Virchows Archiv A Pathol Anat (1993) 423:281–285

Virchows Archiv A
Pathological Anatomy and Histopathology

Nasopharyngeal angiofibroma: an immunohistochemical study of 32 cases

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Received May 21, 1993 / Received after revision June 30, 1993 / Accepted July 1, 1993

Abstract. Thirty-two cases of nasopharyngeal angiofibroma, including 2 recurrences, all of which had been excised from males between 7 and 25 years, were subjected to systematic immunohistochemical study. Most of the tumour vessels, which lacked elastic laminae, were characterized by vascular walls of irregular thickness and variable muscle content. In places endothelial cells were only separated from the stroma by a single attenuated layer of contractile cells, whereas elsewhere the same vessel walls showed pad-like thickenings of their muscle coat. All cells of the vessel walls showed immunoreactivity for vimentin and smooth muscle actin, whereas desmin-positive cells were present only in small numbers in some vessels, generally those with thicker muscle coats. The stromal cells were decorated by vimentin antibodies only; however, in some more fibrotic hyaline areas the stromal cells displayed also reactivity for smooth muscle actin. In most cases S-100 protein-staining disclosed many nerves, and this accentuated their partial distortion by tumour tissue. Our findings provide an extended insight to the morphology of angiofibromas at this site, particularly highlighting the irregularity of their vascular walls, which, taken together with the lack of elastic laminae and elastic stromal fibres, can be held responsible for the typical pronounced tendency for haemorrhage in these lesions.

Key words: Nasopharyngeal angiofibroma – Blood vessel – Smooth muscle cell – Immunohistochemistry – Actin

Introduction

Nasopharyngeal angiofibromas are uncommon tumours, typically arising in adolescent males, which are histologically composed of a proliferated vascular component set in a fibrous stroma. The former is characterized by variably sized, disorganised vessels of varying thickness. The stroma consists of plump spindle, angular or stellate shaped cells and a varying amount of collagen fibres (Stiller and Küttner 1988). Reviewing the literature on the morphology of nasopharyngeal angiofibromas, as demonstrated by special techniques, many electron microscopical studies can be found (for review see Stiller et al. 1976; Taxy 1977; Arnold and Huth 1978). Moreover, recently the presence of oestradiol in stromal fibroblasts (Kumagami 1991) and the localization of an angiogenic growth factor in the endothelium (Schiff et al. 1992) has been shown immunohistochemically.

In contrast, there has been no systematic immunohistochemical evaluation of the typical components in a large series of these lesions. This may be in part due to the relative paucity of cases in a single centre, as well as to their characteristic histological pattern, which usually causes no difficulty in diagnosis. We have examined immunohistochemically 32 cases of nasopharyngeal angiofibroma with special emphasis on the structure of the vessel walls.

Materials and methods

Thirty-two cases of nasopharyngeal angiofibroma were collected retrospectively from our files. These lesions were found only in male patients, with an age range between 7 and 25 years. Two cases represented tumour recurrence after 2 and 4 years respectively.

After surgical removal in each case tissue was fixed in 10% formalin, routinely processed and embedded in paraffin wax. Consecutive sections, 4 μm thick, were stained with haematoxylin and eosin, and with the elastic-van Gieson stain.

For immunohistochemical studies the avidin-biotin-peroxidase (ABP) technique was performed, using antibodies to S-100 protein (polyclonal, 1:2000, Dako), Factor VIII RAG (polyclonal, 1:200, Dako), desmin (monoclonal, 1:50, Dako), pan-muscle actin (HHF 35, monoclonal, 1:20, Enzo), alpha smooth muscle actin (monoclonal, 1:5000, Sigma), vimentin (monoclonal, 1:50, Progen) and anticytokeratin (Lu5, monoclonal, 1:50, Boehringer Mann-

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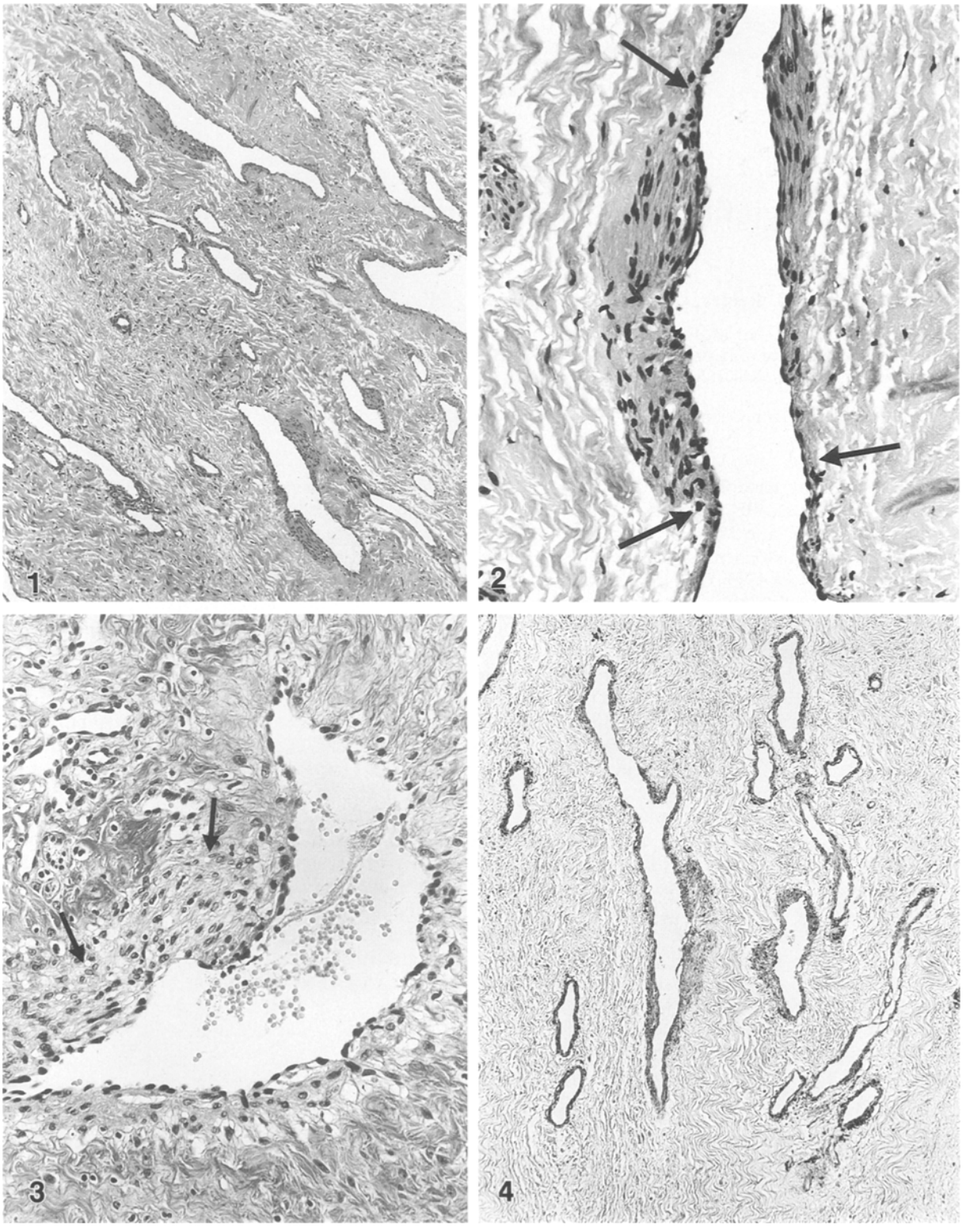

Fig. 1. Tumour vessels showing focal pad-like thickening of their wall. H&E, ×63

Fig. 2. In a single vessel pad-like thickenings of the wall change abruptly to very thin areas (*arrows*). H&E, ×250

Fig. 3. Vessel wall exhibiting focal pad-like thickening as reflected by the presence of several muscle layers (*arrows*); in addition, note the absence of elastic laminae. Elastic van Gieson, ×250

Fig. 4. All cells of the vascular muscle wall are immuno-reactive for smooth muscle actin, thus demonstrating the irregular architecture of the vessel walls. ABP, ×100

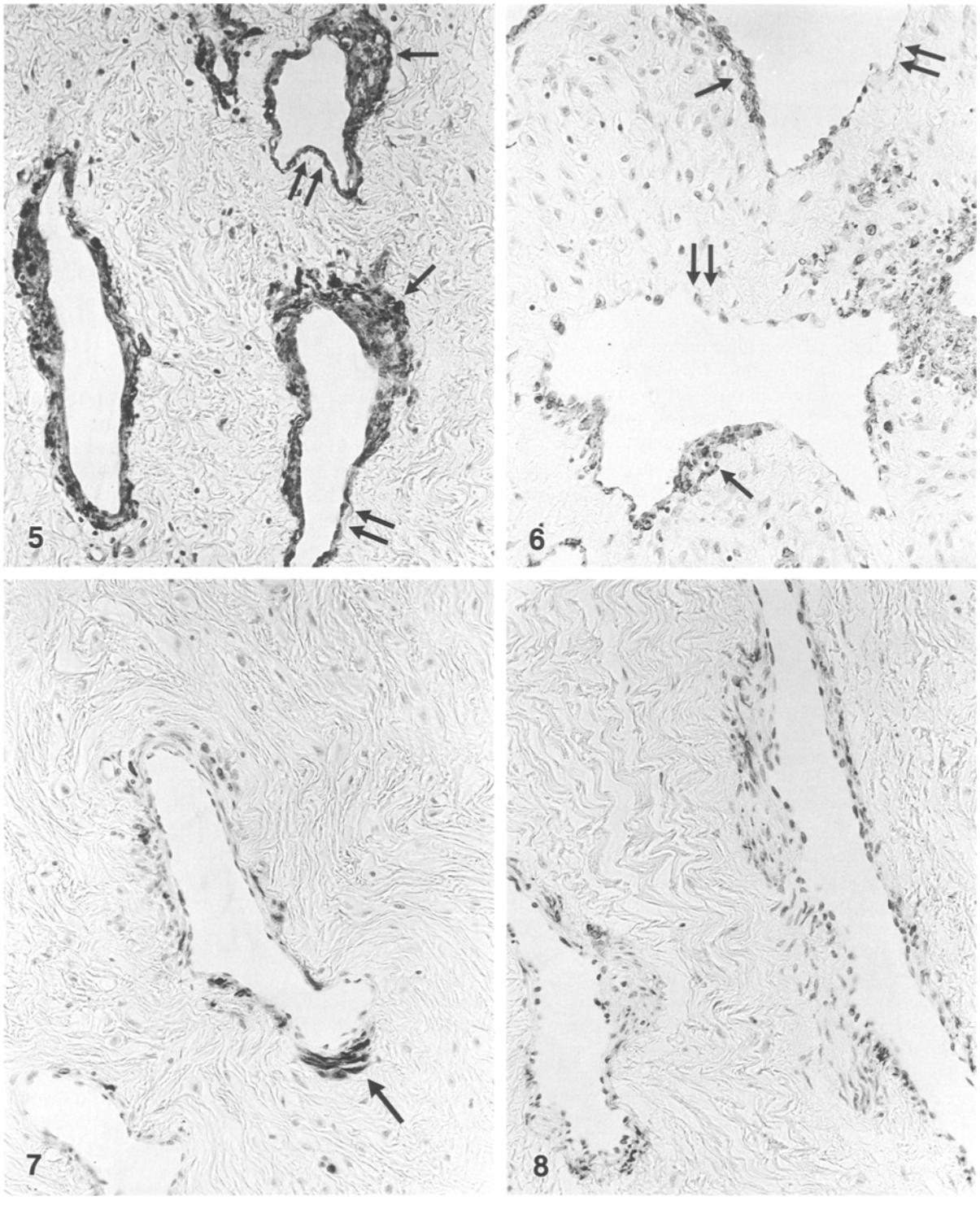

Fig. 5. Immunostaining of the vessel walls for smooth muscle actin elucidates the simultaneous occurrence of pad-like thickening (*one arrow*) and thin areas (*two arrows*) in a single vessel. ABP, ×250

Fig. 6. In places, there are no smooth muscle actin-reactive cells beneath the endothelium (*two arrows*); smooth muscle actin-positive cells are marked by *one arrow*. ABP, ×250

Fig. 7. The wall of this tumour vessel exhibits desmin-positive cells in a thickened area (*arrow*). ABP, ×250

Fig. 8. In contrast to Fig. 7, pad-like thickened areas of vessel walls reveal no desmin-reactive muscle cells. ABP, ×250

heim). For control purposes, tissues known to contain the respective antigens were included (positive controls). Replacement of the primary antibody by normal serum always revealed negative results (negative controls).

Results

By conventional histology all tumours displayed similar characteristic microscopic features to those which have been described in detail elsewhere in the literature (Stiller and Küttner 1988). Briefly, the lesions consisted of a fibrous stroma which was composed of collagen fibres and a varying number of cells of plump spindle, angular or stellate shape. These cells were characterized by one, or rarely multiple hypochromatic nuclei with distinct nucleoli and showing occasional normal mitoses. In places, the stroma showed hyalinization, fibrosis or myxoid change. Moreover, the tumours contained many blood vessels of different size and configuration. The vessels were mostly thin-walled, slit-like or dilated with calibres ranging from capillary size to large diameter. In addition, in each case, there was a varying number of tumour vessels exhibiting a more or less circumscribed, pronounced pad-like muscular thickening of their wall (Fig. 1), a feature which has not been particularly emphasised by previous descriptions. Individual vessel walls showed striking variation in thickness, even over short distances, such that pad-like thickenings could be adjoined by an almost absent vessel wall (Fig. 2). Examining the vessel walls for the presence of elastin by the elastic van Gieson stain, the typical tumour vessels were found to be devoid of all elastic tissue (Fig. 3). However, in the periphery of most lesions there were a few regular vessels of arterial type (feeding arteries, probably representing branches of a larger pre-existing artery) which contained elastic laminae. No elastic fibres could be demonstrated within the fibrous stroma.
Generally, each case revealed a similar immunohistochemical pattern with little or no variation.

The lumen of all tumour vessels and feeding arteries was lined by a single layered endothelium which showed inconspicuous positivity for F VIII RAG. The endothelial cells also showed cytoplasmic immunoreactivity for vimentin, the staining intensity of which often exceeded that of the other cells in the vessel wall or the stroma.

The wall of the feeding arteries, characterized by a regular architecture and the presence of elastic laminae, revealed uniform positivity of each cell with antibodies to vimentin and smooth muscle actin. Moreover, all cells were stained for pan-actin, whereas desmin reactivity could be found only in single cells of peripheral and medial layers of the muscle coat. These normal vessel walls were always invested by hypocellular stromal tissue.

In every neoplastic vessel, all cells of the muscle wall (independent of thickness) displayed immunostaining for vimentin and smooth muscle actin. Thus, the irregular architecture of the muscle wall was highlighted impressively (Fig. 4). In some of the larger vessels, the simultaneous occurrence of very thin, and focally pad-like thickened areas of the muscle coat was noted at different points of the same vessel wall (Fig. 5). Independent of vessel size, focally there were no immunoreactive cells beneath the endothelium, indicating the apparent absence of either smooth muscle cells or pericytes (Fig. 6). In some vessels, mostly of smaller calibre but occasionally of larger size, the wall was composed only of a single, vimentin and smooth muscle actin-positive cell layer.

The immunoreactivity for desmin was variable, did not always correlate with wall thickness, and could be detected in only a few tumour vessels. Generally, more peripherally located cells of thickened vessel walls were stained, but in some places also the cells of inner layers were positive (Figs. 7, 8). The staining pattern for pan-actin was roughly similar to that for smooth muscle actin, but the cells of thin-walled, mainly small and medium sized vessels, were not decorated by this antibody.

The stromal cells showed positivity only for vimentin. In this way, their different shapes, whether spindly, angular or stellate, were accentuated. Only in more fibrotic hyaline areas, possibly reflecting fibrosis consequent upon previous thrombosis and infarction, the stromal cells were also stained by the antibody to smooth muscle actin. In addition, in most cases, the stroma contained many S-100 protein positive nerve fibres. The nerves were either normal or appeared distorted by tumour tissue.

Discussion

In presenting the first systematic immunohistochemical study of nasopharyngeal angiofibromas, our interest has been focussed mainly on the structure of the tumour vessels. It is well-known, from the literature, that in normal vessels the immunophenotype of smooth muscle cells depends on the location of these cells with regard to the vascular lumen and on the anatomical site of the blood vessel (Kocher and Gabbiani 1986; Osborn et al. 1987).

As shown by their reactivity for smooth muscle actin, a reliable marker of cells showing smooth muscle differentiation (Skalli et al. 1986), the vascular walls displayed pronounced variability of their smooth muscle cell layers, even within a single vessel. In this context it is of particular interest that we demonstrated an indisputable, though often irregular, muscle coat in many tumour vessels, which is in contrast to the findings of Neel et al. (1973) and Bremer et al. (1986). Surveying 120 and 150 patients with nasopharyngeal angiofibroma respectively, these authors reported that "microscopically endothelial cells lie directly against stromal cells" and that there is "a lack of intervening smooth muscle between these two cell types". Our alternative findings are supported by the basic ultrastructural study of Svoboda and Kirchner (1966) on nasopharyngeal angiofibromas, which illustrated the presence of vascular smooth muscle cells.

In some places the muscle coat was characterized focally by pad-like thickenings, a finding which has not been mentioned in previous reports, which have usually

described the muscle layer as narrow (Enzinger and Weiss 1988; Stiller and Küttner 1988). Independent of the thickness of the muscle wall, all its cells were immunoreactive for vimentin and smooth muscle actin. If there is one cell layer only, this staining pattern indicates the presence of pericyte-like cells. Normally, pericytes have been found beneath the endothelium of every capillary and postcapillary venule thus being the only investing and contractile element in vessels without muscle coat formation. Since pericytes exhibit an immunophenotype quite similar to vascular smooth muscle cells (Skalli et al. 1989a), we can not be really dogmatic about whether these cells are proper pericytes or simply represent vascular smooth muscle cells in a tumour with irregular vessel wall formation on the basis of immunohistology. However, the presence of pericytes in tumour vessels of nasopharyngeal angiofibroma has been demonstrated electron-microscopically (Stiller et al. 1976; Taxy 1977).

In most tumour vessels the cells of the muscle wall did not show desmin expression, but, occasionally, in areas with a thickened muscle coat, cells of the peripheral layers were desmin-positive, thus paralleling the findings in the feeding arteries. Therefore, these areas can be regarded as showing a complete muscle wall, whereas the other parts correspond to incomplete or abortive muscle coat formation. At one extreme, the vessel wall consisted of one investing cell layer suggestive of pericytes only or, in small foci, was even devoid of these cells. It was most striking that there were frequently abrupt and dramatic changes in vessel wall thickness, as we have illustrated. These local variations must have a significant influence on vessel wall strength and integrity.

In 1966 Svoboda and Kirchner published an ultrastructural study of nasopharyngeal angiofibromas, describing numerous smooth muscle cells in the stroma independent of vessels. These cells, based on the description of intracytoplasmic "fibrillar material" and "fusiform densities", are very likely to correspond to myofibroblasts, which have also be found in the stroma in other studies (Stiller et al. 1976; Taxy 1977). This view is supported by our immunohistochemical study which demonstrated co-expression of both actin and vimentin in some cells. Nevertheless, we are aware that stromal cells showing only vimentin positivity may ultrastructurally represent myofibroblasts, as these cells have turned out to have a very variable phenotype (Skalli et al. 1989b; Skalli and Gabbiani 1990).

The irregularity in structure and thickness of the vessel walls in nasopharyngeal angiofibromas shown in this study, in combination with the known absence of elastic laminae, may explain the striking propensity for haemorrhage in these lesions.

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