Differences in Lesion Severity and Cellular Composition between in vivo Assessed Upstream and Downstream Sides of Human Symptomatic Carotid Atherosclerotic Plaques

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Key Words

Atherosclerosis • Regional blood flow • Magnetic resonance • Carotid artery • Stenosis

Abstract

Background: The heterogeneous structure of carotid atherosclerotic plaques may be better understood if it is related to blood flow variations, influencing gene expression and cellular functions. Upstream of the maximum stenosis there is laminar blood flow and high shear stress, downstream there is turbulence and low shear stress. We studied if these variations were associated with differences in plaque morphology and composition between sites located up- and downstream of the maximum stenosis in symptomatic carotid plaques. **Methods:** Patients with symptomatic carotid stenosis were examined with magnetic resonance angiography to localize the maximum stenosis in-vivo, prior to endarterectomy. In 41 endarterectomized specimens, transverse tissue sections prepared up- and downstream of the maximum stenosis were compared using histopathology and immunohistochemistry. **Results:** The location of maximum stenosis relative the carotid bifurcation varied considerably between plaques. Compared with the downstream side, the upstream side of the stenosis had higher incidence of severe

lesions with cap rupture and intraplaque hemorrhage, more macrophages, less smooth muscle cells and more collagen. **Conclusions:** The up- and downstream sides of symptomatic carotid plaques differed in plaque morphology and composition. This implies that the intraplaque location of sampling sites may be a confounding factor in studies of atherosclerotic plaques.

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Introduction

Studies of symptomatic carotid artery disease based upon endarterectomy specimens are hampered by the considerable heterogeneity of these lesions. Mechanical factors related to blood flow are of great importance for variations in the plaque microenvironment. Wall shear stress, a biomechanical force imposed directly on the vascular endothelium, affects endothelial structure and function by activating transcription factors that modulate gene expression [1]. Stenotic atherosclerotic plaques are associated with laminar blood flow and high wall shear stress upstream of the point of maximum stenosis, as well as low shear stress, turbulent flow and vortex shedding on the downstream side [2]. Plaques often rup-

Table 1. Patient characteristics (n = 40)

	Study sample
Age, years	68 (16)
Gender	
Male	32 (80%)
Female	8 (20%)
Type of clinical event ¹	
Stroke	27 (66%)
TIA	10 (24%)
AF	4 (10%)
Days since last clinical event	84 (70)
Cardiovascular risk factors	
Diabetes	10 (25%)
Hypertension	28 (70%)
Smoking ²	16 (40%)
Medication	
Statin	31 (78%)
ACE-I or ARB	15 (38%)
Anti platelet	37 (93%)
Warfarin	3 (8%)

Figures in parentheses are IQR, unless indicated otherwise. ACE-I = Angiotensin-converting enzyme inhibitor; AF = amaurosis fugax; ARB = angiotensin receptor blocker.

¹ n = 41; 1 patient was operated bilaterally.

² Within the same year as the clinical event.

ture on the upstream side [3–5], whereas plaque growth seems to occur on the downstream side [6]. Low shear stress may also induce upregulation of cell adhesion molecules [7].

In atherogenesis, low shear stress is considered a pro-inflammatory stimulus [1]. However, animal experiments [8] and studies of mixed samples of symptomatic and nonsymptomatic human plaques [4, 5] have suggested a predominant macrophage accumulation in high shear stress regions. While invasion of macrophages is associated with symptomatic carotid artery disease [9, 10], the longitudinal distribution of macrophages in symptomatic plaques is not known.

We hypothesized that plaque sites upstream of the point of maximum carotid stenosis in symptomatic patients have more severe lesions and a different cellular composition compared with plaque sites downstream of the maximum stenosis. Here, we defined the point of maximum stenosis in vivo, and present detailed histopathological and immunohistochemical analyses of symptomatic carotid plaques at different sites along the longitudinal axis relative to the point of maximum stenosis.

Materials and Methods

Study Population

The study sample consisted of symptomatic carotid atherosclerotic plaques obtained from the Göteborg Atheroma Study Group biobank of patients who underwent carotid endarterectomy at the Sahlgrenska University Hospital (Göteborg, Sweden). Criteria qualifying for surgery were minor ischemic stroke, transient ischemic attack (TIA) or amaurosis fugax and a high-grade carotid stenosis ($\geq 70\%$ determined with the European Carotid Surgery Trial method [11]). For this study, we consecutively included those patients who had undergone pre-operative imaging by magnetic resonance angiography (MRA), and from whom serially sectioned paraffin-embedded endarterectomies were available (n = 41). Patients undergoing MRA were those in whom further clinical information was needed in order to reach a final decision on treatment. Comparison of the present patient group with the entire patient cohort undergoing endarterectomy during the corresponding time period showed no differences in age, sex or days since last clinical event. However, in the present patient group ischemic stroke was more (65 vs. 50%) and amaurosis fugax was less (10 vs. 22%) common as qualifying event, compared with the entire patient cohort. Clinical data were obtained through patient questionnaires and medical records. Patient characteristics are presented in table 1. During the time course of the study, clinical practice shifted towards more statin medication and shorter waiting times before surgery. As a consequence, patients with statin treatment had significantly shorter times between last clinical event and operation than those without statin treatment [median 77 days (interquartile range, IQR, 42–101) versus 145 (IQR 74–205), $p = 0.028$]. Study approval was obtained from the regional ethics committee, and all subjects gave informed consent to participate.

Preoperative Imaging

Duplex ultrasound was done using an Acuson Sequoia 512 (Siemens Acuson Corp., Mountain View, Calif., USA) with a 6-MHz linear transducer for initial determination of the degree of carotid stenosis. Intrastenotic peak systolic blood flow velocities were measured and converted to degrees of stenosis using the European Carotid Surgery Trial method [11]. Color Doppler imaging confirmed the upstream laminar flow associated with high shear stress levels, accelerating into the area of maximum stenosis, and the turbulent flow downstream of the maximal stenosis associated with low shear stress levels (fig. 1a and b). No numeric measurements of flow velocity were available from areas up- or downstream of the point of maximum stenosis.

MRA examinations were performed on a Philips Gyroscan Intera 1.5, Release 9 unit with a head and neck coil (Philips Medical Systems, Best, The Netherlands), using standard settings. The MRA images were used to localize the point of maximum stenosis and to measure the distance between this point and the carotid bifurcation (fig. 1c).

Tissue Processing

When the endarterectomies were obtained from the surgeon in the operating room, they were marked with an ink line on their medial side to ensure that correct orientation and rotation were maintained throughout further processing, and then immediately fixed in formalin. The specimens were then divided transverse-

ly at the point of the flow divider in the bifurcation, and from this starting point, approximately 3-mm-thick tissue blocks were prepared along the full length of the plaque. The thickness of each tissue block was measured in order to enable calculations of its exact distance to the bifurcation. The tissue blocks were then embedded in paraffin. There are diverging reports on the effect of formalin fixation on atherosclerotic tissue size, but the changes in tissue volume appear to be minor. One investigation reported tissue expansion by 2–3% [12]. In the current work, we did not compensate for any effect of formalin fixation. In order to examine whether this may have confounded the results, we recalculated the data after adjusting for an increase in size by 3%, and the results remained unchanged for differences in up- and downstream regions regarding all analyzed parameters.

Knowing the distance between the bifurcation and the maximum stenosis, which was determined from the MRA (fig. 1c), each tissue section from the endarterectomized specimens could then be related to the point of maximum stenosis (fig. 1d). Serial transverse 4- μ m sections were taken from each paraffin block for histopathological classification and immunohistochemical analysis.

We also examined if the locations of the point of maximum stenosis, as identified by MRA, were in accordance with the point of maximum stenosis as found in the endarterectomies used in the histopathological analyses. There was a good agreement between the point of maximum stenosis measured by MRA and the narrowest lumen in the specimens, assessed by lumen area measurements on the tissue sections (data not shown).

Histopathological Classification

Sections were deparaffinized and stained with Mayer's hematoxylin and eosin (Histolab, Göteborg, Sweden). Two investigators, blinded for the section identity, classified each section according to the AHA classification [13] using a Zeiss Axio Imager M1 microscope (Göttingen, Germany). Further characterization was made regarding presence of a thin fibrous cap, intraplaque hemorrhage, plaque rupture and/or intraluminal thrombosis (fig. 2a–d). The definitions used were adopted from Lovett et al. [14]: ruptured cap: clear communication between the lipid core and the lumen with a break in the fibrous cap, usually in an area of thinning of the cap (shoulder) and inflammation, and where the break in the cap did not appear to have been caused by surgery; surface thrombus: an organized collection of fibrin and red blood cells in lumen; thin fibrous cap: thin or ruptured layer of fibrous tissue separating the necrotic core from the lumen; intraplaque hemorrhage: area of erythrocytes within the plaque causing disruption of plaque architecture, including recent and old hemorrhage.

To test the reproducibility of the classification, 89 sections from 30 carotid plaques outside this study were analyzed by the same investigator on 2 occasions with an interval of 2 weeks, showing a good reproducibility ($r_s = 0.82$, $p < 0.001$). The second readings were blind to the first readings.

The tissue sections were grouped according to their distance to the maximum stenosis, and the frequencies of the histopathological features were assessed at different sites along the longitudinal axis. For comparisons between the up- and downstream sides, we assessed and compared the presence of each histopathological feature among sections obtained 1.5–7.5 mm proximal to the maximum stenosis (upstream) and sections obtained 1.5–7.5 mm distal to this point (downstream).

Fig. 1. **a** Schematic representation of the laminar flow with high wall shear stress upstream of the maximum stenosis, and the turbulent flow with low wall shear stress downstream of the maximum stenosis. **b** Representative Color Doppler image of a stenotic internal carotid artery (flow direction from left to right), showing the upstream laminar flow pattern accelerating into the area of maximum stenosis. The mosaic pattern downstream of the maximum stenosis illustrates the turbulent flow pattern in this region. **c** Pre-operative MRA image of a stenotic internal carotid artery (ICA). From the MRA image, the point of maximal stenosis (MaxS) was determined and its distance to the carotid bifurcation (Bif) measured, for each plaque. ECA = External carotid artery; CCA = common carotid artery. **d** Endarterectomized and formalin-fixed specimen of the same plaque depicted in the MRA image. The plaque was divided into approximately 3-mm-thick tissue blocks from which serial transverse sections were prepared. Using the bifurcation as a reference level, the endarterectomy could be related to the MRA image and the distance to the maximum stenosis for each tissue section thus calculated.

Fig. 2. Histopathological assessment of hematoxylin and eosin-stained tissue sections, with examples of thin fibrous cap (a), intraplaque hemorrhage (b), plaque rupture (c) and surface thrombus formation (d). Scale bars = 100 μ m.

Immunohistochemistry

Sections were deparaffinized and treated with an antigen retrieval reagent (Dako, Glostrup, Denmark) before application of antibodies. The sections were stained with mouse monoclonal antibodies against CD68 (Novocastra Laboratories, Newcastle, UK; 1:500 dilution) and smooth muscle-specific α -actin (SMA; Enzo Diagnostics, Farmingdale, N.Y., USA; 1:1,500). Immune complexes were visualized using the LSAB system HRP kit (Dako). Incubations with primary antibodies were performed over night in a humid chamber at 20°C. As negative controls, PBS buffer or a negative control mouse antibody (Dako) was substituted for primary antibody. Collagen was stained with Sirius Red F3B (HistoLab). Elastin was stained using a modified protocol for Russel-Movat Pentachrome staining [15]. Examples of immunohistochemically stained sections are given in figure 3.

Stained sections were digitized through standardized microscopy using a Zeiss Axioplan 2 imaging microscope with a top-mounted Zeiss Axiocam digital camera. Digital images were analyzed using the BioPix software (BioPix AB, Göteborg, Sweden) as previously described [16]. Staining was expressed as a percentage of the total section area. For comparisons between the upstream and downstream sides, the mean stained areas among sections obtained 1.5–7.5 mm proximal to the maximum stenosis (upstream) and sections obtained 1.5–7.5 mm distal to this point (downstream) were calculated and compared.

Statistical Analysis

SPSS 15.0 for Windows (SPSS Inc., Chicago, Ill., USA) was used for statistical analyses. Results are given as medians and IQR for continuous variables or as percentage for categorical variables. Statistical significance was assessed using the McNemar, Wilcoxon

on rank and Mann-Whitney U tests, and Spearman correlation. Two-sided p values <0.05 were considered statistically significant.

Results

Characteristics of the Stenoses

Pre-operative Doppler ultrasound examinations were used to determine systolic flow velocities. The flow velocity at the point of maximum stenosis ranged from 2.0 to 9.5 m/s with a median velocity of 4.7 m/s (IQR 1.4). Pre-operative MRA imaging was used to localize the level of maximum stenosis in vivo. A majority of maximum stenoses were found to be located at or just downstream of the carotid bifurcation, but the variation was large with locations ranging from 3.9 mm upstream of the bifurcation to 16.5 mm downstream of the bifurcation (fig. 4).

More Severe Lesions Upstream than Downstream of the Maximum Stenosis

The most severe lesion phenotype (AHA VI) was most common on the upstream side of the maximum stenosis (fig. 5a). Separate analyses of individual histopathological features showed that thin fibrous caps (fig. 5b), intraplaque hemorrhage (fig. 5c) and ruptured caps (fig. 5d)

Fig. 3. Immunohistochemical staining with hematoxylin and eosin (**a**), anti-CD68 antibodies (**b**), anti-SMA antibodies (**c**), elastin (**d**) and Sirius Red photographed in bright-field (**e**) and polarized (**f**) light. This section was located 8 mm upstream of the maximal stenosis and displays a large necrotic core separated from the lumen with a thin fibrous cap, highly invaded with macrophages.

were all more frequent upstream than downstream of the maximum stenosis, while there was no statistically significant difference in the incidence of surface thrombosis (fig. 5e).

The incidence of a thin fibrous cap at the maximum stenosis was higher in plaques from patients operated within 100 days from the last clinical event compared with longer times (88 vs. 54%; $p = 0.02$). No other significant associations were found between the histopathology and

either blood flow velocity at the maximum stenosis, time since clinical event or statin treatment (data not shown).

More Macrophages, Less Smooth Muscle Cells and More Collagen Upstream than Downstream of the Maximum Stenosis

The CD68-stained area (macrophages) gradually increased along the longitudinal axis in the flow direction, reached its highest levels 3 mm upstream of the maxi-

Fig. 4. Histogram demonstrating the location of maximum stenosis in 41 symptomatic carotid atherosclerotic plaques relative to the carotid bifurcation, as determined using MRA. The bifurcation was set to 0 mm. Positive distances were used for locations downstream of the bifurcation, and negative distances used for locations upstream of the bifurcation.

Fig. 5. Histopathology in 41 symptomatic carotid atherosclerotic plaques, in relation to the distance to the maximum stenosis. **a** Frequencies of AHA classes, where AHA VI represents complicated lesions with ruptured fibrous cap and/or surface thrombus, AHA IV + V represents fibroatheromas, and AHA III pre-atheromas. **b** Frequencies of thin fibrous caps. **c** Frequencies of intraplaque hemorrhage. **d** Frequencies of ruptured fibrous caps. **e** Frequencies of surface thrombosis. ** $p < 0.01$; *** $p < 0.001$; n.s. = not significant; from the McNemar test of difference between the upstream and downstream sides, using the assessed presence of each feature for each plaque among sections located 1.5–7.5 mm up- and downstream of the maximum stenosis.

Fig. 6. Macrophage, smooth muscle cell and collagen distribution in 41 symptomatic carotid atherosclerotic plaques. Transverse tissue sections were immunohistochemically stained for macrophages (a), smooth muscle cells (b) and collagen (c). The bars show the mean stained area in tissue sections, organized according to their distance to the maximum stenosis. The error bars indicate the standard deviation. ** $p < 0.01$; *** $p < 0.001$; from Wilcoxon rank test of difference between the up- and downstream sides, using the mean stained area in each plaque among sections located 1.5–7.5 mm upstream and downstream of the maximum stenosis.

imum stenosis, and then gradually decreased on the downstream side (fig. 6a). The CD68-stained areas were significantly larger in a 6-mm interval upstream of the maximum stenosis compared with the corresponding downstream interval (fig. 6a). Sixty-eight percent of the plaques had more macrophages upstream. SMA-stained areas (smooth muscle cells) were larger on the downstream side than on the upstream side of the maximum stenosis (fig. 6b). Sixty-eight percent of the plaques had more smooth muscle cells downstream. The commonly used ratio between CD68- and SMA-stained areas was significantly higher on the upstream than on the downstream side (3.1 vs. 0.89, $p = 0.004$). The Sirius Red staining indicated a gradual decrease in collagen content along the entire plaque, with significantly higher content on the upstream than on the downstream side of the maximum

stenosis (fig. 6c), and 71% of the plaques having more collagen upstream.

No significant associations were found between the immunohistochemically measured plaque components and either blood flow velocity at the maximum stenosis, time since clinical event or statin treatment (data not shown).

Discussion

We used preoperative Doppler ultrasound and MR angiography to identify high-grade stenoses and to localize the point of maximum stenosis in patients with symptomatic carotid atherosclerotic plaques. The corresponding endarterectomized plaques were then used to analyze

plaque morphology and composition at sites located up- and downstream of the maximum stenosis. We found that severe lesions according to the AHA classification, based on features such as thin fibrous cap, intraplaque hemorrhage, plaque rupture and thrombosis, were more common on the upstream side. Macrophage and collagen contents were higher on the upstream side, whereas the smooth muscle cell content was higher on the downstream side. This is the first study that preoperatively, *in vivo*, identified the location of the most narrow stenosis in patients operated for symptomatic stenosis and made a comprehensive examination of plaque morphology and structure along the entire endarterectomy, relating the findings to the point of maximum stenosis.

Some potential limitations of the study should be addressed before relating our results to observations in previous studies. Firstly, the most narrow lumen according to MRA could tentatively be explained by thrombosis, secondary to plaque rupture. In the individual case it is obviously difficult to clarify if, and how, thrombosis on a ruptured lesion has contributed to the degree and location of stenosis, especially given the possibility of fibrinolysis or thrombus organization. The crucial question in the present analysis is whether the locations of the point of maximum stenosis, as identified by MRA, were in accordance with the point of maximum stenosis in the endarterectomies analyzed by histopathology. Indeed, we found a good agreement between the maximum stenosis measured by MRA and the narrowest lumen in the specimens, when the lumen area was measured on the tissue sections. Secondly, the rotation of the specimen and shrinkage during the handling and preparations of the endarterectomies may have confounded the results. However, correct orientation and rotation of the specimens were assured by marking the medial side of the plaque during the operation, and adjustment for changes in specimen size after formalin fixation did not affect the results. Shrinkage secondary to paraffin embedding was no confounder as the measurements of the distances between the bifurcation and the different sections were performed prior to this step. Thirdly, a majority of patients in our study were on statin treatment and the median duration between last clinical event and endarterectomy was about 3 months. Previous studies have shown that the inflammatory component in carotid plaques is reduced during the first 3 months after a minor ischemic stroke caused by carotid plaques [17, 18]. Statins may also reduce inflammation in plaque tissue [19]. However, no confounding effects were observed, as we found no associations between our results and neither statin treatment

nor duration from clinical event. This discrepancy to the previous reports might be explained by suppression of inflammation in the majority of patients due to the statin treatment, and also by the fact that the patients without statin treatment in our study sample had significantly longer waiting times before their operations. Finally, we can only infer our results to patients operated with endarterectomy due to stroke or TIA, as patients with amaurosis fugax were examined with MRA to a lesser extent than patients with stroke or TIA.

Blood flow-induced mechanical stress acting on the vessel wall encompasses vessel wall shear stress and transmural pressure perpendicular to the blood flow [20, 21]. The parallel frictional drag force of wall shear stress is determined by blood flow, vessel geometry and fluid viscosity [21]. Experimental studies of carotid atherosclerotic plaques have identified high wall shear stress regions upstream and low wall shear stress regions downstream of the maximum stenosis [2]. The transmural pressure is greatest upstream of the maximum stenosis [20]. The mechanical impact of wall shear stress and pressure may cause plaque rupture by direct physical impact, or induce compositional changes that may contribute to plaque rupture.

A few previous studies have compared up- and downstream sides of carotid stenoses [3–5], using various techniques and different types of atherosclerotic plaques. Analyzing 531 angiograms of symptomatic carotid high-grade stenoses, ulcerations were more frequently observed upstream than downstream of the maximum stenosis (in 15 vs. 2% of the patients) [3]. Microscopic examinations of a mixed sample of symptomatic and asymptomatic plaques in the same study supported this observation, although assessment of vessel stenosis is unreliable in surgically excised plaques, as the authors pointed out [3]. Dirksen et al. [4] defined up- and downstream areas in the outer borders of longitudinal sections of carotid arteries collected during autopsy, without information on degree of stenosis or related symptoms. Nine of forty-two plaques showed evidence of rupture, 6 of these ruptures were found in the upstream area. A similar definition of up- and downstream areas was used by Yilmaz et al. [5] in a study of longitudinal sections of endarterectomized high-grade stenotic plaques. In their mixed sample of symptomatic (22%) and nonsymptomatic plaques, cap rupture was more frequent upstream than downstream (13 vs. 4%) [5]. Our study had the advantage that it did not mix symptomatic and nonsymptomatic plaques. Furthermore, the point of maximum stenosis was carefully localized using pre-operative imaging, and we made a

comprehensive analysis of plaque morphology along the entire longitudinal axis of the plaque. Our results supported the previous reports of plaque rupture being more frequent in upstream than downstream areas. However, we observed higher frequencies of cap rupture in our sample. Most likely, this reflects a higher incidence of plaque rupture in symptomatic plaques, but could also be attributed to difficulties in detecting small ulcers by angiography and microscopy [3]. Further, we observed a highly significant difference in the incidence of intraplaque hemorrhage between the up- and downstream sides, in contrast to what Yilmaz et al. [5] reported (22% upstream vs. 23% downstream). As the increased prevalence of intraplaque hemorrhage in our sample mainly occurred on the upstream side, this could indicate an association between hemorrhage, plaque rupture and onset of symptomatic events. Our observation of very high proportions of thin caps, approaching 90% upstream compared with 60% downstream to the point of maximum stenosis, must be related to the fact that this included both ruptured and unruptured sections, and that all included patients had symptomatic lesions. None of the previously published studies have examined the occurrence on thin caps longitudinally and in relation to the point of maximum stenosis [3–5].

Our observation of higher macrophage content upstream than downstream of the maximum stenosis in symptomatic plaques is in line with previous studies [4, 5] which used mainly asymptomatic plaques. A noteworthy difference is that we analyzed complete transversal sections of the endarterectomies, whereas the previous studies examined limited areas of the cap shoulders [4, 5]. Anyhow, there is a striking consistency between these studies in the proportion of plaques with more macrophages upstream than downstream (68% in our study, 67% in [4] and 69% in [5]), despite using different types of plaques and different techniques. Hence, unlike plaque rupture and intraplaque hemorrhage, there does not seem to be a difference in the longitudinal distribution of macrophages between symptomatic and asymptomatic plaques.

The longitudinal distribution of collagen within atherosclerotic plaques has not been studied before, but since collagen is considered to stabilize the plaque, it was unexpected to observe that the amount of collagen, unlike smooth muscle cells, was higher on the upstream side, where plaque rupture often occurs. Our results indicate a more complex regulation and role of collagen in the atherosclerotic plaque than generally anticipated. This is supported by the observation of collagen being expressed

by macrophages in atherosclerotic lesions [22]. Further, while the macrophage and collagen contents were both significantly higher in the upstream area, there was a clear difference in their longitudinal distribution patterns. Macrophage content was focused around an area on the upstream side, just proximal of the maximum stenosis, while collagen content gradually decreased along the entire longitudinal axis. This suggests different regulatory mechanisms of these plaque components, and also that the origin of collagen may differ locally within the plaque.

In studies of mechanisms linking macrophages to plaque growth and rupture, entire carotid plaques obtained by endarterectomy have been used for isolation of macrophages by either microdissection [23, 24] or precipitation by macrophage-specific antibodies for expression studies [25], and also for genome-wide expression studies [26]. However, the differences in the longitudinal distribution of macrophages over small distances, together with the variations in the microenvironment implicated by local differences in wall shear stress, indicate that studies of cellular and molecular mechanisms of carotid plaque growth and rupture should take sampling sites up- and downstream of the maximum stenosis into account. Our results also emphasize that studies comparing imaging with histology as gold standard need to take care to match the imaging location with that on histology, also discussed by Lovett et al. [27].

In conclusion, we demonstrated differences in plaque morphology and composition between the up- and downstream sides of the point of maximum stenosis in symptomatic carotid atherosclerotic plaques. The upstream side, subjected to high wall shear stress, was associated with higher macrophage and collagen content and an increased incidence of severe lesions with cap rupture, intraplaque hemorrhage and thin fibrous caps. The downstream side, subjected to low shear stress, was associated with higher smooth muscle cell content.

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