Assessment of 'on-treatment platelet reactivity' and relationship with cerebral microembolic signals in asymptomatic and symptomatic carotid stenosis

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Abstract

Introduction: The relationship between on-treatment platelet reactivity and cerebral micro-embolic signals (MES) on transcranial Doppler ultrasound (TCD) is unknown and has not been previously simultaneously assessed in asymptomatic and symptomatic carotid stenosis patients.

Methods: Consecutive eligible patients with ≥50% asymptomatic or recently symptomatic carotid stenosis (≤4 weeks following TIA/ischaemic stroke) were recruited to this pilot study. Symptomatic patients were followed up to the 'late' phase (≥3 months) following symptom onset or carotid intervention; longitudinal data from symptomatic patients with data available at both time-points were compared. Platelet function/reactivity was assessed using the PFA-100® to measure collagen-ADP (C-ADP) and collagen-epinephrine (C-EPI) closure times in citrate-anticoagulated whole blood. Bilateral simultaneous 1-hour transcranial Doppler ultrasound monitoring of the middle cerebral arteries was performed to classify patients as MES-positive or MES-negative.

Results: 31 patients with asymptomatic and 46 with early symptomatic carotid stenosis or occlusion were included. 35 symptomatic patients were followed up to the late phase (23 following carotid intervention). Prevalence of 'high on-treatment platelet reactivity' (HTPR) on the C-EPI cartridge did not differ between asymptomatic and symptomatic patients overall, but was lower in 'symptomatic post-intervention' than asymptomatic patients on aspirin monotherapy (10% *vs*. 50%; P=0.03). The prevalence of HTPR on the C-EPI cartridge significantly decreased between the early versus late phase in symptomatic patients (63% *vs*. 34%; P=0.017), including those on aspirin monotherapy (P = 0.016).

Discussion: Carotid interventional treatment, presumably in combination with resolution of the acute phase response, may decrease the prevalence of HTPR in patients with recently symptomatic carotid stenosis over time. Preliminary subgroup data suggest that successful interventional treatment may even reduce the prevalence of aspirin-HTPR in symptomatic patients to lower levels than in asymptomatic patients on aspirin monotherapy. Larger, longitudinal studies are warranted to reassess the impact of more intensive secondary preventive treatment on *ex vivo* platelet function at different levels of shear in patients with carotid stenosis.

Introduction

There is evidence that platelets are excessively activated or 'hyper-reactive' in patients with TIA and ischaemic stroke versus controls,[1-7] and excessively activated in patients with recently symptomatic versus asymptomatic carotid stenosis.[2-4;8,9] There is also evidence that short-term treatment with aspirin-clopidogrel combination therapy is more effective than aspirin monotherapy at preventing microembolic signals (MES) on TCD,[10] and that combination therapy with aspirin-dipyridamole appears equally effective as aspirin-clopidogrel at reducing MES in recently symptomatic carotid stenosis patients.[11]

There is an emerging literature to suggest that data from platelet function/reactivity monitoring may enhance our ability to predict the risk of recurrent vascular events and functional outcome in patients with vascular disease.[8,12] Ischaemic heart disease patients on antiplatelet therapy deemed to have 'high on-treatment platelet reactivity' (HTPR) or 'non-responsiveness' on an *ex vivo* test of platelet function have been shown to have a higher risk of clinical outcome events than those without HTPR.[13-15] However, the definition of HTPR on various platelet function devices varies between studies.[13-18] Preliminary, hypothesis-generating, subgroup data analysis from one study suggested that, compared with controls, the prevalence of HTPR reduced in patients with severe carotid stenosis who were followed up from the early ($\leq$4 weeks) to late phase ($\geq$ 3 months) after symptom onset or intervention.[7] To our knowledge, no adequately powered studies have compared the prevalence of *ex vivo* HTPR in whole blood between asymptomatic and early and late phase symptomatic carotid stenosis patients.

Prior studies have illustrated the potential role of MES detection on TCD in identifying asymptomatic and symptomatic carotid stenosis patients who may benefit most from enhanced medical or surgical therapy to prevent TIA or stroke.[19-24] To our knowledge, simultaneous measurement of HTPR on a point-of-care (POC) device, the PFA-100, has not been performed in patients with asymptomatic versus symptomatic carotid stenosis, in conjunction with simultaneous quantification of cerebral MES.

The aims of this component of the **P**latelets **A**nd **C**arotid **S**tenosis (**PACS**) study were to determine whether HTPR on a moderately high shear stress test of platelet reactivity was more common in patients with recently symptomatic than asymptomatic carotid stenosis, and to longitudinally assess HTPR status in symptomatic patients and specific subgroups. We also aimed to determine whether there was any relationship between HTPR status and the presence of MES detected on TCD (MES +ve) versus those without (MES -ve). We prospectively planned to assess whether there was any relationship between HTPR and the risk of recurrent vascular events during follow up in symptomatic patients. **We hypothesised** that recently symptomatic patients were more likely to have an increased prevalence of HTPR than their asymptomatic counterparts, and that the prevalence of HTPR would decrease in symptomatic patients during follow up after intensive medical and/or surgical intervention. We also hypothesised that HTPR status might be informative in certain patient subgroups stratified according to MES status (MES +ve *vs*. MES -ve).

Methods

Pilot 'symptomatic case' *vs*. 'asymptomatic case/control', and 'nested longitudinal studies in symptomatic patients' with moderate-severe carotid stenosis was performed. Consecutive eligible patients > 18 years old with asymptomatic or symptomatic moderate or severe carotid artery stenosis or carotid occlusion, identified on colour Doppler ultrasound using standardised velocity criteria,[25,26] were recruited from the Rapid Access Stroke Prevention (RASP) Service, vascular surgery or general neurology clinics, and the neurology and vascular surgery wards and stroke service at AMNCH and St James's Hospitals between August 2007 and February 2010. Patients were **included** in the '**asymptomatic carotid stenosis group'** if they were incidentally noted to have moderate (50 - 69%) or severe (≥ 70%) carotid stenosis on colour Doppler ultrasound imaging (CDUS), e.g. after noting an audible carotid bruit or during work up for coronary artery disease.[9,19] Subjects were considered to be asymptomatic if they never had a prior TIA or stroke in any vascular territory, or had not had a carotid-territory TIA or stroke within the preceding three years. All demographic and vascular risk factors, and information regarding medication intake was recorded prospectively.

Patients were **included** in the '**symptomatic carotid stenosis' group** if they had a TIA or ischaemic stroke in the vascular territory supplied by a moderate or severe ipsilateral carotid stenosis or carotid occlusion within the preceding 4 weeks and the symptoms was attributed to the stenosed carotid artery of interest (**early phase**). These patients were reassessed at least three months after symptom onset or after surgical or endovascular intervention (**late phase**).

Exclusion criteria for patients included active infection, inflammation including vasculitis, neoplasia, platelet count < 120 or > 450 x 10^9/L, recurrent TIA, stroke, myocardial infarction, pulmonary embolism, deep vein thrombosis or major bleeding requiring transfusion or major surgery within the preceding 3 months; prior history of primary intracerebral haemorrhage; a known bleeding or clotting diathesis or platelet disorder; ongoing unstable coronary or peripheral arterial disease; renal impairment (urea > 10 mmol/l); or other nonsteroidal anti-inflammatory drug (NSAID) intake within 2 weeks (apart from aspirin). Symptomatic patients were also excluded if there was evidence of a potential cardio-embolic source detected within 3 months of recruitment.

All patients underwent a detailed general and neurological assessment by the Clinical Neurology Research Registrars (JAK or WOT) or Consultant Vascular Neurologist (DJHM) participating in the study, to confirm that the asymptomatic patients met inclusion criteria, and to confirm a diagnosis of atherothrombotic TIA or ischaemic stroke in the symptomatic cohort. Information regarding vascular risk factors and medication intake, including anti-plalelet, anti-coagulant, statin and anti-hypertensive therapy, dose and duration of therapy were also recorded prospectively at each visit.

CT and/or MRI brain was performed in all symptomatic patients and magnetic resonance angiography (MRA) or CT angiography (CTA) was performed where deemed appropriate by the treating physician to establish concordance between CDUS and another imaging modality. TIA and stroke work-up was performed according to European Stroke Organisation guidelines.[27] All late stage symptomatic carotid stenosis patients were phoned before their appointment to stress the importance of medication adherence in the week prior to assessment. Patients who were not adherent

to their antithrombotic regimen were invited back for reassessment after 14 days. All patients were advised to immediately contact their attending physician and to inform the PACS research study staff if they had any recurrent vascular events whilst awaiting study follow up. Data on recurrent TIA, stroke, angina, MI, worsening symptomatic peripheral vascular disease, the need for coronary or peripheral arterial interventional treatment or vascular death were recorded prospectively in all symptomatic subjects at their last follow up visit.

To establish the normal range of platelet function assays in the laboratory, a group of **healthy controls** of similar age and sex, with no history of known cerebrovascular disease, were recruited from the local population and from amongst the family members of the participating subjects. Controls had colour Doppler ultrasound of carotid and vertebral arteries (CDUS) to exclude asymptomatic ≥ 50% carotid stenosis prior to inclusion. Subjects were also excluded from the control group if they were on antiplatelets or NSAIDs, or had any other exclusion criteria that applied to patients.

Blood sampling and laboratory tests:

All subjects were rested for at least 20 minutes, and careful venepuncture was performed from a free-flowing vein using a sterile 21G Butterfly needle (Venisystems™, Abbott, Ireland) and a Vacutainer® system with a luer adaptor (Becton Dickinson Vacutainer Systems, UK). Venepuncture was performed using a standardised manner as described previously.[19] Platelet function/on-treatment reactivity was assessed with the PFA-100®, to measure C-ADP and C-EPI closure times in citrate-anticoagulated whole blood between 2 and 2.5 hours after

venepuncture. The PFA-100 activates platelets by exposure to moderately high shear stress (5000 - 6000 s^{-1}) and biochemical stimulation with collagen and either epinephrine (C-EPI cartridge) or ADP (C-ADP cartridge).[28,29] The time taken for activated platelets to occlude an aperture in the cartridge is called the closure time; the maximum closure time recorded by the device is 300 s, and we arbitrarily defined closure times above 300 s as 301 s for statistical analyses.

Transcranial Doppler ultrasound:

Bilateral simultaneous 1 hour-TCD recordings of the MCA were performed by one of two highly-experienced operators (JAK or WOT) with a Viassys Pioneer TC8080, as described previously.[19]

Statistical Methods

Descriptive statistical calculations were performed to calculate the percentage of patients on different antiplatelet regimens. Paired or unpaired t-tests were used for comparison of paired and unpaired parametric variables, respectively. The Wilcoxon signed rank test and the Wilcoxon rank sum test were used for comparison of paired and unpaired non-parametric variables, and the Kruskal-Wallis rank sum test for comparison of multiple non-parametric variables, where appropriate. Chi-squared or Fisher exact tests were used to compare changes in proportions between subject groups.

We established laboratory normal ranges for the PFA-100 assays in 18 healthy controls (mean age 62 years; 72% male), as outlined above. For the purpose of this study, we considered patients to have *ex-vivo* HTPR on the PFA-100 if they had

evidence of platelet ‘hyper-reactivity’ on the relevant PFA-100 cartridge despite treatment with their prescribed antiplatelet regimen. Therefore, (a) *ex-vivo* HTPR on aspirin was defined as failure to prolong the C-EPI closure time beyond the mean + 2 standard deviations of our control range (162s) in patients on aspirin monotherapy, aspirin-dipyridamole or aspirin-clopidogrel combination therapy. (b) *Ex-vivo* HTPR on clopidogrel was defined as failure to prolong the CADP closure time beyond the mean + 2 standard deviations of our control range (165s) in patients on clopidogrel monotherapy, or clopidogrel in combination with aspirin as per usual ‘cross sectional, case-control’ definitions in the literature at the time of planning this study.[7,30] P <0·05 was considered to be statistically significant. All statistical calculations were performed using R, version 2.11.0.[31]

The study was approved by the local Research Ethics Committee at St James’s Hospital / AMNCH (Project/REC Reference: 2007/03/01). Written informed consent, or assent from a relative, where appropriate, was obtained from all participants.

Results

31 asymptomatic and 46 patients with early (≤4 weeks) symptomatic carotid stenosis or occlusion had platelet function data available for analysis. Thirty-five of these symptomatic patients had follow up data at the late stage after symptom onset or carotid intervention (Table 1), 22 of whom had undergone CEA and 1 had endovascular treatment with stenting. As reported previously, two patients had recurrent 'perioperative' ischaemic stroke following carotid endarterectomy: one awoke following endarterectomy with a new ischaemic stroke, and one developed symptoms 48 hours postoperatively.[32] Moderate to severe carotid restenosis was noted in 3 patients on follow-up CDUS.[32]

Assessment of Platelet Reactivity:

There were no significant differences in median C-EPI or C-ADP closure times between the entire asymptomatic *vs*. early or late symptomatic groups, regardless of antiplatelet treatment regimens (Table 2). There were no differences in the prevalence of HTPR between the subgroups of asymptomatic *vs*. early or late phase symptomatic patients who were on aspirin or clopidogrel overall. However, the prevalence of aspirin-HTPR on the C-EPI cartridge was lower in the 'late symptomatic post-intervention subgroup' than in the asymptomatic carotid stenosis subgroup on aspirin monotherapy (10% *vs*. 50%, p = 0.03; Table 2). There were no significant differences in vascular risk factors between these subgroups.

Amongst *all symptomatic patients* with longitudinal data in both the early and late phases after symptom onset or intervention, median C-EPI closure times increased from the early to late phases (143s *vs*. 203s, p = 0.03; Table 3a). *Amongst*

symptomatic patients on aspirin, alone or in combination with other antithrombotic therapy, median C-EPI closure times also increased (p = 0.023), and the proportion of patients with HTPR fell from the early to late phases after symptom onset or intervention (p = 0.01). Similar results were seen in *symptomatic patients on aspirin monotherapy*, with a reduction in the prevalence of aspirin-HTPR on the C-EPI cartridge during follow up from the early to late phases (p = 0.016; Table 3a). There was a significant reduction in aspirin-HTPR between the early and late post-intervention phases in symptomatic patients with matched data who were on aspirin monotherapy (50% vs. 0%; p=0.02), but the number of subjects in this subgroup analysis was very limited (N = 8; Table 3b).

Platelet Reactivity in MES-positive and MES-negative subgroups:

Twenty-five asymptomatic, 31 early symptomatic and 27 late symptomatic patients had TCD data available for analysis.[19] As reported previously, 12% of asymptomatic *vs*. 32% of early symptomatic (p=0.02) and 19% late symptomatic patients (p=0.2) were MES +ve.[9,19,32] There were no significant differences in median C-EPI or C-ADP closure times between MES + ve *vs*. MES -ve subjects within the asymptomatic, early symptomatic, or late symptomatic subgroups ($p \geq 0.16$). There were no significant differences in HTPR status between asymptomatic *vs*. early or late symptomatic MES +ve patients, or between asymptomatic *vs*. early or late symptomatic MES -ve patients ($p \geq 0.32$).

Relationship between HTPR status and clinical outcome events:

Interestingly, one of the 2 symptomatic patients who developed perioperative recurrent stroke was taking aspirin-clopidogrel combination therapy and displayed

HTPR on both the C-ADP and C-EPI cartridges in the early stage. The other symptomatic patient with perioperative stroke did not have HTPR on aspirin-dipyridamole in the early stage. Therefore, one could not comment on any clear association between HTPR status and the incidence of recurrent vascular events due to the limited number of outcome events in this study.

Discussion

This novel, pilot study has revealed several interesting findings. The lack of differences in on-treatment platelet reactivity / platelet adhesion-aggregation on this moderately high shear stress device in asymptomatic *vs*. early symptomatic patients overall may partly reflect the fact that the stenosing atherosclerotic carotid plaque exposed circulating platelets to similar levels of shear stress *in vivo* in both patient groups initially [7,33-36], and the PFA-100 was not sensitive enough at detecting differences between groups. The lack of significant differences in C-EPI closure times between asymptomatic and early symptomatic patients also likely reflects that fact that similar antiplatelet regimens were used in each group initially (predominantly aspirin), and C-EPI closure times are highly sensitive to the effects of aspirin.[7,37] Although there were no statistically significant differences in on-treatment platelet reactivity between asymptomatic and late symptomatic patients overall, this likely reflects a type II error because there were non-significant trends towards more prolonged median C-EPI closure times and a lower prevalence of HTPR in late symptomatic compared with asymptomatic patients (table 2). We do not think that these late symptomatic C–EPI results were likely to have been significantly influenced by the more frequent use of aspirin and dipyridamole combination therapy

in late symptomatic than asymptomatic patients, because previous data from our group have shown that the addition of dipyridamole to aspirin may prolong C-ADP, but not C-EPI closure times following TIA or ischaemic stroke.[38] Our preliminary subgroup data suggest that successful interventional treatment may even reduce the prevalence of aspirin-HTPR, as measured on the C-EPI cartridge, in symptomatic patients to lower levels than in asymptomatic patients on aspirin monotherapy. However, one must emphasise that this latter finding is subject to a type I error because the number of subjects included in this latter subgroup analysis was far too small to make any definitive conclusions; larger longitudinal studies are warranted to confirm or refute these potentially important subgroup findings.

The longitudinal C-EPI data in the symptomatic group are also interesting, and indicate that the prevalence of antiplatelet-HTPR falls as one is followed from the early to the late phase after symptom onset or intervention, including those on aspirin monotherapy. These results most likely partly reflect the effects of successful removal/treatment of the stenosing carotid plaque in the majority of symptomatic patients, as well as resolution of the acute phase response over time in patients treated with modern secondary preventive treatment. Larger, longitudinal studies assessing the same patients before and after changing antiplatelet therapy [38] are needed to adequately assess the impact of changing antiplatelet therapy on HTPR status in patients with carotid stenosis. Such studies will allow one to determine whether patients who exhibit a reduction in on-treatment platelet reactivity in response to commencing or changing antiplatelet treatment will have a lower risk of recurrent vascular events than patients who do not exhibit such a dynamic change.

Data from the C-ADP cartridge in patients on clopidogrel were not informative in this study. Our group and others have since shown that this cartridge is not sensitive to the anti-platelet effects if clopidogrel *ex vivo* when one uses a cross-sectional definition of clopidogrel-HTPR.[12,30,37]

We chose to initially assess platelet function with the PFA-100 in this novel pilot study because platelets in patients with ≥50% carotid stenosis are believed to be exposed to at least moderate-high levels of shear stress *in vivo*.[33,35], and we wanted to mimic these shear stress conditions *ex vivo*. It is possible that one might derive more informative data on HTPR if one were to use an *ex vivo* test of platelet function that exposed platelets to low shear stress, variable levels of shear stress, or simply stirred the platelets in solution, to avoid excessive exposure of platelets to high shear stress both *in vivo* and *ex vivo*. These experiments are ongoing in our lab, and data are awaited.

We did not find significant differences in on-treatment platelet reactivity in MES +vs subjects when compared with MES -ve subjects with asymptomatic or symptomatic carotid stenosis. This may reflect a type I error because the number of subjects included in this study was relatively small, but as stated above, this device may not be sensitive enough at detecting differences in HTPR status between asymptomatic and symptomatic patients overall before they undergo intervention.

In conclusion, these pilot, proof-of-concept studies have shown that platelet function/reactivity monitoring with a moderately high shear stress testing platform may identify dynamic changes in HTPR status in symptomatic *vs*. asymptomatic

moderate-severe carotid stenosis subgroups, and in symptomatic patients over time. Larger, longitudinal studies are warranted to reassess the impact of more intensive secondary preventive and interventional treatment on *ex vivo* platelet function at different levels of shear stress to determine whether monitoring HTPR status may facilitate optimised, individualised stroke prevention in patients with carotid stenosis.

All named collaborators qualify for authorship and contributed to the manuscript as follows:

Study design; collection, analysis and interpretation of data; manuscript writing: Kinsella JA, Tobin WO, McCabe DJH.

Study design, Data interpretation; critical revision of the manuscript for important intellectual content: Hamilton G.

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Conflict of interest statement:

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Table 1: Demographic Data and Risk Factor Profile of Patients. P values relate to chi-squared or Fisher exact testing between asymptomatic and symptomatic carotid stenosis groups. Values are Means (±SD) or absolute counts

Parameter	Asymptomatic Carotid Stenosis (N = 31)	Early Symptomatic Carotid Stenosis (N=46)	Late Symptomatic Carotid Stenosis (N=35)
Mean age (years)	68.2 (± 7.95)	65.0 (± 9.58)	65.0 (±9.9)
p value		0.78	0.78
Gender (M; %)	18 (58%)	28 (61%)	20 (57%)
p value		0.8	0.94
Median interval from symptom onset (days; range)	N/A	7.5 (0 - 27)	175 (99 – 360)
Degree of Stenosis:			
(moderate:≥50 – 69%)	11 (35%)	7 (15%)	15 (43%)
p value		**0.039**	0.54
(severe: ≥ 70 – 99%)	20 (65%)	33 (72%)	9 (26%)
p value		0.50	**0.0015**
(occlusion)	0	6 (13%)	4 (11%)
p value		**0.04**	0.07
Antiplatelet therapy:			
Aspirin monotherapy	22 (71%)	35 (76%)	15 (43%)
p value		0.62	**0.02**
Aspirin /Dipyridamole combination	2 (6%)	4 (9%)	11 (31%)
p value		0.54	**0.01**
Clopidogrel monotherapy	5 (16%)	2 (4%)	6 (17%)
p value		0.09	0.6
Aspirin /Clopidogrel combination	2 (6%)	5 (11%)	3 (9%)
p value		0.4	0.6
Ischemic heart disease	7 (23%)	10 (22%)	7 (20%)
p value		0.93	0.8
Hypertension	27 (87%)	29 (63%)	23 (66%)
p value		**0.02**	**0.04**
Diabetes Mellitus	7 (23%)	8 (17%)	6 (17%)
p value		0.57	0.58
Prior TIA/Stroke before index event	8 (26%)	7 (15%)	6 (17%)
p value		0.25	0.39
Family History Stroke	9 (29%)	16 (35%)	12 (34%)
p value		0.6	0.65
Prior venous thromboembolism	1 (3%)	0	0
p value		0.4	0.5
Peripheral Vascular Disease	5 (16%)	5 (11%)	6 (17%)
p value		0.5	0.91
Migraine (with or without aura)	6 (19%)	5 (11%)	5 (14%)
p value		0.3	0.58
Current smokers	5 (16%)	21 (46%)	14 (40%)
p value		**0.007**	**0.03**
Ex-smoker	22 (71%)	17 (37%)	13 (37%)
p value		**0.003**	**0.006**
Never smoker	4 (13%)	8 (17%)	8 (23%)
p value		0.59	0.3
Statin therapy	28 (90%)	33 (72%)	27 (77%)
p value		**0.043**	0.13

Table 2: Comparison of platelet reactivity in asymptomatic versus early symptomatic, late stage symptomatic and late stage symptomatic post-intervention carotid stenosis patients. Values are medians (25^{th} - 75^{th} percentile). Significant p values in bold. HTPR = high on treatment platelet reactivity.

PFA-100 Results	**Asymptomatic (N=31)**	**Early Symptomatic (N=46)**	**Late Symptomatic (N=35)**	**Late Symptomatic post-intervention (N=23)**
Entire Cohort				
C-EPI closure time (sec)	145 (124 – 229)	146 (112 – 240)	203 (123 – 301)	189 (139 – 253)
P value		0.83	0.22	0.36
C-ADP closure time (sec)	95 (80 – 103)	90 (78 – 100)	93 (74 – 109)	97 (79 – 113)
P value		0.56	0.94	0.62
	Asymptomatic (N=26)	**Early Symptomatic (N=40)**	**Late Symptomatic (N=29)**	**Late Symptomatic post-intervention (N=19)**
Subgroup on Aspirin (Alone or in Combination with Dipyridamole or Clopidogrel)				
C-EPI closure time (sec)	164 (131 – 278)	152 (115 – 295)	223 (165 – 301)	204 (166 – 289)
P value		0.55	0.27	0.56
Number (%) with HTPR on C-EPI cartridge	12 (46%)	22 (55%)	8 (28%)	4 (21%)
P value		0.48	0.15	0.08
	Asymptomatic (N=22)	**Early Symptomatic (N=30)**	**Late Symptomatic (N=15)**	**Late Symptomatic post-intervention (N=10)**
Aspirin Monotherapy Subgroup				
C-EPI closure time (sec)	148 (131 – 221)	169 (121 – 301)	205 (166 – 301)	203 (167 – 205)
P value		0.66	0.17	0.27
Number (%) with HTPR on C-EPI cartridge	11 (50%)	15 (50%)	3 (20%)	1 (10%)
P value		1.0	0.09	**0.03**
	Asymptomatic (N=7)	**Early Symptomatic (N=6)**	**Late Symptomatic (N=9)**	**Late Symptomatic post-intervention (N=6)**
Clopidogrel Subgroup (Alone or in Combination with Aspirin)				
C-ADP closure time (sec)	97 (78 – 142)	93 (82 – 106)	120 (100 – 130)	128 (119 – 145)
P value		1.0	0.46	0.25
Number (%) with HTPR on C-ADP cartridge	5 (71%)	5 (83%)	8 (89%)	5 (83%)
P value		1.0	0.55	1.0

Table 3a: Comparison of platelet reactivity between early and late phase symptomatic carotid stenosis patients with longitudinal data at both time points regardless of their prescribed antiplatelet regimen; those on aspirin alone or in combination with dipyridamole or clopidogrel; or aspirin monotherapy. Values are medians (25^{th} - 75^{th} percentile). Significant p values in bold.

PFA-100 Results in Entire Symptomatic Subgroup with Longitudinal Data	**Early Symptomatic (N=35)**	**Late Symptomatic (N=35)**	**P value**
C-EPI closure time (sec)	143 (113 – 223)	203 (123 – 301)	**0.03**
Number (%) with HTPR on C-EPI cartridge	22 (63%)	12 (34%)	**0.017**
C-ADP closure time (sec)	89 (78 – 100)	93 (74 – 109)	0.48
Symptomatic Subgroup on Aspirin (Alone or in Combination with Dipyridamole or Clopidogrel)	**Early Symptomatic (N=27)**	**Late Symptomatic (N=27)**	**P value**
C-EPI closure time (sec)	149 (115 – 263)	205 (150 – 301)	**0.023**
Number (%) with HTPR on C-EPI cartridge	16 (59%)	7 (26%)	**0.01**
C-ADP closure time (sec)	89 (78 – 96)	92 (73 – 99)	0.8
Symptomatic Subgroup on Aspirin Monotherapy	**Early Symptomatic (N=13)**	**Late Symptomatic (N=13)**	**P value**
C-EPI closure time (sec)	152 (118 – 301)	205 (167 – 301)	0.15
Number (%) with HTPR on C-EPI cartridge	8 (62%)	2 (15%)	**0.016**

Table 3b: Comparison of 'matched' platelet reactivity data between early and late phase symptomatic carotid stenosis patients who underwent carotid intervention. Values are medians (25^{th} - 75^{th} percentile). Significant p values in bold.

PFA-100 Results in Symptomatic Subgroup with Longitudinal Data pre- and post-intervention	**Early Symptomatic (N=23)**	**Late Symptomatic Post-intervention (N=23)**	**P value**
C-EPI closure time (sec)	146 (125 – 254)	191 (143 – 277)	0.32
C-ADP closure time (sec)	92 (80 – 104)	97 (79 – 113)	0.53
Symptomatic Subgroup on Aspirin Monotherapy	**Early Symptomatic (N=8)**	**Late Symptomatic Post-intervention (N=8)**	**P value**
PFA-100			
C-EPI closure time (sec)	231 (147 – 301)	204 (181 – 264)	1
Number (%) with HTPR on C-EPI cartridge	4 (50%)	0 (0%)	**0.02**
C-ADP closure time (sec)	89 (77 – 133)	99 (69 – 138)	0.69

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