

Vessel and Mast Cell Densities in Sporadic and Syndrome-associated Peripheral Nerve Sheath Tumors

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Abstract. *Background/Aim:* Peripheral nerve sheath (PNS) tumors constitute a heterogeneous group of solid tumors. Neurofibroma and schwannoma are the most frequently diagnosed entities. Both tumor types occur sporadically and are associated with syndromes. Current strategies to fight PNS progression by means of pharmaceuticals aim to specifically interfere with vascular growth factors identified in PNS. Furthermore, malignant transformation of PNS tumors is known to be associated with a change in vascularization. The aim of the study was to investigate vascularization of different PNS tumors with respect to sporadic or syndromal state of the entities. *Materials and Methods:* One hundred and thirty-two formalin-fixed and paraffin-embedded PNS tissue samples were retrieved from the archives of the Institute of Neuropathology, Eppendorf University Hospital. Lymphatic and blood vessels were immunohistochemically identified and morphometrically analyzed in PNS and controls. *Results:* Blood vessel density in malignant tumors was significantly higher than in benign lesions (30.8/mm² vs. 13.46/mm²). In the latter, the vessel density resembled that of control tissue. Lymphatic vessel supply was significantly higher in cutaneous neurofibroma and diffuse plexiform neurofibroma (PNF) than in intra-neural localized tumors (schwannoma, nodular PNF). Lymphatic vessels showed no marked differences with respect to tumor entity. Prevalence of mast cells differed markedly between tumor types. *Conclusion:* Different vascularization

of PNS may contribute to diverging tumor response following application of anti-neoplastic drugs. Mast cells may have an impact during formation and growth of neurofibroma but are unlikely to be involved in the process of de-differentiation.

Neurofibromatosis (NF) and associated syndromes are neurogenetic diseases constituting a complex phenotype. At present, three types of neurofibromatosis are classified with development of distinct tumors of the nervous system: neurofibromatosis type 1 and type 2 (NF1, NF2) and schwannomatosis (1). Patients with neurofibromatosis are at-risk of developing peripheral nerve sheath tumors (PNST). The predominant tumor is neurofibroma in NF1 (2), and schwannoma in NF2 and schwannomatosis (2, 3). Treatment for PNST is frequently surgical.

NF1 (incidence 1:3,000 to 1: 4,000) is one of the most frequent autosomal dominant transmitted neurogenetic disorders. Penetrance is about 100% (4). New mutations cause about 50% of NF1 cases, the remaining half have affected ancestors (5). Patients with mild course of NF1 due to genetic mosaicism (5-10%) have no germline mutations. The disease was originally described by von Recklinghausen (6). NF1 phenotype presents numerous neurological manifestations, including a broad spectrum of tumors derived from the neural crest. However, the phenotype may vary considerably, even in members of the same affected family. The *NF1* gene is located on chromosome 17q11.2 and is composed of 61 exons coding for a protein of 2,818 amino acids, named neurofibromin. This protein functions as a proliferation inhibitor *via* interaction with the rabbit sarcoma (RAS) protein pathway and also *via* intracellular release of cAMP (7, 8). Schwann cells are one of few cell entities capable of using cAMP as an inducer of proliferation (9).

Diagnosis of NF1 is established according to updated clinical diagnostic criteria (10) (Table I). The prevalence of characteristic clinical findings differs considerably. Penetrance of café-au-lait spots is the most common physical finding, more than 90% of affected individuals develop this pigmentation disorder during their first year of life. Other

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Table I. *Diagnostic criteria of neurofibromatosis type 1 (NF1). Frequencies are stated in parenthesis. At least two criteria have to be found to allow diagnosis [National Institutes of Health Consensus Development Conference 1988 (10)].*

A	At least 6 café-au-lait spots, diameter at least 5 mm prior to puberty and 15 mm after puberty (99%)
B	Axillary or inguinal freckling (90%)
C	At least 2 cutaneous/subcutaneous neurofibroma (90%, after puberty) or 1 plexiform neurofibroma (30%)
D	At least 2 Lisch nodules seen on slit lamp examination (70%, after puberty)
E	Optic pathway glioma (15%)
F	Bony dysplasia [dysplasia of the sphenoid wing, bowing of long bones, pseudarthroses, cortical atrophy of long bones (<1-2%)]
G	First-degree relative with NF1 (50%)

Table II. *Manchester Diagnostic Criteria of neurofibromatosis type 2 (NF2) diagnosis (modified National Institute of Health (USA) criteria, adapted from (1)). Criteria A to C are definitive NF2 criteria, criteria D to F are supporting criteria for NF2.*

A	Bilateral vestibular nerve schwannoma
B	First-degree relatives with NF2 and unilateral vestibular nerve schwannoma prior to 30th year of life
C	First-degree relative with NF2 and two of the following lesions: meningioma, glioma, schwannoma, juvenile subcapsular cataract
D	Unilateral vestibular nerve schwannoma prior to 30th year of life and at least one of the following lesions: meningioma, glioma, schwannoma, juvenile subcapsular cataract
E	Two or more meningioma and unilateral vestibular nerve schwannoma prior to 30th year of life
F	Two or more meningioma and one of the following lesions: glioma, schwannoma, juvenile subcapsular cataract

Table III. *Diagnostic criteria for schwannomatosis in individuals aged 30 years or more, adapted from (3).*

A	Two or more not intracutaneously situated schwannoma, at least one of the tumors histologically verified
B	No evidence for vestibular nerve schwannoma on high resolution magnetic resonance imaging
C	No evidence of NF2 mutation
D	Or one pathological confirmed schwannoma (other than vestibular nerve schwannoma) and first grade relative with confirmed schwannomatosis according to criteria A to C.

cutaneous alterations occur later in life and are chronically progressive (11). In addition to cutaneous findings, neuropsychological manifestations of the disease emerge even during childhood, *e.g.* developmental delay, motor clumsiness, or disturbance of equilibrium in about two-thirds of children. Macrocephalus develops in about 29% to 45% of children with NF1, however, usually without clinical consequence (12). Complications as a consequence of vascular disease are rare, *e.g.* renal artery stenosis and Moyamoya syndrome. The most prominent finding in NF1 is the high frequency of tumors, in particular of cutaneous neurofibroma. Proliferative activity of tumor cells in neurofibroma of patients with NF1 is low (13). The discussion on the cells of origin of tumorous Schwann cells, *i.e.* tumor stem cells, is still ongoing (14-16).

NF2 (connatal incidence 1:35-40,000, symptomatic prevalence 1:60,000 to 1:200,000) is an autosomal dominant genetic disorder (17). The gene locus of NF2 is chromosome 22q11.2. This gene is composed of 17 exons coding for a 595 amino acid protein called merlin (synonymous:

schwannomin). Merlin is a member of the ezrin-radixin-moesin-protein family (ERM). Merlin acts as a tumor suppressor (18). Detailed knowledge regarding the function of merlin is still lacking. Merlin is likely involved in phosphorylation/activation of sarcoma tyrosine kinase (Src) (19). Mosaicism is more frequent in NF2 (30%) than in NF1. The severity of phenotypes may vary considerably, dependent on the type of NF2 mutation. The manifestation of the disease is quite late, in about 80% between the 18th and 24th year of life (12). Therefore, diagnostic prevalence is low in NF2. The main diagnostic criterion is bilateral vestibular schwannoma (BVS). Further tumors are noted in the context of NF2, such as meningioma, spinal ependymoma and schwannoma (in locations other than the cerebellopontine angle). Diagnosis is usually established based on clinical findings. The most frequent clinical finding is hypacusis as a consequence of BVS. Other cranial nerves can also be affected in the case of schwannomas of the cerebellopontine angle. Currently, Manchester criteria are recommended for establishing diagnosis (Table II).

The annual incidence of schwannomatosis is 1:30,000, similar to that of NF2. Both diseases are closely related, but schwannomatosis is a disease without BVS (3). Differentiation between both diseases was established only recently, indicating the close relationship of schwannomatosis to well-described NF2 (Table III). Many peripheral or spinal schwannomas in schwannomatosis exhibit truncating mutations of the *NF2* gene, including complete loss of the second *NF2* allele. Localisations of schwannomas in schwannomatosis are manifold. The most important difference between both diseases is the lack of reduced life expectancy measured in patients with schwannomatosis, whereas this factor is known for NF2.

Sporadic schwannoma occurs in all life phases but a predilection was noted for the third to sixth decade. The head, neck and the flexor sites of extremities are typically affected. Sensorial nerves are more often affected than motor nerves or sympathetic nerves (3).

Histopathology of PNSTs. Schwann cells constitute the most quantitative cellular aspect in schwannoma and neurofibroma. These tumors frequently develop at the interface of the central nervous system (CNS) to the peripheral nervous system.

Schwann cell precursors or a delicate NF2 haploid milieu in the interface of peripheral nervous system and CNS are suspected to give rise to schwannoma (20, 21). The cells of origin are myelinated Schwann cells. Histology of schwannoma is characterized by two growth patterns named Antoni A and B. Tumor growth in Antoni A type regions exhibits numerous cells and fibrillary stroma with cells directed in lines and palisade-like direction of nuclei. The Antoni B growth pattern is a net-like distribution of cells in the matrix without geometrical orientation of cells, resembling in part a neurofibroma (22). Schwannoma are benign neoplasms; however, compression of adjacent structures may cause severe pain and neural dysfunction (1).

PNST in NF1 derive from non-myelinated Schwann cells and differ with respect to tumors in NF2 and schwannomatosis by growth pattern, cellular composition and potential to de-differentiate into malignant tumors (14). Neurofibroma can arise in every body part. Neurofibromas are so-called mixed tumors with spindle-like shaped Schwann cells and thin, wavy nuclei, collagen fibres, fibroblasts, mast cells and numerous vessels (23).

Neurofibromas are classified into different sub-groups. PNST in patients with NF1 present at birth or developing early in life are almost exclusively of the plexiform type. Other neurofibromas, called cutaneous neurofibromas, generally do not develop prior to the 10th year of life (4).

Plexiform nodular neurofibroma (PNF) may develop deep inside the body close to the nerve roots and displays a net-like growth along the distribution of the peripheral nerves.

They often contain more than one fascicle and extend over wide areas (1). About 8-12% of PNFs transform into a malignant PNST (Figure 1). Some diffuse PNFs are superficially located and have indistinct margins. More than one fascicle is usually affected in this type of PNF. Surgical treatment of these PNFs is often difficult due to limitation of resection in cases with wide extension of tumors. Furthermore, superficially extending PNF can have deep compartments involving the fascia and muscles.

The most prominent tumors are cutaneous (synonym: dermal) neurofibromas. Like most PNFs, cutaneous neurofibroma arise from sensitive nerve fibres, grow intracutaneously and only rarely cause neurological dysfunction. They do not exhibit invasive growth and are not regarded as undergoing malignant transformation. However, they can cause severe disfigurement. Furthermore, histological distinction has created the term 'atypical neurofibroma'. Atypical neurofibroma can develop in any other subgroup of neurofibroma. The defining feature for diagnosing atypical neurofibroma is an increased proliferation index, increase of cell-dense areas inside the neurofibroma, and an increase in nuclear atypia (2).

The tumor cells in neurofibromas are Schwann cells (14, 24). Several studies suggest that an NF1-haploinsufficient environment is a prerequisite for tumorigenesis (14, 25). One study supports the hypothesis of an impact of NF1-haploid mast cells on tumorigenesis (16). Neurofibromas express several growth factors and receptors relevant to tumor progression (26).

Malignant PNST in patients with NF1 are life-threatening diseases. Malignant PNST can develop *de novo* or from existing PNF. Beside mutations in the *NF1* gene further mutations, such as of *p53* gene or *p16* gene can occur (28, 29). Malignant PNST grow invasively and develop distant metastases early (30). Recurrence and metastasis are significantly higher in NF1-associated malignant PNST compared to sporadic cases (31). A recent study rates the five-year event-free survival and overall survival as 19% and 28%, respectively (32). In light of the high mortality rate of patients with malignant PNST, the identification of markers and a more detailed description of tumor composition is of major importance. Key features of therapy improvement could be related to intercellular interaction *via* adhesion molecules, cellular composition of tumors, and tumor vascularization. This is why the present study investigated mast cell density and vascular density in a broad spectrum of PNSTs.

The immunohistochemical identification of lymphatic and blood vessels by means of identifying CD34 (33-36) and podoplanin (37) was detailed in a recent study on sporadic and syndrome-associated peripheral nerve sheath tumors (38). We used this analysis to study the vasculature of peripheral nerve sheath tumors more in detail, with special reference to mast cell distribution (39).

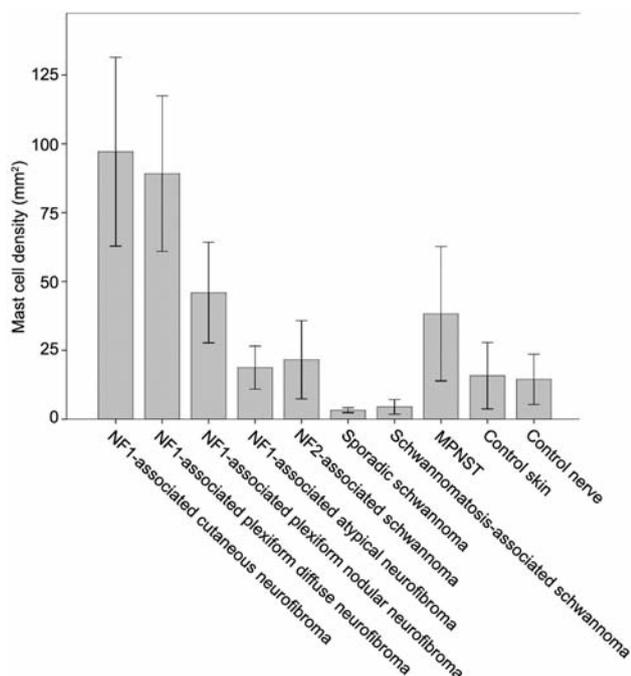


Figure 1. Mast cell density in different tumor types. Mean values and standard error of the mean are shown.

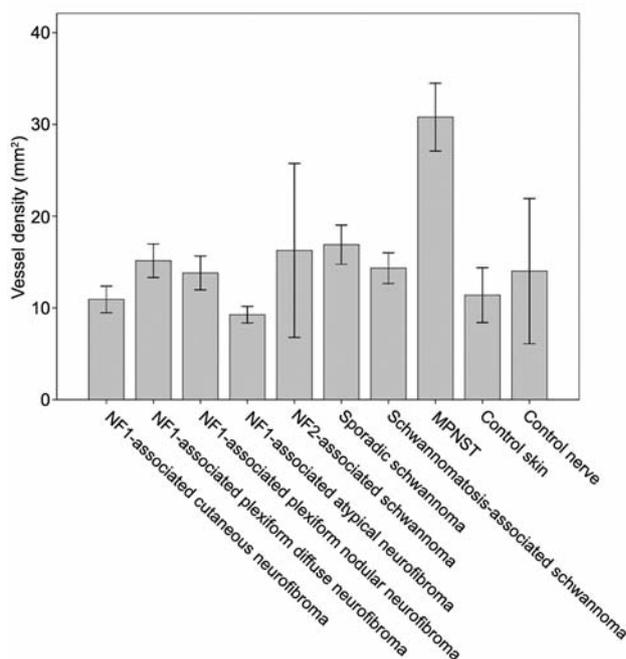


Figure 2. Blood vessel density in different tumor types. Mean values and standard error of the mean are shown.

The aim of the study was to identify possible predictors of tumor progression in tumor tissue by focusing on the vasculature of these tumors. Mast cell density, blood vessel and lymphatic vessel density were measured in different PNSTs. The main questions to be answered were firstly whether there is an increase of vessel density both lymphatic and blood vessels in the course of de-differentiation, and, secondly, whether typical mast cell densities are found in differentiated tumor entities and if there a change of mast cell density in the course of tumor de-differentiation.

Materials and Methods

Patients. Ninety-two patients with NF1 (n=87) or NF2 (n=5) fulfilled NIH diagnostic criteria of NF1 and Manchester criteria of NF2, respectively. Schwannomatosis diagnosis (n=11) was based on clinical findings (two schwannomas minimum, exclusion of NF2) and also with DNA sequencing of *NF2* gene (exclusion of germline mutation). Diagnostic criteria are listed in Tables I to III. Sporadic cases were defined by exclusion of the listed syndromes in each case. All data were anonymized prior to any investigation. The mean (\pm SD) age of patients was 34.02 ± 18.39 years at the time of tissue excision. Seventy-one samples were from female and 61 from male patients. All patients gave informed consent for scientific investigation of their tumor samples (Table IV).

Tissues. One hundred and thirty-two formalin-fixed and paraffin-embedded tissues samples were retrieved from the archives of Institute of Neuropathology, Eppendorf University Hospital. Tissues of patients with neurofibromatosis were received from the

Department of Oral and Craniomaxillofacial Surgery, sporadic schwannomas from the Department of Neurosurgery and Oral and Craniomaxillofacial Surgery, University Medical Center Hamburg-Eppendorf (Table IV). The tissues were routinely processed, cut into 4- μ m slices, fixed on slides and stained with haematoxylin-eosin and elastic van Gieson. Immunohistochemical identification of nerve sheath cells was revealed with anti-S100 immunoreaction (Dako, Hamburg, Germany, No. Z 0311, dilution 1:1000) in all tissues. In schwannomas, nerve fibres were identified with anti-neurofilament antibody (Dako, No. M0762, dilution 1:400). Proliferation of tumor cells was assessed by determining the Ki-67 labelling index (Ki-67 antibody, Ventana, Tucson, USA, No.790-4286, dilution 1: 50) as the ratio of labelled tumor cell nuclei to all nuclei in an area of 0.1 mm². CD34 (CD34 antibody, Dako, No. M7165, dilution 1: 50) and podoplanin (D2-40 antibody, Zymed, San Francisco, USA, No.18-2410, dilution1: 20) antibodies were used to identify lymphatic vessels in tumors. The investigation of immunoreactions [Ki-67, CD34, podoplanin (D2-40)] in nerve sheath tumors were already described in detail (38). This study focuses on the vessel density of these tumors. Following WHO criteria, two neuropathologists reclassified the tissues.

Vascularisation (blood and lymphatics). The software Zeiss Axio-Vison 4.6™ (Zeiss, Oberkochen, Germany) was applied to quantify vascularization. Six adjacent areas were measured at $\times 10$ magnification and consecutively comprised one area for definitive measurement of vessels in each specimen. The final image surface was about 2.5 mm². The lumen of the marked vessels was manually identified. Podoplanin staining identified lymphatic vessels and CD34 staining blood vessels. Only vessels with uniquely defined

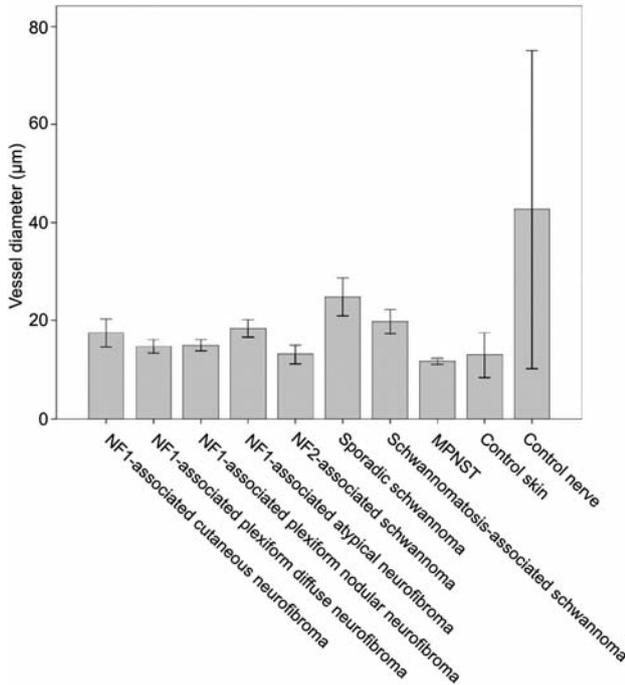


Figure 3. Diameter of blood vessels in different tumor types. Mean values and standard error of the mean are shown. The strong deviation in normal nerve tissue results from an arteriole in the selected area.

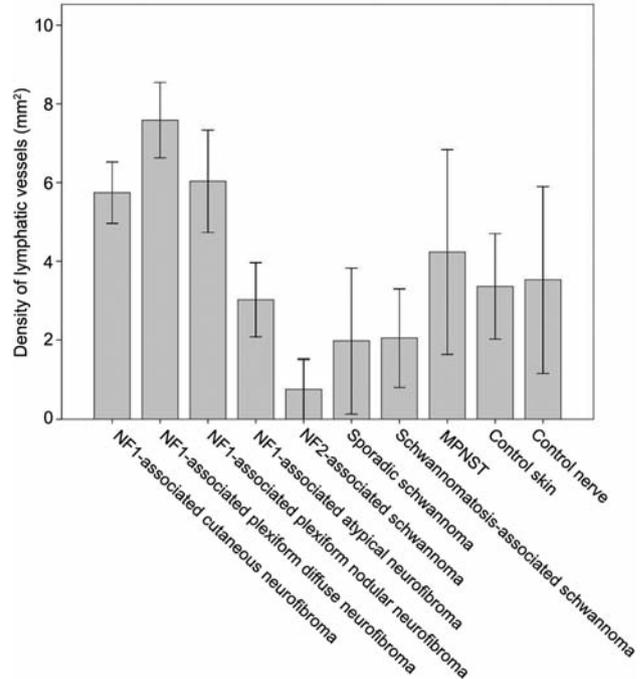


Figure 4. Lymphatic vessels in different tumor types (per mm²). Mean values and standard error of the mean are shown.

lumen were included in the measurement. Vessel density per mm² was calculated with Axio Vision 4.6™ and Microsoft Excel™ (Microsoft Corp., Redmond, VA, USA).

Mast cell density. Periodic-acid-Schiff (PAS) stained specimen were analysed at ×100 magnification for mast cell density. Marked mast cells were evaluated in regions where these cells had the densest accumulation. Four fields of vision of 0.03 mm² each were counted in every specimen.

Results

Histopathology of neurofibroma. Neurofibromas comprise spindle-shaped Schwann cells that exhibit diffuse growth or an arrangement in streams. Scattered within the tumor, few fibroblasts are found and in PNFs, perineurial cells may be encountered. The tissue matrix contains mucous substances and a varying amount of collagen fibres. Mast cells and perivascular lymphocytic infiltrates may be demonstrated within the tumors. Proliferative activity is usually low or absent from both cutaneous and PNF (13).

The morphology of malignant PNST is that of a sarcoma. Malignant PNSTs feature high cellularity, spindle cells arranged in fascicles or in a loose texture, bizarre nuclear atypia, high mitotic rate and necroses.

Histological features of schwannoma. Schwannomas contain round to elongated slender cells arranged in fibrillary

(Antoni A) and reticular (Antoni B) patterns. In fibrillary areas, the nuclei may be arranged in rows (palisading). Atypical nuclei may be present at varying rates, mitoses are rare. Regressive changes are frequently observed and comprise nests of foam cells, haemorrhage, fibrosis and cysts. The vessels are small- to medium-sized and the walls commonly exhibit extensive hyalinization. Upon labelling with the proliferation marker Ki-67, more than 10% of nuclei may be stained in these benign lesions.

Mast cells. The prevalence of mast cells differed markedly between tumor types. It was noticeable that cutaneous neurofibromas in particular exhibited a significantly higher mast cell density than other entities. Malignant PNST and diffuse PNF had the smallest number of mast cells, even lower than that of controls. Figure 1 illustrates mast cell density with respect tumors of this study.

Blood vessels in PNST. Malignant PNSTs exhibited marked neovascularization. The number of vessels in malignant tumors was 30.8/mm² and clearly higher than that in benign tumors (13.46/mm²). Vascularization in benign tumors was virtually identical to normal tissue. In schwannoma and PNF, perineurial vessels were included for measurement of vessel density. Figure 2 illustrates blood vessel density in different tumors. Vessel diameters did not differ significantly. Figure 3

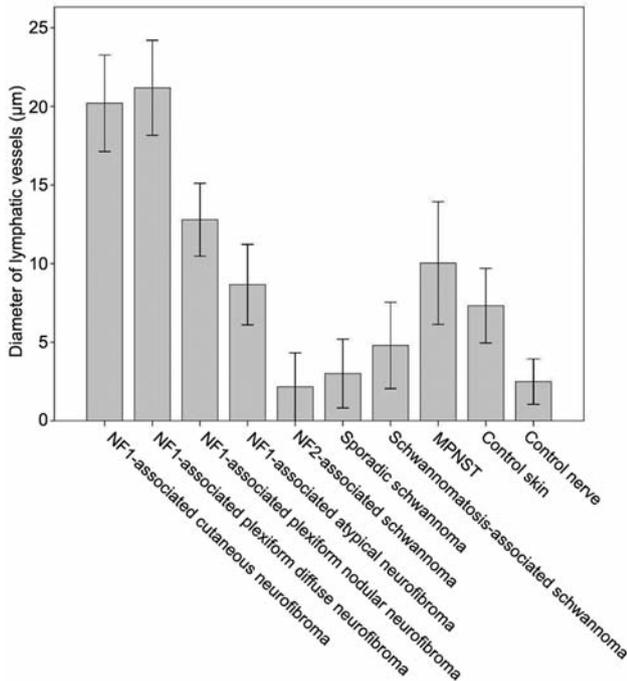


Figure 5. Diameter of lymphatic vessels in different tumor types. Mean values and standard errors of the mean are shown.

illustrates mean values (+/- one standard error of the mean, (SEM)) of vessel diameters. In schwannoma, regressively transformed vessels with large lumen were frequently found. In malignant PNST, vessels were found in the form of small, slit-shaped endothelial tubes.

Lymphatic vessels in PNSTs. Lymphatic vascularization was highest in cutaneous neurofibroma and diffuse neurofibroma. The morphometric values of all other entities did not differ from physiological conditions in controls. Figure 4 shows the lymphatic vessel density with respect to entities of this study. Figure 5 shows the mean diameter of lymphatic vessels. Values for nodular PNF and schwannoma represent epineurial or perineurial lymphatic vessels, respectively.

Discussion

Blood vessels in PNSTs. This study shows remarkable differences of vessel densities in PNSTs. Angiogenesis is an essential capability of multicellular organisms whose vital functions are maintained by blood circulation. In tumors, several factors can induce angiogenesis, e.g. hypoxia of the tumorous microenvironment and signals released by tumor cells. Although not shown in the current investigation, even the extensive oedema seen after debulking procedures for PNF may lead to a hypoxic milieu inducing angiogenesis (31).

In malignant tumors, new vessels fail to mature and form inefficient capillary loops (40). Therefore, increased vessel density can be an indicator of suspected de-differentiation of tissues.

One focus of this study was on intraneurally growing nodular PNF and ‘atypical neurofibroma’. The former are prone to de-differentiation and thus responsible for the majority of malignant PNSTs in patients with NF1, the latter are believed to be a transitional phase to malignant PNST by some authors (41).

A recent study on vascularization of nerve sheath tumors using a comparable study protocol reported similar results on the vascularisation of schwannomas as presented here (42). Compared to the study of Plotkin *et al.* (42), we determined mean diameter 13.1 µm (14.2 µm) and vessel density 16.26/mm² (22/mm²) in NF2-associated schwannomas. In normal nerves, the vessel density was 14/mm² (18/mm²) and the diameter 42.64 µm (7.9 µm). The large difference of vessel diameter in normal tissue is explained by an arteriole lying between nerve bundles in our study (in one of the control cases). After the adjustment of data, the mean diameter was calculated to be 10.29 µm and thus in the range of the former study (42). One earlier study on vessels in nerve tumors determined the mean vessel density to be ~ 30/mm² and thus calculated a higher value than in the recent studies (43). However, the cited study was performed without immunohistochemical verification of vessels. This may have resulted in an erroneous inclusion of lymphatic vessels.

In our study, vessel densities in benign lesions were similar to those previously reported and did not differ markedly from those of normal skin or peripheral nerve. Since the tumors developed within a pre-existing tissue, vessel growth has to be regarded as a regular extension of the local vasculature. In contrast, the vessels of malignant PNST impressed as pathological slit-like endothelial tubes. The vascular density was significantly higher in malignant tumors (30.8/mm²) than in benign ones (13.46/mm², including atypical neurofibroma).

Another study which addressed the issue of angiomas, both in neurofibroma and malignant PNST, described a vascular morphology in the malignant tumors similar to our findings. Vessels in malignant PNST had a criss-cross structure and irregular, difficult-to-differentiate lumen. Vessel density was increased in de-differentiated tissues (44).

Lymphatic vessels in PNSTs. Lymphatic vessels in intraneural tumors (nodular PNF, schwannoma) were exclusively found in the perineurium. This finding is in line with the expectations for physiological conditions. Another study of our group has recently described in detail the differences of oedema formation with respect to peripheral nerve tumors. We showed a distinct delay in the reduction of

Table IV. Number of cases and tumor entities by disease.

Tumor entity	In NF1	In NF2	In Schwannomatosis	Other
Cutaneous neurofibroma	20			
Plexiform diffuse neurofibroma	17			
Plexiform nodular neurofibroma	24			
Atypical neurofibroma	18			
Schwannoma in neurofibroma		2		
Sporadic vestibular nerve schwannoma				20
Schwannoma		3	11	
MPNST	8			
Normal skin (control)				5
Normal nerve (control)				4
Total	87	5	11	29

MPNST: Malignant peripheral nerve sheath tumor; NF1/NF2: neurofibromatosis type 1/type 2.

postoperative oedema in nodular PNF of NF1-affected individuals. Furthermore, these tumors showed an increased amount of water binding extracellular hyaluronic acid. Both factors could have additive effects on long-lasting oedema in surgery of some types of NSTs (47).

Mast cells in PNSTs. Mast cells rank among the most complex cells in the body. Mast cells were discovered by Paul Ehrlich (48) and are present in almost every human tissue, predominantly at the interface of the body with its surroundings such as skin, epithelia of the aerodigestive tract, and the mucosa of the gastrointestinal tract (49). Cross-linked IgE antibodies binding to the IgE receptor of mast cells stimulate their degranulation. This process leads to release of different chemotactic substances into the extracellular space, *e.g.* histamine, serotonin, heparin, prostglandin D2 and leukotriene C4 (49).

In experimental studies, *NF*^{+/-} heterozygous mast cells are required for PNF formation (14). Enhanced proliferative properties of heterozygous mast cells from patients with NF1 and from *Nf1*^{+/-} mice (50) may play a leading part in the initiation of neurofibroma in general, as already suggested by Riccardi (51). In line with the assumption of heterozygous mast cell interaction being confined to nullizygous Schwann cells is the observation that heterozygous (*Nf*^{+/-}) Schwann cells of peripheral nerves do not attract heterozygous mast cells in knockout mice (14). The interaction between mast cells and Schwann cells during neurofibroma formation is probably bi-directional, resulting in *NF1*^{-/-} Schwann cells modifying the extracellular milieu to promote the growth and cell division rate of heterozygous cells pathognomonic for neurofibroma (14, 26). The basis for the interaction between mast cells and Schwann cells can be

dependent on an ontogenetically-determined time frame of the *NF1* mutation (52). Angiogenic factors and receptors are detectable in NF1-associated tumors (53, 54). Furthermore, mast cells are known to produce vascular endothelial growth factor and other angiogenic factors that are released into the extracellular matrix, leading to formation of new vessels (55, 56). The mast cell phenotypes appear to be different with respect to histological subtype of neurofibroma (57). Recently, further immune-competent cells, *e.g.* macrophages, were identified as playing a role in tumor growth in neurofibroma (58).

The few studies published to date on mast cell densities provide different findings for this item in normal skin. While Eady *et al.* (43) published a value of 50 mast cells per mm², a subsequent study noted 77-108/mm² (59). These differences could be attributable to different counting methods. The latter study used mast cell-specific antibodies (mouse ATA clone AA1; Dako) and a standardized computerized counting method. Interestingly, this study also noted an increased mast cell density in more proximal parts of the skin compared to more distal (59).

Our evaluation in particular showed a significantly increased mast cell density in cutaneous neurofibroma and diffuse PNF compared to normal skin (*p*=0.035 and *p*=0.027, respectively). Other benign tumors had mast cell densities similar to those of normal skin and nerve (Figure 1). Apparently, some dysplastic lesions and tumors, particularly skin precancerous lesions, malignant melanoma, breast carcinoma and colorectal carcinoma, are capable of attracting mast cells that are situated in and around these lesions (60). Tissue reactions to tumor growth and localized inflammatory reactions could act as further stimuli for mast cell attraction (61). Current data about the impact of mast

cells on tumor growth revealed ambiguous results. For example, patients with colorectal carcinoma and low mast cell count had better survival rates (62). On the other hand, in patients with breast cancer, an increased mast cell count was associated with a better prognosis (63). Maltby *et al.* reviewed the knowledge about the possible impact of mast cells on cancer and concluded these cells support tumor growth by tissue remodelling and induction of angiogenesis (61). During the course of cancer, mast cells can act statically against the tumor by promoting the immune response, or, alternatively, be tumor-promoting by suppression of the immune system. Suppression of the immune system could be explained by the interaction of mast cells and regulatory T-cells (61). The results of the aforementioned studies were not based on identical counting and staining methods. Therefore, it is not unlikely that mast cells constitute different subgroups with different functions.

The present study was unable to demonstrate an increased number of mast cells in malignant PNST or in PNF. Instead, cutaneous neurofibroma that does not de-differentiate into malignant PNST had the highest mast cell count. Hence our data are compatible with a role for mast cells in neurofibroma formation and growth, but do not suggest an important function of mast cells in malignant progression.

Conclusion

The high variability of blood vessel density especially in schwannoma could contribute to the difficulty to predict response of these tumors to vasoactive anti-neoplastic drugs. With respect to the density of mast cells in PNS, the effect of this cell population on tumor growth is likely to be restricted to cutaneous neurofibroma and seems not to be involved in malignant progression of PNST.

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