Original article

Carotid plaque volume in patients undergoing carotid endarterectomy

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Background: The main indication for carotid endarterectomy (CEA) is severity of carotid artery stenosis, even though most strokes in carotid disease are embolic. The relationship between carotid plaque volume (CPV) and symptoms of cerebral ischaemia, and the measurement of CPV by minimally invasive tomographic ultrasound imaging, were investigated.

Methods: The volume of the endarterectomy specimen was measured using a validated saline suspension technique in patients undergoing CEA. Time from last symptom and severity of stenosis measured by duplex ultrasonography were recorded. Middle cerebral artery emboli were counted using transcranial Doppler imaging (TCD) in a subset of patients.

Results: Some 339 patients were included, 270 with symptomatic and 69 with asymptomatic carotid stenosis. Mean(s.d.) CPV was higher in symptomatic than in asymptomatic patients (0·97(0·43) *versus* 0.74(0·41) cm^3; $P < 0·001$). CPV did not correlate with severity of carotid stenosis ($P = 0·770$). Mean CPV was highest at 1·03(0·46) cm^3 in the 4 weeks following cerebral symptoms, declining to 0·78(0·36) cm^3 beyond 8 weeks. Among 33 patients who had TCD, mean CPV was 1·00(0·48) cm^3 in the 27 patients with ipsilateral cerebral emboli compared with 0·67(0·16) cm^3 in those without ($P = 0·142$). There was excellent correlation between CPV measured by tomographic ultrasound imaging and the endarterectomy specimen in 34 patients ($r = 0·93$, $P < 0·001$).

Conclusion: CPV correlated with symptoms of cerebral ischaemia, but not carotid stenosis. It could be a potential indicator for CEA.

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Introduction

Stroke is the third leading cause of death in the Western world behind ischaemic heart disease and cancer. It is the leading cause of disability in the UK, affecting over 150 000 people per year and costing the UK economy around £7 billion (€7·7 billion; exchange rate 14 August 2017) each year[1,2]. Carotid disease causes 30 per cent of ischaemic strokes[3], although most strokes are caused by atheroembolism. The principal indication for operation on carotid disease remains the severity of stenosis[4–8]. To confer maximal benefit, carotid endarterectomy (CEA) should be performed as soon as possible following symptoms of cerebral ischaemia, and certainly within 2 weeks as 43 per cent of patients suffering stroke had a transient ischaemic attack (TIA) in the preceding 7 days. Patients with 70–99 per cent stenosis have an absolute risk reduction of 23 per cent when CEA is performed within 2 weeks[4,5,9,10]. Although recent symptoms of cerebral ischaemia are a clear indication for early carotid surgery, severity of stenosis alone is a poor predictor of stroke risk as asymptomatic patients with greater than 70 per cent carotid stenosis on best medical care have an ipsilateral stroke risk of under 2 per cent per year[6,11].

Carotid plaque volume (CPV) is the equivalent of atherosclerotic burden and is measured as the volume of atherosclerotic material within a defined length of artery. That stenosis is not inevitable in severe atherosclerotic arterial disease has been known for many years[12]. The importance of atherosclerotic burden has been emphasized

in studies[13–15] reporting substantial plaque burden in angiographically normal arteries; American Heart Association type VI (severe complex) lesions were frequently found in carotid arteries with less than 50 per cent stenosis. The PROSPECT study[16] of coronary atherosclerosis demonstrated that cardiac events were related to high atherosclerotic burden rather than the severity of coronary artery stenosis. It is now accepted that atherosclerotic burden in coronary arteries is more important than stenosis in predicting subsequent cardiovascular events[17–21].

Two recent MRI studies[22,23] of carotid plaque burden have underlined the importance of CPV in patients undergoing CEA within days of acute ischaemic stroke[24]. Patients randomized to early surgery after acute stroke had large-volume unstable plaques, often discharging atherosclerotic material and very different from plaques removed at elective CEA[24].

The aim of this study was to explore the relationship between CPV and symptoms of cerebral ischaemia in patients undergoing CEA. A method to measure CPV accurately in CEA specimens was developed, and the relationship between CPV, the severity of carotid stenosis and recent symptoms of cerebral ischaemia was explored in patients undergoing CEA. The relationships between CPV measured in the endarterectomy specimen and CPV measured by new tomographic ultrasound imaging (tUS) technology and middle cerebral artery emboli counts before surgery were also explored in subgroups of these patients.

Methods

Patients undergoing primary CEA in Greater Manchester were recruited. Local ethics committee approval was obtained, with informed consent in writing obtained from all patients. Patients with atrial fibrillation, a diagnosis or treatment for cancer within 6 months, or unable to give informed consent were excluded.

A detailed medical history was taken, recording cardiovascular risk factors and the timing and nature of any symptoms of cerebral ischaemia. Patients were classified as symptomatic if they had symptoms of cerebral ischaemia in the previous 6 months. Symptoms were further divided into stroke, TIA or amaurosis fugax using established criteria[25]. For symptomatic patients, the time between the onset of the most recent symptom and CEA was also recorded; patients were subdivided into groups with an interval of less than 2 weeks (0–13 days), 2–4 weeks (14–27 days), 4–8 weeks (28–55 days) and at least 8 weeks (56 days or more). The severity of carotid stenosis was measured using peak systolic flow velocity (PSV) on duplex Doppler ultrasound imaging, based on the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria[26,27].

In addition to routine preoperative duplex imaging, a subset of patients underwent tUS within 24 h before surgery, performed by an experienced vascular scientist blinded to the clinical details. A magnetically tracked freehand three-dimensional (3D) ultrasound system (Curefab, Munich, Germany) was attached to a Philips iu22 duplex machine (Philips, Bothell, Washington, USA). Sensors attached to the transducer tracked the transducer orientation and position in time and space. Multiplanar reconstructions were computed to produce 3D ultrasound volumes. System accuracy had been proven previously with phantom studies registered with CT and MRI[28]. CPV was calculated by tracing the luminal surface and the adventitia at an interslice distance of 1 mm. Multiple slices were created along the length of the carotid plaque, over the same length subsequently measured following endarterectomy, and a CPV calculated automatically. There was no attempt to differentiate between plaques mainly involving the bifurcation and those solely within the internal carotid artery. All measurements were repeated by a vascular laboratory scientist and the research fellow; both were trained in the technique, and were blinded to the patient's symptoms and each other's results.

Preoperative transcranial Doppler (TCD) insonation of the ipsilateral middle cerebral artery to count microemboli over 1 h was undertaken in a subgroup of patients less than 24 h before CEA. Microembolic signals were counted by two trained and blinded observers using the 1995 international consensus criteria[29]. Transient, unidirectional signals occurring within the Doppler spectrum, at least 3 dB higher than the background blood flow and lasting less than 300 ms, were counted as emboli.

During CEA, the surgeon was asked to pay particular attention to ensure that the entire carotid plaque specimen was removed *en bloc*, where possible. A member of the research team was present to collect the plaque in a dry pot on ice immediately after endarterectomy to minimize disruption before measuring CPV within the next hour. The full length of the endarterectomy plaque was recorded before the plaque was weighed while suspended below the surface of 110 ml normal saline in a 150-ml polythene beaker placed on an electronic balance. The volume was calculated by dividing the suspended weight by the density of saline at 23 °C. Intraobserver agreement on the measurement of CPV, using data from the first 81 patients, demonstrated a mean bias of only 0·01 cm^3 (95 per cent limits of agreement −0·11 to 0·14 cm^3). Interobserver agreement for CPV had a mean bias of 0·004 cm^3 (95 per cent limits of agreement −0·18 to 0·19 cm^3). These

Table 1 Characteristics of patients undergoing carotid endarterectomy

	Asymptomatic ($n = 69$)	Symptomatic ($n = 270$)	P†
Age (years)*	69·6(8·7)	70·6(8·7)	0·370‡
BMI (kg/m^2)*	27·2(4·1)	27·1(4·9)	0·905‡
Sex ratio (M : F)	47 : 22	177 : 93	0·661
Diabetes	13 (19)	50 (18·5)	0·953
Hypertension	54 (78)	201 (74·4)	0·597
Hypercholesterolaemia	55 (80)	188 (69·6)	0·177
Ischaemic heart disease	14 (20)	63 (23·3)	0·556
Previous myocardial infarct	12 (17)	39 (14·4)	0·567
Previous stroke	22 (32)	52 (19·3)	0·035
Smoking history			0·979
Smoker	16 (23)	62 (23·3)	
Ex-smoker	38 (55)	138 (51·1)	
Never smoked	13 (19)	47 (17·4)	
Missing	2 (3)	23 (8·5)	

Values in parentheses are percentages unless otherwise indicated; *values are mean(s.d.). †χ^2 test, except ‡Student's t test.

demonstrate good reliability and repeatability for the measurement of CPV.

Statistical analysis

Normality of variables was assessed on the basis of skewness and kurtosis measures. Descriptive statistics for CPV are presented as mean(s.d.). Student's t tests and χ^2 tests were used to compare patient characteristics and CPV values between asymptomatic and symptomatic patients. Differences in CPV between the symptom subgroups and at each time interval following the most recent symptoms of cerebral ischaemia were assessed using ANOVA, followed by Scheffé's multiple comparison test. Interobserver and intraobserver agreements for CPV measurement using the suspension hydrostatic weighing technique and tUS images were investigated using the Bland–Altman method of agreement. The conventional two-sided 5 per cent significance level was used for all analyses.

Results

A total of 345 patients were recruited to the study but the endarterectomy specimens in six were removed piecemeal, rendering CPV measurements impossible. This left a total of 339 patients, all with carotid stenosis over 50 per cent (270 symptomatic, 69 asymptomatic) (*Table 1*). Symptomatic patients were more likely also to have had a previous episode of cerebral ischaemia ($P = 0·035$). There were no significant differences in cardiovascular risk factors between asymptomatic and symptomatic patients.

Table 2 Relationship between carotid plaque volume and cardiovascular risk factors

	n	Carotid plaque volume*	P†
Sex			< 0·001
M	221	1·01(0·45)	
F	118	0·76(0·34)	
Diabetes	62	0·95(0·42)	0·772
Hypertension	252	0·93(0·44)	0·573
Hypercholesterolaemia	240	0·94(0·43)	0·728
Ischaemic heart disease	76	0·97(0·49)	0·456
Previous myocardial infarct	50	0·97(0·49)	0·490
Previous stroke	72	0·88(0·42)	0·251
Smoker	78	0·93(0·51)	0·650

*Values are mean(s.d.). †*Versus* carotid plaque volume in absence of risk factor (Student's t test).

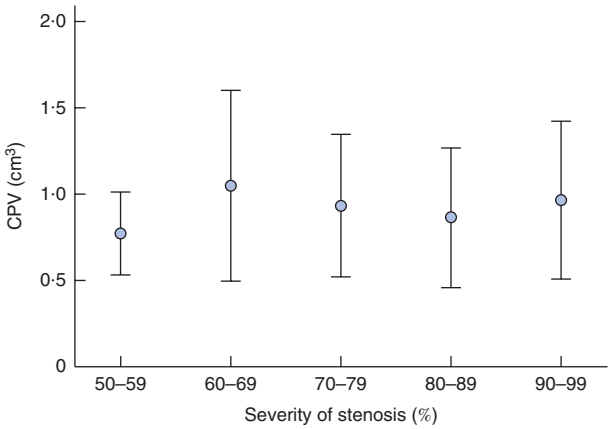

Fig. 1 Mean(s.d.) carotid plaque volume (CPV) in patients undergoing carotid endarterectomy according to severity of stenosis. $P = 0·770$ (ANOVA, followed by Scheffé's multiple comparison test)

Total mean(s.d.) CPV was significantly greater in men than in women (1·01(0·45) *versus* 0·76(0·34) cm^3 respectively; $P < 0·001$). Cardiovascular risk factors were not otherwise significantly related to CPV (*Table 2*). CPV was not associated with the severity of carotid stenosis in these patients, in whom the severity of stenosis was the indication for CEA ($P = 0·770$) (*Fig. 1*).

Mean CPV was significantly greater in the 270 symptomatic patients than in the 69 patients with no symptoms of cerebral ischaemia (0·97(0·43) *versus* 0·74(0·41) cm^3; $P < 0·001$). The difference remained significant following adjustment for sex ($P < 0·001$). The mean CPV was 1·03(0·49) cm^3 in 93 patients following recent stroke, compared with 0·95(0·42) cm^3 for the 141 patients who had a TIA and 0·91(0·28) cm^3 for the 34 patients with amaurosis fugax ($P = 0·254$); the symptom was not recorded for two patients.

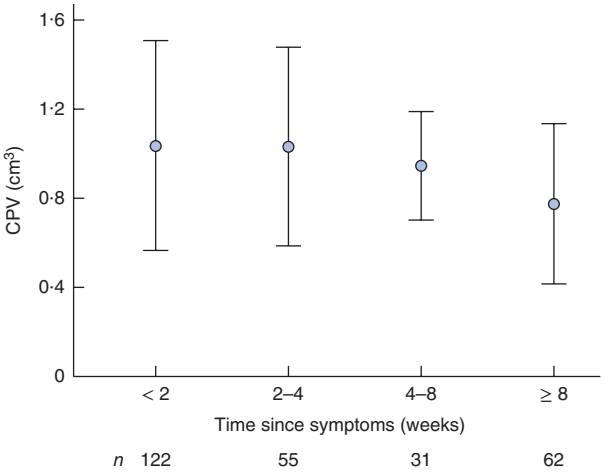

Fig. 2 Mean(s.d.) carotid plaque volume (CPV) in patients undergoing carotid endarterectomy within 2, 2 and less than 4, 4–8 and more than 8 weeks following the most recent symptom of cerebral ischaemia. $P < 0{\cdot}001$ (ANOVA, followed by Scheffé's multiple comparison test)

Among the 270 symptomatic patients, mean CPV was highest in patients undergoing CEA shortly after symptoms of cerebral ischaemia and declined as the interval between symptoms and surgery increased ($P < 0{\cdot}001$) (*Fig. 2*). Mean CPV for patients undergoing CEA within 2 weeks of symptoms (1·039 cm^3) was very similar to that among patients undergoing CEA within 2–4 weeks (1·035 cm^3), so these patients were grouped together for statistical analysis. Mean CPV within 4 weeks following symptoms of cerebral ischaemia was 1·03(0·46) cm^3 compared with 0·95(0·24) cm^3 between 4 and 8 weeks, and 0·78(0·36) cm^3 more than 8 weeks after symptoms. Those who underwent CEA more than 8 weeks following symptoms of cerebral ischaemia had a CPV almost identical to that of asymptomatic patients, but significantly lower than that in patients undergoing CEA less than 4 weeks after symptoms ($P = 0{\cdot}001$, Scheffé's multiple comparison test).

Using PSV as a measure of the haemodynamic severity of stenosis, there were no corresponding differences. Mean(s.d.) PSV was 3·49(1·84) cm/s within 4 weeks of symptoms, 3·88(2·13) cm/s between 4 and 8 weeks, and 3·47(2·09) cm/s more than 8 weeks after the onset of the most recent symptom of cerebral ischaemia ($P = 0{\cdot}570$). Mean PSV was higher in asymptomatic patients undergoing CEA than it was in symptomatic patients (3·99(1·77) *versus* 3·47(1·94) cm/s respectively; $P = 0{\cdot}056$), although this is probably because many surgeons were reluctant to undertake CEA for asymptomatic disease unless the severity of the stenosis exceeded 80 per cent.

Cerebral emboli were counted in the ipsilateral middle cerebral artery over 1 h by TCD before CEA in 33 symptomatic patients. They were detected in 27 of these patients, the mean(s.d.) number of emboli during the hour of monitoring being 3·9(2·7). Mean CPV was 1·00(0·48) cm^3 in the 27 patients with cerebral emboli compared with 0·67(0·16) cm^3 among the six patients in whom no cerebral emboli were detected ($P = 0{\cdot}142$). There was a significant but weak correlation between number of cerebral emboli per h and CPV ($r = 0{\cdot}36$, $P = 0{\cdot}045$). There was a negative but insignificant correlation between number of cerebral emboli per h and PSV ($r = -0{\cdot}255$, $P = 0{\cdot}190$).

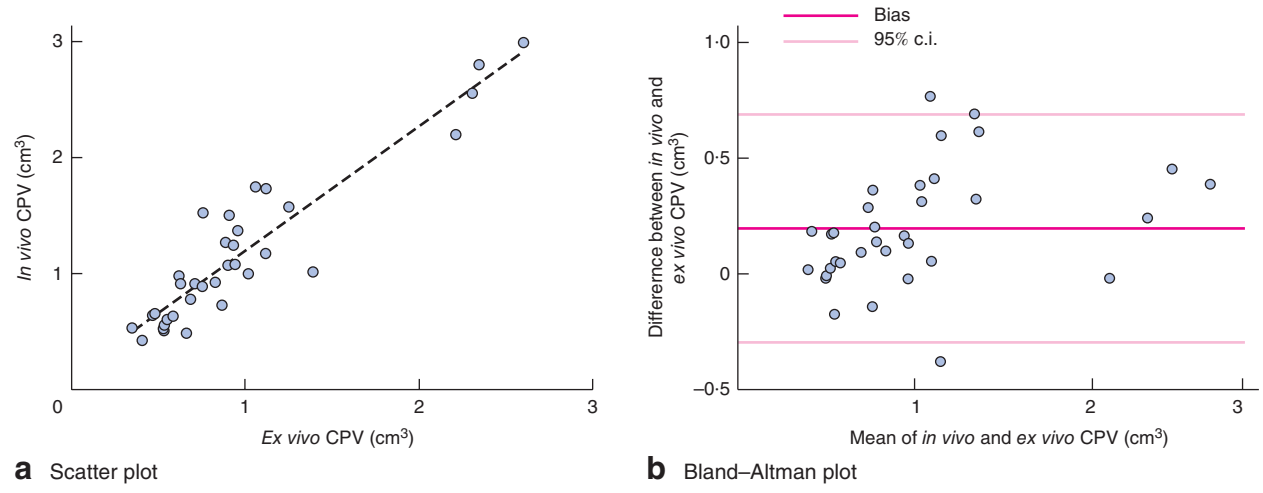

Fig. 3 **a** Scatter plot demonstrating correlation between *in vivo* measurements of carotid plaque volume (CPV) by three-dimensional ultrasonography and *ex vivo* measurements using the immersion technique ($r = 0{\cdot}93$, $P < 0{\cdot}001$). **b** Bland–Altman plot (mean(s.d.) bias 0·20 (95 per cent c.i. −0·29 to 0·69) cm^3)

Of 50 patients who underwent preoperative tUS, results from ten were omitted owing to extensive acoustic shadowing rendering interpretation impossible, and six did not have corresponding surgical specimens. The CPV measured by tUS in the remaining 34 patients correlated closely with the CPV measured in the endarterectomy specimen ($r = 0{\cdot}93$, $P < 0{\cdot}001$), with minimal bias (0·20 (95 per cent c.i. −0·29 to 0·69) cm^3) (*Fig. 3*). Inter-rater analysis demonstrated minimal bias (0·01 (−0·23 to 0·25) cm^3) between the CPV measurements calculated by the two observers, again with excellent correlation ($r = 0{\cdot}98$, $P < 0{\cdot}001$) (data not shown). Intrarater analysis demonstrated minimal bias (0·07 (0·25 to 0·39) cm^3) between the CPV measurements calculated when repeated by the primary observer, again with excellent correlation ($r = 0{\cdot}97$, $P < 0{\cdot}001$) (data not shown).

Discussion

CPV in the endarterectomy specimen was associated with recent symptoms of cerebral ischaemia in patients undergoing CEA. Perhaps more importantly, CPV was markedly higher in the first few weeks following symptoms of cerebral ischaemia, when the risk of stroke is also known to be high. Rothwell and colleagues[10] clearly showed that CEA had the greatest impact on stroke risk if undertaken within days of cerebral ischaemia, and no more impact than it had for asymptomatic patients if undertaken more than 12 weeks following symptoms.

The results suggest that the healing of carotid plaques, following what is presumed to be a discharge of atherosclerotic material at the time of cerebral ischaemia, may be faster than previously recognized. The decline in plaque volume over time following symptoms of cerebral ischaemia was not associated with any significant change in the severity of carotid stenosis. There was no significant relationship between the severity of stenosis and CPV, although this is a population of patients in whom the severity of carotid stenosis was sufficient to justify CEA. It was hardly surprising that the severity of carotid stenosis was marginally higher in the asymptomatic patients as many surgeons are reluctant to undertake carotid endarterectomy in asymptomatic patients unless there is stenosis exceeding 80 per cent.

Middle cerebral artery emboli in patients with carotid disease are known to be associated with recurrent ischaemic events[30]. The Asymptomatic Carotid Emboli Study[31] demonstrated that the annual risk of stroke in patients with embolic signals was 3·6 per cent compared with 0·7 per cent in those without. The significant correlation between CPV and number of cerebral emboli suggests that CPV may be a measure of stroke risk. The absence of a correlation between cerebral emboli and severity of stenosis underlines the low risk of stroke in asymptomatic severe carotid stenosis. Although there has been little previous research on CPV, when measured by ultrasound imaging in 349 patients it was associated with the frequency of subsequent stroke, TIA and death[32]. Both CPV and changes in plaque composition were also reported to be indicators of stroke risk[33].

The concept of the vulnerable plaque was first reported in coronary artery disease, where ruptured, inflammatory and thin cap plaques were associated with acute coronary syndrome[34–36]. The same was assumed to be true for carotid plaques, leading to many attempts to identify carotid plaque characteristics that relate to the subsequent risk of stroke[22,23,37–39]. The present results raise the intriguing possibility that intraplaque haemorrhage[37] and measures of plaque perfusion[38,40] may be the consequence of plaque rupture, rather than risk factors for future stroke. The finding that CPV declines with time following symptoms of cerebral ischaemia suggests a healing process measured in weeks, which may be much shorter than that suggested in the literature. It is possible that, following the discharge of atherosclerotic material from a carotid plaque, the space is filled with blood which has been interpreted as intraplaque or subplaque haemorrhage. Subsequently this haematoma is lysed and progressively replaced by granulation tissue as part of the healing process, with resulting increases in plaque perfusion[38]. The fact there is no difference between CPV within 2 weeks of symptoms and that at 2–4 weeks is consistent with the time required for a haematoma to start to resolve by lysis, and for the resulting inflammation to settle.

Previous studies[33,41,42] measuring CPV by both MRI and CT suggested that CPV was associated with cardiovascular risk factors and symptoms of cerebral ischaemia. If the accuracy of tUS measurement of CPV is confirmed in larger studies, it is possible that the measurement of CPV could replace severity of stenosis as the principal indication for CEA. The ultimate objective could be population screening for carotid disease. 3D ultrasound techniques for the measurement of CPV need to be explored with this in mind[43–45].

Limitations of this study were that only 33 patients of the intended 50 underwent preoperative TCD to detect middle cerebral artery emboli. Neither CT nor MRI of the brain is routine in patients undergoing CEA in Manchester, precluding any investigation of the relationship between CPV and cerebral infarction. The need for urgent CEA following symptoms of cerebral ischaemia has been established by

two major meta-analyses[46,47] demonstrating that the risk of stroke is highest immediately following TIA and then declines over a similar timescale. These studies showed that the stroke risk within 1 week of TIA was approximately 10 per cent and that this risk declined progressively, such that the risk was no higher than in patients with asymptomatic carotid disease by 12 weeks. As CPV declines following symptoms of cerebral ischaemia at a rate remarkably similar to the reduction in stroke risk, this is consistent with CPV being associated with stroke risk. Once a minimally invasive method for measuring CPV accurately in patients has been established, the relationship between CPV and stroke risk needs to be explored in a definitive cohort study.

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