Flow-induced, inflammation-mediated arterial wall remodeling in the formation and progression of intracranial aneurysms

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OBJECTIVE Unruptured intracranial aneurysms (UIAs) are relatively common lesions that may cause devastating intracranial hemorrhage, thus producing considerable suffering and anxiety in those affected by the disease or an increased likelihood of developing it. Advances in the knowledge of the pathobiology behind intracranial aneurysm (IA) formation, progression, and rupture have led to preclinical testing of drug therapies that would prevent IA formation or progression. In parallel, novel biologically based diagnostic tools to estimate rupture risk are approaching clinical use. Arterial wall remodeling, triggered by flow and intramural stresses and mediated by inflammation, is relevant to both.

METHODS This review discusses the basis of flow-driven vessel remodeling and translates that knowledge to the observations made on the mechanisms of IA initiation and progression on studies using animal models of induced IA formation, study of human IA tissue samples, and study of patient-derived computational fluid dynamics models.

RESULTS Blood flow conditions leading to high wall shear stress (WSS) activate proinflammatory signaling in endothelial cells that recruits macrophages to the site exposed to high WSS, especially through macrophage chemoattractant protein 1 (MCP1). This macrophage infiltration leads to protease expression, which disrupts the internal elastic lamina and collagen matrix, leading to focal outward bulging of the wall and IA initiation. For the IA to grow, collagen remodeling and smooth muscle cell (SMC) proliferation are essential, because the fact that collagen does not distend much prevents the passive dilation of a focal weakness to a sizable IA. Chronic macrophage infiltration of the IA wall promotes this SMC-mediated growth and is a potential target for drug therapy. Once the IA wall grows, it is subjected to changes in wall tension and flow conditions as a result of the change in geometry and has to remodel accordingly to avoid rupture. Flow affects this remodeling process.

CONCLUSIONS Flow triggers an inflammatory reaction that predisposes the arterial wall to IA initiation and growth and affects the associated remodeling of the UIA wall. This chronic inflammation is a putative target for drug therapy that would stabilize UIAs or prevent UIA formation. Moreover, once this coupling between IA wall remodeling and flow is understood, data from patient-specific flow models can be gathered as part of the diagnostic workup and utilized to improve risk assessment for UIA initiation, progression, and eventual rupture.

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KEYWORDS intracranial aneurysm; flow; inflammation; remodeling; risk of rupture
Unruptured intracranial aneurysms (UIAs) are found increasingly often as incidental findings during intracranial MR- or CT-angiographic imaging due to better availability of these studies. Because incidentally found UIAs may later rupture, causing devastating aneurysmal subarachnoid hemorrhage (aSAH), many patients with incidental UIAs are anxious and want their aneurysms treated. Current treatment options are all interventions with nonnegligible risk of morbidity and even mortality. As a consequence, physicians treating UIAs are challenged with the assessment of whether the rupture risk of an incidentally found UIA justifies the risks associated with treatment. This task is complex and demanding because multiple factors impacting the risk of UIA rupture have been identified, but no absolute threshold values have been identified for any of these established risk factors to discriminate stable UIAs from those that progress toward rupture.

UIAs are relatively frequent lesions, with 3% or higher prevalence in the population older than middle age. The clearly lower prevalence of UIAs in children or young adults in population-based studies and clinical series, together with the fact that formation of new UIAs (so called de novo aneurysms) is observed during follow-up of patients, demonstrates that UIAs are not innate lesions but develop during life. This implies that UIA formation is the end result of degenerative cerebral arterial wall remodeling. Understanding the biology of this remodeling process is the key to identification and rational management of persons at risk of UIA formation, as well as those who have been diagnosed with UIAs. The fact that many, if not most, UIAs remain unruptured during lifelong follow-up demonstrates that there is also adaptive remodeling that can stabilize the UIA wall and ensure sufficient strength to withstand the mechanical stress imposed on the aneurysm wall by blood pressure and flow. Understanding the mechanisms mediating the destructive and adaptive remodeling of the cerebral artery and aneurysm wall will open the door for the design and development of pharmaceutical or other biological therapies that would inhibit UIA formation and progression toward rupture. This development would thus offer new hope for those at risk of UIA formation (such as persons with familial predisposition to aSAH) and in some cases be an alternative to invasive UIA treatment.

Aneurysm Formation, Active Collagen Remodeling, and Disruption of Elastic Laminas

The wall of normal intracranial arteries is composed of a luminal endothelial cell (EC) layer, underneath which is the basal lamina composed of matrix proteins, then the internal elastic lamina (IEL) composed of elastic fibers, followed by the media layer composed of smooth muscle cells (SMCs), elastic laminas, and collagen fibers. The adventitia layer starts at the outer rim of the media, and is composed of collagen fibers and fibroblast cells. All the components of the arterial wall have specific functions, of which resistance to mechanical stretch and maintenance of structural integrity are almost entirely due to the elastic laminas and the medial and adventitial collagen fibers. Unlike the elastic laminas that distend when loaded to absorb and then release part of the energy of the pulse wave, collagen fibers have little capacity for extension prior to failure. The collagen fibers in the medial and adventitial layers determines how much the artery can distend and protects the artery from overstretching. Loss of elastic laminas is characteristic of aneurysms and shifts greater load bearing to the collagen fibers, which diminishes the tortuosity of the collagen fibers and limits the capacity of the vessel to dilate. Because collagen fibers are relatively inextensible compared with elastin, growth of an aneurysm—sometimes up to a size multiple times the original diameter of the parent artery—requires remodeling of the collagen fibers in the aneurysm wall. Collagen is the main load-bearing structure in the vessel wall once the elastic laminas are lost, therefore the end result of this collagen remodeling determines the strength of the aneurysm wall, and thus also the conditions that suffice for rupture. Not surprisingly, there is high variation in the strength of the aneurysm wall when measured under standardized laboratory conditions, with both robust and vulnerable groups within the UIAs and, on average, ruptured intracranial aneurysms (IAs) having weaker walls than UIAs. Of great interest is the striking observation that remodeled collagen fibers in the aneurysm wall are oriented according to flow and the wall shear stress (WSS) sensed by the EC layer. This raises the question of whether collagen remodeling in the aneurysm wall is guided by flow, a concept further supported by the observation that flow conditions in the aneurysm fundus associate with strength of the aneurysm wall.

What makes these observations especially intriguing is the implication that by guiding the collagen remodeling of the aneurysm wall, flow would eventually determine how prone the aneurysm is to rupture, in addition to its impact on aneurysm formation and growth.

Blood Flow as the Driver of Outward Remodeling in Vessels

The idea that flow would guide the remodeling of blood vessel structure similarly to how mechanical load guides the remodeling of bone trabeculae is not new, and in fact was proposed as early as the 1960s if not earlier. This idea is the underlying premise in studies of growth and remodeling in blood vessels. Surgical arteriovenous fistulas created to enable hemodialysis provide clear evidence that this flow-induced vessel remodeling takes place in humans. In these fistulas, the lack of capillary bed resistance produces an abnormally high flow, which leads to outward remodeling to a size multiple times larger than the original, especially in the draining vein also subjected to higher than intended pressure in addition to abnormally high flow. Experimental models of surgically created arteriovenous fistulas demonstrate that this flow-induced outward remodeling occurs through a coordinated...
sequence of protease expression followed by synthesis of new collagen. Underlying deficiency in elastic laminas accelerates this flow-induced outward vessel remodeling.

**Effect of High Flow in Cerebral Arteries: Focal Outward Remodeling Leading to Aneurysm Formation**

Manipulation of cerebral blood flow by uni- or bilateral ligation of carotid arteries in laboratory animals predisposes to segmental or focal dilation and UIA formation at sites of the cerebral vasculature exposed by the procedure to abnormally high flow, especially if coupled with hypertension and defective collagen synthesis (Fig. 1). As in humans, these induced aneurysms develop preferably at bifurcations (reviewed in Aoki and Nishimura; Fig. 1). Although de novo IA formation is occasionally observed in clinical practice after uni- or bilateral carotid occlusion due to disease or medical procedure, overall the prevalence of UIAs in patients with occlusive carotid artery diseases (3%) seems similar to the general population. This apparent controversy between the animal models and clinical observations can be explained by slower development of the clinical disease, which allows time for collateral circulation to develop. Moreover, differences in the magnitude of flow enhancement obtained with carotid ligation in animals compared to carotid occlusion in humans is likely relevant. A more direct proof of flow-induced vessel remodeling causing aneurysm initiation in humans is obtained from patients with arteriovenous malformations of the brain (bAVMs). Brain AVMs cause similar arteriovenous shunting as surgically created arteriovenous fistulas, and many of the bAVM feeding arteries that are exposed to abnormally high flow develop segmental ectasia or saccular aneurysms. Of special interest is the observation that some of these bAVM-associated IAs spontaneously regress once the shunting lesion is obliterated and flow normalized, demonstrating that these IAs are initiated and maintained by abnormally high flow.

**Inflammation as the Mediator of Flow-Induced Outward Remodeling and Aneurysm Initiation**

Classic work by Langille in the 1980s demonstrated the capacity of the arterial wall to respond to both increases and decreases in flow through an initial acute vasomotor response, followed by wall remodeling. This adaptation to flow requires an intact endothelium. It is now understood that the surface proteoglycan layer (glycocalyx) on
the endothelium surface is the primary sensor of flow-related forces. In particular, flow across the endothelial cells generates a WSS, a frictional force per unit area tangential to the flow.

WSS is primarily dependent on blood viscosity and flow velocity (Fig. 2). If the vessel diameter remains constant, increased volumetric flow rate increases the velocity, which in turn increases WSS (Fig. 2). The largest increases in WSS are generally noted at outer walls of curved vessel segments, highly constricted regions of arteries, and the apex regions of bifurcations. At these sites the local velocity is elevated due to the coupled nature of flow and geometry (reviewed in Tanweer et al.). Using surgically created high-flow bifurcations, Meng et al. showed that the changes predisposing to aneurysm formation, namely IEL disruption and degeneration of the SMC layer, develop at the sites exposed to high WSS and to a positive WSS gradient (WSSG). Since then, several studies using various animal models have concluded that IA initiation occurs in regions exposed to high WSS with a positive WSSG.

Nitric oxide (NO) produced by endothelial NO synthase (eNOS) is the primary mediator of the EC-triggered vasodilation in response to high flow. An increase in WSS induces eNOS production in ECs, in addition to relaxing the medial SMCs. This increase in NO down-regulates the expression of the proinflammatory adhesion molecules macrophage chemotactic protein 1 (MCP1) and vascular cell adhesion molecule 1 (VCAM1) that are otherwise concomitantly upregulated in ECs in response to WSS. Experiments with NOS knockout mice demonstrate how blocking NOS signaling predisposes to IA formation through an increase in macrophage infiltration, presumably through increased MCP1 expression (Fig. 3). Recent results suggest that mechanical stretch can also induce MCP1 expression in adventitial fibroblasts, which promotes the transmural migration of macrophages. Co-localization of high WSS with wall stretch would thus lead to amplified MCP1 expression and macrophage infiltration with increased likelihood of eventual IA initiation.

The crucial role of MCP1 in aneurysm initiation is demonstrated by experiments with MCP1 knockout mice, in which macrophage infiltration was nearly absent in cerebral arteries exposed to high flow, and IA initiation and formation were reduced by more than half. The key role of macrophage infiltration in IA initiation has been confirmed by several other studies using variations of the classic induced-IA formation model and clodronate to deplete macrophages or manipulation of macrophage activation through peroxisome proliferator-activated receptor (PPAR) gamma. In the rodent models of induced-IA formation, macrophages infiltrate the arterial wall through the luminal endothelium during IA initiation. Next, they migrate through the wall, eventually reaching the adventitia following a chemotactic gradient generated by MCP1 expression in adventitial fibroblasts. Because transendothelial migration to the vessel wall has to occur through the IEL.

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**FIG. 2.** WSS is a flow-induced force per unit area tangential to the direction of the flow and dependent on the rate of flow (A). Therefore, WSS at the apices of arterial bifurcations increases if flow increases, as demonstrated by the CFD modeling of WSS in patient-derived geometry of internal carotid artery bifurcation with low (1.86 ml/sec, **B**) and high (2.72 ml/sec, **C**) flows. While the shear stress is a vector that has both magnitude and direction, only one component is nonzero in the case shown in A, and in B and C the magnitude of the vector is shown in the form of contour plots. WSSG is the difference between WSS at two different sites on the vessel wall (WSS1–WSS2).
(Fig. 3) and once in the wall the macrophages produce proteases causing collagen degradation, it is logical that the macrophage infiltration eventually leads to IEL disruption as well as to disruption of the collagen matrix that is the load-bearing structure of the wall once elastic laminae are lost (see above).

Flow-Triggered Inflammation-Mediated Remodeling in Aneurysm Growth

Once the IEL is lost due to the infiltration of inflammatory cells, and the collagen matrix is damaged to the point of allowing the outward bulging of the initial aneurysm, the aneurysm may start to enlarge. This, however, requires that the aneurysm wall actively grows and synthesizes new matrix (discussed above). Mere prolonged proteolytic injury by infiltrating macrophages without concomitant remodeling of the collagen matrix would not lead to substantial growth, but rather a rupture from a small, blister-like bleb sometimes encountered in clinical practice.

SMCs of the IA wall synthesize the new collagen required for this active IA wall growth. These mural cells express receptors for several growth factors secreted by macrophages, e.g., transforming growth factor beta (TGFβ) and platelet-derived growth factor B (PDGF-B), that stimulate SMC matrix synthesis and proliferation. That the macrophage activation constantly stimulates the SMCs, in addition to protease secretion, can explain why the aneurysm wall grows. Once initiated, the macrophage activation in the aneurysm wall amplifies itself through an autocrine feedback loop in which prostaglandin E2 (PGE2) produced by cyclooxygenase 2 (COX2) activates the transcription factor nuclear factor kappa b (NFκB) in other macrophages, leading to increased expression of MCP1 as well as COX2 by these cells (Fig. 4). This stimulates the recruitment of more macrophages to the...
aneurysm wall, as well as NFkB activation in them. This amplification loop may explain the observation that wall remodeling initiated by high-flow conditions can continue even if the pathological flow is normalized.52 Clinically, one of the most pertinent questions regarding the biology of aneurysm formation is how to stabilize small inceptions of aneurysms or small aneurysms and prevent them from growing to a size in which rupture becomes increasingly likely.22,31,72 The presence of MCP1 expression and macrophages9,27,34,55 in human IA walls implies that the same molecular mechanisms as in IA initiation might be involved in IA progression, at least in the formation of daughter aneurysms or so-called “secondary pouches” that tend to form in the IA sacs at regions with high WSS.15 In addition to high-WSS areas, MCP1 can be also induced in IA regions with low WSS.9 This is consistent with the observation that inflammation of the IA wall showed an associative trend with both high WSS and low WSS.11

In animals, activation of NFkB at the site of vessel remodeling is necessary for the IA to form.3 This NFkB activation occurs through the COX2-PGE2-EP2-NFkB pathway as discussed above.2,5 Genome-wide gene expression analysis of ruptured and unruptured human IA walls has shown upregulation of multiple NFkB-regulated genes in ruptured IA walls,46 strongly implying that macrophage-induced NFkB activation is relevant in the wall remodeling of established human IAs, similar to the experimental models of induced-IA initiation and formation.2,5 The presence of COX2 expression in the human IA wall,5,34 as well as of PGE2 receptor subtype EP2, further implies that the COX2-PGE2-EP2-NFkB-COX2 signaling pathway is involved in the growth and wall remodeling of human IAs similar to the experimental models of induced UIA formation. The seminal observation that drugs inhibiting COX2 activity, such as aspirin35 or nonsteroidal antiinflammatory drugs, appear to reduce the growth of UIAs in patients25 further supports this concept.

Hyperplastic Remodeling as a Response to Increase in Mechanical Stretch: Role of Inflammation

Once the initiated aneurysm starts to grow, its wall will be subjected to progressively higher wall tension (WT; Figs. 5–8). For the IA to remain unruptured, the wall has to adapt to the increased mechanical load. In general, the arterial wall adapts to chronically increased mechanical load through proliferation of the medial SMCs and collagen deposition, as noted in wall changes associated with
hypertension. In cases in which this remodeling process is insufficient, the intramural cells may be exposed to supraphysiological stretch. The dynamics of this repair process following overstretch of this kind have been well-studied in arterial balloon dilation injury models and are shown to depend on macrophages and NFκB activation in SMCs. The enlarging IA wall can adapt to this increase in WT through SMC proliferation and collagen remodeling, similar to the way an arterial wall responds to overstretching or chronic high pressure, provided that it has healthy SMCs. Many IA walls, however, have at least focal regions that show loss of SMCs. Moreover, in many IA walls the remaining SMCs turn into foam cells due to lipid ingestion, which impairs their normal function. This can lead to a potentially dangerous scenario in which the aneurysm continues growing but has wall regions that are not able to adapt to the increase in WT caused by growth. The mechanisms of lipid accumulation in the IA wall are discussed in more detail in Ollikainen et al. and Frösen et al. Because endothelial dysfunction appears to be a key factor in promoting the accumulation of lipids in the IA wall, flow that regulates endothelial function is likely to affect the process of lipid-induced IA wall SMC dysfunction as well.

**Clinical Applications: Flow Modulation, Flow Modeling, and Multimodality Diagnostics**

Flow conditions in the human IA sac associate with focal changes in wall structure, as well as with histological changes of the IA wall, including inflammation. Moreover, additional data from a somewhat small number of cases suggest that flow conditions associate with collagen remodeling and strength of the IA wall. Measurement of flow conditions appears to hold great potential for the identification of 1) persons at risk of IA formation, 2) IAs that are likely to grow and should be followed attentively, and 3) unstable IAs that need an intervention. Flow conditions can be estimated with reasonable accuracy from 3D high-resolution angiograms using computational fluid dynamics (CFD), provided that the boundary conditions are well-defined.
FIG. 6. When the aneurysm grows, flow conditions in the aneurysm lumen change because the luminal geometry changes. This is likely to change the flow-induced WSS, especially which areas of the wall are exposed to high WSS (blue line, A–C). Regions exposed to high flow are likely to undergo similar flow-induced inflammation-mediated remodeling as in aneurysm initiation, which in turn can explain focal growth of secondary pouches in the aneurysm wall at sites of high WSS. While in very small aneurysms a larger extent of the aneurysm wall is exposed to high WSS, in general, the more the aneurysm grows less of its surface area is exposed to high WSS. In addition to this, an increase in the aneurysm diameter is prone to cause regions with slower flow, which can predispose to luminal thrombosis (pink, B and C). Luminal thrombosis stimulates aneurysm wall remodeling through multiple mechanisms (reviewed in detail by Frösen et al., 2012). In brief, red blood cells (red), platelets (ellipsoids, red), and inflammatory cells, including neutrophils, are trapped in the thrombus and release cytokines and growth factors that stimulate proinflammatory phenotype and growth of SMCs in the aneurysm wall (D). This promotes wall remodeling that encompasses secretion of proteases that degrade the collagen matrix. FIG. 6. (continued)→
FIG. 6. In addition to SMCs, the inflammatory cells in the luminal thrombus are also a source of collagen-degrading proteases. This thrombus-derived chronic proteolytic injury is especially relevant in aneurysms that have a decellularized wall devoid of mural cells capable of synthesizing new collagen. Flow-related factors predisposing to loss of mural cells include cytotoxic iron released from the degrading red blood cells of the luminal thrombus, as well as lipids that accumulate in the aneurysm wall to mural cells causing foam cell formation and eventually cell death. This lipid accumulation appears to be related to dysfunction of the luminal endothelium, which in turn can be explained by the nonphysiological flow conditions in the aneurysm lumen (E). Overall, through the effects of flow on endothelial function and thrombus formation, flow in the aneurysm lumen also affects wall remodeling by other mechanisms than the ones relevant in aneurysm formation. Consecutive exposure of different aneurysm wall regions to changing flow conditions resulting from aneurysm growth likely explains the high degree of heterogeneity on aneurysm wall structure observed during surgery (F and G). That flow conditions associate with wall structure observed at surgery supports this concept. Asterisk indicates a focal region with a very thin wall. LDL = low-density lipoprotein.

FIG. 7. When aneurysms grow, WT increases. This can be seen from a simple force balance in an idealized spherical shell using the law of Laplace (A). In a sphere, the WT acts around the circumference with similar loads acting on the components of the wall, such as collagen fibers and intramural cells. Although true IAs are rarely close to spherical (B, a sphere superimposed on the 3D geometry of an actual aneurysm in a patient), similar relationships exist between the local WT and geometry. This, in turn, means that in a growing aneurysm, as the diameter increases, the wall has to remodel to withstand higher WT. Moreover, because WT can also be defined through wall thickness and intramural stress (force pulling the wall components apart) as shown, it can be concluded that a simple increase in wall thickness will reduce intramural stress. In a wall with intact functional SMCs (C), for example, this can occur through collagen remodeling and proliferation of SMCs that may lead to significant focal increase in wall thickness (t1 vs t2 in C, H&E-stained aneurysm wall). However, a wall with few healthy SMCs is less able to adapt (H&E-stained example of a decellularized wall in D). This may explain, at least in part, the high variation observed in the strength of aneurysm wall tissue samples when stretched to failure. The stress-strain curve adapted from the measurements performed by Robertson et al. 201558 and applicable for most aneurysm walls is shown in E (intramural stress determined as a first-order Piola-Kirkhoff tensor and the blue area corresponding to the range of curves measured from individual samples with the exception of a few outliers in the original data). Of note is the dependence of intramural stress on the wall thickness, which means that thinner regions (t1) of the same aneurysm wall can be stretched to the maximum and close to rupture while thicker regions (t2) in the same aneurysm wall are not yet maximally loaded.
conditions needed for the flow simulations are determined reasonably (see separate reviews on this topic in this issue). Although several studies have demonstrated how CFD can be used to differentiate ruptured and unruptured IAs with reasonable accuracy (as well as separate publications on the topic in this issue), from the biological point of view it is worth noting that many human IA walls have regions that have completely lost the endothelium. This will significantly impair the normal mechanobiological coupling between flow and wall remodeling, and raises questions as to whether such an IA wall is sensitive to flow-induced remodeling at all. To reliably determine what kind of flow conditions will lead to an unstable UIA, follow-up studies of UIAs with CFD performed at baseline are needed. Currently, there are few of this type of clinical series.

Although IAs in humans form preferentially at sites of high WSS, the fact that they do not form in all bifurcations exposed to high WSS and to all persons does seem to imply that a second factor is needed for IA initiation. In experimental models, concomitant elastase injury significantly promotes aneurysm formation at sites with flow conditions predisposing to IA formation. Because inflammatory cells, especially neutrophils, are an important source of elastase, it seems plausible that such a “second hit” in humans would take effect through activation of inflammatory cells and subsequent increase in elastase activity. Recently, involvement of the gut microbiome in a model of induced-IA formation has been reported. Even more recently, we have shown that severe periodontitis and gingival inflammation predisposes to IA formation.
and eventual aSAH in humans.59 Prior to this, the presence of oral bacteria–derived DNA had been shown in the IA wall.60 The biological mechanism by which the gut microbiome or periodontitis predisposes to IA formation remains to be shown, but clearly other factors besides flow are significant in the initiation and progression of UIAs.

In addition to acquired factors such as periodontitis, in some cases the “second hit” can be genetic, for example in polycystic kidney disease in which mutation in the polycystin gene affects the mechanobiological coupling of flow–endothelial cell interaction.69 Of note, flow-driven vessel remodeling in arteriovenous fistulas was significantly increased in mice heterozygous for defective elastin gene.73 Identification of similar inherited “secondary triggers” will facilitate the identification of persons at risk of developing IAs, especially when coupled with flow modeling (CFD). Knowledge of the mechanobiological coupling between flow and wall remodeling, and identification of these environmental or genetic “secondary triggers,” may also enable more efficient primary prevention of the disease, such as by treatment of periodontitis or by treatment of the flow-induced IA initiation with preventive drug therapy. Results from experimental models suggest that inhibitors of COX2-PGE2-EP2 signaling68 or PARgamma inhibitors,63 for example, could be used for this purpose. Another potential target for drug therapy could be inhibition of the proteases mediating the flow-induced inflammation-driven remodeling.

Conclusions

High flow is an initiator of UIA formation and flow conditions also drive the wall remodeling that determines whether the aneurysm will remain stable, or progress and eventually rupture. Inflammatory cells mediate this flow-induced remodeling. Flow is not, however, the only factor involved in UIA initiation, nor the only factor determining UIA wall remodeling. Understanding the mechanobiological coupling of flow and aneurysm wall remodeling is the key to predicting the clinical course of an aneurysm. This will become increasingly more important because drug therapies that modulate flow-induced inflammatory reaction-driven remodeling are being developed.

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Disclosures
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Author Contributions
Conception and design: all authors. Acquisition of data: Frösen, Robertson, Aoki. Analysis and interpretation of data: all authors. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Frösen. Administrative/technical/material support: Frösen, Cebral, Aoki. Study supervision: Frösen.

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