Nutrition, Metabolism and Cardiovascular Diseases xxx (xxxx) xxx

Contents lists available at ScienceDirect

Nutrition, Metabolism and Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Research Paper

Redox imbalance and metabolic stress in carotid atherosclerosis: Associations with symptomatology and plaque calcification

María Lourdes del Río-Solá [a,b,c,*], Sandra Pérez Fernández [b], Hugo Gonzalo-Benito [d], Rita Losa Rodríguez [e]

[a] Department of Surgery, University of Valladolid, Valladolid, Spain
[b] Department of Vascular Surgery, University Clinical Hospital of Valladolid, Valladolid, Spain
[c] Universidad Europea Miguel de Cervantes, Valladolid, Spain
[d] Biomedical Research Center, University Clinical Hospital of Valladolid, Valladolid, Spain
[e] Department of Clinical Analysis, Santiago Apostle Hospital, Miranda de Ebro, Burgos, Spain

ARTICLE INFO

Handling Editor: Dr L D'Erasmo

Keywords:
Carotid atherosclerosis
Oxidative stress
Antioxidant defenses
Lipid peroxidation
Mitochondrial metabolism
Stroke
Plaque vulnerability

ABSTRACT

Background and aims: Oxidative stress (OS) is a central driver of atherosclerosis, yet its role in carotid plaque vulnerability and neurological symptoms remains insufficiently defined. This study aimed to comprehensively characterize the redox and metabolic profiles of carotid plaques and evaluate their associations with plaque calcification and clinical symptomatology in patients undergoing carotid endarterectomy.

Methods and results: Ninety-two patients were prospectively enrolled. Patients were classified as symptomatic or asymptomatic according to recent neurological events, and plaques were categorized as calcified or non-calcified based on preoperative angio-CT. Excised tissue was analyzed for total antioxidant capacity (FRAP, ABTS), enzymatic defenses (catalase, superoxide dismutase [SOD]), oxidative damage markers (8-hydroxy-2′-deoxyguanosine [8-OHdG], malondialdehyde + 4-hydroxy-2-nonenal [MDA + HNE]), uric acid, and lactate. Non-calcified plaques exhibited reduced antioxidant activity (ABTS: 2635.08 vs. 2803.28 μM, $p = 0.007$), lower SOD activity (1.11 vs. 1.49 U/mL, $p = 0.049$), and higher lactate levels (11.45 vs. 8.57 mg/dL, $p = 0.001$), indicating metabolic instability. Symptomatic patients showed higher uric acid ($p = 0.001$), reduced SOD ($p = 0.009$), and increased lipid peroxidation, while FRAP and ABTS did not differ significantly. The two analytical axes did not fully overlap, as 75 % of non-calcified and 60 % of calcified plaques derived from symptomatic patients ($p = 0.235$).

Conclusion: Carotid plaques associated with symptoms and lacking calcification displayed redox imbalance and metabolic dysfunction, suggesting a more biologically active and rupture-prone phenotype. Importantly, these findings support the integration of tissue oxidative biomarkers with clinical and imaging data to refine stroke risk stratification, guide secondary prevention, and improve postoperative surveillance strategies.

1. Introduction

Cardiovascular diseases (CVD) remain the leading cause of morbidity and mortality in the Western world, accounting for approximately one in every three deaths in developed countries [1]. Among their clinical consequences, ischemic stroke is particularly relevant, as it represents the second leading cause of death in Europe, the primary cause of acquired disability in adults, and a major contributor to vascular dementia [2]. Accurate etiopathogenic classification of ischemic stroke is essential for selecting optimal preventive strategies and reducing recurrence risk [3]. Notably, atherosclerosis of the carotid arteries is among the most frequent causes.

Atherosclerosis is defined by the progressive accumulation of lipids, inflammatory cells, and fibrous elements within the arterial wall. Oxidative stress (OS), resulting from an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense mechanisms, plays a crucial role in plaque development and progression. ROS oxidize lipoproteins, trigger vascular inflammation, and promote the transformation of macrophages into foam cells, a key step in the formation of atheromatous plaques [4,5].

Assessment of redox status—including antioxidant capacity,

* Corresponding author. Department of Vascular Surgery, University Clinical Hospital of Valladolid, University of Valladolid, Valladolid, Spain.
E-mail address: marialourdes.rio@uva.es (M.L. del Río-Solá).

https://doi.org/10.1016/j.numecd.2025.104423
Received 13 May 2025; Received in revised form 3 September 2025; Accepted 17 October 2025
Available online 23 October 2025

List of acronyms:

FRAP	Ferric Reducing Antioxidant Power
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
SOD	Superoxide Dismutase
MDA	Malondialdehyde
HNE	4-Hydroxy-2-Nonenal
8-OHdG	8-Hydroxy-2′-deoxyguanosine

enzymatic defenses, and oxidative damage by-products—provides valuable insight into the biological activity of atherosclerotic lesions. Among the most commonly used methods for evaluating antioxidant capacity are the Ferric Reducing Antioxidant Power (FRAP) and the ABTS radical cation decolorization assay [6]. Superoxide dismutase (SOD) and catalase are the main enzymatic defenses against ROS generation [7,8]. Lipid peroxidation, a central process in plaque instability, generates aldehydic by-products such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), which are recognized biomarkers of oxidative stress [9].

Clinical evidence suggests that the natural history of carotid atherosclerotic lesions differs substantially between symptomatic and asymptomatic patients, even when stenosis severity is similar. Symptomatic individuals face a recurrence risk exceeding 13 %, compared to only 1–2 % in asymptomatic patients [10]. Moreover, plaques with moderate stenosis may evolve rapidly and precipitate ischemic events, while more severely stenotic lesions can remain clinically silent.

These findings imply a disconnect between plaque morphology and its biological behaviour, supporting the concept of two distinct carotid disease phenotypes: a stable form, unlikely to cause embolic events, and an unstable form, more prone to rupture or symptomatic progression regardless of luminal narrowing.

In this context, OS is believed to contribute to endothelial dysfunction and plaque vulnerability. This study aimed to assess carotid plaque vulnerability through a dual analytical approach: (1) comparison of redox and metabolic profiles in symptomatic versus asymptomatic patients, and (2) evaluation of calcified versus non-calcified plaques to identify morphological determinants of oxidative imbalance.

2. Methods

2.1. Study design and population

This was a prospective, longitudinal, observational, and comparative study conducted at the Department of Angiology and Vascular Surgery of the University Clinical Hospital of Valladolid (HCUV) in collaboration with the Biomedical Research Center of Castilla y León, Spain. The study followed a single standardized protocol and included two parallel cohorts: (1) patients with symptomatic carotid stenosis and (2) patients with asymptomatic carotid stenosis. Patients were categorized as symptomatic if they had recent ipsilateral carotid-territory neurological events (amaurosis fugax, transient ischemic attack, or ischemic stroke) and as asymptomatic otherwise. At the time of surgical evaluation and tissue sampling, all patients were receiving antiplatelet therapy with acetylsalicylic acid 100 mg/day according to institutional protocol.

2.2. Inclusion and exclusion criteria

Eligible participants were adults undergoing carotid endarterectomy at HCUV in 2023, with ≥50 % stenosis confirmed by carotid Doppler ultrasound and computed tomography angiography (CTA), according to the NASCET (North American Symptomatic Carotid Endarterectomy Trial) criteria. Patients were categorized as symptomatic or asymptomatic based on the presence of neurological events related to carotid disease.

Exclusion criteria included the presence of advanced systemic conditions (e.g., hepatic, renal, immunological, oncological, neurological, or musculoskeletal disorders), pregnancy, substance abuse, recent participation in other clinical trials, treatment with investigational drugs, or intake of antioxidant supplements within the prior three months.

2.3. Sample size calculation

Assuming a two-tailed α error of 0.05, a β error of 0.20 (power = 80 %), and a common standard deviation of 0.5, the required sample size was 45 patients per group. A 5 % loss to follow-up was anticipated.

2.4. Clinical and demographic variables

The following variables were collected from all patients: age, sex, and cardiovascular risk factors including hypertension, dyslipidemia, diabetes mellitus, and smoking status. Neurological symptomatology was documented, as well as the presence of ischemic brain lesions confirmed by computed tomography (Table 1).

2.5. Biochemical variables (atheroma plaque analysis)

A comprehensive biochemical analysis of carotid atheroma plaques was carried out to characterize oxidative stress and metabolic alterations. Antioxidant capacity was assessed using three complementary methods: the Ferric Reducing Antioxidant Power (FRAP) assay, the ABTS radical cation decolorization assay, and uric acid quantification. Endogenous antioxidant defenses were evaluated through measurement of catalase and superoxide dismutase (SOD) enzymatic activities. Oxidative damage was determined by quantifying DNA oxidation, expressed as 8-hydroxy-2′-deoxyguanosine (8-OHdG), and by assessing lipid peroxidation through the combined levels of malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). Finally, mitochondrial metabolic stress was evaluated by measuring lactic acid concentrations.

Table 1
Baseline clinical and demographic characteristics of the study population.

Characteristic	Symptomatic	Asymptomatic	P-value
Age	75.0 ± 4.6	72.7 ± 6.4	0.11
Sex	61 %	75 %	0.40
Hypertension	83 %	71 %	0.40
Dyslipidemia	58 %	67 %	0.70
Diabetes	36 %	38 %	0.98
Smoking	67 %	58 %	0.70
BMI (kg/m^2)	24.8 ± 2.9	25.2 ± 3.1	0.31
Alcohol consumption	11 %	10 %	0.47
Coronary heart disease	4 %	5 %	0.65
Chronic kidney disease	3 %	2 %	0.38
Stroke	2 %	3 %	0.56
Cancer or malignant tumors	5 %	4 %	0.48
Fatty liver disease	6 %	5 %	0.52
Characteristic	**Calcified**	**Non-calcified**	**P-value**
Age	73.1 ± 5.9	75.3 ± 4.7	0.23
Sex	71 %	62 %	0.54
Hypertension	76 %	81 %	0.44
Dyslipidemia	68 %	54 %	0.67
Diabetes	44 %	27 %	0.96
Smoking	68 %	58 %	0.81
BMI (kg/m^2)	24.5 ± 3.0	25.1 ± 2.8	0.42
Alcohol consumption	12 %	9 %	0.57
Coronary heart disease	5 %	4 %	0.65
Chronic kidney disease	4 %	2 %	0.34
Stroke	3 %	2 %	0.52
Cancer or malignant tumors	6 %	3 %	0.58
Fatty liver disease	5 %	4 %	0.42

2.6. Morphological and hemodynamic variables of carotid stenosis

Preoperative assessment of carotid artery lesions was conducted using duplex Doppler ultrasound and computed tomography angiography (CTA). Carotid plaque morphology was characterized by both imaging modalities, in accordance with the guidelines of the Spanish Society of Angiology and Vascular Surgery (SEACV) – Chapter of Non-Invasive Vascular Diagnosis. CTA was used to document structural features such as calcification, ulceration, and eccentricity. Plaques were classified as calcified when preoperative CTA reported calcific densities within the atheroma and/or duplex ultrasound demonstrated hyperechoic components with acoustic shadowing; plaques lacking these features were considered non-calcified. For this study, the calcification label was abstracted from the final preoperative imaging reports.

Hemodynamic assessment was performed by Doppler ultrasound. Flow velocity measurements were obtained at the point of maximum stenosis, and hemodynamic alterations in the proximal (common carotid artery) and distal (post-stenotic internal carotid artery) segments were also analyzed. The degree of carotid stenosis was classified according to validated Doppler ultrasound criteria (Table 2).

2.7. Sample collection and preservation

Atheroma plaque samples were obtained during carotid endarterectomy procedures. The excised material included the intimal layer and the inner portion of the media of the carotid artery. Samples were immediately snap-frozen in liquid nitrogen and stored at −195.8 °C until biochemical analysis.

2.8. Redox assessment strategy

The redox status of carotid plaques was evaluated across four complementary domains: total antioxidant capacity, enzymatic antioxidant activity, oxidative damage to biomolecules, and mitochondrial metabolic stress. All determinations were performed manually, in duplicate, using commercial colorimetric kits according to the manufacturers' instructions. Absorbance readings were obtained with a SPECTROstar Nano UV/VIS microplate spectrophotometer (BMG Labtech, Ortenberg, Germany).

To assess antioxidant capacity, three complementary assays were applied. The FRAP (Ferric Reducing Antioxidant Power) assay, based on the reduction of ferric to ferrous iron as described by Benzie and Strain [11], was quantified at 595 nm using a Trolox standard curve. The ABTS radical cation decolorization assay, following the method of Re et al. [12], measured the reduction of $ABTS^{+}$ absorbance upon antioxidant action. In addition, uric acid levels were determined according to the method of Trivedi and Kabasakalian, which relies on uricase-mediated oxidation of uric acid to hydrogen peroxide, further reacted with 4-AAP and HMMPS in the presence of peroxidase to yield a chromogenic quinone.

The cellular fraction of plaques was then analyzed to quantify endogenous enzymatic defenses. Superoxide dismutase (SOD) activity was determined using a WST-1–based colorimetric assay forming a soluble formazan dye (Superoxide Dismutase Activity Kit, Arbor Assays). Catalase activity was measured by titration of residual hydrogen peroxide with potassium permanganate (Catalase Activity Assay Kit, Elabscience).

Oxidative injury was evaluated through markers of lipid and DNA damage. Lipid peroxidation was quantified by malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) using the Lipid Peroxidation Assay Kit (Bioquochem, ref. KB03002). DNA oxidative damage was assessed by measuring 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels with the DetectX® DNA Damage Kit (Arbor Assays, ref. K059–H5).

Finally, metabolic imbalance was investigated by determining lactate concentrations, a surrogate marker of mitochondrial dysfunction. Lactate was oxidized to pyruvate and hydrogen peroxide by lactate oxidase; the peroxide reacted with 4-aminophenazone and 4-chlorophenol in the presence of peroxidase to produce a red quinone compound, whose absorbance was proportional to lactate concentration [13].

2.9. Ethical and legal considerations

The study was approved by the Ethics Committee of HCUV. All participants provided written informed consent prior to inclusion. Sample anonymization and confidentiality of personal and clinical data were ensured throughout the study. All procedures complied with the principles of the Declaration of Helsinki and with national regulations, including Organic Law 15/1999 on the Protection of Personal Data, Law 41/2002 on Patient Autonomy and Rights, and General Health Law 14/1986.

2.10. Statistical analysis

Categorical variables were expressed as absolute frequencies and percentages, while continuous variables were presented as mean ± standard deviation. Normality of distribution was assessed using both the Shapiro-Wilk and Kolmogorov-Smirnov tests, with Lilliefors correction when applicable.

Comparisons between categorical variables (e.g., presence of neurological symptoms and plaque calcification) were performed using the chi-square test or Fisher's exact test, as appropriate. For continuous variables, comparisons between two groups (e.g., symptomatic vs. asymptomatic patients or calcified vs. non-calcified plaques) were conducted using Student's t-test for independent samples. Serum lipid

Table 2
Ultrasound criteria used to determine carotid artery stenosis severity.

	<50 %	50–69 %	70–79 %	80–89 %	>90 %	OCLUSION
DIRECT SIGNS						
VSM	<125	125–230	>230	>300	Variable	NA
VDF	<40	40–100	>100	Variable	Variable	NA
INDIRECT SIGNS						
POSTSTENOSIS IN ICA	Normal	Normal	<50	>50	>30	NA
COLATERAL FLOW IN OA	No	No	No/↓/inverted	↓/inverted	↓/inverted	↓/inverted
COLATERAL FLOW IN PW	No	No	No/present	Present	Present	Present
INDICES						
RELATION BETWEEN VSM_{ICA}/VSM_{CCA}	<2	>2	>4	>4	Variable	NA

CCA, common carotid artery; ICA, internal carotid artery; AO, ophthalmic artery; NA, not applicable; PW, polygon of Willis; VDF, end-diastolic velocity; VSM, maximum systolic velocity.

profiles were compared by plaque calcification and by symptom status; results are presented in Supplementary Table S1–S2. In addition, Bayesian analysis was applied to assess the probability distributions of symptom occurrence across groups, complementing frequentist comparisons.

All statistical analyses were performed using SPSS version 27.0 (IBM Corp., Chicago, IL, USA) and R version 4.3.2 (R Core Team). A two-sided p-value <0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics and group distribution

A total of 92 carotid atherosclerotic plaques were analyzed: 46 from asymptomatic patients and 46 from patients with neurological symptoms related to carotid stenosis. The baseline clinical and demographic characteristics were comparable between groups (Table I), with no significant differences observed between symptomatic and asymptomatic patients, nor between calcified and non-calcified plaques.

Regarding plaque morphology, the mean degree of stenosis was 75.8 ± 14.8 % in calcified plaques and 71.6 ± 10.8 % in non-calcified ones (p = 0.143). The proportion of symptomatic patients was 75 % among those with non-calcified plaques and 60 % among those with calcified plaques (p = 0.235) (Fig. 1).

Bayesian analyses showed overlapping posterior distributions of symptom frequency between calcified and non-calcified plaques (Fig. 2), as well as between symptomatic and asymptomatic patients (Fig. 3), supporting the homogeneity of the groups.

Normality tests confirmed that all biochemical parameters—FRAP, ABTS, uric acid, catalase activity, SOD activity, DNA damage (8-OHdG), lipid peroxidation (MDA + HNE), and lactate—followed a Gaussian distribution.

Group comparisons of serum lipid parameters did not show statistically significant differences across calcification or symptom strata (Supplementary Table S1–S2).

3.2. Plaque morphology and redox profile

As shown in Table 3, calcified plaques exhibited higher antioxidant capacity compared to non-calcified ones, with statistically significant differences in ABTS values (2803.28 ± 223.27 vs 2635.08 ± 323.80 µM; p = 0.007), but not in FRAP (181.62 ± 115.68 vs 142.92 ± 73.71 µM; p = 0.285).

Antioxidant enzyme activity was also greater in calcified plaques, with SOD reaching statistical significance (1.49 ± 1.20 vs 1.11 ± 0.53 U/mL; p = 0.049), while catalase activity did not (175.13 ± 102.79 vs 160.73 ± 44.97 U/mL; p = 0.402).

Markers of oxidative damage tended to be higher in non-calcified plaques (8-OHdG: 3318.96 ± 1919.83 vs 2887.15 ± 2052.13 pg/mL; p = 0.336; MDA + HNE: 46.87 ± 30.81 vs 32.45 ± 16.88 µM; p = 0.120), although these differences were not statistically significant.

Lactate levels were significantly higher in non-calcified plaques (11.45 ± 3.28 vs 8.57 ± 4.05 mg/dL; p = 0.001).

3.3. Neurological symptoms and redox profile

Table 4 shows the association between biochemical parameters and clinical symptoms. Asymptomatic patients had higher antioxidant capacity (FRAP: 185.98 ± 110.31 vs 159.11 ± 85.90 µM; ABTS: 2777.18 ± 285.00 vs 2698.00 ± 278.32 µM), although these differences were not significant (p = 0.430 and p = 0.220, respectively).

Symptomatic patients had significantly higher uric acid levels (2.33 ± 1.36 vs 1.48 ± 1.40 mg/dL; p = 0.001) and catalase activity (176.81 ± 92.38 vs 146.79 ± 28.97 U/mL; p = 0.025), whereas SOD activity was higher in asymptomatic patients (1.77 ± 1.15 vs 1.10 ± 0.79 U/mL; p = 0.009).

DNA damage (8-OHdG) was significantly greater in asymptomatic patients (4393.01 ± 2817.94 vs 2476.91 ± 1007.22 pg/mL; p = 0.002), while lipid peroxidation was higher in symptomatic individuals, though not significantly (44.44 ± 28.40 vs 32.20 ± 17.04 µM; p = 0.120).

Lactate levels were similar between both groups (9.74 ± 3.70 vs 9.70 ± 4.34 mg/dL; p = 0.970).

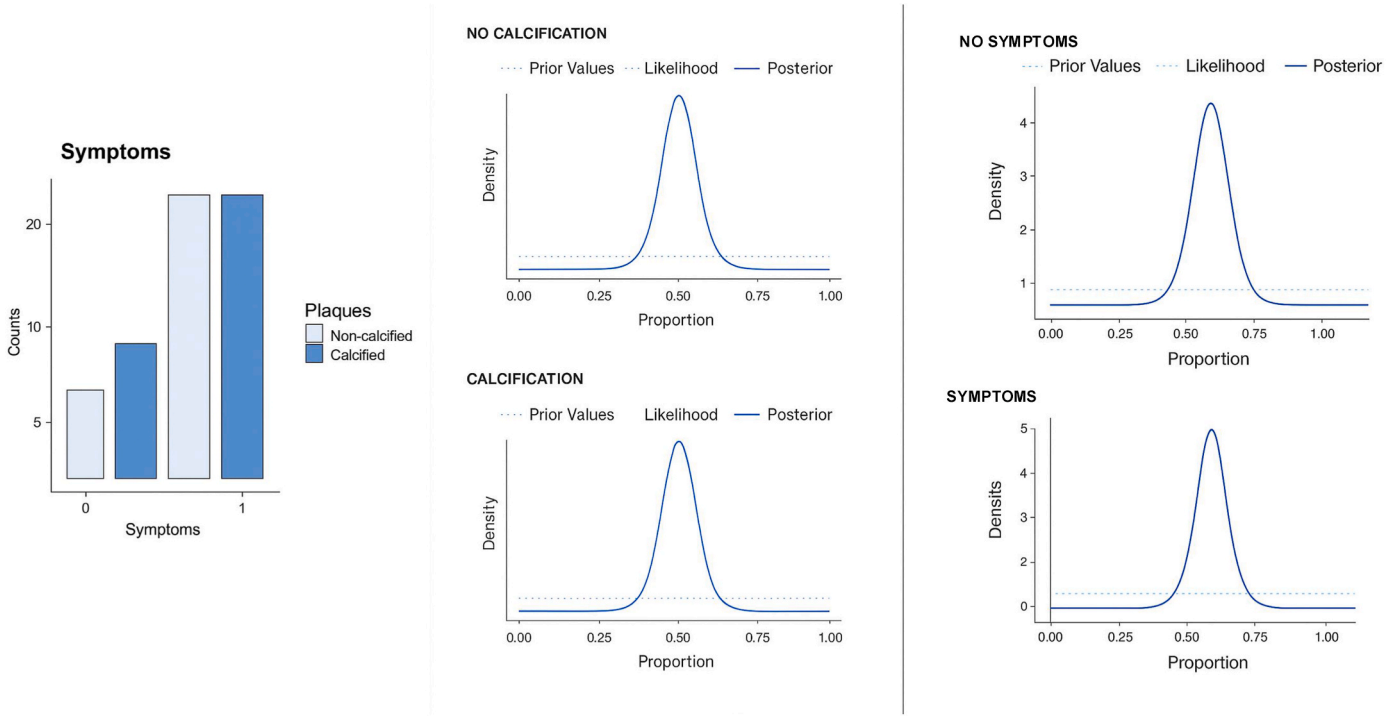

Fig. 1. Frequency of Symptoms by Plaque Type
Bar chart comparing the number of symptomatic and asymptomatic patients across calcified and non-calcified plaque groups.

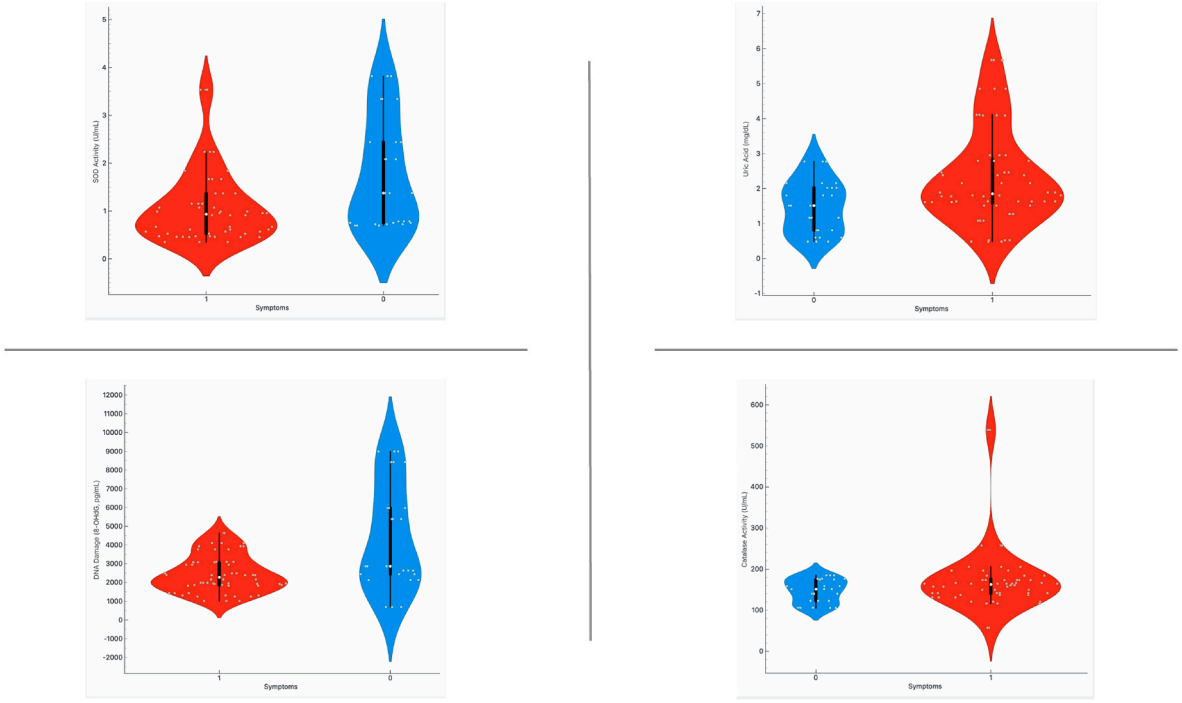

Fig. 2. Bayesian Analysis of Symptom Distribution by Plaque Type
Posterior probability distributions of symptomatic patient proportion in groups with calcified and non-calcified carotid plaques.

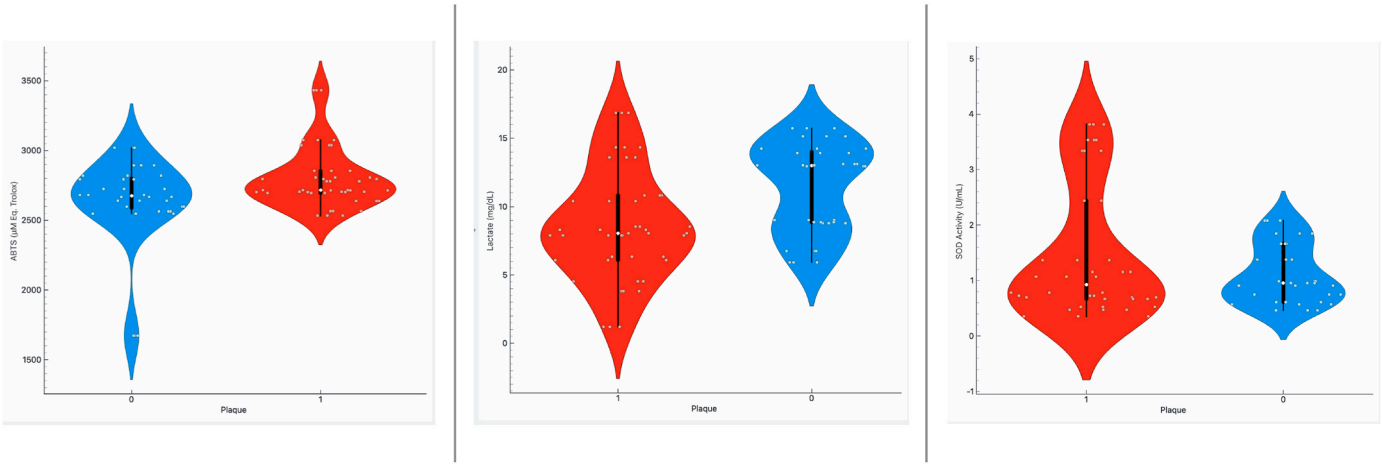

Fig. 3. Bayesian Analysis of Symptom Distribution
Posterior probability distributions comparing symptomatic and asymptomatic patients, irrespective of plaque type.

4. Discussion

The risk of ischemic stroke is known to be higher in patients with hypoechoic and heterogeneous atheroma plaques, as previously reported in large observational studies such as the Cardiovascular Health Study [14] and by authors including Gronholdt et al., Sterpetti et al., and Abu-Rahma et al. [15–17]. However, ultrasound evaluation of plaque morphology is limited by its subjective interpretation.

Our findings support a strong relationship between oxidative stress (OS) and carotid plaque vulnerability. Non-calcified atheroma plaques demonstrated reduced antioxidant capacity, lower enzymatic antioxidant activity, increased oxidative damage, and greater disruption of energy metabolism. These characteristics were more frequently associated with symptomatic patients, reinforcing the idea that OS contributes to clinical instability independent of the degree of stenosis. Importantly, serum lipid parameters did not differ significantly across groups, indicating that the increased oxidative stress observed in symptomatic and non-calcified plaques is not attributable to systemic lipid levels but rather reflects intrinsic plaque biology and downstream inflammatory activation. Taken together, these results indicate that both non-calcified and symptomatic plaques share a redox-imbalanced phenotype. Moreover, plaque calcification status and neurological symptomatology appear to represent related but non-overlapping dimensions of carotid disease biology, which justifies their parallel evaluation within the same study framework.

OS has been widely implicated in the initiation and progression of atherosclerosis via direct damage to the vascular endothelium. Reactive oxygen species (ROS) contribute to lipid peroxidation, endothelial dysfunction, and inflammatory activation, all of which promote plaque development and rupture. In humans, OS is involved in the

Table 3
Redox biomarkers and metabolic parameters in calcified vs. non-calcified plaques.

	No calcification	Calcification	p-value
Antioxidant capacity			
FRAP (μM Eq TROLOX)	142.92 ± 73.71	181.62 ± 115.68	0.285
ABTS (μM Eq. TROLOX)	2635.08 ± 323.80	2803.28 ± 223.27	0.007
Uric Acid (mg/dL)	2.22 ± 1.40	1.81 ± 0.95	0.135
Antioxidant defenses			
Catalase activity (U/mL)	160.73 ± 44.97	175.13 ± 102.79	0.402
SOD Activity (U/mL)	1.11 ± 0.53	1.49 ± 1.20	0.049
Study of oxidative damage			
DNA damage (pg/mL)	3318.96 ± 1919.83	2887.15 ± 2052.13	0.336
[MDA + HNE] (μM)	46.87 ± 30.81	32.45 ± 16.88	0.12
Energy metabolism			
Lactate (mg/dL)	11.45 ± 3.28	8.57 ± 4.05	0.001

Abbreviations: FRAP, ferric reducing antioxidant power; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); SOD, superoxide dismutase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MDA, malondialdehyde; HNE, 4-hydroxynonenal.

Table 4
Redox biomarkers and metabolic parameters in symptomatic vs. asymptomatic patients.

	No symptoms	Symptoms	p-value
Antioxidant capacity			
FRAP (μM Eq TROLOX)	185.98 ± 110.31	159.11 ± 85.90	0.43
ABTS (μM Eq. TROLOX)	2777.18 ± 285.00	2698 ± 278.32	0.22
Uric Acid (mg/dL)	1.48 ± 1.40	2.33 ± 1.36	0.001
Antioxidant defenses			
Catalase activity (U/mL)	146.79 ± 28.97	176.81 ± 92.38	0.025
SOD Activity (U/mL)	1.77 ± 1.15	1.10 ± 0.79	0.009
Study of oxidative damage			
DNA damage (pg/mL)	4393.01 ± 2817.94	2476.91 ± 1007.22	0.002
[MDA + HNE] (μM)	32.20 ± 17.04	44.44 ± 28.40	0.12
Energy metabolism			
Lactate (mg/dL)	9.70 ± 4.34	9.74 ± 3.70	0.97

Abbreviations: FRAP, ferric reducing antioxidant power; ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); SOD, superoxide dismutase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MDA, malondialdehyde; HNE, 4-hydroxynonenal; U/mL, units per milliliter; pg/mL, picograms per milliliter; μM, micromolar; mg/dL, milligrams per deciliter.

pathophysiology of more than 100 major diseases, including cardiovascular disease and aging [18].

Mitochondria are a key source of ROS, especially the superoxide anion, which originates from electron leakage at complexes I and III of the respiratory chain [19]. Although rapidly converted to hydrogen peroxide by superoxide dismutase (SOD), superoxide can initiate extensive oxidative damage to proteins, DNA, and lipids. The imbalance between ROS production and antioxidant defenses is central to the pathogenesis of vulnerable plaques [20,21].

Rafiei et al. demonstrated that reduced FRAP levels are associated with greater coronary artery disease severity [22]. Feki et al. further highlighted the link between DNA damage and oxidative stress in myocardial infarction models. Consistent with those studies, our data showed reduced antioxidant capacity in symptomatic patients, along with elevated lipid peroxidation and altered redox profiles in their carotid plaques [23].

Interestingly, uric acid levels were significantly higher in symptomatic patients and in non-calcified plaques, in contrast with other antioxidant markers. Uric acid may act as both an antioxidant and a prooxidant, depending on the redox context. Elevated uric acid could reflect a compensatory mechanism aimed at counteracting oxidative stress, as suggested in previous research [24,25].

SOD dysfunction has been linked to an increased risk of cardiovascular events, including stroke [26,27]. Experimental studies indicate that the combined administration of catalase and SOD reduces infarct volume and blood-brain barrier permeability more effectively than either enzyme alone [28].

The lack of statistical significance in lipid peroxidation markers may reflect technical limitations in quantification methods. MDA and HNE are often bound to macromolecules and require HPLC-based separation for more specific measurement. Standard commercial kits may underestimate true levels of oxidative damage [18,29].

Lactate accumulation is a recognized marker of altered mitochondrial metabolism. In our study, lactate levels were significantly higher in non-calcified plaques, supporting their greater metabolic instability. While Juraschek et al. [30] previously linked lactate with hypertension and diabetes, our data extend this association to carotid plaque phenotype.

This study provides insight into the redox environment of atherosclerotic plaques and adds prognostic information beyond the anatomical degree of stenosis. We demonstrate that oxidative imbalance in carotid tissue correlates with clinical symptoms, suggesting a mechanistic link between redox disruption and plaque vulnerability.

Our findings support a pragmatic framework in which tissue redox profiling of endarterectomy specimens provides biological information complementary to duplex/CTA morphology and stenosis degree. In symptomatic patients or in those with non-calcified phenotypes—where vulnerability is biologically driven—integrating a small panel (e.g., SOD activity, MDA + HNE, and lactate) with standard factors could refine post-operative risk stratification, prioritize aggressive secondary prevention, and inform closer surveillance for the contralateral carotid.

This study has several limitations. It included only patients with advanced stenosis requiring surgical treatment and thus does not reflect earlier-stage disease. The single time-point design precludes longitudinal interpretation. Future studies should evaluate systemic redox markers in parallel and assess temporal dynamics of oxidative stress. Larger cohorts may also allow for multivariate analysis and subgroup stratification.

Despite these limitations, our results support the clinical relevance of redox biomarkers for identifying vulnerable plaques. The development of a standardized panel of oxidative stress markers could aid in the early identification of high-risk patients and inform targeted therapeutic interventions aimed at stabilizing atherosclerotic disease.

5. Conclusion

This study demonstrates that oxidative stress plays a key role in the biological vulnerability of carotid atherosclerotic plaques. Non-calcified plaques and those associated with neurological symptoms exhibited a consistent pattern of reduced antioxidant capacity, diminished enzymatic defenses, increased oxidative damage, and impaired mitochondrial metabolism. These findings suggest that oxidative stress markers in plaque tissue may serve as useful indicators of clinical instability, regardless of the anatomical severity of stenosis.

The incorporation of oxidative stress profiling into the assessment of carotid atherosclerosis could improve risk stratification and support the development of novel diagnostic and therapeutic strategies aimed at preventing ischemic events in high-risk patients.

Author Contributions

María Lourdes del Río-Solá: Conceptualization, Methodology, Investigation, Writing – Original Draft, Project Administration, Supervision. Sandra Pérz Fernández: Resources, Investigation, Visualization. Hugo Gonzalo-Benito: Validation, Supervision, Writing – Review & Editing. Rita Losa Rodríguez: Formal Analysis, Data Curation, Writing –

Review & Editing.

Declaration on the use of Artificial intelligence (AI) tools

Artificial intelligence tools, including ChatGPT (OpenAI) and Grammarly, were used to support language editing and improve the clarity and readability of the manuscript. The authors reviewed and verified all content to ensure accuracy and scientific integrity.

Declaration of generative AI

Artificial intelligence tools were used to support language editing. The authors reviewed and verified all content to ensure accuracy and scientific integrity.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements *(opcional)*

The authors thank the staff of the Department of Angiology and Vascular Surgery at the University Clinical Hospital of Valladolid for their assistance in sample collection and clinical coordination.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2025.104423.

References

[1] Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, et al. Heart disease and stroke statistics—2009 update: a report from the American heart association statistics committee and stroke statistics subcommittee. Circulation 2009;119(3):e21–181. https://doi.org/10.1161/CIRCULATIONAHA.108.191261.

[2] Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. Eur Heart J 2016;37(42):3232–45. https://doi.org/10.1093/eurheartj/ehw334.

[3] Gökçal E, Niftaliyev E, Asil T. Etiological classification of ischemic stroke in young patients: a comparative study of TOAST, CCS, and ASCO. Acta Neurol Belg 2017;117(3):643–8. https://doi.org/10.1007/s13760-017-0813-8.

[4] Rai V, Agrawal DK. The role of damage- and pathogen-associated molecular patterns in inflammation-mediated vulnerability of atherosclerotic plaques. Can J Physiol Pharmacol 2017;95(10):1245–53. https://doi.org/10.1139/cjpp-2016-0664.

[5] Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol 2013;13(10):709–21. https://doi.org/10.1038/nri3520.

[6] Schlesier K, Harwat M, Böhm V, Bitsch R. Assessment of antioxidant activity by using different in vitro methods. Free Radic Res 2002;36(2):177–87. https://doi.org/10.1080/10715760290006411.

[7] Aruoma OI. Characterization of drugs as antioxidant prophylactics. Free Radic Biol Med 1996;20(5):675–705. https://doi.org/10.1016/0891-5849(95)02110-8.

[8] Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. Mutat Res 2004;567(1):1–61. https://doi.org/10.1016/j.mrrev.2003.11.001.

[9] Mas-Bargues C, Escrivá C, Dromant M, Borrás C, Viña J. Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Arch Biochem Biophys 2021;709:108941. https://doi.org/10.1016/j.abb.2021.108941.

[10] Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. Stroke 2000;31(3):774–81. https://doi.org/10.1161/01.str.31.3.774.

[11] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. Anal Biochem 1996;239(1):70–6. https://doi.org/10.1006/abio.1996.0292.

[12] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26(9–10):1231–7. https://doi.org/10.1016/S0891-5849(98)00315-3.

[13] Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 1972;97(151):142–5. https://doi.org/10.1039/an9729700142.

[14] Polak JF, Shemanski L, O'Leary DH, Lefkowitz D, Price TR, Savage PJ, et al. Hypoechoic plaque at US of the carotid artery: an independent risk factor for incident stroke in adults aged 65 years or older. Radiology 1998;208(3):649–54. https://doi.org/10.1148/radiology.208.3.9722841.

[15] Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. Circulation 2001;104(1):68–73. https://doi.org/10.1161/hc2601.091704.

[16] Sterpetti AV. Eversion endarterectomy of the internal carotid artery combined with open endarterectomy of the common carotid artery. Am J Surg 2010;200(3):e44–7. https://doi.org/10.1016/j.amjsurg.2009.12.029.

[17] AbuRahma AF, Wulu JT, Crotty B. Carotid plaque ultrasonic heterogeneity and severity of stenosis. Stroke 2002;33(7):1772–5. https://doi.org/10.1161/01.str.0000019127.11189.b5.

[18] Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, et al. Clinical relevance of biomarkers of oxidative stress. Antioxidants Redox Signal 2015;23(14):1144–70. https://doi.org/10.1089/ars.2015.6317.

[19] Gilgun-Sherki Y, Melamed E, Offen D. Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood-brain barrier. Neuropharmacology 2001;40(8):959–75. https://doi.org/10.1016/S0028-3908(01)00019-3.

[20] Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. Circulation 2003;108(14):1664–72. https://doi.org/10.1161/01.CIR.0000087480.94275.97.

[21] Summerhill V, Karagodin V, Grechko A, Myasoedova V, Orekhov A. Vasculoprotective role of olive oil compounds via modulation of oxidative stress in atherosclerosis. Front Cardiovasc Med 2018;5:188. https://doi.org/10.3389/fcvm.2018.00188.

[22] Rafiei A, Ferns GA, Ahmadi R, Khaledifar A, Rahimzadeh-Fallah T, Mohammad-Rezaei M, et al. Expression levels of miR-27a, miR-329, ABCA1, and ABCG1 genes in peripheral blood mononuclear cells and their correlation with serum levels of oxidative stress and hs-CRP in patients with coronary artery disease. IUBMB Life 2021;73(2):223–37. https://doi.org/10.1002/iub.2421.

[23] Feki A, Ben Saad H, Bkhairia I, Ktari N, Naifar M, Boudawara O, et al. Cardiotoxicity and DNA damage induced by thiamethoxam: protective role of *Trigonella foenum-graecum*. Environ Toxicol 2019;34(3):271–82. https://doi.org/10.1002/tox.22682.

[24] Kimura Y, Tsukui D, Kono H. Uric acid in inflammation and the pathogenesis of atherosclerosis. Int J Mol Sci 2021;22(22):12394. https://doi.org/10.3390/ijms222212394.

[25] Nieto FJ, Iribarren C, Gross MD, Comstock GW, Cutler RG. Uric acid and serum antioxidant capacity: a reaction to atherosclerosis? Atherosclerosis 2000;148(1):131–9. https://doi.org/10.1016/S0021-9150(99)00214-2.

[26] Yang X, Yang S, Xu H, Liu D, Zhang Y, Wang G. Superoxide dismutase gene polymorphism is associated with ischemic stroke risk in the China dali region Han population. Neurol 2021;26(2):27–31. https://doi.org/10.1097/NRL.0000000000000301.

[27] Otaki Y, Watanabe T, Nishiyama S, Takahashi H, Arimoto T, Shishido T, et al. The impact of superoxide dismutase-1 genetic variation on cardiovascular and all-cause mortality in a prospective cohort study: the Yamagata (Takahata) study. PLoS One 2016;11(10):e0164732. https://doi.org/10.1371/journal.pone.0164732.

[28] Davis SM, Pennypacker KR. Targeting antioxidant enzyme expression as a therapeutic strategy for ischemic stroke. Neurochem Int 2017;107:23–32. https://doi.org/10.1016/j.neuint.2016.12.007.

[29] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11(1):81–128. https://doi.org/10.1016/0891-5849(91)90192-6.

[30] Juraschek SP, Miller ER, Weaver CM, Appel LJ. Effects of sodium reduction and the DASH diet in relation to baseline blood pressure. J Am Coll Cardiol 2017;70(23):2841–8. https://doi.org/10.1016/j.jacc.2017.10.011.