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Original Investigation

# Preoperative Arterial Microcalcification and Clinical Outcomes of Arteriovenous Fistulas for Hemodialysis

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**Background:** Arteriovenous fistulas (AVFs) often fail to mature, but the mechanism of AVF nonmaturation is poorly understood. Arterial microcalcification 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 microcalcification 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 microcalcification (≥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 post-operatively), AVF nonmaturation (inability to cannulate with 2 needles with dialysis blood 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 microcalcification was present by histologic evaluation 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 microcalcification (32% vs 42%; OR, 0.65; 95% CI, 0.28-1.52; $P = 0.3$). AVF nonmaturation was observed in 29%, 33%, 33%, and 33% of patients with <1%, 1% to 4.9%, 5% to 9.9%, and ≥10% arterial microcalcification, respectively ($P = 0.9$). Sonographic arterial microcalcification was found in 39% of patients and was associated with histologic calcification ($P = 0.001$), but did not predict AVF nonmaturation. Finally, among AVFs that matured, unassisted AVF maturation (time to first intervention) was similar for patients with and without preexisting arterial microcalcification (HR, 0.64; 95% CI, 0.35-1.21; $P = 0.2$).

**Limitations:** Single-center study.

**Conclusions:** Arterial microcalcification is common in patients with advanced CKD, but does not explain postoperative AVF stenosis, AVF nonmaturation, or AVF failure after successful cannulation.

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***INDEX WORDS:*** 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 dialysis,[1] the high frequency of nonmaturation, which occurs in 20% to 60% of patients, limits their use.[2,3] The mechanism of AVF nonmaturation remains elusive despite considerable efforts to understand its pathogenesis.[4-6] In recent years, there has been 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 microcalcification, venous intimal hyperplasia, and venous calcification.[7-12] Nonmaturing AVFs frequently are associated with a significant stenosis near the arteriovenous anastomosis.[13,14] However, little has been published about whether AVF nonmaturation is affected by preexisting vascular pathologies. We previously have reported that preexisting arterial medial fibrosis is not associated with AVF nonmaturation and preexisting venous intimal hyperplasia is not associated with postoperative AVF stenosis or AVF nonmaturation.[7,8]

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There is marked vascular dilatation after AVF creation, resulting in a 10- to 20-fold increase in the arterial flow present in the native artery.[15] Arterial microcalcification, 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 nonmaturation.[16] A pilot study from our medical center reported a trend of higher AVF nonmaturation in patients when preexisting arterial microcalcification was scored semiquantitatively, but the patient sample was too small to show statistical significance.[7] In the present study, we prospectively studied a large cohort of patients with chronic kidney disease receiving a new AVF to determine whether preexisting arterial microcalcification contributes to early postoperative juxtaanastomotic AVF stenosis, AVF nonmaturation, or AVF failure following maturation.

## METHODS

### Study Overview

Patients with chronic kidney disease scheduled for creation of an AVF were invited to participate in a prospective study approved by the University of Alabama at Birmingham (UAB) 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 October 8, 2008, and April 30, 2012, with prospective follow-up of AVF events through October 30, 2012. This study cohort included a pilot group of patients reported previously.[7] Patients underwent postoperative ultrasonography of the AVF 4 to 6 weeks after its creation to assess its maturation. Tissue samples were stained with von Kossa, and arterial microcalcification was quantified by a pathologist (S.L.) who was unaware of clinical patient information. Two experienced radiologists (M.L.R. and H.R.U.) who were unaware of clinical AVF outcomes independently scored arterial microcalcification using the preoperative sonographic vascular mapping images. Arterial microcalcification by histology was considered the primary exposure of interest. All histologic and radiologic calcification measurements were performed after clinical AVF outcomes were determined. Finally, we evaluated the association between preexisting arterial microcalcification and clinical AVF outcomes.

### Study Population

The 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 accesses. All medical information is recorded in an electronic medical record. All access procedures are scheduled by 2 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.[18,19] The minimum criteria for creation of an AVF was arterial diameter of 2 mm, 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 1 of 4 experienced transplant surgeons (C.J.Y., M.H.D., J.G., and 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 $\sim$5 mm in length.

### Postoperative Management of AVFs

Patients underwent postoperative ultrasonography of the AVF 4 to 6 weeks after its creation.[20] This ultrasound, which was the standard of care at our medical center, was used to quantify AVF diameter, blood flow rate, and the presence of stenosis at the juxtaanastomotic 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 maturation.[21]

### Pathologic Studies of the Arterial Specimen

All arterial specimens obtained during AVF surgery were fixed immediately in 10% neutral buffered formalin. They were cut at the 50% plane from paraffin-embedded blocks using a Leica 2265 microtome and 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 (Fig 1). After being placed in sodium thiosulfate and 1% gold chloride, specimens were counterstained with van Gieson picrofuchsin for contrast.

A single pathologist (S.L.) who had no knowledge of the patient's clinical characteristics or AVF outcomes quantified the arterial microcalcification. Microcalcification was localized almost exclusively to the media, with very little found in the intima or adventitia. Histomorphometry was performed using the Bioquant Image Analysis software, version 14.1.60 (R&M Biometrics). Two types of primary measurements were performed: total media area and area of calcified tissue in the media. Total tissue area was measured by outlining the entire media while avoiding void space. Calcified tissue measurements were made by selecting only the black pixels on the tissue. Finally, the 2 primary measurements were used to calculate the percentage of calcified tissue versus total tissue. On the basis of histologic quantification of arterial microcalcification by von Kossa stain, patients were subdivided into 2 groups based on calcification distribution. Because 60% of the patient population had $<$1% calcification, patients were divided into the group with $\geq$1% calcification and those with $<$1% calcification, hereafter described as "no calcification." In additional analyses, arterial specimens were subdivided into 4 categories: $<$1%, 1% to 4.9%, 5% to 9.9%, and $\geq$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 microcalcification and agreed on a semiquantitative arterial microcalcification score (1, none; 2, mild to moderate; or 3, severe; Fig 2). Images were available for 119 of 127 (94%) study patients. Because only 5 ultrasounds were scored as having severe