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Fibrotic Venous Remodeling and Nonmaturation of Arteriovenous Fistulas

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ABSTRACT

The frequency of primary failure in arteriovenous fistulas (AVFs) remains unacceptably high. This lack of improvement is due in part to a poor understanding of the pathobiology underlying AVF nonmaturation. This observational study quantified the progression of three vascular features, medial fibrosis, intimal hyperplasia (IH), and collagen fiber organization, during early AVF remodeling and evaluated the associations thereof with AVF nonmaturation. We obtained venous samples from patients undergoing two-stage upper-arm AVF surgeries at a single center, including intraoperative veins at the first-stage access creation surgery and AVFs at the second-stage transposition procedure. Paired venous samples from both stages were used to evaluate change in these vascular features after anastomosis. Anatomic nonmaturation (AVF diameter never ≥6 mm) occurred in 39 of 161 (24%) patients. Neither preexisting fibrosis nor IH predicted AVF outcomes. Postoperative medial fibrosis associated with nonmaturation (odds ratio [OR], 1.55; 95% confidence interval [95% CI], 1.05 to 2.30; P=0.03, per 10% absolute increase in fibrosis), whereas postoperative IH only associated with failure in those individuals with medial fibrosis over the population's median value (OR, 2.63; 95% CI, 1.07 to 6.46; P=0.04, per increase of 1 in the intima/media ratio). Analysis of postoperative medial collagen organization revealed that circumferential alignment of fibers around the lumen associated with AVF nonmaturation (OR, 1.38; 95% CI, 1.03 to 1.84; P=0.03, per 10° increase in angle). This study demonstrates that excessive fibrotic remodeling of the vein after AVF creation is an important risk factor for nonmaturation and that high medial fibrosis determines the stenotic potential of IH.

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Our poor understanding of the biologic processes leading to arteriovenous fistula (AVF) nonmaturation limits our ability to predict or prevent this complication. Previous research has focused primarily on the role of preexisting and postoperative intimal hyperplasia (IH) in producing flow-limiting stenosis that impairs AVF maturation. However, this hypothesis was challenged by recent observational clinical studies that found no association between the thickness of preexisting IH and the occurrence of postoperative AVF stenosis,[1] or between postoperative IH and the likelihood of AVF nonmaturation.[2]

More recently, investigators have highlighted the importance of expansive (outward) wall remodeling for AVF maturation.[3] Experimental models showed

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that outward remodeling is highly dependent on the composition and proper reorganization of the extracellular matrix (ECM).[4–9] Excessive collagen deposition (fibrosis) has been detected in the adventitia of stenotic AVFs[10] and, as a preexisting condition, in native veins and arteries used for access creation.[11,12] Interestingly, clinical studies noted that whereas preexisting arterial fibrosis was associated with maturation, preexisting venous fibrosis was not.[12] However, it is unknown whether native veins develop more fibrosis during early AVF remodeling, or whether postoperative fibrosis after anastomosis affects outward remodeling and maturation outcomes.

This observational study enrolled patients undergoing a planned two-stage upper arm AVF creation surgery, permitting us to obtain venous samples at the time of AVF creation and during AVF transposition several weeks later. We quantified medial fibrosis and IH in both native veins and subsequent AVF venous samples, and investigated the associations of preexisting, postoperative, and change in these vascular features with AVF nonmaturation. In a secondary analysis, we used state-of-the-art second harmonic generation microscopy and image analysis to characterize the organization of collagen fibers in the media and investigate its association with AVF nonmaturation.

Significance Statement

Maturation failure is a common complication after arteriovenous fistula (AVF) creation. The lack of preventive therapies reflects our poor understanding of the biologic mechanisms that control venous outward remodeling. This prospective study quantifies the progression of medial fibrosis, intimal hyperplasia, and collagen fiber organization in native vein and fistula biopsy specimens obtained from patients undergoing surgeries for two-stage AVF creation. Medial fibrosis increased significantly after AVF creation, independent of the preexisting level and of baseline clinical factors. Postoperative medial fibrosis and circumferential alignment of collagen fibers were associated with nonmaturation. Postoperative intimal hyperplasia was only associated with failure in the presence of high medial fibrosis. This work identifies medial fibrosis as a major risk factor of AVF maturation failure.

RESULTS

Demographic, Clinical, and AVF Characteristics of the Study Population

A total of 165 patients with planned two-stage AVF creation surgeries were enrolled in the study. Patients were asked to consent before each of the surgeries. With the exception of two patients who died before AVF transposition and two lost to follow-up, all other 161 participants underwent first-stage (arteriovenous anastomosis) and second-stage surgeries (AVF transposition if the fistula matured [n=122] or a salvage procedure if it failed [n=39]). Figure 1A and Supplemental Figure 1 illustrate the surgery and tissue collection procedures. An end sample of the vein used for the AVF creation was collected intraoperatively before the arteriovenous anastomosis during the first surgery, whereas a juxta-anastomotic venous sample was obtained from both mature and failed AVFs during the second-stage procedure. Tissue sampling was not possible in 47 first-stage surgeries and 32 transpositions due to lack of one of the patient consents or insufficient vessel length.

Three study groups were formed for the analysis of preexisting, postoperative, and change over time in vascular fibrosis, IH, and collagen fiber organization on the basis of the availability of vein and AVF samples (Figure 1B). The first group (118 patients from whom vein specimens were obtained during the first-stage surgery) was used to evaluate the relationship of preexisting vascular features with anatomic AVF nonmaturation. Eight patients were excluded before data analysis due to poor tissue quality (n=4) or loss of clinical follow-up (n=4). The second study group (133 individuals from whom AVF venous samples were collected during the second-stage surgery) was used to analyze the association of postoperative vascular features with AVF outcomes. Eighteen patients were excluded in this group before data analysis due to poor tissue quality. The third group (86 patients from whom both a native vein and an AVF venous sample were obtained) allowed us to assess the association of change in vascular features over time with AVF nonmaturation. We excluded 16 patients due to poor tissue quality in one of the paired tissues.

Table 1 summarizes the demographic, clinical, and vascular access characteristics of the entire patient cohort and the three study subgroups (veins, AVFs, and pairs) after the necessary exclusions. A description of the patient cohort is found in the Supplemental Material.

AVF Outcomes and Association with Baseline Clinical Covariates

Nonmaturation occurred in 39 of 161 AVFs (24%), after excluding four patients due to loss of follow-up (including two participants who died before the second-stage surgery). Additional clinical outcomes are found in the Supplemental Material. Female sex was associated with an increased risk of anatomic AVF nonmaturation (odds ratio [OR], 2.47; 95% confidence interval [9%% CI], 1.17 to 5.20; P=0.02; Supplemental Table 1) using a stepwise logistic regression model with four baseline covariates (age, sex, diabetes, and history of previous AVF).

The median increase in internal vein diameter from first-stage to second-stage surgery was 3 mm (interquartile range [IQR] 2–4), with lower values associated with female sex (Supplemental Figure 2). The time interval between surgeries (median 76 days [IQR 56–112]) was not associated with nonmaturation (OR, 1.00; 95% CI, 0.95 to 1.04; P=0.85, per 10-day increment), nor with the increase in vein diameter (Supplemental Figure 2).

Preexisting Medial Fibrosis, IH, and Association with AVF Outcomes

Figure 2 displays two representative vein samples obtained during first-stage surgery, one with severe medial fibrosis

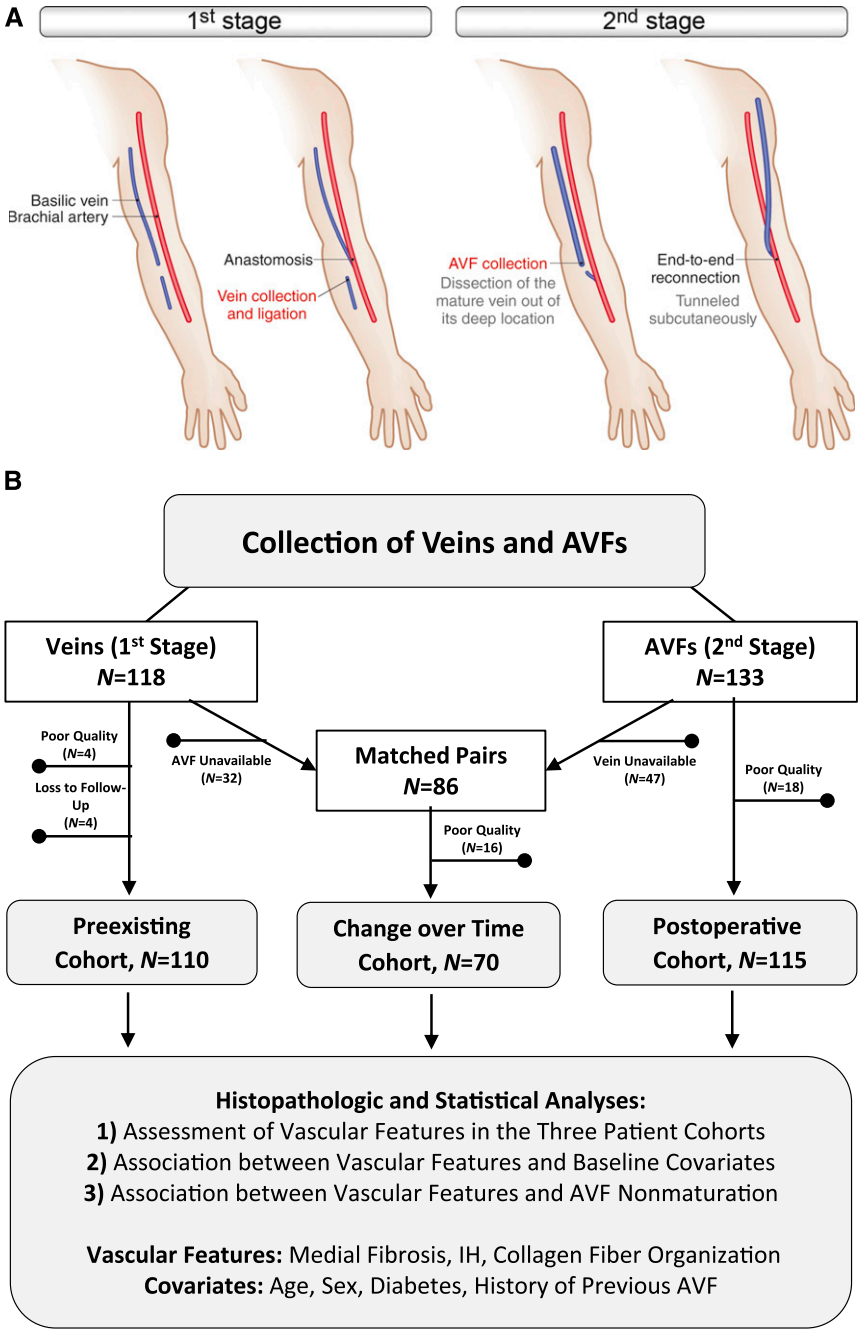

Figure 1. Surgeries, sample collection, and flow diagram of the study design. (A) Schematic representation illustrating the two surgical steps involved in creating a two-stage brachio-basilic AVF: (*1*) creation of the arteriovenous anastomosis (first-stage surgery), and (*2*) transposition of the remodeled AVF (second-stage surgery). The anatomic locations for the collection of native veins (first-stage) and AVF samples (second-stage) are shown. (B) Flow diagram indicating the sample collection algorithm, exclusion criteria, and subsequent histopathologic and statistical analyses. A total of 165 patients undergoing two-stage AVF creation were enrolled in the study. Native vein and AVF venous samples were obtained intraoperatively before the first-stage and second-stage procedures, respectively. If tissue sampling was not possible, the event was designated as vein or AVF unavailable.

(Figure 2A) and one with mild medial fibrosis (Figure 2B). The mean medial fibrosis in native veins was 41.8%±9.6% (Figure 2C), whereas the median intima/media (I/M) area ratio was 0.32 (IQR 0.22–0.52). Age was positively associated with both preexisting medial fibrosis and I/M ratio (Table 2). Logistic regressions, unadjusted (Figure 2D) and adjusted for sex (Table 3), demonstrated no association between preexisting fibrosis or IH and AVF nonmaturation.

Table 1. Baseline characteristics of the patient population and study cohorts of vascular fibrosis and IH

Characteristic	All Patients (*n*=165)	Veins (*n*=110)[a]	AVFs (*n*=115)[a]	Pairs (*n*=70)[a]
Demographics				
Age, yr	56.0±13.3	56.3±13.4	56.0±13.0	56.7±13.2
Women	67 (41)	38 (35)	47 (41)	23 (33)
Ethnicity				
Non-Hispanic Black	104 (63)	67 (61)	74 (64)	43 (61)
Hispanic	57 (35)	41 (37)	40 (35)	27 (39)
White	4 (2)	2 (2)	1 (1)	0 (0)
Comorbidities				
Diabetes	76 (46)	52 (47)	51 (44)	31 (44)
Hypertension	160 (97)	107 (97)	110 (96)	67 (96)
CAD	37 (22)	28 (25)	23 (20)	15 (21)
CHF	14 (8)	11 (10)	10 (9)	8 (11)
Medications				
Antiplatelet agents	102 (62)	70 (64)	68 (59)	42 (60)
Statins	103 (62)	71 (65)	71 (62)	45 (64)
ACE-I or ARBs	62 (38)	39 (35)	40 (35)	22 (31)
Vascular access				
AVF type[b]				
Brachio-basilic	137 (83)	101 (92)	95 (83)	66 (94)
Brachio-brachial	18 (11)	4 (4)	14 (12)	2 (3)
Brachio-cephalic	6 (4)	2 (2)	4 (3)	1 (1)
Aberrant radial-basilic	4 (2)	3 (3)	2 (2)	1 (1)
Diameter, mm				
Anastomosis	4.5 (4.5–4.5)	4.5 (4.5–4.5)	4.5 (4.5–4.5)	4.5 (4.5–4.5)
Native vein	4.0 (4.0–4.0)	4.0 (4.0–4.0)	4.0 (4.0–4.0)	4.0 (4.0–4.0)
AVF	7.0 (6.0–8.0)	7.0 (7.0–8.0)	7.0 (6.0–8.0)	7.0 (6.0–8.0)
Time interval[c], d	76 (56–112)	68 (55–105)	65 (56–96)	64 (55–95)
Prior catheter use	109 (66)	70 (64)	79 (69)	46 (66)
Previous AVF	35 (21)	21 (19)	26 (23)	14 (20)

Values for categoric variables are given as number (%); values for numeric variables are expressed as mean±SD or median (IQR). CAD, coronary artery disease; CHF, congestive heart failure; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

[a]Number of veins, AVFs, and paired tissue samples included in the analyses after the necessary exclusions (see Figure 1). Native veins and AVFs were collected during the first-stage and second-stage surgeries, respectively. A tissue pair was formed when both a patient's vein and subsequent AVF sample were available.

[b]Created at or above the elbow.

[c]Time between first-stage and second-stage surgeries.

Postoperative Medial Fibrosis, IH, and Association with AVF Outcomes

The mean postoperative medial fibrosis in the AVF samples was 48.4%±12.1% (Figure 3A), whereas the median I/M ratio was 0.77 (IQR 0.48–1.30). Both postoperative medial fibrosis (Supplemental Figure 3) and IH demonstrated a significant increase compared with the corresponding values in native veins ($P<0.001$ for both). Contrary to the findings with the preexisting vascular features, patient age was not associated with AVF medial fibrosis or I/M ratio (Table 2). Instead, a history of a previous AVF was significantly associated with the postoperative I/M ratio (Table 2). The I/M ratio, but not the percentage of medial fibrosis, was associated with the time interval between surgeries (Supplemental Figure 4). Even then, the correlation coefficient between time interval and IH was low ($R^2=0.10$). Logistic regression models, unadjusted (Figure 3B) and adjusted for sex (Figure 3C, Table 3), indicated that high postoperative medial fibrosis but not IH was associated with AVF nonmaturation. Medial fibrosis >60% was also associated with a lower increment in internal vein diameter during early remodeling compared with samples with fibrosis<40% (Supplemental Figure 5).

Figure 4 shows representative microphotographs of AVF venous samples obtained during second-stage surgery, which had failed to mature during remodeling or had achieved successful maturation. Regardless of the degree of IH, there was significant collagen deposition in the media of AVFs with nonmaturation (Figure 4, A and B). In contrast, there was better preservation of the muscular layer in mature AVFs (Figure 4, C and D).

Fibrosis is commonly associated with vascular stiffness and impaired outward remodeling.[13] Therefore, a hyperplastic intima is likely to be more occlusive in a vessel with reduced distensibility.[2] Although the degree of IH did not predict AVF outcomes in the overall postoperative cohort (Table 3), we tested its association with AVF nonmaturation in the presence of high medial fibrosis (Figure 5A). Postoperative IH was associated with nonmaturation in patients with postoperative medial fibrosis over the population's median (≥48%; OR, 2.63; 95% CI, 1.07 to 6.46; $P=0.04$, per increase of 1 in the

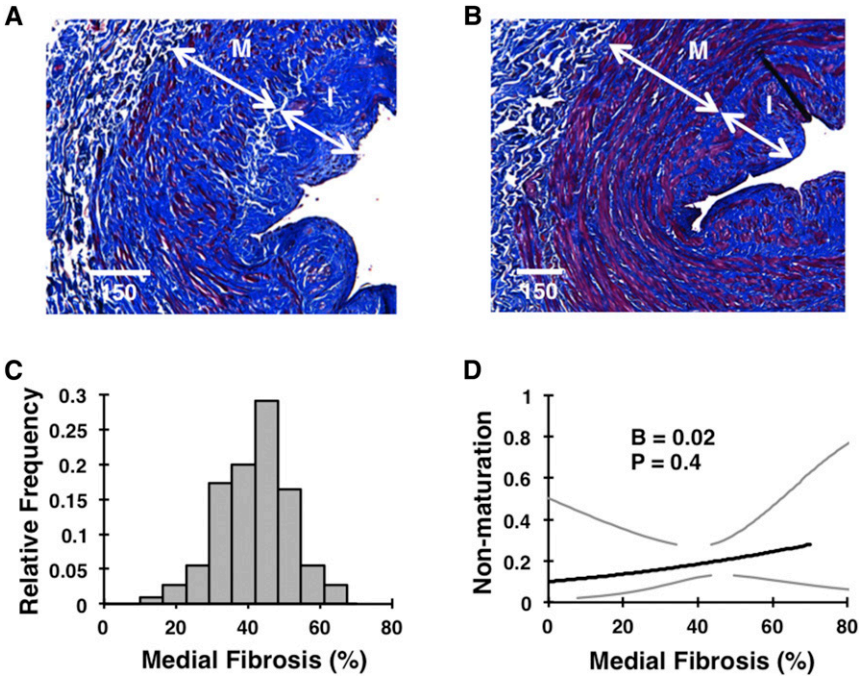

Figure 2. Preexisting medial fibrosis is not associated with AVF nonmaturation. (A and B) Close-up photographs of Masson's trichrome–stained cross-sections of native veins with (A) high and (B) low degree of medial fibrosis. Cells stain in red/pink, whereas collagen is shown in blue. Distances are in micrometers. I, intima; M, media. (C) Distribution of preexisting medial fibrosis in the first-stage veins cohort. (D) Probability of anatomic nonmaturation as predicted by preexisting medial fibrosis using logistic regression analysis. The black line represents the model, whereas gray lines indicate the upper and lower levels of the 95% CI.

I/M ratio), but not in the subgroup with fibrosis<48% (OR, 1.27; 95% CI, 0.56 to 2.89; P=0.57, per increase of 1 in the I/M ratio; Figure 5, A and B). In addition, the product term of postoperative IH×postoperative medial fibrosis was highly associated with AVF nonmaturation, unadjusted (Figure 5C) and adjusted for sex (Table 3). A lower increase in vein diameter during remodeling was also observed in AVFs with an IH×medial fibrosis product >0.8 compared with the group <0.2 (Supplemental Figure 5).

Change in Vascular Fibrosis, IH, and Association with AVF Outcomes

Longitudinal change in medial fibrosis was assessed in patients from whom both native vein and AVF venous samples were obtained (tissue pairs). Supplemental Figure 6A demonstrates extensive heterogeneity in the AVF remodeling process among individual patients. Medial fibrosis increased in 72.9% of the patients, and decreased up to 20% in the rest of the individuals. Similarly, the I/M ratio increased in 84.3% of the patients. Pairwise comparisons demonstrated no correlation between the degrees of preexisting and postoperative fibrosis (R^2=0.01), or between preexisting and postoperative IH (R^2<0.01).

None of the tested baseline covariates (age, sex, diabetes, and history of previous AVF) were associated with the change in fibrosis or IH over time (Table 2). Similarly, neither change in fibrosis nor change in IH predicted AVF nonmaturation (Supplemental Figure 6B, Table 3). This analysis indicates that the risk of anatomic nonmaturation is determined by the absolute postoperative value.

Orientation of Collagen Fibers and Association with AVF Outcomes

The distensibility of the vascular wall may be influenced by the orientation of nonelastic collagen fibers in the media.[14] Therefore, we evaluated the pattern of medial collagen deposition (Supplemental Figure 7) and the orientation angle and anisotropy index of collagen fibers with respect to the lumen (Figure 6, A and B, Table 2). Each fiber's orientation angle is given as the angle between the fiber's main axis and the adjacent lumen's perimeter, ranging from 0° (perpendicular to the lumen) to 90° (circumferential around the lumen). Anisotropy index is the degree of fiber alignment, ranging from "0" for totally random to "1" for totally aligned.

The frequency distribution of collagen deposition patterns was similar between veins and AVFs (Supplemental Figure 7). The median angle of collagen fibers in native veins and AVFs was 32.6 (IQR 17.3–57.9) and 39.7 (IQR 24.8–73.6), respectively, with 66.0% of patients presenting an increase in angle (or tendency to align circumferentially around the lumen) during early remodeling. The mean anisotropy index in native veins and AVF samples was 0.21±0.08 and 0.21±0.09, respectively. Female sex and history of a previous AVF were associated with higher angles in native veins but not in AVFs (Table 2). Similarly, age was associated with lower anisotropy indices (more random

Table 2. Association between baseline covariates and vascular features in native veins and AVFs

Vascular Feature	Age	Female Sex	Diabetes	Previous AVF
Veins (preexisting)				
Medial fibrosis	0.3 (0.1 to 0.5) *P*=0.003	0.02 (−0.2 to 0.2) *P*=0.8	0.1 (−0.05 to 0.3) *P*=0.2	−0.01 (−0.2 to 0.2) *P*=0.9
IH	0.2 (0.01 to 0.4) *P*=0.04	−0.02 (−0.2 to 0.2) *P*=0.8	0.1 (−0.06 to 0.3) *P*=0.2	0.1 (−0.06 to 0.3) *P*=0.2
Collagen organization				
Angle	0.1 (−0.1 to 0.4) *P*=0.3	0.3 (0.06 to 0.5) *P*=0.01	0.08 (−0.2 to 0.3) *P*=0.5	0.3 (0.02 to 0.5) *P*=0.03
Anisotropy index	0.08 (−0.2 to 0.3) *P*=0.6	−0.05 (−0.3 to 0.2) *P*=0.7	0.07 (−0.2 to 0.3) *P*=0.6	0.08 (−0.2 to 0.3) *P*=0.5
AVFs (postoperative)				
Medial fibrosis	0.07 (−0.1 to 0.3) *P*=0.5	0.003 (−0.2 to 0.2) *P*>0.99	0.09 (−0.1 to 0.3) *P*=0.4	−0.04 (−0.2 to 0.1) *P*=0.7
IH	0.1 (−0.06 to 0.3) *P*=0.2	0.09 (−0.1 to 0.3) *P*=0.4	0.05 (−0.1 to 0.2) *P*=0.6	0.2 (0.06–0.4) *P*=0.01
Collagen organization				
Angle	0.05 (−0.2 to 0.3) *P*=0.7	0.05 (−0.2 to 0.3) *P*=0.7	−0.08 (−0.3 to 0.2) *P*=0.5	−0.03 (−0.3 to 0.2) *P*=0.8
Anisotropy index	−0.2 (−0.5 to −0.01) *P*=0.04	−0.04 (−0.3 to 0.2) *P*=0.7	0.02 (−0.2 to 0.3) *P*=0.9	−0.2 (−0.4 to 0.03) *P*=0.08
Pairs (change over time)				
Medial fibrosis	−0.06 (−0.3 to 0.2) *P*=0.7	−0.05 (−0.3 to 0.2) *P*=0.7	−0.08 (−0.3 to 0.2) *P*=0.5	0.07 (−0.2 to 0.3) *P*=0.6
IH	−0.01 (−0.3 to 0.2) *P*=0.9	0.04 (−0.2 to 0.3) *P*=0.7	0.2 (−0.02 to 0.5) *P*=0.07	−0.1 (−0.3 to 0.1) *P*=0.4
Collagen organization				
Angle	−0.05 (−0.3 to 0.2) *P*=0.7	−0.09 (−0.4 to 0.2) *P*=0.5	−0.3 (−0.5 to 0.01) *P*=0.06	−0.1 (−0.4 to 0.1) *P*=0.3
Anisotropy index	−0.3 (−0.6 to −0.06) *P*=0.02	−0.05 (−0.3 to 0.2) *P*=0.7	0.07 (−0.2 to 0.3) *P*=0.6	−0.1 (−0.4 to 0.2) *P*=0.4

Preexisting and postoperative vascular features were assessed in native veins and AVFs collected during the first-stage and second-stage surgeries, respectively. Change in vascular features over time was evaluated in tissue pairs when both a patient's vein and AVF sample were available. Medial fibrosis was expressed as % area of collagen; IH was expressed as I/M area ratio. The angle of collagen fibers ranged from 0° (perpendicular to the lumen) to 90° (circumferential direction around the lumen); the anisotropy index ranged from 0 (random alignment of fibers) to 1 (totally aligned in one direction). Change over time was calculated by subtracting the preexisting value from the postoperative measurement in tissue pairs. Associations are presented as standardized β coefficient (95% CI) and *P* value in multivariate general linear regression models adjusted for age, sex, diabetes, and previous AVF. The reference status for binary covariates is male sex, no diabetes, and no history of a previous AVF.

alignment of fibers) in AVFs, and with a decrease in anisotropy index during remodeling in tissue pairs (Table 2).

Neither the categoric patterns of collagen deposition nor the anisotropy index were associated with AVF outcomes (Supplemental Table 2, Table 3). In contrast, the postoperative angle of collagen fibers was positively associated with AVF nonmaturation (Figure 6C, Table 3). Both excessive fibrosis and circumferential alignment of collagen fibers can be limiting factors for vessel distensibility.[13,15] Interestingly, the product term of postoperative angle×postoperative medial fibrosis was highly associated with anatomic nonmaturation (Figure 6D, Table 3).

DISCUSSION

Medial fibrosis is a common condition in the vasculature of patients with CKD.[11,12,16] Accumulation of collagen and disorganization of the muscular layer have been observed in the media of native veins used for AVF creation.[11] However, little is known about the consequences of venous fibrosis for AVF outcomes and the effects of remodeling on fibrosis progression. This study reveals that (*1*) preexisting medial fibrosis in native veins is not associated with AVF nonmaturation; (*2*) medial fibrosis increases in most patients after arteriovenous anastomosis independent of the preexisting level; (*3*) a high percentage of postoperative medial fibrosis and circumferential alignment of collagen fibers are associated with AVF nonmaturation; and (*4*) postoperative IH predicts nonmaturation only in patients with medial fibrosis over the population's median.

Our results confirm the presence of significant medial fibrosis in native veins. The magnitudes of preexisting fibrosis and IH were directly related to patient age, likely representing a gradual response to elevated pulse pressure, mechanical stress, and vasoactive mediators. In agreement with published data,[12] we found that preexisting venous fibrosis had no effect on AVF nonmaturation.

Table 3. Association between AVF nonmaturation and vascular features in native veins and AVFs

Predictor[a]	Odds Ratio[b]	95% CI	*P* Value
Veins (preexisting)			
Medial fibrosis	1.22	0.74 to 2.02	0.4
IH	0.74	0.15 to 3.64	0.7
Collagen organization			
Angle	1.01	0.76 to 1.35	0.9
Anisotropy index	0.48	0.18 to 1.34	0.2
AVFs (postoperative)			
Medial fibrosis	1.55	1.05 to 2.30	0.03
IH	1.63	0.93 to 2.86	0.09
Collagen organization			
Angle	1.38	1.03 to 1.84	0.03
Anisotropy index	0.70	0.33 to 1.50	0.4
Pairs (change over time)			
Medial fibrosis	1.23	0.79 to 1.93	0.4
IH	1.20	0.45 to 3.21	0.7
Collagen organization			
Angle	1.13	0.87 to 1.48	0.3
Anisotropy index	1.10	0.56 to 2.16	0.8
Postoperative product terms[c]			
Medial fibrosis×IH	4.56	1.36 to 15.27	0.01
Medial fibrosis×angle	1.09	1.03 to 1.16	<0.01

[a]Preexisting and postoperative predictors were assessed in native veins and AVFs collected during the first-stage and second-stage surgeries, respectively. Change over time was evaluated in tissue pairs when both a patient's vein and AVF sample were available. Medial fibrosis was expressed as % area of collagen; IH was expressed as I/M area ratio. The angle of collagen fibers ranged from 0° (perpendicular to the lumen) to 90° (circumferential direction around the lumen); the anisotropy index ranged from 0 (random alignment of fibers) to 1 (totally aligned in one direction). Change over time was calculated by subtracting the preexisting value from the postoperative measurement in tissue pairs.
[b]Odds ratio per 1-unit increase of the predictor variable. One unit is defined as 10% of medial fibrosis, 10° of angle, 0.1 of anisotropy index, and 1 in the I/M area ratio and product terms. All logistic regression analyses were adjusted for sex, except in models that included collagen organization variables (angle, anisotropy index, and medial fibrosis×angle) due to lower power.
[c]% medial fibrosis was expressed as a decimal in the calculation of product terms.

In contrast to the association between age and preexisting fibrosis and IH, our results showed that the development of postoperative medial fibrosis was independent of the baseline covariates tested (age, sex, diabetes, and history of previous AVF). Similarly, the degree of preexisting medial fibrosis did not determine the severity of the postoperative lesion in AVFs, which suggests that ECM turnover after the arteriovenous anastomosis is dependent on the AVF remodeling process. Postoperative IH was associated with a history of a previous AVF, possibly as a result of genetic or patient-specific predisposition for vascular proliferative lesions.

Unlike preexisting fibrosis, the degree of postoperative fibrosis was associated with AVF nonmaturation. Those AVF with a more fibrotic media had a higher risk for failure, particularly in the female subgroup where other, so far unknown, additive risk factor(s) are present. Fibrosis in the AVF wall can be attributed to excessive ECM synthesis by activated smooth muscle cells and myofibroblasts and/or deficient ECM degradation by metalloproteinases. Excessive fibrosis may decrease wall compliance, as indicated by a lower increase in vein diameter during remodeling in AVFs with medial fibrosis >60%. The time interval between surgeries was neither associated with nonmaturation nor with the increase in vein diameter, in agreement with studies indicating that AVF maturation occurs within the first 2 weeks after access creation.[17,18] The time interval was also not associated with the degree of postoperative fibrosis, which suggests that most collagen deposition occurs during early remodeling of the fistula. The mechanisms involved in the development of AVF venous fibrosis are complex and poorly studied. TGF-β is considered an important trigger leading to ECM accumulation.[19] Interestingly, patients receiving hemodialysis with a TGF-β1 high-producer genotype are prone to earlier AVF failure.[20]

This study highlights the importance of a proper balance between remodeling of the media layer and IH. Using a larger patient cohort, these analyses confirmed our previously published data that postoperative IH alone is not sufficient to predict AVF nonmaturation.[2] However, we now demonstrate that postoperative IH increases the risk of AVF failure in individuals with high medial fibrosis. This evidence suggests that AVF nonmaturation is more likely to occur in the setting of both high postoperative IH and medial fibrosis than with high medial fibrosis alone. It is possible that when the wall has lost its compliance due to excessive ECM deposition, a hyperplastic intima becomes obstructive (possibly less compressible under high blood flow) and stenotic.

Medial collagen fibers are organized into lamellar units and oriented circumferentially in healthy blood vessels.[21] However, circumferential arrangement of fibers can limit vessel compliance and may be associated with stiffness.[15] We quantified two important components of fiber organization: orientation angles and anisotropy index (degree of alignment). We found that a circumferential orientation of postoperative fibers around the vascular lumen was associated with AVF nonmaturation. Of note, collagen fibers in healthy blood vessels have crimps that can extend and straighten in the circumferential direction when the wall dilates. In contrast, circumferential collagen fibers in AVFs had minimal or no crimps. It is possible that, as a result of postoperative fibrosis, new collagen fibers are deposited in a nearly fully prestretched conformation, thus preventing any further distention to allow outward remodeling.

The limitations of this study include the low frequency of nonmaturation that reduces the power to find statistical associations, the lack of arterial information, the evaluation of only upper-arm AVFs, and the risk of sampling error by focusing only in the juxta-anastomotic AVF area. The reduced power also prevented us from studying the statistical interactions between IH and medial fibrosis and between collagen orientation angles and fibrosis. In addition, although the use of a single vascular surgeon prevents the confounding effects related to

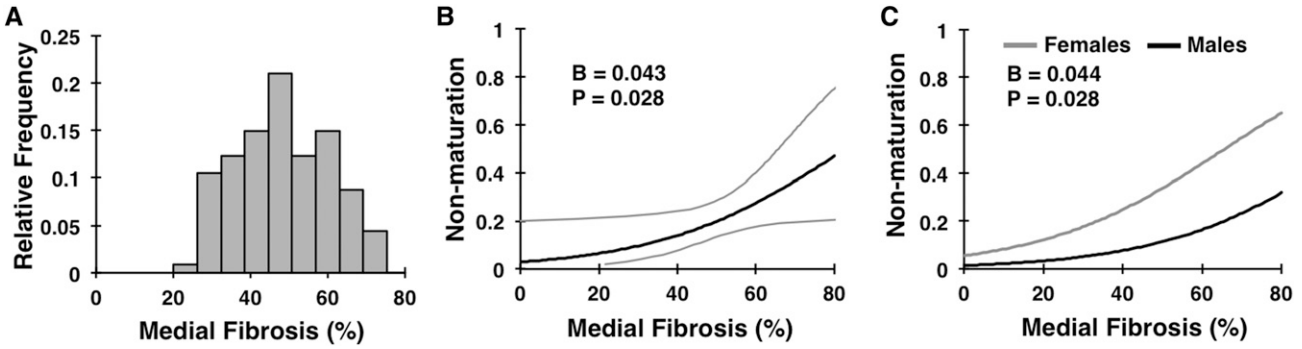

Figure 3. Postoperative medial fibrosis predicts AVF nonmaturation. (A) Distribution of medial fibrosis in the second-stage AVFs cohort. (B) Probability of anatomic nonmaturation as predicted by postoperative medial fibrosis using logistic regression analysis. The black line represents the model, whereas gray lines indicate the upper and lower levels of the 95% CI. (C) Anatomic nonmaturation as predicted by postoperative medial fibrosis using a logistic regression model adjusted for sex. The combined nonlinear B coefficient (for both sexes) is shown in the graph.

different surgical techniques, it also decreases generalizability. Despite these shortcomings, this study demonstrates the advantages of two-stage AVFs over other types of fistulas for the purpose of research. Although forearm AVFs come first in the order of preference for AVF placement, a postoperative sample can only be obtained in those fistulas that require a surgical intervention, limiting our ability to systematically compare with AVFs that mature successfully. Using the two-stage AVF study design, we present new evidence of postoperative histologic markers of AVF outcomes and identify a biologic process that can be potentially targeted to improve AVF maturation.

CONCISE METHODS

Study Design

Patients with CKD aged 21 years and older scheduled for two-stage AVF creation surgery (arteriovenous anastomosis during the first stage and AVF transposition during the second stage) at Jackson Memorial Hospital and University of Miami Hospital were invited to participate in the study. Patients were consecutively enrolled and samples were collected from January of 2012 to July of 2016. Enrolled patients were consented before each of the first-stage and second-stage surgeries. The study was performed according to the ethical principles of the Declaration of Helsinki and regulatory requirements at both institutions. The ethics committee and Institutional Review Board at the University of Miami approved the study.

A single surgeon (M.T.) performed all surgical procedures, using preoperative vascular mapping of the upper extremities to plan the AVF (Supplemental Material).[2] The surgeon collected vascular samples at two time points: a segment of the vein used to create the arteriovenous anastomosis during the first-stage surgery and a sample of the juxta-anastomotic area of the AVF during the second-stage procedure (Figure 1A). When both vascular samples from the same patient were available, a tissue pair was formed to evaluate change in vascular characteristics over time. Patient comorbidities, previous vascular access history, and medications taken in the 6 months before AVF creation (Table 1) were obtained from the patient's medical record.

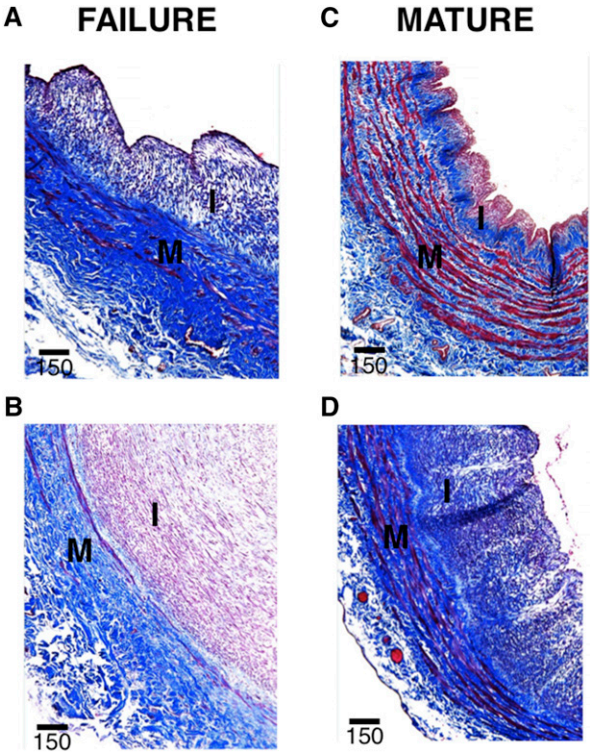

Figure 4. Postoperative histology shows increased fibrosis in failed AVFs. Close-up photographs of Masson's trichrome–stained cross-sections of AVFs with (A and B) anatomic nonmaturation and (C and D) successful maturation. Cells stain in red/pink, whereas collagen is shown in blue. Distances are in micrometers. I, intima; M, media.

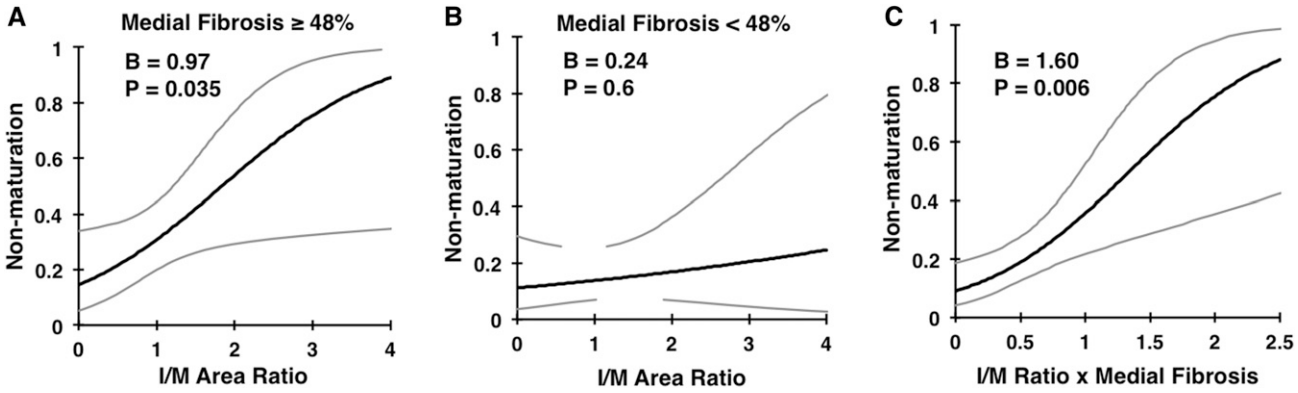

Figure 5. Postoperative IH is associated with AVF nonmaturation in the presence of high medial fibrosis. (A and B) Probability of anatomic nonmaturation as predicted by postoperative IH (expressed as I/M area ratio) in AVFs with postoperative medial fibrosis (A) over and (B) under the median value in the second-stage AVFs cohort. (C) Probability of anatomic nonmaturation as predicted by the product of IH (expressed as I/M area ratio) and percent medial fibrosis (expressed as a decimal) in AVFs using logistic regression analysis. The black lines represent the models, whereas gray lines indicate the upper and lower levels of the 95% CIs.

Anatomic AVF nonmaturation was defined prospectively as an AVF that never achieved an internal cross-sectional luminal diameter ≥6 mm as determined intraoperatively using intravascular probes. These failed AVFs underwent a shorter transposition, graft extension, or ligation during the second-stage surgery (Supplemental Figure 1). The definition of nonmaturation used in this study is a simplified version of the "rule of sixes" for two-stage AVFs. It is specifically suited to the evaluation of early vascular remodeling, when cannulation-related confounders have still not played a role.

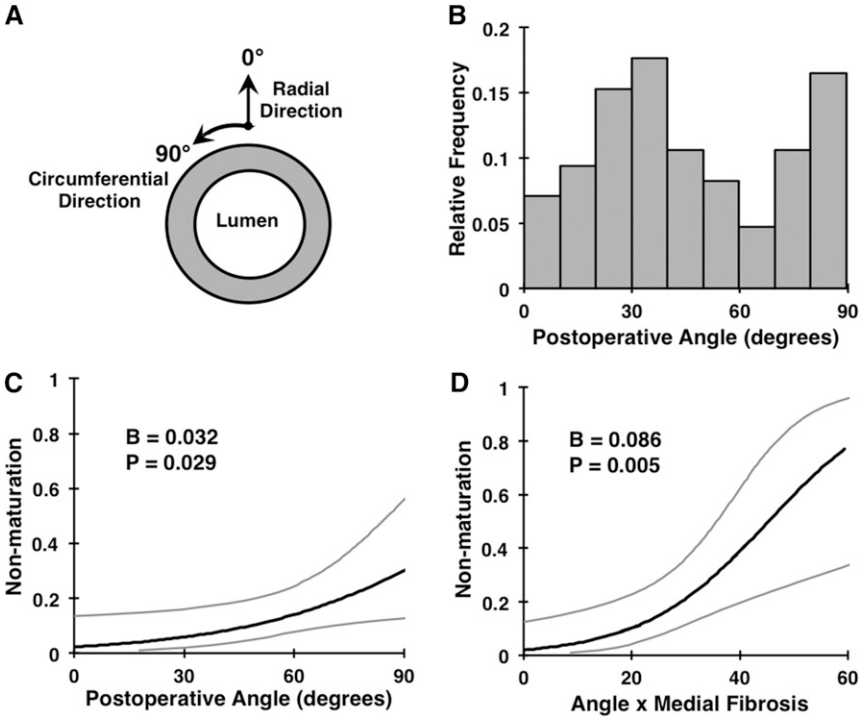

Figure 6. Circumferential orientation of medial collagen fibers predicts AVF nonmaturation. (A) Angle of collagen fibers relative to the lumen, ranging from 0° (perpendicular to the lumen, radial direction) to 90° (circumferential direction). (B) Distribution of postoperative orientation angles in the second-stage AVFs cohort. (C and D) Probability of anatomic maturation failure as predicted by (C) the postoperative angle of medial collagen fibers and (D) the product of the postoperative angle and percent medial fibrosis (expressed as a decimal) using logistic regression analyses. The black lines represent the models, whereas gray lines indicate the upper and lower levels of the 95% CIs.

Specimen Collection and Tissue Processing

The native vein and AVF venous samples were 1–2 mm in length and were collected at the site of transection during the first-stage anastomosis surgery and the second-stage transposition procedure (see Supplemental Material for detailed methodology). Tissues were fixed in formalin after collection, paraffin-embedded, and sectioned for histology.

Histologic and Morphometric Analysis

Tissue sections were stained with Masson's trichrome for gross histomorphometric analysis (see Supplemental Material for detailed methodology). The intima and media areas were delineated to calculate percentages of medial fibrosis and IH (defined as I/M area ratio).

Second Harmonic Generation Microscopy and Collagen Fiber Analysis

Nonlinear second harmonic generation microscopy analysis was performed on 70 vein and 85 AVF samples (see Supplemental Material for detailed methodology). Acquired fields were analyzed for fiber patterns and orientation angles.

Statistical Analyses

The variables of interest in this study included venous medial fibrosis, IH, and collagen fiber organization (angle and anisotropy index) that were determined in the first-stage native vein and the second-stage AVF venous samples. The primary outcome was anatomic maturation of the AVF. The secondary outcome was the increase in internal vein diameter from the first-stage to the second-stage surgery (*i.e.*, the AVF diameter minus the native vein diameter). The Supplemental Material includes a full description of the statistical analyses.

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DISCLOSURES

A.K.C. was a member of the Data and Safety Monitoring Board for a trial on vascular grafts cosponsored by Humacyte, Inc. and the National Heart, Lung, and Blood Institute. He was also a member of the Clinical Events Committee and Data Safety and Monitoring Board for the Novel Endovascular Access Trial sponsored by TVA Medical, Inc.

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