Angiopoietin-2 serum levels correlate with severity, early onset and cardiovascular disease in patients with rheumatoid arthritis

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Abstract

Objectives

Rheumatoid arthritis (RA) is an inflammatory disease associated with accelerated atherosclerosis and high risk of cardiovascular (CV) disease. Angiopoietin-2 (Angpt-2), a marker of endothelial cell activation, has been proposed as a mediator of angiogenesis, which might play an important role in the regulation of endothelial integrity and inflammation. Therefore, the aim of this study was to determine whether Angpt-2 is related to severity and CV disease in RA patients.

Methods

Angpt-2 serum levels were measured by enzyme linked immunosorbent assay (ELISA) in 290 patients with RA. A control group of 100 individuals frequency matched by age and sex and classic CV risk factors and CV disease was also assessed.

Results

Eighty-four patients with RA (28.9%) had experienced CV events. Also, extra-articular manifestations were present in 41 (14%) of these patients. Although there were not significant differences between patients and controls, a correlation between age at the time of disease onset and Angpt-2 was observed in RA patients (r=-0.31; p=0.02). Angpt-2 serum levels also correlated positively with extra-articular disease (mean±standard deviation in RA patients with and without extra-articular manifestations were 2476±1716 pg/ml and 1897±1228 pg/ml, respectively; p=0.01). Moreover, after adjustment for sex, age at RA diagnosis and CV risk factors, Angpt-2 levels were higher in RA patients with CV disease than in RA patients without CV complications (2472±1826 pg/ml vs. 1875±1101 pg/ml; p=0.05). Angpt-2 serum levels remained significantly higher in RA patients with CV disease compared to those without CV disease after additional adjustment for extra-articular manifestations (p=0.04).

Conclusion

Our results show that Angpt-2 serum levels correlate with disease severity, early onset and CV disease in RA patients.

Key words

Angiopoietin-2 (Angpt-2), atherosclerosis, cardiovascular disease, inflammation, rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a complex autoimmune disease characterised by persistent synovitis, joint damage and disability (1). Up to 30% of RA patients with this pathology can be affected by extra-articular manifestations and many of them are related to more active and severe disease (2).

RA is also associated with accelerated atherosclerosis, which is the main cause of increased cardiovascular (CV) morbidity and mortality in patients with this disease (3). Besides traditional CV risk factors (4), recent studies have revealed that genetic factors play a pivotal role in the susceptibility to develop accelerated atherosclerosis in RA patients. In this context, associations of CV mortality with several gene polymorphisms located inside (5-7) and outside (8-13) the HLA region have been analysed. However, it is well known that the chronic systemic inflammatory state present in RA patients is critical for the development of the accelerated atherogenesis (6), a process that can be further reversed by cytokine inhibition (14, 15). The inflammatory response observed in RA is often accompanied by imbalanced angiogenesis. In fact, an interaction between proinflammatory and angiogenic agents at the site of chronic inflammation has been suggested (16). During the last years, much effort has been put into trying to discover new markers, which could identify RA patients at risk of CV disease.

In this context, Angiopoietin 2 (Angpt-2) could be a potential new marker for the diagnosis of CV disease in RA. Angpt-2 belongs to the Angiopoietins family, which are required for the formation of blood vessels. Specifically, these proteins bind to their receptor (Angiopoietin receptor tyrosine kinase-Tie2) and participate in the communication of endothelial cells with the surrounding mesenchyme to establish stable cellular interactions (17). Several studies have postulated that these proteins, as well as the Vascular Endothelial Growth Factor (VEGF), are mediators of angiogenesis and it has been described that the balance of these growth factors may play an important role in the regulation of endothelial integrity and inflammation (18), and facilitate the endothelial cell migration and proliferation (19, 20). This protein causes vascular destabilisation, thereby rendering the endothelium responsive to stimulation by inflammatory and angiogenic cytokines (21, 22). Recently, Angpt-2 levels have been associated with autoimmune pathologies such as Crohn's disease (17). In this context, Angpt-2 levels have been correlated with RA activity and they have also been described as a potential predictive marker for CV disease in recent-onset RA (16). Therefore, we hypothesise that the increased CV mortality observed in RA patients due to accelerated atherogenesis (3, 6) might be induced by increased endothelial activation (23).

Taking these considerations into account, the aim of this study was to investigate whether Angpt-2, a marker of endothelial cell activation, is related to the presence of CV disease in RA patients.

Patients and methods

Patients and study protocol

A set of 290 Caucasian Spanish patients with a diagnosis of RA recruited from Hospital Universitario Marqués de Valdecilla (Santander, Cantabria, Spain) were included in the present study. All the patients fulfilled the 1987 American College of Rheumatology (ACR) classification criteria for RA (24) and they were all assessed for Angpt-2 serum levels. Moreover, Angpt-2 serum levels were also assessed in a control group of 100 individuals from the Cantabria region that were frequency matched by age, sex, classic CV risk factors, prevalence of CV disease and ethnicity. Controls had no family history of RA, polymyalgia rheumatica, psoriatic arthritis, or any connective tissue disease. Controls were community based. They were recruited from family physician health centres of the Cantabria region.

A subject's written consent was obtained in all the cases and the study was approved by the local ethics committee.

Clinical evaluations

Definitions of CV events and classic

CV risk factors were established as previously described (6, 7). However, for the present study, hypertension was defined by using the conventional cut-off value of blood pressure ≥140/90 mmHg (or if they had been diagnosed as having hypertension by their family physicians). The disease activity score (DAS28) (25) was also calculated. The Systematic Coronary Risk Evaluation (SCORE) and the modified EULAR SCORE (mSCORE) were evaluated. The mSCORE was obtained by the application of a multiplier factor of 1.5 in those patients with two of the following three criteria: disease duration >10 years, rheumatoid factor (RF) or anti-cyclic citrullinated protein/peptide antibodies (αCCP) positivity, and presence of certain extra-articular manifestations (26).

Serology and biochemistry

Data on C-reactive protein (CRP) measured by latex immunoturbidity, erythrocyte sedimentation rate (ESR) measured by Westergren, serum glucose, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and triglycerides (in all cases fasting overnight determinations) performed at the time of the study were assessed. RF measured by nephelometry and αCCP levels measured by enzyme-linked immunosorbent assay (ELISA) were also evaluated.

Angpt-2 ELISA

Angpt-2 serum levels were determined by commercially available ELISA ("Angiopoietin 2 Human ELISA Kit", Abcam-AB99971; assay sensitivity=10 pg/ml; intra-and interassay coefficients of variation were <10% and <12%, respectively) (Human Immunoassay Quantikine, R&D Systems, Cambridge, UK), according to the manufacturer's instructions.

Statistical analysis

The results were displayed as mean and standard deviation (SD), median and interquartile range (IQR) or absolute frequency and percentage. The reltionship between Angpt-2 and continuous variables was tested using partial correlation, adjusting for the remaining continuous variables in the model. The association between binary variables and Angpt-2 levels was tested using Student *t*-test with Welch correction for unequal variances when needed. Comparison of means was adjusted for sex, age at RA diagnosis, classic CV risk factors and extra-articular manifestations using analysis of covariance (ANCOVA). Statistical significance was defined as $p \leq 0.05$. Finally, we performed logistic regression models in order to identify the relationship between classic CV risk factors and CV events in patients with very high Angpt-2 levels (included in the fourth quartile); results from these models were displayed as OR.

Table I. Demographic, clinical and laboratory data of 290 patients with RA and controls.

Variable	Patients[¶] n=290	Controls[¶] n=100
Mean age (years) ±SD		
At the time of study	62.5 ± 13.9	63.4 ± 12.4
At the time of disease onset	50.1 ± 16.0	
Duration of disease prior to the study visit	12 ± 8.3	
Percentage of women, n (%)	220 (76)	76 (76)
Rheumatoid factor positive, n (%)	160 (55)	
Anti-CCP antibodies positive, n (%)	125 (43)	
History of classic CV risk factors, n (%)		
Hypertension	95 (33)	32 (32)
Dyslipidemia	88 (30)	31 (31)
Obesity	60 (21)	23 (23)
Current smokers	95 (33)	30 (30)
Diabetes mellitus	10 (3)	5 (5)
Prevalence of CV disease (patients, n [%])		
Ischaemic heart disease	29 (10)	9 (9)
Heart failure	28 (10)	8 (8)
Cerebrovascular accident	18 (6)	6 (6)
Peripheral arteriopathy	9 (3)	2 (2)
Extra-articular manifestations*, n (%)	41 (14)	
Joint erosions in hands, n (%)	62 (21)	
Joint erosions in feet, n (%)	57 (20)	
Mean CRP (mg/l) ±SD** at the time of disease diagnosis	20.4 ± 31.2	
Mean ESR (mm/1st hour) ±SD§ at the time of disease diagnosis	28.8 ± 23.3	
Mean DAS28 ±SD+	3.0 ± 1.4	
Mean blood pressure (mm Hg) ±SD+		
Systolic	135.8 ± 19.4	
Diastolic	80.3 ± 8.0	
Mean cholesterol or triglycerides (mg/dl) ±SD+		
Total cholesterol	210.8 ± 37.3	
HDL cholesterol§	62.9 ± 17.7	
LDL cholesterol§	125.9 ± 31.2	
Triglycerides	105.8 ± 53.4	

*Extra-articular manifestations of the disease (if RA patients experienced at least one of the following manifestations: nodular disease, Felty's syndrome, pulmonary fibrosis, rheumatoid vasculitis, or secondary Sjögren's syndrome).
**Normal value <5 mg/l. § Normal value <20 mm/1st hour.
SD: standard deviation; Anti-CCP: anti-cyclic citrullinated peptide antibodies; CV: cardiovascular; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS: disease activity score; HDL: high-density lipoprotein; LDL: low-density lipoprotein. + At the time of the study. ¶No significant differences in the variables assessed between patients and controls were found. §Available at the time of Angiopoietin-2 serum level analysis in 228 patients.

Results

Demographic, clinical and laboratory data of patients with RA and controls

The main clinical features of the patients and controls are shown in Table I. At the time of the study, extra-articular RA manifestations, identified using previously reported definitions (6, 27), were present in 41 (14%) of the 290 patients. Eighty-four (28.9%) of the RA patients included in the study had experienced CV events (ischaemic heart disease, heart failure, cerebrovascular accident or peripheral arteriopathy).

Table II. Relationship between Angiopoietin-2 and selected variables in patients with RA.

a) Partial correlation of serum Angiopoietin-2 with selected continuous variables

Variable	r	*p*-value
Age at the time of disease onset	-0.310	0.02
Follow up	-0.004	0.98
DAS28	0.198	0.14
CRP	0.159	0.23
ESR	-0.210	0.11
Systolic blood pressure	0.191	0.15
Diastolic blood pressure	-0.140	0.29
Total cholesterol	0.097	0.47
HDL-cholesterol	-0.140	0.30
LDL-cholesterol	-0.074	0.58
Triglycerides	-0.180	0.18

b) Mean values and standard deviations of Angiopoietin-2 according to categorical variables

Variable	Yes (mean±SD)	No (mean±SD)	*p*-value
Sex: female	2024 ± 1331	1872 ± 1106	0.27
Hypertension	1982 ± 1033	1915 ± 1261	0.72
Diabetes mellitus	2330 ± 1163	1924 ± 1199	0.46
Obesity	1713 ± 927	2000 ± 1262	0.16
Dyslipidemia	1840 ± 1056	1988 ± 1264	0.42
Smoking	2066 ± 1225	1869 ± 1183	0.28
Rheumatoid factor	1936 ± 1143	2086 ± 1517	0.36
Anti-CCP antibodies	1956 ± 1190	1896 ± 1081	0.69
Extra-articular disease	2476 ± 1716	1897 ± 1228	0.01
Joint erosions in hands	2047 ± 1104	1992 ± 1389	0.78
Joint erosions in feet	1900 ± 1138	2033 ± 1375	0.50

DAS: disease activity score; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SD: standard deviation.

Differences in Angiopoietin-2 serum levels between RA patients and controls

Angpt-2 concentrations did not differ between patients (mean±SD: 2024±1331 pg/ml; median [IQR]: 1660 [1186-2461]) and controls (mean±SD: 2041±915 pg/ml; median [IQR]: 1938 [1385-2608]) (p=0.91).

Relationships of demographic, clinical and laboratory characteristics with Angpt-2 concentrations in patients with RA

No significant correlations between Angpt-2 serum levels and CRP, ESR, DAS28, follow up, systolic or diastolic blood pressure, total cholesterol, HDL and LDL-cholesterol and triglycerides levels were found in this series of RA patients. Nevertheless, a significant negative correlation between age at the time of disease onset and Angpt-2 serum levels was observed (Angpt-2 concentration was higher in early onset RA patients; r=-0.31; p=0.02) (Table IIa).

No differences in Angpt-2 concentration between men and women, classic CV risk factors and joint erosions in hands and feet were detected. A significant correlation between Angpt-2 serum levels and extra-articular manifestations was observed (Angpt-2 concentration was higher in RA patients with extra-articular complications than in RA patients without; mean±SD: 2476±1716 pg/ml and mean±SD: 1897±1228 pg/ml respectively; p=0.01) (Table IIb).

Differences in Angpt-2 concentrations between RA patients with and without CV events

After adjustment for sex, age at RA diagnosis and traditional CV risk factors, Angpt-2 serum levels were significantly higher in RA patients with CV disease than in RA patients without CV complications (mean±SD: 2472±1826 pg/ml and mean±SD: 1875±1101 pg/ml, respectively; p=0.05) (Fig. 1). Angpt-2 serum levels still remained significantly higher in RA patients with CV disease compared to those without CV disease after additional adjustment for extra-articular manifestations (p=0.04).

To further explore the relationship between Angpt-2 levels and CV events, we aimed to identify factors associated with very high Angpt-2 levels (*i.e.* levels over the third quartile) in patients with CV events. With respect to this, current smokers and patients with extra-articular manifestations showed a higher probability of elevated Angpt-2 levels (OR=1.88), although without reaching statistical significance (data not shown).

Relationships of SCORE risk, modified EULAR SCORE (mSCORE) risk and Angpt-2 serum levels in patients with RA

In a further step, we assessed whether a correlation between Angpt-2 levels and the 10-year risk of fatal atherosclerotic CV event assessed by the SCORE evaluation may exist. For this purpose, we checked data on age, sex, smoking history, systolic blood pressure and lipids. Since the European task force proposed the use of atherogenic index instead of total cholesterol for the calculation of the mSCORE for RA, data on atherogenic index were evaluated instead of using total cholesterol. Regrettably, HDL-cholesterol was not available in some of the patients at the time of the Angpt-2 assessment. Due to this, results on mSCORE were obtained in 228 patients.

As shown in Table III and as recently reported, the use of the multiplying factor proposed by the EULAR task force to calculate the mSCORE (26) did not increase substantially the number of patients with RA that changed from the category of moderate to high or very high CV risk (28). Nevertheless, a positive trend towards an increase in Angpt-2 serum levels according to the severity of the mSCORE was observed. In this regard, RA patients with very high CV risk (mSCORE ≥10%) had much higher Angpt-2 serum levels (median [IQ range]: 2835 [2653–2891] pg/ml) than those with low risk (mSCORE <1%) (median [IQ range]: 1406 [857–2712] pg/ml). Similarly, those with high CV risk (mSCORE ≥5% and <10%) also had higher Angpt-2 serum levels (1675 [1253–2939]) than those

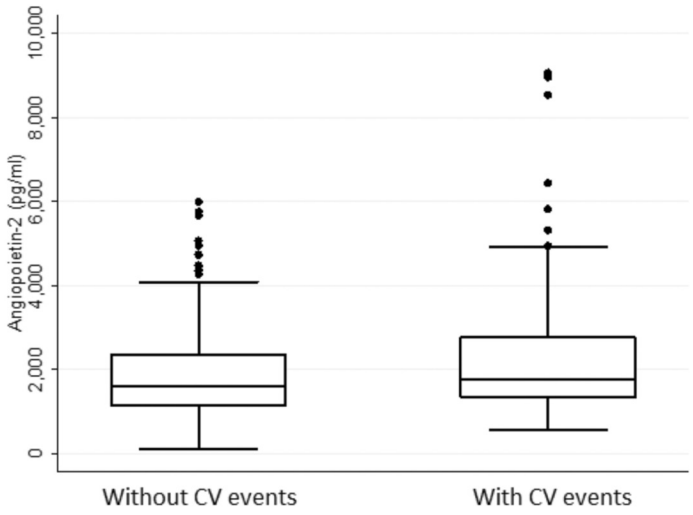

Fig. 1. Box plot showing differences in Angiopoietin-2 serum levels between RA patients with and without CV events, adjusted for sex, age at RA diagnosis and traditional CV risk factors.

Table III. SCORE risk, Modified SCORE risk*, and Angiopoietin-2 serum levels in 228 patients with rheumatoid arthritis.

Cardiovascular risk	SCORE	Modified SCORE*	
	n.	n.	Angiopoietin-2 serum levels median (IQ range)
Low (<1%)	51 (22.4)	51 (22.4)	1406 (857–2712)
Moderate (≥1% and <5%)	146 (64.0)	140 (61.4)	1630 (1228–2276)
High (≥5% and <10%)	27 (11.8)	31 (13.6)	1675 (1253–2939)
Very high (≥10%)	4 (1.8)	6 (2.6)	2835 (2653–2891)

*According to the EULAR recommendations.

with low CV risk. However, due to the relatively small number of patients with high and with very high CV risk, the distribution did not achieve statistical significance (p=0.53) (Table III).

Discussion

RA is an inflammatory autoimmune disease associated with an increased CV morbidity and mortality (3) due to the accelerated atherosclerosis process characteristic of this pathology. A better knowledge of the mechanisms associated with the increased risk of CV disease in RA is of main importance to establish predictive models of CV disorder in RA and, consequently, measures aimed at decreasing the risk of CV complications in RA patients. In fact, many recent studies focused on the search of new markers that may help to improve our understanding of the risk of CV disease in RA. In this context, Angpt-2 is critical in the inflammatory process and acts as a marker of endothelial cell activation. In the last years, this protein was associated with several autoimmune pathologies such as Crohn's disease (17) and increased levels of Angpt-2 were reported in RA patients (16). In this regard, Westra *et al*. have reported that Angpt-2 is highly correlated with disease activity and it could be predictive for CV disease in recent-onset RA (16). In keeping with these data, our study suggests that Angpt-2 serum levels are higher in RA patients with CV disease and that the concentration of this protein correlates with age at the time of disease onset (being higher in early onset RA patients). Interestingly, Takahashi *et al*. have recently found that increased leptin levels were associated with higher levels of angiogenic factors such as Angpt-2 in patients with coronary artery disease (29).

RA is not a disease just limited to the articular tissue, but a range of extra-articular features are also associated with this pathology. Up to 30% of RA patients can be affected by extra-articular manifestations and they are more severe in individuals with active disease (1, 2). In this context, our results describe a significant correlation between Angpt-2 serum levels and disease severity of RA.

Conclusion

At present, it is difficult to know whether Angpt-2 is a better marker of endothelial dysfunction than other biomarkers, such as VEGF or sVCAM-1. However, data shown in patients with early RA suggest that Angpt-2 may be more useful as a predictor of further development of CV disease in RA (16). The clinical applicability of the results shown in this study in the daily clinical practice needs further elucidation. However, we feel that in the assessment of patients with chronic inflammatory rheumatic diseases that have high CV risk, according to different guidelines such as the mSCORE, the determination of Angpt-2 levels could be of some help to further define a subgroup of patients with high risk of CV events.

In conclusion, our results suggest that Angpt-2 serum levels are markers of severity and CV disease in RA.

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