ADIOSURGERY is effective in obliterating cerebral arteriovenous malformations (A VMs). Magnetic resonance (MR) imaging and angiographic studies show that there is a detectable decrease in blood flow through an A VM a few months after radiosurgery, which gradually progresses so that by 2 to 3 years, approximately 75% of A VMs are completely obliterated.4,7,9,15,16 Although these hemodynamic changes have been well documented by angiography and MR imaging, the underlying histopathological processes have not been well characterized. The present study describes the pathological findings in A VMs following gamma knife radiosurgery (GKRS) and correlates these findings with the posttreatment interval and with imaging studies.

Clinical Material and Methods

Clinical Features

Nine patients with cerebral AVMs were treated with GKRS, eight at the University of Virginia in Charlottesville, Virginia, and one in Sheffield, England. The clinical data from these cases are summarized in Table 1. All radiation treatments were given in a single session. Maximum doses to the AVM ranged from 30 to 50 Gy, and minimum doses administered to the AVM nidus ranged from 15 to 25 Gy. Most patients in this study suffered hemorrhages before angiographic obliteration was complete and came to surgery or autopsy at times ranging from 10 to 60 months after undergoing GKRS. Follow-up imaging films or reports were available for seven patients. Complete obliteration was achieved in one patient who did not suffer posttreatment hemorrhage but committed suicide 53 months after the first treatment. An A VM specimen from an untreated patient (Case 10) who died during angiography was included in the histopathological correlation analyses as a sample obtained at Time 0 postradiosurgery.

Specimen Preparation

Ten AVM specimens obtained at autopsy or surgical resection were processed using formalin fixation and paraffin embedding. Additional specimens of untreated AVMs were obtained from our surgical pathology files and were used for qualitative pathological comparisons. Five-micron-thick sections were stained with hematoxylin and
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**TABLE 1**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Pre-GKRS AVM Size (mm)</th>
<th>Location of AVM</th>
<th>Dose (Gy) Max/Min</th>
<th>Follow-Up Imaging Results (imaging grade)</th>
<th>Post-GKRS Imaging Interval (mos)</th>
<th>AVM Specimen Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43, F</td>
<td>25 x 18 x 15</td>
<td>callosum</td>
<td>40/20</td>
<td>NP</td>
<td>10</td>
<td>autopsy</td>
</tr>
<tr>
<td>2</td>
<td>39, M</td>
<td>26 x 34 x 24</td>
<td>lt frontal</td>
<td>30/15</td>
<td>NP</td>
<td>14</td>
<td>surgery</td>
</tr>
<tr>
<td>3</td>
<td>46, F</td>
<td>41 x 27 x 25</td>
<td>callosum</td>
<td>34/17</td>
<td>NC (0)</td>
<td>18</td>
<td>autopsy</td>
</tr>
<tr>
<td>4</td>
<td>65, M</td>
<td>10 x 8 x 5</td>
<td>rt frontal</td>
<td>50/25</td>
<td>&gt;75% reduction (3)</td>
<td>18</td>
<td>surgery</td>
</tr>
<tr>
<td>5</td>
<td>28, M</td>
<td>29 x 25 x 14</td>
<td>lt temporal</td>
<td>36/18</td>
<td>&gt;75% reduction (3)</td>
<td>21</td>
<td>surgery</td>
</tr>
<tr>
<td>6</td>
<td>37, F</td>
<td>30 x 50 x 30</td>
<td>lt frontal</td>
<td>30/15</td>
<td>50–70% reduction (2)</td>
<td>24</td>
<td>surgery</td>
</tr>
<tr>
<td>7</td>
<td>40, F</td>
<td>16 x 12 x 10</td>
<td>callosum</td>
<td>30/25</td>
<td>&gt;75% reduction (3)</td>
<td>34</td>
<td>surgery</td>
</tr>
<tr>
<td>8</td>
<td>29, M</td>
<td>10 x 10 x 10</td>
<td>brainstem</td>
<td>40/20</td>
<td>&gt;75% reduction–obliteration (4)</td>
<td>53</td>
<td>autopsy</td>
</tr>
<tr>
<td>9</td>
<td>25, M</td>
<td>17 x 12 x 10</td>
<td>lt frontal</td>
<td>50/25</td>
<td>&gt;75% reduction (3)</td>
<td>60</td>
<td>surgery</td>
</tr>
<tr>
<td>10†</td>
<td>47, M</td>
<td>50 x 40 x 30</td>
<td>lt cerebellum</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td>autopsy</td>
</tr>
</tbody>
</table>

* Max = maximum; min = minimum; NC = no change; NP = not performed.
† This patient was not treated but is included to show a sample obtained at Time 0 post-GKRS.

Histological Grading

Gamma knife radiosurgery–induced changes in AVM vessels were assessed using two parameters: a histological grade “H,” used to describe the severity of radiation change in the affected vessels, and a frequency grade “F,” used to estimate the percentage of vessels showing histological evidence of radiation change. Grading was performed independently in a blinded fashion by two of the authors (D.A.E. and B.F.S.). All available tissue was examined.

The histological grade H was determined by assigning a score to each affected vessel in a specimen, based on specific histological changes: 1, endothelial damage or intimal separation; 2, concentric intimal–medial proliferation; 3, concentric wall thickening with loss of cellularity and hyalinization; or 4, hyaline obliteration of vessel structure. Scores assigned to affected vessels were summed and averaged to give a histological grade H for each case. The frequency grade F was assigned to each case, based on the overall percentage of vessels showing any of the specific histological changes just described: 0, 0% to rare; 1, less than 10%; 2, 10 to 40%; 3, 40 to 80%; 4, 80 to 100%. Vessels showing no evidence of radiation damage were excluded from the analysis. The F and H grades for each case were correlated with time after irradiation and with degree of reduction in the size of the AVM shown on imaging studies (described in the following section). Correlation coefficients were calculated using linear regression analysis \(^1,5\) and compared statistically using the one-sample t-test.

Imaging Studies

Follow-up MR images or angiograms were available for seven patients. The AVM size reduction on these imaging studies was graded as: 0, no change (<25% reduction); 1, mild (25–50% reduction); 2, moderate (50–75% reduction); 3, marked (> 75% reduction); or 4, complete obliteration. This is a grading scheme that has been used by others to assess the radiographic response in AVMs following radiosurgery. \(^1,5\)

Results

Pathological Findings

Vessels comprising the vascular lesions in all cases displayed features characteristic of AVMs, which have been described in detail. \(^10\) The vessels of untreated AVMs were relatively thin walled with absent, discontinuous, or reduplicated elastic laminae. The GKRS-treated specimens regularly showed a number of changes that appeared to be progressive, producing vessel-wall thickening with stenosis or obliteration of the lumen. The earliest or least severe change was endothelial damage. This was followed by cellular proliferation and extracellular matrix (ECM) expansion in the intimal layer; then loss of cellularity and hyaline transformation of the wall matrix; and, finally, an end-stage appearance showing total obliteration of the vessel wall structure. These changes occurred in muscularized vessels throughout the AVM, with smaller vessels being more severely affected than larger ones. Affected and apparently unaffected vessels were intimately admixed, and zonal differences in GKRS effects within the AVMs were not noticeable.

Endothelial or Subendothelial Damage. This damage was evidenced by a denuded endothelium or by disruption and separation of the endothelial lining from the underlying vessel wall (Fig. 1A). The intimal spaces thus created appeared mostly empty except for a few leukocytes, some proteinaceous material or debris, and spindle cells beginning to proliferate or migrate. These spaces probably re-
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resulted from leakage of fluid across the damaged endothelium into the intima. Separation of the endothelial–intimal or intimal–medial layers was frequently circumferential, resulting in a “double-barreled” appearance of vessel cross sections. Occasionally vessels were thrombosed.

Proliferation of Intimal Smooth-Muscle Cells. Proliferation of these cells (Fig. 1B and C) around most or all of the wall circumference produced concentric or eccentric narrowing of the vessel lumina. The neoproliferating spindle cells within the intimal layer showed a relatively loose, disorganized arrangement in an abundant delicate ECM, in contrast to the more closely packed parallel layers of medial smooth-muscle cells. Variable degrees of medial and adventitial thickening were seen as well, and the transitions between the various layers ranged from distinct to indistinct. Sometimes the entire wall thickness had a “neoproliferative” appearance. Immunohistochemically, the proliferating intimal cells were consistently positive for SMA (Fig. 1C and E) and negative for factor VIII–related antigen and *Ulex europaeus* binding. This identified these cells as smooth-muscle cells or myofibroblasts rather than endothelial cells. The ECM of the proliferative zones stained lightly with collagen stains (hematoxylin-von Gieson and Masson’s trichrome), in contrast to the dense staining seen in the media, which presumably reflected the content of the fibrillar structural collagen types I and III. Immunohistochemical analysis for collagen type IV (basement membrane type) demonstrated strong positivity in the neoproliferating zone ECM (Fig. 1D), whereas the medial layers were negative. In normal cerebral muscular arteries, the intima contains collagen type IV and the media do not.

Cellular Degeneration and Increased Matrix Density. Progressive cellular degeneration and increased matrix density in thickened vessel walls appeared to represent later stages in the evolution of GKRS effects (Fig. 1F and G). Cellular degeneration was evidenced by nuclear pyknosis, decreased cell number, and loss of SMA immunoreactivity. The associated ECM displayed increasing density, hyaline change, and intensified hematoxylin von Gieson staining, indicating deposition of dense fibrillar collagen; this was accompanied by loss of type IV collagen immunoreactivity. In some vessels, these changes preferentially affected the inner or, more often, the outer wall layers. End-stage vessels displayed obliteration of the entire vascular structure, with more or less uniform dense hyalinization throughout, and with few remaining cell nuclei (Fig. 1H). Occasionally, end-stage vessels contained one or multiple endothelium-lined small lumina containing erythrocytes (Fig. 1I).

Grading and Correlational Analyses

The pathological findings suggested a sequence of changes initiated by GKRS that result in progressive stenosis and obliteration of AVM vessels. Semi-quantitative grading scales were developed to determine if these changes were correlated with time after irradiation and with reduction in AVM size observed on imaging studies (see *Clinical Material and Methods*). The data for correlation analysis are listed in Table 2.

The posttreatment interval *t* was positively correlated with both the frequency of affected vessels *F* (*r* = 0.728; *p* < 0.02) and the severity of changes in affected vessels *H* (*r* = 0.762; *p* < 0.02). A combined pathological grade, *F × H*, yielded a stronger correlation with *t* (*r* = 0.790, *p* < 0.01). The pathological grades were not significantly correlated with patient age, a time variable independent of

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Post-GKRS Interval (mos)</th>
<th>F</th>
<th>H</th>
<th>F × H</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1</td>
<td>2.0</td>
<td>0.76 (8)</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>4</td>
<td>2.88 ± 0.95 (101)</td>
<td>11.5</td>
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<tr>
<td>3</td>
<td>18</td>
<td>2</td>
<td>2.53 ± 0.69 (45)</td>
<td>5.06</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>3</td>
<td>3.22 ± 0.54 (67)</td>
<td>9.7</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>3</td>
<td>3.03 ± 0.74 (32)</td>
<td>9.1</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>2</td>
<td>3.2 ± 0.79 (10)</td>
<td>6.4</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>4</td>
<td>3.6 ± 0.48 (34)</td>
<td>14.4</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>4</td>
<td>3.08 ± 0.90 (34)</td>
<td>12.3</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>4</td>
<td>3.76 ± 0.7 (21)</td>
<td>15.0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1.8 ± 0.98 (6)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*F = frequency grade, based on the percentage of AVM vessels showing radiation changes; H = histological grade, describing the severity of radiation change in AVM vessels. See *Clinical Material and Methods* for details of H and F grading.

‡ This patient was not treated but is included to show a sample obtained at Time 0 post-GKRS.

Fig. 1. Photomicrographs displaying transverse sections of AVM vessels after GKRS. The photomicrographs are arranged (from A to I) to illustrate the chronology of the occlusive process. A: Separation of the endothelial lining from the vessel wall, creating a subendothelial space containing proteinaceous material. H & E, original magnification × 20. B: Eccentric, proliferative thickening of the intimal layer and reduplication of the elastica. Elastin von Gieson, original magnification × 20. C: Robust reaction product within the cells of the thickened intimal layer in the same vessel shown in B, demonstrated by immunohistochemical staining for SMA, original magnification × 20. D: Reaction product is deposited in a diffuse extracellular pattern within the thickened intimal layer, shown by immunohistochemical staining for type IV collagen, original magnification × 20. E: In contrast to the extracellular pattern of collagen immunostaining (shown in D), SMA reaction product appears to be intracellular. Original magnification × 50. F: Markedly thickened wall with cellular degeneration evidenced by nuclear pyknosis, decreased cellularity, and a mild lymphocytic infiltrate. The lumen is occluded by a fibrin thrombus. H & E, original magnification × 50. G: Intensified intimal–medial staining, indicating deposition of dense fibrillar collagen following cellular degeneration. Hematoxylin von Gieson staining for collagen, original magnification × 50. H: Obliteration of the entire vascular structure with dense hyalinization, few remaining cell nuclei, and nuclear debris. H & E, original magnification × 33. I: End-stage vessel whose wall structure has been obliterated by dense hyalinization. Multiple endothelial cell–lined channels containing erythrocytes indicate recanalization. H & E, original magnification × 66.
the posttreatment interval (vs. F; r = −0.386, p < 0.3; vs. H, r = −0.348, p < 0.4; vs. F × H, r = −0.403, p < 0.3).

The histopathological grades of the GKRS-induced changes in the lesions correlated well with size reduction on imaging (vs. F; r = 0.864, p < 0.01; vs. H, r = 0.794, p < 0.02). Serial imaging studies have demonstrated that in a given patient AVM size decreases over time after GKRS.1,5,9,13,14 However, using correlation analysis in the present small series, the association of the size reduction shown on imaging studies with the posttreatment interval (r = 0.709, p < 0.05) was not as strong as the associations of either of these variables with the histopathological changes.

**Discussion**

In this study we examined the histopathological changes in AVMs to elucidate the processes leading to AVM obliteration after GKRS. The sequence of changes described by the histopathological H grade—endothelial–intimal damage, intimal smooth-muscle cell proliferation and elaboration of collagen, and hyaline sclerosis with ultimate obliteration of vessel wall structure—correlated well with time after irradiation and with size reduction shown on imaging, and thus appears to represent a progressive process leading to AVM obliteration. An important feature of the radiation-induced effects is that they were concentric or eccentric, involving all or nearly all of the vessel wall circumference. Vessels in untreated AVMs sometimes display focal mural proliferations, forming thickenings or cushions.19 These generally do not cause significant stenosis of the lumen, and marked thickening of the entire wall circumference in untreated AVMs is rare. The relative numbers of noticeably affected vessels in the treated AVMs (F grades) also increased with time after treatment. This may reflect the amount of cell proliferation needed to cause obvious wall thickening and luminal stenosis in small versus larger vessels—that is, the latter require more time to develop significant changes. However, adjacent vessels of similar size sometimes showed marked variation in radiation effects, suggesting that different vessels may have different radiation susceptibilities or develop changes at different rates. This has also been observed in normal cerebral vessels after conventional irradiation.6

There are few previous descriptions of radiosurgery-induced histopathological changes in AVMs. Yamamoto, et al.,15 presented a case in which angiographic obliteration was achieved before the patient’s death. The pathological findings were very similar to those seen in our advanced cases. The occluded vessels in that case were immunopositive for collagen type III, consistent with our observation that end-stage vessels lost collagen type IV immunoreactivity and acquired staining characteristics of dense collagen. Adams1 briefly described thickening, fibrosis, and occlusion by connective tissue in proton beam–treated AVMs, consistent with our findings. Conventional fractionated radiotherapy, which is ineffective in treating AVMs, did not produce these pathological changes.5

The effects of GKRS on AVMs appear to be similar to those described in normal arteries after high-dose irradiation, which have been well documented in humans and in experimental animals.2,6 The earliest lesion appears to be endothelial damage, which is evident within days after treatment. Months to years later, vessels develop concentric or eccentric narrowing of their lumina, with intimal fibrous proliferation, foam cell accumulation, and hyalinization of vessel walls. These changes represent nonspecific responses to vascular injury and, indeed, resemble certain aspects of atherosclerotic and traumatic lesions. The consequences of radiation-induced endothelial damage depend on the size of the involved vessel. Endothelial cells undergo swelling, loss, and compensatory proliferation after irradiation. Endothelial proliferation in the parenchymal microvasculature may cause ischemia and contribute to the development of radiation necrosis.3 In larger vessels, the proliferative response may serve to repair the endothelial lining, but occlusion of these vessels results from intimal smooth-muscle cell proliferation secondary to endothelial injury.2,6

Clinical data indicate that patients treated with radiosurgery may no longer be at increased risk of hemorrhage once angiographic obliteration is achieved.13 Our cases and that of Yamamoto, et al.,15 reveal that some, otherwise obliterated, vessels within the AVM contain small capillary-like spaces that are lined by a single layer of endothelial cells and contain red blood cells (as shown in Fig. 1H). It is impossible to determine from our specimens if these represent recanalization of end-stage occluded vessels or if recanalization occurred at an earlier stage (for example, after thrombosis) in vessels that subsequently developed progressive sclerosis.6 In the latter case, residual recanalized lumina thus would not indicate reversal of the treatment effect. It is noteworthy that in none of our cases was angiographic obliteration of the AVM documented. The finding of such recanalized vessels was a rare occurrence. Based on their small caliber relative to other vessels of the AVM, it is likely that their presence could not be detected using angiography, as has been pointed out previously.16 Functionally, such small vessels are probably of little significance and, because of their low flow, are probably at little risk for rebleeding; however, this may only be determined definitively by using histopathological follow up of AVMs many years after radiosurgery in individuals dying from other causes.

**References**


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