age (45.2±9.8 years, range 27–57 vs. 45.3±10.0 years, range 29–58 in RA), menopausal status (31.3% vs. 35.7%), body mass index (25.1±4.1% vs. 24.2±4.5 kg/m²), smoking habits, and serum lipid levels.

Subjects with the following conditions were excluded: history of CVD, hypertension, diabetes mellitus, hyperlipidemia, premature menopause, and treatment with biologic drugs and/or steroids >10 mg/day as defined in our previous study [13].

**Laboratory Analyses**

The erythrocyte sedimentation rate (ESR) was determined using the modified Westergren method, fibrinogen, glycaemia, total-, high-density, low-density cholesterol, and triglycerides were measured according to the established methods. C-reactive protein (CRP) and rheumatoid factor (RF) were determined by nephelometry, anti-CCP antibodies using ELISA. The vWF activity and PAI-1 were determined by a BC von Willebrand Reagent and Berichrom PAI on coagulation analyzer BCS-XP (Dade Behring/Siemens, Germany) [14], and D-dimer by immunochemistry (D-dimer PLUS reagent, BCS-XP analyzer).

**Carotid Ultrasound**

Carotid IMT was measured using a high resolution B-mode (9 MHz) ultrasound (Toshiba SSA370A, Japan). The IMT was defined as the distance between edges of the lumen-intima and the media-adventitia echos, in a plaque-free section. We measured IMT bilaterally, at the levels of common carotid (CCA), carotid bifurcation (BF) and internal carotid artery (ICA), as in the ARIC study [15]. Total of 18 measurements were performed in each subject and mean values were calculated for all segments (CCA, BF, ICA). Values at any point above mean IMT+2SD of the controls were defined as subclinical atherosclerosis, while atherosclerotic plaque as IMT>1.5 mm [16]. To avoid interobserver variability, all measurements were performed by the same experienced sonographer (TL), blinded for the clinical characteristics of the subjects.

**Statistical Analysis**

All findings were analysed in the entire study population and RA group separately. Values were expressed as means±SD or percentages as appropriate. Student’s t-test or Mann-Whitney U tests were applied for continuous variables, Chi-square or Fisher’s exact test for categorical variables. Simple and multiple logistic regression analysis were performed to identify predictors of subclinical atherosclerosis. Spearman’s correlation coefficient was used to determine the association between vWF and traditional-, as well as RA-related risk factors for atherosclerosis. All data were analysed using SPSS 15.0, considering a 2-tailed level of p<0.05 as significant.

**Results**

**Association of Subclinical Atherosclerosis or Plaques with (Non) Traditional Risk Factors and von Willebrand Factor Activity**

According to IMT values in control subjects (CCA mean 0.62±0.09 mm; BF mean 0.80±0.12 mm; ICA mean 0.54±0.08 mm), subclinical atherosclerosis (IMT mean+2SD of controls) was determined if IMT was higher than: 0.79 mm at CCA, 1.05 mm at BF, and 0.69 mm at ICA. **Subclinical atherosclerosis** on at least one level was demonstrated in 21.6% of all participants (16/74), in 35.7% of RA patients (15/42), and in 3.1% of controls (1/32) (p<0.01). Atherosclerotic plaques (IMT>1.5 mm) were revealed only on carotid BF in 20.3% of all participants (15/74), in 28.6% of RA patients (12/42) and 9.4% in controls 3/32 (p<0.05).
In the entire study population, participants with subclinical atherosclerosis were older than those without (52.7±5.5 vs. 43.2±9.8), had a higher fibrinogen (3.7±0.9 vs. 2.9±0.8), ESR (35.0±24.5 vs. 16.9±15.3) and were more frequently RF positive (81.3% vs. 32.8%) (p<0.01 for all). The univariate regression analysis confirmed association between abovementioned risk factors and presence of subclinical atherosclerosis (Table 1). The same was true for participants with atherosclerotic plaques who were older (53.0±5.7 vs. 43.3±9.7), had a higher fibrinogen (3.8±0.9 vs. 3.0±0.8), ESR (34.4±25.1 vs. 17.6±15.9) and were more frequently RF positive (66.7% vs. 37.3%) than those without plaques (p<0.01 for all) and univariate regression verified this association.

There was no significant difference between the groups regarding all lipid parameters and blood glucose levels. In the multiple regression analysis only age and RA itself were independent risk factors for subclinical atherosclerosis and plaque (p<0.01).

Subjects with subclinical atherosclerosis or plaque had significantly higher vWF activity than those without (130±68 vs. 97±38, p = 0.026; for plaque 123±57 vs. 99±45, p = 0.028) and this association was verified in the logistic regression analysis (Table 1). Among other haemostatic factors, D-dimer was higher in subjects with subclinical atherosclerosis or plaques than those without, but the difference was not significant. No difference was present regarding PAI-1 activity.

In participants with subclinical atherosclerosis or plaque, all smoking parameters were higher, but statistical significance was not reached. In subjects with and without subclinical atherosclerosis the following smoking habits were noted: smokers 68.8% vs. 55.2%, duration of smoking 22±8.8 vs. 19.4±8.2 years, cigarettes per day 20±7.7 vs. 15.4±8.2. In subjects with and without atherosclerotic plaques parameters regarding smoking were as follows: smokers 66.7% vs. 55.9%, duration of smoking 22±9.1 vs. 19.6±8.2 years, cigarettes per day 20±9.4 vs. 15.6±7.9. Univariate regression analysis did not reveal significant association of subclinical atherosclerosis/plaques and smoking habits. Only duration of smoking was close to significant association with subclinical atherosclerosis (β 0.182, p = 0.060).

Table 1. Univariate and multivariate logistic regression analysis of association between traditional or RA related cardiovascular risk factors with the presence of subclinical atherosclerosis (mean IMT+2SD of the controls) or atherosclerotic plaque (IMT>1.5 mm) in the entire study group and patients with rheumatoid arthritis.

|                  | All participants | Patients with RA |
|------------------|------------------|------------------|
|                  | With subclinical atherosclerosis N = 16/74 | With plaque N = 15/74 | With subclinical atherosclerosis N = 15/42 | With plaque N = 12/42 |
| Age (years)      | 0.145 a 0.003 | 0.152 a 0.003 | 0.202 | 0.002 | 0.154 a 0.008 |
| ESR (mm/h)       | 0.045 0.005 | 0.038 0.010 | 0.027 ns | 0.002 | 0.030 ns |
| Fibrinogen (g/l) | 1.006 0.005 | 1.061 0.004 | 0.666 ns | 0.002 | 0.852 0.041 |
| RF positive (%)  | 2.185 0.002 | 1.213 0.047 | 1.497 ns | 0.002 | 1.063 ns |
| vWF (% activity) | 1.290 0.026 | 0.897 ns | 1.535 0.045 | 0.263 ns |
| Smoking habits (cigarettes/day) | 0.065 ns | 0.062 ns | 0.181 0.037 | 0.072 ns |
| Rheumatoid arthritis presence | 2.846 0.008 | 1.352 0.049 | NA | NA |

β—regression coefficient in the univariate analysis, NA—not applicable, vWF—Von Willebrand factor, RF—rheumatoid factor.

Also significant in multiple regression analysis, which included parameters showing significant difference in the univariate analysis (p<0.01 for all, except for smoking habits and subclinical atherosclerosis, where p<0.05)
In the univariate analysis of the impact of smoking habits on vWF activity, statistical significance was obtained only for the duration of smoking ($\beta = 0.026, p = 0.039$). There was no significant difference in vWF activity between smokers (30/74) and non-smokers (44/74): 106±53 vs. 102±45, $p = 0.895$. Importantly, vWF activity was significantly higher in smokers with subclinical atherosclerosis (6/30) than in those without (24/30) (166±82 vs. 91±31, $p = 0.006$). RA patients with subclinical atherosclerosis, compared to those without, were older (52.2±5.4 vs. 40.9±9.3 years) and have smoked more cigarettes/day (20±7.7 vs. 12.6±6.4) ($p < 0.01$ for both), as confirmed by univariate analysis (Table 1). Multivariate analysis revealed that only smoking ($p < 0.05$) was an independent risk factor for subclinical atherosclerosis. RA patients with atherosclerotic plaques, compared to those without, were older (52.7±6.1 vs. 42.4±9.7 years) and had a higher fibrinogen (3.9±0.9 vs. 3.2±0.9) ($p < 0.05$ for both), as verified in the univariate analysis. Multivariate analysis demonstrated that only age had predictive value for plaque ($p < 0.01$).

Patients with subclinical atherosclerosis were more frequently RF positive but this was not statistically significant (86.7% vs. 59.3%, $p = 0.065$) and there was no predictive value in simple regression model ($p = 0.079$). Patients without atherosclerosis had longer RA duration than those with atherosclerosis (8.2±5.8 vs. 5.2±4.2 years, $p = 0.085$), but duration of their combined methotrexate/chloroquine therapy was more than twice longer (3.0±1.9 vs. 1.4±0.5 years, $p = 0.065$). In regression model a negative association was found between atherosclerosis and duration of this therapy ($p = 0.069$).

In the RA group, patients with subclinical atherosclerosis had significantly higher vWF activity compared to those without (134±69 vs. 95±37, $p = 0.024$) and this association was validated by the logistic regression. Patients with atherosclerotic plaques had higher vWF activity but the difference was not significant. No significant difference between the groups was present regarding D-dimer and PAI-1 levels.

Correlation of von Willebrand Factor Activity and Other Haemostatic Factors with Clinical, Laboratory Features, and Anti-Rheumatic Treatment in RA Patients

There was a significant correlation of vWF activity with age, duration of smoking, number of cigarettes/day, markers of inflammation, RF concentration and anti-CCP antibodies, and all parameters of RA activity (Table 2). However, a negative correlation of vWF activity with anti-inflammatory treatment was non-significant. Fibrinogen correlated with disease activity, other markers of inflammation, and RF concentration. D-dimer correlated well with markers of inflammation and disease activity.

Discussion

Our study demonstrated significant association of vWF activity and the presence of subclinical atherosclerosis in young, non-diabetic, normotensive, female RA patients, with no dyslipidemia. We have also shown its significant correlation with inflammation markers, all parameters of disease activity, RF concentration, and anti-CCP antibodies.

Only few studies assessed relationship between haemostatic factors and subclinical atherosclerosis in RA [8–12] and two of them analysed RA patients without atherosclerotic risk factors [11, 12]. Our results are consistent with the report by Daza et al. [11] who evaluated very similar RA group. Södergren et al. [10] revealed significant correlation between CCA-IMT and vWF activity in RA patients and in multiple regression analysis vWF activity was the best predictor for increased IMT. However, their study group was not free from confounding atherosclerotic risk factors.
The major pitfalls in interpreting vWF activity impact on cardiovascular risk arise from the fact that its levels are strongly influenced by age. Poor arterial compliance associated with aging causes increased endothelial vWF secretion which then contributes to the advanced cardiovascular risk [17]. We also found highly significant correlation of vWF activity with age in RA patients, which was probably the reason for the loss of independent predictive value of vWF activity for subclinical atherosclerosis in multiple regression models. Recently, Fan et al. [18] reported significant correlation of vWF with arterial stiffness (assessed by pulse wave velocity) that also disappeared after adjustment for age. Although arterial stiffness represents potentially reversible atherosclerotic changes, while increased carotid IMT and plaque signify irreversible lesions, both are considered surrogate markers of atherosclerosis [16, 19, 20]. According to these results, vWF might be important in very early stages of atherosclerosis, as well as in the later subclinical stage with increased IMT and formation of plaques. Both stages are facilitated by chronic inflammation in RA.

We obtained significant positive correlation of vWF activity with ESR, fibrinogen, and all parameters of disease activity. It was previously demonstrated that several inflammatory cytokines, including tumour necrosis factor (TNF)-α, interleukin (IL)-8, and IL-6 stimulate vWF release from endothelial cells in a dose-dependent manner [21]. Moreover, TNF-α and IL-6 are the key inflammatory cytokines in RA, which could explain correlation between vWF and RA activity. Due to its association with inflammation vWF may be considered as acute phase protein and pathophysiological link between RA activity and endothelial damage.

The association of vWF with inflammation was also reflected by the negative correlation of vWF activity with duration of anti-inflammatory therapy. However, these correlations were not statistically significant, probably due to the cohort size.

Table 2. Correlation of haemostatic factors with clinical, laboratory features, and treatment in patients with rheumatoid arthritis.

| Clinical and laboratory features of patients with rheumatoid arthritis | vWF     | Fibrinogen | D-dimer | PAI-1 |
|---------------------------------------------------------------|---------|------------|---------|-------|
| Age (years)                                                   | 0.490***| 0.272      | 0.023   | 0.056 |
| Smoking (years)                                               | 0.581*  | 0.280      | -0.009  | -0.010|
| Smoking (cigarettes/day)                                      | 0.536*  | 0.123      | -0.545* | 0.407 |
| Erythrocyte sedimentation rate (mm/h)                         | 0.498***| 0.751***   | 0.376*  | 0.109 |
| Fibrinogen (g/l)                                              | 0.466** | /          | 0.124   | 0.009 |
| C reactive protein (mg/l)                                     | 0.247   | 0.636***   | 0.463** | 0.193 |
| Rheumatoid factor (IU/ml)                                     | 0.421*  | 0.440*     | 0.162   | 0.100 |
| Anti-cyclic citrullinated peptide antibodies (IU/ml)          | 0.586** | 0.146      | 0.148   | 0.084 |
| Visual analogue scale general health patient (mm)             | 0.439** | 0.465 **   | 0.215   | 0.107 |
| No of swollen joints, 28 assessed                             | 0.365*  | 0.523 ***  | 0.245   | 0.242 |
| No of tender joints, 28 assessed                              | 0.476***| 0.527 ***  | 0.271   | 0.137 |
| Modified disease activity score (mDAS28-ESR)                  | 0.552***| 0.778***   | 0.377*  | 0.007 |
| Modified disease activity score (mDAS28-CRP)                  | 0.446** | 0.672***   | 0.418** | 0.009 |
| Modified Health Assessment Questionnaire                      | 0.406** | 0.450**    | 0.300   | 0.114 |
| Duration of methotrexate/chloroquine combined therapy (years) | -0.18   | -0.19      | 0.01    | 0.06  |
| Methotrexate therapy—average weekly dose (mg/week)            | -0.32   | 0.06       | 0.14    | -0.11 |

All values are Spearman's correlation coefficients.
*p<0.05;  ** p<0.01;  *** p<0.001  
vWF—von Willebrand factor, PAI—plasminogen activator inhibitor.

doi:10.1371/journal.pone.0130462.t002
In contrast to our study, Södergren et al. found significant relation between disease activity, tPA, and PAI-1 mass, but not with vWF activity [10]. However, their study group was not free from traditional atherosclerotic risk factors.

Wållberg-Johnson et al. also found correlation of vWF, D-dimer, and PAI-1 with ESR, while PAI-1 and D-dimer correlated with accumulated disease activity [22]. Foster et al. demonstrated significantly higher levels of vWF in RA group, but revealed no correlation between vWF and inflammation or disease activity [23, 24]. Yet, both studies used vWF concentration instead its activity and included elderly patients with cardiovascular risk factors.

However, Veselinovic et al. [12] were not able to confirm predictive value of vWF activity for IMT changes over time, although RA patients had significantly higher vWF activity in comparison with controls at initial and repeated measurements. Paradoxically, predictive value for differences in IMT over time was also not shown for age and lipid parameters. These findings could be explained by a complex interplay between traditional risk factors, inflammation-mediated metabolic, atherothrombogenic processes, and anti-inflammatory therapy, which all have influence on atherosclerotic process in RA patients.

Unexpectedly, we found significant correlation of vWF activity with RF concentration as well as with anti-CCP antibodies. Tomasson et al. [25] have revealed that seropositivity was associated with increased cardiovascular mortality even in general population without joint symptoms. In RA patients, seropositivity is frequently associated with extra-articular manifestations including rheumatoid vasculitis. Importantly, these patients have significantly higher plasma levels of vWF than patients without vasculitis and the normal subjects.

Among traditional atherosclerotic risk factors in the entire study population there was no significant difference between the groups with and without subclinical atherosclerosis/plaque, regarding lipids, blood glucose levels, and smoking habits, although all smoking parameters were higher in participants with atherosclerosis/plaque. In the univariate analysis smoking duration was the most relevant factor significantly associated with vWF activity. This result is in concordance with the previous reports that smoking increased levels of vWF [26, 27] while smoking cessation has an opposite effect [28]. There was no significant difference in vWF activity between smokers and non-smokers. However, vWF activity was significantly higher in smokers with subclinical atherosclerosis, than in those without. This finding could be a consequence of a cumulative effect of chronic inflammation and smoking that potentiate each other in advancing atherosclerosis. Importantly, in RA patients we found that smoking duration and number of cigarettes/day significantly correlate with vWF activity. On the other hand, for subclinical atherosclerosis smoking even outweighs the importance of age in multivariate analyses. Among studies that included smokers [9, 22, 29, 30] only two reported significant impact of smoking [10, 31].

Among RA-related atherosclerotic risk factors, seropositivity and disease duration of more than 10 years are considered the most important [32]. In our study, patients with subclinical atherosclerosis were more often RF positive than those without, but its predictive value was not significant. Association with disease duration was also not confirmed. Patients without subclinical atherosclerosis even had RA longer than those with atherosclerosis, but this group had more than twice longer duration of combined methotrexate/chloroquine therapy. Importantly, the number of patients on combined therapy was higher in group with normal IMT values. Accordingly, we found negative association between the presence of subclinical atherosclerosis and duration of this therapy, but statistical significance was not reached. Our results are consistent with previous studies in which methotrexate treatment decreased IMT [9, 13, 33], confirming the importance of inflammatory burden for accelerated atherosclerosis in RA [34]. Sustained control of inflammation and reduced disease activity was demonstrated to also reduce the risk for cardiovascular events in a recent large-scale prospective study [35, 36].
Conclusions

Significant association of vWF activity and subclinical atherosclerosis in RA patients with low cardiovascular risk as well as its correlation with inflammation markers and disease activity implicates a pathophysiological link between RA and endothelial damage. Correlation of vWF and RF may be a manifestation of a process contributing to the accelerated atherosclerosis in RA. Therefore, vWF activity might be a valuable marker of early atherosclerosis in RA patients who may benefit from prevention strategies.

Supporting Information

S1 Table. Database underlying the findings in the manuscript.
(XLSX)

Acknowledgments

The authors would like to express their gratitude to the hospital staff of the Military Medical Academy in Belgrade that volunteered as participants of the control group, as well as to the nurses and laboratory technicians that kindly assisted in the study protocol.

Author Contributions

Conceived and designed the experiments: GGR BG MP DZS. Performed the experiments: GGR VS TL. Analyzed the data: GGR DS ADR. Contributed reagents/materials/analysis tools: VS DS. Wrote the paper: GGR VS TL DS BG ADR MP DZS.

References

1. Avina-Zubieta JA, Thomas J, Sadatsafavi M, Lehman AJ, Lacaille D. Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis. 2012; 71: 1524–9. doi:10.1136/annrheumdis-2011-200726 PMID: 22425941

2. Corrales A, González-Juanatey C, Peiró ME, Blanco R, Llorca J, González-Gay MA. Carotid ultrasound is useful for the cardiovascular risk stratification of patients with rheumatoid arthritis: results of a population-based study. Ann Rheum Dis. 2014; 73: 722–7. doi:10.1136/annrheumdis-2012-203101 PMID: 23905241

3. Martín-Martínez MA, González-Juanatey C, Castañeda S, Llorca J, Ferraz-Amaro, Fernández-Gutiérrez B, et al. Recommendations for the management of cardiovascular risk in patients with rheumatoid arthritis: Scientific evidence and expert opinion. Semin Arthritis Rheum. 2014; 44: 1–8. doi: 10.1016/j.semarthrit.2014.01.002 PMID: 24560170

4. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how “high-grade” systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003; 108: 2957–63. PMID: 14676136

5. Vischer M. von Willebrand factor, endothelial dysfunction, and cardiovascular disease. J Thromb Haemost. 2006; 4: 1186–93. PMID: 16709957

6. Van Schie MC, Van Loon JE, De Maat MP, Leebeek FW. Genetic determinants of von Willebrand factor levels and activity in relation to the risk of cardiovascular disease: a review. J Thromb Haemost. 2011; 9: 899–908. doi: 10.1111/j.1538-7836.2011.04243.x PMID: 21342431

7. Páramo J, Beloqui O, Colina I, Diez J, Orbe J. Independent association of von Willebrand factor with surrogate markers of atherosclerosis in middle-aged asymptomatic subjects. J Thromb Haemost. 2005; 3: 662–4. PMID: 15842351

8. Jonsson SW, Backman C, Johnson O, Karp K, Lundström E, Sundqvist KG, et al. Increased prevalence of atherosclerosis in patients with medium term rheumatoid arthritis. J Rheumatol. 2001; 28: 2597–602. PMID: 11764203

9. Wällberg-Jonsson S, Ohman MI, Rantapaa-Dahlqvist S. Which factors are related to the presence of atherosclerosis in rheumatoid arthritis? Scand J Rheum. 2004; 33: 373–9. PMID: 15794194

10. Södergren A, Karp K, Boman K, Eriksson C, Lundström E, Smedby T, et al. Atherosclerosis in early rheumatoid arthritis: very early endothelial activation and rapid progression of intima media thickness. Arthritis Res Ther. 2010; 12: R158. doi: 10.1186/ar3116 PMID: 20712865
11. Daza L, Aguirre M, Jimenez M, Herrera R, Herrera R, Bollain JJ. Common carotid intima-media thickness and von Willebrand factor serum levels in rheumatoid arthritis female patients without cardiovascular risk factors. Clin Rheumatol. 2007; 26: 533–7.

12. Veselinovic M, Jakovljevic V, Jurisic-Skevin A, Toncev S, Djuric DM. Carotid enlargement and serum levels of von Willebrand factor in rheumatoid arthritis: a follow-up study. Clin Rheumatol. 2012; 31: 1727–32. doi: 10.1007/s10067-012-2079-0 PMID: 22960771

13. Ristic GG, Lepic T, Glicic B, Stanisavljevic D, Vojvodic D, Petronijevic M, et al. Rheumatoid arthritis is an independent risk factor for increased carotid intima-media thickness: impact of anti-inflammatory treatment. Rheumatology (Oxford). 2010; 49: 1076–81.

14. Veyradier A, Fressinaud E, Meyer D. Laboratory diagnosis of von Willebrand disease. Int J Clin Lab Res. 1998; 28: 201–10. PMID: 9879492

15. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, et al. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke. 1995; 26: 386–91. PMID: 7886711

16. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. American Society of Echocardiography Carotid Intima-Media Thickness Task Force: Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr. 2008; 21: 93–111. doi: 10.1016/j.echo.2007.11.011 PMID: 18261694

17. Vischer UM, Herrmann FR, Peyrard T, Nzietchueng R, Benetos A. Plasma von Willebrand factor and arterial aging. J Thromb Haemost. 2005; 3: 794–5. PMID: 15842373

18. Fan F, Galvin A, Fang L, White DA, Moore XL, Sparrow M, et al. Comparison of inflammation, arterial stiffness and traditional cardiovascular risk factors between rheumatoid arthritis and inflammatory bowel disease. J Inflamm (Lond). 2014; 11:29.

19. Yamashina A, Tomiyama H, Ariai T, Hirose K, Koji Y, Hirayama Y, et al. Brachial-ankle pulse wave velocity as a marker of atherosclerotic vascular damage and cardiovascular risk. Hypertens Res. 2003; 26:615–622. PMID: 14567500

20. De Groot E, Hovingh GK, Wiegman A, Duriez P, Smit AJ, Fruchart JC, et al. Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. Circulation. 2004; 109: I113–38. PMID: 15198964

21. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. Blood. 2004; 104: 100–6. PMID: 15026315

22. Wallberg-Jonsson S, Cvetkovic JT, Sundqvist KG, Sundqvist KG, Lefvert AK, Rantapää-Dahlqvist S. Activation of the immune system and inflammatory activity in relation to markers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis. J Rheumatol. 2002; 29: 875–82. PMID: 12022343

23. Foster W, Carruthers D, Lip GY, Blann AD. Relationships between endothelial, inflammatory and angiogenesis markers in RA: implications for cardiovascular pathophysiology. Thromb Res. 2009; 123: 659–64. doi: 10.1016/j.thromres.2008.06.014 PMID: 18692223

24. Foster W, Carruthers D, Lip GY, Blann AD. Inflammatory cytokines, endothelial markers and adhesion molecules in RA: effect of intensive anti-inflammatory treatment. J Thromb Thrombolysis. 2010; 29: 437–42. doi: 10.1007/s11239-009-0370-y PMID: 19578810

25. Tomasson G, Aspelund T, Jonsson T, Valdimarsson H, Felson DT, Gudnason V. Effect of rheumatoid factor on mortality and coronary heart disease. Ann Rheum Dis. 2010; 69: 1649–5. doi: 10.1136/ard.2009.10536 PMID: 19628821

26. Kumari M, Marmot M, Brunner E. Social determinants of von Willebrand factor—the Whitehall II Study. Arterioscler Thromb Vasc Biol. 2000; 20: 1842–7. PMID: 10894827

27. Prisco D, Fedi T, Brunelli L, Chiarugi L, Lombardi A, Gianni R, et al. The influence of smoking on von Willebrand factor is already manifesting in healthy adolescent females: the Floren-Teen (Florence Teenager) study. Int J Clin Lab Res. 1999; 29: 150–4. PMID: 10784376

28. Caponnetto P, Russo C, Di Maria A, Morjaria JB, Barton S, Guarino F, et al. Circulating endothelial-coagulative activation markers after smoking cessation: a 12-month observational study. Eur J Clin Invest. 2011; 41: 616–26. doi: 10.1111/j.1365-2362.2010.02449.x PMID: 21198559

29. Grover S, Sinha RP, Singh U, Tewari S, Aggarwal A, Misra R. Subclinical atherosclerosis in rheumatoid arthritis in India. J Rheumatol. 2006; 33: 244–7. PMID: 16465564

30. Gonzalez-Juanatey C, Llorca J, Testa A, Revuelta J, Garcia-Porrua C, Gonzalez-Gay MA. Increased prevalence of severe subclinical atherosclerotic findings in long-term treated rheumatoid arthritis patients without clinically evident atherosclerotic disease. Medicine (Baltimore). 2003; 82: 407–13.
31. Gerli R, Sherer Y, Vaudo G, Schiliaci G, Gilburd B, Giordano A, et al. Early atherosclerosis in rheumatoid arthritis: effects of smoking on thickness of the carotid artery intima media. Ann N Y Acad Sci. 2005; 1051: 281–90. PMID: 16126969

32. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. Ann Rheum Dis. 2010; 69: 325–31. doi: 10.1136/ard.2009.113696 PMID: 19773290

33. Westlake SL, Colebatch AN, Baird J, Kiely P, Quinn M, Choy E, et al. The effect of methotrexate on cardiovascular disease in patients with rheumatoid arthritis: a systematic literature review. Rheumatology (Oxford). 2010; 49: 295–307.

34. Choy E, Ganeshalingam K, Semb AG, Szekanecz Z, Nurmohamed M. Cardiovascular risk in rheumatoid arthritis: recent advances in the understanding of the pivotal role of inflammation, risk predictors and the impact of treatment. Rheumatology (Oxford). 2014; 53: 2143–54.

35. Solomon DH, Reed G, Kremer JM, Curtis JR, Farkouh ME, Harrold LR, et al. Disease activity in rheumatoid arthritis and the risk of cardiovascular events. Arthritis Rheumatol. 2015; 67: 1449–55. doi: 10.1002/art.39098 PMID: 25776112

36. Nurmohamed MT. Treat to target in rheumatoid arthritis: Good for the joints but also for the heart? Arthritis Rheumatol. 2015; 67: 1412–5. doi: 10.1002/art.39096 PMID: 25779912