Journal of the American Heart Association

BRIEF COMMUNICATION

Plaque Vulnerability Index Predicts Cardiovascular Events: A Histological Study of an Endarterectomy Cohort

Isabel Goncalves, MD, PhD; Jiangming Sun, PhD; Christoffer Tengryd, MD, PhD; Mihaela Nitulescu; Ana F. Persson; Jan Nilsson, MD, PhD; Andreas Edsfeldt, MD, PhD

BACKGROUND: The balance between stabilizing and destabilizing atherosclerotic plaque components is used in experimental studies and in imaging studies to identify rupture prone plaques. However, we lack the evidence that this balance predicts future cardiovascular events. Here we explore whether a calculated histological ratio, referred to as vulnerability index (VI), can predict patients at higher risk to suffer from future cardiovascular events.

METHODS AND RESULTS: Carotid plaques and clinical information from 194 patients were studied. Tissue sections were used for histological analysis to calculate the VI (CD68 [cluster of differentiation 68], alpha-actin, Oil red O, Movat pentachrome, and glycophorin A). Postoperative cardiovascular events were identified through the Swedish National Inpatient Health Register (2005–2013). During the follow-up (60 months) 45 postoperative cardiovascular events were registered. Patients with a plaque VI in the fourth quartile compared with the first to third quartiles had significantly higher risk to suffer from a future cardiovascular event (P=0.0002). The VI was an independent predictor and none of the 5 histological variables analyzed separately predicted events. In the 13 patients who underwent bilateral carotid endarterectomy, the VI of the right plaque correlated with the VI of the left plaque and vice versa (r=0.7, P=0.01).

CONCLUSIONS: Our findings demonstrate that subjects with a high plaque VI have an increased risk of future cardiovascular events, independently of symptoms and other known cardiovascular risk factors . This strongly supports that techniques which image such plaques can facilitate risk stratification for subjects in need of more intense treatment.

Key Words: atherosclerosis ■ histopathology ■ plaque rupture ■ vulnerable plaque

Approximately 70% of all myocardial infarctions and ≈15% of all ischemic strokes are caused by atherosclerotic plaque ruptures.[1,2] Although the cause of rupture remains unknown, such features as a large lipid core, hemorrhage, inflammatory cells, and thin fibrous cap have been associated with rupture-prone or high-risk plaques.[3] As the individual use of each feature is insufficient to identify high-risk plaques, it is more plausible that a feature combination is needed. Therefore, a ratio between the destabilizing components and stabilizing plaque components has been used to calculate the vulnerability index (VI) in both experimental atherosclerotic plaque studies and to validate novel imaging technologies.[4–6] The rationale behind VI is to capture the exquisite balance between plaque destabilizers (macrophages, hemorrhage, and lipids) and stabilizers (smooth muscle cell and collagen), which is ultimately decisive for rupture. In support of the importance of plaque composition assessment, a recent meta-analysis including data from over 20 000 patients, showed that plaques classified as high risk (based on lipid core, neovascularization, echolucency, and thin cap) were associated with higher risk to suffer from future stroke.[7] Importantly, these high-risk plaque features were not associated to the

Correspondence to: Andreas Edsfeldt, MD, PhD, Clinical Research Center, Jan Waldenströmsg 35, 60-12, Skåne University Hospital, SE-21428 Malmö, Sweden. E-mail: andreas.edsfeldt@med.lu.se

For Sources of Funding and Disclosures, see page 5.

JAHA is available at: www.ahajournals.org/journal/jaha

Table 1. Clinical Characteristics of the Patients Included in the Follow-Up Analysis and by Comparing Patients With Plaques in First to Third Quartiles of the Calculated Vulnerability Index to Patients With Plaques With the Highest Vulnerability Index in the Fourth Quartile

	All (n=181)	First–Third Quartile (n=136)	Fourth Quartile (n=45)
Age, y	70 (64.5–74)	69 (64–74)	72 (66.5–78)*
Symptomatic plaques, n (%)	84 (46)	54 (40)	30 (67)†
Sex, male, n (%)	126 (70)	91 (67)	35 (78)
Smoking current smokers, n (%)	61 (34)	54 (40)	7 (16)†
Degree of stenosis, n (%)	90 (80–95)	90 (81–95)	90 (80–95)
Diabetes mellitus, n (%)	58 (32)	39 (29)	19 (42)
Body mass index	27 (24–29)	27 (24–29)	27 (24–30)
Hypertension, n (%)	136 (75)	104 (79)	28 (62)
Medical treatment			
Blood pressure lowering, n (%)	145 (80)	110 (81)	35 (78)
Lipid lowering, n (%)	160 (88)	119 (88)	41 (91)
Blood markers			
High-sensitivity C-reactive protein, mg/L	4 (2.0–6.4)	3.9 (2–6.2)	4.0 (1.7–6.5)
HbA1c, mmol/mol§	44 (38–56)	42 (38–52)	48 (39–64)
Total cholesterol, mmol/L	4.3 (3.6–5.1)	4.3 (3.5–5.1)	4.3 (3.7–5)
Low-density lipoprotein, mmol/L	2.5 (2–3.2)	2.4 (2–3.2)	2.6 (2–3.2)
High-density lipoprotein, mmol/L	1.1 (0.9–1.3)	1.1 (0.9–1.3)	1.1 (0.9–1.3)
Triglycerides, mmol/L	1.3 (0.9–1.8)	1.2 (0.9–1.8)	1.6 (1–2)

Categorical variables are expressed in total amount and percentages. Continuous variables as median and interquartile range. Symptomatic plaques are defined as transient ischemic attack, stroke, or amaurosis fugax <1 month before surgery.

Level of significance between patients in the first to third quartiles and the fourth quartile of the calculated vulnerability index is marked by *P<0.05, †P<0.01, and ‡P<0.005.

§Hypertension defined as antihypertensive treatment or systolic pressure >140 mm Hg.

degree of stenosis, today the main criterion for surgery beyond medical treatment.

Whether a histological VI can determine if a plaque is high risk has to be investigated in a longitudinal study, which had until now never been performed. Therefore, here we tested whether VI can predict cardiovascular events in a cohort undergoing carotid endarterectomy because of significant carotid stenosis.

METHODS

The data sets generated and/or analyzed during the current study are not publicly available because of the sensitive nature of the data; requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to Isabel Goncalves at Lund University.

Clinical Information

This study was approved by the local ethical board and complies with the Declaration of Helsinki; patients provided written informed consent. The study included patients with cerebrovascular symptoms (stroke/transient ischemic attack/amaurosis fugax) and carotid plaques with >70% stenosis, or no symptoms and plaques with >80% stenosis (verified by duplex-ultrasound and neurological evaluation).[8] Clinical characteristics and blood (Table 1) were collected before surgery. The plaques (n=194) were biobanked (between 2005 and 2011) as part of the Carotid Plaque Imaging Project. Thirteen patients had bilateral stenosis and donated plaques at 2 separate time points. The second plaques to be operated were excluded from survival analysis and were used only for the VI comparison between right vs left plaques (or vice versa) of the same patient.

Histological Information

Sections (8 μm) from the plaque most stenotic part were stained for smooth muscle cells (alpha-actin), lipids (Oil Red O), macrophages (CD68 [cluster of differentiation 68]), and hemorrhage (glycophorin A), as previously described.[4] Russell-Movat pentachrome was used to detect plaque collagens. The stained plaque area of each component (%) was analyzed using Biopix iQ 2.1.8 (Gothenburg, Sweden). The VI was calculated as a ratio between the sum of %Oil Red O, %CD68, and %glycophorin A, and the sum of %alpha-actin and %collagen (Figure [A]).

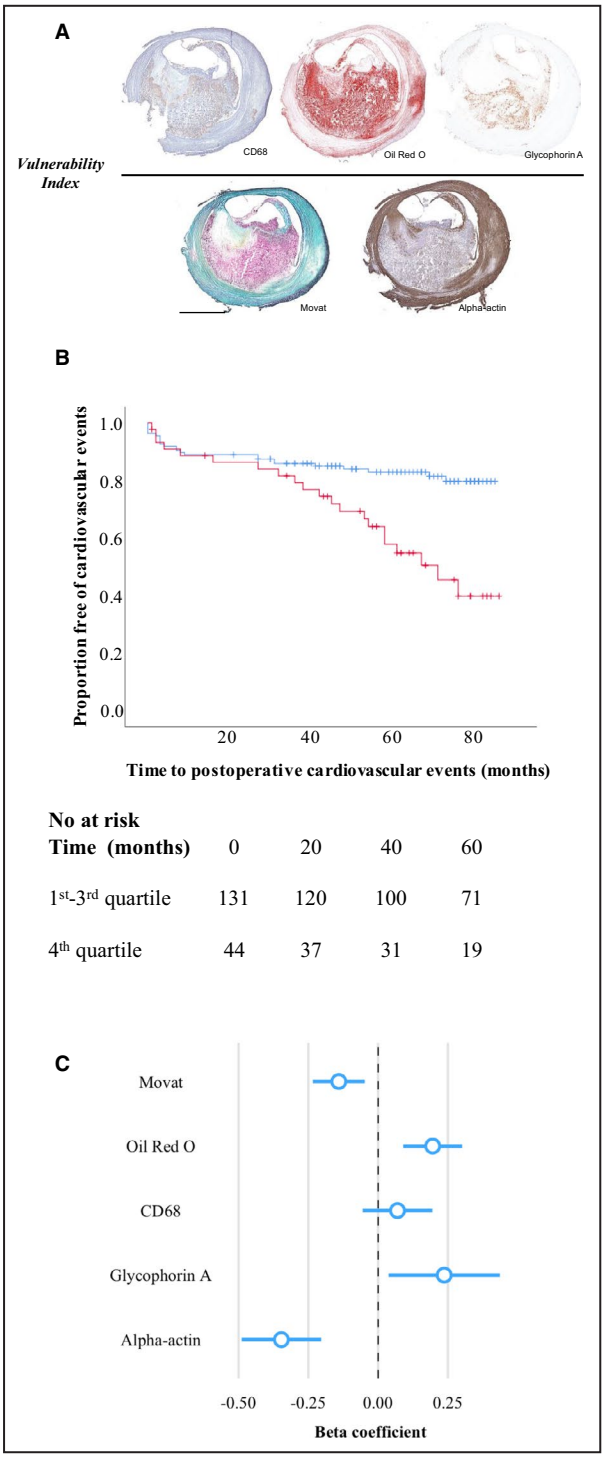

Figure. Patients with carotid plaques with high vulnerability index have higher risk for future cardiovascular events. **A**, Histological stains used for vulnerability index: ratio between the sum of cluster of differentiation 68 (CD68; macrophages stained in brown), Oil red O (ORO; neutral lipids stained in red), glycophorin A (intraplaque hemorrhage stained in brown), and the sum of alpha-actin (vascular smooth muscle cells stained in brown) and collagen (stained in yellow). All histological components were assessed as % of total plaque area. Scale bar, 2 mm. **B**, Kaplan-Meier curve showing the higher risk for cardiovascular events for patients with highest plaque vulnerability index in fourth quartile (red) compared with the first–third quartile (blue, log-rank test, *P*=0.0002). **C**, Multiple linear regression analysis showing the influence of the 5 different histological components on the vulnerability index. Vulnerability index was used as the dependent variable.

Survival Analysis

For the survival analysis 181 patients (unilateral plaques) were studied and divided in quartiles based on the VI. Postoperative cardiovascular events (a composite variable including myocardial infarction, unstable angina, stroke [ipsilateral and contralateral events], transient ischemic attack, amaurosis fugax, vascular interventions [including carotid endarterectomy/stenting, coronary artery bypass grafting/percutaneous coronary artery intervention], and cardiovascular death) were identified through the Swedish National Inpatient Health Register (2005–2013). Death due to other causes than cardiovascular and loss of follow-up (ie, emigration from Sweden or loss of follow-up) were censored from the analysis. Data were analyzed using SPSS 24.0 (IBM Corp., Armonk, NY). Spearman's correlation statistics was used. A nonparametric technique for modelling the survival function by Kaplan-Meier estimator was employed to measure the fraction of cardiovascular free participants with highest plaque VI in the fourth quartile versus the first to third quartile of VI. A logistic regression was used to examine the contribution of VI in predicting cardiovascular events adjusted for covariates.

RESULTS

In the study population, the median age was 70 (interquartile range 65–74) years, 70% male, 88% were statin treated, and 47% had symptoms. We registered 45 events (cerebrovascular events, n=12; cardiovascular events, n=17 and cardiovascular death, n=16) during follow-up (median 60 months). No significant differences in clinical characteristics were seen when comparing the 4 quartiles.

The Kaplan-Meier curve revealed that the fourth or highest quartile of VI was separated from the first to third quartile of VI, even though no dose response effect was identified in the first to third quartile. Therefore, the first to third quartiles were combined and used as reference in comparison with the fourth quartile.

Indeed, the risk of future postoperative cardiovascular events was 2 times higher among patients with a plaque VI in the fourth quartile than those with a lower plaque VI (*P*=0.0002, Figure [B]). Supporting the importance of the balance of the various components, none of the 5 histological variables individually predicted cardiovascular events.

Further, we found that the VI was an independent predictor of cardiovascular events in a logistic regression model including age, sex, diabetes mellitus, hypertension, smoking, cholesterol, and stroke/transient ischemic attack/amaurosis fugax as covariates (Table 2).

In the subgroup of 13 patients who underwent bilateral carotid endarterectomy (surgery at 2 different occasions, removing both the right and left carotid plaques), the VI of the 2 plaques correlated with each other (r=0.7, P=0.01).

Finally, using a multiple linear regression model we investigated which of the 5 histological components had the greatest influence on the VI. Interestingly, plaque area stained for alpha-actin (vascular smooth muscle cells, β=−0.35, P=1.7×10^{-7}) followed by glycophorin A (intraplaque hemorrhage/neovascularization, β=0.24, P=0.001), oil red O (lipids, β=0.20, P=0.007), and Movat (collagen, β=−0.14, P=0.015) showed the strongest associations to the VI. Plaque area of CD68 (macrophages) was not significantly associated with VI (P=0.3; Figure [C]).

DISCUSSION

Here we show for the first time that a calculated histological ratio, the VI, predicts future cardiovascular events independently of other well-known risk factors. This provides evidence for VI as tool for experimental and clinical studies to validate novel plaque imaging technologies.

Until now studies attempting to predict cardiovascular events using plaque histology have focused on either stabilizing or destabilizing components. As the individual use of each histological feature is insufficient to identify rupture prone plaques, it is plausible that a combination of several features is needed instead. Therefore, a ratio as the VI was calculated using histology features.

Table 2. Logistic Regressions Identifying Vulnerability Index as an Independent Predictor of Cardiovascular Events

	Cardiovascular Events	
	OR (95% CI)	*P* Value
VI (fourth quartile)	3.88 (1.72–8.77)	0.001
Age, y, per SD	0.91 (0.60–1.39)	0.67
sex (female)	0.69 (0.29–1.66)	0.41
Diabetes mellitus, yes	1.55 (0.70–3.43)	0.28
Hypertension, yes	1.63 (0.67–3.99)	0.28
Smoking, current or previous	0.83 (0.29–2.34)	0.72
Total cholesterol, per SD	1.20 (0.82–1.76)	0.35
Cerebrovascular symptoms (stroke, transient ischemic attack, or amaurosis fugax)	1.43 (0.64–3.21)	0.38

OR indicates odds ratio; and VI, vulnerability index.

The rationale behind VI is to capture the delicate balance between components involved in plaque instability (macrophages, hemorrhage, and lipid content) and stability (smooth muscle cells and collagen content), which in the end determine if the plaque will rupture or not.[1]

The VI has previously been described and used with minor modifications in animal studies, as well as in studies exploring human plaque composition by ultrasound, magnetic resonance imaging, and human plaque cell metabolism.[4–6,9,10] It has been shown that patients with one vulnerable plaque are likely to have vulnerable plaques in other arteries as well.[11] In line with this, it is well described that patients who already suffered from a cerebrovascular or cardiovascular event are at higher risk to suffer from a new event.[12-15] We now show for the first time using a longitudinal design that the histological VI is a reliable ratio to predict events, proving the importance of the balance of various plaque components in plaque stabilization. Furthermore, our data support that a patient with one vulnerable plaque is at higher risk to have vulnerable plaques in other vascular locations as well.

Our findings also support a recent large meta-analysis by Kamtchum-Tatuene et al, showing that plaques classified as high risk by imaging technologies had higher risk to cause symptoms. Importantly, the high-risk features were not associated with the degree of stenosis, again pointing out the importance to improve our clinical approach to detect high-risk plaques components beyond the degree of stenosis.[7]They identified neovascularization, lipid core, and echolucency as the most common high-risk features. In the present study intraplaque hemorrhage/neovascularization, lipids, collagen, and smooth muscle cells showed indeed strong associations with VI. Taken together these studies indicate that imaging methods able to identify these components and their balance could greatly improve the identification of patients with high-risk plaques.

Limitations

The patients in the present study were not consecutively but randomly selected among patients included between 2005 and 2011, which could potentially bias the results. However, by including plaques over a longer period of time we have reduced other effect such as changes in clinical treatments (ie, more potent statins or intensified treatment strategies), which in turn may affect outcome. Furthermore, the number of patients included in the present may seem relatively small. However, considering the nature of the study and the well-characterized study cohort (both clinical variables and histological plaque

phenotyping) the outcome of the study suggests that the calculated VI is a reliable index to identify high-risk individuals even in a smaller study cohort.

CONCLUSIONS

In conclusion, our findings demonstrate that subjects with one plaque with a high VI have a high risk to have another plaque with a high VI in the carotid arteries. More important, patients with carotid plaques with high VI have higher risk for future cardiovascular events. This supports that techniques imaging such plaques can help to detect subjects in need of more intense treatment. This suggests that VI is a reliable ratio to identify high-risk plaques in experimental studies, validating novel imaging techniques, even though our findings need to be validated in larger and more diverse cohorts.

ARTICLE INFORMATION

Received February 11, 2021; accepted June 14, 2021.

Affiliations

Clinical Sciences Malmö, Lund University, Malmo, Sweden (I.G., J.S., C.T., M.N., A.F.P., J.N., A.E.); Department of Cardiology, Skåne University Hospital, Lund/Malmö, Sweden (I.G., A.E.); and Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden (A.E.).

Sources of Funding

The work was supported by the Swedish Society for Medical Research, Emil and Wera Cornell Foundation, the Swedish Research Council, Crafoord Foundation, The Swedish Society of Medicine, Diabetes Foundation, Swedish Heart and Lung Foundation, Southern Sweden Regional Research Funding, Åke Wiberg Foundation, SUS Foundations and Funds, Lund University Diabetes Center (Swedish Research Council - Strategic Research Area Exodiab Dnr 2009-1039, Linnaeus grant Dnr 349-2006-23, and the Swedish Foundation for Strategic Research Dnr IRC15-006). The Knut and Alice Wallenberg Foundation, the Medical Faculty at Lund University, and Region Skåne are acknowledged for generous financial support.

Disclosures

None.

REFERENCES

1. Hong M-K, Mintz GS, Lee CW, Kim Y-H, Lee S-W, Song J-M, Han K-H, Kang D-H, Song J-K, Kim J-J, et al. Comparison of coronary plaque rupture between stable angina and acute myocardial infarction: a three-vessel intravascular ultrasound study in 235 patients. *Circulation*. 2004;110:928–933. DOI: 10.1161/01.CIR.0000139858.69915.2E.
2. Flaherty ML, Kissela B, Khoury JC, Alwell K, Moomaw CJ, Woo D, Khatri P, Ferioli S, Adeoye O, Broderick JP, et al. Carotid artery stenosis as a cause of stroke. *Neuroepidemiology*. 2013;40:36–41. DOI: 10.1159/000341410.
3. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. *Circulation*. 2003;108:1664–1672. DOI: 10.1161/01.CIR.0000087480.94275.97.
4. Erlöv T, Cinthio M, Edsfeldt A, Segstedt S, Dias N, Nilsson J, Gonçalves I. Determining carotid plaque vulnerability using ultrasound center frequency shifts. *Atherosclerosis*. 2016;246:293–300. DOI: 10.1016/j.atherosclerosis.2016.01.019.
5. Tomas L, Edsfeldt A, Mollet IG, Perisic Matic L, Prehn C, Adamski J, Paulsson-Berne G, Hedin U, Nilsson J, Bengtsson E, et al. Altered metabolism distinguishes high-risk from stable carotid atherosclerotic plaques. *Eur Heart J*. 2018;39:2301–2310. DOI: 10.1093/eurheartj/ehy124.
6. Shiomi M, Ito T, Hirouchi Y, Enomoto M. Fibromuscular cap composition is important for the stability of established atherosclerotic plaques in mature WHHL rabbits treated with statins. *Atherosclerosis*. 2001;157:75–84. DOI: 10.1016/S0021-9150(00)00708-5.
7. Kamtchum-Tatuene J, Noubiap JJ, Wilman AH, Saqqur M, Shuaib A, Jickling GC. Prevalence of high-risk plaques and risk of stroke in patients with asymptomatic carotid stenosis a meta-analysis. *JAMA Neurol*. 2020;77:1524–1535. DOI: 10.1001/jamaneurol.2020.2658.
8. Hansen F, Bergqvist D, Lindblad B, Lindh M, Mätzsch T, Länne T. Accuracy of duplex sonography before carotid endarterectomy—a comparison with angiography. *Eur J Vasc Endovasc Surg*. 1996;12:331–336. DOI: 10.1016/S1078-5884(96)80252-8.
9. Hartwig H, Silvestre-Roig C, Hendrikse J, Beckers L, Paulin N, Van der Heiden K, Braster Q, Drechsler M, Daemen MJ, Lutgens E, et al. Atherosclerotic plaque destabilization in mice: a comparative study. *PLoS One*. 2015;10:e0141019. DOI: 10.1371/journal.pone.0141019.
10. Tang D, Yang C, Zheng J, Woodard PK, Saffitz JE, Petruccelli JD, Sicard GA, Yuan C. Local maximal stress hypothesis and computational plaque vulnerability index for atherosclerotic plaque assessment. *Ann Biomed Eng*. 2005;12:1789–1801. DOI: 10.1007/s10439-005-8267-1.
11. Goldstein JA, Demetriou D, Grines CL, Pica M, Shoukfeh M, O'Neill WW. Multiple complex coronary plaques in patients with acute myocardial infarction. *N Engl J Med*. 2000;343:915–922. DOI: 10.1056/NEJM200009283431303.
12. Touzé E, Varenne O, Chatellier G, Peyrard S, Rothwell PM, Mas JL. Risk of myocardial infarction and vascular death after transient ischemic attack and ischemic stroke: a systematic review and meta-analysis. *Stroke*. 2005;36:2748–2755. DOI: 10.1161/01.STR.0000190118.02275.33.
13. Mohan KM, Wolfe CD, Rudd AG, Heuschmann PU, Kolominsky-Rabas PL, Grieve AP. Risk and cumulative risk of stroke recurrence: a systematic review and meta-analysis. *Stroke*. 2011;42:1489–1494. DOI: 10.1161/STROKEAHA.110.602615.
14. Boulanger M, Bejot Y, Rothwell P, Touze E. Long-term risk of myocardial infarction compared to recurrent stroke after transient ischemic attack and ischemic stroke: systematic review and meta-analysis. *J Am Heart Assoc*. 2018;7:e007267. DOI: 10.1161/JAHA.117.007267.
15. Wang Y, Li J, Zheng X, Jiang Z, Hu S, Wadhera RK, Bai X, Lu J, Wang Q, Li Y, et al. Risk factors associated with major cardiovascular events 1 year after acute myocardial infarction. *JAMA Netw Open*. 2018;1:e181079. DOI: 10.1001/jamanetworkopen.2018.1079.