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Effect of the veins histopathological characteristics and preexisting medical conditions on arteriovenous fistula maturation and primary patency in patients with end-stage renal disease: an observational, prospective study

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Abstract

We assessed the veins histopathological characteristics and preexisting medical conditions before arteriovenous fistula (AVF) creation, and their correlation with AVF outcome and primary patency in patients with end-stage renal disease (ESRD). In this observational, prospective, mono-center study in Romania, patients with artery and venous diameters ≥2 mm and ≥2.5 mm, respectively, were enrolled. Vein specimens were harvested at AVF creation and evaluated by Hematoxylin and Eosin, Masson's trichrome and Orcein stainings, in terms of intimal hyperplasia, elastic fibers disposition, medial hypertrophy and smooth muscle cell disorganization and fibrosis (graded from mild to severe). Venous diameters and blood flow one/two-months post-AVF creation, AVF maturation at dialysis start, two-year primary patency were assessed. Of 115 examined patients, 50 were enrolled and underwent AVF creation. Of six (12%) patients with no vein morphological changes, 11 (22%) with mild histopathological changes, 19 (38%) with moderate and 14 (28%) with severe histopathological changes, four (67%), eight (73%), 17 (89%) and 12 (86%), respectively, had mature AVF. Regardless of histopathological characteristics, non-mature AVF were recorded in older patients and with smaller venous diameter. One/two-months post-AVF creation, in all patients with mature AVF, venous diameter and ultrasonographic blood flow were similar. Two years post-AVF creation, 26 patients had functional AVF; non-functional AVFs were recorded more likely in women and functional AVFs were most likely located on forearm. The veins histopathological modifications may not negatively influence AVF maturation in ESRD patients. AVF maturation failure may most likely be related to age and venous diameter at AVF creation.

Keywords: arteriovenous fistula, end-stage renal disease, vein, histopathological characteristics, pre-existing medical conditions.

Introduction

Chronic kidney disease (CKD) is still an important public health problem worldwide, with 10% of the globe population being affected by this disease. Million persons die annually due to the unaffordable treatment options, especially in middle-income and developing countries [1]. In patients with end-stage renal disease (ESRD), hemodialysis remains the most frequent approach for renal replacement therapy [2].

The first *Kidney Disease Outcome Quality Initiative* (KDOQI) guidelines recommended timely placement of a native arteriovenous fistula (AVF) to prevent the complications that may occur following the Gore-Tex graft or permanent catheter usage in patients with long-term hemodialysis. In addition, early AVF creation is important in patients that are not transplant candidates, when there is a lack of potential kidney donors and for whom the peritoneal dialysis is not a treatment option. Given this, KDOQI guidelines recommend AVF use as vascular access in all suitable patients [3]. Several clinical trials have shown that AVF is the preferred hemodialysis access because of the expected long-term patency of the fistula, decreased complication rates and low maintenance costs compared to other types of vascular access [2, 4, 5].

In the past decades, with the increasing number of patients that underwent native AVF creation, it has been observed that, surprisingly, maturation rate dramatically decreased. If 30 years ago it was reported a maturation rate of 83%, in the last decade AVF maturation rate was less than 60% [6–8]. Although the interest in this pathology has significantly increased to overcome AVF non-maturation, the major obstacle in AVF use, the results obtained still did not lead to a significant improvement. A better preoperative assessment of patients, by using Doppler ultrasound, increased the number of AVF placement, but their maturation rate remained low [9, 10].

The use of antiplatelet and lipid-lowering therapies in patients with native AVF has not increased the rate of maturation [7, 11, 12]. Given these unfortunate results, a better understanding of the pathogenic mechanisms that lead to native AVF non-maturation has become an imperative need [13].

In 2006, Wali *et al.* suggested that the histopathological modifications of the veins used for AVF creation, such as intimal hyperplasia (IH), medial fibrosis, mural calcifications, etc., as well as preexisting disease of the cephalic vein in patients with renal failure have a negative influence on AVF maturation and patency [14]. However, since then, many conflicting studies have been published suggesting that the presence of these vascular abnormalities in veins may have or not an effect on AVF maturation rate [15–21].

Given the lack of understanding the factors that may be responsible for AVF non-maturation, the aim of this study was to assess the preexisting histopathological characteristics of the veins before AVF creation and preexisting clinical conditions in patients with ESRD and to evaluate their correlation with AVF outcome and primary patency.

Patients, Materials and Methods

Study design and population

This was a mono-center, observational, prospective study, conducted between January 2014 and August 2014, in the Department of Cardiovascular Surgery, "Niculae Stăncioiu" Heart Institute, Cluj-Napoca, Romania. Patients with ESRD who required vascular access during the study period were planned to be enrolled.

All study participants had to meet the following eligibility criteria for inclusion in the study: previous diagnosis of CKD, clinical status suitable for AVF creation (as established by medical examination and ultrasonography), artery and venous diameter ≥2 mm and ≥2.5 mm, respectively, and written informed consent obtained before enrollment. Any of the following was considered as reason for exclusion from the study: diagnosis of stenosis or thrombosis in the draining veins planned to be used for AVF creation, concomitant diagnosis of clinical/subclinical bacterial infection, concomitant medication that may influence AVF maturation (*e.g.*, anticoagulants), if physician considered that patients may not comply with the study procedures and refusal to be included in the study.

At baseline, demographic characteristics and clinical data of interest such as age, gender, race, presence or absence of diabetes mellitus (DM), arterial hypertension (AHT), ischemic cardiomyopathy (IC) and peripheral arterial disease (PAD), cholesterol level, body mass index (BMI), smoking status, the presence or absence of AVF on forearm and if AVF was created before dialysis initiation were recorded.

Preoperative vascular assessment was performed by the vascular surgeon for all study participants. At the time of AVF creation, specimens of veins were harvested and were examined by a pathologist. At one and two months following AVF creation, all patients were clinically examined and Doppler ultrasonography was performed. At two years after AVF creation, the patency of mature AVFs was evaluated. Postoperative outcomes were statistically analyzed to identify the factors that may be involved in AVF non-maturation of this study population.

The study protocol was approved by the Ethics Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, and in accordance with the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Preoperative vascular assessment

For all study patients, arterial and venous Doppler ultrasound evaluation was performed by the vascular surgeon. The NextGen LOGIQ ultrasound machine with a broad-spectrum linear transducer of 7–15 MHz was used.

Vascular measurements were performed on the patients in sitting position, after a tourniquet was applied and the arm was rested on an adjustable supporting stand. The arterial diameters were computed in real-time by analyzing its longitudinal sections at the level of wrist and antecubital fossa for the radial and brachial artery, respectively, and diameters of artery location selected for the anastomosis were recorded. The veins were evaluated through the forearm and upper-arm trajectory to identify stenosis or thrombosis if any, and the venous diameters were measured in the location elected for AVF creation. We considered a minimum artery diameter of 2 mm and vein diameter of 2.5 mm suitable for AVF creation. If the forearm vessels were assessed as unsatisfactory for AVF creation, the upper-arm was selected for AVF creation if feasible.

Surgical procedure

All surgical procedures were performed under local anesthesia (10 mL of 2% Xylocaine). Depending on the preoperative findings, either a radial-cephalic, brachial-cephalic or transposed brachial-basilic AVFs were created. A direct anastomosis was performed between the side of the selected artery and the end of the vein. Before performing the anastomosis, a circumferential section from the respective vein was harvested and fixed in formalin. Vein specimens were collected from all participants without resulting in postoperative complications. Intraoperative intravenous heparinized saline infusion (20 mL) was administered for vein dilation. Postoperative, heparin or antiplatelet therapy was recommended only if the patient was already receiving this medication.

Histopathological and immunohistochemical assessment of the vein specimens

Circumferential segments of the veins were excised at the time of surgery, prior to AVF creation, fixed in 10% formalin and then processed for light microscopy. Following paraffin embedding, each vein specimen was cut into 5 μm sections and prepared for Hematoxylin and Eosin (HE), Masson's trichrome, Orcein stainings and for immunohistochemistry. A pathologist performed all the histological evaluations on Axioskop 40 microscope. Masson's trichrome staining was performed to visualize the vein fibrosis, smooth muscle cells medial disposition and the elastic fibers of the vein. In the Masson's trichrome staining, the collagen fibers are green, the smooth muscle fibers are red and the elastic fibers are orange. As the elastic lamellae are not evident in HE staining, we used Orcein staining. In Orcein staining, the elastin fibers are

brown. Immunohistochemical stainings were performed to evaluate the expression of alpha-smooth muscle actin (α-SMA), desmin and vimentin on the vein walls. Primary antibodies were used to identify the phenotypes of the cells involved in the vein morphological changes (the myofibroblastic phenotypes).

The HE staining was performed to assess venous IH; IH was evaluated by the thickness of the tunica intima on the luminal side of the internal elastic lamina.

Masson's trichrome staining was performed for the assessment of medial fibrosis, smooth muscle disposition and internal elastic lamina. The collagen fibers are stained in green, the smooth muscle cells in red and the elastic lamina in orange-red.

Orcein staining was performed to optimize visualization of the internal elastic lamina, which delimitates the border between intima and media and it is less visible by HE. The elastic lamella is colored in dark brown.

Immunohistochemistry was performed to evaluate α-SMA, desmin and vimentin expression in the vein specimens. The primary antibodies were applied in the following dilutions: vimentin, clone 9 – 1:100, desmin clone D33 – 2:100, α-SMA, clone A4 – 1:100. The technique followed the steps of the Labeled Streptavidin–Biotin system (LSAB universal kit) and the chromogenic base for peroxidase was 3,3'-Diaminobenzidine (DAB). The positive cells are brown; the counterstaining was made with watery Hematoxylin that gives the bluish color in the background.

The objectives of the histological study were to evaluate the intimal vein hyperplasia, the disposition and the patterns of the elastic vein fibers, medial vein hypertrophy, smooth muscle cells disposition and disorganization in the venous wall. The cases of smooth muscle cells hypertrophy at the level of vein valves followed by thrombosis and fibrosis with severe reduction of the vein lumen are excluded from the study.

Postoperative vascular and AVF assessment

All patients were examined clinically and by ultrasonography (as previously described) at one and two months following AVF creation. AVF maturation was assessed at the time of dialysis start and their patency, defined as functional patency with adequate dialysis, was assessed at two years after AVF creation. The arterial and vein diameters were measured at one and two months, and AVF patency was assessed by blood flow measurement, using the methodology recommended by Napoli (2011) for both diameters and patency assessments [22]. AVF was considered suitable for dialysis (mature) if it was successful cannulated with two needles and permitted the circulation of a blood flow ≥300 mL/min for six consecutive dialysis sessions starting with one month and up to six months after its creation. Criteria used in our study for mature AVF assessment is in accordance with the recommendations provided by the *Committee on Reporting Standards for Arterio-Venous Accesses of the Society for Vascular Surgery* and the *American Association for Vascular Surgery* [23], and widely used in studies on arteriovenous hemodialysis accesses [8, 15, 16, 18, 19].

Statistical analysis

Statistical analyses were performed using the Epi Info software. Descriptive statistics were tabulated as prevalence and percentages for continuous data, and as mean with standard deviation (SD) or 95% confidence intervals (CI) for categorical variables. Analysis of Variance (ANOVA) Parametric Test for Inequality of Population Means was used to compare the continuous variables and χ^2 (chi)-square test was used to examine the association between categorical variables; p-value was considered statistically significant if <0.05. To identify the possible correlation between two variables, the Pearson's correlation coefficient was calculated; a strong (positive/negative) correlation was considered if R=1, a medium (positive/negative correlation) if R=–/+0.5, and a weak (positive/negative) correlation if R=–/+0.25.

Results

Study population

Out of 115 patients with ESRD examined during January 2014 and August 2014, 50 met the eligibility criteria and were enrolled in this study. Twenty-six patients refused to be included in the study, 12 had the venous diameters <2.5 cm, six had artery diameters <2 cm and 21 did not comply with study procedures.

All enrolled patients underwent native AVF creation. The distribution of AVF procedures was as follows: 20 (40%) radial-cephalic, 23 (46%) brachial-cephalic and seven (14%) brachial-basilic.

Histopathological and immunohistochemistry outcomes

Based on the histopathological outcomes, the pathologist graded the morphological changes on a semi-quantitative scale, as shown in Table 1.

Table 1 – ***Semi-quantitative scale for assessment of the venous morphological changes***

Scale	Morphological features
None	▪ no pathological changes.
Mild	▪ concentric intimal hyperplasia; ▪ medial hypertrophy; ▪ enrichment of the elastic fibers.
Moderate	▪ intimal hyperplasia with intimal cellular eccentric plaques; ▪ enrichment of the elastic tissue at the intimo-medial level with focal thickening of the elastic fibers, especially under the intimal plaques; ▪ medial hypertrophy; ▪ hyperplasia and hypertrophy of the smooth muscle cells at the vein valve with its projection in the lumen; ▪ sparse fibrosis between the intimo-medial cells and minimal muscle cells disorganization.
Severe	▪ intimal hyperplasia with fibrous plaques; ▪ intimo-medial vein fibrosis affecting the intimal plaques; ▪ focal replacement of the muscular medial structure and elastic fibers by fibrosis; ▪ disorganization of the smooth muscle cells.

Regardless of AVF type, patients were distributed in four groups, based on the severity level of the histopathological modifications at the time of AVF creation: six (12%) patients with no morphological changes (Control group, Figure 1A), 11 (22%) patients with mild morphological changes (MI-AVF group, Figure 1B), 19 (38%) patients with moderate morphological changes (MO-AVF group, Figure 1C) and 14 (28%) patients with severe morphological changes (SE-AVF group, Figure 1D).

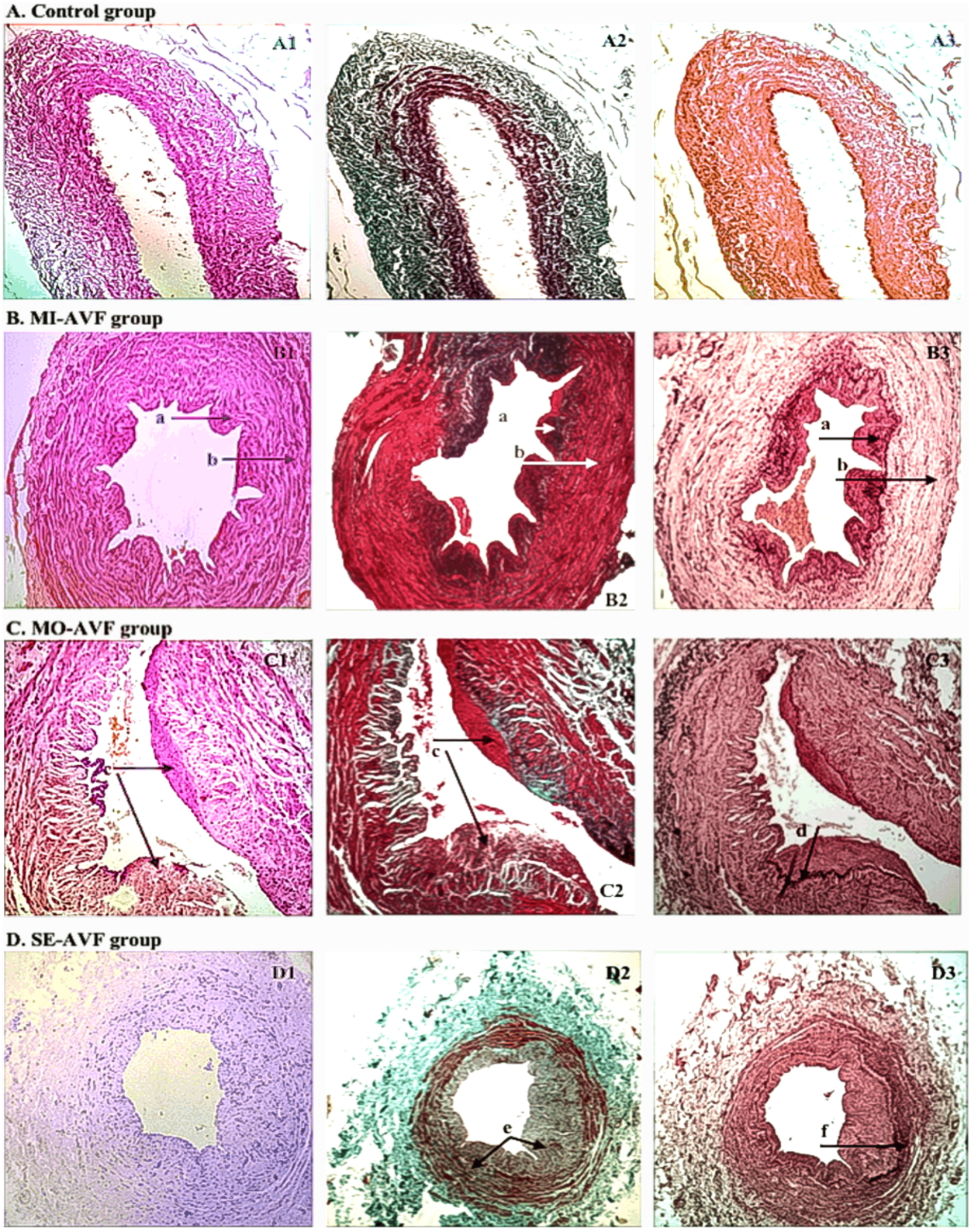


Figure 1 – ***Venous histological sections at the time of AVF creation by group: (1) HE staining, (2) Masson's trichrome staining and (3) Orcein staining. (A1) HE staining (×200): no venous morphological changes observed; (A2) Masson's trichrome staining (×200): less than four smooth muscle cells layers are observed; (A3) Orcein staining (×200): elastic fibers of the normal vein. (B1) HE staining (×100): vein with mild changes; (B2) Masson's trichrome staining (×100): (a) Concentric intimal hyperplasia (arrow); (b) Medial hypertrophy (arrow); (B3) Orcein staining (×100): enrichment of the elastic tissue; elastic lamina thin and discontinued under the intimal hyperplasia. (C1) HE staining (×100): vein with multiple intimo-medial changes, including (c) intimal cellular plaques (arrow); (C2) Masson's trichrome staining (×100): vein with medial hypertrophy, cellular plaques and sparse fibrosis; (C3) Orcein staining (×100): (d) Elastic lamina with focal increased thickness. (D1) HE staining (×100): vein with severe changes; (D2) Masson's trichrome staining (×100): (e) Intimal fibrotic plaque and medial fibrosis (arrow); (D3) Orcein staining (×100): (f) Increased thickness of the elastic lamina below the intimal plaque (arrow).***

IH of the vein was frequently recorded. IH of the vein was concentric in 22% of patients or eccentric determining the histological pattern of intimal plaques in 66% of patients. The intimal cellular plaques are visible in 38% of cases (Figure 1C) and the intimal fibrous plaques in 28% of cases (Figure 1D).

Medial hypertrophy of the vein was considered when the medial thickness of the vein was more than four layers of smooth muscle cells. Medial hypertrophy of the vein was observed in the cases with mild and moderate changes (Table 1), in 60% of patients.

Disorganization of the smooth muscle cells disposition was sparse in 38% cases and substantial in 28%. This pattern is better observed in the media under intimal plaques. Smooth muscle cells hypertrophy and hyperplasia at the level of the vein valve was a rarely recorded image (Figure 2).

The vein elastic tissue was enriched by segmental thickness of the elastic fibers especially under the intimal plaques in 28% of cases. The elastic fibers were segmentally effaced by fibrosis in the group with severe vein changes (Table 1).

The immunohistochemistry showed the presence of α-SMA-positive cells, desmin-positive cells and vimentin-positive cells in the intimal plaques observed in veins with IH (Figure 3). In our study, the cellular phenotype of the intimal cellular plaques suggests the presence of cells with contractile phenotype: α-SMA, desmin-positive cells and vimentin-positive cells (Figure 3).

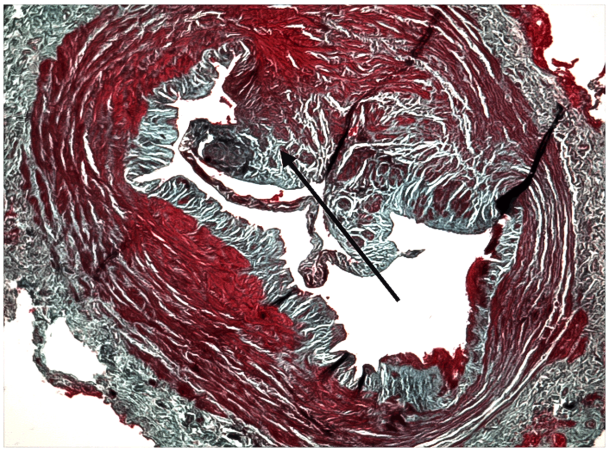


Figure 2 – ***Venous valve with smooth muscle cell hypertrophy in a patient with moderate histopathological vein modification; vein valve with hypertrophy (arrow). Masson's trichrome staining (×100).***

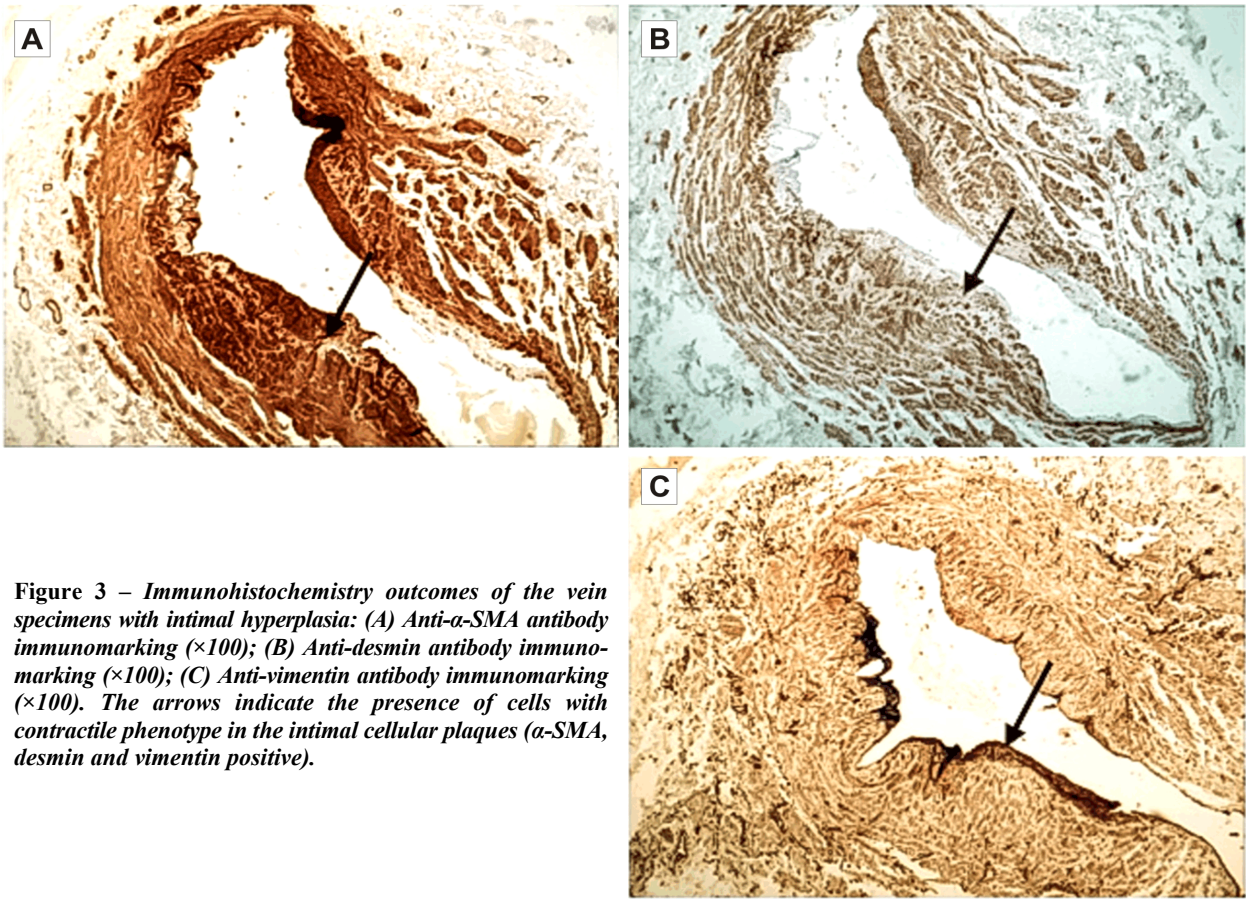


Figure 3 – ***Immunohistochemistry outcomes of the vein specimens with intimal hyperplasia: (A) Anti-α-SMA antibody immunomarking (×100); (B) Anti-desmin antibody immunomarking (×100); (C) Anti-vimentin antibody immunomarking (×100). The arrows indicate the presence of cells with contractile phenotype in the intimal cellular plaques (α-SMA, desmin and vimentin positive).***

Demographic and clinical characteristics by group at baseline

All patients were of European–White Caucasian heritage. The mean age (years±SD) at the time of AVF creation ranged between 58.7±8.7 years and 61±13.2 years in all groups, and no statistically significant difference was observed between the Control group *versus* all groups with histopathological modifications. The percentage of women ranged between 7.1% (n=1) and 45.5% (n=5). The prevalence of AHT, IC, PAD, high cholesterol, smoking status and BMI was similar between groups. DM prevalence tended to be higher in the MI-AVF *versus* Control group (borderline statistical significance, p=0.05). The number of patients with AVF located on

forearm and AVF creation after dialysis initiation was comparable between groups. The detailed description of the patients' demographic and clinical characteristics is shown in Table 2.

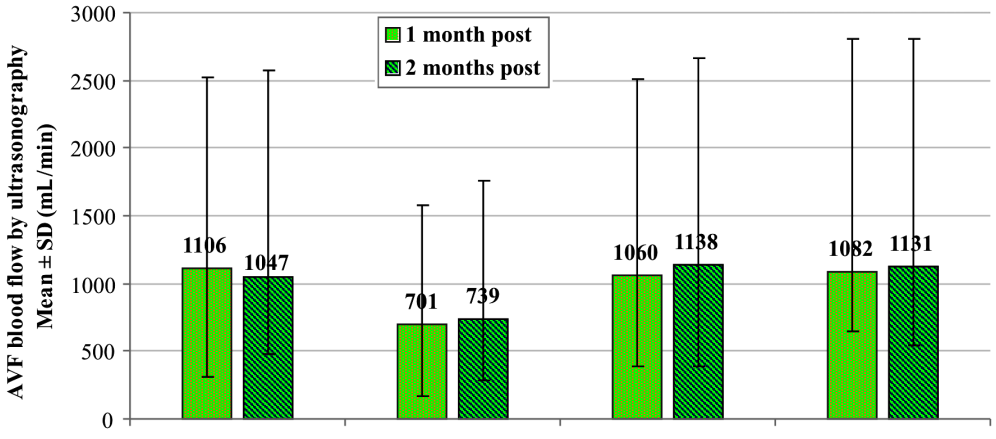
Table 2 – ***Baseline characteristics of the study participants***

Category	Control group *N=6*	MI-AVF group *N=11*	*p*	MO-AVF group *N=19*	*p*	SE-AVF group *N=14*	*p*
Age [years], mean±SD	61±13.2	58.7±8.7	0.39	60.3±12.5	0.6	59.4±10.3	0.49
Women, *n* (%) (95% CI: LL–UL)	2 (33.3) (4.33–77.72)	5 (45.5) (16.75–76.62)	0.62	7 (36.9) (6.29–61.64)	0.87	1 (7.1) (0.18–33.87)	0.13
DM, *n* (%) (95% CI: LL–UL)	0	5 (45.5) (16.75–76.62)	0.05	6 (31.6) (12.58–56.55)	0.11	5 (35.7) (12.76–64.86)	0.09
AHT, *n* (%) (95% CI: LL–UL)	6 (100)	10 (90.9) (57.19–98.22)	0.44	15 (79) (54.43–93.95)	0.22	12 (85.7) (57.19–98.22)	0.32
IC, *n* (%) (95% CI: LL–UL)	3 (50) (11.81–8.19)	6 (54.6) (23.38–83.25)	0.85	5 (26.3) (9.15–51.2)	0.27	2 (14.3) (1.78–42.81)	0.09
PAD, *n* (%) (95% CI: LL–UL)	0	3 (27.3) (6.02–60.97)	0.15	3 (15.8) (3.38–39.58)	0.29	4 (28.6) (8.39–58.1)	0.14
Cholesterol ≥200 mg/dL, *n* (%) (95% CI: LL–UL)	2 (33.3) (4.33–77.72)	3 (27.3) (6.02–60.97)	0.79	2 (10.5) (1.30–33.14)	0.18	4 (28.6) (8.39–58.1)	0.79
Smoker, *n* (%) (95% CI: LL–UL)	0	4 (36.4) (10.93–69.21)	0.09	4 (21.1) (6.05–45.57)	0.22	3 (21.4) (4.66–50.8)	0.21
BMI [kg/m^2], mean±SD	29.6±9.4	30.9±4.8	0.31	26.2±4	0.4	28.9±6.3	0.39
AVF located on forearm, *n* (%) (95% CI: LL–UL)	3 (50) (11.81–88.19)	6 (54.6) (23.38–83.25)	0.4	6 (31.6) (12.58–56.55)	0.07	5 (35.7) (12.76–64.86)	0.52
AVF created after dialysis initiation, *n* (%) (95% CI: LL–UL)	1 (16.7) (0.42–64.12)	3 (27.3) (6.02–60.97)	0.62	8 (42.1) (20.25–66.5)	0.25	5 (35.7) (12.76–64.86)	0.39

The difference between groups was considered statistically significant if $p<0.005$. *N*: Total number of patients included in each group; SD: Standard deviation; *n* (%) (95% CI): Number (percentage with 95% confidence interval) of patients included in a given category; DM: Diabetes mellitus; AHT: Arterial hypertension; IC: Ischemic cardiomyopathy; PAD: Peripheral arterial disease; BMI: Body mass index; AVF: Arteriovenous fistula; MI: Mild morphological changes; MO: Moderate morphological changes; SE: Severe morphological changes; CI: Confidence interval; LL: Lower limit; UL: Upper limit. Note: All comparisons were made *versus* the control group. No statistically significant difference ($p<0.05$) was recorded between the MI-AVF, MO-AVF and SE-AVF groups *versus* Control group.

Hemodialysis access outcomes

AVF maturation was recorded in four (67%) patients in the Control group, eight (73%) in the MI-AVF group, 17 (89%) in the MO-AVF group and 12 (86%) in the SE-AVF group and were successfully used for dialysis (Table 3). At the time of dialysis start, the dialysis blood flow in patients with mature AVF was 375.5±29.9, 316.7±76.3, 311.3±56.5 and 325.5±38 mL/min in the Control, MI-AVF, MO-AVF, SE-AVF groups, respectively; no statistically significant difference was observed between groups. In these patients, the venous diameter ranged between 5.95 mm and 6.8 mm at one month after AVF creation, and between 6.55 mm and 7.93 mm at two months after AVF creation; no statistically significant difference was observed between groups. The ultrasound blood flow at one and two months after AVF creation ranged from 701 to 1106 mL/min and from 739 to 1138 mL/min, respectively, with no statistically significant difference observed between MI-AVF, MO-AVF, SE-AVF groups and Control groups (Figure 4).



Venous diameter (mean ± SD, mm)	Control (N=4)	MI-AVF (N=8)	MO-AVF (N=17)	SE-AVF (N=12)
1 month post	6.18 ± 1.13	5.95 ± 1.69	6.80 ± 1.81	6.63 ± 1.62
2 months post	6.90 ± 1.22	6.55 ± 1.26	7.93 ± 2.15	7.50 ± 2.52

Figure 4 – ***Postoperative ultrasonographic blood flow and venous diameter in patients with mature AVF. AVF: Arteriovenous fistula; MI: Mild morphological changes; MO: Moderate morphological changes; SE: Severe morphological changes; N: Number of patients; SD: Standard deviation; post: After AVF creation.***

Table 3 shows the patients' demographic and clinical characteristics by AVF outcome at dialysis start in the Control, MI-AVF, MO-AVF and SE-AVF groups. Even if no correlation was observed between the venous histopathological features and clinical characteristics at baseline (Table 2), when comparing patients' characteristics by AVF outcome, regardless of the histopathological characteristics, it was observed that patients with non-mature AVF were more likely to be older and to have a smaller venous diameter, but were similar to patients with mature AVF in gender, DM, AHT, PAD and IC concomitant illness, cholesterol level, smoking status, AVF location on forearm and creation after dialysis initiation (Table 3).

The Pearson's correlation test also showed a weak negative correlation between age and maturation (R= –0.28) and a weak positive correlation between venous diameter and maturation (R=0.27).

Table 3 – ***Participants characteristics by AVF outcome***

Category	Control group		MI-AVF group		MO-AVF group		SE-AVF group		Total		*p*
	Non-mat N=2	*Mat N=4*	*Non-mat N=3*	*Mat N=8*	*Non-mat N=2*	*Mat N=17*	*Non-mat N=2*	*Mat N=12*	*Non-mat N=9*	*Mat N=41*	
Age [years], mean±SD	70.5±7.78	56.3±13.4	53.7±10	60.6±8.1	73	58.8±12.4	74.5±9.2	56.8±8.3	66.3±11.6	58.3±10.4	0.04*
Women, *n* (%) (95% CI: LL–UL)	1 (50) (1.3–98.7)	1 (25) (0.6–80.6)	2 (66.7) (9.4–99.2)	3 (37.3) (8.5–75.5)	0	7 (41.2) (18.4–67.1)	0	1 (8.3) (0.2–38.5)	3 (33.3) (7.5–70.1)	12 (29.3) (16.1–45.5)	0.8
DM, *n* (%) (95% CI: LL–UL)	0	0	1 (33.3) (0.8–90.6)	4 (50) (15.7–84.3)	2 (100)	4 (23.5) (6.8–49.9)	0	5 (41.7) (15.2–72.3)	3 (33.3) (7.5–70.1)	13 (31.7) (18.1–48.1)	0.92
AHT, *n* (%) (95% CI: LL–UL)	2 (100)	4 (100)	2 (66.7) (9.4–99.2)	8 (100)	2 (100)	13 (76.5) (50.1–93.2)	2 (100)	10 (83.3) (51.6–97.9)	8 (88.9) (51.8–99.7)	35 (85.9) (10.9–69.2)	0.72
IC, *n* (%) (95% CI: LL–UL)	2 (100)	1 (25) (0.6–80.6)	2 (66.7) (9.4–99.2)	4 (50) (15.7–84.3)	0	5 (29.4) (10.3–56)	2 (100)	2 (16.7) (2.1–48.4)	4 (44.4) (13.7–78.8)	12 (29.3) (16.1–45.5)	0.38
PAD, *n* (%) (95% CI: LL–UL)	0	0	0	3 (37.5) (8.5–75.5)	2 (100)	1 (5.6) (0.2–28.7)	1 (50) (1.3–98.7)	3 (25) (5.5–57.2)	3 (33.3) (7.5–70.1)	7 (17.1) (7.2–32.1)	0.27
Cholesterol ≥200 mg/dL, *n* (%) (95% CI: LL–UL)	0	2 (50) (6.8–93.2)	0	3 (37.5) (8.5–75.5)	0	2 (11.8) (1.5–36.4)	0	4 (33.3) (9.9–65.1)	0	11 (26.8) (14.2–42.9)	0.07
Smoker, *n* (%) (95% CI: LL–UL)	0	0	2 (66.7) (9.4–99.2)	2 (25) (3.2–65.1)	0	4 (23.5) (6.8–49.9)	0	3 (25) (2.8–60)	2 (22.2) (2.8–60)	9 (22) (10.6–37.6)	0.98
BMI [kg/m^2], mean±SD	22.6±3.1	33.14±3.75	28.6±3.1	31.8±5.1	28.4	25.9±4.1	25.7±3.8	29.4±6.6	26.6±3.4	28.9±6.2	0.28
AVF located on forearm, *n* (%) (95% CI: LL–UL)	1 (50) (1.3–98.7)	2 (50) (6.8–93.2)	2 (66.7) (9.4–99.2)	4 (50) (15.7–84.3)	1 (50) (1.3–98.7)	5 (29.4) (10.3–56)	0	5 (41.7) (15.2–72.3)	4 (44.4) (13.7–78.8)	16 (39) (24.2–55.5)	0.93
AVF created before dialysis initiation, *n* (%) (95% CI: LL–UL)	1 (50) (1.3–98.7)	0	1 (33.3) (0.8–90.6)	2 (25) (3.2–65.1)	0	8 (46.5) (23–72.2)	2 (100)	3 (25) (5.5–57.2)	4 (44.4) (13.7–78.8)	13 (31.7) (18.1–48.1)	0.46
Venous diameter [mm], mean±SD	2.55±0.07	3.52±0.59	2.96±0.23	3.7±1.19	3.65±0.49	4.06±1.25	3.25±0.5	3.75±1.1	3.09±0.49	3.84±1.13	0.048*

AVF: Arteriovenous fistula; MI: Mild morphological changes; MO: Moderate morphological changes; SE: Severe morphological changes; *N*: Number of patients by AVF outcome; SD: Standard deviation; *n* (%) (95% CI): Number (percentage with 95% confidence interval) of patients included in a given category; LL: Lower limit; UL: Upper limit; DM: Diabetes mellitus; AHT: Arterial hypertension; IC: Ischemic cardiomyopathy; PAD: Peripheral arterial disease; BMI: Body mass index. Note: Comparisons were made between total non-mature and mature AVFs; **p*-value statistically significant (p<0.05).

At two years after AVF creation, of the 41 patients with mature AVF, 26 patients had functional AVF, six had non-functional AVF, four underwent kidney transplantation and five died. When comparing patient's characteristics by AVF primary patency, regardless of the histopathological characteristics, it was observed that patients with non-functional AVF were more likely to be women and patients with functional AVF had most likely AVF located on the forearm (Table 4).

The Pearson's correlation test also showed a weak negative correlation between women and non-functional AVF (R=–0.38) and a weak positive correlation between venous AVF forearm location and patency (R=0.45).

Table 4 – ***Participants characteristics with mature AVFs by AVF patency at two years after dialysis start***

Category	Control group		MI-AVF group		MO-AVF group		SE-AVF group		Total		*p*
	Non-functional N=1	*Functional N=2*	*Non-functional N=1*	*Functional N=6*	*Non-functional N=3*	*Functional N=11*	*Non-functional N=1*	*Functional N=7*	*Non-functional N=6*	*Functional N=26*	
Age [years], mean±SD	58	65±2.8	74	57±5.1	70.3±3.8	58.7±12.7	64.1±11.7	58±9.7	64.1±11.7	58±9.7	0.16
Women, *n* (%) (95% CI: LL–UL)	1 (100)	0	1 (100)	2 (33.3) (4.3–77.7)	1 (33.3) (0.8–90.6)	4 (36.4) (10.9–69.2)	1 (100)	0	4 (66.7) (22.3–95.7)	6 (23.1) (9–43.7)	0.03*
DM, *n* (%) (95% CI: LL–UL)	0	0	0	3 (50) (11.8–88.2)	0	4 (36.4) (10.9–69.2)	0	3 (42.9) (9.9–81.6)	0	10 (38.5) (20.2–59.4)	–
AHT, *n* (%) (95% CI: LL–UL)	1 (100)	2 (100)	1 (100)	6 (100)	3 (100)	8 (72.7) (39–94)	0	6 (85.7) (42.1–99.6)	5 (83.3) (35.9–99.6)	22 (84.6) (65.1–95.6)	0.94
IC, *n* (%) (95% CI: LL–UL)	0	1 (50) (1.3–98.7)	0	3 (50) (11.8–88.2)	1 (33.3) (0.8–90.6)	3 (27.3) (6–61)	0	1 (14.3) (0.4–57.9)	1 (16.7) (0.4–64.1)	8 (30.4) (14.3–51.8)	0.49
PAD, *n* (%) (95% CI: LL–UL)	0	0	0	2 (33.3) (4.3–77.7)	1 (33.3) (0.8–90.6)	0	0	1 (14.3) (0.4–57.9)	1 (16.7) (0.4–64.1)	3 (11.5) (2.5–30.2)	0.37
Cholesterol ≥200 mg/dL, *n* (%) (95% CI: LL–UL)	1 (100)	1 (50) (1.3–98.7)	0	3 (50) (11.8–88.2)	0	1 (9.1) (0.2–41.3)	0	2 (28.6) (3.7–71)	1 (16.7) (0.4–64.1)	7 (26.9) (11.6–47.8)	0.6
Smoker, *n* (%) (95% CI: LL–UL)	0	0	0	2 (33.3) (4.3–77.7)	2 (66.7) (9.4–99.2)	1 (9.1) (0.2–41.3)	0	1 (14.3) (0.4–57.9)	2 (33.3) (4.3–77.7)	4 (15.4) (4.4–34.9)	0.31
BMI [kg/m^2], mean±SD	32.8	35.6±15.9	27.7	33.2±5.1	24.7±2.4	26.9±4.2	28.2	30.6±7.7	27.6±3.5	30.2±6.8	0.42
AVF located on forearm, *n* (%) (95% CI: LL–UL)	0	1 (50) (1.3–98.7)	0	2 (33.3) (22.3–95.7)	1 (33.3) (0.8–90.6)	3 (27.3) (6–61)	0	4 (57.4) (18.4–90.1)	1 (16.7) (0.4–64.1)	12 (46.2) (26.6–66.6)	0.01*

Category	Control group		MI-AVF group		MO-AVF group		SE-AVF group		Total		*p*
	Non-functional N=1	***Functional N=2***	***Non-functional N=1***	***Functional N=6***	***Non-functional N=3***	***Functional N=11***	***Non-functional N=1***	***Functional N=7***	***Non-functional N=6***	***Functional N=26***	
AVF created before dialysis initiation, *n* (%) (95% CI: LL–UL)	0	0	0	2 (33.3) (4.3–77.7)	0	5 (45.5) (16.8–76.6)	0	2 (28.6) (29–96.3)	0	9 (34.6) (17.2–55.7)	0.08
Venous diameter [mm], mean±SD	4.4	3.25±0.07	3.6	3.7±1.41	3.73±1.47	4.3±1.37	3.1	3.6±1.07	3.71±1.02	3.89±1.25	0.75

AVF: Arteriovenous fistula; MI: Mild morphological changes; MO: Moderate morphological changes; SE: Severe morphological changes; *N*: Number of patients by AVF outcome; SD: Standard deviation; *n* (%, 95% CI): Number (percentage with 95% confidence interval) of patients included in a given category; LL: Lower limit; UL: Upper limit; DM: Diabetes mellitus; AHT: Arterial hypertension; IC: Ischemic cardiomyopathy; PAD: Peripheral arterial disease; BMI: Body mass index. Note: Comparisons were made between total non-mature and mature AVFs; **p*-value statistically significant ($p<0.05$).

Discussion

Our study reported a high AVF maturation rate (41/50 [82%]) in patients with ESRD, regardless of the histopathological veins modification before AVF creation. This finding suggests that the veins with histopathological modifications such as IH, medial hypertrophy with a severity level from mild to severe at the time of AVF creation may not have a decisive influence on the AVF maturation. In addition, even if in some patients from the SE-AVF group, vein stenosis before AVF creation was recorded, this seems to not influence AVF maturation (of 14 patients with severe modifications, 12 [85%] had mature AVFs).

A medium vein, as the ones included in our study, is structurally composed of three layers: intima, media and adventitia. At intimal level, the elastic lamella of the veins was thin and often incomplete. Beneath the endothelial layer, were observed some collagen fibers. Frequently, the vein intima formed valves. The medial layer was composed of smooth muscle cells with a network loosely organized. The muscle fibers were poorly oriented in circular and longitudinal layers. Adventitial layers are the thickest of the three layers consisting mostly of elastic fibers and collagen, occasionally with smooth muscle cells [24–26].

Some studies deny the presence of the venous IH in patients with renal chronic insufficiency that need hemodialysis access [15]. Other authors consider the presence of the IH anterior to the surgery poorly understood [21]. The presence of the vein IH in ESRD patients could be related to dedifferentiation and migration of the smooth muscle cells in the subendothelial space because of the endothelial vascular dysfunction present prior to surgery. Smooth muscle cells proliferate and undergo a hypertrophic remodeling; they remain in the media of the vein as activated smooth muscles cells [21]. Skartsis *et al.* (2011) showed that the activated smooth muscle cells of the vein media participated to neointima formation after the AVF creation [27]. Surprisingly, there are other studies suggesting that the presence of the preoperative IH does not correlate with IH evolution at the AVF level. The separation between these two entities could not be artificial, and different production mechanisms are suggested by Vazquez-Padron & Allon (2016). More than that, the authors eliminate the role of preoperative IH in fistula maturation [21].

Medial fibrosis and medial smooth muscle cells disorganization, especially fibrosis, is related with the smooth muscle cells transformation into a myofibroblastic phenotype and a migratory phenotype that directly influence the IH at fistula level [14]. The mechanism by which fibrosis act on the distension and maturation of the fistula is insufficiently known. The correlation between the presence of medial fibrosis in veins used for AVF creation and AVF maturation and patency is still uncertain. This could be the subject for further investigations, designed to explain the remodeling following AVF creation.

Overall, in our study, IH, medial hypertrophy and medial fibrosis do not seem to have a predictive role in the evolution of the fistula, suggesting that further investigations are needed to complete the gap regarding vein pathological changes involved in AVF maturation and patency. Poor data is available in the published literature on vein histology in patients with ESRD and the published studies include a low number of patients.

Lee *et al.* (2014) suggest that the vascular injury caused by uremia and inflammation may influence the migration into the media and intima of adventitial fibroblasts. At intimal level, the fibroblast acquires a myofibroblastic phenotype. Additionally, it seems that smooth muscle cells from the media migrate into the intima, dedifferentiating into myofibroblasts [18].

The myofibroblast was discovered in 1971 on the electron microscopy images of granulation tissue. It is a cell present in the human body, normally, being identified as a participant cell, among others, in the repair processes. It is unclear how the myofibroblasts migrate into the intima of the vein, but myofibroblasts seem to be directly involved in the lesions that occur in the AVF, and it may become a possible therapeutic target [18]. In our study, the cellular phenotype of intimal cellular plaques shows the presence of cells with contractile phenotype, α-SMA, desmin-positive and vimentin-positive (Figure 3). Myofibroblasts seem to be the predominant cellular phenotype in IH of the vein anterior to the surgery. We are not able to demonstrate if the IH in the veins prior to surgery is the result of adventitial or of the medial cell migration. We think that further studies can explain the process with possible therapeutic target identification.

Despite different grades of pathological changes in veins specimens, the rate of AVF maturation ranged between 73% and 89% in patients with ESRD.

We also assessed if the baseline characteristics and clinical status may be related with the development of preexisting vascular pathologic changes in patients with ESRD, but no statistical significance difference was observed between MI-AVF, MO-AVF and SE-AVF groups when compared to the Control group ($p>0.05$), in terms of age, gender and clinical characteristics, suggesting that

histopathological modifications in patients with ESRD are not related to age, gender, DM, AHT, IC, PAD, cholesterol level, smoking status, BMI, AFV forearm location or if AVF was created after dialysis initiation (Table 2).

Furthermore, given that the preexisting histopathological vascular conditions did not seemed to influence AVF maturation and patency in our study, we assessed the role of age, gender, underlying diseases or other medical conditions that may interfere with the normal vascular response and make some patients more likely to develop non-mature AVFs. We found that patients with non-mature AVFs were more likely older (patients of 66.3±11.6 years old with non-mature AVFs *versus* patients of 58.3±10.4 years old with mature AVFs) and had a smaller venous diameter (3.09±0.49 mm and 3.84±1.13 mm in patients with mature and non-mature AVFs, respectively). Our results are in line with a study that showed a negative effect of the increased age on the AVF patency [28], but subsequent studies reported conflicting outcomes [29]. Interestingly, even if in our study were enrolled patients with venous diameter ≥2.5 mm, we found a negative correlation between veins of around 3 mm and AVF outcome, while previous studies reported that a venous diameter <2 mm have and influence on AVF patency [30]. We did not find any correlation between AVF maturation and gender (33.3% of women with non-mature AVF and 29.3% of women with mature AVF). Even if in women it is expected to have lower AVF patency because they have smaller venous calibers, Caplin *et al.* showed no difference between the arterial and venous diameter in man and women [31]. Our results are in line with this study and another study that had similar findings [32], but in contrast, Allon *et al.* (2011) reported an increase in non-maturation rate in women [15]. DM (33% non-mature *versus* 29% mature AVFs), AHT (89% non-mature *versus* 86% mature AVFs), IC (44% non-mature *versus* 29% mature AVFs), PAD (33% non-mature *versus* 17% mature AVFs) did not influence AVF maturation in our study, which is in line with the results published by Allon *et al.* (2011) [15]. However, other studies suggest that DM have an influence on AVF maturation, but its effect may be minimized by careful preoperative vessel imaging and AVF site selection [29]. Regarding the smoking status, previous studies reported higher incidence of AVF failure in smokers than non-smokers [33]. In our study, no statistically significant difference was recorded (22% non-mature *versus* 22% mature AVFs), but this finding is most likely due to the low number of smokers that were included in our study (two of nine patients with non-mature AVFs and nine of 41 patients with mature AVF). Regarding BMI, in our study BMI was 26.6±3.4 kg/m^2 in patients with non-mature AVFs *versus* 28.9±6.2 kg/m^2 in patients with mature AVFs (p=0.28) and no correlation between BMI and AVF outcome was detected. These results are in line with a previous study that showed no influence of BMI on AVF outcome in patients with a BMI of <27 kg/m^2 *versus* >27 kg/m^2 [34]. Regarding the AFV location on forearm (44% non-mature AVFs *versus* 39% mature AVFs) and whether it was created after dialysis initiation (44% non-mature AVFs *versus* 32% mature AVFs), according to our results no influence on AVF maturation was observed. Similar results have also been found by Allon *et al.* (2011) [15].

In addition, we assessed the two years primary patency in mature AVFs, and the factors that may influence their durability. We observed that patients with non-functional AVF were more likely to be women (67% non-functional AVF *versus* 24% functional AVF) and patients with functional AVF had most likely AVF located on the forearm (17% non-functional AVF *versus* 46% functional AVF). No correlation with age, DM, AHT, PAD and IC concomitant illness, cholesterol level, smoking status and creation after dialysis initiation was found (Table 3).

Our study has several limitations. Study duration and the criteria for participants' inclusion in the study permitted the inclusion of a limited number of patients within the defined study period. Given the high number of patients excluded from the study, especially for non-compliance with the study protocol, the sample size is insufficient to reach definitive conclusions about the effect of veins histopathological changes and preexisting medical conditions on the AVF outcome and two years primary patency of mature AVF.

Conclusions

In summary, our results suggest that in patients with ESRD, the veins with histopathological modifications such as IH, medial hypertrophy and smooth muscle cell disorganization and fibrosis used for AVF creation may not negatively affect AVF maturation. We support the involvement of myofibroblasts in the vein remodeling after AVF creation, which could constitute a future therapeutic target. AVF maturation failure is most likely related to age and venous diameter at the time of AVF creation, and its patency may be prolonged by the forearm location. However, we would suggest further studies to be performed on a bigger size population.

Conflict of interests

The authors declare that they have no conflict of interests.

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