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Concise report

Evidence of subclinical atherosclerosis in eosinophilic granulomatosis with polyangiitis

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Abstract

Objectives. Patients affected by eosinophilic granulomatosis with polyangiitis (EGPA) display an increased risk of atherothrombotic events compared with the general population. An increased frequency of subclinical markers of atherosclerosis has been observed in other ANCA-associated vasculitis, but no specific study focused on EGPA. We therefore evaluated subclinical atherosclerosis in EGPA patients and in a control population.

Methods. Forty EGPA patients and 80 controls matched by age, sex and traditional cardiovascular risk factors underwent sonographic assessment of common carotid artery (CCA) intima–media thickness (IMT). The presence of plaques of the CCA was also investigated. The correlation between CCA-IMT and clinical and laboratory features was also assessed.

Results. Median CCA-IMT was significantly higher in EGPA patients compared with controls ($P = 0.002$). Also, the proportion of subjects with increased CCA-IMT and with presence of plaques was significantly higher among EGPA patients ($P < 0.001$ for both). Moreover, within the EGPA cohort, CCA-IMT tended to increase with disease duration ($P = 0.034$) and corticosteroid cumulative dose ($P = 0.004$). No significant associations were found between CCA-IMT, ANCA status, other clinical features and therapeutic regimens. Notably, the prevalence of traditional cardiovascular risk factors was comparable in patients with *vs* without an increased CCA-IMT.

Conclusion. Ultrasound markers of subclinical atherosclerosis are increased in EGPA patients as compared with controls, independently of traditional cardiovascular risk factors.

Key words: eosinophilic granulomatosis with polyangiitis, ANCA, eosinophils, vasculitis, atherosclerosis, intima–media thickness, cardiovascular risk

Rheumatology key messages

- Carotid intima–media thickness and the frequency of plaques are increased in EGPA patients.
- In EGPA ultrasound markers of atherosclerosis are increased independently from traditional cardiovascular risk factors.
- EGPA *per se* represent a risk for accelerated atherosclerosis.

Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare systemic necrotizing vasculitis characterized by adult-onset asthma, blood eosinophilia, and eosinophilic and granulomatous inflammation affecting different organs [1]. EGPA is classified among ANCA-associated vasculitis (AAV), together with granulomatosis with polyangiitis and microscopic polyangiitis [2].

Granulomatosis with polyangiitis and microscopic polyangiitis are associated with a significantly higher

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incidence of cardiovascular events compared with the general population [3]. Moreover, an increased frequency of surrogate markers of atherosclerosis, such as intima–media thickness (IMT) and presence of carotid plaques, was found in granulomatosis with polyangiitis patients compared with controls matched by age, sex and traditional cardiovascular risk factors [4]. Accordingly, patients suffering from AAV are thought to develop an 'accelerated atherosclerosis', which evolves independently from traditional cardiovascular risk factors, similarly to other chronic inflammatory diseases, such as SLE, aPL syndrome and RA [5].

We recently showed that patients with EGPA also display a significantly higher incidence not only of venous thrombotic events, but also of arterial ones, mostly in the period of higher disease activity [6]. Interestingly, accumulating evidence suggests that eosinophils participate in the atherosclerosis process, thus potentially contributing to cardiovascular disease progression in EGPA [7].

A previous study by Pacholzak *et al.* evaluated the laboratory and ultrasonographic parameters of endothelial dysfunction in EGPA patients, suggesting the presence of endothelial injury, despite no significant IMT difference being found between cases and controls [8]. However, common carotid arteries (CCA)-IMT measurement was not the primary objective of Pacholzak *et al*.'s study, and to date no specific data exists on subclinical atherosclerosis in EGPA.

On these bases, the present study aimed to evaluate ultrasound markers of subclinical atherosclerosis in a cohort of EGPA patients as compared with a reference population.

Methods

A cross-sectional study was conducted. The research study was carried out according to the Declaration of Helsinki, and the study protocol was approved by the local Ethical Committee (CEAVC).

Consecutive adult patients who met the American College of Rheumatology classification criteria for EGPA [9] or the criteria proposed in the MIRRA trial [10] were enrolled at the Vasculitis Centre of the Careggi University Hospital, Florence (Italy), between 2016 and 2021. The control group was recruited from the hospital staff of the two participating centres (Florence and Naples). Exclusion criteria for cases and controls were history of stroke or ischaemic heart disease (occurring before EGPA diagnosis for cases), ongoing malignancy or haematological disease, unstable medical conditions, or ongoing pregnancy. Controls were matched 2:1 with EGPA patients by age, gender and major traditional cardiovascular risk factors (hypertension, hypercholesterolaemia, obesity, diabetes and smoking). All participants signed a written informed consent. For both EGPA patients and controls, demographic, clinical and therapeutic data were retrospectively collected at the time of enrolment.

An ultrasound assessment of CCA-IMT was performed in cases and controls as described by Di Minno *et al.* [11]. Briefly, IMT was measured in the near and far walls of both right and left common carotid arteries (CCA) at three different positions (anterior, lateral and posterior). IMT measurement was performed at a distance of at least 5 mm below the distal end of CCA. For each subject, IMT was defined as the mean value among the three measures. Ultrasound studies were performed with an Esaote MyLab 25 Gold (Florence, Italy), using a 7.5–12 MHz linear-array transducer duplex scanner with an appropriate depth of focus and optimal frame rate, in a darkened and quiet room, after subjects rested for 5 min in a supine position. All the ultrasound examinations were performed by trained operators, specifically with 15 years (N.D.D.M.), 15 years (M.M.) and 6 years (D.M.) of ultrasound experience. The inter-operator reproducibility of the vascular measurements was previously confirmed in 20 subjects and the overall Pearson's *r*-value for the IMT measurements was 0.91 ($P < 0.001$).

IMT was defined as increased when it exceeded 0.9 mm. The presence of carotid plaques was defined as an IMT $\geq$1.3 mm [11–14].

Differences in CCA-IMT and the presence of carotid plaques were evaluated between EGPA patients and healthy controls. In the EGPA group, CCA-IMT was further stratified based on ANCA status, disease duration, cumulative steroid dose, immunosuppressive treatment, number of disease relapses, disease severity at diagnosis [assessed by the Five-Factor Score (FFS) [15]], and chronic organ damage [evaluated by the Vasculitis Damage Index (VDI) [16]]. Disease relapse was defined as the presence of signs and symptoms suggestive for active vasculitis or asthma exacerbations, leading to an increase in the glucocorticoid dose to $>$4.0 mg per day of prednisolone or equivalent, an initiation of or an increase in immunosuppressive therapy, or requiring hospitalization [10]. The association with traditional cardiovascular risk factors was also investigated.

Continuous data were expressed as median and interquartile range (IQR) and were compared using the Mann–Whitney test. Categorical variables were expressed as absolute numbers and percentages and compared by Fisher's exact test. All the results are presented as two-tailed values with statistical significance if *P*-values $<$0.05. The dataset generated during the current study is available from the corresponding author on reasonable request.

Results

Patients' characteristics

A total of 40 EGPA patients and 80 controls were included. The demographic and clinical characteristics of the two groups are summarized in Table 1. Gender distribution and median age at enrolment were comparable between the two groups. Seventeen percent and 16% of EGPA cases and controls were current or past smokers, respectively. The presence of hypertension,

Table 1 Demographic table of cases and controls and clinical features of EGPA patients

Characteristic	EGPA patients ($n = 40$)	Controls ($n = 80$)	*P*-value
Demographic characteristics			
Age at disease onset, median (IQR), years	53.0 (43.0–62.0)		
Age at sonographic IMT assessment, median (IQR), years	59 (48–71)	56.0 (45.0–69.0)	0.229
Female sex, *n* (%)	24 (60.0)	47 (58.8)	1.000
Smoking habit, *n* (%)	7 (17.5)	13 (16.3)	1.000
Comorbidities, *n* (%)			
Hypertension	20 (50.0)	35 (43.8)	0.563
Hypercholesterolaemia	17 (42.5)	34 (42.5)	1.000
Diabetes	2 (5.0)	5 (6.3)	1.000
Obesity	0	0	—
Atrial fibrillation	4 (10.0)	0	n.a.
Cancer	3 (7.5)	0	n.a.
Disease manifestations, *n* (%)			
Asthma	40 (100.0)		
ENT	33 (82.5)		
Peripheral nervous system	24 (60.0)		
Cardiac	13 (32.5)		
Gastrointestinal	8 (20.0)		
Cutaneous	8 (20.0)		
Renal	2 (5.0)		
Articular	1 (2.5)		
Ocular	1 (2.5)		
ANCA positivity, *n* (%)	18 (45.0)		
Immunosuppressive treatment at the time of IMT evaluation, *n* (%)			
Corticosteroid therapy	36 (90)		
Corticosteroid monotherapy	13 (32.5)		
Intravenous immunoglobulins	7 (17.5)		
Rituximab	7 (17.5)		
Azathioprine	5 (12.5)		
Ciclosporin	4 (10.0)		
Mycophenolate	4 (10.0)		
Methotrexate	3 (7.50)		
Omalizumab	1 (2.5)		
Cardiovascular treatment at the time of IMT evaluation, *n* (%)			
Anti-hypertensive	7 (17.5)	27 (33.7)	0.085
Statin	5 (12.5)	23 (28.7)	0.066
Oral anticoagulant	4 (10.0)	0	n.a.

EGPA: eosinophilic granulomatosis with polyangiitis; IMT: intima–media thickness; IQR: interquartile range; n.a.: not applicable.

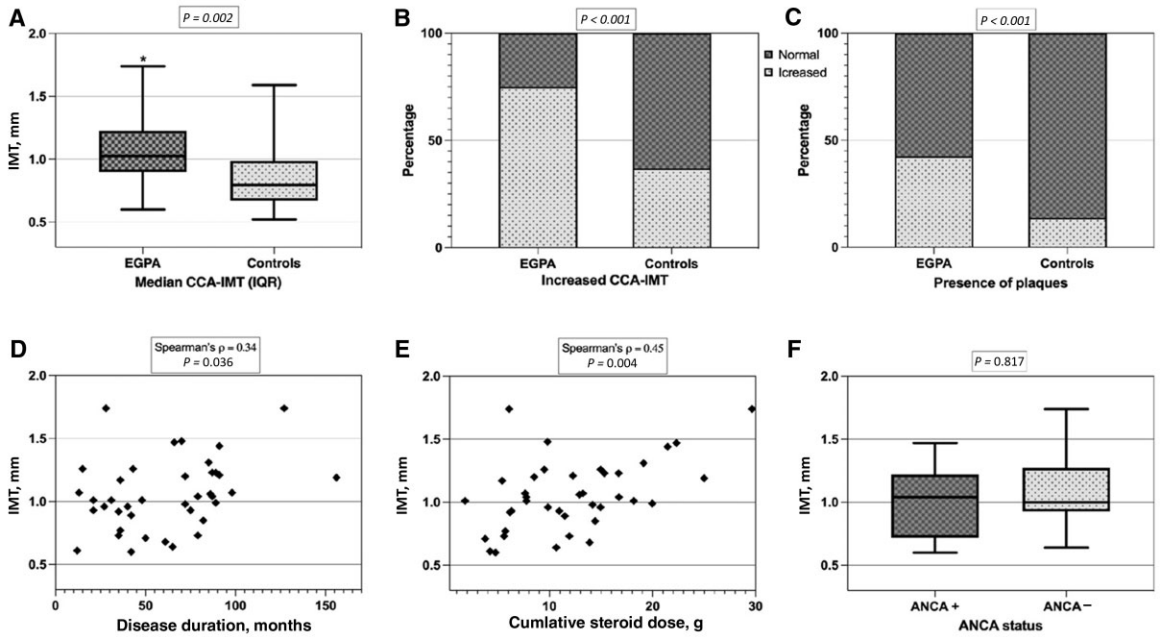
hypercholesterolaemia and diabetes was also comparable between cases and controls: hypertension was reported in 50.0% and 43.8% of EGPA cases and controls, respectively; hypercholesterolaemia in 42.5% of subjects in both groups; diabetes in 5.0% and 6.3% of subjects, respectively. A previous history of cancer was reported in three EGPA patients (7.5%), while none of the controls reported a history of malignancy.

In the EGPA cohort, all patients had asthma; other common manifestations were ENT (82.5%), peripheral nervous system (60.0%) and cardiac involvement (32.5%). ANCA positivity was found in 18 patients (45%) at diagnosis. At the time of enrolment most patients were receiving corticosteroids ($n = 36$; 90%), as monotherapy in 12 patients (30%). Sixteen patients (40%) were receiving traditional DMARDs, while 15 were receiving biologic DMARDs. As for cardiovascular therapy, at the time of CCA-IMT evaluation seven patients were receiving anti-hypertensive drugs (17.5%), five statins (12.5%), while oral anticoagulants were used in four patients (10%). No significant differences were present in terms of cardiovascular therapy among cases and controls (Table 1).

Ultrasound assessment of subclinical atherosclerosis

Median CCA-IMT was significantly greater in EGPA patients than in controls [median, 1.03 mm (IQR 0.91–1.23) *vs* 0.79 mm (IQR 0.67–0.98); $P = 0.002$] (Fig. 1A). Coherently, the proportion of subjects with increased

FIG. 1 CCA-IMT and plaque comparison between EGPA patients and controls and analysis of CCA-IMT according to EGPA clinical, therapeutic and laboratory features



(**A**) Median CCA-IMT is increased in EGPA patients in respect to controls. (**B**) The proportion of subjects with increased CCA-IMT is higher among patients than controls. (**C**) The presence of carotid plaques is more frequent among EGPA cases. (**D**, **E**) Within the EGPA cohort, CCA-IMT directly correlates with disease duration (**D**) and with corticosteroid cumulative dose (**E**). (**F**) No difference in CCA-IMT is found based on ANCA status. CCA: common carotid artery; EGPA: eosinophilic granulomatosis with polyangiitis; IMT: intima–media thickness.

CCA-IMT levels was significantly higher in EGPA patients than in controls [75% *vs* 37%; $P < 0.001$] (Fig. 1B). Moreover, carotid plaques were more frequently detected among EGPA cases [42.5% *vs* 13.75%; $P < 0.001$] (Fig. 1C).

Within the EGPA cohort, median CCA-IMT directly correlated with disease duration (Spearman's $\rho = 0.34$, $P = 0.036$) and with corticosteroid cumulative dose (Spearman's $\rho = 0.45$, $P = 0.004$) (Fig. 1D, E).

Conversely, no association was found between CCA-IMT and ANCA status [median CCA-IMT 1.05 mm (IQR 0.73–1.23) for ANCA+ *vs* 1.00 mm (IQR 0.93–1.26) for ANCA−; $P = 0.817$] (Fig. 1F).

Also, no significant differences in CCA-IMT were found with respect to immunosuppressive treatment (steroids alone *vs* DMARDs plus steroids) [median 1.02 mm (IQR 0.89–1.23) *vs* 1.04 (IQR 0.93–1.23); $P = 0.887$], disease severity at diagnosis [median 1.02 mm (IQR 0.94–1.2) for patients with FFS = 0 *vs* 1.01 mm (IQR 0.77–1.26) for patients with FFS ≥ 1; $P = 0.833$], number of disease relapses (Spearman's $\rho = 0.042$; $P = 0.802$) and VDI (Spearman's $\rho = 0.054$; $P = 0.774$).

Moreover, the proportion of patients with an increased CCA-IMT was comparable in patients with *vs* those without traditional cardiovascular risk factors (Supplementary Fig. S1, available at *Rheumatology* online).

Discussion

Atherosclerosis is an inflammatory process that begins early in life and leads to remodelling of the arterial wall. An early sign of this phenomenon is the increase in IMT, which can be assessed by high-resolution B-mode ultrasound. CCA-IMT measurements have been demonstrated to mirror the atherosclerosis process in other body sites and to correlate with the risk of cardiovascular events [17].

In the present study, we show that CCA-IMT is increased in EGPA patients compared with controls matched by age, sex and traditional cardiovascular risk factors. Furthermore, CCA-IMT positively correlates with disease duration, suggesting that disease-related or treatment-related damage might predispose to the development of accelerated atherosclerosis.

Our results are in line with those from previous studies on other AAV. In AAV the oxidative stress from ANCA-primed neutrophils is thought to be a central event in endothelial dysfunction [18]. In EGPA, eosinophils are key mediators of tissue damage and—similar to neutrophils—they might play a role in cardiovascular disease [18].

Also, higher CCA-IMT was not associated with the presence of predictors of adverse prognosis at diagnosis (expressed by the FFS), nor with a more severe course of the disease (e.g. higher number of disease relapses,

higher VDI scores at the time of examination). This might suggest that atherosclerosis is related to EGPA *per se*, independently of the disease course.

If systemic inflammation and eosinophils play a central role in the atherosclerosis process, early immunosuppression should be able to restore endothelial function and prevent vascular damage [19]. Glucocorticoids still remain the cornerstone of EGPA therapy, but they may not be curative. In our study, the cumulative steroid dose positively correlates with CCA-IMT. Indeed, while in the short-term period the anti-inflammatory effects of glucocorticoids prevail, chronic corticosteroid therapy can exacerbate the traditional risk factors, and glucocorticoid-related toxicity may emerge several years after diagnosis. Despite no differences in markers of subclinical atherosclerosis being found between patients receiving glucocorticoids alone or in combination with DMARDs, a reduction of glucocorticoid exposure is indeed advocated in long-term EGPA management. Nevertheless, the possible confounding effect of older age and longer disease duration on the correlation between cumulative steroid dose and CCA-IMT cannot be excluded.

Our study has some limitations. Firstly, although the more recent consensus on CCA-IMT measurement defines a carotid plaque as IMT $\geq$ 1.5 mm [20], the definition of a plaque is still debated; in this study, we used a cut-off of 1.3 mm, to detect an early subclinical marker of atherosclerosis. Nevertheless, the over-estimation of plaque presence cannot be excluded, and thus our data on carotid plaques need cautious interpretation. A second limitation is that it was not possible to compare the duration of traditional cardiovascular risk factors (namely hypertension, hypercholesterolaemia, diabetes, overweight and smoking) between cases and controls, due to the retrospective nature of the study.

Despite these limitations, our results suggest that EGPA might be associated with accelerated subclinical atherosclerosis, reflected by the CCA-IMT increase. Although these findings were independent from traditional cardiovascular risk factors, these must nevertheless be abated in all patients with EGPA.

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Data availability statement

The data underlying this article are available in the article and in its online supplementary material. The dataset generated during the current study is available from the corresponding author on reasonable request.

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Fagni F, Bello F, Emmi G. Eosinophilic granulomatosis with polyangiitis: dissecting the pathophysiology. Front Med (Lausanne) 2021;8:627776.

2 Jennette JC, Falk RJ, Bacon PA *et al.* 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013;65:1–11.

3 Morgan MD, Turnbull J, Selamet U *et al.* Increased incidence of cardiovascular events in patients with antineutrophil cytoplasmic antibody-associated vasculitides: a matched-pair cohort study. Arthritis Rheum 2009; 60:3493–500.

4 de Leeuw K, Sanders JS, Stegeman C *et al.* Accelerated atherosclerosis in patients with Wegener's granulomatosis. Ann Rheum Dis 2005;64:753–9.

5 Gasparyan AY, Stavropoulos-Kalinoglou A, Mikhailidis DP *et al.* The rationale for comparative studies of accelerated atherosclerosis in rheumatic diseases. Curr Vasc Pharmacol 2010;8:437–49.

6 Bettiol A, Sinico RA, Schiavon F *et al.* Risk of acute arterial and venous thromboembolic events in eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome). Eur Respir J 2021;57:2004158.

7 Xia GL, Wang YK, Huang ZQ. The correlation of serum myeloid-related protein-8/14 and eosinophil cationic protein in patients with coronary artery disease. Biomed Res Int 2016;2016:4980251.

8 Pacholczak R, Bazan-Socha S, Iwaniec T *et al.* Endothelial dysfunction in patients with eosinophilic granulomatosis with polyangiitis. Clin Rheumatol 2019; 38:417–24.

9 Masi AT, Hunder GG, Lie JT *et al.* The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum 1990;33:1094–100.

10 Wechsler ME, Akuthota P, Jayne D *et al.* Mepolizumab or placebo for eosinophilic granulomatosis with polyangiitis. N Engl J Med 2017;376:1921–32.

11 Di Minno MND, Emmi G, Ambrosino P *et al.* Impact of cardiovascular and immunologic variables on subclinical carotid atherosclerosis in subjects with anti-phospholipid antibodies. Data Brief 2018;19:1799–803.

12 Yerly P, Rodondi N, Viswanathan B *et al.* Association between conventional risk factors and different ultrasound-based markers of atherosclerosis at carotid and femoral levels in a middle-aged population. Int J Cardiovasc Imaging 2013;29:589–99.

13 Petta S, Valenti L, Marchesini G *et al.* PNPLA3 GG genotype and carotid atherosclerosis in patients with non-alcoholic fatty liver disease. PLoS One 2013;8:e74089.

14 Zhang M, Liu Y, Xu M *et al.* Carotid artery plaque intervention with Tongxinluo capsule (CAPITAL): a multicenter randomized double-blind parallel-group placebo-controlled study. Sci Rep 2019;9:4545.

15 Guillevin L, Lhote F, Gayraud M *et al.* Prognostic factors in polyarteritis nodosa and Churg-Strauss syndrome. A prospective study in 342 patients. Medicine (Baltimore) 1996;75:17–28.

16 Exley AR, Bacon PA, Luqmani RA *et al.* Development and initial validation of the Vasculitis Damage Index for the standardized clinical assessment of damage in the systemic vasculitides. Arthritis Rheum 1997;40:371–80.

17 de Groot E, van Leuven SI, Duivenvoorden R *et al.* Measurement of carotid intima-media thickness to assess progression and regression of atherosclerosis. Nat Clin Pract Cardiovasc Med 2008;5:280–8.

18 Stark K, Massberg S. Interplay between inflammation and thrombosis in cardiovascular pathology. Nat Rev Cardiol 2021;18:666–82.

19 Raza K, Thambyrajah J, Townend JN *et al.* Suppression of inflammation in primary systemic vasculitis restores vascular endothelial function: lessons for atherosclerotic disease? Circulation 2000; 102:1470–2.

20 Johri AM, Nambi V, Naqvi TZ *et al.* Recommendations for the assessment of carotid arterial plaque by ultrasound for the characterization of atherosclerosis and evaluation of cardiovascular risk: from the American Society of Echocardiography. J Am Soc Echocardiogr 2020;33: 917–33.