

Review Article

Vasa vasorum and atherosclerosis – Quid novi?

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Summary

The role of vasa vasorum (VV) in atherosclerosis is hotly debated, and new experimental techniques have recently opened an opportunity to take a fresh look at this important topic. Although the proliferation of VV due to atherogenic stimuli is controversial, experimental and clinical evidence strongly suggest the potential of VV in vascular proliferative disorders. In the past, paradigms of atherosclerosis and restenosis have excluded the adventitia and VV in the artery wall due, in part, to a lack of i) appropriate animal models featuring adventitial VV neovascularization, ii) imaging technologies to quantitate adventitial VV and

plaque neovascularization and iii) its consequences, concerning information on detectable plaque substrate in vulnerable lesions. VV proliferation is associated with increasing plaque burden and is linked to cellular processes which are critical during the development of atherosclerotic plaques such as inflammation, plaque perfusion and concomitant intraplaque hemorrhage – but the regulation and induction of VV based on pathological settings are poorly understood. This review discusses the current scientific status and its controversies and identifies open research questions.

Keywords

Vasa vasorum, inflammation, atherosclerosis, intraplaque hemorrhage

Thromb Haemost 2007; 97: 873–879

Vasa vasorum

Various studies in different species demonstrated development of vasa vasorum (VV) in the first weeks of gestation and during growth (1–3). In these studies it has been reported that the increasing volume of VV match the increasing needs of the growing vessel wall. With advancing age, the VV tree structure expands and covers a larger volume of vessel wall instead of only deploying more branches in the same volume of the vessel wall.

Wolinsky and Glagov (4) reported that the extent and the distribution of vascularization of the vessel by VV depends on the vessel wall thickness. In blood vessels larger than 0.5 mm in diameter nourishment of the media is supplemented by VV.

Three different types of VV have been described by Schoenenberger and Mueller (5): the vasa vasorum externae (VVE), the vasa vasorum internae (VVI) and the venous vasa vasorum (VVV) (Fig. 1). Schoenenberger and Mueller defined the VVE as originated from major branches and the VVI as originated from the main lumen of the aorta (5). The VVV drain the arterial wall in concomitant veins. These findings were supported by Gossl et al. (6) where VV in porcine coronary arteries were

studied. In this study, it was found that the volume of vessel wall tissue perfused or drained by the VV tree correlated well with the cross-sectional area of the root segment of the VV tree.

VV have been described as a network or plexus of microvessels in the wall of arteries by several investigators (7–11). A network or plexus of vessels is defined as a connection of arteries to other vessels' perfusion territories, both antegrade or retrograde perfused with each other. Using microembolization techniques, Gossl et al. (12) demonstrated for the first time that VV are functional endarteries.

Stimuli of vasa vasorum neovascularization

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor in physiological and pathophysiological conditions. However, little is known regarding the changes in adventitial VV and the mechanism of the formation in hypertensive arteries. Recently, it has been shown that hypertension not only induced medial and adventitial thickening but also significantly increased adventitial VV. Furthermore, concomitant expression of VEGF and a hypoxia-inducible transcription factor was observed in the outer layers of medial smooth muscle (13). Using

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Received December 29, 2006
Accepted after resubmission April 3, 2007

Prepublished online May 3, 2007
doi:10.1160/TH06-12-0742

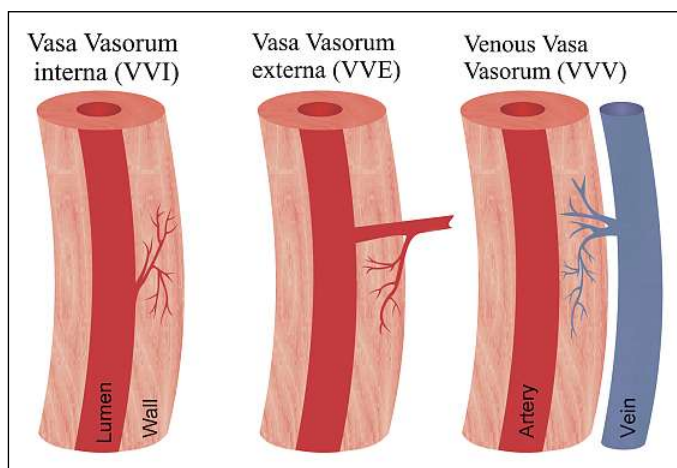


Figure 1: Different types of vasa vasorum (VV). Arterial VV (VVI and VVE) are branching into the arterial wall. The venous VV are draining in the concomitant venous system (Reprinted with permission from Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc. from: Gössl M, Rosol M, Malyar NM, et al. Functional and hemodynamic characteristics of vasa vasorum in the walls of porcine coronary arteries. *Anat Rec* 2003; 272A: 526–37).

recombinant human VEGF, Celletti et al. (14) demonstrated an increase in the rate and degree of atherosclerotic plaque formation in the thoracic aorta in a cholesterol-fed rabbit model. Moreover, VEGF significantly increased macrophage levels in bone marrow and peripheral blood and increased atherosclerotic plaque area significantly compared with controls (15).

In many developing tissues, local angiogenesis may be regulated by tissue oxygen levels (16). Consequently, diffusion length increase due to atherosclerotic lesions may produce a relatively hypoxic state which in turn triggers VV formation by the production of angiogenic factors. Moreover, it has been reported that acute vessel wall hypoxia increased the conductance of VV in arteries and veins in dogs (17). In hypoxia-induced pulmonary artery hypertension, adventitial VV neovascularisation was reported in rats (18).

Other pro-atherogenic stimuli, i.e. hypertension, have been demonstrated to induce VV growth in rats (19). Controversial data exist about the influence of hypercholesterolemia and VV. Recently, Gössl et al. (20) demonstrated in coronary arteries no increase of VV total luminal surface area due to high plasma cholesterol in pigs. This is in contrast to previous findings reporting an increase in VV density in hypercholesterolemic coronary arteries (7, 21). Additionally, hypercholesterolemia does not alter the delivery of oxygen to the artery wall prior to the formation of atherosclerotic lesions (22). Several studies (23–25) concerning diet-induced hypercholesterolemia in pigs and its impact on VV reported an increased VV neovascularization in the adventitia layer before the development of vascular lesions. Arterial remodelling due to hypercholesterolemia causes thickening of the vascular wall in C57/BL mice (26) at an age of 24 weeks, and resembles the structural changes present in the early phase of atherosclerotic development. However, C57/BL mice do not develop VV in the aorta. Consequently, it raises the question whether hypercholesterolemia per se is the factor stimulating angiogenesis of VV.

Vasa vasorum and their contribution to inflammation

Recent studies have emphasized the involvement of VV in inflammation and atherosclerosis. Although the proliferation of VV due to atherogenic stimuli is controversial, experimental and clinical evidence strongly suggest the potential of VV in vascular proliferative disorders.

VV may be suitable therapeutic targets, because they may contribute to plaque development in several different ways: through (1) alterations of arterial blood, oxygen, and nutrient supply to the plaque; (2) changes in venous drainage of venous VV; (3) their role as a conduit for inflammatory cells; and (4) via their influence on plaque stability and instability. However, in addition to inflammation, a key process involves angiogenesis and proliferation of VV. In normal arteries adventitial inflammation is absent, but once atherosclerosis occurs, adventitial inflammation increases with plaque development (27, 28). Supporting these findings, Moos et al. (29) reported that the lamina adventitia is the major site of immune cell accumulation in apolipoprotein E (apoE)^{-/-} deficient mice thereby exceeding those of the neo-intima by 85-fold in animals at the age of 78 weeks.

Inflammatory reactions due to vasculitis, i.e. Takayasu arteriitis, appear to begin in the adventitia where inflammatory cells are located close to the VV (30, 31). The inflammatory process within the vascular wall comprises a complex pathophysiological interplay between endothelial cells, circulating blood cells and humoral mediator systems and involves the activation of endothelial cells and leukocytes followed by the expression of adhesion molecules, enhanced endothelial-leukocyte-interaction, release of cytokines and subsequent activation of the coagulation and fibrinolytic system and the complement cascade. From its own nutritive vascular system, the vessel wall is infiltrated by inflammatory cells which destroy medial and intimal structures (32, 33). Furthermore, the inflammatory process within the VV may lead to the stasis of blood flow, loss of endothelial barrier function, increased vascular permeability and subsequent extravasation of fluid and proteins. Consequently, ischemia and fibrinoid necrosis of the “macrovesel” wall occur. On the one hand, the progression of the inflammatory process may lead to the formation of aneurysms causing rupture or dissection of the vessel wall. On the other, endothelial and intimal structures of the “macrovesels” are involved, leading to intimal thickening and endothelial activation with subsequent luminal narrowing and thrombus formation (34). However, intimal and medial changes due to “vasa vasoritis” resemble inflammatory reactions within the vascular wall due to atherosclerosis.

The exact function of VV in inflammation due to atherosclerosis is still subject of debate. In early atherosclerosis, VV may contribute to lesion development through nutrient and oxygen supply to the plaque and through their role as a conduit of inflammatory cells (Fig. 2). Moreover, blocking plaque neovascularization by angiostatin reduces progression of advanced atherosclerosis in apoE^{-/-}-knockout mice (35). Moreover, this study indicates that VV density correlates highly with the extent of inflammatory cells, not the size of atheromas in apoE-deficient mice. This effect was accompanied by reduced frequency and density of VV in advanced atherosclerotic lesions.

In animal models of hypercholesterolemia-induced atherosclerosis (36) as well as in human atherosclerosis (37), advanced

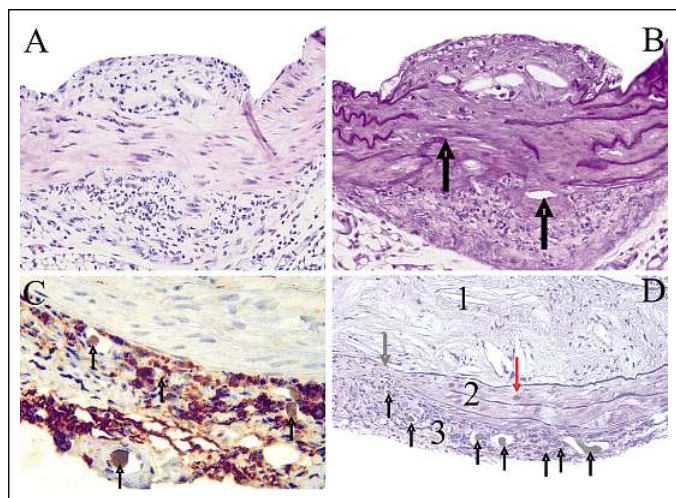


Figure 2: Adventitial Inflammation and VV in apoE^{-/-}/LDL^{-/-} double knockout mice. A) Intimal macrophage/foam cell plaque containing mononuclear inflammatory cells, mild fibrosis of the media and marked mononuclear infiltrating cells within the adventitia accompanied by some polymorphonuclear leukocytes (HE, original magnification x40). B) Same area as in panel A showing transmurular inflammatory-type disruption of elastic lamellae and intimal, medial and adventitial fibrosis (HE & Elastica, original magnification x40). C) Contrast-enhanced VV are continuously present at the border to the external elastic layer (C, black arrows) surrounded by CD11b-positive cells. D) Media atrophy and degradation of elastic laminae (2) with dense adventitial inflammation and adventitial VV (3) (black arrows) as well as contrast enhanced microvessel in the media (red arrow) (HE, original magnification x20). Note, the inner elastic layer is intact indicating an “outside-to-inside” inflammation.

plaque vascularization may give rise to a substantial percentage of the VV that contribute inflammation and lesion formation.

VV neovascularisation and its contribution to aneurysm in different vascular beds

There is an increasing body of evidence that the vessel wall is infiltrated by inflammatory cells from its own nutritive vascular system, destroying medial and intimal structures (32, 33). Therefore, the progression of the inflammatory process may lead to the formation of aneurysms causing rupture or dissection of the vessel wall (Fig. 3). Recent studies have shown that increases in proteolytic activity are associated with abdominal aortic aneurysm. Herron et al. (38) identified tissue inhibitor of metalloproteinase (TIMP) and gelatinase by immunoperoxidase staining in human dilated aortic wall. Moreover, this connective tissue proteinases and inhibitors were spatially localized to adventitial VV, suggesting involvement of VV in the maintenance and possibly the genesis of abdominal aortic aneurysm.

The pathogenesis of intracranial arterial aneurysms remains unclear. It has been demonstrated that human intracranial arteries in neonates, children and adults do not have VV (39). In contrast, Connolly et al. (40) identified endothelial-lined channels in the proximal intracranial vessels in humans. These vessels might represent intracranial VV, which in turn might play a role in intracranial dissection and aneurysms. Supporting this study, VV have been found to be spatially and frequently associated with intrinsic atheromatous plaques and large thick-walled

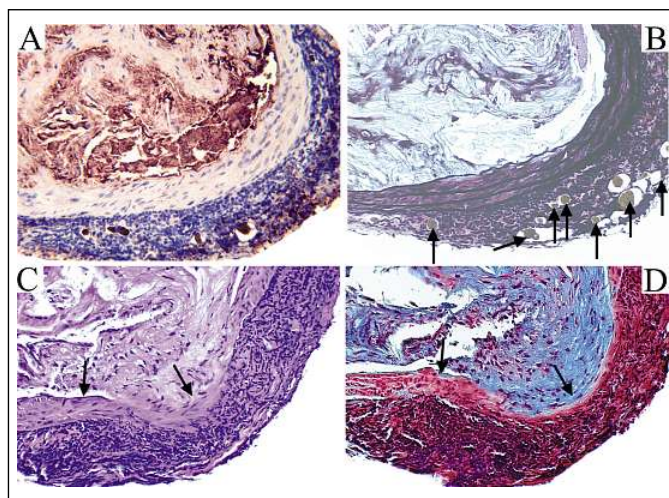


Figure 3: Intraplaque hemorrhage in the descending aorta in apoE^{-/-}/LDL^{-/-} double knockout mice at the age of 80 weeks. Serial sectioning (A, E, glycoporphin A; B, F, Movat Penachrome, C, G, H&E, D, H, Masson Trichrome; magnification x40). A) Intense staining for glycoporphin A together with cholesterol clefts and severe adventitial inflammation with monocytic cells correlates with the prevalence of adventitial VV. VV are continuously present at the border to the external elastic layer (B, black arrows). C, D) Media atrophy and degradation of all elastic laminae (black arrows) with dense adventitial inflammation (with permission from Ref. [72]).

aneurysms in human intracranial arteries (41). Adventitial inflammation leads to a weakening of the media from the abluminal part of the vessel wall due to the release of proinflammatory factors that invade the media, thereby degrading the extracellular matrix, the elastic lamina of the vascular wall, and, finally, the integrity of the vessel lumen. This in turn results in a dilation of the vessel and aneurysm formation. Indeed, this hypothesis and the empirical use of anti-inflammatory drugs in giant intracranial aneurysms have been confirmed by recent studies (42) reporting that an enzyme involved in the inflammatory cascade (5-lipoxygenase or 5-LO) promotes the pathogenesis of specific aneurysms in humans.

VV neovascularisation and its contribution to atherosclerosis in different vascular beds

The arterial vasculature presents substantial variation in its susceptibility for development of atherosclerotic lesions. The distribution and location of plaques throughout different vascular beds exhibits different clinical presentations, the most severe consequences being myocardial infarction, stroke and claudication. A significant difference also exists in the prevalence and severity of atherosclerotic lesions in coronary, cerebrovascular and peripheral circulation based on clinical (43–45), autopsy (46, 47), and animal studies (23, 48). Although a variety of different effects may contribute to lesion formation in different vascular beds, the underlying mechanism(s) of this heterogeneous response is not yet defined.

More than three decades ago, the heterogeneity of VV distribution of the human aortic wall was demonstrated (10), but this was not correlated to the regional propensity for plaque

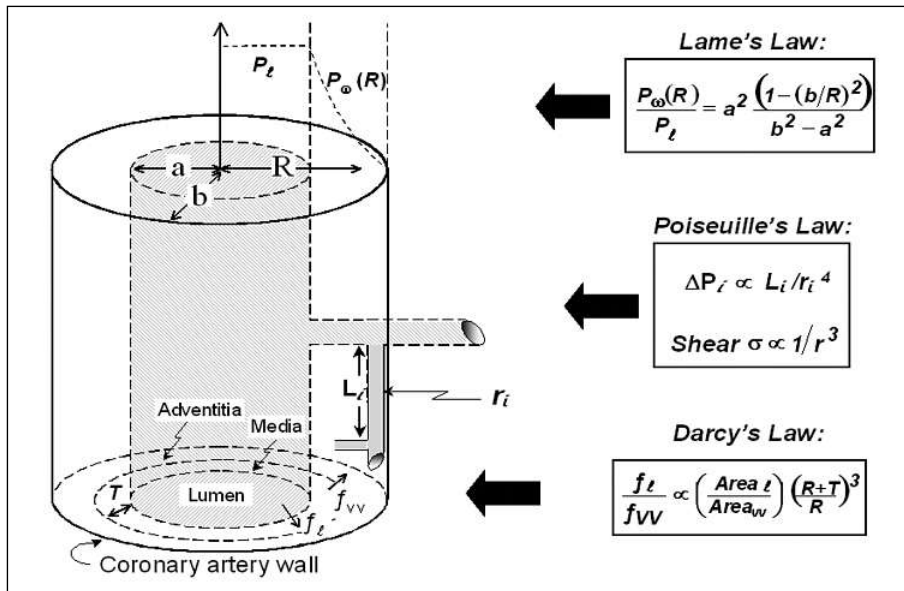


Figure 4: Analytic model of mechanical and fluid dynamic forces affecting arterial wall perfusion by vasa vasorum (VV). P_ω is intramural pressure at distance R from the center of the coronary artery lumen, P_ϵ is the pressure in the coronary artery lumen, P_l is pressure in lumen of VV at distance L from the origin of the VV. 'a' is radius of coronary artery lumen, 'b' is the radius of the outer adventitia, R is the radial distance of a point within the coronary arterial wall, f_ϵ and f_{VV} are the fluxes of solutes from the coronary arterial lumen and into the vasa vasorum respectively, as determined by Darcy's Law. 'r_i' is the radius of the 'proximal' vasa vasorum lumen. Area ϵ is the coronary arterial endothelial surface area across which solute flux occurs and Area_v being the area of the vasa endothelial surface within the wall at distance R within the wall (with permission from Dr. E. L. Ritman, Mayo Clinic College of Medicine, Rochester, MN, USA; figure reprint from Current Cardiology Reviews Vol. 3, No. 2, 2007; in press).

formation. Recently, the distribution of VV in different vascular beds has been shown (23), with the limitation that those vessels did not show any atherosclerotic lesions. However, those studies did not address the issue of local variations in the transmural pressure gradient among different vascular beds. A wall that is not supported at its abluminal surface would have a large pressure gradient which would drive diffusion into the wall via Darcy's law (49) as well as compress some of the arterial and venous VV. The latter effect is conceivably the most important, as the compression of the venous VV might prevent removal of solute that entered the wall at a rate equal to the influx from the arterial lumen. As illustrated schematically in Figure 4, an important consequence of the anatomic location and branching architecture of the VV, which enter the arterial wall via the adventitia, is that flow through the VV cannot proceed far into the media due to the compressive force within the arterial wall as described by Lamé's law.

However, arteries that are completely surrounded by an incompressible tissue, such as intra-cerebral arteries (which are surrounded by solid tissue which is constrained within the liquid shell), would also have reduced transmural pressure gradients. This reduced transmural pressure gradient would diminish solute transport into the wall. Similarly, arteries that are supported by surrounding muscle, such as intramyocardial vessels, could also have reduced transmural pressure gradient so that solute transport into the wall could also be reduced. In mice, vessels become intramyocardial within the first 120 μm in average of their origin from the sinus. It has been reported that apoE-deficient mice develop atherosclerotic lesions in the epicardial coronary arteries and within the myocardial branches, but do not develop myocardial infarctions (50). In contrast, intramural branches of the human coronary arteries do normally not show atheromatous lesions (51). Moreover, in human coronary arteries, several investigations confirmed that plaque microvessels originate from adventitial VV and are strongly positively correlated with intimal thickness and negatively correlated with relative lumen size,

indicating that intimal microvessels may play an important role in the development of atherosclerosis (52–54).

The cause for the absence of lesions in the intramyocardial vasculature is still under debate, but it has been hypothesized that freedom from atherosclerosis of the intramyocardial coronary arteries is due to the lack of transmural stress, i.e. the lower or absent transmural pressure gradient (55). Previously, it has been demonstrated that the intracerebral as well as the intra- and extramyocardial arteries did not develop VV, although the myocardial vessels (but not cerebral arteries) showed atherosclerotic lesions (56). The observed variability of VV neovascularization in different vascular beds supports the hypothesis that i) local vessel lesion heterogeneity relates to vessel luminal diameter, and ii) only advanced atherosclerotic lesions are related to VV neovascularization. The different distribution of VV may play a critical role in the progression of atherosclerotic lesions and may therefore contribute to the patchy manifestation of this systemic disease in different sized vessels.

Consistent with these data, Moghadasian et al. (57) confirmed the lack of atherosclerotic lesions in cerebral arteries. No pathological changes were observed in the brain from apoE-deficient and wild-type mice. This finding is of particular interest, because it is consistent with the hypothesis that VV neovascularization is not necessarily obligatory for each stages of lesion formation. Recently, Okuyama et al. (58) showed anatomical differences between human and mouse cerebral arteries which have different hemodynamic and pathophysiological implications in addition to the differences in their evolutionary development. The authors hypothesized that several possible variations in vessel anatomy may cause differences in flow and pressure gradients in different cerebral arteries, because the circle of Willis in the mouse does not complete a loop of cerebral arteries. On the contrary, one can argue that intracerebral arteries do not develop atherosclerotic lesions; the forming of a complete loop of the cerebral arteries during ontogenesis has no survival value.

Data, published by Sollberg (59) and Weber (47) indicate that atherosclerosis affects different vascular beds to a different extent. The aorta and proximal coronary arteries frequently have atherosclerotic changes. Vink et al. (60) showed severity of atherosclerosis to be highest in the coronary and lowest in the brain-supplying arteries.

The different patterns of VV neovascularization in different vascular beds may be useful for testing other hypotheses believed to explain the different plaque distribution in various vascular beds, such as systemic inflammatory factors.

VV and their contribution to intraplaque hemorrhage

Intraplaque hemorrhage represents an event in the induction and/or as a consequence of instability of advanced atherosclerotic lesions. Intraplaque hemorrhage is believed to arise from the disruption of thin-walled microvessels that are lined by a discontinuous endothelium without supporting smooth-muscle cells (61). Iron should accumulate in a lesion after hemorrhage (62), as part of each erythrocyte, whether isolated or within macrophage-derived cells that have phagocytosed the red blood cells (63). Supporting this idea, Lee et al. (64) suggested that hemoglobin/haeme released from the phagocytosed erythrocytes may contribute to at least part of iron deposition in atherosclerotic lesions in apoE-deficient mice.

Recently, neoangiogenesis as a source of intraplaque hemorrhage has been reported in carotid arteries in apoE-deficient mice (65). In this study, CD31 (PECAM-1) staining did reveal colocalization of plaque microvessels and sites of extravasated and degraded erythrocytes, consistent with intimal neo-angiogenesis as a feasible source for intraplaque hemorrhage.

These data are consistent with the findings of Kolodgie et al. (62) who showed that the degree of reactivity of glycophorin A and that the magnitude of iron accumulation corresponded to the size of the necrotic core. The increase in these variables paralleled the increase in the density of macrophages, raising the possibility that the hemorrhage itself serves as an inflammatory stimulus (Fig. 3).

Capillary-like microvessels were shown in very early atherosclerotic lesions (type II) in human carotid artery samples, associated with accumulations of macrophages, mast cells, and T cells, indicative of local inflammatory reactions (66). Evidence of local microvascular damage with subsequent intraplaque hemorrhage within the shoulder regions in human carotid arteries are demonstrated by extravascular red blood cells, macrophages containing haemosiderin, and perivascular fibrin deposition (67). Such findings indicate that plaque neovascularization is an important feature of plaque development and may provide an important source of intraplaque hemorrhage (68).

Moreno et al. (69) demonstrated the correlation of the presence of VV and inflammatory cells in human aortas. Lesions with intraplaque hemorrhage also had increased numbers of intraplaque microvessels, i.e. microvessel density was low in lesions with mild plaque inflammation and increasingly higher in lesions with moderate or severe inflammation.

It has been reported that patients with symptomatic atherosclerosis had a denser network of VV than patients with asymptomatic disease, and that this denser network of VV was accompanied by a strong inflammatory reaction within the vascular

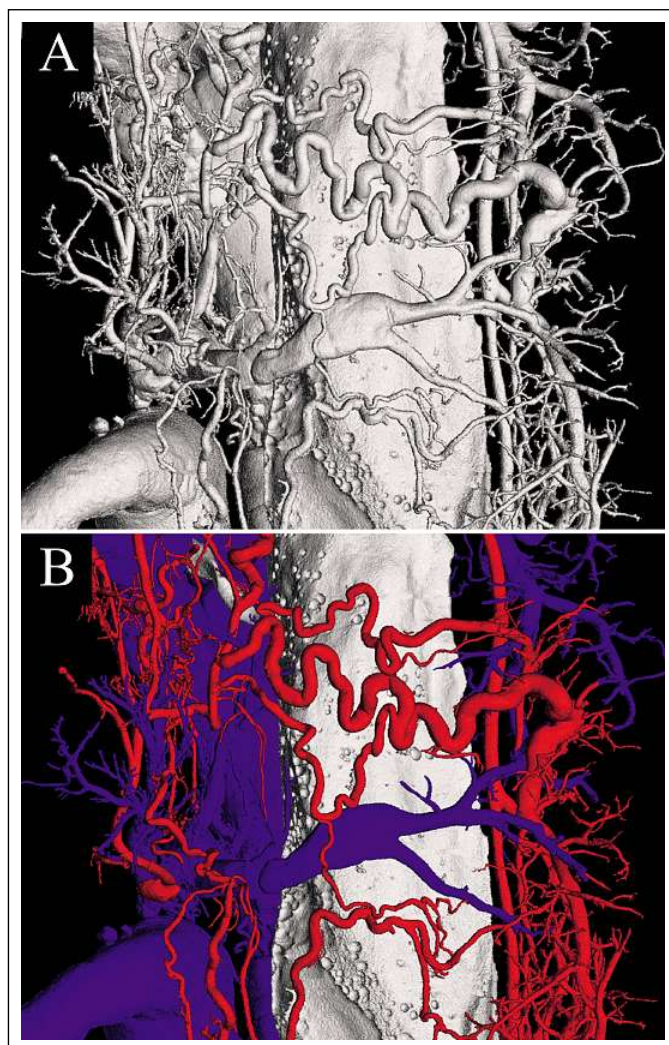


Figure 5: Distribution of VV in apoE^{-/-}/LDL^{-/-} double knockout mice at the age of 80 weeks. A) Volume rendered high-resolution synchrotron-based Micro-CT image of the descending aorta in apoE^{-/-}/LDL^{-/-} mice demonstrating VV (2 μ m voxel size). B) The 3D relationship of the arterial (red) and venous (blue) VV perfusion territories is demonstrated.

wall (37). These findings are supported by several investigations showing that the inflammatory response in the adventitia (increased expression of interleukin-6, tumor necrosis factor- α , monocyte chemoattractant protein-1, vascular cell adhesion molecule-1) could also alter atherogenesis (70, 71).

Supporting the link of VV proliferation and inflammation is the observation that VV density correlates highly with the extent of inflammatory cells within the plaque, not the size of atheromas in apoE-deficient mice (35). Previously, it has been demonstrated that adventitial VV density and adventitial inflammation correlates in different advanced atherosclerotic lesions (72) and in different vascular beds (56) in apoE^{-/-}/LDL^{-/-} mice. In this study, it was reported that the spatial location and magnitude of VV density and adventitial inflammation were strongly correlated in advanced atherosclerotic lesions and identified as an independent correlate to different categories of advanced lesion

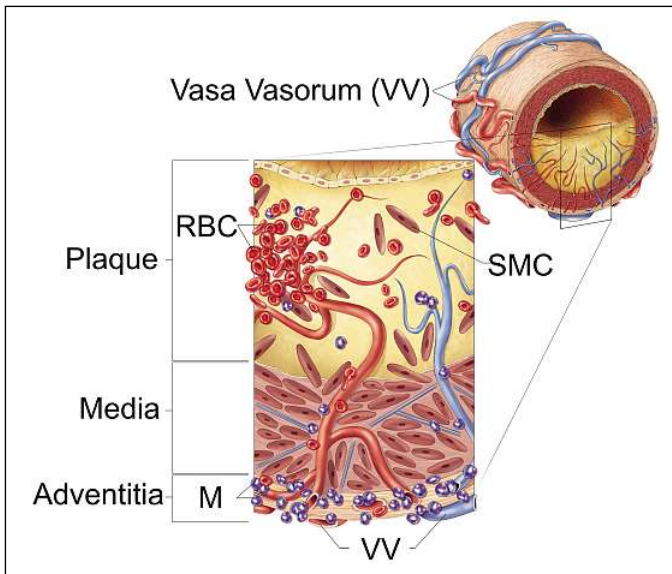


Figure 6: Schematic illustration summarizing conclusions as to the contribution of vasa vasorum to advanced atherosclerotic lesion provoking intraplaque hemorrhage. The red blood cells and/or macrophages are detectable by their iron deposits left in their place. RBC, red blood cells; M, monocytes; SMC, smooth muscle cells; VV, vasa vasorum.

types. Synchrotron-based micro-computed tomography imaging was used to demonstrate and quantitate arterial and venous VV neovascularisation along the aorta in apoE^{-/-}/LDL^{-/-} mice (Fig. 5). High-resolution imaging modalities demonstrated the distribution of iron deposits within plaques which also show evidence of prior intraplaque hemorrhage (73). In this study, the strong spatial, punctate colocalization of the elements iron and

calcium within the advanced atherosclerotic lesion with intraplaque hemorrhage, indicating that iron within the lesion results from intraplaque hemorrhage. This study supports the idea that plaque progression, plaque vascularization and VV neovascularization are seemingly inseparably linked, triggered and perpetuated by inflammatory reactions within the vascular wall. Figure 6 summarizes conclusions as to the contribution of VV to advanced atherosclerotic lesion provoking intraplaque hemorrhage.

Conclusion

The present data indicate that VV neovascularisation and atherosclerosis are seemingly inseparably linked, triggered and perpetuated by inflammatory reactions within the vascular wall. The ultimate test of any hypothesis about plaque vulnerability and plaque rupture will depend on technologies that would allow us to serially image advanced lesions by non-invasive studies in appropriate animal models and humans. Consequently, the combined imaging of plaque perfusion (as an index of VV density) and plaque substrate may form a basis for computed tomography imaging-based identification of vulnerable plaques.

Because VV and plaque neovascularization contribute to plaque growth and are associated to different lesion types it may be possible to convert unstable lesions to fibrocalcified (“stable”) lesions by inhibiting plaque perfusion, i.e. by anti-angiogenic drugs. Moreover, because monocytes/macrophages conceivably use VV as their means of transport into the arterial wall, blocking VV neovascularization may also have an indirect effect on plaque inflammation. Therefore, blocking VV neovascularization, to reduce/inhibit hemorrhage, and, to reduce plaque inflammation should restrict plaque growth and may result in plaque stabilization.

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