

Endothelial dysfunction and subclinical atheromatosis in gout: A Spanish multicenter cross-sectional study

Objectives: To assess subclinical vascular features in gout patients using carotid ultrasound and flow mediated vasodilation as measures of cardiovascular risk.

Methods: Cross-sectional study over a cohort of 150 patients diagnosed with gout, on whom a vascular study was performed to assess angiodynamic parameters measured by flow mediated vasodilation in brachial artery and pathological findings in carotid ultrasound: carotid intima-media thickness and atherosclerotic plaques. Classical cardiovascular risk factors were also assessed, as well as main features of gout in terms of joint involvement and inflammation parameters.

Results: 150 patients with gout were recruited. 81% of patients had endothelial dysfunction, being severe in 51%. Carotid intima-media thickness was pathological in 66% and atherosclerotic plaques were present in 38%. 73% of patients had low vitamin D levels and elevation of homocysteine was present in 38% of the sample. A negative correlation between vitamin D levels and endothelial dysfunction was obtained (OR= 0.27; CI 95% 0.09-0.83). No other features related to gout (serum uric acid levels, joint involvement, inflammatory parameters or treatments) were associated to vascular damage in this cohort.

Conclusions: Patients with gout have a great prevalence of subclinical vascular involvement, mainly endothelial dysfunction. So a correct characterization of vascular damage is important in these patients to better assessment and management of cardiovascular risk.

Keywords: gout • uric acid • cardiovascular risk • endothelial dysfunction • atherosclerotic plaques

Introduction

Gout is the most frequent cause of arthritis in developed countries. Its prevalence and incidence have increased globally in recent decades, possibly owing to changes in factors such as lifestyle and eating habits [1-3]. Damage caused by gout and hyperuricemia is not only limited to joints, and it also may affect the vascular endothelium, so several studies performed in recent years have revealed a major increase in Cardio Vascular Risk (CVR) and even in cardiovascular-related mortality in patients with gout [4-7] as occurs in other inflammatory rheumatic diseases [8-15].

Despite its leading position among the tools used to estimate CVR in rheumatic diseases such as rheumatoid arthritis, ankylosing spondylitis or systemic lupus erythematosus [16-18] in recent years, it is striking that research with vascular ultrasound in patients with gout has received little attention [19-27], especially given that the prevalence of gout is estimated to be much higher than that of other rheumatic diseases.

Nevertheless, the management of CVR in gout remains suboptimal in that the disease is

underestimated or not correctly stratified using appropriate tools [14,28]. For this reason, the objective of this study was to assess vascular hemodynamics in patients with gout to optimize the cardiovascular diagnostic-therapeutic approach and, therefore, improve morbidity and mortality.

Methods

In this cross-sectional study, we evaluated a cohort of 150 adult patients diagnosed with gout (American College of Rheumatology criteria) [29] attended in the Rheumatology Departments of a hospital group (Madrid, Spain) between May 2014 and September 2015. CVR assessment was performed according to the established protocol between Rheumatology and Vascular Surgery Units in our Center. The study was developed pursuant to the *Declaration of Helsinki principles* and informed consent was obtained from all patients.

Clinical features were collected in Rheumatologist baseline visit. We evaluated the presence of traditional Cardio Vascular Risk Factors (CVRF) such as hypertension,

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diabetes mellitus, dyslipidemia, and smoking, as well as personal and family history of Cardio Vascular Disease (CVD), i.e. acute myocardial infarction, stroke, or peripheral arterial disease. We also recorded heart rate, blood pressure, and anthropometric data. Regarding to gout, we evaluated the disease duration, treatments received, clinical joint involvement and also joint ultrasound examination (MyLab 25 Gold, by Esaote) was performed to determine the microcrystalline aggregates following the four-joint protocol proposed by Peiteado et al [30]. Blood sample were obtained to determine a series of variables: complete blood count, biochemistry for detection of liver or kidney disorders, Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), homocysteine, vitamin D, ferritin, and serum Uric Acid (sUA).

A preliminary ultrasonographic protocol was designed for the vascular study, which established the CVR variables to be studied: carotid Intima-Media Thickness (cIMT) (pathological if >0.9 mm) (Figure 1), Atherosclerotic Plaques (AP) (cIMT greater than 1.5 mm) (Figure 2), and Endothelial Dysfunction (ED) measured by Flow Mediated Vasodilation (FMD) of post-ischemic Brachial Artery (BA) (considered pathological <10% and severe <5%). Ultrasonographic measurements were performed in all patients by a vascular surgeon experienced in these techniques and blinded for patient clinical information, using a high frequency linear transducer (>7,5 MHz; Samsung US System SONOACE R7), following international recommendations [31-35]. The examination is performed in a quiet environment with dimmed light, with the patient lying down in a supine position. First, with the neck extended and slightly rotated to the opposite side of the carotid axis to be explored, a morphological examination is performed in B mode with the probe, placing it in a transverse

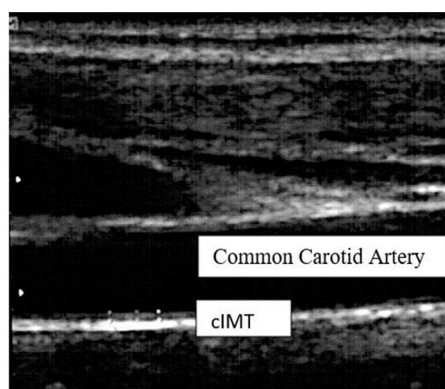


Figure 1. Ultrasonographic measurement of carotid Intima-Media Thickness (cIMT).

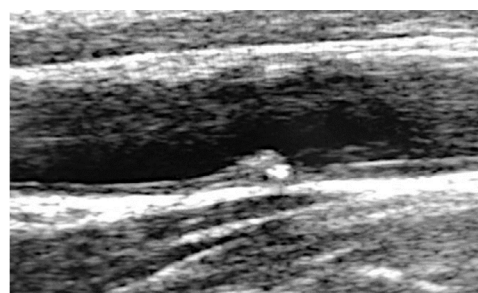


Figure 2. Evaluation of atherosclerotic carotid plaque using ultrasound imaging.

position to obtain a preliminary impression on the anatomy and possible morphological alterations. Subsequently, a longitudinal scan is performed from the clavicle to the jaw, as distally as possible. A complete visualization is performed in which the Common Carotid Artery (CCA), the carotid bifurcation, the Internal Carotid Artery (ICA) and the External Carotid Artery (ECA) are identified, locating the plaques and areas of obstruction, if any. IMT of CCA and carotid bulb is measured, describing AP morphologically, if any. IMT is measured manually on the posterior side of the CCA, in its middle segment, 1 cm from the bulb. Three measurements are taken in a 1 cm segment and the average is calculated. Subsequently, the IMT is measured in both carotid axes at the level of the bulb, on the posterior side, with a single measurement. After that, the non-invasive study of endothelial function was performed, evaluating the vasodilation response of the endothelium in the BA after a period of transient ischemia of 5 minutes. With the patient in a comfortable supine position, right upper limb extended, the scan of the BA is performed in the longitudinal plane, 4 cm proximal to the cubital fossa, marking the place of application of the probe. At baseline, blood flow is measured with pulsed Doppler and with an angle of incidence of 60°. To measure the arterial diameter, we set the image in B mode, manually taking three measurements of the intima-media interface at three nearby points and the mean is calculated. To create the stimulus of the flow in the BA we place the pressure cuff above the cubital fossa, which is inflated above the systolic pressure to occlude the artery. When the cuff is deflated, the diameter of the BA is measured again to assess the response to hyperemia. Two measurements are made: one immediately after the cuff is deflated and another about 60-75 seconds later. All measurements are made in diastole. FMD, as a percentage change from baseline measurements,

Table 1. Baseline patients characteristics and cardiovascular risk factors.

N=150	N (%)
Male gender	147 (98%)
Family history of cardiovascular disease	65 (43.3%)
Obesity (BMI>30.0)	47 (32%)
Hypertension	76 (50.6%)
Diabetes mellitus	8 (5.3%)
Smoking habit	
• Former-smoker	59 (39.3%)
• Current smoker	30 (20%)
Previous stroke	1 (0.7%)
Ischemic heart disease	
• Angina without infarction	11 (7.3%)
• Episode of acute myocardial infarction	7 (4.7%)
Thrombotic phenomena	4 (2.6%)
• Pulmonary thromboembolism	1 (0.7%)
• Lower limb thrombosis	1 (0.7%)
• Central nervous system thrombosis	1 (0.7%)
• Thrombosis in other territories	1 (0.7%)
Hyperhomocysteinemia	49 (37.7%)
Family history of gout	67 (44.6%)
Renal lithiasis caused by uric acid	17 (11.3%)
Presence of tophi	15 (10%)
Articular involvement pattern	
• Monoarticular	99 (66%)
• Polyarticular	42 (28%)
Ultrasound elementary lesions in gout	
• 1 joint	33 (26.6%)
• ≥ 2 joints	91 (73.4%)
Study of synovial fluid with PLM	92 (61.3%)
Elevated CRP levels > 5 mg/dl	18 (12.7%)
Levels of low 25-hydroxy vitamin D (<30)	96 (73.8%)
Elevated ESR > 20 mm/h	14 (10.9%)
BMI: Body Mass Index; PLM: Polarized Light Microscope; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate	

is calculated using the following formula: FMD (%) = $\frac{([Post\text{-}ischemic\ Peak\ Diameter - Baseline\ Diameter] / Baseline\ Diameter) \times 100}$.

Statistical analysis

Descriptive analysis was made using mean and Standard Deviation (SD) or median and Inter Quartile Range (IQR) for quantitative variables and frequencies for qualitative variables. The association between different vascular and clinical variables defined by the different result measures was studied using *Chi-square* test for categorical data and *t*-test for continuous variables. Significant variables from bivariate analysis were selected to assemble a multivariate logistic regression model and linearity was checked in the logistic function using a linear trend test over included continuous variables. Differences were considered significant at $p < 0.05$. Statistics software was STATA version 14. Results

Out of the 150 patients included, 98% were

middle-aged men (55.9 ± 12.7 years) and the duration of gout was 6 years (IQR 1-15).

Classic CVRF distribution was: 50.6% hypertension, 5.3 % diabetes mellitus, 32% obesity, 57.3 dyslipidemia and 59.3% ever-smokers. Regarding CVD history, one patient previously suffered stroke and 18 patients' ischemic coronary disease. Suboptimal levels of vitamin D were observed in 73% of patients, high levels of homocysteine in 38% and regarding to acute phase reactants, CRP was >5 mg/dl in 12.7% and ESR >20 mm/h in 10.9%. Mean sUA levels of 6.8 ± 1.7 mg/dl. 78% of patients received urate-lowering therapy (ULT). Table 1 shows the clinical characteristics of patients and Table 2 treatments received.

The vascular study revealed ED in 80.5% of the patients, which was severe (FMVD <5%) in 51.6%. Mean value of FMD was -0.60 ± 0.52 . A negative association (OR= 0.27; CI 95% 0.09-0.83) was found between ED and vitamin D

Table 2. Vascular hemodynamics features.	
Vascular features (N=150)	N (%)
Atherosclerotic plaques	
• Unilateral	28 (18.7%)
• Bilateral	29 (19.3%)
Atherosclerotic plaques	
• Unilateral or bilateral	57 (38%)
Pathological carotid intima-media thickness	99 (66%)
Endothelial dysfunction	120 (80.5%)
Endothelial dysfunction: categories of involvement	
• < 5%	77 (51.6%)
• 5-10%	43 (28.8%)
• > 10%	29 (19.5%)

Table 3. Treatments.	
Treatment	N (%)
Use of corticosteroids (N=150)	
• Never	117 (78%)
• Previously	25 (16.7%)
• Currently	8 (5.3%)
Doses of corticosteroids (N=33)	
• <10 mg/day	14 (42.4%)
• 10-30 mg/day	12 (36.4%)
• >30 mg/day	7 (21.2%)
Duration of treatment with corticosteroids (N=33)	
• <1 month	12 (36.4%)
• >1 month and <1 year	20 (60.6%)
• >1 year	1 (3%)
Use of NSAIDs (N=148)	
• Never	11 (7.3%)
• Previously	134 (89.3%)
• Currently	3 (1.8%)
Doses of NSAIDs (N=118)	
• Under recommended dose	6 (4%)
• Recommended dose	112 (74.7%)
Duration of treatment with NSAIDs (N=142)	
• <1 month	57 (40.7%)
• >1 month and <1 year	59 (42.1%)
• >1 year	16 (11.4%)
Use of urate-lowering treatments (N=150)	
• Never	15 (10%)
• Currently allopurinol	98 (65%)
• Currently febuxostat	20 (13.3%)
• Sometimes allopurinol	17 (11.3%)
Doses of urate-lowering treatments (N=135)	
• 100 mg/day of allopurinol	63 (46.7%)
• >100-300 mg/day of allopurinol	50 (37%)
• >300 mg/day of allopurinol	2 (1.5%)
• <80 mg/day of febuxostat	6 (4.4%)
• 80 mg/day of febuxostat	14 (10.4%)
NSAIDs: Non-Steroidal Anti-Inflammatory Drugs	

levels. This difference was statistically significant ($p=0.023$). cIMT presented pathological values in 66% and AP were observed in 38% of patients, which were bilateral in half of the cases (Table 3).

Inflammatory parameters (CRP and ESR), hyperhomocysteinemia and sUA levels were not significantly associated with any of the vascular variables studied.

Discussion

Our study provides a complete clinical, cardiovascular and laboratory characterization of patients with different degrees of gout severity by multidisciplinary cooperation. Patients were included from everyday clinical practice, so our cohort was not extent from several patients with comorbid conditions such as chronic kidney disease, established CVD or patients aged >65 despite the influence of age on vascular parameters. These features were considered as exclusion criteria in patient selection in some studies [19-21,27] but in others, as in ours, were included [22,23,25]. While our sample size could be larger, it contains the second highest number of vascular examinations at present (after *Gancheva* et al cohort).

Our findings revealed the high prevalence of subclinical cardiovascular involvement in patients with gout, as well as classic CVRF and CVD. The most relevant result of this study was the elevated percentage of ED (81% of patients), which was severe in 51.6%. The mean value of FMD was negative, thus indicating, albeit paradoxically, a vasoconstrictive response. In our patients, the only factor that presented correlation with ED was deficiency of vitamin D, no other laboratory parameter such as hyperuricemia was associated with this finding, which is in line with *Brook* et al, although several studies observed that hyperuricemia may damage endothelium and reduce arterial responsiveness leading to ED [36,37]. Other studies reported a higher rate of ED in gout, [26] not only due

to hyperuricemia, but also associated to elevated CRP in untreated patients [38]. In our study, only 12.7% of patients presented CRP levels >5 mg/dl, so it could be the reason why CRP is not associated to ED in our cohort.

Regarding cIMT, we found lower values than *Gancheva* et al, *Cukurova* et al and *Uyanik* et al, which reported comparable results. But we found a higher percentage of patients with pathological cIMT (66%) than *Andrés* et al (45.1%) and *Il'ina* et al (41.6%). In our cohort, cIMT was not related to any parameter, in contrast with the studies of *Lapkina* et al and *Andres* et al, which found a significant correlation between serum uric acid levels and cIMT. On the other side, the percentage of AP in our study was 38%, which has also heterogeneous results between different studies, *Gancheva* et al reported AP in 50% of non-tophaceous gout patients and 46.7% in tophaceous gout patients, and *Andrés* et al reported 46.5%. However, *Cukurova* et al and *Uyanik* et al reported respectively 29.5% and 28.9% of patients with AP (Table 4).

Other interesting point is the fact that 38% of patients presented hyperhomocysteinemia, which has been classically related to CVR [39], but in our study, homocysteine levels were not related to any vascular parameter.

Our results should be interpreted with caution, because this study is limited by its cross-sectional design, and no causal relationship can be established between the development of CV events in patients with gout. The absence of an age-matched control group, which is

Table 4. Analysis of associations with pathological IMT.

	Raw Estimates (Bivariate Study)		Adjusted Model (Multivariate Study)	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Serum uric acid levels	0.54 (0.27-1.08)	0.084	0.74 (0.31-1.76)	0.505
Presence of tophi	1.47 (0.44-4.86)	0.529		
CRP	1.44 (0.48-4.33)	0.508		
Waist circumference	2.02 (0.99-4.12)	0.052		
Homocysteine	0.66 (0.32-1.38)	0.277	0.46 (0.18-1.15)	0.097
25-OH-Vitamin D	1.01 (0.44-2.27)	0.99		
SCORE indicating CVR	10.79 (4.46-26.07)	<0.0001	13.02 (5.05-33.5)	<0.0001
Use of corticosteroids previous/current	0.53 (0.24-1.17)	0.119		
Use of urate-lowering treatment previous/current	1.05 (0.85-1.30)	0.651		
Ultrasound gout lesions	1.20 (0.53-2.72)	0.665		
Use of colchicine previous/current	0.46 (0.94-2.28)	0.344		
Ferritin	0.13 (0.65-2.67)	0.436		
Time since onset				
• Early (<2 years)	1	0.05		
• Established	2.02 (0.99-4.11)			

OR: Odds Ratio; CI: Confidence Interval; CRP: C-Reactive Protein; CVR: Cardio Vascular Risk

also important, mostly when studying cIMT and carotid plaques. However, this has been partially compensated by benchmarking against samples from other studies, some of which were performed in our Country [40,41]. Nevertheless, there is a prospective ongoing follow-up of patients to fill data gaps over time and determine the evolution of CV parameters in gout.

Conclusion

Vascular assessment in patients with gout enables us to establish a correct characterization of the scope of CVR. Ultrasound study provides a more comprehensive estimation of CVR, mainly due to the early identification of pathologic vascular features (ED and cIMT) which are considered the previous stage to AP. This can be useful to establish better preventive strategies and

prescribe more individualized and comprehensive treatment for these patients because up to date, CVR may be underestimated when classic tools were used.

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Disclosure of interest

Dr. Enrique Calvo Aranda has received speaking and consulting fees from: Menarini and Grünenthal labs. None of the other authors has any conflict of interest.

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