# Giant schwannoma of the cheek – a comprehensive histological and immunohistochemical description of a rare tumour

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Schwannoma is a benign tumour originating from Schwann cells forming sheaths of peripheral nerves. Its location in the oral cavity, and particularly in the cheek, is very rare. A large tumour (5 cm) was surgically removed from the left cheek of a fifty-five-year-old man and pathological examination revealed schwannoma with Antoni A and B patterns. The tumour was investigated using immunofluorescence and histochemical stainings. It showed positive immunostaining for S-100, PGP 9.5, NSE, collagen IV, laminin, merosin and vimentin. No immunofluorescence for GFAP, NPY and CGRP was observed. Cells of the macrophage family (CD68-immunopositive) were scattered in the connective tissue. Neither B (CD20+) nor T (CD3+, CD8+) lymphocytes were found. The capillary network was revealed by CD34 immunostaining. SMA immunoreactivity was observed in walls of larger blood vessels but not in tumour cells. The tumour contained numerous mast cells visualized by thionin staining and an abundance of collagen fibres revealed by picrosirius red.

## Introduction

Benign peripheral nerve tumours of the oral cavity include schwannoma, neurofibroma, nerve sheath myxoma, mucosal neuroma, traumatic neuroma and granular cell tumour [1, 2]. Schwannoma (neurilemmoma) is an encapsulated perineural tumour originating from Schwann cells associated with motor, sensory or autonomic peripheral nerves [3]. There is no age or gender predilection [4]. Schwannomas are occasionally found in the region of the head and neck but oral localization is uncommon (about 10% of head and neck schwannomas) [1]. The most frequently involved regions are, in decreasing order: mobile portion of the tongue, palate, cheek mucosa, lips and gingiva [1]. The tumour shows two different histological patterns, Antoni type A and Antoni type B [4].

Routine histopathological procedure is usually sufficient to differentiate benign neural tumours. However, some cases require more data to establish the proper diagnosis. This case report documents an uncommon presentation of a large schwannoma in the cheek mucosa and comprehensively describes immunohistochemical properties of the tumour.

## Material and methods

A fifty-five-year-old male complained of a large tumour growing on the inner side of the left cheek. The patient reported a 20-year history of gradually increasing difficulty in chewing caused by the mass. No symptoms of generalized neurofibromatosis were noticed. Functionality of face muscles was normal. No sensory abnormalities in the region supplied by

the trigeminal nerve were observed prior to surgery. The tumour was removed under local anaesthesia after dissection of the mucosa of the cheek. During surgery no definitive nerve of origin was identified. The lesion was hard, encapsulated, 50 mm in diameter. Material was fixed in 4% buffered paraformaldehyde. One part was routinely processed and paraffin sections were stained with Meyer's haematoxylin and eosin for general morphology, with thionin for mast cells, with alcian blue for matrix proteoglycans and with picrosirius red for collagen fibres. The second part of the tumour was snap-frozen and 10 μm-thick cryosections were subjected to indirect immunofluorescence, applying primary rabbit or mouse antibodies (Table I). For visualization of the reaction, Cy3-conjugated goat anti-rabbit antiserum (Jackson IR, West Grove, PA; 111-165-144; diluted 1 : 600) or Cy3-conjugated goat anti-mouse antiserum (Jackson IR; 115-165-146; diluted 1 : 600) was used (this step was omitted in the case of Cy3-conjugated anti-smooth muscle actin antibody). In the controls, the primary or secondary antibodies were replaced by noni-mmune serum. An Olympus BX50 bright field/epifluorescence microscope (Olympus, Tokyo, Japan) equipped with U-MNG filter set was used to examine histologically stained and immunostained sections, respectively. Images were recorded using an Olympus DP71 digital CCD camera.

## Results

The patient has been followed up for 12 months. Discrete sensory insufficiency has persisted in the region of the left cheek over the tumour site half a year after surgery. No motor disturbances or other complaints were observed. Histopathological examination of the tumour revealed schwannoma surrounded by thick connective tissue capsule and containing spindle-shaped cells forming typical Antoni A and Antoni B zones. Antoni A tissue was dominant in the lesion, showing characteristic palisade arrangement and Verocay bodies. Interestingly, also neurofibroma-like regions were occasionally seen (Fig. 1). Sirius red staining revealed an abundance of collagen fibres in the stroma of the tumour. Moreover, collagen was present not only in the capsule and connective tissue but also within Antoni A and Antoni B areas. In thionin-stained sections, numerous mast cells were visualized in the connective tissue areas of the lesion. There was weak and uniform alcian blue staining in the tumour. The tumour cells showed positive immunostaining for S-100 protein in both schwannoma and neurofibroma-like areas. Virtually all spindle-shaped cells were S-100 immunopositive (Fig. 2). Similarly, PGP 9.5 and NSE were abundantly present and they displayed a similar distribution pattern in neoplastic cells (Figs 3, 4). However, NSE was also present in a population of oval cells in the connective tissue

**Table I.** List of primary antibodies used in the study

| Antigen | Host/type | Dilution | Vendor; catalogue number |
|---|---|---|---|
| S-100 | mouse/monoclonal | 1 : 100 | Chemicon, Temecula, CA; MAB079-1 |
| PGP 9.5 | rabbit/polyclonal | 1 : 2000 | Chemicon; AB1761 |
| NSE | mouse/monoclonal | 1 : 200 | Novocastra, Newcastle, UK; NCL-NSE-435 |
| NPY | rabbit/polyclonal | 1 : 2000 | Biomol (Europe), Exeter, UK, NA1233 |
| CGRP | rabbit/polyclonal | 1 : 4000 | Chemicon; AB5920 |
| GFAP | mouse/monoclonal | 1 : 50 | DAKO, Glostrup, Denmark; M0761 |
| CD3 | mouse/monoclonal | 1 : 100 | Novocastra; NCL-CD3-565 |
| CD8 | mouse/monoclonal | 1 : 1 | Chemicon; ICHR2114-6 |
| CD68 | mouse/monoclonal | 1 : 1 | Chemicon; ICHR2113-6 |
| CD20 | mouse/monoclonal | 1 : 100 | Novocastra; NCL-CD20-7D1 |
| CD34 | mouse/monoclonal | 1 : 50 | Novocastra; NCL-END |
| Collagen IV | mouse/monoclonal | 1 : 100 | Novocastra; NCL-COLL-IV |
| Laminin | rabbit/polyclonal | 1 : 50 | Sigma, St. Louis, MO; L9393 |
| Merosin | mouse/monoclonal | 1 : 4000 | Chemicon; MAB1922 |
| Vimentin | mouse/monoclonal | 1 : 100 | Novocastra; NCL-VIM |
| SMA-Cy3 | mouse/monoclonal | 1 : 800 | Sigma; C6198 |

(Fig. 3). No immunofluorescence for GFAP, NPY and CGRP was observed. Numerous cells of the macrophage family (CD68-immuno-positive) were scattered in the connective tissue (Fig. 5). Neither B (CD20+) nor T (CD3+, CD8+) lymphocytes could be seen in the tumour. CD34 immunoreactivity was observed mainly in the stroma, displaying a characteristic appearance of capillary meshwork (Fig. 6). Smooth muscle actin (SMA) immuno-

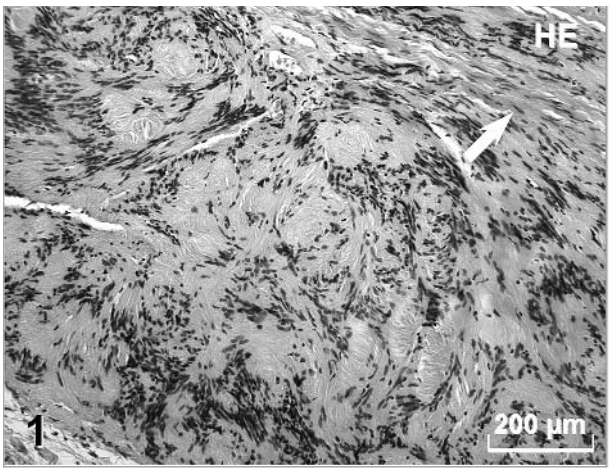


**Fig. 1.** Haematoxylin and eosin staining of the tumour displaying mostly Antoni A component. Neurofibroma pattern marked with arrow

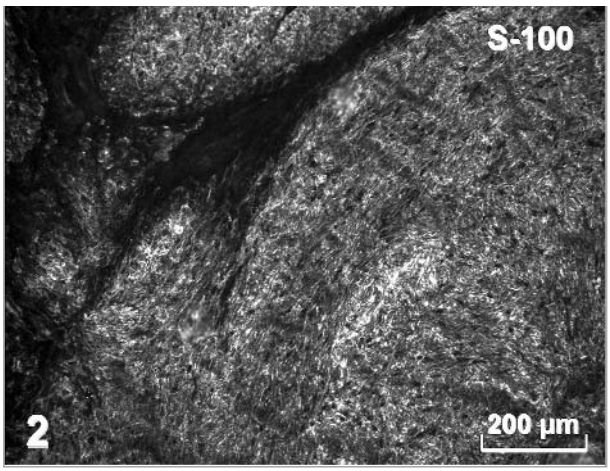


**Fig. 2.** Positive immunohistochemical staining for S-100 protein in the tumour

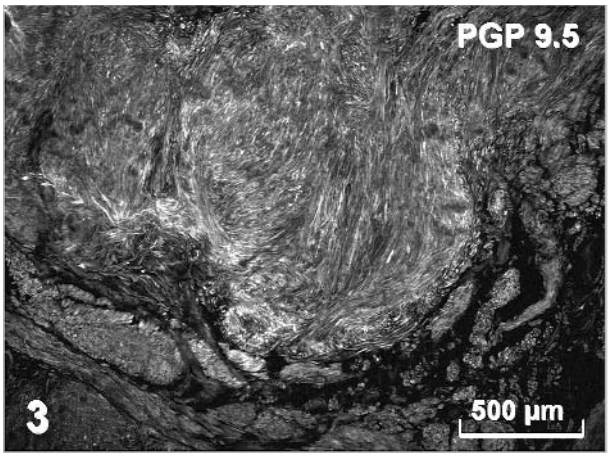


**Fig. 3.** Positive immunohistochemical staining for PGP 9.5 in the tumour

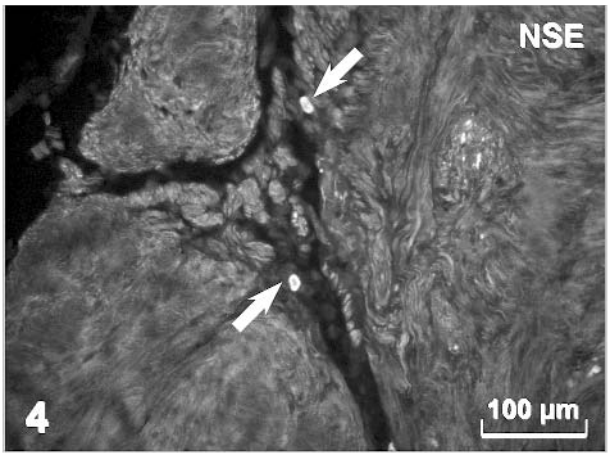


**Fig. 4.** Immunohistochemical staining for NSE showing positive cells in the connective tissue (arrows)

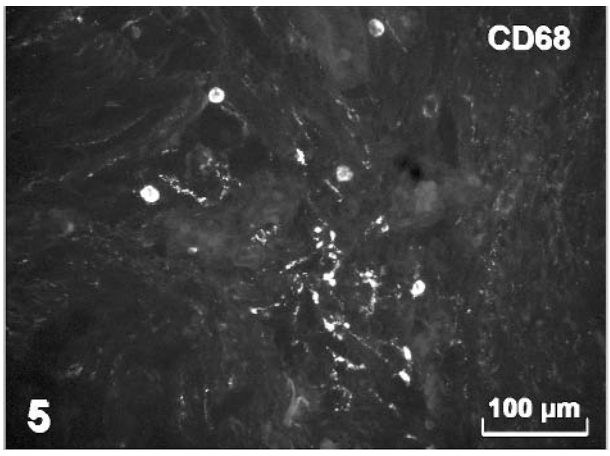


**Fig. 5.** Macrophages visualized by CD68 immunohistochemistry in the connective tissue

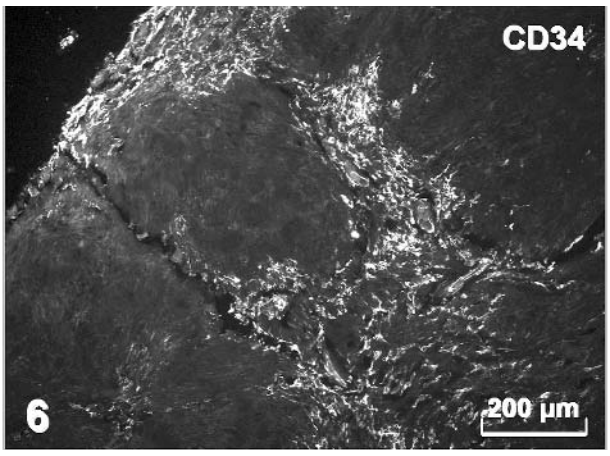


**Fig. 6.** Microvasculature of the tumour demonstrated by CD34 immunostaining

reactivity was not found in tumour cells, but larger blood vessels were labelled. Vimentin immunostaining was expressed mostly in areas of the connective tissue. Immunofluorescence for collagen IV was relatively weak in Antoni A and B areas; much higher immunoreactivity was observed in the capsule and in blood vessels. The tumour showed positive immunostaining for laminin (Fig. 7) as well as merosin (Fig. 8), although laminin was more widespread and abundant judging from reaction intensity.

## Discussion

Schwannoma, particularly a tumour of this size, is a very rare finding in the discussed site [1]. The present paper reports for the first time results of immunocytochemical assays employing a broad panel of primary monoclonal and polyclonal antibodies in order to describe the biological nature of the tumour. Selected histochemical stainings were applied to further characterize components of the extracellular matrix in the tumour and to detect mast cells.

Positive immunostaining for S-100 protein is a characteristic feature of schwannomas. This protein is expressed by Schwann cells, but not by perineurial cells and endoneurial fibroblasts. The pan-neuronal marker protein gene product 9.5 (PGP 9.5) is localized in both central and peripheral neurons and it has also been found in neuroendocrine cells. Zarfoss *et al.* obtained positive immunostaining for PGP 9.5 in 7 out of 9 canine anterior uveal spindle cell tumours (consistent with human schwannomas) [5]. In the tumour presented in this study, PGP 9.5 immunoreactivity was also confirmed in tumour cells. It is interesting that a typical marker of nerve and neuroendocrine cells is expressed by neoplasms originating from Schwann cells. NSE immunoreactivity, observed within the discussed tumour, was also seen in some cells located in the connective tissue, probably belonging to the macrophage family, particularly in view of the fact that CD68-positive cells were recognized in this location and NSE was reported to be produced and released by activated macrophages [7]. NPY and CGRP are physiological neuronal markers, characteristic of autonomic and sensory nerve fibres, respectively. They were not seen in the described schwannoma, making it impossible to determine the origin of the tumour from a specific type of peripheral nerve. The apparent absence of nerve fibres within the tumour is typical for schwannomas, since they displace nerve bundles rather than invade the nerve, the latter feature being characteristic for neurofibromas. However, axons in schwannomas were occasionally detected by neurofilament immunoreactivity and Bielschowsky silver impregnation [2, 6]. Inflammatory and immune cells are present in the microenvironment of most, if not all, tumours. They include macrophages, mast cells and T lymphocytes. In our case we showed numerous mast cells which have long been known to drive angiogenesis as well as to promote new blood vessel formation and tumour growth. In addition, tumours are frequently infiltrated by regulatory T cells, which suppress both adaptive and innate immune responses [8]. However, B (CD20+) and T (CD3+, CD8+) lymphocytes were not seen in the tumour described in this study. The presence of CD68-positive macrophages distributed in the connective tissue of the investigated tumour has also been described in schwannomas by Chrysomali and coworkers, who reported CD68-positive cells being more numerous in Antoni B than Antoni A regions [2].

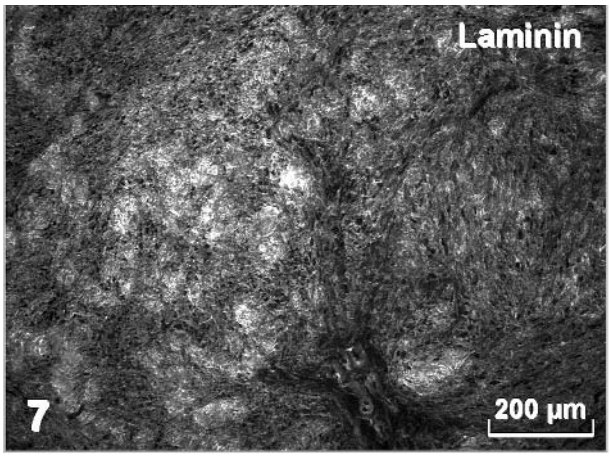


**Fig. 7.** Positive immunostaining for laminin in Antoni A

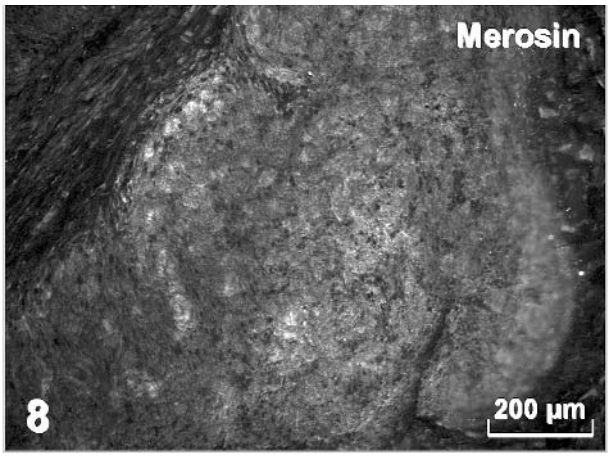


**Fig. 8.** Positive immunostaining for merosin in Antoni A

Components of the basal lamina, including laminin, merosin and type IV collagen, are synthesized by Schwann cells and are therefore present in schwannomas [9]. Expression of type IV collagen is generally a feature of all benign neural tumours and schwannomas show immunoreactivity for collagen type IV in association with most of the tumour cells [2]. The capsule around schwannomas also shows positive immunostaining for collagen type IV and these findings were confirmed in our case [2, 10]. In human Schwann cell neoplasms, laminin was seen in all tumour cell basement membranes. In contrast, merosin was found exclusively in areas where schwannoma cells were in contact with stromal or vascular tissue [11]. It is present almost exclusively in highly differentiated Schwann cell neoplasms and its distribution is more restricted than that of laminin, which was confirmed in the tumour described in this study.

CD34 is typically expressed by bone-marrow stem cells and endothelial cells but it has also been detected in dendritic/spindle cells in mucosa, dermis, and in endoneurium [2]. In the tumour studied, CD34 immunostaining was consistently present only in endothelial cells of the capillary vessels. All schwannomas express vimentin immunoreactivity, paradoxically suggesting mesenchymal phenotype of the tumour, although Schwann cells originate from the neural crest. SMA immunoreactivity is typical for smooth muscle cells. All canine anterior uveal spindle cell tumours were negative for SMA [5]. In our tumour, SMA immunoreactivity was only found in the walls of large blood vessels, reflecting the presence of vascular smooth myocytes. In the present study we used histochemical stainings to characterize components of the tumour stroma. Collagen fibres (visualized by picrosirius red) were present not only in the capsule and larger connective tissue areas, but formed a dense stroma throughout the tumour. Matrix proteoglycans (stained with alcian blue) were less abundant. Small areas showing a neurofibroma-like pattern were observed in the studied tumour. Similar findings were reported by Nascimento and coworkers [6]. Moreover, hybrid tumours displaying both patterns, even with prevalence of neurofibroma, were also described [10].

Although there are characteristic histological features that allow one to distinguish between morphologically similar lesions, such as leiomyoma, schwannoma, benign fibrohistiocytoma, neurofibroma and myoepithelioma of the salivary glands, the differential diagnosis can be difficult in the case of small or fragmented biopsies [12]. Thus, immunohistochemistry and other stainings seem to be a valuable tool in pathomorphological diagnosis of benign oral tumours.

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