Intimal Hyperplasia, Stenosis, and Arteriovenous Fistula Maturation Failure in the Hemodialysis Fistula Maturation Study


*Division of Nephrology and Hypertension, University of Utah, Salt Lake City, Utah; †Medical Service, Veterans Affairs Salt Lake City Healthcare System, Salt Lake City, Utah; ‡Department of Nephrology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, People's Republic of China; §Department of Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, Ohio; ¶Department of Quantitative Health Sciences, Cleveland Clinic, Cleveland, Ohio; ‡‡Department of Pathology, University of Washington Medical Center, Seattle, Washington; **Department of Radiology and ††Division of Nephrology, University of Alabama at Birmingham, Birmingham, Alabama; †††Renal-Electrolyte and Hypertension Division, Department of Medicine,¶¶Center for Clinical Epidemiology and Biostatistics, and Departments of ¶¶Biostatistics and Epidemiology and ¶¶¶Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania; ‡‡‡Department of Population Health Sciences, University of Utah School of Medicine, Salt Lake City, Utah; §§§Department of Medicine, Kidney Research Institute, University of Washington, Seattle, Washington; ††††Division of Nephrology, University of Arizona Health Sciences and Banner University Medical Center, Tucson, Arizona; †††‡‡‡Medical Service, Southern Arizona Veterans Affairs Healthcare System, Tucson, Arizona; §§§Division of Nephrology, University of Texas Southwestern Medical Center, Dallas, Texas; and |||Division of Kidney, Urologic and Hematologic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland

ABSTRACT

Intimal hyperplasia and stenosis are often cited as causes of arteriovenous fistula maturation failure, but definitive evidence is lacking. We examined the associations among preexisting venous intimal hyperplasia, fistula venous stenosis after creation, and clinical maturation failure. The Hemodialysis Fistula Maturation Study prospectively observed 602 men and women through arteriovenous fistula creation surgery and their postoperative course. A segment of the vein used to create the fistula was collected intraoperatively for histomorphometric examination. On ultrasounds performed 1 day and 2 and 6 weeks after fistula creation, we assessed fistula venous stenosis using pre-specified criteria on the basis of ratios of luminal diameters and peak blood flow velocities at certain locations along the vessel. We determined fistula clinical maturation using criteria for usability during dialysis. Preexisting venous intimal hyperplasia, expressed per 10% increase in a hyperplasia index (range of 0%–100%), modestly associated with lower fistula blood flow rate (relative change, −2.5%; 95% confidence interval [95% CI], −4.6% to −0.4%; P=0.02) at 6 weeks but did not significantly associate with stenosis (odds ratio [OR], 1.07; 95% CI, 1.00 to 1.16; P=0.07) at 6 weeks or failure to mature clinically without procedural assistance (OR, 1.07; 95% CI, 0.99 to 1.15; P=0.07). Fistula venous stenosis at 6 weeks associated with maturation failure (OR, 1.98; 95% CI, 1.25 to 3.12; P=0.004) after controlling for case mix factors, dialysis status, and fistula location. These findings suggest that postoperative fistula venous stenosis associates with fistula maturation failure. Preoperative venous hyperplasia may associate with maturation failure but if so, only modestly.


Received December 22, 2016. Accepted May 13, 2017. Published online ahead of print. Publication date available at www.jasn.org.
Although the arteriovenous fistula (AVF) is the preferred type of vascular access for maintenance hemodialysis, many AVFs fail to mature.1–4 The pathogenic processes leading to maturation failure have not been clearly delineated. Although de novo outflow venous stenosis is recognized as an important cause of failure of synthetic arteriovenous grafts,5–7 the roles of preexisting and newly occurring venous stenosis in AVF maturation failure remain unclear. Vascular access stenosis is predominantly caused by intimal hyperplasia, and preexisting venous intimal hyperplasia before AVF creation has been frequently observed.8–10 In the Hemodialysis Fistula Maturation (HFM) Study, a prospective, multicenter cohort study of 602 men and women with newly placed AVFs, we previously found that intimal hyperplasia occupied ≥20% of the luminal cross-sectional area in most (57%) vein samples for which this could be quantified.11

We extend our HFM Study findings by examining (1) the association of preexisting venous intimal hyperplasia with subsequent AVF stenosis identified from serial ultrasound measurements after AVF creation surgery and (2) the associations of both preexisting venous intimal hyperplasia and postoperative AVF vein stenosis with clinical AVF maturation failure. Our hypotheses are that preexisting intimal hyperplasia in the vein used for AVF creation promotes postoperative AVF stenosis and subsequent maturation failure and that stenosis developing after AVF creation by any mechanism also contributes to maturation failure.

RESULTS

The HFM Study

Detailed baseline characteristics of the HFM Study cohort have been previously published.12 Six hundred and two participants were enrolled from March of 2010 to September of 2013 and followed prospectively. At baseline, the mean age was 55.1 ± 13.4 (SD) years old, 70% were men, 44% were blacks, 59% self-reported diabetes, and 64% were receiving maintenance hemodialysis. Forty-eight percent were taking an antiplatelet agent, 7% were taking an anticoagulant, 50% were taking a renin-angiotensin system blocker, 28% were taking a statin, and 25% were taking an active vitamin analog. Four hundred fifty-seven (76%) of 602 participants had their AVFs created in the upper arm.

Vein samples were obtained from 554 (92.0%) participants. A complete circumferential vein section unobscured by valves was observed histologically in 365 (65.9%) of these samples. The numbers of postoperative AVF ultrasound measurements available for evaluation at 1 day, 2 weeks, and 6 weeks after surgery were 550 (91.4%), 537 (89.2%), and 517 (85.9%), respectively.

Less than one half (263 of 602; 43.7%) of the participants had AVFs that achieved unassisted maturation; approximately one quarter (166 or 27.6%) had AVFs that matured with assistance, and approximately one quarter (133 or 22.1%) had AVFs that failed to mature. Unassisted and overall maturation status could not be determined for 4.3% and 6.6% participants, respectively, primarily because follow-up was terminated administratively before dialysis was required or the maturation status could be resolved or the participant received a kidney transplant before the resolution of maturation status. Maturation status stratified by sex and arm location is presented in Supplemental Table 1. Patients with indeterminate AVF maturation status, missing ultrasound measurements, and/or incomplete vein samples from which the hyperplasia index could not be adequately assessed were retained in the analysis by multiple imputation of such missing data from ultrasounds at other time points and additional patient attributes as previously described.13

Frequency, Characteristics, and Time Course of Stenosis on Postoperative Ultrasounds

Postoperative ultrasound measurements were analyzed for the presence or absence of venous stenosis and other features. Stenoses meeting the criteria described in Concise Methods were classified as either juxta anastomotic (juxta-anastomotic stenosis) or AVF draining vein (distal stenosis) on the basis of whether the distance from the anastomosis in the AVF draining vein was ≤2 or >2 cm, respectively. As shown in Figure 1, the prevalence of stenosis detected on ultrasound was 14% at
1 day, doubled to 28% at 2 weeks, and increased slightly to 30% from 2 to 6 weeks. Juxta-anastomotic stenosis was observed more frequently than distal stenosis at 1 day (61 [11.1%] versus 23 [4.2%] participants, including three with both types) and 2 weeks (92 [17.1%] versus 60 [11.2%], including four with both types), but these were similar in frequency (75 [14.5%] versus 80 [15.5%], including six with both types) at 6 weeks. Supplemental Table 2 shows, separately, the prevalence of juxta-anastomotic stenosis and distal stenosis for forearm and upper arm AVFs. The increase in prevalence of stenosis was not cumulative, because 37% of study participants who exhibited stenosis on the 1-day ultrasound did not exhibit stenosis on the 2-week ultrasound, and virtually the same fraction (36%) of those who exhibited stenosis on the 2-week ultrasound did not exhibit stenosis on the 6-week ultrasound. Conversely, among participants with ultrasounds that did not reveal stenosis at 1 day, a new stenosis was identified in 21% at 2 weeks; for those with no stenosis at 2 weeks, a new stenosis was identified in 16% at 6 weeks (Figure 1).

The mean AVF vein inner diameter was lower in participants with a stenosis than those without a stenosis detected by ultrasound at 1 day (4.48 versus 4.84 mm; \( P = 0.02 \)), 2 weeks (5.44 versus 5.88 mm; \( P < 0.001 \)), and 6 weeks (5.86 versus 6.57 mm; \( P < 0.001 \)). The mean blood flow rate was also lower in participants with a stenosis (495 versus 726 ml/min at 1 day; 714 versus 962 ml/min at 2 weeks; and 764 versus 1124 ml/min at 6 weeks; \( P < 0.001 \) for each).

**Association of Preexisting Venous Intimal Hyperplasia with Stenosis on Postoperative Ultrasounds**

As previously reported, 88% of the 365 complete vein samples examined showed some degree of intimal hyperplasia. However, 42.7% of these samples showed intimal hyperplasia indices \( \geq 20\% \), and only 24.1% showed indices \( > 50\% \). Table 1 summarizes associations of the preexisting venous intimal hyperplasia with the presence of stenosis identified on each postoperative ultrasound examination expressed as odds ratios (ORs) comparing samples differing in their intimal hyperplasia indices by 10% of maximal luminal area (Concise Methods). The OR for the 6-week ultrasound, designated a priori as the primary ultrasound examination for this report, was 1.07 (95% confidence interval [95% CI], 1.00 to 1.16), which approached but did not achieve statistical significance (\( P = 0.07 \)). The associations of the intimal hyperplasia index with stenosis at 1 day and 2 weeks were weaker and also statistically nonsignificant. The associations of the intimal hyperplasia index with juxta-anastomotic stenosis and distal stenosis are reported separately in Supplemental Table 3, where the associations with juxta-anastomotic stenosis seem weaker than those with distal stenosis.

**Associations of Preexisting Venous Intimal Hyperplasia with Postoperative Vein Diameter and Blood Flow Rate**

Greater preexisting venous intimal hyperplasia was significantly associated with lower AVF blood flow rates assessed by ultrasound at 6 weeks (2.5% reduction per 10% increase in intimal hyperplasia index; \( P = 0.02 \) (Table 1) but was not significantly associated with the 2-week or 1-day measurements. Associations between preexisting venous intimal hyperplasia and vein diameters were not statistically significant at any postoperative time point examined.

**Associations of Preexisting Venous Intimal Hyperplasia with Clinical Maturation Failure**

The association of increasing venous preexisting hyperplasia with clinical AVF maturation failure that occurred in the absence of assistance did not achieve statistical significance (OR, 1.07 per 10% increase in hyperplasia index; 95% CI, 0.99 to 1.15; \( P = 0.07 \)). Results were similar for overall maturation failure (i.e., failure that occurred regardless of whether interventional procedures were used to assist maturation; OR, 1.08; 95% CI, 0.98 to 1.19; \( P = 0.11 \)). If the association of intimal hyperplasia with postoperative variables was mediated by

---

**Table 1.** Associations of preexisting venous intimal hyperplasia index with AVF stenosis, venous blood flow rate, and mean venous diameter on three postoperative ultrasounds

<table>
<thead>
<tr>
<th>Postoperative Ultrasound Time Point</th>
<th>Stenosis Prevalence</th>
<th>Venous Blood Flow Rate</th>
<th>Mean Venous Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (^{a})</td>
<td>95% CI</td>
<td>( P ) Value (^{b})</td>
</tr>
<tr>
<td>6 wk(^{1})</td>
<td>1.07</td>
<td>1.00 to 1.16</td>
<td>0.07</td>
</tr>
<tr>
<td>2 wk</td>
<td>1.00</td>
<td>0.93 to 1.09</td>
<td>0.91</td>
</tr>
<tr>
<td>1 d</td>
<td>1.04</td>
<td>0.94 to 1.16</td>
<td>0.49</td>
</tr>
</tbody>
</table>

\(^{a}\)OR of developing stenosis per 10% increase in intimal hyperplasia index adjusted for age, sex, black race, chronic dialysis status at time of AVF creation, and AVF location (upper arm versus forearm) as well as inflow artery diameter, mean vein diameter, and brachial artery blood flow rate on preoperative ultrasound, with clinical center modeled as a random variable.

\(^{b}\)\( P \leq 0.05 \) is nominally considered to be statistically significant.

\(^{c}\)Adjusted for age, sex, black race, chronic dialysis status at time of AVF creation, and AVF location (upper arm versus forearm), with clinical center modeled as a random variable

\(^{d}\)Percent relative change in AVF blood flow rate per 10% increase in intimal hyperplasia index.

\(^{e}\)Change in mean AVF vein diameter (millimeters) per 10% increase in intimal hyperplasia index.

\(^{1}\)Week 6 results were identified a priori as the primary ultrasound outcomes among the three time points. Hence, these \( P \) values were not corrected for testing at multiple time points.
Association of Stenosis Determined by Postoperative Ultrasound Measurements with Clinical Maturation Failure

Stenosis identified on ultrasound at each postoperative time point was directly associated with failure to achieve unassisted maturation and failure to achieve overall maturation after adjustment for case mix and preoperative ultrasound measures. The ORs of unassisted maturation failures for stenosis identified by the 1-day, 2-week, and 6-week ultrasounds were 1.86 (95% CI, 1.09 to 3.18; \( P = 0.02 \)), 1.47 (95% CI, 0.96 to 2.25; \( P = 0.08 \)), and 1.98 (95% CI, 1.25 to 3.12; \( P = 0.004 \)), respectively. The corresponding ORs of overall maturation failures were 2.24 (95% CI, 1.27 to 3.93; \( P = 0.005 \)), 1.66 (95% CI, 1.04 to 2.65; \( P = 0.04 \)), and 1.98 (95% CI, 1.26 to 3.13; \( P = 0.004 \)), respectively (Table 2).

We investigated whether the association of stenosis with AVF clinical maturation failure varied depending on the location of the stenosis by comparing juxta-anastomotic stenosis with distal stenosis. For ultrasounds at each time point, the associations of unassisted maturation with distal stenosis were stronger than those with juxta-anastomotic stenosis, although the difference was statistically significant only at 2 weeks (Supplemental Table 4).

Previously, we have shown in the HFM Study that the combination of AVF vein diameter, blood flow rate, and depth on ultrasound predicted both AVF unassisted maturation and overall maturation failure moderately strongly. Because these measures are conceptually related to, albeit not directly used in, the criteria for defining stenosis, we investigated whether the associations of stenosis with maturation failure remained detectable after adjustment for concurrent ultrasound measures of vein diameter, blood flow rate, and depth. Accordingly, alternative analyses were performed with adjustments for concurrent mean AVF vein diameter, blood flow rate, and depth instead of adjustment for preoperative vein diameter and blood flow rate. In these alternative analyses, associations of stenosis with maturation failure were greatly attenuated and no longer statistically significant (Table 2). In contrast, the associations of maturation failure with lower concurrent vein diameter and blood flow rate and higher depth remained largely statistically significant (i.e., in five of six [equals two types of maturation outcomes \( \times \) three ultrasound examination time points] analyses for vein diameter, five of six analyses for blood flow rate, and six of six analyses for vein depth; data not shown). Results of sensitivity analyses replacing mean vein diameter with minimum vein diameter were similar (Supplemental Table 5).

**DISCUSSION**

Although stenosis is established as a common cause of failure of synthetic arteriovenous grafts, its role in AVF maturation failure is unclear. In the absence of external compression, which is probably rare, stenosis is likely to be caused by intimal hyperplasia. In this study, we examined the associations of

---

Table 2. Associations of stenosis on ultrasound at three postoperative time points with clinical maturation failure outcomes

<table>
<thead>
<tr>
<th>Postoperative Ultrasound Time Point and Ultrasound Adjustment*</th>
<th>Unassisted Maturation Failure</th>
<th>Overall Maturation Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI P Value</td>
<td>OR 95% CI P Value</td>
</tr>
<tr>
<td>Week 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative ultrasound measures</td>
<td>1.98 1.25 to 3.12 0.004</td>
<td>1.98 1.26 to 3.13 0.004</td>
</tr>
<tr>
<td>Concurrent ultrasound measures</td>
<td>1.19 0.67 to 2.08 0.55</td>
<td>1.24 0.73 to 2.11 0.42</td>
</tr>
<tr>
<td>Week 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative ultrasound measures</td>
<td>1.47 0.96 to 2.25 0.08</td>
<td>1.66 1.04 to 2.65 0.04</td>
</tr>
<tr>
<td>Concurrent ultrasound measures</td>
<td>0.89 0.54 to 1.45 0.64</td>
<td>1.12 0.67 to 1.88 0.66</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative ultrasound measures</td>
<td>1.86 1.09 to 3.18 0.02</td>
<td>2.24 1.27 to 3.93 0.005</td>
</tr>
<tr>
<td>Concurrent ultrasound measures</td>
<td>1.14 0.65 to 2.00 0.66</td>
<td>1.43 0.78 to 2.61 0.25</td>
</tr>
</tbody>
</table>

All analyses involved multiply imputed data.

*Models adjusting for preoperative ultrasound measures included inflow artery diameter, mean vein diameter, and brachial artery blood flow rate. Models adjusting for concurrent ultrasound measures instead included mean AVF venous diameter, blood flow rate, and depth obtained from the same ultrasound as the stenosis assessment. All models included additive adjustments for age, sex, black race, chronic dialysis status at time of AVF creation, AVF location (upper arm versus forearm), and Gaussian clinical center random effects.

<sup>b</sup>Week 6 results were identified a priori as the primary ultrasound outcomes among the three time points.
preexisting intimal hyperplasia in vein samples obtained at the time of AVF creation surgery and postoperative AVF venous stenosis detected by serial ultrasounds with each other and AVF clinical maturation failure.

We recently reported that preexisting intimal hyperplasia was highly prevalent (88%) in veins used for AVF creation in the HFM Study. One might expect that such preexisting hyperplasia would be manifested as stenosis on ultrasound soon after AVF creation surgery. However, there was no significant association between the presence of preexisting hyperplasia assessed by histomorphometry and the presence of AVF venous stenosis on postoperative ultrasounds (Table 1). In fact, in the complete case (nonimputed) data, nine of 33 (27.3%) AVFs created using veins with preexisting hyperplasia index >80% achieved unassisted clinical maturation. A plausible explanation for this lack of significant association is that the degree of the postoperative stenosis, even if present, was too modest to satisfy the diameter and peak flow velocity ratio criteria (Concise Methods). Moreover, if the degree of preexisting venous hyperplasia varies substantially along the vessel, our single-vein sample might have inadequately represented the overall level of hyperplasia along the entire length of the vein. Other available data also suggest that preexisting venous hyperplasia does not progress significantly after AVF creation. For example, proliferative activity, as indicated by the paucity of Ki67-positive cells, was low in the hyperplastic lesions in the samples of vein used for the anastomosis in the HFM Study. In a single-site study of 113 participants, Allon et al. found no difference in the frequency of stenosis on ultrasounds performed 4–6 weeks after AVF creation in veins with preexisting maximal intimal thickness above the median of 22.3 μm compared with those with preexisting maximal intimal thickness below. A recent single-center study of 56 patients by Tabbara et al. also showed that preexisting venous intimal hyperplasia did not correlate with increases in the hyperplasia observed in vein samples collected at the second-stage AVF transposition surgery.

Our study does not show statistically significant associations of preexisting venous intimal hyperplasia with unassisted or overall AVF maturation failure. This does not, however, definitively exclude a role of preexisting hyperplasia in AVF maturation failure, because the HFM Study was insufficiently powered to detect modest relationships in the multifactorial setting of AVF development. Nonetheless, these results are generally consistent with the results from the study by Allon et al., in which preexisting venous hyperplasia did not correlate with AVF clinical maturation failure, and that by Tabbara et al., in which preexisting venous hyperplasia was not associated with AVF anatomic maturation failure. Together, these data suggest that the clinical significance of any relationship of preexisting hyperplasia to clinical maturation failure is not great.

Five of six associations relating stenosis detected on postoperative ultrasounds to unassisted maturation failure or overall maturation failure were statistically significant, with ORs from 1.47 to 2.24, even after adjustment for preoperative arterial and venous ultrasound features and various potential confounders (Table 2). This observation is also in general agreement with the study of Allon et al., in which maturation failure was approximately four times (30% versus 7%; P=0.001) more common in participants with juxta-anastomotic stenosis than those without stenosis on ultrasound at 4–6 postoperative weeks. It is, however, in apparent contrast to the results of Allon et al. on more distant stenosis, in which no association with maturation failure was found, and the study of Tabbara et al., in which postoperative AVF venous hyperplasia was not associated with AVF anatomic maturation failure. There are a number of differences between the HFM Study and the studies by Allon et al. and Tabbara et al. including sample size, single center versus multicenter, the methods used to determine intimal hyperplasia, the numbers of postoperative ultrasound examinations, and the criteria for AVF maturation.

The associations of stenosis with maturation failure were attenuated and no longer statistically significant after adjusting for concurrent mean AVF venous diameter, blood flow rate, and depth (Table 2), which collectively predict maturation failure more accurately than stenosis per se. Thus, from a prognostic perspective, the maturation prospects of an AVF that satisfies the definition of stenosis seem no worse than those of any AVF with similar mean diameter, depth, and AVF flow. It should be noted that the stenosis definition reflects a localized variation as assessed by ratios in diameter and flow (through peak systolic velocity) along the length of the vessel, whereas the parameters used for adjustments in the statistical model were blood flow rate, mean diameter, and depth of the AVF vein, which numerically need not reflect such localized variation. Hence, this empirical result cannot be presumed a priori. Our results are consistent with stenosis as an important cause of clinical maturation failure mediated by effects on venous diameter, blood flow rate, and less likely, depth of the AVF. Our results do not support a contribution of stenosis to maturation failure via other pathways. It is also possible that stenosis is not a cause of maturation failure but is simply a consequence or correlate of small AVF vein diameter and low blood flow rate.

The apparent changes in the prevalence of stenosis on postoperative AVF ultrasounds over time are interesting observations. Overall stenosis prevalence increased over time (14%, 28%, and 30% in the 1-day, 2-week, and 6-week ultrasounds, respectively). As discussed above, it seemed unlikely that preexisting venous hyperplasia progressed significantly after AVF creation. Alternatively, the new lesions detected by postoperative ultrasounds could have resulted from de novo intimal hyperplasia formation induced by various factors, such as highly aberrant mechanical forces on the venous wall after AVF creation. Perhaps more intriguing was that some stenotic lesions detected in an earlier ultrasound were no longer present in subsequent ultrasounds (Figure 1). There are several potential explanations for this loss of detectable stenotic lesions over time: (1) technical variability in ultrasound
measurements resulting in reclassification of patients across the dichotomization criteria for stenosis; (2) expansion of the lumen and consequently, decrease in peak systolic blood flow velocity ratio, such that these parameters no longer satisfied the criteria of stenosis on ultrasound defined a priori; and (3) actual regression of intimal hyperplasia.

This study has a number of strengths. The HFM Study is the largest multicenter prospective study of AVF, including detailed information on a broad range of measures, such as histology, vascular functions, ultrasound, and clinical attributes. Standardized protocols were used for vein sample collection and centralized morphometric analysis of the vein histologic sections by the Histology Core. We used standardized training for data collection and ultrasound measurements, a central facility for the reading of ultrasound images, and uniform criteria for defining stenosis. Serial postoperative research ultrasounds at prespecified time points were performed to examine the evolution of AVF development. Clinical data were concealed from the pathology personnel analyzing the venous histologic sections and radiologists reading the ultrasound images. Clinical AVF maturation was assessed using rigorous, standardized criteria. Statistical treatment included a comprehensive multiple imputation process for missing data, adjustment for a suite of potential confounding variables using generalized linear mixed models (GLMMs), and investigation of the incremental contribution of stenosis to clinical maturation prediction over prediction by AVF vein diameter, blood flow rate, and depth.

Our study also has the following limitations. (1) The sample size, although considerable, was too low for adequate statistical power against modest rather than strong associations with binary outcomes, such as clinical maturation. (2) A single-vein sample may not adequately reflect the degree of hyperplasia along the entire length of the vein. (3) Although clinical usability as an outcome has the advantage of clinical relevance, the many factors that may affect it, such as processes of care in the dialysis unit and variability in decisions to intervene surgically, can partially obscure its association with AVF properties. (4) Our results pertain only to preexisting hyperplasia that was mostly mild and may not be applicable to more severe hyperplasia that would likely preclude use of the vein for AVF creation. (5) There are no consensus criteria for stenosis on ultrasound in the literature, and other criteria may yield different results. (6) The HFM Study did not include regularly scheduled ultrasound examination after 6 weeks postoperatively; therefore, stenotic lesions occurring later were not included in this analysis. (7) The lack of repeated vein tissue sampling and histologic examination precludes tracking of the postoperative evolution of intimal hyperplasia and further understanding of its role in AVF development. (8) Intimal hyperplasia or postoperative stenosis of the feeding artery, not included in our analyses, may also be important for AVF development. (9) The observational study design precludes the establishment of causality. (10) Many other factors, such as medications, precise AVF configuration, aberrant shear stress along the vascular wall, and other abnormal mechanical forces, potentially modulate AVF development. Some of these factors will be topics of other analyses.

In summary, our data are consistent with but insufficient to confirm a modest association of preexisting venous intimal hyperplasia with subsequent AVF maturation failure. Stenosis after detected AVF creation was clearly associated with clinical maturation failure, but its implications for maturation were not distinguishable from those of other ultrasound measures, such as mean diameter, blood flow rate, and depth of the AVF vein.

**CONCISE METHODS**

**Participant Cohort**

The design of the HFM Study has been previously reported. Briefly, the HFM Study is a prospective cohort study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases conducted at seven clinical sites located in United States academic institutions, designed to identify predictors and elucidate underlying mechanisms of AVF maturation failure. The study enrolled 602 participants who were scheduled to undergo a single-stage AVF creation surgery in an upper extremity as recommended by their respective own surgeons. All clinical decisions regarding the AVF, including timing of the creation surgery, location and configuration, potential remedial intervention, and timing of cannulation for dialysis, were determined by the clinical team and were not determined by the investigators. The participants were followed for a median of 2.1 years (range of 0.2–4.1 years) until abandonment of the AVF, kidney transplant, death, or administrative end of the HFM Study. The institutional review boards of each clinical institution and the Data Coordinating Center approved the study. Informed consent was obtained from each participant before enrollment.

**AVF Creation Surgery**

Only one-stage AVF creation surgeries in the arm were eligible for inclusion in the HFM Study. Of these upper arm AVFs, 62% and 38% had brachial-cephalic and brachial-basilic configurations, respectively. A 5- to 10-mm segment of the vein used for the anastomosis was excised for research purposes immediately before the anastomosis creation as previously described.

**AVF Ultrasonography**

Figure 2 depicts the study schedule for ultrasound sample collection and ultrasound. Before AVF creation surgery, all participants underwent detailed ultrasound examination of the arteries and veins in the extremity to be used for AVF creation. The HFM Study protocol also mandated ultrasonography examination of the AVF within 3 days and preferably, at 1 day, 2 weeks, and 6 weeks after AVF creation. For individual logistical reasons, 32%, 48%, 13%, and 2% of the 1-day ultrasounds were actually performed on postoperative days 0, 1, 2, and 3, respectively, with 4% missing from this 3-day window.

Preoperative vascular mapping and postoperative AVF ultrasonography were performed by clinical site personnel trained by the
HFM Study Ultrasound Core using standardized protocols, and all images were read centrally by one of three core radiologists specializing in hemodialysis vascular access ultrasound as described. For the postoperative AVF ultrasounds, the internal diameter of the brachial or radial artery (depending on the AVF configuration) was measured 2 cm upstream from the anastomosis. The internal diameter of the AVF draining vein was measured at 2, 5, 10, and 15 cm, and the blood flow rate through the AVF was measured in triplicate 10 cm downstream from the anastomosis in a straight section of the vein. In reporting results, diameter refers to the mean of the diameters at these four locations along the length of the vein, and blood flow rate refers to the mean of the triplicate blood flow rate measurements.

The algorithm for defining an AVF venous stenosis on ultrasound in the HFM Study has been previously described. Stenosis detection was initially triggered by gross visualization or elevation of peak systolic velocity. The stenosis was confirmed if the suspicious location satisfied both of the following criteria: (1) the vein diameter was <50% of the measured diameter upstream of the narrowing and (2) the ratio of the peak systolic blood flow velocity at this location to the peak systolic velocity measured 2 cm upstream exceeded 3.0 when the location was within 2 cm of the anastomosis or exceeded 2.0 when the location was farther from the anastomosis. The stenosis was described as juxta anastomotic in the former case and distal in the latter case.

Results of the 1-day and 2-week postoperative ultrasounds were not available to clinicians or the HFM Study investigators unless there was a finding that threatened the participant’s immediate health (e.g., impending rupture of a pseudoaneurysm) as determined by the Central Core radiologist. Results of the 6-week postoperative ultrasounds were available to local clinicians only if a 6-week AVF ultrasound was part of routine clinical practice at that clinical center.

Vein Morphometry
A segment of the vein used for the anastomosis harvested during the AVF creation surgery was processed by the HFM Histology Core as previously described. Morphometric analysis of intimal hyperplasia was performed on a slide in all 365 patients in whom a complete circumferential histologic section of the vein was present and was not obscured by valves. Cross-sectional open luminal area and maximal luminal area were measured using the ImagePro Plus software. Maximal luminal area was defined as the entire area within the internal elastic lamina (i.e., the open luminal area plus the intimal hyperplasia area). The intimal hyperplasia index was defined as the ratio of open area to maximal area, subtracted from 1.0, and valued between 0 and 1.0.

Definitions of AVF Clinical Maturation
The primary HFM Study outcome was unassisted clinical maturation defined as use of the AVF with two needles for ≥75% of dialysis sessions over a continuous 4-week period, including either four consecutive sessions during the 4-week period in which two needles were used with mean dialysis machine blood pump speed ≥300 ml/min, or any session with a single-pool urea Kt/V ≥1.4 or urea reduction ratio >70%. Unassisted maturation was considered to have been achieved on the date of the first of the consecutive sessions with pump speed ≥300 ml/min, or the date of the first qualifying Kt/V or urea reduction ratio, provided that this date was within 9 months of AVF creation or 4 weeks of hemodialysis initiation without any preceding endovascular or surgical interventions on the AVF. Overall maturation was fulfillment of these same criteria, regardless of whether it was preceded by such interventions. Treating clinicians, not HFM Study personnel, made all decisions regarding the initiation of chronic dialysis, whether and when the AVF would be used, and the need for diagnostic and interventional procedures to assist maturation.

Statistical Analyses
GLMMs, with random Gaussian clinical center effects, were used to assess (1) the associations of the preexisting venous intimal hyperplasia index with the diameter, blood flow rate, and stenosis in the AVF vein on each postoperative ultrasound and with both unassisted and overall clinical maturation; (2) the associations of stenosis on each ultrasound with both types of clinical maturation; and (3) whether associations of stenosis with clinical maturation varied with the location of the stenosis. The intimal hyperplasia index was treated as a predictor of each outcome in 1, whereas stenosis was treated as a predictor for each outcome in 2 and 3. Additive linear models were used for vein diameters and analogous logistic regression models were used for stenosis and each maturation outcome. We modeled blood flow rates using a γGLMM with log link function due to their skew.

The 6-week ultrasound outcomes were a priori considered primary among the three postoperative ultrasounds. This was the latest regularly scheduled ultrasound mandated by the HFM Study protocol and, therefore, closest to clinical utilization, and it was only rarely preceded by procedural interventions on the AVF, which were discouraged in the protocol before 6 weeks after surgery. Unless otherwise noted, all models included adjustment for a set of case mix variables: sex, age, black race, whether the patient was on maintenance dialysis at the time of AVF creation surgery, AVF location (forearm versus upper arm) as well as preoperative ultrasound measures of inflow artery diameter, vein diameter, and brachial artery blood flow rate.

The above models were fit by maximum likelihood, using numerical integration, to a dataset consisting of ten imputations of (1) missing values of the venous intimal hyperplasia index; (2) missing ultrasound measures before AVF thrombosis or death of the
ACKNOWLEDGMENTS

We thank the patients for their participation in the Hemodialysis Fistula Maturation (HFM) Study: We acknowledge Lin Belt, Carl Abts, and Lauren Alexander for their invaluable expertise at the HFM Ultrasound Core.

The HFM Study is funded by National Institute of Diabetes, Digestive and Kidney Disease (NIDDK) grants U01DK082179, U01DK082189, U01DK082218, U01DK082222, U01DK082232, U01DK082236, and U01DK082240.


REFERENCES


This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2016121355/-/DCSupplemental.