

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

**A Microsurgical Arteriovenous Malformation Model
on the Saphenous Vessels in the Rat**

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**UNIVERSITY OF DEBRECEN
KÁLMÁN LAKI DOCTORAL SCHOOL**

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1. INTRODUCTION

AVMs are anomalous vascular structures and are made up of a draining vein and a supply artery. This configuration facilitates a high blood flow rate between the artery and vein with no intermediate capillary bed, creating a complex, entangled structure known as a nidus. Research suggests AVMs may develop during embryogenesis, specifically in the third week, when the primordial vascular system fails to evolve into a proper capillary network. Additionally, AVMs can emerge postnatally and exhibit growth following surgical resection or radiotherapy.

AVMs can manifest various symptoms based on their location. The high arterial pressure flowing through the nidus and draining veins makes these vessels prone to rupture, potentially leading to severe outcomes, including a considerable percentage (38-68%) of new cases exhibiting intracranial hemorrhage, disability, or death. Additionally, AVMs are associated with a range of neurological symptoms, from chronic headaches and migraines to seizures and strokes. AVMs can also occur outside the brain, affecting the spine, head, neck, limbs, and other organs.

The study of AVM hemodynamics and rheology is vital, as it is believed that alterations in these areas, especially in venous drainage, may contribute to the formation of AVMs. Chronic venous hypertension and elevated cerebral venous pressure can result from abnormalities in the cerebrovascular system, such as venous occlusions, narrowing, or absence, which can arise during embryogenesis or after birth. HIF, VEGF, and the angiogenic process can all be triggered by this illness, which can also reduce tissue perfusion. Postnatally, the venous blockage might reactivate dormant arteriovenous connections, potentially leading to AVM formation. AVM development appears to persist past the embryonic period, as evidenced by the process of spontaneous growth and regression. Factors such as chronic hypoxia, shear stress, exercise, hormonal changes, and direct trauma can influence AVM morphology, impacting these lesions irrespective of their anatomical position.

The existence of arteriovenous shunts of various sizes and locations can impact micro-rheological parameters. Past studies have shown a significant increase in red blood cell aggregation and reduced deformability, changes that continue to evolve in the weeks following the creation of artificial arteriovenous shunts, such as carotid-jugular, femoral, saphenous, and portocaval shunts. The size, shape, and location of the artificial shunt influence the extent of these changes. In the case of AVMs, which also vary in size, the specific size and vascular structure that led to both hemodynamic and hemorheological disturbances are not yet fully understood.

Diagnosis of cerebral AVMs often occurs incidentally or follows the investigation of acute cerebral hemorrhage, seizures, or chronic headaches. These malformations vary in size, location, and vascular structure, necessitating the use of grading scales for classification and management. AVMs are graded according to size, location, and venous drainage using the widely used Spetzler-Martin score system; a higher score denotes a higher risk of microsurgical excision. A 10-point scale that took into account variables including age and hemorrhagic appearance was added to the Spetzler-Martin system in 2010. Another grading system, the Toronto scale, predicts neurological outcomes post-resection.

Treatment approaches for AVMs vary based on their grading and include radiosurgery, microsurgical resection, and endovascular embolization. Surgical removal remains the preferred method for most lesions, though recurrence post-intervention is not uncommon, often due to reperfusion from recanalization or undetected vascular contributions. Despite significant advancements in research and clinical care, the management of AVMs continues to pose challenges, primarily due to the limited understanding of AVM behavior and progression, especially regarding the risk of rupture and aneurysm formation.

Various animal models have been employed to study the hemodynamics of AVMs, each with differing vascular arrangements. These include several anastomosis types between arteries and veins, radiosurgery, gene manipulation, and telangiectasia models. However, these models face challenges, including inaccurate AVM representation and limited data on their role in AVM rupture. Establishing a model that closely mirrors clinical AVM cases is crucial for better understanding their pathophysiology.

2. OBJECTIVES

1. Our main goal was to establish an arteriovenous malformation (AVM) model in rats that doesn't cause prompt significant hemodynamic and cardiac alterations but is feasible for follow-up of the AVM's progression.
2. In a 12-week follow-up period to study hemodynamic parameters, echocardiography variables.
3. To analyze micro-rheological and hematological alterations in the presence of the artificial AVM during the follow-up period.
4. At the end of the 3-month follow-up period we conducted a detailed morphological and histomorphological analysis of the AVM with a focus on vascular regeneration/remodeling processes.

3. MATERIALS AND METHODS

3.1. Experimental Animals

In compliance with national (Act XXVIII of 1998 on the protection and sparing of animals) and EU (Directive 2010/63/EU) regulations, the experiment was registered and approved by the University of Debrecen Committee of Animal Welfare and by the National Food Chain Safety Office (registration Nr. 25/2016/UDCAW, 25/2022/UDCAW).

In this investigation, sixteen female Sprague Dawley rats (10–12 weeks old, body weights: 293.21 ± 10.33) were used. A group with arteriovenous malformation (AVM, $n = 8$) and a sham-operated control group (Sham, $n = 8$) were randomly assigned to them. The rodents were housed in typical cages with free access to commercial rat chow and water, and they have exposed today and night light cycles every twelve hours.

3.2. Operative techniques

We ensured a sterile surgical environment for each rat by setting up sterilized instruments and keeping the operating space clean. A combination of 0.05 mg/kg atropine, 10 mg/kg xylazine, and 100 mg/kg of ketamine was administered intraperitoneally to anesthetize the rats. The rats also had an intravenous infusion of heparin at the recommended 80 IU/kg dosage to avoid thrombotic events. The lateral vein of the tail was fitted with a safe, 26-gauge cannula in the event that perioperative medication requirements existed.

The lower abdomen, inner thighs, and upper legs were shaved and treated with betadine for infection after anesthesia. Betadine and sterile gauze were used to prepare the surgery site carefully. A 3-centimeter incision was made obliquely over the right knee. The right saphenous veins and arteries in the AVM group were the targets of the intervention.

Their sizes ranged from 0.3 to 0.4 mm. Exact dissection was used to reveal the veins. Cutting the vascular was made easier by performing proximal and distal ligation of the vein and artery. The vein was cut farther than the artery to give room and flexibility for the next surgical steps. Heparin was used to clean the vessels, and they were then cautiously moved into their proper location.

A successful end-to-end anastomosis was achieved by using three 3/8 serosa (taper) needles with 10/0 non-absorbable monofilament polyamide-6 sutures (Daclon, SMI, Saint Vith, Belgium) simultaneously. Because of the vessels' small dimensions and propensity to collapse, this method was found to be less stressful and time-efficient. Without knotting, the first needle was used to position the first suture in both vessels at the 12 o'clock position. After that, the identical procedure was carried out again, this time inserting the needle into

the opposite end of the vessel at the six o'clock position, creating a wrapped shape. Midway between the first two entry locations was where the third needle was put next. The front wall of the anastomosis was sealed by tying and knotting the three threads after they had been secured. The vessels were then turned over, and the anastomosis was completed by tying a fourth knot in the center of the back wall.

After the anastomosis was finished, we checked for flow leakage. Any defects in the anastomosis were noted when the proximal microvascular clip was taken out of the venous side. In order to improve coagulation, subcutaneous fat may need to be temporarily placed on the anastomosis after the arterial microvascular clip was removed if no issues were detected. Anastomosis patency was assessed using the twofold occlusion test or the milking test. In order to close the skin, a continuous suture with a 3/8 cutting needle and 5/0 absorbable polyglycolic acid thread was used.

The sham-operated group had an identical skin incision made after anesthesia, which was followed by dissection. The rats were sedated for sixty minutes, which is the same amount of time needed for the anastomosis in the AVM group to be finished. Similar to the AVM group, the sham-operated group's skin closure was carried out.

Tramadol (15 mg/bwkg, i.p.) was administered for analgesic during the first three postoperative days. Regular surveillance and wound care were conducted over the 12-week follow-up period.

3.3. Ultrasound examination

On the third, seventh, and twelfth postoperative weeks, echocardiography was carried out using a high-frequency transducer (MX250, 14–28 MHz) connected to a Vevo 3100 Ultrasound Imaging System (Fujifilm VisualSonics Inc., Toronto, ON, Canada). Rats were given an intraperitoneal ketamine-xylazine solution (50/5 mg/kg) to induce anaesthesia. After shaving, the rats were put onto a VisualSonics SR200 platform, which monitors HR and the ECG in real time.

The American Society of Echocardiography's guidelines for evaluating cardiac function were followed. The following views were used in the construction of the database: B-, 2D-, M, Doppler modes; parasternal long and short axis; suprasternal (aortic arch); and apical four-chamber views (PSLAX, PSAX, SST, and A4C, respectively), B-, 2D-, M-, and Doppler modes. Using the VevoLAB programme (version 5.1, Fujifilm VisualSonics Inc., Toronto, ON, Canada), a blinded reader examined data offline after acquisitions. The

recorded ECG's R-R intervals during a 5-second period were automatically used to compute heart rate.

At the midpoint of the papillary muscles, wall thickness and chamber sizes were determined in M-mode using PSLAX and PSAX views. M-mode pictures were also used to calculate the maximal diameter of the left atrium (LA) in millimeters and the diameter of the aortic root (Ao) in millimeters. M-mode tracing in PSLAX view was used to assess the left ventricle's internal diameter in end-diastole (LVIDd, mm) and end-systole (LVIDs, mm), as well as the anterior and posterior LV wall thickness (mm, LVAWd, LVAWs, and LVPWd, LVPWs, respectively).

The software computed the left ventricular volume in end-diastole and end-systole (LVVOLD, LVVOLs, respectively; μL). The formula for calculating the left ventricular ejection fraction (LVEF, %) was $100 \times (\text{LVVOLD} - \text{LVVOLs}) / \text{LVVOLD}$. Automatic calculations were made using $\text{SV} \times \text{HR}$ for cardiac output (CO, mL/min) and LVIDd-LVIDs for stroke volume (SV, L).

Pulsed-wave Doppler (PW) and tissue Doppler imaging (TDI) from apical four-chamber views were used to assess diastolic function. PW Doppler echocardiography was utilised to calculate the E wave deceleration time (DecT, ms) and the ratio of the transmitral early (E) and atrial (A) peak flow velocities (E/A ratio). Using PW Doppler imaging in the left ventricular cavity, four parameters were measured: left ventricular ejection time (LVET, ms), isovolumic contraction time (IVCT, ms), isovolumic relaxation time (IVRT, ms), and c (MCOT, ms).

The formula used to calculate the cardiac performance index (MPI) was $(\text{MCOT} - \text{LVET}) / \text{LVET}$. Additionally, aortic flow (Ao) characteristics were evaluated using the modified suprasternal view in the PW Doppler mode. The programme was used to manually trace the boundaries of the Doppler jet before measuring the aorta velocity time integral (Ao VTI, mm), pressure gradients (Ao mean Grad, Ao peak Grad, mmHg), and aortic flow velocities (Ao mean Vel, Ao peak Vel, mm/s).

A modified PSLAX image was used to see the pulmonary vein. Measurements included pulmonary vein atrial reversal peak velocity (PV Ar, mm/s) and duration (PV ARdur, ms), as well as systolic and diastolic deflections (PV S, mm/s and PV D, mm/s). At the septal annulus, tissue Doppler imaging (TDI) was used to assess peak tissue velocities at systole (s' , mm/s), as well as in early (e' , mm/s) and late (a' , mm/s) filling. The ratio of e' to a' was used to define wall motion velocity. Additionally, the E/ e' ratio was established. Every metric was averaged over three cardiac cycles, and the results are shown as the mean $\pm$ S.D.

3.4. Hemodynamic measurements

In our study, we conducted hemodynamic measurements encompassing systolic and diastolic blood pressure and heart rate. For the twelve-week follow-up, we utilized an invasive approach, inserting a catheter into the left femoral artery. The equipment used was the LD-01 system from Experimetria Ltd., Hungary, with a G20 cannula serving as the catheter. This system allowed us to measure SBP, DBP, and heart HR. During the invasive measurements, we analyzed data spanning 10 seconds for each case. The average of the highest pressure readings was taken as the SBP value, while the average of the lowest pressure readings was used to determine the DBP value. Additionally, the HR value was calculated as the average of seven measured HR values. After the hemodynamic measurements, the animals were over anesthetized.

3.5. Blood sampling protocol

First, third, fifth, seventh, ninth, and twelfth postoperative weeks (PO1, PO3, PO5, PO7, PO9, and PO12) were among the times when blood samples were taken from the tail cannula, in addition to both before and after the procedure. For hematological and hemorheological measures, 300 μ l of the collected blood was obtained each time and deposited in Vacutainer tubes (BD Vacutainer® tubes, 5.4 g K3-EDTA, 3 ml). The rats underwent further postoperative weeks whereby the lateral tail vein was cannulated for the purpose of taking samples after they were sedated using the same anesthetic combination.

3.6. Hematological measurements

Hematological parameters were analyzed using the A Sysmex K-4500 automate (TOA Medical Electronics Co., Ltd., Japan). This analysis requires approximately 70 μ l of blood. The parameters evaluated included: white blood cell count (WBC [10^9 /L]), red blood cell count (RBC [10^{12} /L]), hemoglobin concentration (Hgb [g/L]), hematocrit percentage (Hct [%]), mean corpuscular hemoglobin (MCH [pg]), mean corpuscular volume (MCV [fl, femtoliter]), mean corpuscular hemoglobin concentration (MCHC [g/dl]), red blood cell size distribution (RDW-CV%), platelet count (Plt [10^9 /L]), and mean platelet volume (MPV [fl]).

3.7. Hemorheological measurements

The deformability of RBCs in our study was assessed using the laser diffraction ektacytometry technique. This method enables the measurement of RBC elongation under specific shear stress through laser diffraction patterns.

We use the LoRRca MaxSis Osmoscan, a device produced by Mechatronics BV in the Netherlands, also known as Lorrca® (Laser Optical Rotational Red Cell Analyzer). The test requires a highly diluted blood sample: 10 µl of blood is mixed with 2 ml of PVP in PBS, having a viscosity of 27 mPas, osmolarity of 300 mOsm/kg, and pH of approximately 7.3. RBCs are subjected to increasing levels of SS, and their elongation is measured across a range of shear stresses from 0.3 to 30 Pa. The results are depicted as EI-SS (elongation index - shear stress) curves.

An individual curve represents each sample, and comparisons between cases are made by analyzing these curves. We assess these curves using parameters such as the EI_{max} , the shear stress at half of the maximum elongation index ($SS_{1/2}$ [Pa]), and the ratio of EI_{max} to $SS_{1/2}$. These parameters are determined through Lineweaver-Burke analysis, using the formula: $1/EI = (SS_{1/2}/EI_{max}) \times (1/SS) + (1/EI_{max})$.

During the follow-up period, we studied RBC aggregation and RBC deformability. A Myrenne MA-1 erythrocyte aggregometer device (Myrenne GmbH, Germany) uses the light-transmittance method to express the aggregation index values in order to measure erythrocyte aggregation. The M1 5s and M1 10s aggregation indices were determined at a shear rate of 3 s^{-1} , and M 5s and M 10s were examined at a share rate of 0 s^{-1} .

3.8. Histological investigation

The heart and blood vessels were among the histology samples that were dissected under an operating microscope and preserved for a week in 4% formalin. The samples were embedded and sectioned (5 µm thickness) after fixation. The sections were then stained with H&E. The morphology of the heart was next examined using a Ventana DP200 scanner (Hoffman-La Roche, Roche Holding AG, Basel, Switzerland) in order to look for any structural or morphological variations between the two groups' cardiomyocytes and blood vessels.

Three different parts of the heart were measured: the basal, middle, and apex, to guarantee a comprehensive inspection of the histology slides. In this study, there were two groups: the control group (which included eight healthy hearts and twelve intact saphenous vessels) and the AVM group (which had eight hearts, twelve intact saphenous arteries, and eight anastomosed saphenous vessels).

Each animal's heart had its left ventricle's wall diameter measured, and each section's 100 cardiomyocytes were evaluated, yielding 12 wall diameters and 300 cardiomyocytes for each animal's heart. These measurements were made at several points around the left

ventricle. The cells were viewed horizontally, and their diameters were measured after zooming in.

Both the control and AVM groups removed saphenous vessels from the left non-operated leg and the right operated leg in addition to the heart analysis. The AVM group also performed an examination of the anastomosis location. The control group also had saphenous vessels taken from both legs. Samples of vessels were dissected in conjunction with the surrounding tissue in order to maintain their geometry. Ten measurements were made for each of the three layers—tunica externa, tunica media, and tunica intima—in each vessel. As a result, each histology slide had a total of thirty measurements.

With the aid of the 3DHistech Slide Viewer programme, which allowed for exact measurements, the histology slides of the hearts and blood arteries were assessed. The outcome's data were entered into an Excel document and thereafter examined.

3.9. Statistical analysis

The sample size was ascertained by applying Mead's resource equation approach. The significance level was chosen at $p < 0.05$, and the statistical analysis was performed utilizing GraphPad Prism 10 software. All data distributions had their normalcy examined. ANOVA tests in both directions, non-parametric tests like Wilcoxon or Mann-Whitney, and the Student t-test were used, depending on the findings. Also, for intra-group comparison Kruskal-Wallis and Dunn's method of ANOVA tests were applied.

4. RESULTS

4.1. Echocardiography

Preoperative and the third, seventh, and twelfth postoperative weeks were used for LV ultrasound evaluations.

In terms of the posterior wall's thickness during systole, the control group showed an initial decrease from 3.43 ± 0.41 mm to 3.13 ± 0.51 mm, then a rise to 3.57 ± 0.54 mm, followed by a drop to 3.24 ± 0.55 mm. The AVM group followed a similar trajectory, initially decreasing from 3.57 ± 0.54 mm to 3.33 ± 0.68 mm, then increasing to 3.46 ± 0.67 mm, and eventually decreasing to 3.09 ± 0.39 mm. Regarding the posterior wall's thickness during diastole, it remained relatively consistent throughout the duration of the study for both control and AVM groups.

During systole, the anterior wall thickness in the control group showed a reduction in the 3rd postoperative week, followed by a return to initial values later in the study. In the

AVM group, there was an initial decrease in anterior wall thickness from 3.72 ± 0.57 mm before surgery to 3.29 ± 0.32 mm in the 7th postoperative week, with an eventual increase to 3.47 ± 0.21 mm by the 12th week. Regarding the diastolic thickness of the anterior wall, the control group experienced a thickness peak in the 7th postoperative week, followed by a decrease in the 12th week. On the other hand, the AVM group showed only slight changes, maintaining a relatively steady thickness of around 2 ± 0.13 mm. None of the observed changes in anterior and posterior wall thickness during both systole and diastole, whether comparing between groups or within the same group before surgery and during the follow-up period, demonstrated statistical significance.

In terms of LV mass, the control group saw a slight increase from 863.15 ± 78.7 mg before surgery to 997.8 ± 143.4 mg in the 3rd postoperative week, with values remaining fairly stable thereafter. The LV mass in the AVM group remained consistently around 1000 ± 41.1 mg throughout the follow-up period. The aortic diameter exhibited an increase in both groups as the animals aged. Additionally, both groups' body weight increased by the 12th postoperative week as the rats aged (control group: $18.56 \pm 6.16\%$, AVM group: $16.56 \pm 3.22\%$).

Heart rate in the control group experienced a slight rise from 217 ± 40.5 beats per minute (min^{-1}) before surgery to 231 ± 36.32 min^{-1} in the 3rd week, followed by a decrease to 200.8 ± 14.68 min^{-1} by the 12th week. Conversely, in the AVM group, the heart rate started at 255 ± 53.66 min^{-1} and gradually declined significantly throughout the study to 164.2 ± 27.71 min^{-1} . These findings provide a comprehensive view of heart structure and function changes over time in both the control and AVM groups.

Ejection fraction in the control group commenced at $88.03 \pm 11.1\%$, experienced a 10-point drop in the 3rd week, peaked at $89.5 \pm 5.29\%$, and subsequently declined to $80.4 \pm 6.93\%$. In the AVM group, ejection fraction began at $89.85 \pm 10.21\%$ and decreased in the PO weeks to $84.09 \pm 11.37\%$, $84.07 \pm 10.82\%$, and $84.58 \pm 4.76\%$.

Regarding stroke volume, the control group exhibited a steady increase from 175.2 ± 32.8 μL , 198.7 ± 33.77 μL to 202.6 ± 48.91 μL , and to 203.5 ± 51.6 μL . In contrast, the AVM group initially increased from 192.4 ± 60.83 μL to 201.5 ± 33.94 μL , subsequently declined, and finally rose to 199.1 ± 51.61 μL .

In the control group, the E/A ratio peaked at 1.97 ± 0.36 in the 3rd week but dropped dramatically by the 7th week. By the end of the study period, it had returned to a normalized value of 1.75 ± 0.29 . The AVM group had varying values for this parameter during the follow-up period. Both groups exhibited minimal fluctuations in the LA/Ao ratio.

The peak gradient in the control group demonstrated minimal fluctuations. In contrast, the AVM group began at 2.7 ± 0.84 mmHg, increased to 3.17 ± 1.3 mmHg, experienced a slight decline to 2.93 ± 1.02 mmHg, and finally peaked at 3.44 mmHg.

In terms of deceleration time, the control group peaked in the seventh postoperative week at 67.08 ± 7.61 ms, but by the end of the study period, it had normalized to 54.5 ± 7.54 ms. On the other hand, the AVM group experienced no notable changes during the third post-operative week, a minor increase in the seventh week, and a peak of 61.8 ± 5.75 ms in the 12th week, following an initial value of 51.3 ± 6.11 ms before to surgery. The AVM group had a substantial increase in aortic mean velocity.

4.2. Weight and Hemodynamic parameters

Our findings revealed a minor decrease in the body weight of rats in the AVM group, possibly due to post-operative stress. However, rats regained and continued to gain weight during the 12-week follow-up. Both groups showed an increase in body weight over this period (Control group: from 299.2 ± 4.55 g to 355 ± 13.88 g, an increase of $18.56 \pm 6.16\%$; AVM group: from 287.25 ± 8.66 g to 334.89 g, an increase of $16.56 \pm 3.22\%$).

In the 12th week, prior to tissue sampling and extermination, tests on hemodynamic parameters (heart rate [bpm], systolic and diastolic blood pressure, and mean arterial pressure [mmHg]) did not reveal any significant differences.

4.3. Hematological parameters

The majority of the noteworthy alterations in the haematological parameters were noted during the PO1, when the red blood cell count, hematocrit, and haemoglobin concentration levels significantly decreased in both groups. Nonetheless, by the end of the PO12, these numbers returned to normal. When comparing the platelet count during PO5 to the preoperative status, the AVM group's was significantly greater than the control group's. Furthermore, even while both groups' leukocyte counts raised during surgery, the AVM group's leukocyte count continued to rise until the PO7. On the other hand, at the conclusion of the study period, the WBC count normalized in both groups.

4.4. Hemorheological alterations

The cumulative EI and SS curves for both the Control and AVM groups were presented. The EI values' standard deviation was minimal, resulting in curves that nearly coincided and exhibited no significant differences. A comparative analysis of the EI-SS

curves was provided. In the AVM group, there was a minor, non-significant reduction in EI at 3 Pa by the first postoperative week, accompanied by a moderate rise in $SS_{1/2}$, leading to a lower $EI_{max}/SS_{1/2}$ ratio. Apart from these observations, the values between the two groups were largely comparable. The variations in RBC aggregation indices throughout the study period. Observations of indices such as M 5s, M1 5s, M 10s, and M1 10s showed inconsistent changes over time, marked by fluctuations and broad individual differences, leading to significant data variance. In the AVM group, these values often saw an increase, remaining high until the 9th postoperative week, yet these variations were not statistically significant.

4.5. Morphology of the shunts

An overview of the vessel diameters was provided. Only the arterial side of the fistula showed a significant difference ($p=0.011$ vs. preoperative condition, $p=0.025$ vs. control side) by the 12th postoperative week, despite a noticeable increase in both the venous and arterial branches.

4.6. Histomorphology

At the end of the 12-week observation period, the heart wall and myocyte thicknesses in the basal, middle, and apical incision planes showed comparable changes, which were most effectively represented by their combined average. With respect to wall thickness, there was a notable divergence between the AVM and control groups ($p=0.0057$). In the AVM group, there was a significant rise in the average myocyte thickness ($p=0.0001$).

In none of the groups did the qualitative evaluation show any signs of cardiac fibrosis resulting from microvascular ischemia or subsequent cellular death. Cardiomyocytes in the AVM group were notably hypertrophic, with abundant eosinophilic cytoplasm, nuclei that resembled boxes, and occasionally unusual forms like Y-shaped branching. Myocardial disarray was evident in the architectural arrangement of the myocardium, where cardiomyocyte bundles were placed at perpendicular and oblique angles. When compared to the control saphenous veins, the tunica intima and media layers of the saphenous vein at the anastomosis location dramatically enlarged.

5. DISCUSSION

A comprehensive AVM model replicating human AVMs' anatomical, physiological, biological, and clinical characteristics has yet to exist. Even the transgenic mice model, though featuring spontaneous systemic vascular malformations, fails to fully mimic the sporadic nature predominantly observed in clinical settings. Despite these constraints, these diverse models have been instrumental in addressing specific research queries in AVM studies.

AVM is challenging to emulate surgically due to the intricate nidus linking arteries and veins. Nevertheless, approximating its physiological aspects is feasible by constructing conditions akin to AVMs, particularly using vessels of similar diameter. This approach enables the examination of hematology and hemorheology in the anastomosis, correlating it with AVMs. Surgical vascular anastomosis typically involves side-to-end, end-to-end, or end-to-side configurations. In model construction, anastomosis is not restricted to suture methods or peripheral vessels; many models utilize central AVFs employing alternative techniques. This choice is influenced by factors like the small size of peripheral vessels and the meticulous effort required for microscopic suturing. One such method is the puncture technique, where adjacent vessels are pierced to form a channel, maintained open by arterial-venous pressure differences. Other documented techniques include arterial insertion into veins, the cuff technique, and various anastomosis forms.

For our study, we selected the suturing technique to create anastomoses between the relatively small peripheral saphenous vessels, aiming for long-term observation with minimal complications. The two pivotal principles for anastomosis under microscopy are ensuring no tension on the anastomosis line and maintaining a moist working environment.

Certain tips can minimize frustration and time during surgery. Initially, it's essential to determine the correct incision site. Draw an imaginary line from the medial inguinal ligament to the knee joint; appropriate skin shaving reveals the saphenous vessels where the incision should be made in the mid-point. However, it's crucial to recognize that the saphenous vessels typically emerge after the epigastric vessels' origin. To avoid misidentification, atraumatic dissection should be used to trace these vessels precisely and carefully.

Once the vessels are located, check for any small muscular branches emanating directly from them. If present, either cut distally to these branches or ligate them. Planning subsequent steps is vital, particularly in deciding the anastomosis location. This decision should consider not only the vessels but also the workspace relative to surrounding structures. For example, cutting the saphenous vessel on its proximal half could restrict working space,

complicating the procedure and extending its duration. Therefore, we recommend a distal cut on the saphenous vessel. Given that the saphenous vein and artery run parallel, and the goal is a U-shaped anastomosis, it's advisable to cut the artery more distally than the vein. This positioning facilitates an easier end-to-end anastomosis with the vessels facing each other.

Anastomosing such small vessels is a complex task that requires thorough training and patience. If difficulties arise, particularly with vessel collapse after the initial suture placement, a technique involving multiple needles simultaneously can be beneficial. This method avoids finalizing knots immediately. Begin by using one needle to create the distal corner suture, leaving it untied initially while maintaining thread tension. Follow this with a second needle to secure the proximal corner. Again, do not tie a knot initially, but apply tension on the thread opposite to the first needle's direction. Then, use a third needle for the mid-section suture.

Once all three needles/threads are correctly positioned, proceed to knot them in sequence: the distal, the proximal, the middle. Keeping the long ends of the thread on the proximal and distal sutures can assist in flipping the vessels for the back wall knot. This staged approach helps manage the procedure's delicacy and ensures the anastomosis's stability.

Simulating AVM hemodynamics is crucial for understanding its pathophysiology and enhancing endovascular treatment methods. Typically, models comprise an arteriovenous shunt and a nidus. The swine rete mirabile, with its network of interconnected micro-arteries resembling an AVM nidus angiographically, has been a model of choice. Yet, its arterial structure differs from the arterial-to-venous nature of human AVMs. A temporary arteriovenous shunt was established between the rete, the internal carotid artery, and the cavernous sinus to overcome this. Despite its structural similarity to human AVMs, this model causes several side effects in animals, such as proptosis and sub-conjunctival hemorrhage. It remains viable for about a week before spontaneous occlusion. While not located in the brain, this model is invaluable for refining endovascular techniques and testing new embolic materials.

Hemodynamic models are crucial for investigating aspects such as perfusion pressure breakthrough, venous hypertension, and thrombosis in AVMs. Rats are commonly used for these studies. These models typically involve an anastomosis between the common carotid artery and the external jugular vein in the neck region. Here, the common carotid artery serves as the feeding artery, and the external jugular vein becomes the arterialized vein. For instance, one model connects the rostral carotid artery to the caudal jugular vein, leading to

intracranial hypoperfusion similar to the "steal" phenomenon in AVMs, which is useful for studying chronic ischemia effects.

One of the earliest models using CJF for studying AVMs, was initially introduced by Spetzler and colleagues. Their work focused on examining the NPPB theory, which is associated with swelling and hemorrhaging in brain tissue surrounding AVM lesions after surgical intervention. Developed in cats, this model involved creating an anastomosis between the common carotid artery CCA and EJV. The purpose of this anastomosis was to induce non-infarction cerebral hypoperfusion by reversing blood flow from the circle of Willis. Despite its innovative approach, subsequent research reported only minimal and temporary changes in cerebrovascular hemodynamics following CJF formation, casting doubts on the model's effectiveness in fully exploring the underlying mechanisms of NPPB.

Morgan et al.'s carotid jugular fistula model, which involved an end-to-end anastomosis between the common carotid artery and the external jugular vein, was specifically employed to study hypoperfusion. This model's integration of intracranial arterial and venous systems with extracranial venous systems is a notable characteristic. Moreover, Morgan and his team developed an altered CJF model in rats, involving an end-to-end anastomosis of the rostral ends of both CCA and EJV. This model demonstrated a notable decrease in cerebral blood flow (CBF) on the side of the fistula over a period of 8-12 weeks. Sealing the fistula resulted in a significant increase in CBF, leading to the breakdown of the blood-brain barrier in hypertensive conditions. Rats were selected for this model primarily due to their cost-effectiveness and availability despite their anatomical variances from humans. Efforts have been made to replicate CJF models in monkeys, but these are fraught with challenges related to management, high costs, and complex ethical issues.

In some models, the anastomosis involves a side-to-end connection between the common carotid artery and the rostral external jugular vein. In this configuration, arterial blood is directed through the external jugular vein and its branches, functioning as the "nidus". The blood then exits through the transverse sinus and the contralateral jugular vein, which acts as the draining vein. This model has been instrumental in exploring the molecular and morphological alterations in AVMs following radiosurgery treatments. Notably, it generates an endothelial phenotype similar to that observed in human AVMs, vital for examining the varied endothelial responses to radiation across different phenotypes. Additionally, it has been observed that blood flow in this model increases over time, persisting at least up to 42 days post-surgery. In many AVM models that purport to replicate human AVM blood flow, the location is typically extracranial rather than intracranial.

We aimed to develop an AVM model that avoids the immediate and substantial hemodynamic and cardiac changes seen in other arteriovenous shunt models with larger calibers. Working with rat saphenous vessels requires advanced microsurgical techniques, yet this model has the potential to replicate a small, asymptomatic AVM. This offers an opportunity to delve into the factors influencing AVM progression, enlargement, and destabilization.

AVMs, with their complex network of feeding arteries and draining veins, are known to cause a range of symptoms, sometimes leading to severe neurological complications, disabilities, or even death due to hemorrhage. Therefore, there's a critical need for an effective AVM model that closely mirrors human AVMs in anatomical, physiological, biological, and clinical aspects to enhance our understanding of their pathogenesis, behavior, and treatment responses. A major drawback of most arteriovenous shunt models is the immediate impact on hemodynamics and cardiac function following arterial and venous connections. These models fail to represent the 'silent', asymptomatic AVMs and cannot track their gradual enlargement and progression.

Our study findings indicated that in the AVM group, hematological and hemorheological parameters remained largely unchanged compared to the sham-operated control group. However, likely due to acute phase reactions, there was an increase in leukocyte count in the early postoperative phase. A slight decline in red blood cell count, hematocrit, and hemoglobin was also noted, a trend observed in the sham-operated group as well. Factors such as repeated anesthesia during follow-ups for sampling, immobilization, recovery, blood sampling, and surgical procedures (like skin incisions, tissue handling, suturing, and wound healing) in both groups could account for these variations. This includes the irregular, minor rise in RBC indices and the temporarily moderate reduction in RBC deformability.

The hemorheological changes were more pronounced and significant in other rat models employing artificial arteriovenous shunts in larger vessels. For instance, in the carotid-jugular shunt model, which is technically simpler than the recent saphenous AVM model, RBC deformability was notably compromised by the end of a 6-week observation period. Mechanical stability tests revealed significantly lower deformability under stress than the sham-operated group, indicating that this model may not be suitable for studying AVM progression due to its immediate and substantial hemodynamic and hemorheological effects. The carotid-jugular fistula model was originally intended to monitor shunt maturation, not as an AVM model.

In another rat model, the superficial inferior epigastric vein was used to create an arteriovenous connection in the femoral region. Here, hematocrit, red blood cell count, and hemoglobin levels significantly dropped by the first postoperative week, and RBC aggregation notably increased by the fifth week. In these cases, the underlying causes of micro-rheological disturbances include acute phase-related immune/inflammatory processes, changes in oxygenation levels (dependent on the caliber and extent of blood mixing), and altered shear stress at and near the shunt sites.

The cardiac echography results from our study could be partially attributed to the aging of the animals, as changes were also noted in sham-operated controls. Moderate yet progressive changes were observed in cases of slowly developing and enlarging AVMs. By the 12th postoperative week, significant histological changes were evident in heart samples. The thickening of the shunt vessels' walls was consistent with findings in other arteriovenous shunt models of varying locations and sizes. For example, carotid-jugular fistula models showed immediate hemodynamic alterations and significant left ventricular hypertrophy within six weeks. Similarly, arteriovenous fistulas with different geometries in the femoral region led to notable hemodynamic shifts after five weeks, with vessel histology showing uneven thickening due to varying intraluminal pressure and wall shear stress. Asymmetric shear stress distribution correlated with spots of intima hyperplasia. These vascular wall changes are attributable to altered local blood flow and shearing forces, as supported by numerous studies.

Interestingly, the changes observed were neither uniform nor continuously progressive across all parameters. Adaptation mechanisms in hemodynamics and cardiovascular function, along with the animals' aging and growth, might play a role.

AVF models have demonstrated a consistent increase in heart and lung weights proportional to the fistula's diameter. The impact of arteriovenous fistulas on body weight varies and is influenced by the fistula size and the post-surgery follow-up duration. Rapid weight gain post-surgery often indicates fluid retention. Some studies suggest that an increase of 50 grams or more within seven days is indicative of severe heart failure.

Our results align with other studies showing a temporary post-surgery weight decrease followed by normal weight gain, with no significant difference from the control group after a month. Unlike other models, the arteriovenous fistula in this study did not result in significant left ventricular hypertrophy compared to the control group. This, combined with the high survival rate and lack of evident cardiac pathology, suggests that using saphenous vessels effectively avoids systemic circulatory effects.

The histological examination showed no evidence of myocardial fibrosis due to microvascular ischemia or resultant cellular death in any group. In the AVM group, the cardiomyocytes displayed hypertrophy, characterized by abundant eosinophilic cytoplasm, square-shaped nuclei, and at times, unusual shapes like Y-shaped branching. The structural organization of the myocardium was disrupted, as observed by the disordered arrangement of cardiomyocyte bundles, positioned perpendicularly and obliquely to each other, suggesting myocardial disarray. An additional observation was intimal hyperplasia in the venous limb of the fistula in the operated group, as opposed to the non-operated vein in the sham group, which was supported as well by the diameter of the operated artery and vein compared to the control group at the 12th week post-operatively. The diameter of the operated arteries at 12th post-operative week were significantly larger than the diameter of the same arteries pre-operatively and compared to the control group at 12th week post-operatively. This model is therefore recommendable for studies investigating the rheology and hematology of AVMs, which can contribute to predicting AVM behavior.

Observing the systemic circulation and cardiac changes in carotid jugular fistula cases, we found contrasting results in our saphenous AVM model. Unlike the former studies, where significant alterations were evident after 6 weeks, in our study the rats were followed up for 12 weeks, and we observed no notable impact on systolic or diastolic function. In contrast to the structural changes seen in the left ventricle and reductions in ejection fraction and fractional shortening reported in the other articles, our murine AVF model did not replicate these findings.

Specifically, while the heart rate and systolic blood pressure significantly decreased in the CJF group, our study did not reveal similar effects. Moreover, our comparative morphological analysis did not indicate significant increases in left ventricular hypertrophy based on echocardiogram examinations. Additionally, the EF reduction observed in the AVF group in the previous studies was not replicated in our model. Left ventricular perivascular staining did not show a notable increase in fibrosis in the LV vessel areas compared to the sham-operated group as reported in the other articles.

6. Main Findings and Conclusions

1. We could successfully establish a small-caliber AVM model in rats using the saphenous vessels.
2. During the 12-week follow-up period, shunts remained unobstructed. Systolic, diastolic, mean arterial pressure, and heart rate values slightly and non-significantly decreased in the AVM group. Echocardiogram results indicated minor systolic function impact, with slight and insignificant changes in aortic pressure and blood velocity, and minimal left ventricular wall enlargement. The small-caliber saphenous AVM model does not cause acute hemodynamic changes.
3. Moderate but progressive vascular and histological alterations, as well as venous dilatation, confirmed AVM-like features.
4. Compared to the sham-operated control group the AVM group did not show important alterations in hematological parameters nor in erythrocyte aggregation and deformability. However, slightly increased aggregation and moderately decreased deformability values were found, without significant differences. The changes normalized by the 12th postoperative week. The alterations found were most likely related to the acute phase reactions and not to the presence of a small-caliber shunt.
5. The model seems to be suitable for studying further the progression, enlargement, or destabilization of AVM.

7. Certified publication list



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Doctoral School: Kálmán Laki Doctoral School

List of publications related to the dissertation

1. **Al-Smadi, M. W.**, Fazekas, L., Varga, Á., Mátrai, Á. A., Aslan, S., Beqain, A., Al-Khafaji, M. Q. M., Baráth, B., Novák, L., Németh, N.: Minor micro-rheological alterations in the presence of an artificial saphenous arteriovenous shunt, as an arteriovenous malformation model in the rat. *Clin. Hemorheol. Microcirc.* [Epub ahead of print], 2024.
DOI: <http://dx.doi.org/10.3233/CH-231825>
IF: 2.1 (2022)
2. **Al-Smadi, M. W.**, Fazekas, L., Aslan, S., Bernát, B., Beqain, A., Al-Khafaji, M. Q. M., Priksz, D., Orlik, B., Németh, N.: A Microsurgical Arteriovenous Malformation Model on Saphenous Vessels in the Rat. *Biomedicines*. 11, 1-14, 2023.
DOI: <http://dx.doi.org/10.3390/biomedicines11112970>
IF: 4.7 (2022)

List of other publications

3. Aslan, S., **Al-Smadi, M. W.**, Kozma, I., Viola, Á.: Enhanced Precision and Safety in Thermal Ablation: O-Arm Cone Beam Computed Tomography with Magnetic Resonance Imaging Fusion for Spinal Column Tumor Targeting. *Cancers (Basel)*. 15 (24), 1-12, 2023.
DOI: <http://dx.doi.org/10.3390/cancers15245744>
IF: 5.2 (2022)
4. **Al-Smadi, M. W.**, Kozma, I., Aslan, S., Bölöni, B., Viola, Á.: Percutaneous Superimposed O-Arm-MRI-Navigated Biopsy for Spinal Column Pathologies. *Diagnostics*. 13 (13), 1-9, 2023.
DOI: <http://dx.doi.org/10.3390/diagnostics13132252>
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5. Szabó, B., Gasz, B., Fazekas, L., Varga, Á., Kiss-Pápai, L., Matolay, O., Rezsabek, Z., **Al-Smadi, M. W.**, Németh, N.: Heterogeneous Maturation of Arterio-Venous Fistulas and Loop-Shaped Venous Interposition Grafts: a Histological and 3D Flow Simulation Comparison. *Biomedicines*. 10, 1-14, 2022.
DOI: <http://dx.doi.org/10.3390/biomedicines10071508>
IF: 4.7
6. Szabó, B., Tanczos, B., Varga, Á., Baráth, B., Ghanem, S., Rezsabek, Z., **Al-Smadi, M. W.**, Németh, N.: Micro-rheological changes of red blood cells in the presence of an arterio-venous fistula or a loop-shaped venous graft in the rat. *Front. Physiol.* 11, 1-12, 2020.
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