Eur J Vasc Endovasc Surg (2022) 63, 800—806

Flow Cytometry Based Platelet Reactivity Testing to Predict the Occurrence of Per-operative Solid Microemboli During Carotid Endarterectomy

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WHAT THIS PAPER ADDS

This study shows that platelet reactivity testing using a flow cytometry assay can predict the peri-operative occurrence of the microembolic signals that may cause thrombotic cardiovascular events. Despite antiplatelet therapy to prevent secondary thrombotic events, non-responders to antiplatelet agents still have a poorer outcome. Ideally, these high risk patients are identified in an early stage. Flow cytometry base platelet reactivity testing might be used to identify these patients.

Objective: Peri-operative antiplatelet therapy (APT) aims to prevent thrombotic events such as stroke. High platelet reactivity ,despite the use use of APT, increases the risk of thrombotic events. Transcranial Doppler imaging (TCD) is used to detect peri-operative microembolic signals (MES) during carotid endarterectomy (CEA). Peri-operative MES are associated with an increased risk of procedural stroke and new silent lesions on diffusion weighted magnetic resonance imaging following surgery. The main components of TCD detected MES are platelet aggregates, and therefore patients displaying multiple MES during surgery could have benefited from more stringent APT. This study investigated whether the use of flow cytometry based platelet reactivity measurements were correlated with the incidence of pre-operative MES and thereby in the future suitable to predict patients at increased risk of peri-operative thrombotic events.

Methods: Bilateral TCD with MES detection was performed in 197 patients undergoing CEA. Platelet reactivity was assessed with a flow cytometry based platelet reactivity assay measuring platelet response in whole blood. High on treatment platelet reactivity status was assessed for all patients. The secondary outcome was major adverse cardiovascular events (MACE) within one year.

Results: In total, 197 patients were included, 49 had peri-operative MES. The platelet response to adenosine diphosphate (ADP) correlated with MES ($p = .021$), and high on treatment platelet reactivity after adenosine diphosphate stimulation was associated with MACE (OR 2.34, 95% confidence interval 1.126 − 4.890, $p = .023$).

Conclusion: Pre-operative platelet reactivity determined by flow cytometry after ADP stimulation correlated with the occurrence of intra-operative MES and post-operative MACE. Clopidogrel treatment showed the most substantial effect on reducing MES frequency and platelet reactivity measured by flow cytometry.

Keywords: Brain infarction, Carotid artery disease, Diffusion magnetic resonance imaging, Embolism and thrombosis, Platelet function tests
Article history: Received 21 June 2021, Accepted 5 March 2022, Available online 10 March 2022

INTRODUCTION

Post-operative silent ischaemic brain lesions shown on diffusion weighted magnetic resonance imaging occur in up to 17% of patients after carotid endarterectomy (CEA) and correlate with an unfavourable neurological prognosis.[1,2] These lesions are regarded as a surrogate marker of cerebral ischaemia resulting from peri-operative haemodynamic changes and arterial microemboli. These microemboli can be detected as microembolic signals (MES) by transcranial Doppler imaging (TCD) during surgery. The main component of these MES are platelet aggregates.[3—5]

Antiplatelet therapy (APT) is the cornerstone of thrombosis prevention for patients with peripheral arterial disease and carotid artery disease (CAD).[6—8] The P2Y12 inhibitor clopidogrel lowers platelet reactivity and is associated with a reduction in thrombotic events. Despite a clear thromboembolic preventive benefit, APT is also

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https://doi.org/10.1016/j.ejvs.2022.03.005

associated with an increased risk of bleeding or thrombosis in a subset of patients.[9] To attain optimal efficacy, monitoring of APT is needed. Patients at risk of either secondary thromboembolic cardiovascular events or bleeding can be identified by platelet function tests (PFTs). High on treatment platelet reactivity (HTPR), determined by platelet function testing, predicts clinically silent new ischaemic lesions in patients undergoing carotid artery stenting.[10,11] This substantiates the theory that these ischaemic cerebral lesions have a thromboembolic pathogenesis and that PFTs might be capable of predicting their occurrence.

Optical light transmission aggregometry (LTA) is still considered the gold standard PFT. A meta-analysis identified the VerifyNow (Accumetrics, Inc., San Diego, CA), an aggregation based assay, as a promising point of care test to predict bleeding by analysing platelet reactivity in patients using aspirin or P2Y12 inhibitors. Unfortunately, VerifyNow has a moderate predictive value for thromboembolic events.[10] Suitable cut off values for (non-)responders need to be determined, and reproducibility and interpretability need to be improved.[12] Meanwhile, new tests have become available that might predict thromboembolic events more accurately. Although flow cytometry is still laborious and not yet a point of care test, it was previously shown that flow cytometry based assays are more sensitive for detecting platelet reactivity changes than aggregation based assays.[13] In this study, the aim was to investigate the predictive value of platelet reactivity measurements with flow cytometry on the occurrence of MES during CEA.

MATERIALS AND METHODS

A single centre non-randomised cohort study in a consecutive series of patients with symptomatic CAD undergoing CEA was performed. Patients were recruited at the University Medical Centre Utrecht (UMCU) between 2012 and 2015. Patients were eligible for inclusion after meeting the following inclusion criteria: age $>$ 18 years, an indication for CEA according to current guidelines,[14–16] and a temporal skull window that allows for TCD registration. Exclusion criteria were the need for blood transfusion prior to surgery, a temporal skull window unsuitable for TCD, vitamin K inhibitor or other anticoagulant use, or artificial cardiac valve. The institutional review board approved the study, and all patients provided written informed consent in compliance with the declaration of Helsinki.

The UMCU is a large tertiary vascular referral centre. Around 70% of CEA patients are referred from primary centres. APT was accepted and continued as originated in the referring centre in all patients. Patients underwent CEA under general anaesthesia using a longitudinal arteriotomy with simultaneous electroencephalographic neuromonitoring and TCD monitoring.[17] All patients had preoperative TCD imaging. An experienced vascular surgeon or a supervised vascular trainee executed the surgery. A shunt or patch was used if necessary, and all patients received heparin (5 000 IU) three minutes prior to cross clamping. Data on patient characteristics, pre-operative physical status as defined by the American Society of Anesthesiologists (ASA classification), comorbidities, and clinical follow up were collected from patient records.

The primary outcome was the number of solid MES during CEA recorded by TCD. Secondary endpoints were thromboembolic stroke/transient ischaemic attack (TIA) (a clinical diagnosis by a neurologist, either direct or within one day post-operatively), post-operative troponin elevation (at least one measurement $>$ 0.06 μg/L less than three days post-operatively), post-operative bleeding and re-intervention, ipsilateral re-stenosis (on Duplex or CT angiography where available), major adverse cardiovascular events (MACE, defined as stroke, TIA, or amaurosis fugax, myocardial infarction, and surgical or endovascular peripheral intervention) and death. Clinical outcome is presented for three months of follow up and complete follow up (13.4 months). Follow up data were obtained from electronic patient files.

A Delica UMS-9UA system equipped with a 1.6 MHz probe in a head frame (SMT Medical, Wurzburg, Germany) allowed hands off monitoring of the ipsilateral middle cerebral artery. TCD monitoring started prior to induction of anaesthesia and ended when the patient was fully awake. Experienced clinical neurophysiology technicians performed all recordings. Two blinded experts (T.C.L. and D.v.V.) identified the solid emboli following the International Consensus Criteria.[18] Classification of solid emboli was performed once for each patient.[19]

Before induction of anaesthesia, arterial blood was collected into 0.105 M trisodium citrate vacuum tubes (Becton Dickinson, Temse, Belgium) for flow cytometric platelet reactivity analysis.

Flow cytometry

Measurement of platelet reactivity with flow cytometry was performed with platelet agonists adenosine diphosphate (ADP) and PAR-1 activating peptide (SFLLRN).[13] Whole blood samples were processed between one and six hours after blood collection. Platelet fibrinogen binding was used as a proxy of integrin αIIbβ3 activation. Data are presented per agonist as the sum of the median fluorescent intensity of each agonist concentration (ΣMFI). HTPR is defined as the ΣMFI exceeding the 75th percentile of the ΣMFI of all included patients.

All analyses were performed in SPSS Statistics 25 for Windows (IBM, Armonk, USA). Correlation between platelet reactivity measured with flow cytometry and the number of solid MES was assessed with Spearman's rho and linear regression analysis. The predictive value of the PFT for patients at risk of MES was evaluated with a logistic regression analysis. The effect of clopidogrel on platelet reactivity and the number of solid MES was calculated with the non-parametric Wilcoxon signed rank test. The occurrence of MACE was analysed with Fisher's exact test. Data are available on request.

RESULTS

After meeting the inclusion and exclusion criteria, 197 patients were included. The mean age at inclusion was 70

years, and 75% were male. In total, 106 patients had peri-operative MES. Baseline characteristics are shown in Table 1. The majority of patients received either aspirin and dipyridamole (39.6%) or a single P2Y12 receptor inhibitor (mainly clopidogrel, 34.5%). Oral anticoagulants were prescribed to 11.7% of all patients, and 6.6% received aspirin monotherapy. Oral anticoagulants were prescribed after inclusion in the study due to the patient's medical condition. Flow cytometry data were available for all patients. The mean follow up was 13.4 months (SD 10.9).

Table 1. Baseline characteristics of 197 patients undergoing carotid endarterectomy under bilateral transcranial Doppler imaging for peri-operative microembolic signals

	Patients (n = 197)
Gender	
Female	49 (24.9)
Male	148 (75.1)
Age − y	70 ± 1
History	
MI, CAD and or coronary interventions	68 (34.5)
History of peripheral intervention*	38 (19.3)
Renal failure, GFR < 50 mL/min	30/179 (16.1)
Risk factors	
Current smoker	64 (32.5)
Diabetes mellitus	46 (23.4)
Hypertension	149 (75.6)
Hypercholesterolaemia	124 (62.9)
BMI − kg/m^2	26.85 ± 0.34
eGFR − mL/min/1.73 m^2	64.61 ± 2.32
ASA classification	
ASA 1	6 (3.0)
ASA 2	109 (55.3)
ASA 3	80 (40.6)
ASA 4	2 (1.0)
Beta blockers	72 (36.5)
Statins	171 (86.8)
Antithrombotic therapy	
Monotherapy	13 (6.6)
Aspirin and clopidogrel	15 (7.6)
Aspirin and dipyridamole	78 (39.6)
Aspirin and oral anticoagulant	–
Dipyridamole monotherapy	–
Clopidogrel monotherapy	68 (34.5)
Oral anticoagulant monotherapy	23 (11.7)
Ipsilateral stenosis	
0–50%	3 (1.5)
50–70%	16 (8.1)
70–99%	177 (89.8)
Dissection	1 (0.5)
Symptoms	
Asymptomatic	23 (11.7)
TIA	71 (36.0)
Stroke	64 (32.5)
Ocular	39 (19.8)
Indication	
De novo	183 (92.9)
Restenosis	14 (7.1)
Contralateral stenosis	
0–50%	118 (63.1)
50–100%	68 (36,4)
Occlusion	1 (0.5)

Data are presented as n (%) or mean ± standard error of mean. ASA = American Society of Anesthesiologists; MI = myocardial infarction; CAD = coronary artery disease; BMI = body mass index; GFR = glomerular filtration rate; TIA = transient ischaemic attack.
* Including interventions in carotid and upper limb arteries

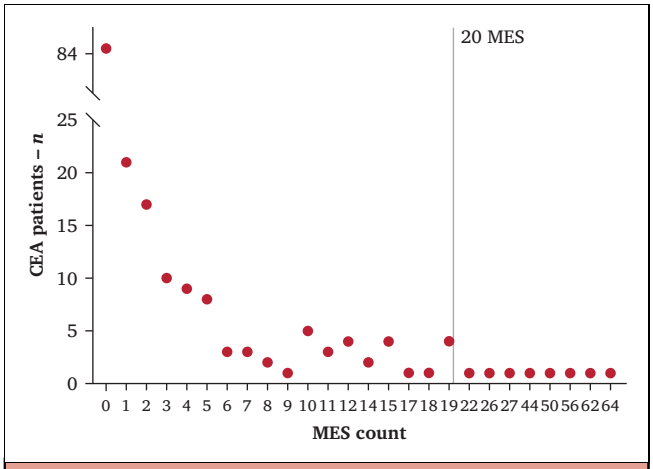

Figure 1. Prevalence of peri-operative solid microembolic signals (MES) among 197 patients undergoing carotid endarterectomy (CEA) under bilateral transcranial Doppler imaging.

Valid TCD recordings were available for 190 patients. A median of one (IQR 5) solid MES was observed peri-operatively (Fig. 1). Eighty-four of 190 patients (44.2%) did not display any solid MES. Several studies identified patients suffering from ≥ 20 solid MES as at high risk of peri-operative stroke.[19,20] Within the study, eight of 190 patients met this criterium. They were prescribed aspirin monotherapy (n = 1), aspirin and dipyridamole (n = 5), clopidogrel (n = 1), or aspirin and an oral anticoagulant (n = 1). One of these patients suffered immediate post-operative stroke and one patient needed re-operation of their CEA.

Platelet reactivity assessed with flow cytometry showed a weak correlation with the number of solid MES, with ρ = .217 for the response to ADP (p = .003) and ρ = .214 for

Table 2. Correlation between the number of peri-operative solid microembolic signals (MES) and flow cytometry (FC) measured platelet reactivity for adenosine diphosphate (ADP) and thrombin receptor activation peptide 6 (TRAP-6) in 190 patients undergoing carotid endarterectomy under bilateral transcranial doppler imaging

	FC-ADP (n = 190)*	FC-TRAP-6 (n = 190)*	Solid MES
FC-ADP			
Correlation coefficient			
Significance, 2-tailed†			
FC-TRAP-6			
Correlation coefficient	.897‡		
Significance, 2-tailed†	< .001	–	
Solid MES			
Correlation coefficient	.217*,‡	−.214‡	
Significance, 2-tailed†	.003	.003	–

* Only patients with P2Y12 inhibitor treatment. Invalid transcranial Doppler registration for seven patients in total
† Spearman's rho.
‡ Correlation is significant at the .01 level (two tailed).

the response after thrombin receptor activating peptide (TRAP-6, $p = .003$) (Table 2). MES frequency could only be predicted with the response to ADP determined with flow cytometry with $\beta < .001$ (95% CI .000 − .000, $p = .021$), whereas the response to TRAP-6 did not ($p = .15$).

The association of HTPR identified by flow cytometry with being at "high risk of stroke" (i.e., displaying > 20 solid microemboli) was investigated. HTPR was defined as a platelet response > 75th percentile of the platelet response of all included patients towards either ADP or TRAP-6. Five of eight patients with > 20 MES were identified with HTPR by flow cytometry in response to ADP. Two of eight patients with > 20 MES were identified with HTPR by flow cytometry in response to TRAP. HTPR determined with flow cytometry in response to ADP stimulation was associated with the occurrence of ≥ 20 solid microemboli (OR 5.732, 95% CI 1.314 − 25.003, $p = .020$) (Table 3, Fig. 2), but HTPR determined with flow cytometry in response to TRAP-6 was not. Thirty-four of 83 clopidogrel users had MES and 72 non-clopidogrel users had MES. After correction for clopidogrel use, the correlation disappeared (adjusted odds ratio 3.898, 95% CI .803 − 18.932, $p = .092$). Clopidogrel use correlated with fewer solid microemboli during CEA ($\rho = -.254$, $p < .001$). Clopidogrel users had fewer MES than non-users ($\beta = -.215$, 95% CI −7.161 to −1.500, $p = .003$).

Within 30 days post-operatively, five patients (2.5%) suffered a stroke. The 30 day mortality rate was 0.5% (1/197). This patient died 12 days after the CEA due to an immediate post-operative stroke, followed by an intervention related bleed that required re-intervention and subsequent palliative medical care. Four patients (2.0%) suffered an intervention related bleed with the need for re-intervention less than two days after CEA.

The crude mortality rate during the total follow up was 6.1% (12/197). Within the follow up period of just over one year, 42 major adverse events occurred of which there were 14 (33.3%) cardiac, 19 (45.2%) cerebral (stroke, TIA or AF), and nine (21.4%) peripheral events. Recurrent ipsilateral stenosis of 51% − 70% occurred in eight patients (4.1%), re-stenosis of 71% − 99% occurred in seven patients (3.6%), and total occlusion in two patients (1.0%). Five patients required a re-CEA. The mean number of MES in patients that developed MACE was significantly higher than in patients without MACE (4.11 *vs.* 7.17, $p = .018$). From the patients who displayed ≥ 20 MES, 37.5% suffered from MACE compared with 23.8% in patients with < 20 MES ($\rho = .238$). Only an HTPR status determined by flow cytometry ADP showed an increased risk of MACE during follow up (OR 2.346, 95% CI 1.126 − 4.890, $p = .023$) (Tables 3 and 4).

Table 3. Post-operative clinical events in 197 patients undergoing carotid endarterectomy under bilateral transcranial doppler imaging for peri-operative microembolic signals

Clinical events	Patients (n = 197)
Immediately post-operative	
Stroke	4 (2.0)
Transient ischaemic attack	0 (0.0)
After symptom free period < 1 day	
Stroke	1 (0.5)
Transient ischaemic attack	4 (2.0)
Hyperperfusion syndrome	
BP based	23 (11.7)
BP and symptomatology	5 (2.5)
Troponin elevation	14/194 (7.2)
Bleeding	4 (2.0)
Re-intervention	5 (2.5)
Bleeding	4 (2.0)
Direct stenosis	1 (0.5)
3 month follow up	
Death	1 (0.5)
Ipsilateral restenosis	
0−30%	3/193 (1.6)
31−50%	13 (6.7)
51−70%	6 (3.1)
71−99%	5 (2.6)
Occlusion	1 (0.5)
Total follow up	
Time − months	13.4 ± 10.9
Mortality	12/196 (6.1)
Cardiovascular related death	4 (2.0)
Major adverse events	
Cardiac	14/196 (7.1)
Cerebral (Stroke, TIA, AF)	19 (9.7)
Peripheral	9 (4.6)
Ipsilateral restenosis	
0−30%	6/193 (3.1)
31−50%	16 (8.3)
51−70%	8 (4.1)
71−99%	7 (3.6)
Occlusion	2 (1.0)
Re-operation due to stenosis	
< 1 year	3/195 (1.5)
≥ 1 year	2 (1.0)

Data are presented as n (%) or mean ± standard deviation. BP = blood pressure; TIA = transient ischaemic attack; AF = amaurosis fugax.

DISCUSSION

This is the first study correlating flow cytometry analysis of platelet reactivity to thrombotic events. It showed that

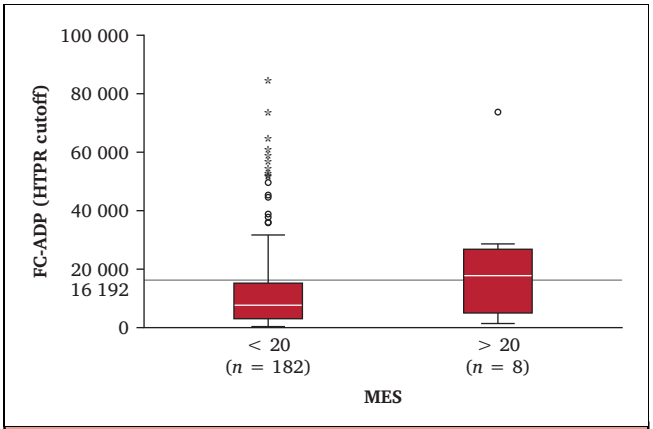

Figure 2. Flow cytometry (FC) measured platelet reactivity for adenosine diphosphate (ADP) for high on treatment platelet reactivity (HTPR) status and the number of peri-operative solid microembolic signals (MES) in 190 patients undergoing carotid endarterectomy under bilateral transcranial Doppler imaging.

Table 4. Patients at risk of stroke and major adverse cardiovascular events (MACE) predicted by high on treatment platelet reactivity (HTPR) status among 190 patients undergoing carotid endarterectomy under bilateral transcranial doppler imaging for peri-operative microembolic signals

	n	HTPR status defined by	Univariable analysis	
			OR (95% CI)	*p* value
20 solid microemboli	8	FC-ADP	5.732 (1.314–25.003)	.020
		FC-TRAP-6	1.045 (0.204–5.367)	.96
Major adverse cardiovascular events	42	FC-ADP	2.346 (1.126–4.890)	.023
		FC-TRAP-6	1.765 (0.837–3.721)	.14

ADP = adenosine diphosphate; TRAP-6 = thrombin receptor activating peptide-6; CI = confidence interval; FC = flow cytometry.

platelet reactivity measured by flow cytometry could predict the number of MES measured by TCD during CEA. Platelet reactivity measured with flow cytometry after stimulation with ADP correlated with MES frequency. Platelet reactivity in response to ADP stimulation, measured with flow cytometry, could identify patients at risk of post-operative silent ischaemic brain lesions (displaying $\geq$ 20 microemboli).

Although the VerifyNow is the most commonly used test for monitoring APT, reports on the association between VerifyNow results and clinical outcome are inconsistent. HTPR, according to the VerifyNow P2Y12 assay, was related to stent thrombosis, myocardial infarction, or cardiovascular death in large trials studying more than2 500 patients undergoing percutaneous coronary interventions.[21,22] Some studies found an association with clinical outcome in high risk patients with stable CAD with diabetes and chronic kidney failure.[23] Other trials did not show a predictive value.[24,25] There is no proven benefit from adjusting APT based on platelet reactivity test outcome.[26,27]

Flow cytometry is possibly a more sensitive way of testing platelet reactivity because it has the advantage of quantifying platelet activation on a wide scale. Flow cytometry based assays are more sensitive than aggregation based assays to detect platelet reactivity changes.[13] Therefore, more nuanced differences in platelet activity can be detected. Further studies are needed to verify this hypothesis.

The correlation between MES and the occurrence of cerebral symptoms is firmly grounded.[28] During CEA, peroperative MES occur due to manipulation of the atherosclerotic plaque (during dissection) and activation of platelets by the exposed collagen below the injured endothelium of the endarterectomy and clamp site after restoring the blood flow. The process of microemboli formation is pluricausal. Given the weak correlation between platelet reactivity assessed with flow cytometry and the number of solid MES, it is hypothesised that the influence of pre-existing (elevated) platelet reactivity plays a role as one of the factors in the formation of microemboli. The differences in response towards ADP and TRAP support this hypothesis. MES can be either gaseous or solid, bearing different clinical consequences. Classifying MES as being solid or artefacts is mentally demanding, limiting the availability of the procedure and potentially leading to less reliable results. Thus far, human experts are the gold standard.[29] The benefit of pre-operative blood tests to predict MES instead of a time consuming TCD measurement is therefore substantial.

Since only five patients were identified as non-responders to clopidogrel by flow cytometry (ADP), additional studies will have to investigate the influence of HTPR on MES frequency. For the TCD registration, only peroperative measurements were available; no data concerning post-operative embolisation were available. The association of the flow cytometry assay with MES warrants further investigation of the usability of this test.

Clopidogrel use was a strong predictor of low platelet reactivity towards ADP and TRAP-6, measured with flow cytometry. Clopidogrel reduces the occurrence of MES, possibly explaining the low mean MES frequency in this study.[20,30] Previous studies on P2Y12 inhibition and MES frequency have demonstrated that platelet response towards ADP is significantly higher in patients with $>$ 25 MES, measured with both LTA and flow cytometry.[31] A small cohort of 100 patients on aspirin were randomised to clopidogrel (n = 46) or placebo (n = 54) prior to surgery. Clopidogrel use reduced the platelet response towards ADP, resulting in a ten fold relative risk reduction of displaying $>$ 20 post-operative microemboli.[20] This was confirmed by a retrospective cohort study investigating 270 patients on aspirin and 75 mg of clopidogrel compared with a historical control cohort.[30] Embolisation occurred more frequent in the historical cohort (3.2% *vs.* 0.4%, p = .006). However, another study found no difference in MES between the antiplatelet regimens Asasantin (dipyridamole 200 mg/aspirin) 25 mg twice daily, Asasantin plus 75 mg clopidogrel once daily or Asasantin plus Rheomacrodex (dextran 40) 100 g/L intravenously (500 mL).[32] In practice, despite the protective effect of clopidogrel, only 31% of the centres participating in the Asymptomatic Carotid Surgery Trial (ACST)-2 study treated patients undergoing CEA with dual antiplatelet therapy prior to intervention and only 24% post-intervention.[33] As DAPT is now increasingly incorporated in guideline recommendations; patients might currently be better protected against the occurrence of MES and by extension against silent lesions on diffusion weighted imaging.[34] After inclusion, oral anticoagulants

were prescribed in 14.7% of patients possibly resulting in more MES (i.e., advanced disease).

There are some limitations to the study. First, guidelines switched to advising clopidogrel over acetylsalicylic acid, yet new studies propose a low dose antiplatelet agent combined with a direct acting oral anticoagulant.[35,36] The influence of these strategies on platelet reactivity and the predictability of MES is uncertain. No details about the influence of *CYP2C19* polymorphisms, a gene involved in the metabolism of clopidogrel, on the platelet reactivity test outcome in the study can be given.[23] Platelet reactivity might be affected both in carriers of slow metaboliser and ultra rapid metaboliser genes. In patients with CAD, slow metabolisers showed a genotypic hazard ratio for MACE of 1.41 (95% CI 1.06 − 1.89, $p = .010$). Easier accessibility and lower cost of genomic analysis would allow for assessment of abnormal responses to APT and the risk of thrombotic complications in non-responders. Last, this is a tertiary medical centre with high comorbidity rates and patients presenting with complex anatomy and late stage disease. Therefore, MES rates could be above average.

Despite clopidogrel use, there remains a group of patients that have more MES and are prone to a poorer outcome. Notwithstanding that the effect of clopidogrel shown in the study might suggest that TCD monitoring, or platelet function testing, is not necessary anymore, flow cytometry testing might be of value to identify these ultra high risk patients pre-operatively at an early stage of their treatment. Further exploration of the nuances of platelet function test results using flow cytometry will uncover the potential of these tests.

Conclusion

Platelet reactivity towards ADP, assessed by flow cytometry can predict the occurrence of per-operative solid MES measured by TCD during CEA and post-operative MACE. Clopidogrel treatment showed the most substantial effect on reducing microemboli frequency and platelet reactivity measured by flow cytometry.

FINANCIAL DISCLOSURES FUNDING

Professor Heimburger award, CSL Behring.

ACKNOWLEDGEMENTS

We thank all clinical neurophysiology technicians of the UMC Utrecht.

REFERENCES

1 Traenka C, Engelter ST, Brown MM, Dobson J, Frost C, Bonati LH. Silent brain infarcts on diffusion-weighted imaging after carotid revascularisation: a surrogate outcome measure for procedural stroke? A systematic review and meta-analysis. *Eur Stroke J* 2019;**4**:127–43.

2 Rots ML, van der Lugt A, de Borst GJ. Surrogate markers and reporting standards for outcome after carotid intervention. *Eur J Vasc Endovasc Surg* 2019;**58**:794–5.

3 Heider P, Poppert H, Wolf O, Liebig T, Pelisek J, Schuster T, et al. Fibrinogen and high-sensitive C-reactive protein as serologic predictors for perioperative cerebral microembolic lesions after carotid endarterectomy. *J Vasc Surg* 2007;**46**:449–54.

4 Ritter MA, Jurk K, Schriek C, Nabavi DG, Droste DW, Kehrel BE, et al. Microembolic signals on transcranial Doppler ultrasound are correlated with platelet activation markers, but not with platelet-leukocyte associates: a study in patients with acute stroke and in patients with asymptomatic carotid stenosis. *Neurolog Res* 2009;**31**:11–6.

5 Kinsella JA, Tobin WO, Tierney S, Feeley TM, Egan B, Collins DR, et al. Increased platelet activation in early symptomatic vs. asymptomatic carotid stenosis and relationship with microembolic status: results from the Platelets and Carotid Stenosis Study. *J Thromb Haemost* 2013;**11**:1407–16.

6 Murphy SJX, Naylor AR, Ricco JB, Sillesen H, Kakkos S, Halliday A, et al. Optimal antiplatelet therapy in moderate to severe asymptomatic and symptomatic carotid stenosis: a comprehensive review of the literature. *Eur J Vasc Endovasc Surg* 2019;**57**:199–211.

7 Aboyans V, Ricco JB, Bartelink MEL, Bjorck M, Brodmann M, Cohnert T, et al. Editor's Choice − 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS). *Eur J Vasc Endovasc Surg* 2018;**55**:305–68.

8 Conte MS, Bradbury AW, Kolh P, White JV, Dick F, Fitridge R, et al. Global vascular guidelines on the management of chronic limb-threatening ischemia. *Eur J Vasc Endovasc Surg* 2019;**58**(1S): S1–109.

9 Aradi D, Kirtane A, Bonello L, Gurbel PA, Tantry US, Huber K, et al. Bleeding and stent thrombosis on P2Y12-inhibitors: collaborative analysis on the role of platelet reactivity for risk stratification after percutaneous coronary intervention. *Eur Heart J* 2015;**36**:1762–71.

10 Wisman PP, Roest M, Asselbergs FW, de Groot PG, Moll FL, van der Graaf Y, et al. Platelet-reactivity tests identify patients at risk of secondary cardiovascular events: a systematic review and meta-analysis. *J Thromb Haemost* 2014;**12**:736–47.

11 Song TJ, Suh SH, Min PK, Kim DJ, Kim BM, Heo JH, et al. The influence of anti-platelet resistance on the development of cerebral ischemic lesion after carotid artery stenting. *Yonsei Med J* 2013;**54**:288–94.

12 Leunissen TC, Peeters Weem SM, Urbanus RT, den Ruijter HM, Moll FL, Asselbergs FW, et al. High on-treatment platelet reactivity in peripheral arterial disease: a pilot study to find the optimal test and cut off values. *Eur J Vasc Endovasc Surg* 2016;**52**: 198–204.

13 Leunissen TC, Wisman PP, van Holten TC, de Groot PG, Korporaal SJ, Koekman AC, et al. The effect of P2Y12 inhibition on platelet activation assessed with aggregation- and flow cytometry-based assays. *Platelets* 2017;**28**:567–75.

14 Naylor AR, Rothwell PM, Bell PR. Overview of the principal results and secondary analyses from the European and North American randomised trials of endarterectomy for symptomatic carotid stenosis. *Eur J Vasc Endovasc Surg* 2003;**26**:115–29.

15 Mayberg MR, Wilson SE, Yatsu F, Weiss DG, Messina L, Hershey LA, et al. Carotid endarterectomy and prevention of cerebral ischemia in symptomatic carotid stenosis. Veterans Affairs Cooperative Studies Program 309 Trialist Group. *JAMA* 1991;**266**:3289–94.

16 European Carotid Surgery Trialists' Collaborative G. Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST). *Lancet* 1998;**351**:1379–87.

17 Pennekamp CW, Moll FL, de Borst GJ. The potential benefits and the role of cerebral monitoring in carotid endarterectomy. *Curr Opin Anaesthesiol* 2011;**24**:693–7.

18 Ringelstein EB, Droste DW, Babikian VL, Evans DH, Grosset DG, Kaps M, et al. Consensus on microembolus detection by TCD.

International Consensus Group on Microembolus Detection. *Stroke* 1998;**29**:725–9.
19 Leunissen T, van Vriesland D, den Ruijter H, Moll F, Mess W, de Borst GJ. Validation of the automated electronic microemboli detection system in patients undergoing carotid endarterectomy. *Ultraschall Med* 2018;**39**:198–205.
20 Payne DA, Jones CI, Hayes PD, Thompson MM, London NJ, Bell PR, et al. Beneficial effects of clopidogrel combined with aspirin in reducing cerebral emboli in patients undergoing carotid endarterectomy. *Circulation* 2004;**109**:1476–81.
21 Stone GW, Witzenbichler B, Weisz G, Rinaldi MJ, Neumann FJ, Metzger DC, et al. Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study. *Lancet* 2013;**382**:614–23.
22 Price MJ, Angiolillo DJ, Teirstein PS, Lillie E, Manoukian SV, Berger PB, et al. Platelet reactivity and cardiovascular outcomes after percutaneous coronary intervention: a time-dependent analysis of the Gauging Responsiveness with a VerifyNow P2Y12 assay: Impact on Thrombosis and Safety (GRAVITAS) trial. *Circulation* 2011;**124**:1132–7.
23 Viviani Anselmi C, Briguori C, Roncarati R, Papa L, Visconti G, Focaccio A, et al. Routine assessment of on-clopidogrel platelet reactivity and gene polymorphisms in predicting clinical outcome following drug-eluting stent implantation in patients with stable coronary artery disease. *JACC Cardiovasc Interv* 2013;**6**:1166–75.
24 Paulu P, Osmancik P, Tousek P, Minarik M, Benesova L, Motovska Z, et al. Lack of association between clopidogrel responsiveness tested using point-of-care assay and prognosis of patients with coronary artery disease. *J Thromb Thrombol* 2013;**36**:1–6.
25 Legrand V, Cuisset T, Chenu P, Vrolix M, Martinez C, Dens J, et al. Platelet reactivity and cardiovascular events after percutaneous coronary intervention in patients with stable coronary artery disease: the Stent Thrombosis In Belgium (STIB) trial. *EuroIntervention* 2014;**10**:204–11.
26 Price MJ, Berger PB, Teirstein PS, Tanguay J-F, Angiolillo DJ, Spriggs D, et al. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. *JAMA* 2011;**305**:1097–105.
27 Montalescot G, Range G, Silvain J, Bonnet JL, Boueri Z, Barthelemy O, et al. High on-treatment platelet reactivity as a risk factor for secondary prevention after coronary stent revascularization: a landmark analysis of the ARCTIC study. *Circulation* 2014;**129**:2136–43.
28 King A, Markus HS. Doppler embolic signals in cerebrovascular disease and prediction of stroke risk: a systematic review and meta-analysis. *Stroke* 2009;**40**:3711–7.
29 Zivari Adab H, Chalavi S, Beets IAM, Gooijers J, Leunissen I, Cheval B, et al. White matter microstructural organisation of interhemispheric pathways predicts different stages of bimanual coordination learning in young and older adults. *Eur J Neurosci* 2018;**47**:446–59.
30 Sharpe RY, Dennis MJ, Nasim A, McCarthy MJ, Sayers RD, London NJ, et al. Dual antiplatelet therapy prior to carotid endarterectomy reduces post-operative embolisation and thromboembolic events: post-operative transcranial Doppler monitoring is now unnecessary. *Eur J Vasc Endovasc Surg* 2010;**40**:162–7.
31 Hayes PD, Box H, Tull S, Bell PR, Goodall A, Naylor AR. Patients' thromboembolic potential after carotid endarterectomy is related to the platelets' sensitivity to adenosine diphosphate. *J Vasc Surg* 2003;**38**:1226–31.
32 de Borst GJ, Hilgevoord AAJ, de Vries JPPM, van der Mee M, Moll FL, van de Pavoordt HDWM, et al. Influence of antiplatelet therapy on cerebral micro-emboli after carotid endarterectomy using postoperative transcranial doppler monitoring. *Eur J Vasc Endovasc Surg* 2007;**34**:135–42.
33 Huibers A, Halliday A, Bulbulia R, Coppi G, de Borst GJ, Group A-C. Antiplatelet therapy in carotid artery stenting and carotid endarterectomy in the Asymptomatic Carotid Surgery Trial-2. *Eur J Vasc Endovasc Surg* 2016;**51**:336–42.
34 Brand AR, de Borst GJ. Evidence for periprocedural antiplatelet therapy, heparinization and bridging of coumarin therapy in carotid revascularization. *J Cardiovasc Surg (Torino)* 2017;**58**:143–51.
35 Naylor AR, Ricco JB, de Borst GJ, Debus S, de Haro J, Halliday A, et al. Editor's Choice – Management of atherosclerotic carotid and vertebral artery disease: 2017 clinical practice guidelines of the European Society for Vascular Surgery (ESVS). *Eur J Vasc Endovasc Surg* 2018;**55**:3–81.
36 Hiatt WR, Bonaca MP, Patel MR, Nehler MR, Debus ES, Anand SS, et al. Rivaroxaban and aspirin in peripheral artery disease lower extremity revascularization: impact of concomitant clopidogrel on efficacy and safety. *Circulation* 2020;**142**:2219–30.