Neointimal Hyperplasia in Early Arteriovenous Fistula Failure

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Background: Hemodialysis vascular access dysfunction currently is a huge clinical problem. Although arteriovenous fistulas (AVFs) are the preferred form of permanent dialysis access, they continue to have significant problems with early AVF failure. Although inadequate dilatation of the venous segment was believed to have a role in early AVF failure, the exact pathogenesis of early AVF failure is unknown despite the magnitude of the clinical problem.

Study Design: Case series.

Setting & Participants: Hemodialysis patients.

Outcomes & Measurements: Stenotic venous segments from 4 patients with early AVF failure were subjected to a detailed histological, morphometric, and immunohistochemical analysis.

Results: All 4 patients had significant luminal stenosis, primarily as a result of eccentric neointimal hyperplasia. This was confirmed through morphometric analysis, which documented intima-media area and thickness ratios that were greater than unity. Cellular phenotyping studies showed that the majority of cells within the region of neointimal hyperplasia were myofibroblasts, with smaller numbers of contractile smooth muscle cells.

Limitations: We described only a limited number of specimens.

Conclusions: We show for the first time that aggressive neointimal hyperplasia is present in venous segment specimens from patients with early AVF failure. Future therapies to address this problem will need to target this pathogenetic pathway.


INDEX WORDS: Arteriovenous fistula; early failure; vascular stenosis; neointimal hyperplasia.

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H emodialysis vascular access dysfunction currently is a huge clinical problem.1,2 It is an important cause of hospitalization in the hemodialysis population, is associated with very significant morbidity, and has an economic cost of approximately $1 billion per annum.3-5 Although arteriovenous fistulas (AVFs) are the preferred form of permanent dialysis access, they have significant problems with early failure because of nonmaturation, reported to vary from 23% to 46%. In addition, recent attempts to increase the AVF prevalence rate in the United States through the Fistula First initiative appear to have increased the early failure rate, perhaps as a result of AVF placement in patients with inadequate vessels and/or such risk factors as female sex, African-American ethnicity, and obesity.6-10

Early fistula failure was defined as a fistula that never developed adequately for dialysis use or that failed within the first 3 months of use.11 At a radiological level, early fistula failure was characterized by tight stenosis in the juxta-anastomotic region of the venous segment.11,12 However, reasons for early AVF failure in hemodialysis patients are unclear at the present time. Based on relatively sparse experimental13,14 and clinical15 data, we and others suggested that early AVF failure was likely to be caused by inadequate venous dilatation in combination with neointimal hyperplasia.16,17 However, it is important to emphasize that currently no data are available that clearly document the presence or absence of neointimal hyperplasia at a histological level in clinical AVFs that fulfill this defini-
tion of early AVF failure. The few available studies of histological characteristics of AVF stenosis either focused on late AVF failure or did not define the period for specimen collection.18-20

The aim of the present study therefore is to examine tissue specimens from the venous segment of AVFs with early failure to identify the presence or absence of venous neointimal hyperplasia.

METHODS

Definitions

For the purpose of this study, we defined early fistula failure as a fistula that never developed adequately to support dialysis. Our definition is slightly different from that of Beathard et al11 in that we excluded AVFs that had been used for dialysis, even if the duration of use was less than 3 months. We also excluded all patients who had undergone an endovascular or surgical intervention on the target vein within 3 months of fistula placement. Patients with AVF failure as a result of infection or other local complications were not defined as early AVF failure. Patients with a fistula that never developed adequately to support dialysis were defined as early AVF failure. Our definition is slightly different from that of Beathard et al11 in that we excluded AVFs that had been used for dialysis, even if the duration of use was less than 3 months. We also excluded all patients who had undergone an endovascular or surgical intervention on the target vein within 3 months of fistula placement. Patients with AVF failure as a result of infection or other local complications were not defined as early AVF failure. Patients with a fistula that never developed adequately to support dialysis were defined as early AVF failure.

Demographics and Specimen Collection

Venous tissue specimens (which would ordinarily have been discarded) from 4 patients with early AVF failure as defined previously were collected at the time of surgical revision. Basic demographic data that included age, sex, ethnicity, type of AVF, time after fistula placement, and absence of interventional procedures between initial surgery and the time of surgical revision were collected. Institutional review board approval was obtained.

Tissue Processing

Each tissue specimen was fixed in formalin and cut into 2 to 3 tissue blocks of 4 mm, depending on the size of the specimen. Pieces were embedded in paraffin and cut into 3-μm sections for histological, immunohistochemical (cellular phenotyping), and morphometric analyses.

Histological and Immunohistochemical Analyses

Sections from each tissue block were assessed using hematoxylin and eosin stain and also for expression of α-smooth muscle actin (SMA; a smooth muscle cell and myofibroblast marker; Dako, Carpinteria, CA; 1A4, 1:200), desmin (a marker of differentiated contractile smooth muscle cells; Dako; 1:400), vimentin (a marker of fibroblasts, myofibroblasts, and, in some cases, smooth muscle cells; Dako; V9, 1:200), and proliferating cell nuclear antigen (PC10; a marker of cellular proliferation). Briefly, after deparaffinization and hydration, slides were washed and underwent protease digestion (if required for a particular primary antibody). Slides then were incubated with the primary antibody for 1 hour, with the biotinylated secondary antibody blend (antirabbit immunoglobulin, antimouse immunoglobulin G, and antimonkey immunoglobulin M) for 30 minutes and with streptavidin/horseradish peroxidase for 30 minutes. All incubations were performed at room temperature with appropriate washes between each step. Slides then were developed with a diaminobenzidine/hydrogen peroxide mixture for 4 minutes, counterstained with hematoxylin, dehydrated with graduated alcohols and xylene, and mounted using a xylene-based medium. A brown coloration indicated a positive stain. Negative controls were performed on each run by omitting the primary antibody. In addition, positive control tissue (gut, lymph node, or spleen) was used to document the efficacy of each antibody.

Morphometric Analysis

Video images of hematoxylin and eosin–stained venous sections were projected at a final magnification of ×20 (Fig 1). Image J software was used for morphometric analyses. Area measurements were made of the luminal area (area enclosed by the black line, region A; Fig 1), area enclosed by the neointima (area enclosed by the red line, region B; Fig 1), and area enclosed by the media (area enclosed by the blue line, region C; Fig 1). Percentage of luminal stenosis was calculated for each tissue block using the formula (1 − A/C) × 100. The ratio of intimal area (Ia) to medial area (Ma) was calculated using the formula Ia/Ma = (B − A)/(C − B). Results for these parameters from all tissue blocks from each specimen were averaged to obtain mean values for percentage of luminal stenosis and intimal-medial area ratios for individual patients, which were then averaged to obtain a mean value for all patients used in this analysis. We previously described this approach for measuring luminal stenosis.21

After area measurements, a point was placed visually in the center of the lumen (Fig 1). Four lines extending from the center of the lumen to the endothelial border were drawn at each of the 4 quadrants (0°, 90°, 180°, and 270°). At the point where each of these lines intersected with the lining of the lumen, a line that was approximately perpendicular to the black and red lines at that point was drawn. This was used as a measure of intimal thickness (eg, line It [black double-headed arrow; Fig 1] would be a measure of intimal thickness for the 90° measurement). A second line was drawn perpendicular to the red and blue lines starting at the point where line It touched the red line. This was used as a measure of medial thickness (thus, line Mt [white double-headed arrow; Fig 1] would be a measure of medial thickness for the 90° measurement). These lines were used to derive an intimal thickness (It) to medial thickness (Mt) ratio (It/Mt ratio) at each of the 4 quadrants; the mean was defined as average intima to media thickness for a particular section (Avg It/Mt). Finally, a line was drawn visually for each section at the point of maximal intimal thickness (Max It), and the ratio of maximal intimal thickness to medial thickness (Mt) was measured in an identical fashion to It/Mt. This ratio was referred to as the maximal intima to media thickness (Max It/Mt). Comparison of average intima to media thickness with maximal intima to media thickness allowed us to make a scientific assessment of the eccentricity of
neointimal distribution within a single cross section. Results for these parameters from all tissue blocks from each specimen were averaged to obtain mean values for average intima-media thickness ratio (It/Mt) and maximal intima-media thickness ratio (Max It/Mt) for individual patients. These individual patient values were averaged to obtain a mean value for all patients studied in this analysis.

**Immunohistochemical Analysis**

To obtain information about actual cellular phenotypes within the region of neointimal hyperplasia, neointimal expression of SMA, desmin, vimentin, and proliferating cell nuclear antigen for each patient was scored separately by 2 observers (L.A. and P.R.-C.) using a semiquantitative scoring scale from 0 to 4\(^H1\), depending on the percentage of total neointimal cells that were positive for the specific marker (0 indicates 0% to 10% positive; 1, 11% to 25% positive; 2, 26% to 50% positive; 3, 51% to 75% positive; and 4, 76% to 100% positive). Mean values for SMA, vimentin, desmin, and proliferating cell nuclear antigen for all 4 patients were calculated. These 3 markers were used to identify the relative contribution of myofibroblasts, fibroblasts, and contractile smooth muscle cells within the region of neointimal hyperplasia by using the schema listed in Table 1 (based on data previously presented by Shi et al., Kalra and Miller, and Owens). Although we accept there could be some overlap of these markers, it is important to note that desmin is the predominant intermediate filament present in smooth muscle cells in the smaller arteries and therefore is a reasonable marker for these cells in the context of this particular study. Finally, because our scoring system was based on percentage of total cells that express a specific marker, differences in staining intensity (weak to strong) are unlikely to affect the final results.

**RESULTS**

**Demographics**

Four patients were assessed in this study (2 men, 2 women). Average age was 69 years (range, 62 to 84 years). One patient had a Brescia-Cimino fistula at the wrist (patient A) and the other 3 had brachiocephalic fistulas. Mean interval between AVF placement and surgical revision was 133 days (114, 115, 198, and 304 days). In all cases, no endovascular or surgical interventions were performed between AVF placement and surgical revision (time of harvest of specimens). In 3 patients, the AVF was found to be nondeveloped, but patent, at the time of surgical revision. In 1 patient (114-day patient; patient D), the fistula was found to be thrombosed (no flow) at the time of surgical revision, albeit with documented evidence that the thrombus occurred after an episode of hypotension 75 days after AVF placement.

**Histological Analysis**

Patients A and C had 2 tissue blocks that were suitable for analysis, whereas patients B
and D had 3 such tissue blocks. Examination of tissue specimens showed significant neointimal hyperplasia and some medial hypertrophy in all 4 patients (Fig 2). To show the aggressiveness of neointimal hyperplasia in early AVF failure, Fig 3 shows normal intima-media thickness in a similar-sized human vein that was not associated with an AVF. The intimal region in the normal vein is only 1 to 2 cell layers thick (cf, 20 to 30 cell layers in the stenotic AVF specimens) and the media is only 3 to 4 cell layers thick (cf, 10 to 15 cell layers in the stenotic AVF specimens).

**Morphometric Analysis**

Table 2 lists data for percentage of luminal stenosis, intimal-medial area ratio, average intima-media thickness, and maximal intima-media thickness, measured as described in Methods. All specimens had a very significant degree of luminal stenosis (>80%) predominantly caused by neointimal hyperplasia as opposed to medial hypertrophy in view of: (1) the increased intimal-medial area ratio (1.67 times) and (2) the increased ratio of average intimal thickening to medial thickening (any value > 1 suggests a predominance of neointimal hyperplasia compared with medial hypertrophy). Figure 4 shows percentages of luminal stenosis in these 4 patients, whereas Fig 5 shows intima-medial area ratios. Figure 6 shows pooled data for both average and maximal intimal to medial thickening. The marked difference between average intimal to medial thickness and maximal intimal to medial thickness in the same specimen suggests that the lesion of neointimal hyperplasia in

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**Figure 2.** (A to C) Neointimal hyperplasia in representative sections from 3 patients with early arteriovenous fistula failure. All specimens have significant neointimal hyperplasia (length of double-headed arrow). (Smooth muscle actin [SMA]; original magnification ×20.)

**Figure 3.** Histological characteristics of normal human vein not associated with arteriovenous fistula (AVF) stenosis. Note the 1 to 2 cell layer thick intima and the 3 to 4 cell layer thick media compared with the 20 to 30 cell layer thick intima and the 15 to 20 cell layer thick media in the specimens with early AVF failure (Fig 2). Note also that smooth muscle actin (SMA) and desmin have similar staining intensities within the venous media.
the setting of primary fistula failure is an eccentric lesion. These overall statistical data are in keeping with the representative examples of vascular stenosis described previously in Fig 2.

**Immunohistochemical Studies**

Figure 7 shows representative specimens from a patient who underwent surgical revision at 114 days. In addition to standard hematoxylin and eosin analysis, these specimens were stained with antibodies against SMA, desmin, and vimentin to phenotype specific cell types within the region of neointimal hyperplasia. Figure 8 quantifies the pattern of immunohistochemical staining within the neointima in these patients. Note that both SMA and vimentin have scores higher than 3, indicating that the majority of cells express these markers (score of 3 indicates 51% to 75% of cells are positive for the specific marker). As shown in Fig 7B and C, most cells within the neointima are positive for both SMA and vimentin, suggesting they are myofibroblasts (Table 1). However, Fig 7D clearly documents that at least some cells within the neointima are desmin positive (arrows), suggesting they still retain their contractile smooth muscle cell phenotype (possibly as a result of migration from the media). Vimentin and SMA stains also showed the presence of microvessels (angiogenesis) within the adventitia, media, and neointima of these specimens with early fistula failure.

**DISCUSSION**

This report shows for the first time that tissue specimens from the venous segment of nondeveloped AVFs (which fulfill the clinical definition of early AVF failure) are characterized by an aggressive neointimal hyperplasia consistent with the angiographic demonstration of tight stenosis in the juxta-anastomotic region reported by others in patients with maturation failure.11,12 The neointimal hyperplasia in this specific clinical setting is eccentric in orientation and primarily made up of myofibroblasts. Although our numbers are small, we believe this is an important finding because it: (1) could shed light on some of the pathogenetic processes responsible for early AVF failure and (2) suggests that therapies to decrease neointimal hyperplasia could be effective in decreasing the huge clinical and economic burden of this complication.

**Table 2. Mean Values for Patients With Early Fistula Failure**

<table>
<thead>
<tr>
<th>Stenosis (%)</th>
<th>Intimal-Medial Area Ratio</th>
<th>Average Intimal-Medial Thickness</th>
<th>Maximal Intimal-Medial Thickness</th>
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<tbody>
<tr>
<td>85.8 ± 3.4</td>
<td>1.67 ± 0.1</td>
<td>3.12 ± 0.43</td>
<td>7.77 ± 1.49</td>
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*Note: Values expressed as mean ± SE.*

![Figure 4](image.png)

*Figure 4. Percentage of luminal stenosis in early arteriovenous fistula (AVF) failure: note the very significant luminal stenosis present in this condition. A, B, C, and D indicate the 4 individual patients.*
burden currently associated with early AVF failure.

After creation of an AVF, there are 2 important and concurrent processes that contribute to the appropriate maturation of an AVF.\textsuperscript{16,17} These include: (1) venous dilatation in response to increased flow and shear stress in the venous segment\textsuperscript{25-27} and (2) venous wall thickening (primarily medial hypertrophy) caused by an increase in circumferential pressure.

Based on our presented results, we therefore hypothesize that the early and aggressive occurrence of neointimal hyperplasia and medial hypertrophy could be an important mechanism in the pathogenesis of early AVF failure. In addition, the eccentric pattern of neointimal hyperplasia seen in our tissue specimens (Figs 2 and 7) suggests differences in the pattern of vascular injury, even within a single cross section. This unusual pattern of vascular injury is most compatible with local differences in shear-stress profiles. This is in keeping with our earlier preliminary results documenting differences in shear-stress profiles within a single venous segment cross section.\textsuperscript{28}

\textbf{Figure 5.} Intima-media area ratios in early arteriovenous fistula (AVF) failure: ratios greater than 1 indicate that neointimal hyperplasia, rather than medial hypertrophy, is the primary cause of vascular wall thickening in early AVF failure.

\textbf{Figure 6.} Intima-media thickness ratios in early arteriovenous fistula (AVF) failure: both average and maximal intima-media thickness ratios are greater than 1, indicating the intima is thicker than the media. The larger values for maximal (red) compared with average (black) intima-media thickness ratios suggest an eccentric development of neointimal hyperplasia (see Discussion).
It is important to emphasize that we attempted to exclude all possible confounding factors, such as the impact of vascular injury caused by dialysis needles, angioplasty, or revision surgery, on neointimal hyperplasia in this study by excluding all such patients. Although this significantly lim-

**Figure 7.** Neointimal hyperplasia in early arteriovenous fistula (AVF) failure. (Panel A) Hematoxylin and eosin (H and E), (Panel B) smooth muscle actin (SMA), (Panel C) vimentin, and (Panel D) desmin stains on sequential sections of the venous segment of an AVF that was never used for dialysis and was revised 114 days after initial placement. Note the very significant degree of neointimal hyperplasia ([Panel A and B] black double-headed arrows) with relatively less medial hypertrophy ([Panel A and B] white double-headed arrows). Note also that although most cells within the region of neointimal hyperplasia appear to be SMA-positive, vimentin-positive, desmin-negative myofibroblasts, there also are some SMA-positive desmin-positive contractile smooth muscle cells present within the neointima ([Panel D] small black arrows).

**Figure 8.** Cellular phenotyping in early arteriovenous fistula (AVF) failure: note that most neointimal cells in early AVF failure appear to be smooth muscle actin (SMA)-positive vimentin-positive myofibroblasts, with smaller numbers of SMA-positive desmin-positive contractile smooth muscle cells. A large number of these neointimal cells (mean score, 1.75) appear to be actively proliferating (proliferating cell nuclear antigen [PCNA] positive).
ited the number of specimens we could use, we believe our data are a direct reflection of surgical and hemodynamic stressors on early AVF failure. However, we recognize that recent reports documented the presence of preexisting intimal hyperplasia within both the cephalic veins and radial arteries used for AVF creation in hemodialysis patients. Whether our patients had preexisting venous neointimal hyperplasia at the site of AVF placement is unknown.

Our results also document that the predominant cell type within the lesion of neointimal hyperplasia in patients with early AVF failure is the myofibroblast. However, it is not known whether these myofibroblasts are transformed fibroblasts that developed \(\alpha\)-SMA expression or dedifferentiated medial smooth muscle cells that lost desmin expression. Although we cannot rule out the possibility that even a small number of cell types other than myofibroblasts within the neointima could have important paracrine effects on migration and proliferation, the predominance of myofibroblasts suggests that focused targeting of this cell type (rather than fibroblasts or contractile medial smooth muscle cells) could result in novel therapies for early AVF failure. Finally, it is important to mention that the cellular composition of neointimal hyperplasia in AVFs is similar to that within stenotic proximal (downstream) vein beyond the graft-vein anastomosis in the setting of polytetrafluoroethylene grafts. However, the pseudointima in association with PTFE grafts tends to be less cellular (more extracellular matrix components) with a complete absence of contractile smooth muscle cells (no desmin staining).

In conclusion, we show the presence of both eccentric neointimal hyperplasia and medial hypertrophy in AVFs that failed to mature adequately (early AVF failure). Our studies suggest that a multipronged approach that includes the use of pharmacological intervention to enhance dilatation and minimize neointimal hyperplasia is needed urgently to address the clinical problem of early AVF failure.

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