Endovascular Aneurysm Repair
Magnetic Resonance Monitoring of Histological Organization Processes
in the Excluded Aneurysm

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Background—The purpose of the present study was to systematically analyze the histopathologic organization processes in excluded aneurysms after endovascular stenting and to develop a noninvasive monitoring method for these processes using MRI.

Methods and Results—In 36 mongrel dogs, autologous aortic aneurysms were created. Endovascular treatment was performed using covered stents. Follow-up was after 1 week, 6 weeks, and 6 months. MRI was performed with T2-weighted turbo-spin-echo sequences and T1-weighted spin-echo sequences and was repeated after contrast bolus with gadolinium. Histopathologic findings were correlated to signal intensities (SIs) of MRI images. SIs of distinct areas were analyzed and related to the SI of the reference tissue (SI ratio). The histological organization process was gradated in the following 4 classes: class 0, detritus without organization; classes I and II, connective tissue proliferation with increasing fiber synthesis; and class III, dense fibrous connective tissue. The SI ratios of T2-weighted images were significantly reduced from 4.76 in detritus (0) to 1.70 in dense fibrous connective tissue (III) as a function of histopathologic classes. SI ratios of T1-weighted images were reduced from 1.84 (0) to 1.12 (III). Contrast bolus with gadolinium-DTPA showed no change of SI ratio in detritus (0.99) but an increase from 1.12 (I) to 1.70 (III) as organization increased.

Conclusions—The histological organization of excluded aneurysms can be monitored by MRI. Progressive organization is indicated by decreasing SIs in T2- and an increasing signal increase in T1-weighted images after gadolinium bolus.

Key Words: aneurysm ▪ grafting ▪ stents ▪ magnetic resonance imaging ▪ aorta

The era of endovascular aneurysm treatment was started in 1990 by Parodi et al.1 The technique has since evolved as an alternative to open surgery. Medium-term results have been encouraging in cases fit for this treatment,2–14 and complication rates have been moderate by comparison with standard surgery.7,15–26 Effective endovascular treatment results in a significant pressure reduction27 and a shrinkage of the excluded aneurysm. It is proposed that aneurysm exclusion and aneurysm shrinkage are accompanied by histological connective tissue organization,28–33 but data on the progression of these processes are only inconclusive and in part contradictory. Because stability and shrinkage of the excluded aneurysm are important therapeutic objectives of the treatment mode, knowledge of histological transformation as a function of time and the development of a noninvasive method of monitoring this process would be desirable. The present study systematically analyzes the histological transformation of the excluded aneurysmal sacs in an animal experiment. In particular, it classifies distinct histological organization levels in the aneurysms and correlates histological findings with MRI to develop a noninvasive follow-up method.

Methods
According to institutional guidelines, all invasive procedures were performed in general anesthesia using morphine (2 mg/kg) and atropine (0.5 mg/kg IM) for premedication (30 minutes before) and general anesthesia induced by pentobarbital (30 mg/kg IV) maintained by the gaseous anesthetic isoflurane (0.8% to 1.8%) and muscle relaxation by alcuronium (0.1 mg/kg). According to national law on the care and use of laboratory animals, the local committee for medical ethics and the district government approved the protocol.

In 36 mongrel dogs (age 11 to 13 months, 26 to 33 kg; Winkelmann, Borchen, Germany), autologous aortic aneurysms were created surgically using an oversized patch of the sheath of the rectus abdominis muscle. After exposure of the infrarenal aorta, the oversized patch (55 mm long and 30 mm wide) was sutured using Prolene 5-0, resulting in an aneurysmal vessel segment. After 12

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weeks (median), endovascular treatment was performed by transfemoral cut down using covered Nitinol stents (Passager, Boston Scientific Corp). The maximum size of the aneurysms was measured by spiral CT PQ 5000 (Picker International) before stent grafting and during follow-up. Postinterventional survival and radiological follow-up were 1 week, 6 weeks, and 6 months for 12 animals each. Three hours before euthanization, MRI was performed (Magnetom Vision, Siemens; 1.5 Tesla) to characterize tissue organization in the excluded aneurysm. Transverse images were obtained to scan the whole aneurysmal sac using T1- and T2-weighted turbo-spin-echo sequences (TR 4000 ms, TE 96 ms, 2 acquisitions, 4-mm slice) and T1-weighted fat-saturated spin-echo sequences (TR 784 ms, TE 12 ms, 4-mm slice) before and after injection of contrast medium (0.2 mL/kg gadolinium-DTPA, Magnevist, Schering). Images were transferred to a workstation for additional analysis.

To prepare and fix the aneurysm, a left lateral thoracotomy was performed in deep anesthesia and the descending aorta was exposed. After an arteriotomy distal to the left subclavian artery, the descending aorta was cannulated using a 34-Charriere orotracheal tube. For anticoagulation, 10,000 IU heparin was injected, and cardiac arrest was induced by intravenous KCl infusion. For hemostasis, the cuff around the aorta was cannulated using a 34-Charriere orotracheal tube. For additional fixation, the specimens were immersed in formalin for at least 8 weeks. For histological evaluation, different staining methods were used (H&E, Elastica-van Gieson, Azan, Masson-Goldner, and Sirius Red).

### Histological Definition

<table>
<thead>
<tr>
<th>Class</th>
<th>Histological Definition</th>
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<tbody>
<tr>
<td>Class 0</td>
<td>Exclusively cell detritus (+ + +)</td>
</tr>
<tr>
<td>Class I</td>
<td>Only soft fibrous connective tissue (+ to + +) with various quantities of detritus (+ to + + +), often with inflammatory cells (+ to + + +) and capillary vessels (+)</td>
</tr>
<tr>
<td>Class II</td>
<td>Soft to dense fibrous connective tissue (+ + to + ++), with capillary vessels (+ to + +) and various quantities of inflammatory cells (+ to + +)</td>
</tr>
<tr>
<td>Class III</td>
<td>Dense fibrous connective tissue (+ + + to + +++), always with capillary vessels (+ + to + + +) rarely with inflammatory cells (+)</td>
</tr>
<tr>
<td>Patch</td>
<td>Dense fibrous connective tissue (+ +++ +), with various quantities of capillary vessels (+ to + ++ +) and various quantities of inflammatory cells (+ to + ++ +)</td>
</tr>
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</table>

For macropathologic evaluation, the specimens were incised lengthwise, and both halves of the stented aneurysms were documented photographically. The metallic stent struts were removed, and both halves were put together and embedded in paraffin. The aneurysmal sac was sectioned in the transverse direction (3.5 μm) in planes corresponding to the MR slices. For histological evaluation, different staining methods were used (H&E, Elastica-van Gieson, Azan, Masson-Goldner, and Sirius Red).

### Statistical Analysis

For statistical analysis, the nonparametric Wilcoxon test for independent samples was selected. It was performed using SAS version 6.12. Differences were considered significant if $P<0.05$.

### Results

Before stent grafting, CT scans of experimental aneurysms revealed aneurysms with sagittal diameters of 28.8 mm (23.5/33.0) (median± quartile). The proximal aortic diameter was 9.0 mm (8.3/10.0). Aneurysm diameters were not significantly different during follow-up, although a trend to diameter reduction was identified at 6 months (1 week, 29.5 [28.0/34.0] versus 28.5 mm [26.5/34.0]; 6 weeks, 26.0 [24.0/36.0] versus 25.0 mm [23.5/36.0]; 6 months, 24.3 [20.0/28.0] versus 28.0 mm [20.0/32.0]; $P<0.05$). The macropathologic evaluation of the specimens provides a fair impression of histological organization. Deep-red material indicates cell detritus and unorganized thrombus, and signal intensities (SIs) of all 3 MR sequences were measured in these areas. SIs were referred to an internal standard. The patch tissue of the rectus sheath, which consisted of dense fibrous connective tissue, served as reference. All SIs were thus related to the signals of the patch (SI ratio). The SI ratios of $T_1$ and $T_2$-weighted images were compared with the histological classes for statistical analysis.
bus, whereas light-brown material typically indicates advanced tissue organization (Figure 1).

A total of 187 specimens from 36 canines were analyzed and compared with the corresponding SIs. Low-power magnifications of the slices were in good agreement with the corresponding areas of MR slices with their different SIs (Figure 2). The patch reference tissue showed constant signal characteristics during follow-up with moderate SI in T1-weighted images (SI 245) and low SI in T2-weighted images (SI 117) (Tables 2 and 3). At 1 week of follow-up, the aneurysms consisted mostly of red blood cell detritus with no evidence of histological organization. The corresponding ROIs in MR slices showed high SIs in T2-weighted images (SI 604) and high SI ratios (4.76) relative to the internal reference tissue (Tables 2 and 3). The SI ratios of T1-weighted images were only moderately increased. There was no significant signal increase after contrast medium (Tables 2 and 3). As histological organization increased, SI decreased successively. At 6 weeks of follow-up, the aneurysmal sac again contained nonorganized detritus in more than four fifths (median) of the cross-sectional surface. Small parts of the aneurysms showed signs of beginning tissue organization (class 1). The examination identified loose or reticular collagenous fibers, which were distributed heterogeneously in all

| TABLE 2. Signal Intensities of Distinct Histological Classes in T1- and T2-Weighted Sequences (T1, T2) and T1-Weighted Sequences After Contrast Bolus (T1, Km; T1, Km/T1) |
|-----------------|----------|----------|----------|----------|----------|
|                 | T2       | T1       | T1 Km    | T1 Km/T1 |
| Class 0         | 604 (452, 685) | 441 (367, 505) | 436 (364, 526) | 0.99 (0.93, 1.04) |
| Class I         | 445* (371, 538) | 397 (333, 469) | 460 (359, 547) | 1.12* (1.04, 1.20) |
| Class II        | 239† (186, 368) | 324‡ (281, 366) | 476 (404, 554) | 1.40† (1.19, 1.91) |
| Class III       | 161 (132, 201) | 257‡ (232, 311) | 440 (325, 544) | 1.70‡ (1.37, 2.06) |
| Patch           | 117 (92, 139) | 245 (217, 268) | 409 (353, 456) | 1.68 (1.53, 1.90) |

Q1 and Q3 indicate first and third quartile.

*P<0.001; †P<0.01; ‡P<0.05.

Significance compared with next lower class.

Figure 2. Different classes of histological organization at 6 months of follow-up. Histological specimens with the different organization classes (E) were indexed with A through D according to the following definition: class I (B, yellow dotted line); class II (C), with capillary vessel (red dotted line) and fibrous connective tissue (white dotted-line); class III (D, orange dotted line); and class 0 (A, blue dotted line), detritus. Note the tight connective tissue of the patch (F). Corresponding T2-weighted MR image indicates the different signal classes (F).
areas of the aneurysm. However, at 6 months of follow-up, microscopic analysis detected a mixture of all histological classes, indicating progressing tissue organization. About two thirds of the aneurysm volume consisted of connective tissue (about one third detritus [median], one third organization class I, less than one third class II, and only a few percent of dense fibrous connective tissue [class III]). However, in some individual cases, histological organization was more advanced (predominantly class II and III). In one case where the aneurysm was small, the detritus was completely phagocytosed, and the aneurysmal sac consisted of a mixture of different histological classes. The heterogenous organization resulted in a corresponding mixture of signal classes in the MR slices. As collagenous fiber content increased and red blood cell detritus decreased, the SI in T2-weighted images was significantly reduced in class 1 (P<0.001), class 2 (P<0.01), and class 3 (P<0.05) compared with the next lower organization class. A similar signal reduction occurred in T1-weighted images (Tables 2 and 3), but selectivity and discrimination between the classes were significantly better in T2-weighted sequences.

In contrast, an increasing SI after contrast bolus administration was found in areas with a higher degree of tissue organization. Detritus (class 0) showed no contrast effect. As organization progressed, an increasing angiogenesis in the excluded aneurysms was observed. Increasing amounts of capillaries and arterioles penetrated the organizing tissue, indicating the progressive organization. Apparently, this new vessel formation was responsible for the significant signal increase in the ROIs of MR images after gadolinium-DTPA, predominantly distinguishing the lower classes 0 and I from classes II and III. The maximum signal increase was recorded in class III with a median of 1.70. Decreasing SIs in T2-weighted images and an increasing effect of contrast bolus administration were found in areas with a higher degree of tissue organization (from detritus via soft fibrous connective tissue to dense fibrous connective tissue) and the correspondence between histopathologic findings and MR findings to develop a noninvasive monitoring method for clinical patients.

The aneurysm model used consisted of autologous material to prevent any kind of foreign-body reaction or influence on the histological organization. According to some authors, connective tissue organization is suggested by aneurysm shrinkage, which is one of the objectives of endovascular treatment.24–28 In our study, the patch material from the sheath of the rectus abdominis muscle consisted of dense fibrous connective tissue. It was therefore fit to serve as an internal reference for the SIs in MR images. It is well known that there is not an appropriate animal model that reproduces the characteristics of humans, because tissue reaction varies in different species. Moreover, the perfusion disturbances in the wall of arteriosclerotic vessels of a human aneurysm are not represented in the aneurysm model. However, the way of tissue organization (from detritus via soft fibrous connective tissue to dense fibrous connective tissue) and the corresponding SIs in MRI can be expected to be similar.

The data from this study clearly demonstrate that the organization of tissue in aneurysms takes at least several months, and it may be put forward that these histological transformations are decisive for stable treatment results and aneurysm shrinkage. One week after stent grafting, the aneurysms consisted mostly of detritus without evidence of fibroblast invasion and collagenous fiber synthesis. At 6 weeks of follow-up, a small amount of collagenous fiber synthesis (class I and II) had occurred, but most parts of the aneurysmal sac still consisted of detritus. At 6 months, the aneurysms were characterized by a mixture of different histological classes, indicating progressive histological organization. Even at this time, a substantial part of the aneurysm (about one third) still consisted of detritus. The present data do not describe the complete histological aneurysm transformation process, which would apparently take much more time. This observation is consistent with the fact that no significant shrinkage of the aneurysms was found even at 6 months of follow-up. The present study confirms the experimental data of Eton et al28 and others24–29 who also described such long organization processes, even if the different organization levels were not as clearly defined. Moreover, the present data are consistent with the experience in clinical patients where aneurysm shrinkage takes many months. On the other hand, the present study contradicts several reports that describe histological organization within a few months30–33 without demonstrating and quantifying microscopical findings.

### Discussion

Endovascular treatment of aortic aneurysms is becoming a widespread alternative to open surgery. However, several methodological problems and questions remain to be solved. One of these questions is whether and under which conditions the excluded aneurysm develops histological organization and how this process can be monitored in patients. The present study investigates the histopathologic organization processes of excluded experimental aneurysms at defined follow-up intervals. It focused on the correlation of histopathologic findings and MR findings to develop a noninvasive monitoring method for clinical patients.

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### Table 3. T1 to T2 Ratio: Signal Intensity Relative to Reference Tissue (Patch)

<table>
<thead>
<tr>
<th>Class</th>
<th>Median</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>Median</th>
<th>P1</th>
<th>P2</th>
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<th>P4</th>
<th>Median</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
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<tr>
<td>Class 0</td>
<td>4.76</td>
<td>1.84</td>
<td>0.99</td>
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<td>Class I</td>
<td>3.35</td>
<td>1.72</td>
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<td>Class II</td>
<td>2.24</td>
<td>1.40 *</td>
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<tr>
<td>Class III</td>
<td>1.70</td>
<td>1.12</td>
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*P<0.001; †P<0.01; ‡P<0.0001; §P<0.05.
The differentiation of the tissue organization was predominantly based on collagenous fiber content and interlacing. This definition was used, because increasing stability of the connective tissue presupposes increasing quantities of collagenous fibers. The advancing organization was accompanied by progressive neoangiogenesis, which begins in an early stage (class I) and is a condition of a higher degree of tissue organization. It controls signal increase after contrast medium injection (T1-weighted) obviously is of secondary interest for the purposes of the classification. The content of round and polygonal inflammatory cells was of secondary interest for the purposes of the classification. These distinct histological classes resulted in MR image signal classes, which are predominantly a function of the fiber content.

In a clinical study, Engellau et al.44 discovered that increasing organization of thrombus in excluded aneurysms resulted in decreasing SIs in T1-weighted images. In the present study, the SIs of T2-weighted sequences were the best tool to distinguish the different classes. Probably, the reason is the decreasing fluid content as tissue organization progresses. Detritus organization and low-class organization were characterized by high SIs. As organization levels increased, SIs declined significantly, in keeping with the histological classification. The overlapping quartiles of distinct signal classes, for example, in T1-weighted images, could be explained by the continuity of the organization process. The signal increase after contrast bolus injection (T1-weighted) obviously is related to angiogensis and distinguishes detritus and low-class organization from advanced classes. Although these relationships are not specific, they describe and quantify the expected ingrowth of connective tissue. The method investigated therefore seems to be fit for the noninvasive characterization of these processes in clinical patients.

The study protocol provided for follow-up intervals of at most 6 months. Because histological organization was not completed at this time, shrinkage of the aneurysm could not be expected to occur. The contrast bolus induced signal increase in class III (T1-weighted) is likely to decline after longer follow-up periods, because vascularity will be reduced when histological organization is complete. However, this prediction is an assumption based on the behavior of scar tissue.

References

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