Analysis of spinal cord blood supply combining vascular corrosion casting and fluorescence microsphere technique: A feasibility study in an aortic surgical large animal model

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Abstract

Introduction: Spinal cord ischemia after cardiovascular interventions continues to be a devastating problem in modern surgery. The role of intraspinal vascular networks and anterior radiculomedullary arteries (ARMA) in preventing spinal cord ischemia is poorly understood.

Materials and methods: Landrace pigs (n = 30, 35.1 ± 3.9 kg) underwent a lateral thoracotomy. Fluorescent microspheres were injected into the left atrium and a reference sample was aspirated from the descending aorta. Repeated measurements of spinal cord and renal cortical blood flow from the left and right kidneys with three different microsphere colors in five pigs were taken to validate reproducibility. Spinal cord blood flow to the upper thoracic (T1–T4), mid-thoracic (T5–T8), lower thoracic (T9–T13), and lumbar (L1–L3) levels were determined. After euthanasia, we carried out selective vascular corrosion cast and counted the left and right ARMAs from levels T1–T13.

Results: Blood flow analysis of the left and right kidneys revealed a strong correlation ($r = .94, p < .001$). We detected more left than right ARMAs, with the highest prevalence at T4 ($p < .05$). The mean number of ARMAs was 8 ± 2. Their number in the upper thoracic region ranged from 2 to 7 (mean of 5 ± 1), while in the lower thoracic region they ranged from 0 to 5 (mean of 3 ± 1 [$p < .001$]).

Conclusions: This study shows that combining fluorescence microsphere technique and vascular corrosion cast is well suited for assessing the blood flow and visualizing the arteries at the same time.

Keywords
collateral blood flow, fluorescence microspheres, spinal cord blood flow, spinal cord vasculature, vascular corrosion casting, watershed infarction
1 | INTRODUCTION

1.1 | Microspheres

Since its introduction by Rudolph and Heymann in 1967, microsphere technique has undergone continuous development (Rudolph & Heymann, 1967). Today, it is the gold standard for measuring regional organ perfusion. Microspheres for blood flow studies are typically 15-μm-diameter particles labeled with colored, radioactive, or fluorescent substances. When injected into the left atrium, they mix into the central circulation and trigger microembolization in small capillaries (“trapping”). Blood flow is proportional to the number of microspheres in the region of interest. Following introduction of the reference sample method, it became possible to calculate absolute blood flow in ml/min/g by comparing the number of microspheres in the reference sample, aspirated at a predefined rate downstream to the injection site, with the number of microspheres in the region of interest (Malik, Kaplan, & Saba, 1976). However, the radioactive microspheres that were first introduced were hazardous for both humans and animals because of the radiation burden. Their expense, especially due to their high disposal costs and large animal experimental models, led to new methods (Prinzen & Bassingthwaighte, 2000). Fluorescent microspheres have the advantage of great accuracy, very good spectral separation, high reliability, and low-cost compared to radioactive microspheres (Glenny, Bernard, & Brinkley, 1993; Van Oosterhout, Willigers, Reneman, & Prinzen, 1995).

1.2 | Vascular corrosion casting and spinal cord anatomy

Vascular corrosion casting has a long history in describing the morphology of vessels and visualizing small vessels that remain otherwise undetectable by the human eye. With the invention of low viscosity resin in 1970, it became possible to study the microvasculature and distribution of small vessels (Bielke, Nagle, Trump, & Bulger, 1976; Dollinger & Armstrong, 1974; Fujita & Murakami, 1973). Modifications in resin’s viscosity helped to obtain highly detailed vascular castings. In combination with scanning electron microscopy, this method can provide a precise image of the endothelial surface of the vessels (Murakami, 1971).

In contrast to these advantages, there are certain sources of error that can affect the casts’ reliability. Although modern polymers have improved the quality of casts, there is still some shrinkage. For example, the average shrinking of the polyurethane-based resin (the same one that we used in our experiments) is reported to be 6.8% after one week (Krucker, Lang, & Meyer, 2006). Furthermore, extravasation and changes in the surface and surrounding tissue have been mentioned (Aharinejad et al., 1990). However, these observations have not been made with the resin we used (Krucker et al., 2006). Although combining the fluorescence microsphere technique and vascular corrosion cast is a useful method to describe the anatomy of the vasculature and determine the tissue perfusion in the same model, no simultaneous usage has been reported to our knowledge.

We are the first to combine fluorescence microsphere technique and vascular corrosion casting in an experimental porcine model to determine spinal cord perfusion and visualize anterior radiculomedullary arteries (ARMA). The ARMA are branches from segmental intercostal arteries supplying the anterior spinal artery, and vary in number and distribution. Thirty-one somites are formed during embryological development and receive blood from the corresponding segmental arteries through ARMA, most of which degenerate, and only 4–8 of them remain feeding the anterior spinal artery (Bosnia, Hogan, Loukas, Tubbs, & Cohen-Gadol, 2015). They are, therefore, crucial for supplying adequate blood flow to the anterior two-thirds of the spinal cord and thus motor functions. Our group’s recent investigations suggest that ARMA play a key role in preventing ischemia after cardiovascular surgery interventions (Kari et al., 2017). The Collateral Network Concept introduced by Griep describes intraspinal vascular networks that can prevent acute ischemic conditions if segmental arteries become occluded (Griep & Griep, 2010). ARMA in this case connect the intraspinal collateral system and extraspinal vessels with the anterior spinal artery, thus their number and the maximum distance between them could be an important preoperative risk predictor in aortic surgery (Kari et al., 2016a; Kari et al., 2016b). Figure 1 illustrates the blood supply’s schematic to the spinal cord.

2 | MATERIALS AND METHODS

This study was conducted at the University Medical Center Freiburg, Freiburg, Germany. Institutional Review Board approval was obtained before beginning any experiment. The study animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals and in compliance with the guidelines established by the local German government (Protocol number G 14/39). An experienced veterinarian carried out anesthesia, pain control, perioperative monitoring, and euthanasia.

2.1 | Preparation for surgery

The pigs were housed in ventilated rooms and fasted 18 hr before surgery. Water was provided ad libitum. Premedication was performed with an intramuscular injection of ketamine (20 mg/kg) and 0.5 mg/kg of body weight (BW) midazolam. An 18 G intravenous cannula was inserted into an ear and anesthesia was deepened with propofol (2–4 mg/kg BW) intravenously (i.v.). Orotracheal intubation was carried out with a 6.5 Fr tracheal tube. Adequate ventilation and oxygenation was ensured by ventilation with a positive end-expiratory pressure of 5 cm H2O, respiratory frequency of 12–14 min⁻¹, and a tidal volume of 8 ml/kg BW. Anesthesia was maintained with isoﬂurane 1.5–2% in O2/room air (FiO2 = 0.6) in combination with fentanyl (5–10 μg/kg/hr) and vecuronium (0.2–0.4 mg/kg/hr). Electrocardiogram, pulse oximetry, and temperature monitoring was performed. Vet ointment was used on eyes to prevent dryness under anesthesia. Adequate pain control was carried out with fentanyl (5–10 μg/kg/hr) i.v. and heart rate and pain reactions were monitored. Under sterile conditions, the common carotid artery
and external jugulary vein were dissected free using scissors, and cannu-
lated with three-French-catheter using the Seldinger technique
(Seldinger, 1953). This step was taken to monitor central venous and
mean arterial pressure via pressure transducer and amplifier.

2.2 | Microsphere injection

While maintaining sterile conditions, we carried out a left posterolat-
eral thoracotomy by an incision in the 5/6 intercostal space using a
#10 scalpel blade for the initial incision. The situs was opened with
scissors and fingers. The parietal pleura was opened by an incision
and the intercostal nerves were anesthetized by injecting 1–2 ml
mepivacaine (2%, 20–40 mg). The situs was opened by introducing a
rib spreader. Subsequently, we dissected the thoracic aorta free using
scissors, tweezers, and fingers, and introduced a 3-French-catheter
into the aorta to withdraw microsphere reference samples. Finally, we
connected a three-way-stop-cock for blood sampling.

The pericardium was opened using a scissor and a 14-G-cannula
was inserted into the left atrium through the left atrial appendage.

FIGURE 1  (a) Schematic illustrations of the blood supply to the intraspinal and paraspinal vascular system of the spinal cord. Branch points of
segment arteries connect the paraspinal with the intraspinal system and consecutive intraspinal systems. ARMAs vary in number and distribution
and connect the intraspinal and paraspinal system with the anterior spinal arteries. The paraspinal system is the "sleeping reserve" of blood supply
activated by arteriogenic stimuli. It serves as a long-term back-up system, as opposed to the intraspinal collateral system, which is the spinal
cord's emergency back-up system as described in reference (Meffert et al., 2014). Cervicothoracic and lumbosacral inflows to the spinal cord are
parts of the Collateral Network Concept (Griep & Griep, 2010). (b) Watershed = Classical Watershed areas of the poor collateralized mid-
thoracic spinal cord at T4/T5 and T8/T9 (Cheshire, Santos, Massey, & Howard, 1996; Shamji, Maziai, Shamji, Ginsberg, & Pon, 2003; Zülch,
1976). GARMA, great anterior radiculomedullary artery [Color figure can be viewed at wileyonlinelibrary.com]
The cannula was secured with a 4-0-prolene suture for microsphere injections. We rinsed with approximately 10 ml saline to maintain patency for microsphere injection.

Calculation of the minimum number of microspheres to be injected was performed using the formula:

\[
N_{\text{min}} = 400 \times \frac{n}{Q_{\text{organ}}/Q_{\text{total}}}
\]

where, \(N_{\text{min}}\) = minimum number of microspheres required for the injection, \(n\) = total number of organ pieces, \(Q_{\text{organ}}/Q_{\text{total}}\) = fraction of total cardiac output supplying the organ of interest.

There should be a minimum number of microspheres in the region of interest to ensure highly accurate measurements (Buckberg et al., 1971). Here, 2.5 million microspheres were used for the injection. The vials containing the fluorescent microspheres were stored in a refrigerator at 2–8°C and protected from light. We vortexed the fluorescence microsphere vials containing 10 ml solution (1 million microspheres per ml) for 20 s and placed them in a cold ultrasonic water bath for 5 min. Because the heat generated might damage the microsphere particles, one should not leave them in the ultrasonic bath for too long. Of note, 2.5 ml (2.5 million) of microspheres was diluted with 7.5 ml sodium chloride in a 10-ml plastic syringe. Injection was carried out immediately after aspiration into the syringe, because aggregation of microspheres leads to inaccurate measurements. The microsphere solution was injected into the previously introduced left atrial cannula at a steady injection rate lasting 60 s. Injection was only performed in hemodynamically stable pigs to ensure good microsphere distribution in the cardiovascular system and accurate measurements. The reference blood sample was aspirated through the aortic catheter with a withdrawal pump at a predefined aspiration rate of 4.55 ml/min into a 20-ml syringe. Aspiration was started 15 s before the microsphere injection, and continued for an additional 180 s for a total of 195 s. This step guaranteed that all microspheres were "trapped" in the tissue and reference sample, and could be used to calculate blood flow.

The blood samples were transferred into tubes. The syringe was rinsed with 5 ml 2% Tween 80 solution and the solution was added to the tube.

These steps were performed with fluorescent microspheres of different colors that do not exhibit spectral overlap of excitation and emission wavelengths to obtain blood flow at different time points. The time points can be chosen according to the experimental setup. Up to seven different microsphere colors can be used in the same model without performing correction calculations (Schimmel, Frazer, & Glenny, 2001). Euthanasia was performed using thiopental (20 mg/kg BW i.v.), potassium (5 mmol/kg BW i.v.), and exsanguination by cutting the inferior vena cava under isoflurane anesthesia.

### 2.3 Vascular corrosion casting

Immediately after sacrifice, the animals were placed in supine position. A 14-French-catheter was placed in the descending aorta, fixed with a 4-0-prolene suture and flushed with 500 ml saline with heparin.
50 mg blue vascular casting pigment was mixed in 50 g casting resin until a dark blue solution was obtained. The dilution solution (74.1 ml ethanol, 10 ml 2-propanol, and distilled water in 100 ml solution) and 5 g hardener were mixed into the resin. The amount of casting material and solvent needed for injection depends on the experimental setup. Ethylmethylketone or dichloromethane can also be used as a dilution solution. A dilution of up to 40% is recommended. The prepared casting material was drawn into a 50-ml plastic syringe and immediately injected manually with high pressure into the aortic catheter. The use of an injection apparatus enables pressure monitoring. A physiological pressure of 90–120 mmHg is recommended. During injection, a high pressure was maintained by closing the catheter manually. After the injection, the operating table was adjusted for a better distribution into the organ of interest.

2.4 | Autopsy

Autopsy was performed the day after the vascular corrosion casting procedure. The pigs were stored in a freezer overnight at approximately −10°C. The animal was placed in prone position and a longitudinal incision was made above the dorsal spine processes. A midline incision was carried out from the cervical region to the sacrum using a #10 scalpel blade. The paraspinal muscles were dissected off the vertebral column and the spinal cord was exposed via laminectomies using a bone Rongeur. After removing fatty tissue in the spinal canal using an anatomical tweezer, the ARMA from segments T1 to T13 were counted. The segments were identified through the origins of the spinal nerves.

The spinal cord was dissected at each segment for blood flow analysis using a disposable microtome blade, and the tissue was put in 15-ml polypropylene tubes. The dissection was performed in the middle of two consecutive spinal nerves. No polyethylene tubes were used because the digesting solution used in the tissue processing would also digest the tubes.

The left and right kidneys were exposed taking a posterior surgical approach between segment T12 and the iliac crest using a scalpel and scissors. The incision was deepened through the latissimus dorsi muscle, and fat and parts of the lumbodorsal fascia were removed with a tweezer until the renal fossa was reached. After the incision into the renal fascia with a scissor, the kidneys were dissected free using a scissor and fingers, and removed. This step was performed after removing the paraspinal muscles. The outer renal cortical part of the left and right kidneys was dissected using a #11 scalpel, and was put into the tubes for blood flow analysis. The tubes were stored in the dark at room temperature. This step was performed to validate microsphere distribution and reproducibility of blood flow analysis due to the simultaneous usage of casting material in the same tissue.

2.5 | Tissue processing

Blood and tissue samples were processed via a modified sedimentation technique for lipid-rich tissues (Powers, Schimmel, Glenny, & Bernards, 1999). The samples were allowed to rest for 2 weeks in the dark at room temperature (18–22°C) for autolysis to occur. After 2 weeks, 7 ml of 2.3 M KOH with 0.5% Tween 80 was placed into each tube. Afterwards the tubes were vortexed for 20 s, and placed in a 50°C water bath for 48 hr. After 48 hr, the tubes were centrifuged at 2,000g for 20 min at 20°C. The microspheres were pelleted at this step. The supernatant was removed until there is a volume of 1 ml. Next, 7 ml of Triton X-100 was added and the tubes were vortexed again. Subsequently, the tubes were centrifuged at 2,000g for 20 min at 20°C and the supernatant was discarded until a volume of approximately 1 ml was obtained. To neutralize KOH, 7 ml of dilute buffer (5.88 g K2HPO4 in 200 ml distilled water and 22.9 g K2HPO4 in 800 ml distilled water; combine the solutions) was added and the tubes vortexed again. Following the next centrifugation at 2,000g for 20 min at 20°C, all but approximately 150 μl of the supernatant was removed. This step is crucial and should be carried out very carefully to minimize microsphere loss. Finally, exactly 3 ml of 2-ethoxyethylacetate was added and the tubes were allowed to rest for 5 days in the dark at room temperature. This step released the fluorescent dyes from the microsphere particles. An exact volume of 2-ethoxyethylacetate is crucial because the fluorescence-intensity measurements depend on the concentration. The tube should be placed in the dark, because the fluorescent dyes are no longer bound to the particles and the fluorescence intensity will weaken due to light exposure ("quenching").

After the last centrifugation step at 2,000g for 20 min at 20°C, the supernatant was taken out and the fluorescence intensity was measured in the spectrometer. A less time-consuming wavelength program was used to take repeated measurements at different excitation and emission wavelengths according to the microsphere colors used in this experiment.

Excitation (Ex) and Emission (Em) wavelength of red, green, and yellow fluorescent microspheres: Red (Ex/Em), green (Ex/Em), yellow (Ex/Em) = 568/595, 455/482, 508/538, respectively.

Regional blood flow (ml/min/g) was calculated using the following formula:

$$RBF = \frac{Ft}{F_{ref}} \times \frac{R}{g}$$

where RBF: regional blood flow; Ft: fluorescence intensity of tissue sample; Fref: fluorescence intensity of reference sample; R: withdrawal rate of pump; g: tissue weight in grams.

2.6 | Statistical analysis

Statistical analysis was performed using the SPSS software ver. 22.0 (IBM, Armonk, NY). Values were displayed as means ± SD. The student’s t test was used to assess the difference between means. Difference between the three time points were assessed using repeated measures ANOVA. A p value less than .05 was considered statistically significant.
3 | RESULTS

3.1 | Blood flow analysis

We observed no alterations in the blood-flow analysis results and background fluorescence noise when using corrosion-casting material in the same tissue. To prevent background fluorescence with the combined technique, the microsphere colors and inherent background fluorescence of the casting pigment must not exhibit spectral overlap of their excitation and emission wavelengths. Furthermore, spectral overlap with the solvents used for digesting the tissue must be excluded.

Regional blood flow values are shown in Figure 2. Blood flow to the mid-thoracic region in the spinal cord was less pronounced than to the upper thoracic (p < .05), lower thoracic (p < .001), and lumbar levels (p < .05). The absolute blood flow values allow a comparison between different timepoints and regions in in the tissue of interest. Repeated measurements of spinal cord blood flow values are shown in Figure 3. We identified no significant differences in spinal cord perfusion at the three timepoints. The microsphere method thus yielded a reproducible blood flow measurement at different timepoints. Regional cortical renal blood flow in the left and right kidneys is shown in Figure 4. A strong correlation was detected between blood flow values from the left and right kidneys (r = .94, p < .001). There was no difference in regional renal cortical perfusion among repeated measurements. The high correlation and reproducible blood flow measurements of the kidneys reveal good distribution of microspheres in the cardiovascular system and their high yield in the tissue-digesting process. A strong difference between the kidneys is evidence of a hemodynamically unstable pig during injection, bad pipetting during tissue digesting, or a faulty microsphere injection or withdrawal of the microsphere reference samples.

3.2 | Vascular corrosion cast

ARMAs were well perfused with casting material through their entire length (Figure 5). A low penetration of the ARMAs is due to the
casting material’s high viscosity or a low injection pressure. A material of low viscosity will penetrate smaller vessels. The anterior spinal artery, however, exhibited some interruptions in the mid-thoracic region. Such interruption is technical due to the viscosity of the casting material that demonstrates less penetration of the anterior spinal arteries of small diameter in the mid-thoracic region. The ARMAs number and distribution are shown in Figure 6. We counted more left than right ARMAs in all autopsies (p < .05). Mean number of ARMAs in the pigs was 8 ± 2. The number of ARMAs in the upper thoracic region ranged from 2 to 7 with a mean of 5 ± 1, while in the lower thoracic region they ranged from 0 to 5 with a mean of 3 ± 1 (p < .001). Selective vascular casting allowed visualizing the distribution of these small vessels of the spinal cord. The technique can be combined with scanning electron microscopy to analyze morphological alterations of small vessels.

4 | DISCUSSION

We are the first to have combined the fluorescence microsphere technique and vascular corrosion cast in this feasibility study to visualize spinal cord vasculature and determine spinal cord blood flow at the same time.

The spinal cord’s thoracic region has some important features that must be considered in cardiovascular surgery. The anterior spinal artery’s supply of blood through the ARMAs leaves watershed areas with decreased blood flow next to the regions in which extraspinal and intraspinal vessels overlap (Zulch, 1954). The spinal cord’s mid-thoracic region in this case has classic watershed areas vulnerable to ischemic damage around segments T4/T5 and T8/T9 (Cheshire et al., 1996; Shamji et al., 2003; Zülch, 1976). Furthermore, the thoracic region reveals the largest space between the ARMAs, and collateral blood flow in this section is reportedly low (Bosmia et al., 2015; Hickey, Albin, Bunegin, & Gelineau, 1986). The risk for spinal cord ischemia therefore rises when intercostal arteries are occluded, because the collateral system is incapable of providing sufficient blood flow to this area (Gillilan, 1958). Furthermore, the anterior spinal artery is reported to be narrowest in the mid-thoracic region, increasing the risk for infarction in this area (Aminoff, 2008). The casting material we used penetrated the anterior spinal artery in the mid-thoracic area the least, thus verifying previous findings. The interruptions in our experiments were technical in nature due to the anterior spinal artery’s small diameter in the mid-thoracic region and due to the casting material's viscosity. In one study, we carried out a simulated “frozen elephant trunk procedure” (FET) by occluding thoracic segment arteries and interrupting collateral inflow into the epidural arcades to investigate histological changes in the thoracic region (Kari et al., 2017). We found out that the 3 hr postoperative observation of ischemia was too short to permit the observance of any histological tissue changes. Further long-term experiments could help us better understand the findings previously mentioned.

This study supports the existence of a constant anterior radiculomedullary artery, referred to as the “Artery of von Haller” at level T4, as opposed to the “Artery of Adamkiewicz,” which often originates between T8 and L3 (Adamkiewicz, 1881; Gailloud, 2013). This fact should be considered in aortic surgery when segmental arteries in this area are occluded because of the watershed zone downstream of this region (Henson & Parsons, 1967; Zülch, 1976). Furthermore, the upper thoracic region has more ARMAs than the mid-thoracic and lower thoracic segments. These findings support the theory of a higher ischemia risk in these regions, as other studies have documented (Cheshire et al., 1996; Dommisse, 1974; Gailloud, 2013; Perk, 2014; Zülch, 1976). The regression of ARMAs has been described as being prominent in the caudal region, where the Artery of Adamkiewicz often remains as the largest ARMA (Adamkiewicz, 1881; Gillilan, 1958; Suh & Alexander, 1939).

The combined method applied in this study was reproducible in blood flow analyses, although the vascular corrosion cast had been used in the same tissue. The blood flow values we measured in the pig resembled those described before (Etz et al., 2008). The number and distribution of ARMAs in the pig resemble the values documented in humans (Gailloud, 2013). Krucker et al. reported some background fluorescence of casting material, but we detected no spectral overlaps with the microsphere colors used in our experiment (Krucker et al., 2006). However, blue microspheres are not recommended, as background fluorescence noise has been reported when using the digesting solution to release the microsphere dyes from the particles and blue pigment for vascular corrosion cast (Glenny et al., 1993). This combined technique has several limitations that should be addressed. The microsphere method is prone to errors, which can influence the blood flow values calculated. The injection of microspheres in hemodynamically unstable pigs leads to poor distribution of microspheres in the cardiovascular system and therefore inaccurate blood flow values. Furthermore, the reference sample needs to be withdrawn at a steady withdrawal rate for blood flow calculation. Any irregularities in the withdrawal pump lead to inaccurate blood flow calculations. The time-consuming processing of the samples is an important limitation, as each process step can lead to inaccurate blood flow calculations. However, an internal standard can be used with a predefined
number of microspheres colors that are not used in the experiments before beginning processing. Low extraction rates of the internal standard can be due to faulty pipetting and an inaccurate digesting-solution volume (Step 5.7). However, employing the sedimentation technique enables samples to be processed in one tube, minimizing the loss of microsphere particles (Powers et al., 1999). Finally, the microsphere colors used in the experiment must not exhibit spectral overlap with the vascular casing pigment, the tissue of interest, or the solutions used to process the samples. We recommended making "test samples" with tissue and digesting solution without microsphere particles to measure the tissue's background fluorescence.

Improvements in perioperative risk management associated with thoracoabdominal aneurysm repair have significantly reduced the incidence of spinal cord ischemia. Cerebrospinal fluid drainage, neuromonitoring using somatosensory-evoked potentials and motor evoked potentials, monitoring of spinal cord perfusion pressure, distal aortic perfusion, and the preoperative visualization of collateral blood flow and ARMAs are important factors for the improving spinal cord risk management. However, preoperative risk management via magnet resonance angiography (MRA) tends to focus on just one ARMA, the great anterior radiculomedullary artery (GARMA). Our group’s recent investigation found that the distance between ARMAs in the thoracic region plays a key role in neurological outcomes after the FET procedure (Kari et al., 2017). A large distance between ARMAs led to insufficient reactive hyperemia after acute ischemic settings. Furthermore, we discovered that the number of ARMAs correlates with the decrease in spinal cord vascular resistance and therefore reactive hyperemia after ischemic conditions (unpublished data). Physiologically, we detected no significant correlation between the number of ARMAs and spinal cord blood flow. ARMAs seem to be the collateral means of providing rapid and sufficient reactive hyperemia when the spinal cord is affected by ischemia. Preoperative visualization of the number and distribution of ARMAs and the collateral pathways could therefore function as an important preoperative risk predictor. In this case, assessing the spinal cord blood flow and vasculature is essential for determining the risk. This combined method can be used for preclinical work to investigate tissue blood flow and vasculature. It enables the investigation of absolute blood flow at different time points, but not real-time measurements. Intra-arterial catheter angiography makes highly detailed images to visualize collateral pathways and feeding vessels possible (Kieffer et al., 2002). However, it is an invasive method associated with the risk for iatrogenic paraplegia itself. Furthermore, it is incapable of visualizing all collateral pathways and ARMAs simultaneously, making more sessions necessary, thus raising the risk for iatrogenic paraplegia (Kieffer et al., 2002; Williams, Roseborough, Webb, Perler, & Krosnick, 2004). Non-invasive imaging of the GARMA via computer tomography and MRA has improved recently (Hyodoh et al., 2005; Kawaharada et al., 2004; Nijenhuis et al., 2009). The assessment of collateral pathways with MRA allowed the assessment of important relations between collateral pathways and spinal cord functions. Backes et al. found a significant correlation between the existence of collateral pathways visualized with MRA and a negative predictive value of spinal cord function after cross-clamping in the corresponding aortic area (Backes et al., 2008). Further research on collateral pathways and their visualization could help us create individual risk algorithms preoperatively.

We postulate that the combination of fluorescence microsphere technique and vascular corrosion cast can be used in the same tissue to analyze the vascular system and hemodynamics. Furthermore, we call for more research on the role of the ARMA and intraspinal collateral system to lower the rate of spinal cord ischemia after cardiovascular interventions.

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DISCLOSURE OF INTEREST

The authors have no financial or personal relationship to disclose that would create a conflict of interest or bias.

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