Neutrophil Gelatinase Associated Lipocalin (NGAL) for Identification of Unstable Plaques in Patients with Asymptomatic Carotid Stenosis

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WHAT THIS PAPER ADDS

This cross sectional research study presents the evaluation of neutrophil gelatinase associated lipocalin (NGAL) for identification of vulnerable plaques in a selected cohort of asymptomatic patients with carotid artery stenosis. A biomarker for early identification of vulnerable plaques potentially prone to embolisation in otherwise asymptomatic patients could be life saving. NGAL was elevated in asymptomatic but unstable grade VI lesions according to the American Heart Association and correlated with soft plaques in pre-operative duplex sonography. Moreover, NGAL showed a borderline significance in patients with silent brain infarction, indicating the role of NGAL as a potential biomarker, although validation is needed in future studies with large patient cohorts.

Objective: Neutrophil gelatinase associated lipocalin (NGAL) and matrix metalloproteinase (MMP)-9/NGAL complex were investigated in asymptomatic patients with carotid artery stenosis including gender specific differences aiming at vulnerable plaques prone to embolisation.

Methods: Serum NGAL and MMP-9/NGAL levels were analysed in 83 patients with asymptomatic carotid artery stenosis. Pre-operative ultrasound and post-endarterectomy histology of carotid atherosclerotic lesions were evaluated.

Results: Patients with vulnerable plaques, as determined by ultrasound (plaques with decreased echogenicity) and histological analysis (type VI according to the classification of the American Heart Association), displayed the highest levels of NGAL and MMP-9/NGAL complex ($p = .0003$ and $p = .0078$, respectively). Grade VI plaques were primarily detected in patients with "soft" plaques (12 type VI plaques in 25 patients), but also in patients with mixed (four of 19) and calcified (three of 39) plaques according to ultrasound. Higher grade carotid artery stenosis ($\geq$90%) was not associated with elevated NGAL levels. The receiver operating characteristic curve analysis detecting grade VI lesions yields an area under the curve (AUC) = 0.85, with respect to soft plaque on ultrasound the AUC = 0.86. There were no gender specific differences in levels of NGAL 80.9 (37.7) ng/mL in women vs. 76.7 (36.3) ng/mL in men, $p = .607$) nor of MMP-9/NGAL 33.0 (18.2—55.5) ng/mL in women vs. 36.7 (20.2—54.0) ng/mL in men, $p = .969$. Likewise, there were no gender associated differences in vulnerable plaque characteristics: either for grade VI plaques (17.9% vs. 27.3%, $p = .582$) or for the presence of soft plaques as evaluated by ultrasound (35.9% vs. 25%, $p = .503$).

Conclusion: Circulating NGAL and MMP-9/NGAL are significantly increased in asymptomatic patients with vulnerable carotid atherosclerotic plaques independent of gender. Accordingly, serum NGAL may be proposed as a valuable biomarker for the detection of unstable carotid plaques in asymptomatic patients, who can then be selected for early carotid endarterectomy or stenting.

Keywords: Neutrophil gelatinase associated lipocalin, MMP-9/NGAL complex, Asymptomatic carotid atherosclerosis, Vulnerable plaque, Echolucent plaque

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INTRODUCTION

According to the 2011 American Heart Association (AHA) guidelines and the 2017 European Society of Vascular Surgery (ESVS) carotid guidelines, all patients with asymptomatic >70% carotid artery stenosis should be offered risk

factor control and optimal medical therapy.[1,2] Carotid endarterectomy (CEA) should only be considered in highly selected patients provided the procedural risk is <3%.[2,3] The AHA, however, does not define "highly selected". Although stroke rates have declined progressively with modern medical therapy,[4,5] a small proportion of asymptomatic patients are still likely to benefit from CEA. Identifying these patients would be beneficial; however, validated clinical biomarkers are not currently available.[2,3] In addition, no single imaging modality can reliably identify the vulnerable plaque in relation to the development of future stroke.[6]

Neutrophil gelatinase associated lipocalin (NGAL) is found in granules of activated human neutrophils.[7] NGAL is also detected in atherosclerotic plaques with heavy inflammatory cell infiltration.[8–10] The present authors and others have shown that NGAL is expressed by macrophages, smooth muscle cells, and endothelial cells in human carotid atherosclerotic tissue.[8,10] Additionally, NGAL upregulates the production of pro-inflammatory cytokines such as interleukin (IL)-6, IL-8, and monocyte chemoattractant protein (MCP)-1 in vitro in those cell types.[10]

NGAL is also known to form a stable complex with the extracellular matrix degrading enzyme matrix metalloproteinase-9 (MMP-9), preventing its inactivation.[11] Prolonged activity of MMP-9 within a carotid plaque may promote vulnerability to embolisation.[12] Interestingly, serum NGAL levels are higher in patients with symptomatic carotid artery stenosis, compared with asymptomatic patients.[13] Likewise, NGAL mRNA content is higher in atherosclerotic carotid plaques from symptomatic patients.[10] This study addresses the key question of whether unstable plaques prone to embolisation express higher levels of NGAL in the subgroup of asymptomatic patients.

The aims of the current study were firstly to investigate the relationship between circulating NGAL and MMP-9/NGAL levels and plaque morphology as detected by preoperative duplex sonography; secondly, to address the relationship between NGAL and plaque morphology as detected by histological examination specifically in patients with asymptomatic carotid artery stenosis; and thirdly, to correlate plaque histo morphology with ultrasound characteristics. The goal was to evaluate whether NGAL can be used pre-operatively as a biomarker of plaque instability in asymptomatic patients. In addition, possible gender specific differences were analysed.

METHODS

Study population

Eighty-three consecutive patients with asymptomatic ≥70% carotid artery stenosis who underwent CEA between 2011 and 2014 at the Department of Surgery, Division of Vascular Surgery at the General Hospital of Vienna, Medical University of Vienna, Austria were included in the study. Patients suffering from acute infection, autoimmune or neoplastic disease were excluded. Patients were defined as having an asymptomatic carotid stenosis if they reported no ipsilateral neurological symptoms within the preceding six months. All included patients were Caucasian. The study was reviewed and approved by the ethics committee of the Medical University of Vienna (EK 269/2009) and all participants gave their written consent. Indication criteria for surgery included 1) ≥ 70% stenosis with presence of "soft" lesions on ultrasound or 2) ≥ 90% stenosis in line with the recently updated recommendation of the ESVS.[3,4,14] Coronary artery disease (CAD) and arterial hypertension were defined according to the AHA.[14]

Duplex sonography

Pre-operative duplex sonography was used to classify carotid plaques according to echogenicity into "soft" (echolucent > 25%), calcified/hard (echogenic > 25%), and mixed lesions (echolucent < 25%, echogenic < 25%).[15] Furthermore, the extent of carotid artery stenosis (in percentage) and blood velocity (in m/s) was recorded. The ultrasound measurements were cross validated by two technologists blinded to the study and finally approved by a medical doctor.

CT/MRI

Pre-operative computed tomography (CT) or magnetic resonance imaging (MRI) was performed in all patients.

Blood sampling

On the day before surgery, between 10 and 12 am, peripheral venous blood was drawn into serum clot activator tubes. After at least 1 h at room temperature, the blood was processed into serum by centrifugation at 3000 rpm at 4 °C for 15 min. Serum was stored in aliquots at −80 °C until further analysis.

NGAL and MMP-9/NGAL measurement

Serum NGAL and MMP-9/NGAL levels were measured using commercially available enzyme linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA; catalog number DLCN20 and DM9L20, respectively).[13,16] All assays were performed by investigators blinded to patient data.

Carotid endarterectomy

Surgery was performed under general anaesthesia using the "non-touch" technique. All patients were operated on under protective hypertension or shunting if oxygen saturation decreased by more than one third. To prevent embolisation, all patients received standard systemic heparinisation (unfractionated, adjusted to body weight and renal function) at the beginning of the operation. A linear arteriotomy was performed, and the plaque removed and sent for histological and biochemical evaluation. If necessary, distal intimal tacking sutures were applied and all patients underwent routine patching.

Histological investigation

Histological classification of the endarterectomy specimens was performed according to the AHA classification into type

IV (confluent extracellular lipid core), type V (fibroatheroma), type VI (complex plaque with possible surface defect, haemorrhage, or thrombus), type VII (calcified plaque), and type VIII (dominated fibrous tissue).[17] Accordingly, type VI was considered as an unstable or vulnerable atherosclerotic lesion. The histological analysis was performed by trained pathologists, who were blinded to the study, and validated by an experienced colleague. There were no significant inter-observer differences.

Statistical analysis

Normally distributed continuous variables are stated as mean (standard deviation), and non-normally distributed variables as median (interquartile range). Absolute numbers and percentages were used to describe categorical variables. Differences in continuous variables were tested using a two sample *t* test; non-normally distributed variables were compared using the Wilcoxon rank sum test. Log transformed values of MMP-9/NGAL were used because of the skewed distribution. Categorical variables were compared using the chi-square or the Fisher's exact test, as appropriate. Receiver operating characteristic (ROC) curve analyses were performed to evaluate the diagnostic power of NGAL and MMP-9/NGAL in discriminating between ultrasound characteristics, and in detecting grade VI lesions, respectively. Multivariable logistic regression models for detection of grade VI lesions were calculated using NGAL and MMP-9/NGAL levels together with ultrasound characteristics. All p values are the result of two sided tests and $p < .05$ was considered to be statistically significant. SAS software version 9.4 (SAS Institute Inc. 2002–2012; Cary, NC, USA) was used for statistical analyses.

RESULTS

Characteristics of the patients

Patient characteristics are shown in Table 1, stratified according to histological evidence of plaque stability (AHA type $\leq$ V and $\geq$ VII) or instability (AHA type VI). One patient was excluded from the study because of pneumonia two days after surgery. There were no significant differences with regard to demographic or routine laboratory parameters between the two study groups.

Pre-operative ultrasound showed that 25 of 83 patients (30.1%) had echolucent "soft" plaques, 19 (22.9%) were described as "mixed," and another 39 (47%) as "calcified" plaques. Histological analysis revealed 19 of 83 (22.9%) plaques to be unstable type VI plaques. The histological analysis correlated significantly with the previously performed ultrasound examination. According to ultrasound, there were 12 type VI plaques in 25 patients with "soft" plaques. However, grade VI plaques were also found in patients with mixed (4 of 19) and calcified (3 of 39) plaques defined by ultrasound.

Surgical outcome

In this series of patients there was no single patient with peri- or post-operative TIA or stroke. The complication rate was as follows: in total, three patients underwent surgical revision (3.6%). Two patients developed a haematoma post-operatively and were re-operated on as a consequence (2.4%). One patient developed a pseudoaneurysm within the first two weeks after surgery (1.2%) and underwent revision. Two patients showed temporary signs of hypoglossal paresis and problems with swallowing (2.4%). At three months' follow up, there were no neurological symptoms.

NGAL and MMP-9/NGAL serum levels according to histological plaque type

NGAL serum levels in patients with AHA type VI plaques were almost twice as high compared with other plaque types: 116.9 $\pm$ 41.4 ng/mL vs. 67.3 $\pm$ 26.4 ng/mL, $p <$ .0001 (Fig. 1A). The serum levels of the MMP-9/NGAL complex were also significantly higher in patients with type VI plaques: 53.5 (31.8–68.2) ng/mL, compared with patients with other plaque types (29.1 [18.0–45.8] ng/mL, $p = .0078$, Fig. 1B).

NGAL levels according to plaque echolucency

Serum NGAL levels were significantly higher in patients with echolucent, "soft" carotid plaques ($n = 25$) on duplex ultrasound (Fig. 2A) (mean [SD]: 111.7 [36.4] ng/mL), compared with patients with calcified or mixed plaque morphology ($n = 58$, 64.4 [27.1] ng/mL, $p < .0001$). Similarly, patients with "soft," echolucent carotid plaques also had higher MMP-9/NGAL complex levels (median [quartiles]: 46.2 [31.8–77.8] ng/mL), compared with patients with calcified or mixed plaque morphology (27.0 [17.8–42.4] ng/mL, $p = .0003$).

No significant differences were observed for NGAL serum levels, with respect to the degree of carotid stenosis ($\geq$90% vs. $<$ 90%), (mean [SD]: 80.3 [38.1] vs. 72.1 [34.0] ng/mL, $p = .39$, Fig. 3A). Similar results were obtained for MMP-9/NGAL complex levels, which also were not significantly different between these two subgroups (median [quartiles]: 36.7 [20.0–54.1] vs. 29.1 [15.0–50.5] ng/mL, $p = .37$, Fig. 3B).

ROC analysis: logistic regression models

Receiver operating characteristic (ROC) curve analysis was calculated for NGAL levels with respect to detection of grade VI carotid lesions. The ROC showed an area under the curve (AUC) = 0.85 (Fig. 4A). At a cut off level of 87.7 ng/mL, NGAL had a sensitivity and specificity of 78%. Above this threshold, plaques were more likely to be histologically graded as "unstable." In a multivariable logistic regression model with duplex sonography, NGAL did not further improve the detection of grade VI lesions (AUC = 0.86) (Fig. 5A). Odds ratios (95% CI) of the univariable and multivariable models are given in Table 2, identifying NGAL as an

Table 1. Demographics of asymptomatic patients with carotid artery stenosis studied for neutrophil gelatinase associated lipocalin (NGAL) for identification of vulnerable carotid plaques

Patients	Stable plaques (n = 64) AHA types ≤V or ≥VII	Unstable plaques (n = 19) AHA type VI	p value
Age — years	68.5 (7.7)	69.5 (10.1)	.648[a]
Sex, male — n (%)	32 (50)	7 (37)	.313[b]
Median stenosis grade in % (IQR)	90 (90–90)	90 (85–90)	.731[d]
Hypertension — n (%)	55 (86)	16 (84)	1.00[c]
Coronary artery disease — n (%)	19 (30)	4 (22)	.510[b]
Smoker — n (%)	29 (46)	7 (38)	.591[b]
Body mass index — kg/m^2	27.7 (4.4)	26.4 (3.3)	.247[a]
No PAD or Fontaine classification I	53 (83)	18 (95)	.620[b]
Median creatinin (IQR) — mg/dL	0.90 (0.80–1.05)	0.89 (0.82–1.14)	.753[d]
NGAL — ng/mL	67.3 (26.4)	116.9 (41.4)	<.0001[a]
Median MMP-9/NGAL concentration (IQR) — ng/mL	29.1 (18.0–45.8)	53.5 (31.8–68.2)	.008[a]
Ultrasound plaque characteristics — n (%)			<.001[b]
"Soft" (echolucent)	13 (20)	12 (63)	
Calcified (echogenic)	36 (56)	3 (16)	
Mixed	15 (23)	4 (21)	
Antihypertensive medication — n (%)	24 (37)	5 (28)	.466[b]
Anticoagulants and antiplatelet drugs/medication — n (%)	56 (88)	15 (79)	.457[c]
Median total cholesterol level (IQR) — mg/dL	163.5 (141.0–199.0)	166.0 (137.0–174.0)	.559[d]
Median LDL level (IQR) — mg/dL	82.5 (62.4–111.8)	82.0 (70.2–93.6)	.628[d]
HDL — mg/dL	52.1 (14.4)	54.4 (14.0)	.559[a]
Median triglyceride level (IQR) — mg/dL	129.0 (97.0–207.0)	122.0 (79.0–151.0)	.198[d]
Median neutrophil level (IQR) — g/L	5.1 (4.0–6.4)	5.8 (4.4–7.2)	.168[d]
Leukocytes — g/L	8.5 (2.7)	7.9 (2.8)	.399[a]

Data are given as mean (standard deviation) unless otherwise indicated. Antihypertensive medication includes inhibitors of angiotensin converting enzyme, β blocker, calcium channel blockers, and diuretics; antiplatelet drugs/medication and anticoagulants include clopidogrel, acetylsalicylic acid, low molecular weight heparin, and marcoumar. AHA = American Heart Association; HDL = high density lipoprotein; LDL = low density lipoprotein; MMP = matrix metalloproteinase; NGAL = neutrophil gelatinase associated lipocalin; PAD = peripheral artery disease; SD = standard deviation; IQR = interquartile range.

[a] t test.

[b] Chi-square test.

[c] Fisher's exact test.

[d] Wilcoxon rank sum test.

independent predictor of grade VI lesions. The ROC curve for MMP-9/NGAL with respect to grade VI AHA lesions is shown in Fig. 4C, with an AUC = 0.70. Combining MMP-9/NGAL with duplex sonography in a multivariable logistic regression model yields an increased AUC = 0.80 (Fig. 5B). Odds ratios (95% CI) are presented in Table 3.

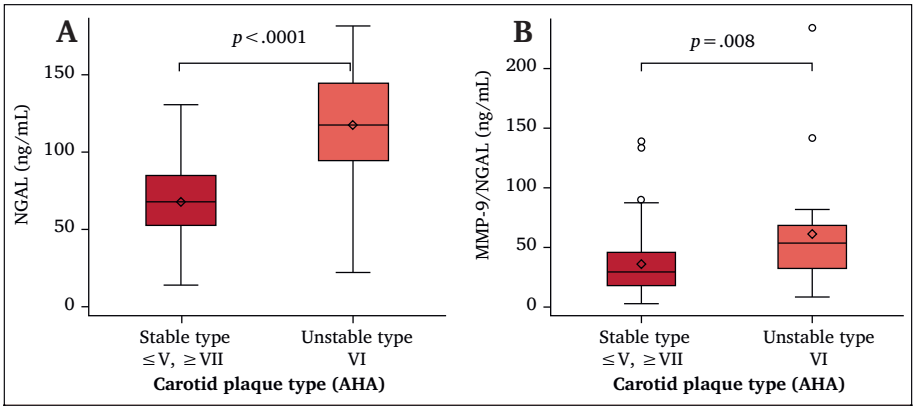

Figure 1. Box plots showing serum neutrophil gelatinase associated lipocalin (NGAL) (A) and matrix metalloproteinase (MMP)-9/NGAL (B) concentrations stratified according to histological American Heart Association (AHA) classification of atherosclerotic carotid artery lesions in patients with asymptomatic carotid stenosis.

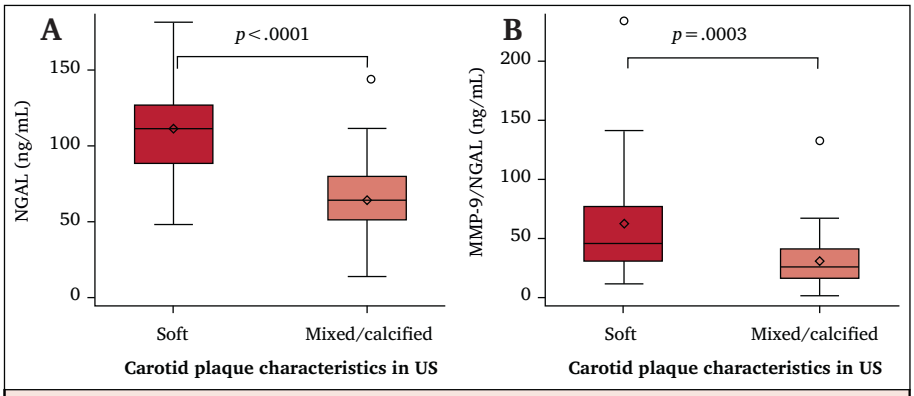

Figure 2. Box plots showing serum neutrophil gelatinase associated lipocalin (NGAL) (A) and matrix metalloproteinase (MMP)-9/NGAL (B) concentrations stratified according to ultrasound (US) characteristics of atherosclerotic carotid artery lesions in patients with asymptomatic carotid stenosis.

The presence of silent cerebral infarction on CT/MRI was also evaluated. Patients with pre-operative evidence of infarction ($n = 14$ vs. $n = 69$) had borderline significantly raised levels of NGAL (mean [SD]: 105.7 [57.1] vs. 73.1 [28.8] ng/mL, $p = .055$), but not of MMP-9/NGAL complex (median [quartiles]: 49.1 [23.0—77.8] vs. 32.9 [19.3—48.3] ng/mL, $p = .14$).

NGAL and MMP-9/NGAL distribution in male and female patients

Age, stenosis grade, and the presence of comorbidities did not differ significantly between women and men, with the exception of CAD, which was predominant in women (Table 4). Women also had significantly elevated levels of triglyceride compared with men. There were no significant gender specific differences in the prevalence of vulnerable plaque type VI ($p = .582$), soft plaques on ultrasound ($p = .503$), serum NGAL (mean [SD]: 80.9 [37.7] vs. 76.7 [36.3] ng/mL, $p = .607$), and MMP-9/NGAL levels (median [quartiles]: 33.0 [18.2—55.5] vs. 36.7 [20.2—54.0] ng/mL, $p = .969$, Table 4).

DISCUSSION

NGAL has been described as a potential diagnostic and prognostic biomarker for various diseases.[18—20] The present study investigated NGAL and MMP-9/NGAL as a potential biomarker of plaque instability in a selected group of asymptomatic patients with carotid atherosclerosis. Several studies have observed that NGAL expression is elevated in atherosclerotic plaques with high inflammatory components.[8,9] MMP-9 and numerous pro-inflammatory cytokines trigger a chronic inflammatory state in atherosclerotic lesions.[21—27]

AHA grade VI carotid lesions were associated with higher NGAL levels in this study. A cut off level of 87.2 ng/mL indicated that the plaque was more likely to be graded as "unstable" in the histological examination. Tissue NGAL and MMP-9/NGAL expression were previously shown to be associated with an unstable phenotype of atherosclerotic plaques.[9,10,28] The findings of the present study support previous publications on the association between NGAL and MMP-9/NGAL expression in carotid atherosclerotic tissue and circulating serum levels with an unstable plaque phenotype,[9,10,13,28] although, to the best of the present

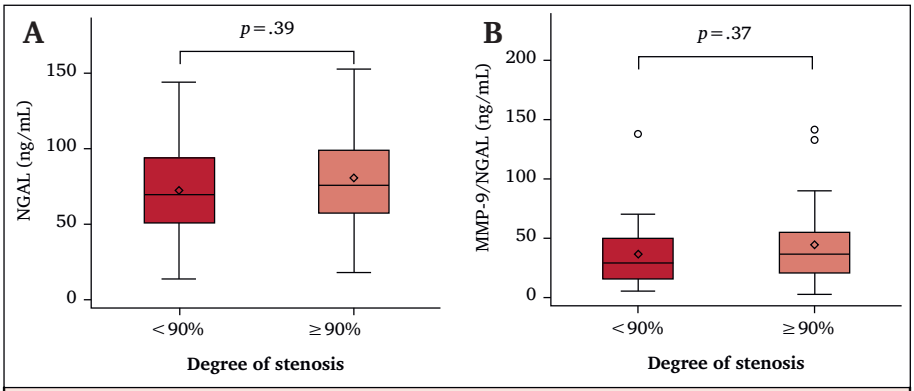

Figure 3. Box plots showing serum neutrophil gelatinase associated lipocalin (NGAL) (A) and matrix metalloproteinase (MMP)-9/NGAL (B) concentrations stratified according to degree of stenosis as < 90% or ≥90% by ultrasound.

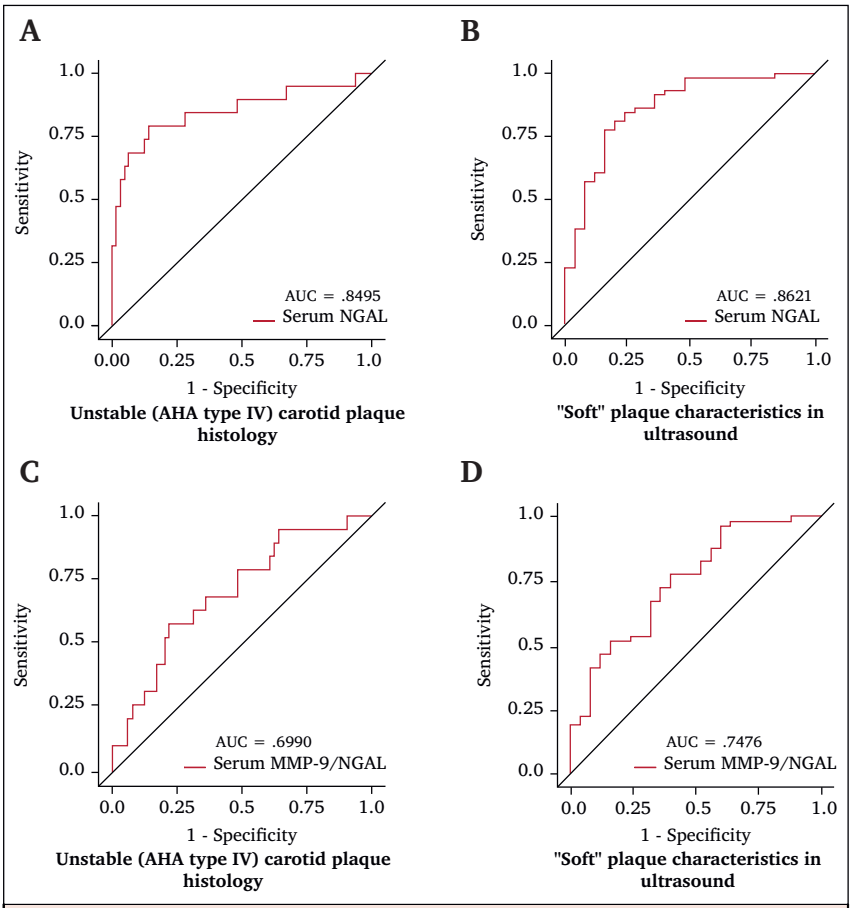

Figure 4. Receiver operating characteristic (ROC) curve analysis showing diagnostic strength of serum neutrophil gelatinase associated lipocalin (NGAL) with respect to American Heart Association (AHA) grade VI carotid lesions (A) and serum NGAL with respect to "soft" plaque ultrasound characteristics (B). ROC analysis showing diagnostic strength of serum matrix metalloproteinase (MMP)-9/NGAL concentrations with respect to AHA grade VI carotid lesions (C) and serum MMP-9/NGAL concentrations with respect to "soft" plaque ultrasound characteristics (D). AUC = area under the curve.

authors' knowledge, this is the first study dealing exclusively with asymptomatic patients. These findings suggest that serum NGAL and MMP-9/NGAL levels might be useful biomarkers for identifying asymptomatic patients with unstable carotid plaques. Previous studies demonstrated that type VI lesions were more likely to have evidence of intraplaque haemorrhage, which can be associated with higher rates of ipsilateral stroke compared with patients with other plaque types.[29,30]

There is little evidence that increasing carotid stenosis severity is associated with an increased risk of late ipsilateral stroke in asymptomatic patients.[3] In line with these findings, the current study found no evidence for an association between stenosis severity and NGAL or MMP-9/NGAL levels. A possible explanation for this is that NGAL, as an acute phase protein, reflects the degree of inflammation related to plaque vulnerability, which is not directly assigned to the degree of stenosis.[13,31]

Ultrasound based characteristics can provide valuable information on plaque vulnerability and stroke risk.[3,32] "Soft" (echolucent) plaques on ultrasound were associated with increased NGAL levels in the current study. ROC analyses identified NGAL to be a potentially useful biomarker for predicting the presence of an echolucent (more unstable) plaque. The presence of an echolucent plaque has been shown to be significantly associated with vulnerable lesions on post-operative histology.[13,29] Of note, less than half of the plaques graded as soft on ultrasound finally turned out to be vulnerable grade VI plaques. In addition, type VI plaques have also been detected in patients, whose plaques had been graded as mixed or calcified. As a consequence, NGAL may actually contribute to a change in selecting patients for surgery. Interestingly, serum NGAL levels in patients with grade VI plaques were comparable irrespective of ultrasound characteristics. Besides the relatively low number of patients, these facts may explain why the combination of duplex sonography and serum NGAL levels did not further improve the diagnostic marker potential. Nevertheless, NGAL may be of great use clinically, as plaque stability changes over time. In future,

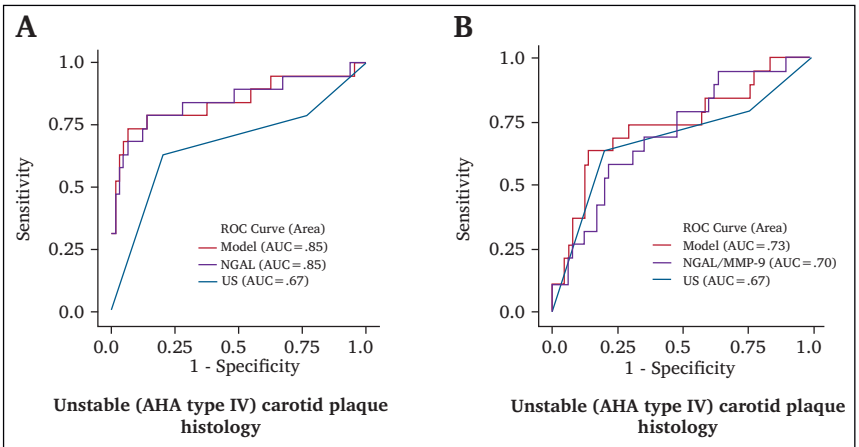

Figure 5. Receiver operating characteristic (ROC) curve analysis showing diagnostic strength of serum neutrophil gelatinase associated lipocalin (NGAL) combined (Model) with ultrasound (US) characteristics with respect to American Heart Association (AHA) grade VI carotid lesions (A) and serum matrix metalloproteinase (MMP)-9/NGAL combined (Model) with ultrasound characteristics with respect to AHA grade VI carotid lesions (B). AUC = area under the curve.

Table 2. Results of univariable and multivariable logistic regression models for serum NGAL and ultrasound in detecting AHA grade VI unstable carotid lesions in patients with asymptomatic carotid stenosis

	Univariable models		Multivariable model	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Neutrophil gelatinase associated lipocalin (NGAL) − ng/mL	1.05 (1.03−1.08)	< .0001	1.05 (1.02−1.08)	.0008
Ultrasound characteristics:				
Mixed vs. soft	0.29 (0.08−1.12)	.003	1.58 (0.28−9.03)	.413
Calcified vs. soft	0.09 (0.02−0.37)		0.48 (0.08−2.74)	

AHA = American Heart Association; CI = confidence interval; NGAL = neutrophil gelatinase associated lipocalin; OR = odds ratio.

Table 3. Results of univariable and multivariable logistic regression models for serum MMP-9/NGAL and ultrasound in detecting AHA grade VI unstable carotid lesions in patients with asymptomatic carotid stenosis

	Univariable models		Multivariable model	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Matrix metalloproteinase (MMP)-9/neutrophil gelatinase associated lipocalin (NGAL) − ng/mL, log transformed	2.84 (1.26−6.38)	.012	1.96 (0.82−4.70)	.131
Ultrasound characteristics:				
Mixed vs. soft	0.29 (0.08−1.12)	.003	0.43 (0.10−1.84)	.026
Calcified vs. soft	0.09 (0.02−0.37)		0.13 (0.03−0.57)	

AHA = American Heart Association; CI = confidence interval; MMP = matrix metalloproteinase; NGAL = neutrophil gelatinase associated lipocalin; OR = odds ratio.

NGAL levels may be measured on follow up visits to identify a "high risk of stroke" group of patients, who might benefit from early surgery.[3]

A trend was observed towards higher NGAL levels in patients with silent infarction on CT/MRI. The underlying cause may be plaques being more prone to embolisation to the brain arteries, as an inflammatory environment may increase the risk of thrombo-embolic events. These findings are in line with a recent publication by the present study group showing that type II diabetic patients with carotid artery stenosis had significantly higher rates of silent infarction compared with those without diabetes.[16]

In the present study, no significant gender specific differences were observed regarding the prevalence of plaque vulnerability as defined by ultrasound or histological

Table 4. Gender characteristics of asymptomatic patients with carotid artery stenosis studied for neutrophil gelatinase associated lipocalin (NGAL) for identification of vulnerable carotid plaques

Patients	Female (n = 39)	Male (n = 44)	p value
Mean age (SD) – years	67.3 (8.8)	70.1 (7.7)	.127[a]
Stenosis grade – %	90 (90–90)	90 (85.0–90)	.745[d]
Hypertension – n (%)	36 (92)	35 (80)	.090[c]
Coronary artery disease – n (%)	16 (41)	8 (18)	.026[b]
Smoker – n (%)	18 (46)	19 (43)	.803[b]
Mean body mass index (SD) – kg/m^2	27.9 (4.0)	26.9 (4.4)	.077[a]
No PAD or Fontaine classification I – n (%)	31 (80)	36 (86)	.562[b]
Creatinin – mg/dL	0.90 (0.86–1.03)	0.89 (0.77–1.23)	.853[d]
Mean NGAL level (SD) – ng/mL	80.9 (37.7)	76.7 (36.3)	.607[a]
MMP-9/NGAL – ng/mL	33.0 (18.2–55.5)	36.7 (20.2–54.0)	.969[a]
Histological classification of plaques based on AHA criteria – n (%)			.582[b]
Type ≤ V	18 (46)	19 (43)	
Type VI	7 (18)	12 (27)	
Type ≥ VII	14 (36)	13 (30)	
Ultrasound plaque characteristics – n (%)			.503[b]
"Soft" (echolucent)	14 (36)	11 (25)	
Calcified (echogenic)	16 (41)	23 (52)	
Mixed	9 (23)	10 (23)	
Statins – n (%)	33 (85)	34 (77)	.397[b]
Antihypertensive medication – n (%)	12 (32)	17 (38)	.542[b]
Anticoagulants and antiplatelet drugs/medication – n (%)	35 (90)	36 (82)	.306[b]
Total cholesterol – mg/dL	174.0 (139.5–201.0)	161.0 (137.8–179.8)	.116[d]
LDL – mg/dL	80.2 (68.8–110.3)	83.1 (60.6–107.6)	.229[d]
Mean HDL level (SD) – mg/dL	51.7 (14.2)	53.5 (14.4)	.595[a]
Triglyceride – mg/dL	179.0 (99.0–254.0)	120.5 (93.8–167.0)	.019[d]
Neutrophils – g/L	5.8 (3.9–6.9)	5.1 (4.3–6.3)	.883[d]
Mean leukocyte level (SD) – g/L	8.5 (2.6)	8.2 (2.8)	.593[a]

Data are presented as n (%) or median (interquartile range), unless indicated otherwise. Antihypertensive medication includes inhibitors of angiotensin converting enzyme, β blocker, calcium channel blockers, and diuretics; antiplatelet drugs/medication and anticoagulants include clopidogrel, acetylsalicylic acid, low molecular weight heparin, and marcoumar. AHA = American Heart Association; BMI = body mass index; CAD = coronary artery disease; HDL = high density lipoprotein; LDL = low density lipoprotein; MMP = matrix metalloproteinase; NGAL = neutrophil gelatinase associated lipocalin; PAD = peripheral artery disease; SD = standard deviation.

[a] t test.

[b] Chi-square test.

[c] Fisher's exact test.

[d] Wilcoxon rank sum test.

characteristics as well as regarding NGAL and MMP-9/NGAL levels. The possible protective influence of oestrogen in pre-menopausal women has not been confirmed in any study to date,[33,34] mainly because most female patients with clinically relevant carotid plaques are post-menopausal.[2]

The limitations of this study include a relatively small sample size, even though it was possible to provide a cut off threshold for NGAL of 87.7 ng/mL. Also, serum NGAL is sensitive to any inflammatory condition, which must be considered when interpreting the results.

In conclusion, NGAL may be a useful biomarker for predicting an increased risk of an unstable carotid plaque in otherwise asymptomatic patients. Unstable carotid plaque is an important prognostic marker for cerebrovascular events and its identification represents a significant challenge in the clinic. In the future, early surgery may be warranted in asymptomatic carotid patients with NGAL values exceeding the cut off threshold of 87.7 ng/mL, especially in combination with echolucent plaques and high degree of stenosis.

CONFLICTS OF INTEREST

None.

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COUP D'OEIL

Surgical Management of a Total Arteriovenous Fistula Aneurysm

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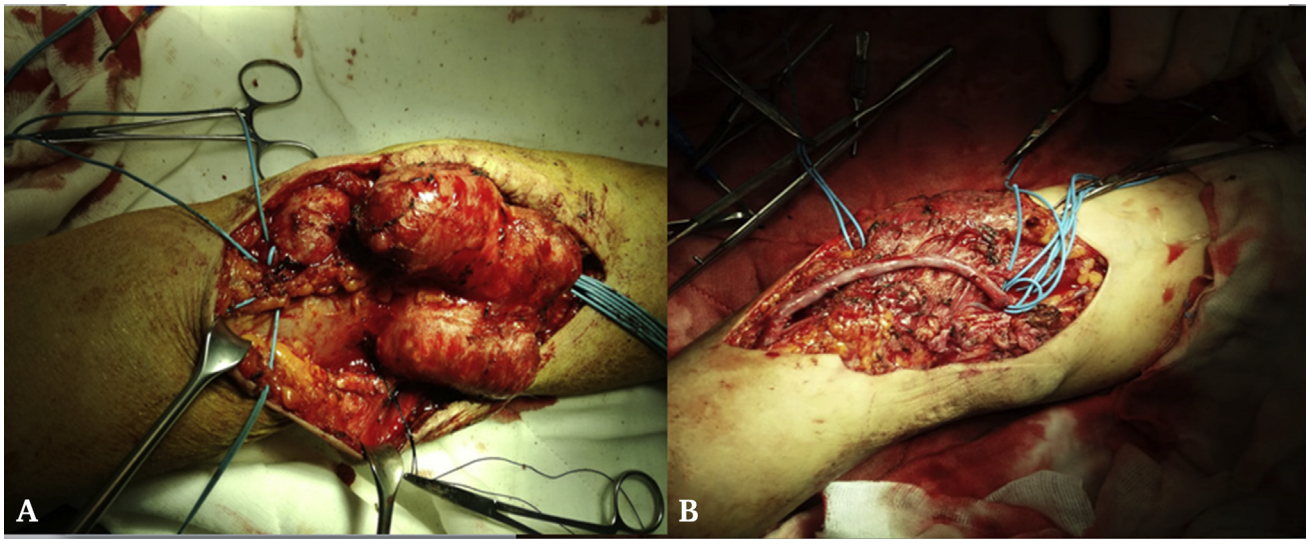

A 40 year old female with dialysis dependent end stage renal failure complained of right arm pain at the level of her brachiocephalic arteriovenous fistula which was no longer in use. The cephalic vein was aneurysmal (3 cm diameter) and completely thrombosed at the level of the dialysis puncture site. The brachial artery was also aneurysmal (4 cm diameter) with monophasic distal flow. Proximal and distal brachial artery control was established and all the aneurysmal segments of brachial artery and cephalic vein were excised. A proximal to distal brachial artery bypass using autologous vein was performed. The pain resolved after surgery.

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