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Preoperative Arterial Microcalcification and Clinical Outcomes of Arteriovenous Fistulas for Hemodialysis

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Abstract

Background—Arteriovenous fistulas (AVFs) often fail to mature, but the mechanism of AVF non-maturation is poorly understood. Arterial micro-calcification is common in patients with chronic kidney disease (CKD), and may limit vascular dilatation, thereby contributing to early postoperative juxta-anastomotic AVF stenosis and impaired AVF maturation. This study evaluated whether preexisting arterial micro-calcification adversely affects AVF outcomes.

Study Design—Prospective study.

Setting & Participants—127 patients with CKD undergoing AVF surgery at a large academic medical center.

Predictors—Preexisting arterial micro-calcification (1% of media area) assessed independently by von Kossa stains of arterial specimens obtained during AVF surgery and by preoperative ultrasound.

Outcomes—Juxta-anastomotic AVF stenosis (ascertained by ultrasound obtained 4-6 weeks postoperatively); AVF non-maturation (inability to cannulate with 2 needles with dialysis blood

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flow 300 ml/min for 6 sessions in 1 month within 6 months of AVF creation); and duration of primary unassisted AVF survival after successful use (time to first intervention).

Results—Arterial micro-calcification was present by histology in 40% of patients undergoing AVF surgery. The frequency of a postoperative juxta-anastomotic AVF stenosis was similar in patients with or without preexisting arterial micro-calcification (32% vs 42%; OR, 0.65; 95% CI, 0.28-1.52; $p=0.3$). AVF non-maturation was observed in 29%, 33%, 33%, and 33% of patients with <1%, 1%-4.9%, 5%-9.9%, and 10% arterial micro-calcification, respectively ($p=0.9$). Sonographic arterial micro-calcification was found in 39% of patients and was associated with histologic calcification ($p=0.001$), but did not predict AVF non-maturation. Finally, among AVFs that matured, the unassisted AVF maturation (time to first intervention) was similar for patients with and without preexisting arterial micro-calcification (HR, 0.64; 95% CI, 0.35-1.21; $p=0.2$).

Limitations—Single center study.

Conclusions—Arterial micro-calcification is common in patients with advanced CKD, but does not explain postoperative AVF stenosis, AVF non-maturation, or AVF failure after successful cannulation.

Keywords

Arteriovenous fistula (AVF); arteriovenous access; vascular access; hemodialysis; vascular calcification; arterial micro-calcification; von Kossa staining; vascular ultrasound; juxta-anastomotic AVF stenosis; AVF non-maturation; AVF survival; cannulation; chronic kidney disease (CKD)

Although arteriovenous fistulas (AVFs) are considered the preferred type of vascular access for dialysis¹, the high frequency of non-maturation, which occurs in 20%-60% of patients, limits their use²³. The mechanism of AVF non-maturation remains elusive, despite considerable efforts to understand its pathogenesis⁴⁻⁶. In recent years there has been a growing interest in preexisting pathologic abnormalities of the veins and arteries used to create an AVF. These vascular abnormalities include arterial medial fibrosis, arterial intimal hyperplasia, arterial micro-calcification, venous intimal hyperplasia, and venous calcification⁷⁻¹². Non-maturing AVFs are frequently associated with a significant stenosis near the arteriovenous anastomosis^{13,14}. However, little has been published about whether AVF non-maturation is affected by preexisting vascular pathologies. We have previously reported that preexisting arterial medial fibrosis is not associated with AVF non-maturation, and preexisting venous intimal hyperplasia is not associated with postoperative AVF stenosis or AVF non-maturation⁷⁸.

There is a marked vascular dilatation after AVF creation, resulting in a 10- to 20-fold increase in the arterial flow present in the native artery¹⁵. Arterial micro-calcification, by making the artery stiffer, may limit its dilatation and resultant increase in blood flow following AVF creation, thereby providing a potential mechanism for AVF non-maturation¹⁶. A pilot study from our medical center reported a trend of higher AVF non-maturation in patients when preexisting arterial micro-calcification was scored semi-quantitatively, but the patient sample was too small to show statistical significance⁷. In the present study we prospectively studied a large cohort of patients with chronic kidney disease

(CKD) receiving a new AVF to determine whether preexisting arterial micro-calcification contributes to early postoperative juxta-anastomotic AVF stenosis, AVF non-maturation, or AVF failure following maturation.

Methods

Study Overview

Patients with CKD scheduled for creation of an AVF were invited to participate in a prospective study approved by the University of Alabama at Birmingham Institutional Review Board. Patients who provided informed consent had a small arterial specimen obtained at the time of access surgery. All study AVFs were created between 10/1/08 and 4/30/12, with prospective follow-up of AVF events through 10/30/12. This study cohort included a pilot group of patients reported previously ⁷. The patients underwent a postoperative ultrasound of their AVF 4-6 weeks after its creation, to assess its maturation. The tissue samples were stained with von Kossa, and arterial micro-calcification quantified by a pathologist (?.?.) who was unaware of clinical patient information. Two experienced radiologists (?.?, ?.?) who were unaware of clinical AVF outcomes independently scored arterial micro-calcification using the preoperative sonographic vascular mapping images. Arterial micro-calcification by histology was considered the primary exposure of interest. All histologic and radiologic calcification measurements were performed after the clinical AVF outcomes were determined. Finally, we evaluated the association between preexisting arterial micro-calcification and clinical AVF outcomes.

Study Population

The University of Alabama at Birmingham (UAB) provides nephrology care to approximately 500 hemodialysis patients treated at several in-center units in the metropolitan Birmingham area. The patients receive their medical care by full-time nephrologists on the UAB faculty, and nearly all their hospitalizations occur at UAB Hospital. In addition, UAB surgeons and radiologists perform surgical and percutaneous procedures on vascular access. All medical information is recorded in an electronic medical record. All access procedures are scheduled by two full-time vascular access coordinators, who also maintain a prospective, computerized database of the access procedures performed ¹⁷.

Preoperative Vascular Mapping

All patients underwent standardized preoperative vascular mapping by ultrasound prior to seeing the surgeon for vascular access creation ¹⁸¹⁹. The minimum criteria for creation of an AVF was an arterial diameter of 2 mm, a vein diameter of 2.5 mm, and no evidence of stenosis or thrombosis in the draining vein. The AVFs were created preferentially in the forearm, but an upper arm AVF was created if the forearm vessels were unsuitable. The ultrasound mapping results were provided to the surgeon prior to the preoperative clinic appointment.

Surgical Procedure

All AVFs were created by one of four experienced transplant surgeons (C.J.Y., M.H.D., J.G., M.H.) by making a direct anastomosis between the end of the vein and the side of the artery. At the time of AVF creation, the surgeon obtained a small specimen of the artery used to create the AVF. The arterial specimen was a partial (elliptical) section approximately 5 mm in length.

Postoperative Management of AVF

The patients underwent a postoperative ultrasound of their AVF 4-6 weeks after its creation²⁰. This ultrasound, which was the standard of care at our medical center, was used to quantify the AVF diameter, blood flow rate, and presence of stenosis at the juxta-anastomotic area, as previously reported. Mature AVFs were cannulated 6 to 8 weeks after their creation. Sonographically immature AVFs with an identifiable anatomic lesion (stenosis or accessory veins) underwent subsequent percutaneous or surgical procedures to promote AVF maturation²¹.

Pathologic Studies of the Arterial Specimen

All arterial specimens obtained during AVF surgery were immediately fixed in 10% neutral buffered formalin. They were cut at the 50% plane from paraffin embedded blocks using a Leica 2265 microtome. They were stained with the von Kossa method for calcium quantification analysis. In this staining procedure the slides were placed in 1% aqueous silver nitrate. During this step the calcium cations are replaced with silver, causing the calcified material to take on a black coloration (**Figure 1**). After being placed in sodium thiosulfate and 1% gold chloride, the specimens were then counterstained with Van Gieson's Picro-fuchsin for contrast.

A single pathologist (S.L.) who had no knowledge of the patient's clinical characteristics or AVF outcomes quantified the arterial micro-calcification. The micro-calcification was localized almost exclusively to the media, with very little found in the intima or adventitia. The histomorphometry was performed using the Bioquant Image Analysis software version 14.1.60 (R&M Biometrics, Nashville, TN). Two types of primary measurements were performed: total media area and area of calcified tissue in the media. The total tissue area was measured by outlining the entire media while avoiding void space. The calcified tissue measurements were made by selecting only the black pixels on the tissue. Finally, the two primary measurements were used to calculate the percentage of calcified tissue vs total tissue. On the basis of histologic quantification of arterial micro-calcification by the von Kossa stain, the patients were subdivided into two groups based on the calcification distribution. As 60% of the patient population had less than 1% calcification, the patients were divided into the groups with 1% calcification and those with less than 1% calcification, hereafter described as "no calcification". In additional analyses, the arterial specimens were subdivided into four categories: <1%, 1%-4.9%, 5%-9.9%, and 10% calcification of the media area.

Assessment of Preoperative Arterial Microcalcification by Imaging Studies

Two experienced radiologists (M.L.R. and H.R.U.) who were not aware of clinical patient characteristics or AVF outcomes retrospectively reviewed the preoperative vascular ultrasound of each study patient to assess the magnitude of arterial micro-calcification, and agreed on a semi-quantitative arterial micro-calcification score (1, none; 2, mild to moderate; or 3, severe)(**Figure 2**). Images were available in 119 of the 127 study patients (94%). Because only 5 ultrasounds were scored as having severe calcification, patients with calcification scores of 2 and 3 were combined. Thus, the final analysis compared patients in whom sonographic arterial micro-calcification was present or absent.

Outcomes

An AVF was considered to have hemodynamically significant juxta-anastomotic stenosis if the 4-6 week postoperative ultrasound revealed focal stenosis within 2 cm of the anastomosis, and the ratio of peak systolic velocity pre- and post-stenosis was 3:1 or greater ²⁰. An AVF was considered to be usable for dialysis if it could be cannulated reproducibly (for at least 6 sessions in one month) for dialysis with 2 needles and a dialysis blood flow 300 ml/min within 6 months of its creation ⁷. If the AVF had been placed prior to initiation of dialysis and the patient had not yet initiated dialysis, maturation was determined within one month of dialysis initiation. Unassisted AVF survival after maturation was calculated as the time from successful cannulation until the first intervention to restore patency (angioplasty, thrombectomy or surgical revision), with patient follow-up censored at the time of death, transplantation or transfer to a non-participating dialysis unit.

Data Analysis

Demographic and clinical characteristics of the study patients were extracted from the electronic medical records. The baseline characteristics of patient subgroups were compared using t-tests for continuous variables and Chi square test for discrete variables. Multiple variable logistic regression was used to assess clinical factors that independently predicted arterial micro-calcification. We compared the histologic arterial micro-calcification scores in patients whose preoperative ultrasound revealed “present” or “absent” calcification using the Mann-Whitney test, as the calcification scores were not normally distributed. The frequency of AVF non-maturation was compared among the four media calcification categories (<1%, 1%-4.9%, 5%-9.9%, and 10% of the media area) using the Chi-square test. Kaplan-Meier survival curves were created, and the difference in AVF survival between groups was analyzed using the log rank test. Multiple variable survival analysis was used to assess clinical factors that independently predicted unassisted AVF survival after maturation. We included in the multiple variable analyses covariates that have previously been associated with AVF non-maturation. A p-value < 0.05 was considered statistically significant.

Results

During the study period, 236 patients were consented for the study, of whom 192 underwent AVF surgery (**Figure 3**). The AVF outcome could not be determined in 47 patients who did not initiate hemodialysis during the follow-up period, or those who died, received a kidney transplant or transferred to an outside dialysis unit before AVF maturation could be

assessed. Thus, there were 145 study AVFs with sufficient follow-up to determine their clinical maturation. The surgeon was able to obtain an adequate arterial sample in 127 patients, or 88% of the cases. This group of patients represents the study population. Patients with arterial micro-calcification represented 40% of the study population (**Table 1**). As compared to patients without arterial micro-calcification, those with calcification were more likely to be female (57% vs 38%) and more likely to have diabetes (69% vs 32%). The two groups were similar in age, race, hypertension, vascular disease, and AVF location (upper arm vs forearm). Using multiple variable logistic regression with sex, age, race, diabetes, and access location in the model, only diabetes predicted arterial micro-calcification ($p<0.001$). In addition, the preoperative arterial and venous diameters were similar for patients with and without preexisting arterial micro-calcification (**Table 2**).

An early (4-6 week) postoperative ultrasound was performed in 99 patients, and revealed juxta-anastomotic AVF stenosis in 38 patients. There was a similar frequency of postoperative juxta-anastomotic AVF stenosis in patients with and without pre-existing arterial micro-calcification (32% vs 42%; odds ratio, 0.65; 95% confidence interval [CI], 0.28-1.52; $p=0.3$)(**Table 3**). This was still the case even when forearm and upper arm AVFs were analyzed individually.

Of the 127 study AVFs, 39 (31%) failed to mature. The frequency of AVF non-maturation was similar for patients in the four categories of arterial micro-calcification ($<1\%$, $1\%-4.9\%$, $5\%-9.9\%$, and 10% of the media area; $p=0.9$)(**Table 4**). Using multiple variable logistic regression analysis with age, sex, diabetes, access location, and arterial micro-calcification in the model, only access location (upper arm vs forearm) predicted AVF non-maturation ($p=0.03$). Specifically, AVF non-maturation was observed in 43% of forearm AVFs vs 24% of upper arm AVFs.

Among the 119 preoperative ultrasounds assessed for arterial micro-calcifications, 47 (39%) revealed some degree of calcification, whereas 72 (61%) had no visible calcification. The magnitude of arterial micro-calcification by histology was associated significantly with the sonographic assessment of calcification. The median histologic calcification was 1.37% (interquartile range [IQR], 0.50%-8.79%) in patients with calcification, as compared to 0.56% (IQR, 0.19%-1.54%) in those without calcification ($p=0.001$)(**Fig 4**). Non-maturation of AVFs was similar for patients with and without sonographic arterial micro-calcification (32% vs 30%; $p=0.9$).

Among the subset of patients whose AVF matured, the unassisted AVF survival after maturation was similar for patients with preexisting arterial micro-calcification and those without calcification (unadjusted hazard ratio, 0.64; 95% CI, 0.35-1.21; $p=0.2$) (**Figure 5**). The median unassisted AVF survivals were 544 and 338 days, respectively. Using multiple variable survival analysis with age, sex, diabetes, access location, and arterial micro-calcification in the model, none of these variables predicted unassisted AVF survival.

Discussion

The presence of calcification in the lower extremity arteries of hemodialysis patients is well known²². However, calcification of upper extremity arteries is less well appreciated. A small Scandinavian study observed histologic evidence of radial artery calcification in 6 of 15 (40%) of patients undergoing placement of a radiocephalic AVF for dialysis²³. More recently, a pilot study of 50 patients undergoing AVF placement at our center reported some degree of pre-existing arterial micro-calcification in 68% of cases⁷. In that study, we observed that the semi-quantitative calcification score tended to be nominally higher in non-maturing AVF than in those that matured, but this result was not statistically significant ($p=0.08$).

In addition, two studies presented only in abstract form have addressed this research question. Shin et al noted preexisting arterial micro-calcification in 49% of 84 patients undergoing AVF surgery²⁴. Early AVF failure was significantly more common in patients with preexisting arterial micro-calcification than in those without calcification.. Heaton et al reported a significant correlation between arterial micro-calcification and small artery elasticity index, suggesting that arterial micro-calcification affects the ability of the artery to dilate after AVF creation²⁵.

The present study is to our knowledge the largest series to date investigating the association between arterial micro-calcification and AVF outcomes. We observed a similar frequency of preexisting arterial micro-calcification by two independent methods in patients receiving an AVF: 40% by histology and 39% by ultrasound. Moreover, there was a significant association between calcification measured by the two methods. The frequency of AVF non-maturation was very similar between patients with or without preexisting arterial micro-calcification, and this lack of association was true regardless of whether calcification was evaluated by histologic or sonographic methods. Moreover, preexisting arterial micro-calcification was not associated with a higher frequency of juxta-anastomotic stenosis in the early postoperative ultrasound, nor was it associated with a shorter unassisted AVF patency after maturation. Of interest, a recent publication using radiography to assess arterial macro-calcification in diabetic hemodialysis patients observed shorter primary patency of radiocephalic fistulas in those patients with preoperative arterial macro-calcification²⁶. In view of the low sensitivity of radiography for arterial micro-calcification²⁷, it may be that only extreme degrees of arterial micro-calcification are likely to impair AVF outcomes.

The strengths of the present study include the routine use of preoperative vascular mapping to plan the AVF surgery, prospective collection of information about AVF outcomes, scoring of preoperative vascular parameters by radiologists and pathologists who were unaware of the AVF outcomes, and the correlation of arterial micro-calcification by ultrasound and pathology. There are also a number of limitations. First, this was a single-center study; thus, the results might not generalize to some dialysis centers. Second, we obtained partial cross-sections of the arterial samples, rather than full cross-sections. Third, the regional degree of calcification assessed at ultrasound may vary from the actual portion of the artery sampled by the surgeons. Finally, AVF surgery was avoided when the

preoperative ultrasound revealed severe concentric arterial micro-calcification (<5% of patients), and the results from the current study would not apply to such patients.

Why isn't arterial micro-calcification associated with AVF non-maturation? There are several possible explanations. First, the minimal degree of calcification required to restrict arterial dilatation may be higher than that observed in the current study. Second, continuous concentric calcification was uncommon in our study, and may be more functionally relevant than the discrete micro-calcifications seen in this study. Finally, venous dilatation may be a more important determinant of AVF maturation than arterial dilatation.

It is tempting to think that preexisting histologic abnormalities of the arteries or veins used to create an AVF will have an adverse effect on AVF maturation. However, as we have previously reported, neither preexisting arterial medial fibrosis nor preexisting venous intimal hyperplasia are associated with AVF non-maturation⁷⁸. The current study now shows that preexisting arterial micro-calcification also doesn't predict clinical AVF outcomes. Taken together, these observations suggest that the structural abnormalities seen in the native vessels used to create an AVF correlate poorly with the functional vascular properties that result in AVF non-maturation.

In summary, arterial micro-calcification is commonly found in the native arteries used to create an AVF. However, preexisting arterial micro-calcification does not explain early postoperative AVF stenosis, AVF non-maturation, or subsequent failure of AVF that achieved maturation for dialysis. From a clinical perspective, AVF surgery should not be avoided when the preoperative ultrasound shows micro-calcification, unless the image shows dense concentric calcification.

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Fig. 1. Representative von Kossa stains of arterial specimens obtained during AVF creation. Calcium stains black, and calcification was quantified as the percent of the total tissue specimen staining black. **A**, an artery with 0.25% calcification. **B**, an artery with 20.7% calcification. **I**, intima; **M**, media; **A**, adventitia.

Fig. 2.

Representative vascular ultrasound images. **A.** Longitudinal high-resolution image of left brachial artery showing good depiction of intimal-medial arterial layers (cursors), without arterial micro-calcification. **B.** Longitudinal high resolution image of left brachial artery in a different subject showing heavy calcifications (small white dots) in the anterior arterial wall which are confluent (arrowheads), limiting visualization of the far wall (cursors).

Fig. 3.
Flow chart illustrating how the patients were selected for the final study sample.

Fig. 4. Distribution of histologic arterial micro-calcification in patients with or without arterial micro-calcification by preoperative ultrasound ($p=0.001$). The solid black lines represent the means.

Fig. 5. Unassisted primary survival after successful cannulation of AVF that achieved maturation for dialysis. The endpoint was the first AVF intervention after maturation (angioplasty, thrombectomy or surgical revision). $P=0.2$ by the log rank test.

Table 1

Clinical features of patients with and without pre-existing arterial micro-calcification at time of AVF creation.

Parameter	Calcification 1%	Calcification < 1%	p-value
No. of patients	51	76	
Age 65 y	11 (22%)	16 (21%)	0.9
Female sex	29 (57%)	29 (38%)	0.04
Black race	31 (61%)	55 (72%)	0.2
Diabetes	35 (69%)	24 (32%)	<0.001
Hypertension	44 (86%)	70 (92%)	0.3
Coronary artery disease	5 (10%)	11 (14%)	0.4
Peripheral vascular disease	5 (10%)	6 (8%)	0.7
Cerebrovascular disease	6 (12%)	5 (6%)	0.3
Heart failure	10 (20%)	12 (16%)	0.6
Upper arm fistula	32 (63%)	51 (67%)	0.6

Note: Unless otherwise indicated, values are given as number (percentage).

AVF, arteriovenous fistula.

Table 2

Preoperative vascular diameters in patients with and without pre-existing arterial micro-calcification.

Parameter	Calcification 1%	Calcification < 1%	p-value
<i>Forearm AVF</i>			
No. of patients	19	25	
Arterial diameter (mm)	2.6 ± 0.4	2.8 ± 0.6	0.2
Venous diameter (mm)	3.2 ± 0.6	3.1 ± 0.7	0.6
<i>Upper arm AVF</i>			
No. of patients	32	51	
Arterial diameter (mm)	4.1 ± 0.7	4.7 ± 1.3	0.04
Venous diameter (mm)	4.1 ± 1.0	4.3 ± 1.3	0.5

Note: Unless otherwise indicated, values are given as mean ± standard deviation.

AVF, arteriovenous fistula.

Table 3

Association between preoperative arterial micro-calcification and early postoperative juxta-anastomotic AVF stenosis.

	Stenosis	No stenosis	p-value
All patients	13 (33)	27	0.3
Arterial micro-calcification 1%	25 (42)	34	
Arterial micro-calcification <1%			
Forearm AVF	6 (40)	9	0.4
Arterial micro-calcification 1%	10 (56)	8	
Arterial micro-calcification <1%			
Upper arm AVF	7 (28)	18	0.5
Arterial micro-calcification 1%	15 (37)	26	
Arterial micro-calcification <1%			

Note: Values are given as number (percentage) or number. An early postoperative ultrasound was not obtained in 28 patients, either because their AVF failed before it could be obtained or because they missed the appointment.

AVF, arteriovenous fistula.

Table 4

Preexisting arterial micro-calcification plus AVF non-maturation

Arterial micro-calcification	Total AVF	Non-maturing AVF
<1%	76	22 (29)
1%-4.9%	27	9 (33)
5%-9.9%	9	3 (33)
10%	15	5 (33)

Note: Values are given as number or number (percentage). P=0.9 for comparison of the patients in the four groups.