Relationships between hemorrhage, angioarchitectural factors and collagen of arteriovenous malformations

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Abstract: Objective While associations between the angioarchitecture of arteriovenous malformations (AVMs) in the brain and pathological features have been described, here we investigated the relationship between the angioarchitecture, the pathological features of the vessel wall, and hemorrhagic events. Methods The study was conducted on 43 patients: 16 with ruptured AVM (rAVM), 15 with non-ruptured AVM (nrAVM), 6 with craniocerebral trauma (control) and 6 with epilepsy (control). The diagnosis of AVM was confirmed by preoperative digital subtraction angiography. Tissues were stained with hematoxylin and eosin and Masson’s trichrome (for collagen fibers) to evaluate the vessel wall structure and endothelial integrity. The content and distribution of collagen types I and III in the vessel wall were assessed by immunohistochemical staining. Results In the nrAVM group, the nidus had more draining veins than the rAVM group (P < 0.05). Severely damaged endothelial cells, significantly fewer smooth muscle cells in the media, and hyperplastic type-I and -III collagen fibers were found in the rAVM group. The content of collagen types I and III in rAVMs was higher than that in the nrAVM (P < 0.05) and control groups (P < 0.01). Conclusion There is an association between angioarchitectural features such as the number of draining veins and the pathological structure of the AVM wall. These abnormalities may contribute to AVM rupture.

Keywords: arteriovenous malformations; angioarchitecture; pathology

1 Introduction

The incidence of arteriovenous malformations (AVMs) in the brain is 1 per 100 000 per year in unselected populations, and the point prevalence in adults is 18 per 100 000[1,2]. AVMs are usually discovered in the search for a potential structural cause of intracranial hemorrhage, epilepsy, headache, or a focal neurological deficit. Among the symptomatic presentations, 60% are due to intracranial hemorrhage[3,4]. Bleeding due to AVMs is the cause in 1% of all strokes and in 15% of patients presenting with spontaneous intracranial hemorrhage, especially those aged <40 years[5]. The risk is highest during the first 5 years after diagnosis. The average annual risk of hemorrhage from AVMs is 2.4%, and that of re-bleeding is 2%–4%[6,7]. The long-term crude mortality appears to be between 1% and 1.5% per annum, although 50%–70% of all deaths are caused by hemorrhage[3]. Among the AVM-hemorrhage subtypes, the parenchymatous subtype is associated with a
higher morbidity.

The risk of brain AVMs is associated with a history of hemorrhage, increasing age, hypertension, and even ethnic differences. Hemodynamic risk factors are also thought to play important roles in the pathogenesis of AVMs, and to be associated with the prevalence of intracranial hemorrhage. Although the hemodynamic factors in AVMs cannot be measured readily, angioarchitectural features such as size, location, pattern of arterial feeding, deep venous drainage, and associated aneurysms can be assessed and used as surrogate parameters.

AVMs are hamartomatous lesions characterized by elongated and enlarged feeding arteries that directly communicate with draining veins and lack intervening resistance vessels or capillary beds. However, there are few studies concerning the pathological structure of the vessel walls of AVMs.

The present study focused on the morphological properties of human AVMs, specifically the pathological structure of the vessel wall, and the distribution of collagens I and III.

2 Methods

2.1 Patients Control vessel specimens consisted of the broken ends of vessels obtained during neurosurgery from six patients with craniocerebral trauma and specimens of histologically normal temporal lobe neocortex from 6 patients undergoing surgery for epilepsy at Beijing Tiantan Hospital (Beijing, China) from September 2009 to July 2010.

The study population consisted of 31 patients (23 males, 74%; and 8 females, 26%; mean age, 28.4 ± 12.2 years; range, 8–52) diagnosed with AVMs from September 2009 to July 2010. All patients were identified by preoperative digital subtraction angiography or magnetic resonance angiography. No patients had a history of cardiovascular disease, diabetes mellitus, or gamma-knife therapy. The AVMs were not embolized before resection.

Patients were divided into a ruptured AVM group (rAVM; n = 16) and a non-ruptured AVM group (nrAVM; n = 15) according to the history of rupture, demonstrated by CT/MRI/spinal puncture before surgery, or intraoperative detection of hemosiderin deposits beside cerebral tissue.

The study protocol was approved by the Ethics Committee of the Capital University of Medical Sciences (Beijing, China). Informed written consent was obtained from all subjects included in the study.

2.2 Criterion of angioarchitecture Based on the appearance of the AVM on digital subtraction angiography, the lesion was evaluated by one investigator to reduce bias. The size, location, shunt vessels, and pattern of venous drainage were analyzed. The presence of an aneurysm associated with the AVM would affect the impact of blood on the vessel wall. In order to avoid this complication, none of the selected AVM patients had an aneurysm.

2.2.1 Size Lesions were classified as small (≤3 cm), medium (>3 cm to ≤6 cm), or large (>6 cm) according to the maximum diameter.

2.2.2 Location Lesions were grouped into frontal, temporal, parietal, occipital, corpus callosum, basal ganglia, insular, brainstem, or cerebellum. The lesions were also grouped into deep (basal ganglia, thalamus, cerebellum, and corpus callosum) or superficial (all other locations).

2.2.3 Pattern of venous drainage The number of draining veins was classified into two groups: a single draining vein and more than one draining vein. The presence of deep venous drainage was also recorded.

2.3 Sample preparation The vessels were harvested immediately after the AVM nidus was dissected away from adjacent brain tissue. After removal of the AVM, we identified the feeding arteries, draining veins, and nidus. The vessels located inside the nidus, without being fulgerized, were defined as shunt vessels. The specimens were immediately fixed in 10% formalin for histological examination.

2.4 Histology

2.4.1 Hematoxylin and eosin (HE) staining After dehydration in graded alcohols (70, 80, 95, and 100%), the specimens were embedded in paraffin and cut into serial sections (5 μm), followed by warming, de-waxing, and washing with distilled water. Then nuclei were stained with hematoxylin, followed by washing, soaking in eosin,
further washing, and dehydration.

2.4.2 Masson’s trichrome staining Slides were routinely de-waxed, and nuclei stained with hematoxylin. Sections were then stained with Masson’s composite dye, acetic acid, phosphomolybdate, and light-green staining fluid. They were then differentiated in 95% ethanol, dehydrated, cleared and mounted. Green denotes collagen fibers, red indicates muscle fibers, and orange identifies red blood cells.

2.5 Immunohistochemistry After routine de-waxing, antigen was retrieved in sodium citrate (pH 6.0), and the slides were washed three times in phosphate-buffered saline. Endogenous peroxidase was inactivated by 3% hydrogen peroxide. Then slides were blocked in 5% bovine serum albumin, and incubated with monoclonal antibody (type-I collagen, 1:500; type-III collagen, 1:600) overnight at 4°C followed by horseradish peroxidase-labeled goat anti-mouse IgG (Beijing Zhongshan Golden Bridge, Beijing, China) at 37°C for 1 h. Slides were visualized using 3,3-diaminobenzidine tetrahydrochloride (Beijing Zhongshan Golden Bridge), dehydrated, cleared and mounted. Immunohistochemically-positive reaction products displayed a brown color under light microscopy. The mean integrated optical density was measured using the Leica QWin image processing and analysis system (Wetzlar, Germany).

2.6 Semi-quantitative immunohistochemical analyses Semi-quantitative immunohistochemical analyses of foci positive for collagens I and III were done using a color video image processor (FW4000-Leica, Wetzlar, Germany). The measured value was obtained using Leica QWin software.

2.7 Statistical analyses Data are presented as mean ± SD. Comparison of age, size and number of draining veins was performed with the unpaired t-test. As enumeration data, AVM location was tested by the \( \chi^2 \) test. The associations between all parameters were analyzed by multivariate linear stepwise regression. Groups were compared by analysis of variance in SPSS software (version 11.0; SPSS Inc., Chicago, IL). \( p < 0.05 \) was considered statistically significant.

3 Results

3.1 Clinical analyses Among the 31 patients who had undergone neurosurgery, 16 (52%) were in the rAVM group and 15 (48%) in the nrAVM group. Unpaired t-test analyses showed that the ages of the two groups were not significantly different (\( t = 0.865, P > 0.05 \)).

3.2 Angioarchitectural features In the nrAVM group, 3 cases (20%) had small lesions and all had superficial lesions. One patient (6.7%) had a single draining vein, 3 (20%) had an arteriovenous shunt, and 2 (13.3%) had deep venous drainage.

In the rAVM group, 13 cases had medium lesions (81.3%) and 1 had a large lesion (6.3%). Three patients (18.8%) had lesions in a deep location; only one patient (6.3%) had a single draining vein. One (6.3%) case had an arteriovenous shunt, and 6 (37.5%) had deep venous drainage (Table 1).

Lesion size was not significantly different between the two groups (unpaired \( t \)-test, \( t = 0.429, P >0.05 \)) and neither was location (\( \chi^2 = 0.267, P >0.05 \)). However, a superficial lesion was more common in the nrAVM group.

The number of draining veins was higher in the nrAVM group (3.9 ± 0.4) than in the rAVM (2.7 ± 0.2) according to the unpaired \( t \)-test (\( t = 18.556, P <0.05 \)) (Fig. 1).

In stepwise multiple regression analysis, the number of draining veins was the factor most associated with rupture, and the remaining data were excluded (Table 2).

3.3 Presentation of gross specimens nrAVMs revealed arteries and veins that communicated directly without a capillary bed. There was a close relationship with surrounding brain tissue but no hemosiderin deposits.

In the rAVM group, the lesions had the same appearance. The nidus had a complex structure and closely adhered to the surrounding brain tissue. Hemosiderin deposits were detected beside the cerebral tissue (Fig. 2).

3.4 Pathological structure

3.4.1 Staining (HE and Masson’s trichrome) HE and Masson’s trichrome staining clearly revealed that all specimens comprised muscular artery. The vascular walls were composed of intima, media and adventitia.
In the control group, optical microscopy showed endothelial cells with a fusiform shape. The internal elastic membrane was normal and wave-like. There were 6–8 layers of concentrically arranged smooth muscle cells (SMCs) in the media. Collagen fibers were present between the layers of SMCs. The adventitia covering the vessel was composed of connective tissue with collagen and elastic fibers (Fig. 3A, B).

In the nrAVM group, some endothelial cells were detached, and others had disappeared. The internal elastic membrane was normal and wave-like. There were 6–8 layers of concentrically arranged smooth muscle cells (SMCs) in the media. Collagen fibers were present between the layers of SMCs. The adventitia covering the vessel was composed of connective tissue with collagen and elastic fibers (Fig. 3A, B).

### Table 1. Clinical summary of data for patients with AVM

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (years)</th>
<th>Size (cm)</th>
<th>Location</th>
<th>Shunt</th>
<th>Deep venous drainage</th>
<th>Deep/Superficial location</th>
<th>No. of DV</th>
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<td>S</td>
<td>2</td>
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<td>36</td>
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<td>-</td>
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<td>5</td>
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<tr>
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<td>47</td>
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<td>5</td>
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<tr>
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<td>-</td>
<td>S</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>46</td>
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<td>R Temporal</td>
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<td>-</td>
<td>S</td>
<td>3</td>
</tr>
<tr>
<td></td>
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<td>R Temporal</td>
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<td>+</td>
<td>S</td>
<td>6</td>
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<td>3</td>
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<td>-</td>
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<td></td>
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<td>S</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>42</td>
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<td>-</td>
<td>S</td>
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</tr>
<tr>
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<td>15</td>
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<td>-</td>
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<tr>
<td></td>
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<tr>
<td></td>
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</tr>
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<td>D</td>
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</tr>
<tr>
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<td>R Parietal</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>2</td>
</tr>
</tbody>
</table>

AVM, arteriovenous malformation; DV, draining vein; D, deep location; S, superficial location; L, left; R, right.
membrane was wave-like and apparently rigid. Proliferating SMCs had degenerated, and were elongated and broken. Collagen fibers in the media had proliferated and appeared rigid (Fig. 3C, D).

In the rAVM group, thrombi were observed in several

Fig. 1. Digital subtraction angiographic image of a ruptured AVM. Male, 15 years old, left parietal lobe. The lesion (arrow) is located superficially, its diameter is 3 cm, and drainage is from 3 superficial veins (asterisks).

Table 2. Multivariate linear stepwise regression analysis of data for patients with AVM

<table>
<thead>
<tr>
<th>Factor associated with rupture</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>t</th>
<th>P value</th>
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<tbody>
<tr>
<td>Model</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (Constant)</td>
<td>1.089</td>
<td>0.224</td>
<td>4.865</td>
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</tr>
<tr>
<td>No. draining veins</td>
<td>−0.176</td>
<td>0.064</td>
<td>−0.455</td>
<td>−2.754</td>
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</table>

Excluded variables

<table>
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<tr>
<th>Variables</th>
<th>Beta In</th>
<th>t</th>
<th>P value</th>
<th>Partial correlation</th>
<th>Co-linearity tolerance</th>
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<td>0.600</td>
<td>−0.100</td>
<td>0.971</td>
</tr>
<tr>
<td>Size</td>
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</tr>
<tr>
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<td>−0.441</td>
<td>0.662</td>
<td>−0.083</td>
<td>0.913</td>
</tr>
<tr>
<td>Deep draining</td>
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<td>1.063</td>
<td>0.297</td>
<td>0.197</td>
<td>0.946</td>
</tr>
<tr>
<td>Deep location</td>
<td>0.038b</td>
<td>0.226</td>
<td>0.823</td>
<td>0.043</td>
<td>0.998</td>
</tr>
</tbody>
</table>

aDependent variable: rupture; bPredictors in the model: (Constant), No. of draining veins. B, partial regression coefficient; Beta In, partial standard linear regression coefficient.
Fig. 3. HE and Masson's trichrome staining in three groups. A: Control group, HE. Continuous arrangement of fusiform endothelial cells (black arrow); internal elastic lamina (white arrow) shows a continuous wave-like pattern with pink staining; the media is composed of 6–8 layers of tightly-packed smooth muscle (arrowhead) in a concentric arrangement; no external elastic lamina. B: Control group, Masson. Continuous wave-like internal elastic lamina (with arrow) with pink staining. Mainly smooth muscle cells (arrowhead) in the media, with collagen fibers running in parallel (smooth muscle cells stain red, collagen fibers stain green). An external elastic lamina is absent. C: Unruptured AVM group, HE. Completely continuous endothelial cells (black arrow), showing some disorder. The internal elastic lamina is not obvious, and the arrangement of smooth muscle cells (arrowhead) is loose, with disordered orientation in 6–7 layers. D: Unruptured AVM group, Masson. Internal elastic lamina (green) appears rigid, and smooth muscle cells (red, arrowhead) are loosely arranged. Green-staining collagen scattered among the smooth muscle layers. E: Ruptured AVM group, HE. A large thrombus (white arrowhead) can be seen within the lumen; endothelial cells cannot be seen clearly; the internal elastic lamina is absent; and the smooth muscle layer (black arrowhead) in the media is loosely arranged. F: Ruptured AVM group, Masson. Endothelial cells are irregular in shape, and unevenly distributed. The internal elastic lamina is stained green and appears rigid, having lost the wave-like form. In the media, 8–10 layers of smooth muscle cells (arrowhead) are loosely arranged, showing breaks. Fibrous tissue is increased significantly, showing a web-like, disordered orientation. Scale bar: 20 µm.
specimens. Endothelial cells were empty, swollen, and other structures could not be visualized clearly. The morphology of the internal elastic lamina changed remarkably, developing into a disrupted or even broken pattern. Microscopic examination revealed another feature: thickened collagen fibers. There were more collagen fibers than in the control and nrA VM groups. SMCs and collagen fibers had a disordered orientation in the media. The outer lamina was apparently normal (Fig. 3E, F).

### 3.4.2 Immunohistochemical detection of collagen types

#### 3.4.2.1 Collagen I

In the control group, collagen I appeared as bulky fiber bundles in a circular orientation. Collagen I fibers were regularly distributed in the media, and more evident in the outer layer. The fibers were arranged in a parallel pattern within the layers of SMCs. Positive staining was detected in the adventitia covering the vessel (Fig. 4A).

In the nrAVM group, collagen I expression was found mainly in the media (particularly in the outer layer) as bulky fiber bundles. There were more collagen I fibers in the media than in the control group (Fig. 4B).

In the rAVM group, type-I collagen was also found in the media. Some fibers were tortuous and irregularly arranged. The fibers were more numerous, had a disordered orientation, and were unequally distributed in the media (Fig. 4C).

The mean grayscale intensity of collagen I in the rAVM group (0.251 ± 0.006) was higher than in the control (0.206 ± 0.005, \( P < 0.01 \)) and nrAVM (0.232 ± 0.006, \( P < 0.05 \)) groups (Fig. 4D).

#### 3.4.2.2 Immunohistochemical detection of collagen III

In the control group, collagen III staining was weak. The filament-like fibers were interlaced, different from the type-I pattern. The fibers were relatively well distributed
in the media, and there were clearly more in the outer layer (Fig. 5A).

In the nrAVM group, type-III collagen was found in the media (particularly in the outer layer). The fibers had disparate thicknesses. Some fibers were reticulated, but most were scattered and straight (Fig. 5B).

In the rAVM group, the type-III collagen fibers had diverse shapes: bunched, filament-like and helical. Nearly all the fibers were scattered and unevenly arranged. The expression of collagen III in the media was continuous (Fig. 5C).

The mean grayscale intensity of collagen III in the rAVM group (0.247 ± 0.004) was higher than in the control (0.210 ± 0.006, \( P < 0.01 \)) and nrAVM (0.228 ± 0.009, \( P < 0.05 \)) groups (Fig. 5D).

4 Discussion

The development and rupture of AVMs is a multifactorial, multistage process. In the present study, we investigated the relationship between the angioarchitecture of AVMs, pathological structures in the vascular wall, and rupture of the lesion, to uncover the possible mechanism underlying this process.

"Angioarchitecture" describes the morphology, internal structure and other characteristics of blood vessels\(^{20}\). In recent years, the relationship between angioarchitectural characteristics (particularly the size and location of lesions, the characteristics of feeding arteries and draining veins, and rupture of AVMs) has been a focus of research\(^{21-24}\). From the clinical data on the characteristics of AVMs, we found no significant difference in lesion size between the non-ruptured and ruptured groups. However, non-ruptured lesions tended to have more draining veins, and all were in a superficial location. Gross samples showed that the arteries and veins communicated directly, and were associated with brain tissue, but without a capillary bed. To reveal...
the underlying causes of rupture, we further clarified the angioarchitecture in the vessel wall by pathological examination and immunohistochemical staining of vascular tissue obtained at surgery. We found that the vessel wall of AVMs showed different orientations of endothelial cells, poor development of the internal elastic lamina, rigidity, a concentric distribution of SMCs in the media, and a loose arrangement of mixed collagen fibers. Another study revealed severe mural fibrosis and a marked arterialization of the vein. Along with loss of the internal elastic membrane, smooth muscle cells are the notable features of AVMs\(^{25,26}\).

The pathologic structure we found was different from the change of cerebral vasculature after subarachnoid hemorrhage. The intima are thickened with swelling and vacuolization of endothelial cells. SMCs are vacuolated and capped by elastin and collagen fibers. The internal elastic lamina is also corrugated and disrupted. The adventitial changes are axonal, cytoplasmic and mitochondrial swelling, with the virtual absence of synaptic vesicles, and disruption of the outer axonal membrane\(^{27,28}\), demonstrating vascular injury. However, the pathological changes of the vascular wall we observed were not only due to repair of a partial rupture, but also the features of the vascular wall before rupture.

AVMs with a single draining vein tended to rupture. This result is in accordance with other clinical studies\(^{12,22,29,30}\). The nidus had a complex structure and closely adhered to surrounding brain tissue in this group. Hemosiderin deposits were detected beside cerebral tissue. The pathological structure of rAVMs revealed that endothelial cells were severely damaged, significantly reduced or even absent. The irregular internal elastic lamina was discontinuous, interrupted, or even absent. The number of SMCs in the media was significantly reduced. The numbers of collagen fibers were considerably increased, loosely arranged and disordered.

All 3 lesions located in the basal ganglia were in the rAVM group. Lesions in the nrAVM group were all superficial. There were more AVMs in a deep location in the rAVM group than in the nrAVM group. We believe that lesions in a deep location have less space in the tissue around abnormal vessels. This means that stress cannot be immediately buffered if there is rapid blood flow through the vessel. Mechanical damage to the abnormal vessel wall is therefore enhanced\(^{22-24}\). According to our results, there were more shunts in the rAVM group. The result could be accounted for by assuming that the arteriovenous shunt disperses the blood pressure in the malformation and lowers the pressure in the nidus, thereby reducing the risk of rupture. More abnormal venous drainage from the nidus helps to share the pressure of blood flow. The mean stress is therefore less than the pressure from the malformation.

In the rAVM group, we found more cases of deep venous drainage than in the nrAVM group. It is speculated that the pressure of deep venous drainage is greater than that of superficial drainage, which increases the risk of a hemorrhage event.

Most importantly, the hemodynamic changes of high blood flow and low resistance from local abnormal angioarchitecture can cause mechanical damage to the vascular wall. This influences the structure of the wall, eventually resulting in rupture.

When damaged, endothelial cells proliferate. Mature endothelial cells secrete collagen, laminin and fibronectin, whereas the surrounding fibroblasts secrete type-III collagen substrate to repair damage. Reconstruction of the vessel wall may involve fibrous repair by collagen proliferation.

Immunohistochemical analyses of type-I and -III collagen revealed significantly higher content in the rAVM group than in the control and nrAVM groups. Type-I and -III collagen are interstitial or fibrous collagen, and the fibers can be highly differentiated, providing structural support. Type-I and -III collagen fibers are connected, entangled and packed to form a stable structure\(^{31}\).

Collagen structure and mechanical load are closely linked. Researchers have found that the preferred orientation of collagen fibers is in the direction of vessel stretch. Vascular collagen fibers arranged normally have a symmetric spiral arrangement\(^{32}\). This can explain the results in the present study: long-term turbulence disturbs the direction of flexibility of the vessel wall and the effect of stretch;
this causes a disordered arrangement of collagen fibers and weakness.

Although the mechanism is unclear, we speculate that the abnormal angioarchitecture contributes to hemodynamic changes such as feeding-artery pressure, draining-vein pressure, and blood-flow velocity. Damage to endothelial cells and SMCs in the media may affect the number and morphology of these cells, leading to apoptosis or autophagy. If the cells are stimulated by injury factors, several growth factors and chemical mediators are released to promote self-healing. The main cause of fibrous repair is the hyperplasia of type-I and -III collagen and their disordered arrangement. The advantage of this process is to fill in the defects, and to maintain the integrity of the vessel wall. The proliferation of collagen fibers offers a degree of tensile strength. We therefore speculate that the long-term impact of mechanical damage and the continuous process of injury and repair ultimately lead to irreversible pathological changes to the wall, leading to a decrease in elasticity, an increase in brittleness, and eventually rupture of the vessel wall. However, the specific mechanism needs to be explored further.

5 Conclusion

Ruptured and non-ruptured AVMs had different angioarchitectural characteristics (especially the number of draining veins). The most remarkable pathological change in the structure of the vessel wall was the proliferation of type-I and -III collagen. We hypothesize that there is a correlation between the two phenomena and that the angioarchitectural characteristics of the AVMs lead to hemodynamic changes. These changes may contribute to further pathological structural changes in the vessel wall.

Acknowledgements: This work was supported by National Natural Science Foundation of China (30973112).

References:


