Novel Paradigms for Dialysis Vascular Access: Downstream Vascular Biology—Is There a Final Common Pathway?

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Summary
Vascular access dysfunction is a major cause of morbidity and mortality in hemodialysis patients. The most common cause of vascular access dysfunction is venous stenosis from neointimal hyperplasia within the perianastomotic region of an arteriovenous fistula and at the graft-vein anastomosis of an arteriovenous graft. There have been few, if any, effective treatments for vascular access dysfunction because of the limited understanding of the pathophysiology of venous neointimal hyperplasia formation. This review will (1) describe the histopathologic features of hemodialysis access stenosis; (2) discuss novel concepts in the pathogenesis of neointimal hyperplasia development, focusing on downstream vascular biology; (3) highlight future novel therapies for treating downstream biology; and (4) discuss future research areas to improve our understanding of downstream biology and neointimal hyperplasia development.

Introduction
A working vascular access is truly the lifeline, but also the Achilles heel, for hemodialysis patients. Seventy percent of patients with ESRD use hemodialysis as their renal replacement modality (1). Thus, the importance of a functioning and durable permanent vascular access is even greater. Vascular access dysfunction continues to remain a major cause of morbidity and hospitalization (2–4) among hemodialysis patients. The estimated annual costs of treating vascular access dysfunction is over US$1 billion (3), in large part because of the high proportion of nonmaturing arteriovenous fistulas (AVFs) and recurrent arteriovenous graft (AVG) stenosis resulting in hemodialysis catheter use. Despite the magnitude of the clinical problem of vascular access dysfunction, the pathogenesis of venous stenosis remains poorly understood. In considering the etiology of vascular access dysfunction, the natural history is defined by a series of progressive vascular injuries (Figure 1) from (1) uremic damage to the vessel beginning during the CKD period before access creation, (2) hemodynamic injury to the vessel after vascular access creation, and (3) endovascular repair to treat venous stenosis. A detailed understanding of the downstream biology (response to vascular injuries) at the various time points in the vascular access development will be essential to develop effective novel therapies and treatment paradigms for AVF nonmaturation and AVG stenosis. Finally, therapies and strategies for treatment of vascular access dysfunction will probably require multiple therapies at multiple time points during the natural history of a patient’s vascular access.

Histopathology of Hemodialysis Vascular Access Dysfunction

AVFs
Nonmaturation of AVFs has been a recent area of significant research and clinical interest as a recent large, multicenter, randomized controlled clinical trial reported that 60% of AVFs placed failed to mature for dialysis (5). The histology of AVF nonmaturation has been demonstrated to be secondary to aggressive venous neointimal hyperplasia (Figure 2, A and B), with the majority of cells within the neointima staining for myofibroblasts, but with contractile smooth muscle cells and fibroblasts also present (6). In addition to aggressive neointimal hyperplasia development, inadequate vasodilation (inward remodeling), resulting in vasoconstriction of the vein, also likely plays an important role in AVF nonmaturation (7–10).

AVGs
Dysfunction of AVGs is most commonly characterized by a venous stenosis occurring at the graft-vein and juxta-anastomotic vein segments (11) (Figure 2, C and D). Venous stenosis in AVGs most commonly arises from progressive neointimal hyperplasia, characterized by the presence of myofibroblasts and an abundance of extracellular matrix components within the neointima (Figure 2C). Angiogenesis (neovascularization) within the adventitia and macrophage layer lining the perigraft region is also present in AVGs,
which is the major difference from venous stenosis in AVFs (12).

**Histopathology Following Endovascular Repair**

The primary therapy available to treat stenosis in AVFs and AVGs is balloon angioplasty. Angioplasty is effective because it causes an aggressive outward remodeling (vasodilation) by rupturing the intima-media junction. Consequently, it also results in significant endothelial and medial smooth muscle cell damage, and the vessel wall invariably responds with an increased cellular proliferation within the neointima and, subsequently, a more aggressive neointimal hyperplasia development (13). Although most of the data on angioplasty-induced neointimal hyperplasia come from studying arterial models (14,15), it is also very likely that there is a deleterious injury from angioplasty to the venous system, especially because the venous tissue has intrinsically high baseline levels of inflammation and oxidative stress (16,17) from the uremic milieu.

**Histopathology of Uremic Vessels Prior to Vascular Access Creation**

Recently, several studies have demonstrated that neointimal hyperplasia is present in the majority of patient veins at the time of surgical creation (16–18) (Figure 1B), suggesting that the health of the vein at the time of AVF creation may also play an important role in AVF nonmaturation. Moreover, initial results from the multicenter National Institutes of Health Fistula Maturation Study have reported that 87.8% of vein samples examined to date (n = 204; total planned enrollment, 600 participants) have neointimal hyperplasia (19). These studies have shown the presence of myofibroblasts or contractile smooth muscle cells within the neointima in all veins with pre-existing neointimal hyperplasia (18,19). However, none of these studies have yet shown an association with pre-existing venous neointimal hyperplasia and worse AVF outcomes, but several of these studies are still in the follow-up period. Of note, in a recent study by Allon et al., pre-existing
neointimal hyperplasia was not present in any vein samples collected at baseline surgery, and this study population had nearly 50% AVF nonmaturation (20). Thus, this opens up the possibility that AVF nonmaturation may not feature neointimal hyperplasia in all cases and outward remodeling (vasodilation) probably plays an important role in AVF maturation.

The arterial inflow is also critical for vascular access success in AVF and AVG (21). Pre-existing arterial neointimal hyperplasia has been described by Kim et al. to be present in most radial arteries used to create new AVFs and to be associated with AVF maturation failure (22). Arterial microcalcification has also been reported to be present in the majority of radial arteries used to create new AVFs and present predominate within the media layer (23). Recently, Allon et al. also reported that most arteries used to create AVFs had microcalcification present, and microcalcification tended to be associated with AVF nonmaturation (20).

Collectively, the above studies demonstrate that vascular changes occur to arteries and veins before vascular access creation, and uremic injury may play an important role in adaptation to future hemodynamic injury after creation of an AV anastomosis. Thus, further investigation into the mechanisms and role of vascular health before AV access creation may be crucial to improve vascular access outcomes and is currently an emerging area of novel research.

**Downstream Biologic Response to Upstream Vascular Injury**

The pathogenesis of venous neointimal hyperplasia in AVF and AVG stenosis involves a number of events that are best divided and explained as upstream and downstream events (Figure 3). Upstream events are the initial vascular injuries responsible for endothelial and smooth muscle cell injury, which lead to a cascade of mediators (downstream events) that regulate mediators of oxidative stress and inflammation and ultimately result in venous neointimal hyperplasia (8,24) (Figure 3). Common upstream events involved in the pathogenesis of neointimal hyperplasia development include (1) surgical trauma at the time of AV surgery, causing vasospasm and ischemia to the vessel; (2) hemodynamic sheer stress at the vein-artery or vein-graft anastomosis, resulting in low sheer stress and turbulent flow; (3) bioincompatibility in polytetrafluoroethylene grafts; (4) vessel injury from routine needle cannulations; (5) uremia resulting in endothelial dysfunction; and (6) repeated balloon angioplasties to repair venous stenosis causing further endothelial injury, as well as smooth muscle injury within the media. Downstream events represent the response to endothelial and vascular injury from upstream events. They result in the activation; proliferation; and migration of fibroblasts, smooth muscle cells, and myofibroblasts from the media to the intima, which produce the products contributing to the lesion of venous neointimal hyperplasia (Figure 3).

Although the majority of research to date studying AVF nonmaturation has focused largely on neointimal hyperplasia development, the ability of the vessel wall to adequately remodel in response to upstream injuries likely is also pertinent to successful AVF maturation. Lack of outward remodeling (vasodilation) of the downstream or the proximal vein may also play an important role in the magnitude of final venous stenosis, particularly in the context of AVF nonmaturation.

**Figure 3.** Upstream and downstream events in hemodialysis vascular access dysfunction. Upstream events result in initial vascular injury. Downstream events are the vascular biologic response to upstream injury. Downstream biology involves mediators of oxidative stress and inflammation that regulate activation, proliferation, and migration of fibroblasts, smooth muscle cells, and myofibroblasts. PTFE, polytetrafluoroethylene.
Experimental Models Exploring Downstream Vascular Biology and Pathways

Models Evaluating Oxidative Stress
Heme oxygenase-1 (HO-1) is an enzyme that catalyzes degradation of heme and is an inducible isoform upregulated in response to vascular injury. Moreover, HO-1 may also have intrinsic protective properties against inflammation, oxidant stress, and vascular proliferation. In a seminal clinical study, Lin et al. demonstrated that patients with gene polymorphisms of HO-1 characterized by long GT repeats (less production of HO-1) were more likely to have worse AVF patency (25). A subsequent experimental study published by Juncos et al. in a murine model of AVF, where the HO-1 gene was knocked out, evaluated the functional and biologic significance of HO-1 in AVF development (26). Key findings from this seminal study include the following (26): (1) HO-1 gene expression was markedly induced in HO-1+/− mice compared with HO-1−/− mice, (2) strong HO-1 protein expression was present in vascular smooth muscle cells in HO-1+/+ mice, (3) patency rates for AVF were significantly higher in HO-1+/+ mice with less vein wall thickness and increased luminal area compared with HO-1−/− mice at 3 weeks, and (4) expression of proinflammatory and prooxidant mediators, such as monocyte chemoattractant protein-1 (MCP-1) and matrix metalloproteinases-2 and 9 (MMP-2 and MMP-9), was considerably greater in HO-1−/− mice compared with HO-1+/+ mice. MMPs play an important role in both inward (vasoconstriction) and outward vascular remodeling because they (1) are key enzymes that cause the breakdown of extracellular matrix proteins, such as collagen and elastin, to promote vasodilation (27,28) and (2) facilitate the proliferation and migration of smooth muscle cells and inflammatory cells, important processes in neointima hyperplasia development and vascular stenosis (29,30). The absence of HO-1 may lead to more prooxidant and proinflammatory effects from MMPs, as demonstrated in this study by Juncos et al. (26). Thus, it appears that HO-1 induction and expression appear to be a critically important regulator in the functional and biologic development of AVF by mediating proinflammatory and prooxidant mediators. From a clinical perspective this is significant because future therapies that can upregulate HO-1 expression may play a valuable role in improving AVF maturation and preventing AVG stenosis.

Production of superoxide anion has been shown to be another key player in AVF dysfunction in experimental models (31). Peroxynitrite, a product of superoxide anion and nitric oxide, is present in endothelial cells and smooth muscle cells in rodent AVF models (31). In rodents models of AVF where tempol, a superoxide anion scavenger, is administered, rodents had decreased neointimal hyperplasia development and improved blood flow (31). Pharmacologic approaches that target inhibition of oxidative stress mediators may serve as potential therapies for AVF maturation and dysfunction.

Models Evaluating Inflammation
MCP-1 is a potent chemokine reported to play an important role in atherosclerosis and other vascular diseases through chemotaxis of monocytes and macrophages, activation and migration of endothelial cells, proliferation and migration of smooth muscle cells, and induction of procoagulant mediators (32–36). Juncos et al. evaluated the role of MCP-1 in AVF development in murine and rodent models (32). The key findings from their seminal study were as follows (32): (1) expression of MCP-1 increased 1 week after AVF creation in mice, (2) MCP-1−/− rodents had increased AVF patency and decreased vein wall thickness compared with MCP-1+/+ rodents at 6 weeks, and (3) MCP-1 expression was localized within the endothelium, smooth muscle cells, and leukocytes in a rodent AVF model. Thus, MCP-1 appears to be upregulated very early after AVF creation and serves as a mediator for AVF dysfunction and failure. In experimental studies in vein graft models, inhibition of MCP-1 significantly reduced neointimal hyperplasia development (37). Therapies targeting MCP-1 inhibition have not been evaluated in vascular access to date but may potentially serve as important targets for AVF maturation and dysfunction.

Models Evaluating Endothelial Dysfunction
Clinical studies evaluating endothelial function in patients with advanced CKD before AVF creation using brachial artery flow-mediated vasodilation (FMD) have shown that low FMD is associated with decreased arterial remodeling and final vein diameter at 3 months, suggesting that the level of endothelial function and nitric oxide production may play an important role in AVF maturation (38). A recent study in a rodent AVF model evaluated inhibition of endothelial and inducible nitric oxide synthase (eNOS and iNOS) with N ω -nitro-L-arginine methyl ester and reported that venous neointimal hyperplasia and MCP-1 were significantly elevated in the group of rodents administrated N ω -nitro-L-arginine methyl ester (39). On a clinical level, asymmetric dimethylarginine, an endogenous inhibitor of NOS, accumulates with progressive CKD, and high levels are associated with aggressive restenosis after angioplasty in AVF (40). Thus, therapies that induce iNOS and eNOS expression may play a beneficial role in AVF development and stenosis.

Cellular Phenotypes and Migration of Cells in Neointima Formation
The development of neointima hyperplasia represents a major mechanism of vascular repair (41). Although this process differs in various pathologic conditions, the traditional paradigm for the pathogenesis of neointimal hyperplasia stresses the migration of smooth muscle cells from the media to the intima (42,43). Recent evidence, primarily from models of arterial injury (coronary angioplasty and saphenous vein bypass grafting), have supported a concept whereby the adventitial layer of the vessel plays an active role in neointima formation characterized by the following events (14,15,44–46): (1) adventitial activation of fibroblasts in response to endothelial cell injury; (2) adventitial proliferation and migration of fibroblasts from the adventitia, through the media, and into the intima, where these cells transform into myofibroblasts and vascular smooth muscle cells controlled by matrix metalloproteinases and local cytokines and growth factors; and (3) further proliferation of myofibroblasts and vascular smooth muscle cells within the intima, and synthesis of new
extracellular matrix to form the lesion of neointimal hyperplasia. In vascular access, where the main interest is venous injury, several experimental studies in AVGs have supported the concept of a migration of adventitial cells into the intima, where they contribute to final neointimal volume (47,48). Of note, a recent study has suggested that bone marrow-derived cells, which differentiate into smooth muscle cells, as a potential origin of neointimal cells (49).

**Common Pathways and Mediators for Neointimal Hyperplasia Development in Vascular Access**

On the basis of the limited clinical and experimental data available to date from vascular access literature described above, it appears that several key mediators and pathways are involved in the vascular injury response after creation of arteriovenous access (Figure 4). First, HO-1 plays a major adaptive role through its regulation of MCP-1 and other oxidative stress mediators. In the clinical setting, it appears that higher HO-1 expression is associated with a better AVF outcome. Next, increased expression and production of MCP-1 appears to play an important role in neointimal hyperplasia development. Regulation of MCP-1 expression appears to depend on the level of expression of HO-1 and eNOS, which in the clinical setting of CKD and uremia may already be compromised. Finally, the endothelial function of the vessel may play one of the most important roles in AVF development and neointimal hyperplasia formation. Adequate nitric oxide probably plays an important role, along with HO-1, in regulating inflammatory and oxidative stress mediators. Inadequate regulation of these inflammatory and oxidative stress mediators may lead to activation and proliferation of fibroblasts, myofibroblasts, and smooth muscle cells.

**Translating the Science of Downstream Biology to Therapies**

Understanding the pathology and pathophysiology of hemodialysis vascular access dysfunction may allow for targeting of specific mechanistic pathways and mediators for further investigation and identify potential therapies to prevent and treat vascular access dysfunction. At the current time, few, if any, therapies effectively treat hemodialysis vascular access dysfunction. Below are several recent examples of how our understanding of downstream biology has translated into potential novel therapies.

**Local Perivascular Delivery Therapies**

Local therapies may be the most beneficial and effective way to successfully modulate the downstream events involved in the pathogenesis of dialysis access dysfunction and deliver drugs to the direct site where local vascular injury occurs. The advantages and rationale behind local therapies in dialysis access (8,24), specifically perivascular therapy, are that (1) drug delivery targets the adventitia and may block adventitial activation and fibroblast migration; (2) small amounts of potentially toxic drugs can be delivered to the site of stenosis without concerns of systemic toxicity; and (3) local therapies, tested in dialysis access patients, who are a captive audience, can be an ideal model for testing perivascular therapies in other diseases, such as coronary artery disease and peripheral artery disease because these therapies can be applied at the time of surgery. Two recent examples of perivascular-delivered therapies include (1) endothelial cell–loaded gel foam wraps and (2) recombinant elastase therapy. Both of these therapies have been tested in early-phase clinical trials and have shown safety and feasibility (50,51). A larger, multicenter, phase II study of endothelial cell–loaded gel foam wraps will be initiated in the United States in 2013.

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**Figure 4.** | **Unifying pathway for downstream vascular biology.** Heme oxygenase-1 (HO-1) plays a major adaptive and protective role to prevent vascular access dysfunction through its regulation of monocyte chemoattractant protein (MCP-1) and other oxidative stress mediators, such as matrix metalloproteinases (MMP-2 and MMP-9) and peroxynitrite. Increased expression and production of MCP-1 and MMPs and peroxynitrite play an important role in neointimal hyperplasia development. Regulation of MCP-1 expression appears to depend on the level of expression of HO-1 and endothelial nitric oxide synthase. HO-1 may inhibit oxidative stress and inflammation following hemodynamic injury and nitric oxide inhibits endothelial dysfunction that may proceed from oxidative stress and inflammation. Inadequate regulation of these inflammatory and oxidative stress mediators results in a cascade of events leading to activation and proliferation of fibroblasts, myofibroblasts, and smooth muscle cells, and subsequently production of neointimal hyperplasia.
Endovascular Therapies
The current standard treatment for AVF and AVG stenosis is primarily percutaneous transluminal angioplasty (PTA). Success rates are dismal (11,21,52–54) because vessel injury to the endothelium and smooth muscle cells within the media that occurs after the PTA leads to further development of neointimal hyperplasia and restenosis (13,55). This suggests that although PTA may be important to treat stenosis in AVF and AVG, drug therapies may need to be applied to the site of angioplasty and endothelial injury to promote vascular healing and prolong vascular access patency.

Drug-Eluting Stents
In experimental models of AVGs, drug-eluting stents reduce neointimal hyperplasia and improve luminal stenosis compared with bare-metal stents (56). Recently, preliminary results from 32 patients with AVG dysfunction randomly assigned to a drug-eluting stent versus angioplasty alone were reported (57). Drug-eluting stents trended toward improving mean primary patency compared with angioplasty, but without a significant effect (163 versus 104 days; P=0.16).

Drug-Coated Balloons
Drug-coated balloons may be a technology that improves outward remodeling and prevents further neointimal hyperplasia development. A recent randomized controlled trial is evaluating the outcomes of angioplasty with a paclitaxel-coated balloon (PCB) versus plain balloon angioplasty (58). Six-month interim data from this study reported that cumulative target lesion primary patency at 6 months, defined as angiographic visualization of a patent lesion or circuit with ≤50% angiographic restenosis and no need for any repeat procedures during the follow-up period, was significantly higher after PCB (70% in PCB group versus 25% in balloon angioplasty group; P<0.001) (58).

Far-Infrared Therapy
Far-infrared therapy (FIR) is an electromagnetic wave with wavelengths that range from 5.6 to 1000 μm. The thermal effect of FIR results in vasodilation and increased blood flow. The nonthermal effects induce eNOS expression, inhibit neointimal hyperplasia, and reduce oxidative stress. In a clinical study in dialysis access, FIR increased access blood flow and unassisted patency through AVFs (59), likely through activation of HO-1 pathways and reduction in endothelial inflammation (60). A recent randomized controlled trial showed that FIR improves the access blood flow, maturation, and long-term patency of newly created AVFs in patients with CKD stages 4 and 5 (61).

Future Perspectives: New Frontiers in Vascular Access Research
Animal Models
Small and large animal models of dialysis access will play an important role in understanding the pathogenesis of dialysis access dysfunction. The main advantages of small animal models are that they allow for evaluation of specific pathways through specific knockout genes. The main small-animal models of dialysis access have been murine and rodents model (26,32,39,62,63). Large-animal models are advantageous because they have vessel anatomy similar to that of humans and provide the opportunity to study in detail hemodynamics and histology, but also allow for testing of experimental drug therapies and devices. The main large-animal models to date in dialysis access have been porcine models (47,64,65). One main disadvantage of both small and large animal models is the inability to simulate the long-term effects and consequences of CKD and endothelial dysfunction. However, recently, several groups have created porcine (66) and murine (67) models of CKD to study dialysis access dysfunction. In a murine model with CKD, accelerated neointimal hyperplasia was present in AVFs compared with non-CKD mice (67). In addition to porcine and murine models of CKD to study vascular access dysfunction, a rat model of CKD has also been superimposed on the rat AVF to demonstrate the damaging effect of uremia on the AVF in rat (39).

Genomics
Recent sequencing of the human genome has opened up fertile areas of investigation for many human diseases. To date, few studies have investigated the genetic underpinnings of vascular stenosis in hemodialysis access dysfunction. A recent study by Verschen et al. evaluated candidate genes associated with important mechanisms in AVF failure, such as inflammation, proliferation, vascular remodeling, and thrombosis (68). This study identified two single-nucleotide polymorphisms, LDL receptor-related protein 1 (rs1466335) and factor V Leiden, that were associated with AVF failure (68). Experimental and clinical studies evaluating arterial and venous tissue samples using gene expression microarrays (RNA, micro RNA sequencing, and epigenetics) may provide new molecular patterns of neointimal hyperplasia development in vascular access as they have in coronary artery disease peripheral artery disease. Fundamental advancements in our knowledge of the molecular pathophysiology of neointimal hyperplasia development and vascular access stenosis using genomic technology represents an unprecedented opportunity to develop new diagnostic, prognostic, and therapeutic interventions for this important clinical problem of vascular access dysfunction.

Is There a Final Common Pathway?
There is likely not one unifying final common pathway to explain the downstream biologic processes in hemodialysis vascular access dysfunction because (1) the natural history of vascular access dysfunction involves upstream injuries that occur before, during, and after access creation, and after endovascular repair and (2) unique pathways are upregulated in response to these various injuries that occur at these different time points in the vascular access process. However, pathways such as oxidative stress, inflammation, and endothelial dysfunction probably all play important and common roles in the downstream vascular biology of hemodialysis access dysfunction (Figure 4) and need further investigation with clinical and animal
models. Finally, given the multiple areas where downstream biology plays a role in the natural history of vascular access dysfunction (Figure 1), multiple therapies will probably be required at multiple time points in the course of a patient’s vascular access history.

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