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D-dimer local expression is increased in symptomatic patients undergoing carotid endarterectomy

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Abstract

Background: Although atherosclerosis is a silent widespread disease, the focal character of the lesions triggering the clinical manifestations is unquestionable. We hypothesized that symptomatic patients with advanced, unstable carotid plaques have increased local intraplaque and circulating levels of fibrin–fibrinogen related products.
Methods: Plaque tissue and plasma samples were studied in 106 patients undergoing endarterectomy for symptomatic and asymptomatic carotid disease. Fibrin–fibrinogen related products were evaluated by ELISA, Western-blotting, and histology. All tested parameters were compared with patient carotid symptomatology, multiple vascular risk factors (VRF), bilateral carotid pathology, ultrasound examination, and previous therapies with statins and/or antiplatelet drugs.
Results: In symptomatic patients, plasma D-dimer was elevated in patients with unstable carotid plaques (UNS) compared with stable (STA) ones (857±121 *vs*. 692±156 ng/ml, $p=0.026$). Furthermore, plasma D-dimer was significantly increased in patients with a coexistence of carotid and coronary artery disease, compared to others (976±325 *vs.* 714±197 ng/ml; $p<0.001$). Intra-plaque D-dimer content was increased in ulcerated-complicated (UC) plaques compared with fibrous non-complicated (F) plaques in symptomatic patients (5.9±1 *vs.* 1.8±1, $p<0.001$), and in patients with hypercholesterolaemia, compared with those with normal cholesterol levels (6.1±1 *vs.* 2.9±0.7; $p=0.027$). However, there was no correlation between D-dimer content in the carotid plaque and plasma D-dimer levels.
Conclusions: Hypercholesterolemia and UC plaques appear to be associated with high fibrin intraplaque turnover as demonstrated by higher intraplaque D-dimer. Plasma markers of fibrin turnover were increased in UNS plaques, and in patients with coexisting carotid and coronary artery disease.

Although, both plasma and plaque D-dimers were associated with unstable carotid disease, the usefulness of the measurement of plasma D-dimer in these patients should be confirmed by prospective studies.

Keywords: D-dimer; Atherosclerosis; Carotid artery disease; Unstable plaque

Abbreviations: FRA, fibrin related antigens; CAD, coronary arterial disease; PAD, peripheral arterial disease; TIA, transient ischaemic attack; AHA, American Heart Association.

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1. Introduction

Almost one-third of patients admitted with cerebral infarction have significant atherosclerotic narrowing of the ipisilateral internal carotid artery [1]. Although the importance of the degree of vessel narrowing to the subsequent risk of stroke remains elusive, and the role of plaque surface morphology is uncertain, plaque progression, ulceration, rupture, local thrombus formation and intraplaque hemorrhage are likely to contribute to symptomatic disease [2,3]. Circulating molecules are associated with increased risk of symptomatic vascular disease in moderate carotid stenosis and with a worse outcome in patients with ischemic stroke [4–6]. The focal character of atherosclerosis is unquestionable, and there is some recent evidence that the plaque wall itself can secrete proteins that could be markers for atherosclerosis [7,8].

Advanced atherosclerotic lesions contain large amounts of plasma macromolecules, including fibrinogen and fibrin related antigens (FRA) formed during local fibrinogenesis and fibrinolysis [9]. Mediators of fibrinolysis may contribute to the development of symptomatic disease in patients with high-grade carotid stenosis [10]. In unstable, active plaques, the intrinsic pathway of coagulation leads to formation of thrombin and fibrin [11]. Local fibrin formation and lysis is part of the inflammatory response, and fibrin degradation products (FDP), including D-dimer [12], a marker of fibrin degradation, have different effects on acute-phase responses [13]. The fate of fibrin/fibrinogen may reflect the current state of coagulation and hemostasis in patients as well as the relative risk of clot formation [14].

It is not known whether hyper-coagulation, caused by increased plasma fibrin levels, is a cause or consequence of atherosclerosis and thrombosis. D-dimer appears to be a consistent marker of the risk of cardiovascular disease. We hypothesized that symptomatic patients with advanced, unstable carotid plaques have increased local intraplaque and circulating levels of fibrin–fibrinogen related products.

2. Patients and methods

We included 62 symptomatic (suffering from TIA or hemispheric stroke in the 6 months prior to surgery) and 44 asymptomatic (at least 6 months without cerebrovascular syndromes prior to surgery) patients undergoing endarterectomy for high grade carotid stenosis (>70%) at our institution [15]. They consisted of 92 men and 14 women, aged 55–79 (mean age 69, S.D.±8.6), and samples were collected over a period of approximately 2 years. Among the ipsilateral symptomatic patients, 24 had hemispheric stroke and 38 had transient ischemic attack (TIA) (lacunar infarcts were excluded from the analysis). All patients underwent MR angiography and the degree of carotid stenosis was calculated by EchoDoppler imaging according to European Carotid Study Group [16]. Patients were also screened for the presence of bilateral pathology (>50% contralateral stenosis). Duplex colour ultrasound analysis was used to classify plaques as clearly hypoechogenic, intermediate, or hyperechogenic (1995) by two independent, trained personnel [16]. The presence of vascular risk factors (VRF) was recorded (Table 1). Previous statin treatment and duration of antiplatelet treatment were recorded and anticoagulated patients were excluded from further analysis. Patients with a history of acute arterial or venous thromboembolism, active infections or inflammatory conditions, neoplasia, recent trauma or surgery were excluded from the quantitative assay of D-dimer.

The study was approved by the local ethical committee in accordance with institutional guidelines and patient's written informed consent was obtained.

2.1. Blood sampling and tests

Plasma samples were prepared from blood collected after overnight fasting and prior to surgery. These were frozen in liquid nitrogen and stored at −80 °C for further analysis. D-dimer was measured in plasma by automated latex enhanced immunoassay on IL Coagulation Systems (IL Test D-Dimer, 20008500, Biokit, SA). Each sample was measured in duplicate, and the absorbance compared with that produced using a range of D-dimer standards. All other standard heamatological and biochemical analyses were performed routinely at the hospital laboratory.

2.2. Histology, Western blotting of carotid specimens and identification of D-dimers

Carotid specimens were excised by the vascular surgeon without damage to the plaque surface. They were immediately rinsed in 0.9% saline and cut longitudinally into two segments. The first one was snap frozen in liquid nitrogen and stored at −80 °C and the second was fixed for 24 h in buffered formalin, cryo-protected in 30% sucrose and frozen in OCT for histology. Plaque morphology was assessed

Table 1
Patients clinical characteristics

Feature	Asymptomatic	Symptomatic
N	44	62
Male/Female	38/6	55/7
Age	69.5±5.5	68.1±7.4
Hypertension	31	39
Diabetes	17	23
Hypercholesterolaemia	26	36
Active smokers	30	47
CAD	7	16
PAD	7	9
UC plaque (histology)	28	45
Bilateral carotid pathology (EchoDoppler)	23	23
Antiplatelets	35	44
Statins	21	21

CAD—coronary artery disease; PAD—peripheral artery disease; UC—ulcerated-complicated plaque on histology.