Preliminary study regarding the utility of certain immunohistochemical markers in diagnosing neurofibromas and schwannomas

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Abstract
The present study shows the histopathological and immunohistochemical aspects encountered in 49 benign tumors with neural origin diagnosed in the Pathology Department of the Emergency County Hospital of Craiova between 2000 and 2007. Histopathological criteria were used for the histopathological diagnosis, having been diagnosed 22 neurofibromas and 27 schwannomas. Histopathological examination was completed by the immunohistochemical examination using anti-S100 and anti-vimentin antibodies, anti-CD34, anti-CD57 and anti-neurofilament antibodies, as well as the Ki67 proliferation marker. Both tumors showed positive immunostaining for S100, CD34, CD57, but of varying intensity and distribution. Schwannomas and neurofibromas showed a low proliferation index (<5%).

Keywords: schwannoma (neurilemoma), neurofibroma, immunohistochemistry, S100, vimentin, CD34, CD57, neurofilaments, Ki67.

Introduction
Schwannomas and neurofibromas are the two most common benign tumors derived from peripheral nerves. Schwannomas are usually solitary, encapsulated tumors of the peripheral nerve neural sheath. Neurofibromas are circumscribed, non-encapsulated tumors that frequently develop from small nerves and extend into the surrounding soft tissue. In case the lesion is limited to the epineur, then the neurofibroma has a true capsule [1].

Although previous data suggested that both represent pure lesions of Schwann cells, recent ultrastructural studies showed that schwannomas exclusively consist of neoplastic cells that simulate differentiated Schwann cells, while neurofibromas present a mixture of cell types: Schwann cells, perineural cells and endoneurial fibroblasts [2, 3].

Although, generally, these tumors are easily differentiated in standard light microscopy, in a number of cases there might be a number of close resemblances between them. Nuclear palisades are not present in all schwannomas and thus the differentiation between some of these lesions and cellular neurofibromas is sometimes difficult. Moreover, schwannomas consisting exclusively of Antoni B areas are slightly cellular and myxoid and might mimic the histological pattern of a neurofibroma on Hematoxylin–Eosin stained samples.

The importance of differentiating these two entities is relevant in the association of some neurofibromas (with diffuse or plexiform pattern) with neurofibromatosis-1 [4]. It is well known that in some neurofibromatosis-1 patients, the malignant peripheral nerve tumors may develop in preexistent neurofibromas, thus emphasizing the necessity for a more accurate diagnosis of schwannoma or neurofibroma [1].

Material and Methods
The studied material consisted out of 49 benign peripheral nerve sheath tumors (22 neurofibromas and 27 schwannomas) from surgical resection specimens or biopsy samples, diagnosed in the Pathology and Cytology Department of the Emergency County Hospital of Craiova between 2002 and 2007. The samples were fixed in 10% formalin and tissue fragments were processed using the classic histopathological paraffin embedding technique and stained with Hematoxylin and Eosin.

Immunohistochemical examination using the three-step Avidin–Biotin technique on sections (3 µm thick) from the paraffin-embedded samples completed the histopathological diagnosis.

This technique included: inhibition of endogenous peroxidase with hydrogen peroxide; antigen unmasking...
The present study consisted of 49 benign peripheral nerve sheath tumors, our study showed that all neurofibromas were superficial, as well as most of the schwannomas (88.89%). There were only three cases (11.11%) with deep schwannomas (Table 3).

Taking into consideration the localization of the benign peripheral nerve sheath tumors according to the patient’s age revealed the aspects presented in Table 4. There was no significant prevalence of neurofibromas in any age group, with the same number of cases (nine cases, 45%) between 20–39-years-old and 50–69-years-old. Only four (20%) patients were between 20 and 29-years-old, and between 60 and 69-years-old, respectively; one 40-year-old woman had a multiple tumor (two neurofibromas) associated with neurofibromatosis-1, and one 59-year-old woman also had a multiple tumor (two neurofibromas), but without associated neurofibromatosis-1.
Table 4 – Distribution of benign peripheral nerve sheath tumors according to patient’s age

<table>
<thead>
<tr>
<th>Age group [years]</th>
<th>Neurofibroma (20 patients)</th>
<th>Schwannoma (26 patients)</th>
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<tr>
<td></td>
<td>No. of cases</td>
<td>%</td>
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<tr>
<td>0–9</td>
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<td>10–19</td>
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<td>20–29</td>
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<td>30–39</td>
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<td>40–49</td>
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<td>50–59</td>
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<td>60–69</td>
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<td>70–79</td>
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<tr>
<td>&gt;80</td>
<td>–</td>
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</tr>
<tr>
<td>Minimum age</td>
<td>14</td>
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<tr>
<td>Maximum age</td>
<td>64</td>
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</table>

In the case of schwannomas, most of the patients were young, between 20 and 29-years-old (seven cases, 26.92%), and six other patients were between 40 and 49-years-old (23.08%). This type of tumor was rarely encountered in elders; there are two cases (7.69%) in each of the following age groups: 60–69, 70–79, and over 80-year-old, respectively. One 68-year-old patient had two schwannomas.

The youngest patient with neurofibroma was a 14-year-old one and the oldest was a 64-year-old one; the youngest patient with schwannoma was a 3-year-old one and the oldest was an 81-year-old one.

The histopathological aspects in neurofibromas varied and depended on the content of cells (Schwann cells, mast cells, lymph cells and xanthomatous cells), extracellular mucoid material and collagen.

From the histopathological point of view, most neurofibromas (16 cases) were represented by the common type, and consisted of fascicles overlapping with elongated cells with rippled dark nuclei, thin collagen fibers and a variable amount of mucoid material. Tumoral stroma consisted of lymph cells, mast cells and rare xanthomatous cells (Figure 1).

However, two highly cellular neurofibromas consisted of islands of Schwann cells in a uniform collagen matrix, with absence of the extra cellular mucoid material. In these tumors, the cells formed fascicles with a storiform-like pattern (Figure 2). Four tumors also showed a significant myxoid pattern with decreased cellularity.

The histopathological aspect of myxoid neurofibromas revealed the presence of distinct lobules separated by connective tissue, each of them containing groups or isolated round or spindle cells, with elongated, often angulated nuclei, dense and homogenous chromatin, pale cytoplasm, in an abundant myxoid matrix with few mast cells and fibroblasts.

Histopathological examination of schwannomas (neurilemomas) revealed two components with varying proportions: a rich, tight cellular component (Antoni A area), and a loose myxoid component (Antoni B area). Antoni A areas consisted of spindle cells forming short strips or overlapping fascicles; nuclear palisading was seen in well-differentiated Antoni A areas. In the more loose Antoni B areas, with lower cellularity, spindle cells were randomly arranged in a loose matrix together with inflammatory cells, thin collagen fibers, hyalinized blood vessels with thick walls and dilated lumens frequently occupied by thrombi in different states of organization (Figures 3 and 4).

These histopathological aspects characteristic for the common type of schwannoma were seen in most schwannomas (23 cases). However, there was one case with a highly cellular schwannoma, with prevalence of Antoni A areas. Degenerative chances were seen in three cases and consisted of cystic masses, hemorrhages, hyalinizations, and the presence of siderophages. Out of the 24 schwannomas of the common type, six tumors showed a prevalence of Antoni B areas leading to myxoid, slight cellular microscopic pattern.

**Immunohistochemistry**

Immunostaining assessment was performed as described in the “Material and Methods” section.

All studied tumors showed positive nuclear and cytoplasmic immunostaining for S100 in the elongated, spindle-shaped cells that form short strips and overlapping fascicles. The immunostaining distribution varied: in schwannomas there was a diffuse, intense and homogenous immunostaining, while neurofibromas showed a variable, focal immunostaining pattern.
for S100. Cytomorphologically and immunophenotypically, these spindle-shaped cells were neoplastic Schwann cells (Figures 5 and 6). Cells from both tumor types showed a diffuse cytoplasmic vimentin immunostaining in both tumor types, with varying intensity in the same tumor group (Figures 7 and 8).

All schwannomas showed a negative immunostaining for neurofilaments, while nine neurofibromas showed a positive immunostaining (41%).

Immunostaining for CD57 was showed positive foci in highly cellular Antoni A areas of schwannomas and rare positive cells in neurofibromas (Figures 9 and 10).

CD34 immunostaining revealed a positive reaction in a small number of schwannoma cells, as opposed to neurofibromas, where the number of CD34-positive cells was significantly greater. These CD34-positive cells with relatively long dendritic projections, round/oval nuclei and fine chromatin, were present in
small numbers in Antoni B areas of schwannomas, but in great numbers in the myxoid areas of neurofibromas and in peripheral areas of both tumor types (Figures 11 and 12). In our study, the Ki67 proliferation index was under 5% in all benign peripheral nerve sheath tumors (Figures 13 and 14).

**Figure 9** – Schwannoma: positive focal immunostaining for CD57 within Antoni A zone (LSAB technique, 40×).

**Figure 10** – Neurofibroma: positive immunostaining for CD57 in rare tumoral cells (LSAB technique, 200×).

**Figure 11** – Schwannoma: positive immunostaining for CD34 within Antoni B zone (LSAB technique, 100×).

**Figure 12** – Neurofibroma: positive immunostaining for CD34 in fibroblast-like cells, within the vessels walls, at the peripheral of the tumor (LSAB technique, 100×).

**Figure 13** – Schwannoma: positive immunostaining for Ki67 in rare tumoral nuclei (LSAB technique, 100×).

**Figure 14** – Neurofibroma: positive immunostaining for Ki67 in rare nuclei of the tumoral cells (LSAB technique, 100×).

**Discussion**

1342 soft tissue tumors were diagnosed in the Pathology and Cytology Department of the Craiova Emergency County Hospital between 2000 and 2007. Forty-nine of them were benign peripheral nerve sheath tumors (3.65%), which correspond to the literature data. Thus, Whitaker and Droulias reported 76 schwannomas in 1500000 admitted patients [5], while Adams (1985) recorded 65 peripheral nerve schwannomas in a series of 1500 primary neural tumors [6].
Similarly to the literature, our study showed that the superficial localization of benign neural tumors prevailed [1]. The most frequent localization of neurofibromas was the head (31.82% of all cases), followed by the upper limbs (27.27%) and the trunk (22.73%). The most frequent localization of schwannomas was equally distributed between the trunk and the head (25.93% each), followed by the upper limbs (22.22%). Data from the literature revealed a higher frequency of soft tissue tumors, generally located in the lower limbs [6].

Our study revealed that the two tumor types were encountered in men as well as in women, but neurofibromas were more frequent in women (12 cases, 60%), while schwannomas were more frequent in men (14 cases, 53.85%).

The distribution of the 46 patients with benign peripheral nerve sheath tumors according to age showed that neurofibromas were more frequent between 30 and 39-years-old (25%) as well as between 50 and 59-years-old (25%), while schwannomas were more frequent in patients between 20–29-years-old (26.92%) and between 40–49-years-old (23.08%). These data agree with the literature, which states that schwannomas may occur at any age, but are more frequent between 20–50-years-old, with equal gender distribution [1]. Data from the literature state that sporadic, localized neurofibromas equally affect both genders preval in patients aged between 20 and 30-years-old and those they have a uniform distribution on the surface of the body.

Neurofibromas are benign neural tumors that may show one of three growth patterns: localized, diffuse and plexiform, with associated neurofibromatosis-1 in the case of the latter ones. The localized (sporadic) type is the most frequent; macroscopically, it is a solitary tumor with superficial localization and it occurs in healthy individuals.

As far as the histopathological aspects of the two tumor types are concerned, they are the same as those described and encountered in tumors reported by other authors [1, 7].

In our study, the histological aspects of neurofibromas varied and depended on cellularity, mucin and collagen content. Histopathologically, most neurofibromas (16 cases, 72.73%) were common type neurofibromas and consisted of fascicles of spindle-shaped cells with rippled dark nuclei, thin collagen fibers, and a variable amount of mucoid material. Two neurofibromas showed a high cellularity, and consisted of islands of Schwann cells in a more uniform collagen matrix, with absence of the extracellular mucoid material. Other four neurofibromas showed a significant myxoid pattern with low cellularity.

In our study, histopathological examination of schwannomas revealed that most of them (23 cases, 85.18%) showed the histopathological aspects of the common type schwannoma (neurilemoma) highlighted by the presence in different proportions of the two components: the tight, highly cellular component (Antoni A area), and the loose, myxoid component (Antoni B area). One case of cellular schwannoma and three cases of degenerative schwannoma were diagnosed after histopathological examination. In six cases with schwannoma – the common type, the loose myxoid component prevailed.

Benign peripheral nerve tumors show a significant microscopic heterogeneity, despite their common origin from the neural crest. The differential diagnosis of these tumors on Hematoxylin–Eosin stained samples may be sometimes difficult, in which case the immunohistochemical examination may contribute to the final diagnosis.

Immunohistochemical examination was performed to complete the histopathological examination for two reasons: first of all, in order to determine the phenotype of the proliferating tumor cells and secondly, in order to highlight the value of a certain marker in the differentiated diagnosis of the two tumor types. We assessed the immunolabeling of tumor cells using the following markers: S100, vimentin, CD57, CD34, neurofilaments, and Ki67 proliferation index for the assessment of tumor proliferation.

S100 protein is a low molecular weight protein found in a great variety of human cells and tissues including glial cells, neurons, chondrocytes, Schwann cells, melanocytes, macrophages, Langerhans cells and different epithelial tissues (especially those in the breast, sudoral glands and female genital tract). S100 immunoreactivity is both nuclear and cytoplasmic [8]. S100 is an acid protein frequently found in the peripheral nervous system. All 49 benign peripheral nerve sheath tumors in our study showed intense S100 positivity. Schwann cells from schwannomas showed diffuse and intense S100 immunolabeling, while Schwann cells from neurofibromas showed variable positive staining with focal distribution. This is due to the fact that neurofibromas contain a variable cell population and consist of mucoid matrix that may contain scattered myelinated and non-myelinated axons together with a heterogenous cell population (Schwann cells, fibroblasts, and perineural cells), which explains the S100 immunoreactivity in some cells as compared to the relatively uniform immunoreactivity in schwannomas [1]. Another observation confirmed by other studies was that Schwannomas showed a higher S100 immunoreactivity in the Antoni A highly cellular areas as compared to the poorly cellular Antoni B areas [9].

Vimentin is a 57-kD protein of the intermediate filaments, which is part of the cytoplasmic cytoskeleton of cells with mesenchymal origin. Vimentin is expressed by the great majority of mesenchymal cells as well as certain epithelial cells [8].

Vimentin immunostaining in schwannomas was positive and had a diffuse distribution pattern. However, the intensity of the reaction varied from intense to slight positive. Antoni B areas showed slight vimentin immunostaining as compared to Antoni A areas.

Neurofibromas also showed diffuse positive vimentin immunostaining.

CD57 is the marker for HSB-2 human lymphoblastic cell line, but may also be encountered in the myelin-associated glycoprotein (MAG). This property provides
the reactivity for normal tissues with neural and Schwann cell origin as well as its applicability in recognizing benign correlated neoplasms, such as the granular cell tumor.

In our study, highly cellular areas (Antoni A) of 15 schwannomas (55.56%) as well as rare scattered cells in six neurofibromas (27.27%) showed focal CD57 immunostaining. The fact that CD57 recognizes the myelin-associated glycoprotein of Schwann cells that form the cell population of schwannomas and are less numerous in neurofibromas can provide an explanation for this distribution of the immunostaining pattern. Data from the literature also showed a variable proportion of CD57-positive cells in schwannomas and neurofibromas ranging from 0.1% to 10% [10].

Neurofilaments are the intermediate filaments of neurons, forming a family of three polypeptides of 68, 150 and 200-kD, respectively. Neurofilaments are expressed by neurons, neuroendocrine cells as well as tumors with neural differentiation [8].

Some neurofibromas showed focal positive neurofilament immunostaining, while schwannomas lacked neurofilament immunostaining. This can be explained by the fact that neurofibromas develop inside the nerve and may later include it, as opposed to schwannomas, which replace the nerve of origin; thus, the presence of neurofilaments may be demonstrated in the remaining nerve included by the neurofibroma, while neurofilaments lack in schwannomas, except rarely in the peripheral region of the lesion [1, 7]. Aberrant axons were observed in the schwannomas associated with NF2/schwannomatosis but not in the solitary schwannomas [11].

CD34 is a 110-kD antigen of the cell surface, which is selectively expressed by progenitor cells of the myeloid and lymphoid lines, small vessel endothelial cells, embryonal fibroblasts and some of the cells from the adult and fetal nervous tissue [8].

Our study revealed only a small number of fibroblast-like CD34-positive cells in Antoni B areas of schwannomas, as opposed to neurofibromas which contained a significantly larger number of CD34-positive cells. Antoni A areas of schwannomas were also CD34-negative. Since collagen-producing fibroblasts are CD34-negative, a conclusion was drawn: it is unlikely that this sub population of CD34-positive cells is fibroblastic, but non-neoplastic cells with support function [12, 13]. It was suggested that CD34 also defines a normal population of dendritic cells that developed on enderve of the peripheral nerves that is different from fibroblasts and conventional Schwann cells [14].

Chaubal A et al. found that cutaneous neurofilaments showed a moderate to great number of CD34-positive spindle-shaped cells, which sometimes were scattered and sometimes formed thick strips concentrated around blood vessels, skin annexes and peripheral regions of tumors [13].

Ki67 protein is a nuclear antigen expressed during the proliferating phase of the cell cycle (G1, S and G2) [8]. In tumors, Ki67 immunostaining provides a measure for the tumor growth fraction, which in turn is an indicator for the mitotic activity of the cells [15, 16]. An increased Ki67 expression indicates an increase in the mitotic activity and cell proliferation [17].

In our study, all benign peripheral nerve sheath tumors showed a Ki67 proliferation index lower than 5%, also seen in other studies in which benign peripheral nerve tumors showed a Ki67 proliferation index no higher than 4% (an average of 1.2%). Other studies have shown that malignant peripheral nerve sheath tumors showed a Ki67 index of 7–38% (with an average of 23%) [18]. Kindblom LG et al. reported a Ki67 index ranging from 5% to 65% in malignant peripheral nerve sheath tumors, as opposed to benign peripheral nerve sheath tumors in which the Ki67 index was lower than 5%. The values of the Ki67 proliferation index, which were generally higher in malignant peripheral nerve sheath tumors, lead to the idea that this immunohistochemical marker may contribute to an earlier detection of malignant transformation of benign neural tumors [19].

Conclusions
S100 is the first choice for the immunohistochemical diagnosis of schwannomas and neurofibromas. As S100 protein demonstrates the neuroectodermal origin of tumors, it becomes an exclusion immunohistochemical marker. Schwannomas showed diffuse, intense and homogenous nuclear and cytoplasmic S100 immunostaining, as opposed to neurofibromas that showed a focal and variable immunostaining pattern, in accordance with the observation that neurofibromas consist of various cell populations.

Both benign neural tumors constantly showed a CD34-positive fibroblast-like cell subpopulation, but with a varying proportion of CD34-positive cells in each tumor type. Taking into consideration the fact that the number of CD34-positive fibroblast-like cells was increased in the myxoid areas of neurofibromas, as opposed to the low number of CD34-positive cells in the Antoni B areas of schwannomas, CD34 appears to play an important role in differentiating neurofibromas from schwannomas.

All benign peripheral nerve sheath tumors in our study showed a Ki67 proliferation index lower than 5%. Ki67 proliferation index is useful for differentiating a neurofibroma with atypical histology from a peripheral nerve sheath tumor with a low degree of malignancy as well as for early detection of malignant transformation of peripheral nerve tumors.

Neurofilaments can be useful for the differential diagnosis of benign neural tumors due to the lack of immunostaining in schwannomas, while CD57 may be a useful tool due to the increased cytoplasmic immuno-reactivity of tumor cells in more than 50% of schwannomas.

References


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Received: December 17th, 2008
Accepted: April 15th, 2009