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Original Article

Vascular alterations in schwannoma

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Abstract: Schwannomas or neurilemmoma are benign peripheral nerve sheath tumors, which most frequently occur at the cerebellopontine angle. This morphologic study examines vascular alterations in these tumors, comparing them to other benign spindle cell neoplasms of the nervous system, while correlating these findings with evidence of vascular permeability. Thirty-four nervous system spindle cell neoplasms, sixteen schwannomas, nine fibroblastic/transitional meningiomas and nine peripheral neurofibromas were stained with H&E, Prussian-blue stain, and immunoreacted for factor VIII-related antigen and interstitial albumin. Schwannomas had focal clusters of vascular proliferation including groups of small thin-walled vessels, as well as larger vessels with extensive hyalinization. Neurofibromas and meningiomas almost uniformly had modest numbers of well-defined, thin walled individual vessels. Free hemosiderin and hemosiderin-laden macrophages were frequently identified in schwannomas. Prussian-blue stain for iron revealed focal or fairly widespread positivity in almost all schwannomas, only one meningioma and none of the neurofibromas. Immunoreaction for albumin demonstrated leakage of vascular proteins into the interstitium confirming tumor vessel permeability in schwannomas. Neither neurofibromas nor meningiomas displayed any detectable interstitial albumin. The above findings confirm a degree of reactive proliferation of vessels in schwannoma along with functional deficits in their vascular integrity with permeability to protein and blood. The presence of hyalinized vessels, hemosiderin, both free and within macrophages, and more readily evident Prussian blue staining, may provide an additional diagnostic clue in discriminating between histologically similar spindle cell lesions. The study however raises the possibility that these changes likely precede or facilitate the degenerative 'ancient change' seen in some schwannoma.

Keywords: Schwannoma, vascular hyalinization, hemosiderin, vascular permeability

Introduction

Schwannomas may be seen in individuals of any age, although a peak incidence between the third and sixth decades has been defined. These tumors are the most common intracranial nerve sheath tumors with the most frequent location being the cerebellopontine angle [1]. These spindle cell neoplasms present a biphasic appearance with both hypercellular areas with a fibrillary or fascicular arrangement, and hypocellular, microcystic or vacuolated areas. These distinct morphologic patterns were first described in 1920 by Antoni and have since been referred to as Antoni A and Antoni B areas [2]. Additionally the presence of a focal palisading of tumor cells, often referred to as Verocay bodies is also considered a diagnostic feature [3] and although quite common in peripheral Schwannomas, is rarely seen in the intracranial variant. Hyalinized vessels are mentioned in occasional histopathologic descriptions of Schwannomas [4-8]. The vessels are described as having thickened walls with a glassy eosinophilic, collagenous wall. Hemosiderin, both free and within macrophages has also been described in Schwannomas particularly in the context of areas of 'ancient change' [5, 6, 9, 10]. Although the presence of hyalinized vessels is mentioned in the context of intracranial, cerebellopontine angle Schwannomas and rarely in examples of peripheral Schwannomas, this histologic feature has generally not been reported in other peripheral nerve sheath tumors such as neurofibromas. Similarly, these changes have not been described in meningiomas, fibroblastic or others, which may also occur at the same intracranial location. In our review of the literature we found

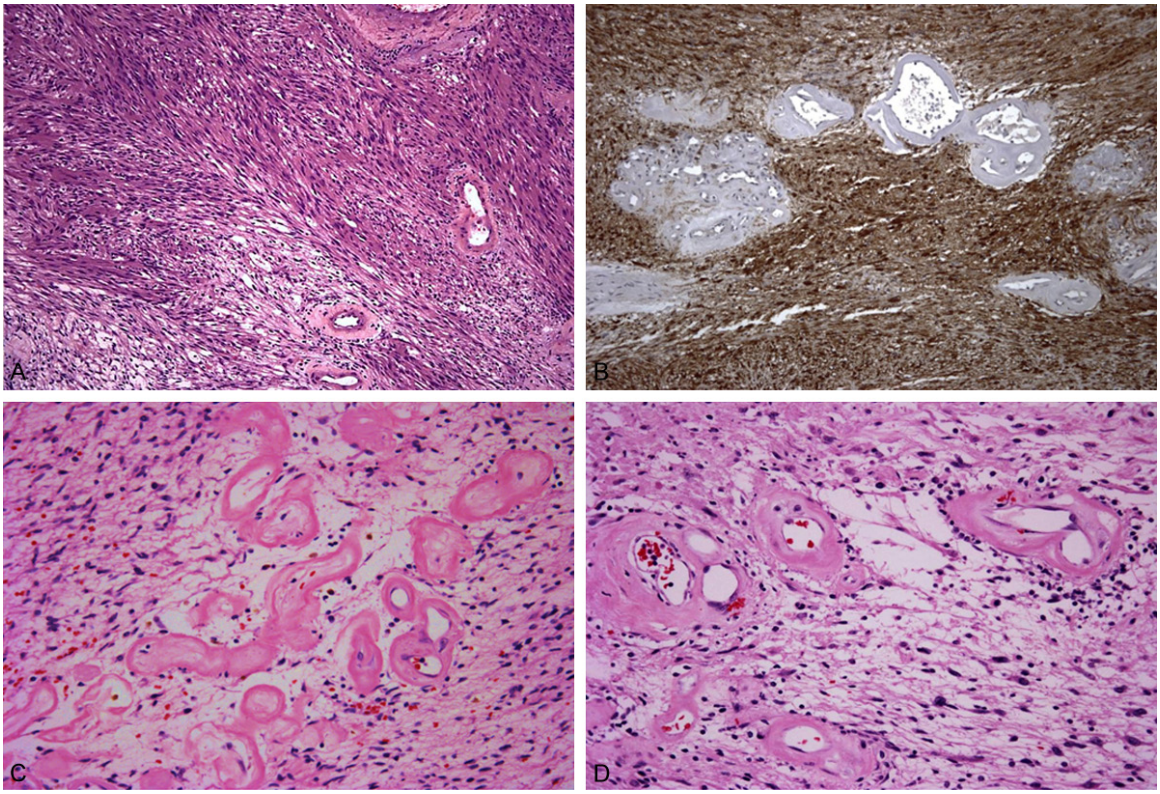

Figure 1. A: Histologic appearance of a typical Schwannoma with more compact spindle cell component (Antoni A area) and a looser, somewhat vacuolated and hypocellular Antoni B area in the lower part of the image. Hyalinized vessels (arrows) can be seen in both areas (Original magnification 100 x); B: All Schwannomas were strongly immunoreactive for S-100. Hyalinized vessels, single and ectatic or small and clustered are seen as nonimmunoreactive, in a background of S-100 immunoreactive Schwann cells (Original magnification 100 x); C: Hyalinized vessels were often seen in clusters and were focally ectatic and tortuous. These vessels are readily identified by their thickened eosinophilic, collagenous walls (Original magnification 200 x); D: Hemosiderin laden macrophages were frequently seen, especially adjacent to vessels with hyaline change along with some free hemosiderin (Original magnification 200 x).

only rare and limited descriptions of these changes, their distribution, possible functional implications or their diagnostic utility in surgical neuropathology. With these considerations in mind, we examined histologic features in a group of Schwannomas, both intracranial and peripheral, and compared them with neurofibromas and meningiomas.

Materials and methods

Archived cases from the surgical pathology files were randomly selected based on prior diagnosis and the availability of adequate tissue for further studies. Fourteen intracranial Schwannomas and 16 peripheral Schwannomas, 9 neurofibromas and 9 fibroblastic/transitional meningiomas were examined. All cases had been fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin. 5 micron sections were examined with H&E and Prussian blue stain for iron.

A very limited number of cases (3 schwannomas) for electron microscopy were fixed in 2.5% glutaraldehye in phosphate buffer, osmicated and embedded in LX112 resin. Ultrathin sections were cut on a Reichert ultramicrotome, stained with uranyl acetate and lead citrate and examined on a Philips CM10 transmission electron microscope.

Immunohistochemistry for factor VIII related antigen (fVIIIra) was performed using a rabbit, anti-human polyclonal antibody (DAKO, Santa Barbara, CA), on a Ventana automated immunostainer with a protease-2 pretreatment for eight minutes, at a dilution of 1:1500. Slides were incubated for 30 minutes and counter-

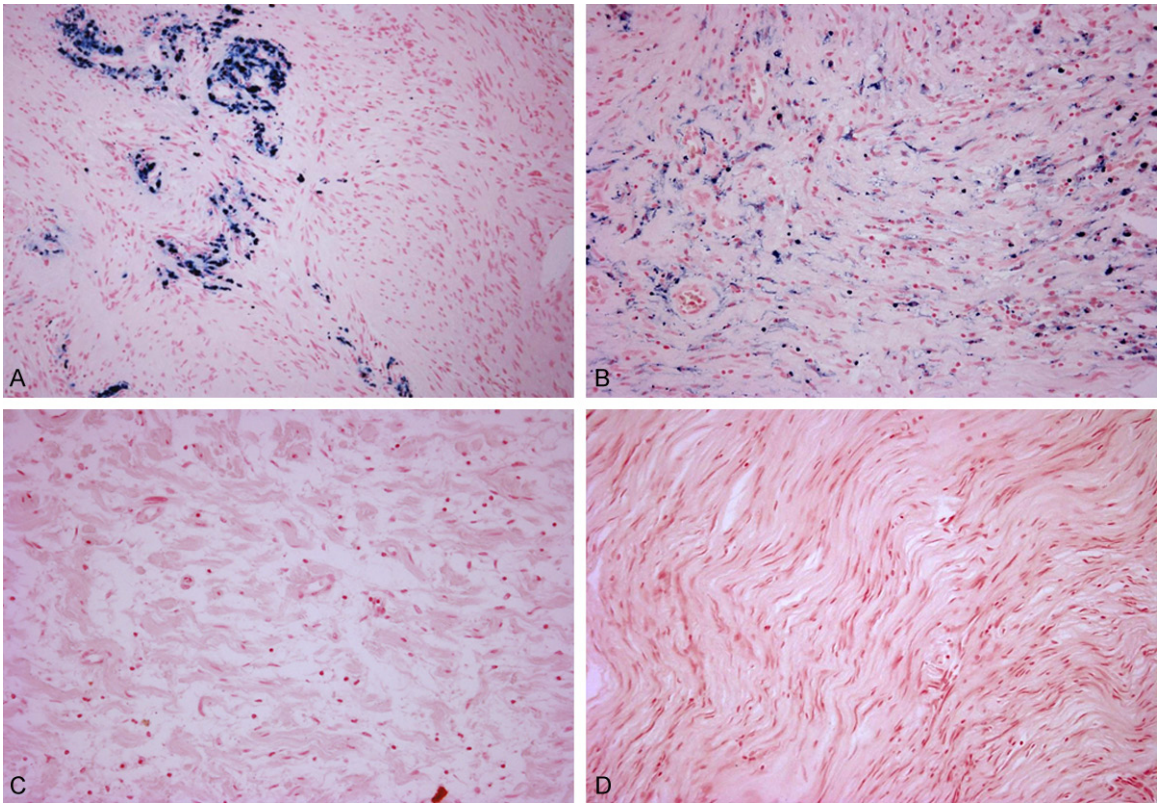

Figure 2. A: Prussian blue staining readily identified hemosiderin laden macrophages as well extracellular iron (Original magnification 40 x); B: Although in most cases brown hemosiderin product was seen predominantly in siderophages and could be identified on H&E stains, in many examples Prussian blue stain identified a more diffuse distribution of extracellular hemosiderin (Original magnification 100 x); C: Neurofibromas did not show any positive staining with the Prussian blue method (Original magnification 100 x); D: Similarly, no Prussian blue positivity was seen in 8 of 9 meningiomas (Original magnification 100 x).

stained with hematoxylin. Positive controls included blood vessels in normal tissues. Negative controls include either omission of primary antibody or nonspecific IgG applied to the sections.

Immunoreaction for the detection of extravasated albumin was carried out with an HRP-conjugated rabbit IgG fraction to albumin. This was visualized with diaminobenzidine as the chromogen. The protocol was as for routine immunohistochemistry, but without secondary antibody.

Results

Tumors were selected based on prior surgical pathology diagnosis. This diagnosis was reconfirmed by histopathologic examination. Typical histologic features of Schwannoma, neurofibroma or meningioma were confirmed on H&E stained sections.

Vascular changes

Special attention was focused on the vasculature in these tumors. Hyalinized vessels i.e. vessels with thickened eosinophilic, collagenous walls were identified in all Schwannomas examined. They occurred either singly or in clusters as illustrated. They were seen both within Antoni A and Antoni B areas (**Figure 1A**). Schwannomas that showed areas of degeneration (ancient change) often had more extensive and diffuse hyalinization of vessels (**Figure 1B**). In some areas the vasculature was relatively normal in appearance, although occasionally they were clustered rather than singly distributed. This is illustrated in **Figure 1B** and also in **Figure 1C** as non-reactive vessels seen in a background of S-100 immunoreactive Schwann cells. Also present around many of these hyalinized vessels were scattered macrophages, some with lipid, especially in areas showing

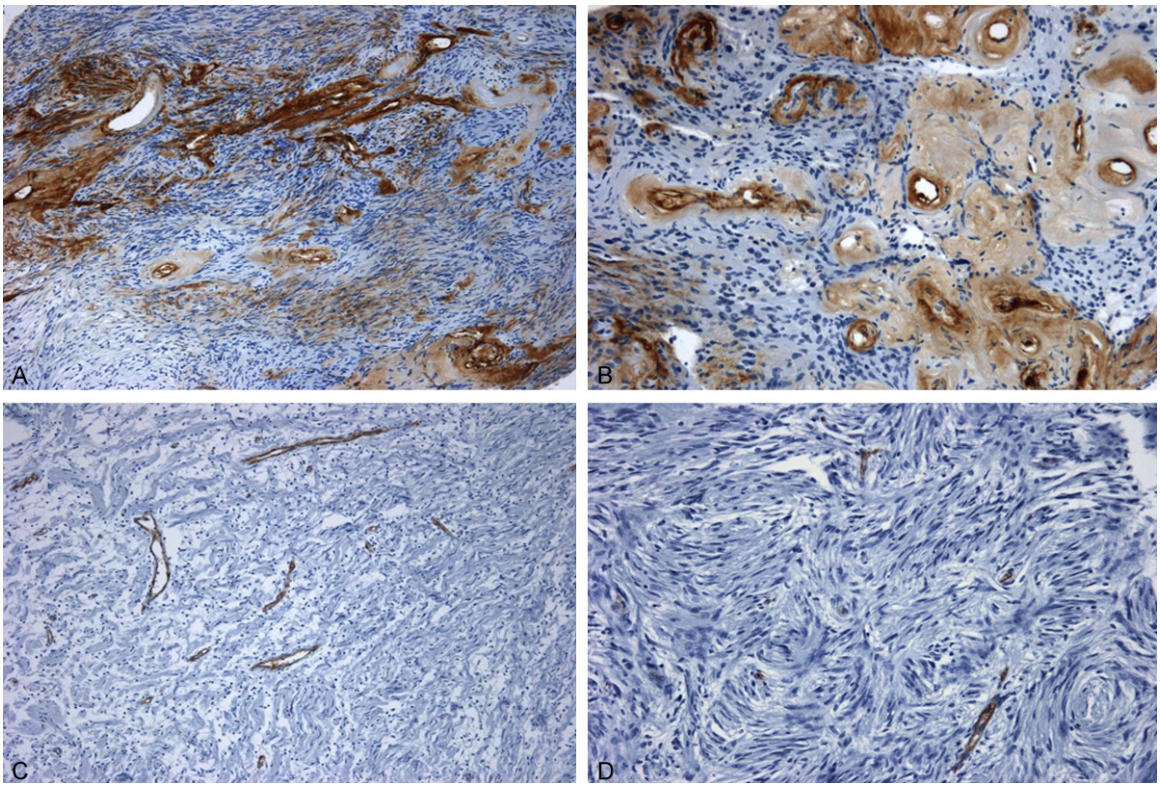

Figure 3. A: Factor VIIIra immunoreactivity highlights blood vessels, with strong positivity within endothelial cells. This staining pattern identifies a larger number of vessels than is readily apparent on H&E stains; B: Vessels with significant hyalinization displayed a moderate, positive intramural blush in addition to their more intensely stained endothelial cells. This staining pattern differed from the typical endothelial immunoreactivity seen on the luminal surface and raises the possibility of diffusion or leakage: C and D: Blood vessels in neurofibroma and meningioma were widely spaced, often single and discrete. Immunoreactivity was sharp and limited to endothelial cells and without any extravascular blush (Original magnifications 100 x and 200 x).

degenerative changes. Hemosiderin laden macrophages were frequently seen, especially in a perivascular distribution; although extracellular hemosiderin was also present (**Figure 1D**). Blood vessels in neurofibromas and meningiomas were typically thin-walled and simple, and in only very rare examples displayed any hyalinization, and even then the changes were minimal.

Prussian blue staining

Prussian blue staining was positive in 29/30 Schwannomas. With the exception of a modest increase (not quantitated) in staining in peripheral tumors relative to intracranial neoplasms no significant morphologic differences were recognized. The staining pattern varied from large or small perivascular foci (**Figure 2A**) to fairly diffuse areas of positive reaction scattered in multiple regions of the tumor (**Figure 2B**). The hemosiderin was seen both within macrophages and extracellularly. None of the neurofibromas examined (0/9) and eight of the nine meningiomas had no Prussian blue positivity (**Figure 2C** and **2D**). Scattered, focal staining was detected in only one of nine meningiomas.

Factor VIIIra immunoreactivity

Positive immunoreaction for fVIIIra was specifically identified in blood vessels. Vessels were often seen in clusters and appeared thick walled (**Figure 3A**). Hyalinized vessels in addition to their intensely stained endothelial cells also revealed a moderate, positive blush in their thickened walls. This staining pattern differed from the typical endothelial immunoreactivity seen on the luminal surface of vessels and suggested diffusion or leakage, (**Figure 3B**). Other changes included clustering of vas-

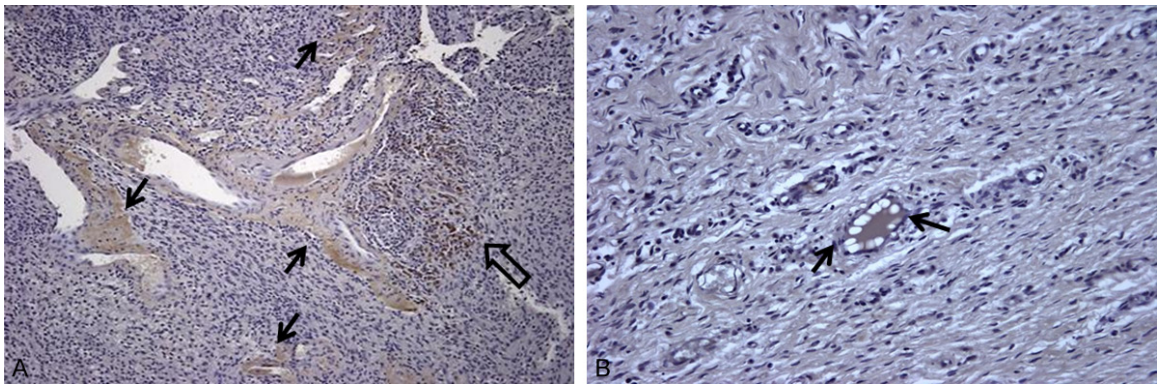

Figure 4. A: Anti-albumin antibody demonstrated leakage of vascular proteins into the interstitium, as diffuse and poorly defined, often close to vessels in Schwannomas (arrows). This could be clearly differentiated from the golden-brown hemosiderin seen in this case (open arrow). (Original magnification 40 x); B: No reactivity was seen in the neurofibromas or meningiomas except as an intraluminal product (arrows) (Neurofibroma, (Original magnification 200 x)).

culature along with groups of thin-walled vessels particularly in Antoni B areas, likely reactive neovascularization. In contrast, blood vessels in neurofibromas and meningiomas were widely spaced, often single and discrete. Immunoreactivity was discrete, limited to endothelial cells and without any extravascular blush (**Figure 3C** and **3D**).

Anti-albumin immunoreactivity

In order to define the vascular integrity of these tumors we sought to detect the presence of extravasated albumin. Using an HRP-conjugated rabbit IgG fraction to albumin we identified positive staining in nine of fourteen Schwannomas examined with this method. The pattern of immunoreactivity was interstitial, often close to vessels, but quite poorly defined and diffuse (**Figure 4A**). Positive intravascular immunoreactivity was also seen within the lumina of multiple vessels. No reactivity was seen in the neurofibromas or meningiomas except as an intraluminal product (**Figure 4B**).

Ultrastructural examination

This study makes no attempt to describe the ultrastructural characteristic of these three neoplasms which have been well described in earlier studies [4, 6, 11-14]. Only a limited number of Schwannomas were examined by electron microscopy, specifically examining vascular profiles only. Typical ultrastructural characteristics including laminin rich profiles were readily seen. Small aggregates of basal lamina-like material were observed around some vessels. However, reduplicated layers of basal lamina seen within areas of the tumor did not appear to contribute to the hyalinized appearance of vessels. Rather, multiple bundles of collagen were readily apparent around many vessels. Endothelial cells revealed no structural abnormalities and no significant alterations were observed that would provide a morphologic substrate for the increased permeability previously identified.

Discussion

Peripheral nerve sheath tumors, whether intracranial or peripheral generally do not present a diagnostic dilemma. Similarly, intracranial fibroblastic or transitional meningiomas are uncommonly of concern with regard to identity. This study however has sought to understand certain morphologic difference between these three neoplasms, with a particular focus on alterations in blood vessels and possibly functional integrity.

Immunoexpression of S-100 protein is readily identified in both Schwannomas and neurofibromas. Schwannomas have a more extensive S-100 distribution as Schwann cells are the predominant histologic element. Neurofibromas however may have less prevalent expression pattern because of other cellular components [15, 16]. The classic histologic pattern seen within most Schwannomas includes Antoni A and Antoni B regions. Antoni A areas are typically described as cellular areas containing

spindle shaped nuclei with tapered ends. These areas have a more densely packed cell population and may display Verocay bodies or palisading clusters of nuclei. Although readily seen in Schwannomas associated with peripheral nerves this curious architectural arrangement is rarely associated with intracranial tumors. Antoni B regions in contrast are relatively hypocellular, often with a vacuolated or microcystic appearance, Ancient change has been well documented in Schwannomas and is generally recognized to be a degenerative change. Hemorrhage and hyalinization, with hemosiderin-laden macrophages are commonly seen. In our study the morphologic changes observed relative to the vasculature were not confined to Antoni B areas or to areas undergoing degeneration (ancient change), although they were undoubtedly more prominent in these areas.

The focal and sometimes diffuse, perivascular iron (Prussian blue positive) deposits confirmed that vessels were permeable to red blood cells and breakdown products. Similar deposits were not present in meningiomas or neurofibromas and there were no significant differences between intracranial and peripheral neoplasms. These vessels are also permeable to vascular proteins as well as red blood cell components as evidenced by immunoreactivity for albumin and fVIIIra. Factor VIIIra immunoreactivity was specific to endothelial cells, however many hyalinized vessels also revealed a strong positive blush in their walls and often in their immediate vicinity. Albumin is the most abundant plasma protein and is responsible for maintaining oncotic pressure. Using an HRP-conjugated anti-albumin antibody, interstitial protein was detected within Schwannomas further supporting the notion that these vessels are also functionally impaired. The distribution and morphologic pattern of fVIIIra immunoreactivity also supports the same observation, while defining the proliferation of blood vessels that is a component of Schwannomas, but not seen in meningiomas or neurofibromas. Leakage of serum components into the neoplasm may be responsible for eliciting immunologic or toxic injury, predisposing to influx of macrophages and release of vasculogenic and proinflammatory cytokines with consequential reactive responses. While much of this remains conjecture the possibility that these vascular changes may be precursors or contributors to the process that results in Antoni B areas or areas undergoing degeneration/ancient change warrants further consideration.

Disclosure of conflict of interest

None.

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