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Elevated EMMPRIN serum levels indicate plaque vulnerability in patients with asymptomatic high grade carotid stenosis

Short Title: EMMPRIN serum levels in asymptomatic carotid stenosis

S. Kampf[1], A. Micko[2,] S. Stojkovic[3], M. Nackenhorst[4], S. Demyanets[5], W. Eilenberg[1], C. Krenn[6], J.Wojta[3,7,8], C. Neumayer[1]

1 Department of Surgery, Division of Vascular Surgery, Medical University of Vienna
2 Department of Neurosurgery, Medical University of Vienna
3 Department of Internal Medicine II, Division of Cardiology, Medical University of Vienna
4 Department of Pathology, Medical University of Vienna
5 Department of Laboratory Medicine, Medical University of Vienna
6 Department of Anesthesiology and General Intensive Care, Medical University of Vienna
7 Core Facilities, Medical University of Vienna
8 Ludwig Boltzmann Institute for Cardiovascular Research, Vienna

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Corresponding author:

Christoph Neumayer, MD
Department of General Surgery, Division of Vascular Surgery
Medical University Vienna
E-Mail christoph.neumayer@meduniwien.ac.at
P: 0043(0)1/40400/69560

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What does this study/review add to the existing literature and how will it influence future clinical practice

This study reports for the first time, that preoperative EMMPRIN serum levels are significantly higher in patients with asymptomatic carotid artery disease.

Abstract

Objectives

Carotid atherosclerosis, is an important cause of cerebral ischemic stroke. Sonographic plaques characteristics are inappropriate for exact prediction of possible future ischemic events. Additional markers are needed to forecast clinical outcome in high grade carotid stenosis. The aim of our study was to test extracellular matrix metalloproteinase inducer (EMMPRIN), due to the involvement in plaque formation/destabilization, as a potential marker of high-risk vulnerable plaques.

Methods:

EMMPRIN was analyzed in preoperative blood serum samples from patients with symptomatic and asymptomatic carotid artery stenosis by specific ELISA. Preoperative duplex sonography classified the atherosclerotic plaque due to echogenicity. Histopathological analysis of vulnerable and non-vulnerable plaques was based on the American Heart Association (AHA) classification.

Results:

265 patients undergoing carotid endarterectomy were included: 90 (m/f = 69/21) patients with symptomatic and 175 (m/f = 118/57) with asymptomatic disease. Analysis of circulating EMMPRIN revealed significantly higher levels in patients with echolucent plaques (4480 (IQR: 3745 - 6144) pg/ml) compared to echogenic plaques (4159 (IQR: 3418 - 5402) pg/ml, p = .025). Asymptomatic patients with vulnerable plaques had significantly higher levels of EMMPRIN (4875 (IQR: 3850 - 7016) pg/ml) compared with non-vulnerable plaques (4109 (IQR: 3433 - 5402) pg/ml, p < .001). In logistic regression analysis, duplex sonography combined with age, sex and clinical risk factors predicted vulnerable plaques in asymptomatic patients with an AUC of 0.71 (95% CI 0.61 – 0.80). EMMPRIN significantly improved the area under the curve in asymptomatic patients (AUC of 0.79 (95% CI 0.71 – 0.87, p = .014).

Conclusion:

Patients with high-risk plaques according to ultrasound and histopathological characteristics demonstrated increased serum EMMPRIN levels. EMMPRIN on top of clinical risk factors including age and gender as well as duplex sonography may be used for preoperative risk stratification in asymptomatic patients.

Introduction

Carotid artery stenosis is responsible for 10-20% of cerebral ischemic strokes in adults. (1) For these symptomatic patients, carotid endarterectomy (CEA) is the primary surgical approach. (2, 3) For investigation and grading of carotid artery stenosis, duplex sonography is the best noninvasive modality. Though, duplex sonography is inadequate for exact prediction of possible future ischemic events, new additional markers are needed to forecast poor clinical outcome in high grade carotid stenosis. (2)

Atherosclerotic plaques, which lead to carotid stenosis, are the main cause for stroke, TIA, retinal ischemia or even vascular death in the western population.(4) Plaque evolution is caused by endothelial dysfunction, accumulation of lipids, inflammatory cells and extracellular matrix (ECM) remodeling. Macrophages have been identified to secrete matrix metalloproteinases (MMPs) and to play a role in the depletion of collagen and inflammation, contributing to plaque vulnerability.(5) Extracellular matrix metalloproteinase inducer (EMMPRIN, CD147, Basigin) is a glycoprotein for induction and secretion of MMPs and thus might contribute to the pathogenesis and progression of atherothrombotic disease and plaque formation.(6-9) EMMPRIN can be found in vascular smooth muscle cells, as well as in human carotid plaques.(7, 10) Elevated expression levels of EMMPRIN and MMP-9 have been correlated with advanced atherosclerotic lesions, followed by plaque rupture, myocardial infarction and ischemic stroke.(11-15) The role of EMMPRIN in acute cerebral ischemia is not clearly understood. A mouse model showed EMMPRIN to participate in neurovascular remodeling after ischemic stroke and elevated levels may modulate MMP-9 activity. (16) Also CD147 expression was found to be rapidly increased directly after acute ischemic cerebral artery occlusion in a mouse model. The inhibition of EMMPRIN reduced the infarct size, improving functional outcome 3 days after the acute event, showing potential neuroprotective effects. (17)

Compared to normal blood vessels, the expression of EMMPRIN is significantly increased in human atherosclerotic vessels. (7)

Furthermore, several studies have implied an important role of EMMPRIN in plaque destabilization, due to the upstream regulation of matrix metalloproteinase. (6-9) However, clinical studies about serum levels of EMMPRIN in patients suffering from carotid artery stenosis are limited.(18)

The primary aim of this study was to evaluate preoperative serum EMMPRIN expression in a cohort of well-characterized patients undergoing CEA, to detect a possible difference in symptomatic and asymptomatic patients, and an association with different plaque characteristics.

Materials and Methods

This retrospective cohort analysis comprises of 265 consecutive patients undergoing CEA with selective intraoperative shunting at the Department of Surgery, Division of Vascular Surgery, Medical

University of Vienna, Austria. All patients underwent general anesthesia (TIVA) with intermittent elevation of blood pressure by vasopressor support. Near-infrared spectroscopy (NIRS) was used to monitor cerebral circulation. Systemic unfractionated heparinization (adjusted to body weight) was administered at the beginning of the CEA to prevent embolization.

For analysis, symptomatic patients with a neurological event (stroke, TIA) directly associated to carotid artery disease, as well as asymptomatic patients with high grade carotid stenosis scheduled for elective CEA were included.

Patients with inflammatory disease, malignant disease, chronic or acute infection or autoimmune disease were excluded.

All blood samples were collected within 24 hours prior to surgery.

The study has been reviewed and approved by the institutional review board of Medical University of Vienna (IRB number: 269/2009) and all participants have given their informed consent.

Patient characteristics

Patients were either transferred to the Division of Vascular Surgery for acute CEA due to a neurological event caused by carotid stenosis, or seen at the outpatient department for planned CEA due to high grade (≥ 70%) carotid stenosis.

Therefore, the study cohort was divided into two groups:

Group 1: symptomatic patients with at least one neurological event directly associated with carotid stenosis within 30 days prior to surgery.

Group 2: asymptomatic patients with high grade carotid stenosis ≥ 70% of luminal diameter.(19)

Additionally, the following parameters were evaluated from patient files: age at time of operation (years); gender; body mass index (BMI); peripheral artery occlusive disease (PAOD); preoperative transient ischemic attack (TIA; symptoms lasting from minutes to a maximum of 24 hours, and without evidence of infarction on imaging) (20) ; cerebral infarction (seen on preoperative computed tomography angiography (CTA), magnetic resonance angiography (MRA)); coronary artery disease (CAD); myocardial infarction (MI); additional preexisting conditions (chronic obstructive pulmonary disease, COPD; diabetes mellitus, insulin dependent/non-insulin dependent diabetes mellitus (DM); hyperlipidemia; hypertension) drug abuse (alcohol; nicotine); antiplatelet therapy; preoperative statins therapy including which statin agent;

Furthermore, preoperative laboratory parameters taken at the time of inpatient admission were investigated: total cholesterol (low-density lipoprotein, LDL; high-density lipoprotein, HDL; triglycerides, TRIG; HDL ratio; Apolipoprotein a1, ApoA1; ApoB); glycated hemoglobin (HbA1c); Vitamin D (25-Hydroxy, 1,25 Dihydroxy); C-reactive protein (CRP); brain natriuretic peptide (BNP); creatinine (CREA);

Ultrasound and histopathological plaque analysis

Preoperative duplex sonography (GE Logiq E9, GE Healthcare GmbH, Beethovenstr. 239, 42655 Solingen) was used to classify carotid plaques according to echogenicity into “soft” (echolucent), “hard” (calcified, echo-rich) and “mixed” lesions. (21) Examinations were performed by 3 trained Medical Technology Assistants (MTA), during every examination 2 MTAs were present for cross-checking results.

The degree of stenosis was defined by the NASCET criteria according to the ESC guidelines and measured by duplex- sonography in the disease-free internal carotid artery (ICA) segment above the stenosis. (20, 22) Velocity waveforms were obtained from the common carotid artery (CCA) and ICA. The angle between the ultrasound beam and the direction of blood flow was maintained at ≤ 60°. Elevated flow-velocity implied high-grade stenosis, diminished flow velocities implied near-occlusion velocity implied high-grade stenosis, no detectable flow implied complete occlusion of the vessel. Percentage diameter stenosis was defined as 1 – (residual lumen diameter (RLD) / vessel lumen diameter (VLD)) x 100. VLD was defined as intima – to – intima measurement, RLD was defined as corresponding measurement at the narrowest section of the vessel wall. (23)

Carotid plaque specimens, taken intraoperatively, were analyzed at the Department of Pathology. Samples were embedded in FFPE (formalin-fixed paraffin-embedded) and 3 µm sections were cut by experienced technicians. Specimen were stained with hematoxylin-eosin and Elastica-van-Gießen staining according to standard in-house procedures. Analysis was performed by light microscopy. Histological specimens were classified according to the modified American Heart Association (AHA) Classification of atherosclerosis. (24, 25) Vulnerable Plaques were defined as Grade VI according to AHA classification.

Tissue processing

Within 24 hours before surgery, blood serum samples were collected and stored at -80 °C for further analysis at the Department of Cardiology.

For EMMPRIN measurements samples and reagents were brought to room temperature and the samples were analyzed with the Quantikine® Human EMMPRIN ELISA kit (R&D System Inc., Minneapolis, MN, USA), following the manufacturer’s recommendations (sensitivity: 9.77 pg/mL, specificity: natural and recombinant human EMMPRIN).

Statistical analysis

Data are presented as means (± standard deviation) as well as median (interquartile range) for continuous variables and as frequencies for categorical variables and evaluated via contingency tables.

To test significance of normally distributed values, an unpaired t-test was used to check for EMMPRIN expression within the defined groups and plaque qualities. For a further binary analysis, mixed plaques were excluded for EMMPRIN evaluation.
For statistical analyses SPSS® version 25.0 software (SPSS Inc., Chicago, IL, USA) was used.
A p-value < .05 was considered significant.

Results

Patient characteristics

Two-hundred-sixty-five consecutive patients were included in this study. Ninety patients with symptomatic (group 1) and 175 with asymptomatic (group 2) carotid stenosis. No significant differences in regard to gender, age or BMI at time of surgical intervention could be identified (**Table 1**).
A history of TIA dating back > 6 months and minor cerebral infarction seen on neuroradiological imaging were found in both in asymptomatic and symptomatic patients. However, a significantly higher rate of preoperative TIA (group 1: 63% vs group 2: 10%, p < .001) and cerebral infarction (group 1: 58% vs group 2: 16%, p < .001) was found in patients with symptomatic disease.
Median time from onset of neurological symptoms to surgery in the symptomatic group was 8 days (IQR: 4 – 27).
PAOD was significantly more often diagnosed in patients with asymptomatic (42%) than in patients with symptomatic carotid stenosis (19%, p < .001). (**Table 1**) Antiplatelet therapy was significantly more often prescribed in the asymptomatic cohort (96%) than in the symptomatic cohort (89%, p = .013). The overall intake of statin therapy was evenly distributed between groups, however there were some differences regarding the intake of exact substance. Atorvastatin was more frequently used within the symptomatic patients (p < .001) and simvastatin within the asymptomatic patients (p = .004). Overall, 12/265 (5%) patients presented with no preoperative intake of statins. (**Table 1**) Pre-existing conditions such as coronary artery disease (CAD), myocardial infarction or others that occurred within patients' cohorts were not significantly different between groups. For further details see **Table 1**.
In case of preoperative laboratory parameters, no significant differences could be determined between groups (**Table 2**).

Plaque characteristics

Sonographic features:

Overall, soft plaques could be found in 122/265 (46%), hard plaques in 98/265 (37%) and mixed plaques in 45/265 (17%) of patients.

In regard to the defined groups, soft plaques (70% vs 46%) and mixed plaques (31% vs 10%) were diagnosed more often in the symptomatic group than in the asymptomatic group, whereas hard plaques were found less often in the symptomatic cohort than in the asymptomatic cohort (22% vs 45%).
In case of preoperatively found minor or major infarction in neuro-radiological imaging (regardless of the time of insult), soft plaques 46/62 (74%) were found significantly more often than hard plaques 16/62 (26%, $p < .001$).
A histopathological examination was possible in 83/90 (92%) of the symptomatic and 157/175 (90%) cases of the asymptomatic group. **(Figure 4 and Figure 5)**
The overall distribution between grade IV to VIII showed no statistically significant differences ($p = 0.53$) between groups (symptomatic vs asymptomatic): grade IV = 1/83 (1%) vs. 1/157 (0.1%); grade V = 22/83 (27%) vs. 31/157 (20%), grade VI = 34/83 (41%) vs. 57/157 (36%), grade VII = 14/83 (17%) vs. 33/157 (21%), grade VIII = 12/83 (14%) vs. 35/157 (22%).

Analysis of EMMPRIN

Overall analysis of EMMPRIN expression revealed a significantly higher levels in patients with soft plaques (4480 IQR: 3745 – 6144 pg/mL) in comparison to patients with hard plaques (4159 pg/mL IQR: 3418 – 5402; $p = .025$), considering duplex sonography (**Figure 1**). Within the groups a statistically significant higher level of EMMPRIN was seen in case of asymptomatic carotid stenosis (soft plaques 4706 pg/mL (IQR:3753 – 6352) vs. hard plaques 4534 pg/mL (IQR: 3452- 5805); $p = .040$).
To rule out a possible influence of the significantly higher rate of PAOD in group 2, patients were excluded in a subgroup analysis, leading to an unchanged statistically significant EMMPRIN level in patients with soft plaques (4285pg/mL, IQR: 3664 – 5260) in comparison to patients with hard plaques (4122pg/mL, IQR: 3418 – 5088; $p = .019$).
There was a trend towards higher levels of EMMPRIN in symptomatic patients with soft plaques, however it did not reach statistical significance (soft plaques: 4245 pg/mL (IQR: 3608 – 5187) vs. hard plaques: 3781 pg/mL (IQR: 3149 - 4805); $p = .055$) (**Table 3**).

Patients with histologically classified plaques as grade VI according to the AHA classification showed the highest levels of EMMPRIN (4682pg/mL, IQR 3611 – 6565). In contrast, patients with grade VIII plaques according to the AHA classification showed the lowest circulating levels of EMMPRIN (4061pg/mL, IQR: 3501 – 5180).
Overall EMMPRIN levels in patients with histopathological vulnerable (grade VI) plaques were significantly higher (4682pg/mL, IQR: 3611 – 6565) than in non-vulnerable (grade IV, V, VII, VIII) plaques (4106pg/mL, IQR: 3522 – 5175; $p < .001$) (**Figure 2**).
Furthermore, patients in the asymptomatic group with vulnerable plaques had significantly higher

EMMPRIN levels (4875pg/mL, IQR: 3850 – 7016) than patients with non-vulnerable plaques (4109pg/mL, IQR: 3433 – 5402; p < .001) according to AHA classification.
In a subgroup analysis of all asymptomatic patients with duplex sonographic "hard" plaques (100/175 57%), 45/100 (45%) were additionally declared as histologically "vulnerable" plaques.
Of these, 60% had EMMPRIN serum levels above the calculated cut-off levels of 4151 pg/ml.

Receiver operating characteristic curve (ROC) was used to predict performance of serum EMMPRIN levels for distinguishing vulnerable and non-vulnerable plaques in the asymptomatic cohort. Based on our data, circulating EMMPRIN levels under the calculated cut-off value of 4151 pg/ml for EMMPRIN indicate highly vulnerable plaques in asymptomatic patients with a sensitivity of 64.5% and a specificity of 54.4% (AUC 0.64).
In the logistic regression analysis, preoperative duplex sonography in combination with age, sex and clinical risk factors including arterial hypertension, hypercholesterolemia, diabetes, coronary and peripheral artery disease, smoking status predicted the occurrence of vulnerable plaque in asymptomatic patients with an AUC of 0.71 (95% CI 0.61 – 0.80). When EMMPRIN was added to the same logistic regression model, it significantly improved the area under the curve for prediction of vulnerable plaque in asymptomatic patients with an AUC of 0.79 (95% CI 0.71 – 0.87, p = .014).

In contrast, EMMPRIN levels in patients with vulnerable plaques showed no statistical difference to patients with non-vulnerable plaques (grade IV, V, VII and VIII) in the symptomatic group (vulnerable plaques 4155 pg/mL (IQR: 3514 – 5199) vs. non-vulnerable plaques 4093 pg/mL (IQR: 3569 – 4913); p = .12) (**Table 4, Figure 2**).

Regarding EMMPRIN distribution among genders, no significant difference of EMMPRIN levels was found between male and female patients in group 1 (p = .31), nor in group 2 (p = .65).
However, male patients with vulnerable plaques had the highest levels of EMMPRIN (4767 pg/mL, IQR: 3917 – 6708) in comparison to female patients with vulnerable plaques (4278 pg/mL, IQR: 3212 – 5288) or non-vulnerable plaques (4543 pg/mL IQR: 3624 – 6070). Additionally, male patients with vulnerable plaques had significantly higher levels of EMMPRIN, than male patients with non-vulnerable plaques (4020pg/mL, IQR: 3334 – 4939; p < .001). **(Table S1)**

Furthermore, no statistical significance could be found considering preoperative serum EMMPRIN levels and patients with/or without statin intake (4311 pg/mL (IQR: 3544 – 5475) vs 5224 pg/mL (IQR: 3569 – 5856); p = .41).
Statins were not associated with symptomatic presentation in univariate analysis (OR 1.6 95% CI, -0.5 – 5.8, p = .42). Previous TIA and cerebral infarction, were significantly associated with

recurrence of TIA and cerebral infarction, as expected (TIA: OR 15.1 95% CI 7.8 -28.8, p < .001; Cerebral Infarction: OR 7.2 95% CI 4.1 – 12.8, p < .001).
In contrast peripheral artery disease and antiplatelet disease was significantly associated with asymptomatic carotid stenosis in univariate regression analysis (PAOD: OR 0.32 95% CI 0.17 – 0.58, p < .001, antiplatelet therapy: OR 0.28 95% CI 0.1 -0.8, p < .001). In multivariate regression model including TIA, cerebral infarction, PAOD and EMMPRIN, EMMPRIN remained an independent significant predictor of vulnerable carotid artery stenosis (p = .043).

Discussion

The preoperative classification of high-risk vulnerable plaques in asymptomatic patients is still challenging. To our knowledge this study is the first and largest in vivo study to evaluate preoperative serum EMMPRIN levels in symptomatic and asymptomatic patients with carotid artery stenosis.

A recent study found increasing serum levels of EMMPRIN in patients with TIA onset < 24h, correlating the serum levels of EMMPRIN with the risk of a possible stroke after a recent episode of TIA.(18) This is comparable to our results considering plaque vulnerability, but not explicit in the symptomatic cohort, where we could only show a trend of higher EMMPRIN levels, but no statistical significance. We hypothesize, that the time of blood drawing could be a possible confounder. Blood samples in our symptomatic cohort were taken > 48h after the acute event, which is not comparable to Xu et al, where patients with an onset time > 24hours were excluded.(18) In average, blood was drawn at 8 days (IQR: 3 -27) after the acute event. Xu et al correlated elevated EMMPRIN serum levels within < 24h of the acute event. The half-life of EMMPRIN has been shown to be between 12-16 h.(26) Therefore, we suspect a possible influence on the non-significant results in our symptomatic group and hypothesize a possible decrease of EMMPRIN after the acute event. Also, the limited number of patients in our symptomatic group could distort these results. Further studies with serial blood sampling after the acute event are needed to role out any possible bias. In addition, the correlation of EMMPRIN with natural cause follow up outcomes in asymptomatic patients might be an interesting topic for future investigations.

Considering gender diversity, asymptomatic male patients with high-risk vulnerable plaques did show the highest levels of EMMPRIN. Especially for this subgroup of patients, increased serum levels may represent a new marker, which could predict the risk for future acute vascular events.

Statins are inhibitors of 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase. Apart from their lipid lowering activity, statins have shown to have immunomodulatory effects in vitro and in animal models and have been shown to inhibit the upregulation of CD147 during monocytic differentiation in vitro. The authors of a recent in vitro study with fluvastatin, atorvastatin and

pravastatin conclude all three statins have an anti-inflammatory effect on monocytes and macrophages by influencing CD147. (27) In our cohorts, the overall intake of statins was not significantly different between the asymptomatic and symptomatic group. Although the symptomatic group showed a significantly higher intake of atorvastatin, and the asymptomatic cohort a significantly higher intake of simvastatin. Randomized placebo-controlled trials suggest pravastatin, simvastatin and atorvastatin, when used at their standard dosages to be equally effective in their long-term cardiovascular prevention. (28) Although, in literature, there is evidence from animal studies of statins having an effect on the downregulation of EMMPRIN expression in atherosclerotic plaques, best to our knowledge, no evidence for the downregulation of in vivo serum EMMPRIN levels has been published by now. As compared with the recent study (27), we do not find EMMPRIN levels to be associated with the intake of either simvastatin or atorvastatin in our cohort. Furthermore, statins were not associated with symptomatic presentation in univariate analysis. In the current literature, only a small case series evaluated the effect of perioperative statin therapy on serum EMMPRIN levels. (29) The authors stated, that intensive statin therapy could improve outcomes in patients undergoing middle cerebral artery stenting after ischemic stroke. The statin-treated group showed both lower EMMPRIN levels and more favorable outcomes.
Prospective studies have to be conducted to further evaluate the effect of various statins on lipid parameters and EMMPRIN levels over time.

Another chinese trial including 68 patients, showed EMMPRIN to have good diagnostic potential for early detection of carotid intraplaque hemorrhage (IPH). (30) IPH was analyzed by MRA scan and serum EMMPRIN levels were significantly higher in patients with vulnerable plaques. This observation also supports our results, relating serum EMMPRIN levels to asymptomatic vulnerable carotid artery plaques.

The therapeutic potential of EMMPRIN against arterial thickening has also been examined in a rat model.(31) An EMMPRIN inhibitor (SP-8356) was evaluated and immunohistochemical analysis did show a significantly reduced MMP activity and therefore reduction of neointimal carotid hyperplasia in rats.
Our results could indirectly show, independent of possible confounders such as coronary artery disease(32), or PAOD(33) a significantly lower level of EMMPRIN in stable, and therefore more inactive plaques. Furthermore, the ROC analysis in asymptomatic patients revealed a moderate correlation (AUC 0.74) of vulnerability and EMMPRIN.
Several markers have been found to be associated with plaque vulnerability in histopathological analysis of patients with carotid artery stenosis. For example, higher circulating levels of neutrophil gelatinase-associated lipocalin (NGAL) and MMP-9/NGAL complex have been shown in a clinical study to be associated with symptomatic carotid stenosis.(34) Furthermore, cathepsin, chitinase 3-like-

1, S100 calcium binding protein A8/A9 have been proven to be upregulated in symptomatic carotid artery disease.(35)

According to our results, overall EMMPRIN levels revealed significantly higher values in duplex sonographic soft, as well as histopathological vulnerable plaques. A significant difference between duplex sonographic "soft' and histologically classified "vulnerable" plaques could be shown in asymptomatic patients, with significantly higher EMMPRIN levels in patients with "soft" lesions in duplex sonography and vulnerable lesions according to histological assessment. Therefore, preoperative analysis of serum EMMPRIN levels may be a useful additional marker to identify vulnerable plaques in neurologically asymptomatic patients more clearly.

In our opinion, the clear advantage of EMMPRIN is, that it has primarily been found to be upregulated in atherosclerotic plaque tissue,(11, 12) and has also been found to show elevated serum levels in patients with soft or vulnerable plaques in both our as well as in a Chinese cohort analysis of patients with TIA and respectively acute cerebral infarction and patients with intraplaque hemorrhage.(18, 30)

Limitations

A possible limitation of the present study is the retrospective character of the study design.

PAOD was found significantly more frequent in asymptomatic patients, however a subgroup analysis, excluding these patients, led to unchanged statistically significant results.

Furthermore, elevated LDL, CRP and high glucose levels can induce EMMPRIN expression in inflammatory cells in vitro.(15, 36, 37) However, no significant difference in LDL or CRP were found between the two defined groups.

The limited number of symptomatic patients and the long time between the acute event and the blood drawings could be a possible confounder for our non-significant results in this group. Therefore, future prospective studies are needed to evaluate a potential clinical application of serum EMMPRIN as an additional biomarker in patients with asymptomatic carotid stenosis.

Conclusion:

Serum EMMPRIN levels in patients with carotid artery stenosis undergoing CEA were significantly increased in patients with high risk soft and vulnerable plaques, especially in men, showing the highest levels. EMMPRIN on top of clinical risk factors including age and gender as well as duplex sonography may be used to improve preoperative risk stratification in the asymptomatic patients.

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Figure Legends

Figure 1. Boxplot of EMMPRIN levels between soft (blue column) and hard plaques (red column) in asymptomatic and symptomatic patients, evaluated by duplex sonography. (asymptomatic soft plaques: n = 80 vs. hard plaques: n = 78; symptomatic soft plaques: n = 42 vs. hard plaques: n = 20)

Figure 2. Boxplot of EMMPRIN levels between vulnerable (blue column) and non-vulnerable plaques (red column) in asymptomatic and symptomatic patients, evaluated by histopathological analysis. (asymptomatic vulnerable plaques: n = 89 vs. non-vulnerable plaques: n = 68; symptomatic vulnerable plaques: n = 57 vs. non-vulnerable plaques: n = 26)

Figure 3. Receiver operating characteristic (ROC) curve analysis, showing significantly improved diagnostic strength of preoperative duplex sonography if EMMPRIN was added to the logistic regression model.
Blue ROC area: preoperative duplex sonography in combination with age, sex and clinical risk factors predicting the occurrence of vulnerable plaque in asymptomatic patients
Red ROC area: preoperative duplex sonography and EMMPRIN in combination with age, sex and clinical risk factors predicting the occurrence of vulnerable plaque in asymptomatic patients

Figure 4. Histological examples for non-vulnerable plaques: A non-vulnerable plaque from an asymptomatic patient with calcification (arrows) is depicted in (a) and (b), HE. (c) and (d) show a non-vulnerable plaque from a symptomatic patient with marked thinning of the intima and areas of calcification (arrows), HE.

Figure 5. Histological examples for vulnerable plaques: vulnerable plaque (a) and (b) from an asymptomatic patient with areas of bleeding (*) and some calcification (arrow), HE. A vulnerable plaque from a symptomatic patient is shown in (c) and (d) with lifting of intimal cap (arrow point), HE.

What does this study/review add to the existing literature and how will it influence future clinical practice

This study reports for the first time, that preoperative EMMPRIN serum levels are significantly higher in patients with asymptomatic vulnerable carotid plaques, compared to patients with non-vulnerable plaques, strengthening the predictive value of carotid duplex sonography and contributing to a better patient selection. EMMPRIN on top of clinical risk factors including age and gender as well as duplex sonography may be of additional value in preoperative risk stratification in asymptomatic patients with vulnerable plaques.

Table 1. Patient characteristics

	Group 1 (symptomatic) n (%)	Group 2 (asymptomatic) n (%)	p-value
Number	90	175	
Age(y) (mean ±SD)	69 (±9)	69 (±10)	0.90
Gender (f:m)	1 : 3.3	1 : 2.1	0.10
BMI	28 (±4)	27 (±4)	0.35
PAOD	17 (19)	74 (42)	< 0.001
TIA (≥ 1 event)*	57 (63)	18 (10)	< 0.001
Cerebral infarction	52 (58)	28 (16)	< 0.001
CAD	24 (27)	64 (37)	*0.09*
Myocardial infarction	12 (14)	37 (22)	0.1
Comorbidities			
COPD	16 (18)	25 (15)	0.54
DM Insulin dependent	7 (8)	18 (10)	0.48
DM Non-insulin dependent	83 (92)	157 (90)	
Hyperlipidemia	65 (72)	121 (69)	0.60
Hypertension	78 (87)	157 (90)	0.48
Drug abuse			
Alcohol	20 (23)	27 (18)	0.40
Nicotine	28 (32)	48 (32)	0.95
Antiplatlet Therapy	80 (89)	169 (97)	0.013
Statin Therapy	84 (93)	169 (97)	0.45
Atorvastatin	58 (69)	76 (45)	< 0.001
Ezetrol	4 (5)	8 (5)	0.99
Fluvastatin	3 (4)	6 (4)	0.99
Pravastatin	-	1 (1)	0.31
Rosuvastatin	5 (6)	21 (12)	0.07
Simvastatin	14 (17)	57 (34)	0.004

*TIA: TIA more than 6 months prior surgery

Table 2. Laboratory values

	Group 1 (symptomatic) mean (±SD)	Group 2 (asymptomatic) mean (±SD)	p-value
Total cholesterol (<160 mg/dL)	157 (±44)	166 (±39)	0.12
LDL (<70 mg/dL)	89 (±32)	84 (±31)	0.34
HDL (f >65 mg/dL, m >55 md/dL)	46 (±13)	50 (±16)	0.07
TRIG (<150 mg/dL)	154 (±96)	161 (±110)	0.63
HDL ratio (<4)	4.8 (±6)	4.2 (±4)	0.37
Apoa1			
f (108 - 225 mg/dL)	141 (±37)	149 (±54)	0.62
m (104 - 202 mg/dL)	129 (±27)	135 (±32)	0.46
Apob			
f (60 - 117 mg/dL)	98 (±37)	82 (±19)	0.39
m (66 - 133 mg/dL)	79 (±17)	89 (±25)	0.07
Hba1c (4.0 - 6.0 %)	6.3 (±1)	6.8 (±5)	0.31
Vitamin D			
25-Hydroxy (nmol/L)	50 (±31)	58 (±27)	0.29
1,25 Dihydroxy (pg/mL)	46 (±19)	46 (±17)	0.98
CRP (<0.5 mg/dL)	0.7 (±1)	1.1 (±3)	0.09
BNP (<125 pg/mL)	603 (±1344)	560 (±1303)	0.89
CREA			
f (0.5 - 0.9 mg/dL)	0.8 (±0.2)	0.8 (±0.2)	0.56
m (0.7 - 1.2 mg/dL)	1.1 (±0.4)	1.2 (±0.8)	0.54

f: female, m:male

Table 3. Correlation of serum EMMPRIN levels with Sonographic Features

	Soft Plaques median (IQR)	Hard Plaques median (IQR)	p-value
Overall - EMMPRIN	4480 (3745 – 6144) pg/ml	4159 (3418 – 5402) pg/ml	0.025
Symptomatic Group	4245 (3608 – 5187) pg/ml	3781 (3149 – 4805) pg/ml	0.055
Asymptomatic Group	4706 (3753 – 6352) pg/ml	4534 (3452 – 5805) pg/ml	0.041

Table 4. Correlation of serum EMMPRIN levels with Histopathologic Features (AHA Classification)

	Vulnerable Plaques median (IQR)	Non-vulnerable Plaques median (IQR)	p-value
Overall - EMMPRIN	4682 (3611 – 6565) pg/ml	4106 (3522 – 5175) pg/ml	<0.001
Symptomatic Group	4155 (3514 – 5199) pg/ml	4093 (3569 – 4913) pg/ml	0.119
Asymptomatic Group	4875 (3850 – 7016) pg/ml	4109 (3433 – 5402) pg/ml	0.001