Atypical Plexiform Neurofibroma in NF1 with High Standardised Uptake Value (SUV) in Positron-Emission Tomography (PET) Expressing Podoplanin

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Abstract. A 19-year-old female with established neurofibromatosis type 1 (NF1) diagnosis and history of malignant peripheral nerve sheath tumour (MPNST) of the lower extremities showed a tumour of her right upper extremity with a maximum standardised uptake value (SUV) of 5.7 on positron emission/computerised tomography (PET/CT) scan. The extirpated tumour proved to be an atypical plexiform neurofibroma. Immunohistochemical investigations revealed a strong positive reaction of tumour cells for S-100 protein. Epithelial membrane antigen (EMA)-positive perineural cells were demonstrated at the border of the tumour. Scattered nerve fibres were labelled with neurofilament antibodies within the tumour. The maximum Ki-67 labelling index reached 9%. Slight to moderate podoplanin expression of the tumour cells was noted in areas of fibrillary growth. Podoplanin expression has been proposed as a differentiation marker for schwannomas and neurofibromas. This atypical neurofibroma showed a high SUV on PET that is indicative for MPNST. It appears likely that subtypes of Schwann cells express podoplanin. This marker may be useful in distinguishing different Schwann cell populations in neurofibromas.

Neurofibromatosis type 1 (NF1) is an autosomal-dominant inherited disorder (1). This diagnosis can be established in about 1 in 3000 individuals living at birth (2). NF1 is a tumour predisposition syndrome. Certain types of neoplasms, both benign and malignant, occur frequently in NF1 patients. The hallmark of NF1 is the existence of neurofibromas, benign nerve sheath tumours that usually develop in large numbers within the skin of the affected individuals. Cutaneous neurofibromas only grow to a maximum diameter of a few centimetres and are judged to maintain a benign biological behaviour throughout life. A subtype of neurofibroma, currently denominated as plexiform neurofibroma, is characterised by a diffuse and invasive growth and has the propensity to dedifferentiate into a malignant peripheral nerve sheath tumour (MPNST) (3). Standardised uptake values (SUV) of tumours depicted in positron-emission tomography (PET) is a well-recognised diagnostic tool in NF1 (4), in particular, in order to differentiate benign and malignant peripheral nerve sheath tumours (5-11). However, in borderline cases it may be difficult to distinguish benign from malignant lesions (7, 12). Recently, the different expression of podoplanin in nerve sheath tumours was recommended as a diagnostic tool to discriminate between schwannomas and neurofibromas (13, 14). This report describes a case with plexiform neurofibroma that showed a distinct podoplanin expression and a remarkably high SUV.

Case Report

A 19-year-old female with NF1 was admitted to the nationwide outpatient center for neurofibromatosis at Eppendorf University Hospital for a check-up. Her first investigation in this institution had taken place when she was ten years of age. She was known to have axillary and inguinally freckling and café-au-lait spots since early childhood. Already at the time of the first investigation glomatous alterations were detected on T2-weighted magnetic resonance imaging (MRI) scans of the brain bilaterally in the region of globus pallidus. Her learning capability was reduced and her articulation was indistinct. The mother of the patient was NF1-affected and her sister also fulfilled the diagnostic criteria for NF1 (15).
At the age of 14 years the patient had developed an MPNST in the right popliteal fossa that was completely resected in another hospital. Two years later a second-look operation was performed due to the development of a small tumour in the same region. The tumour proved to be a lipoma.

On admission, the patient expressed her wish to be relieved of several cutaneous neurofibromas, predominantly of her trunk. During the investigation, the patient pointed to a tumour of her right upper limb that was moderately painful on digital pressure. In contrast to the cutaneous tumours, this tumour was an invisible alteration below the integument and subcutaneously palpable as a solid, oval mass. This mass was slightly laterally moveable, referring to the long axis of the extremity, but not in a proximal or distal direction. She reported the tumour had grown in size over the previous months. No pigmentation disorders were seen in this region.

PET/CT imaging was performed on the Philips Gemini GXL10 (Philips, Best, The Netherlands) hybrid PET/CT scanner consisted of a full-ring, whole-body, 3D-only PET equipped with gadolinium orthosilicate (GSO) crystal detectors with a 10-slice high-resolution spiral CT. The patient was instructed to fast, except for oral intake of glucose-free liquids to maintain oral hydration, at least four hours before the injection of 350 MBq of $^{18}$F-FDG to standardise blood glucose and insulin levels. Sixty minutes after injection of $^{18}$F-FDG, whole body images were acquired from head to foot in a cranio-caudal direction. Emission images were acquired for 1.5 min per bed position (effective axial field of view: 90 cm).

Subsequent to the PET acquisition, a fully diagnostic CT scan was performed with administration of intravenous contrast material (140 ml Imeron 300, Bracco Imaging, Konstanz, Germany) 90 seconds prior to the CT scan. Acquisition parameters were: 120 kV, 150 mAs, slice thickness 5 mm, no gap, pitch 0.9, rotation time 0.74 s, matrix 512x512x512. CT data were used for low noise attenuation correction of PET data and for co-registration with attenuation corrected PET images. PET, CT and fused PET/CT images were available for review and were displayed in axial, coronal and sagittal planes. The PET data were displayed as attenuation-corrected images and also in a rotating maximum-intensity projection.

**PET/CT image analysis.** FDG PET/CT images were analysed by two experienced nuclear medicine physicians using the Extended Brilliance Workstation (EBW, Philips, Best, the Netherlands). Scans were evaluated both qualitatively and semiquantitatively using the maximum standardised uptake value (SUV$_{\text{max}}$). SUV$_{\text{max}}$ was calculated using the single maximum pixel count within the volumes of interest. Tumours with a SUV$_{\text{max}}$ of 3.5 and above were considered malignant, whereas neurofibromas with SUV$_{\text{max}}$ <2.5 were classified as benign (5). Patients with lesions with SUV$_{\text{max}}$ in the range of 2.5 to 3.5 should be followed up clinically (6).

PET demonstrated a site of intense FDG uptake the right upper extremity, close to the elbow region (SUV$_{\text{max}}$=5.7). The lesion had a largest diameter of about 5.5 cm on CT. SUV$_{\text{max}}$ of other lesions ranged between 1.0 (muscles of both legs) and 2.0 (right axillary region). Tentative differential diagnosis was MPNST of the humeral part of the right antecubital fossa.

**Surgery.** It was decided the lesion in the right upper arm and other tumours would be extirpated. After incision of the skin the tumour was localised inside the muscle and appeared as an enlarged nerval cord running from the proximal side into the tumour. No distal cord-like extension of the tumour was identified (Figure 2). The tumour was separated from the muscle by blunt dissection and macroscopically completely excised. The wound was closed by primary intention. Healing was uneventful. Following surgery the patient had no impairment of motor functions but noted a slight numbness of the palmar side of her third and fourth finger pads that diminished within days.

**Histopathology.** Histopathological examination revealed a tumour of medium to high cellularity composed of spindle shaped cells in a myxoid stroma containing a varying proportion of collagen fibres. Frequently atypical large hyperchromatic nuclei were demonstrated and some of the karyomegalies contained amorphic inclusions. Mitoses were rarely seen. No necrosis was observed. Focally dense perivascular lymphocytic infiltration was noted.

For immunohistochemical investigation antibodies against S-100 protein (DAKO, Hamburg, Germany; Z0311, 1:8000), neurofilament (DAKO; M0762, 1:800), epithelial membrane antigen (EMA, DAKO; M0613, 1:200), podoplanin (Invitrogen, Löhne, Germany; 18-2410, 1:20) and the Ki-67 antigen (Lab Vision, Cheshire, UK; RM-9106-S, 1:1000) were used in a Ventana automatic stainer (Ventana Medical Systems, Tucson, AZ, USA) applying diaminobenzidine (DAB) as chromogen. The tumour cells were strongly positive for S-100 protein. At the border of the tumour EMA-positive perineural cells were demonstrated and within the tumour scattered nerve fibres were labelled with neurofilament antibodies. The maximum Ki-67 labelling index reached 9% (19 labelled nuclei
Figure 1. FDG-PET/CT: The tumour is depicted in the right antecubital fossa in close association with the distal humerus. The tumour appears as a distinct and homogeneous nodular mass. The distinction between a subcutaneous/extramural or intramuscular position cannot be made from these images. A: Detail of axial slices of PET (upper image) and CT (lower image) showing the tumour’s high SUV and the small semicircular line demarcating the lesion in close proximity to the humerus (CT). B: Whole-body PET shows one lesion only with high SUV suspicious of a malignant tumour. Arrows point to the tumour.

Figure 2. Photograph of the plexiform neurofibroma. Enlarged intramuscular nerve merges into the nodular tumour but shows no distal extension.

Figure 3. Histopathological features of atypical neurofibroma. a: Neurofibroma with increased cellularity and marked nuclear pleomorphism, H&E stain. b-d, Immunohistochemical demonstration of S-100 protein (b), podoplanin (c), and Ki-67 antigen (d). Chromogen: DAB, scale bar (200 μm) in c applies also for b and d.
of 221 nuclei in a high power field of 0.2 mm²). Slight to moderate podoplanin expression of the tumour cells was noted in areas of fibrillary growth.

The cutaneous neurofibromas showed the typical appearance of this tumour entity with high collagen content and low cellularity. The slender cells had inconspicuous elongated nuclei.

Immunohistochemically the cells stained strongly positive for S-100 protein, whereas EMA, neurofilament and podoplanin were not detected. The Ki-67 labelling index was below 2%.

**Discussion**

The findings in this NF1 patient showed a diffuse expression of podoplanin in a case of atypical neurofibroma, associated with a high SUV in PET that is often detected in MPNST. Podoplanin is rarely expressed in neurofibromas but showed a diffuse staining pattern in this case (14). This quality may be a tool to delineate atypical neurofibromas in NF1 further.

**PET.** The first study on the application of 18FDG-PET for the discrimination of benign and malignant nerve sheath tumours in NF1 was published by Ferner et al. (5). These authors demonstrated that SUV is lower in benign tumours (1.54, SD (standard deviation) 0.7; range 0.56-3.3) and higher in malignant (5.4, SD 2.4; range 2.7-8.4). They found an overlap of SUV between malignant and benign tumours in the range (2.7-3.3) and suggested that the optimum time for calculating SUV is 200 minutes after the injection of 18FDG.

Brenner et al. (9) suggested that SUV in PET may be a better predictive marker for clinical outcome in NF1-patients with MPNST. They used a cut-off level >3 to predict patients with a significantly shorter survival time. These results were not confirmed in a later study (6).

Ferner et al. (6) presented a second study on PET/CT and calculated the mean SUV for plexiform neurofibromas (PNF) as 1.5 (SD 1.06) and for MPNST 5.7 (SD 2.6). These data were based on a long-term follow-up control study of a large group of NF1 patients. Out of 80 patients with PNF 76 were negative on FDG-PET/CT (95%). They diagnosed 5 out of the 80 patients with PNF and MPNST. They used a cut-off level >3 to predict patients with a significantly shorter survival time. These results were not confirmed in a later study (6).

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Ferner et al. (6) detected no MPNST with SUVmax below 2.5, but noted 3 false-positive scans above SUVmax=3.5 in PNF (4.1, 4.8 and 6.4). The SUV max of the present report was in the range of the 3 PNF cases with elevated SUV. Ferner et al. (6) concluded that symptomatic neurofibromas with SUV max=3.5 should be excised. Furthermore, these authors reported on a considerable overlap between MPNST and PNF concerning the increase in size and the change of tumour texture as an indicator of malignant transformation.

Warbey et al. (16) analysed 69 patients by PET/CT and demonstrated a significant difference in SUVmax and tumour grades. The sensitivity of this measurement was 0.97 [95% confidence interval (CI): 0.81-0.99]) and the specificity 0.87 (95% CI: 0.74-0.95) to diagnose an MPNST accurately. These authors recommended a cut-off SUVmax of 3.5 to achieve maximal sensitivity (early 90 min and delayed 4-hour imaging). In this study, 9 out of 10 atypical neurofibromas showed high SUV max.

Mautner et al. (7) demonstrated elevated SUVmax in 4 symptomatic cases and showed associated alterations of the tumours on MRI. Only one case had developed an MPNST despite pathological findings on MRI and elevated SUV in PET. These authors presumed that the neurofibromas with high SUV, conspicuous patterns on MRI (17) and histopathological findings conforming to PNF possibly represent intermediate stages of PNF to MPNST.

Karabatsou et al. (11) investigated NF1 patients with PNF suspected to have undergone malignant transformation to MPNST by PET/CT and studied the resected tumours immunohistochemically. They revealed a positive correlation between the number of proliferating cells in PNF in terms of the proliferating index (Ki-67), and SUV max.

Interestingly, these authors defined an intermediate SUV max value (4.0-7.0) as a transition area between PNF and MPNST. However, their conclusions were based on a small sample size (n=9).

Brinkmann et al. (12) reported on a NF1 patient with clinical findings suspicious for malignant transformation who showed numerous signals on PET but homogeneous cutaneous or subcutaneous lesions on MRI. These authors excised 20 lesions at two time points derived from the patient’s report about pain in the tumoural region. In none of the cases an MPNST was found but 6 out of 20 lesions proved to be PNF. These authors described their PET technique in detail but refrained from calculating SUV. Their semiquantitative 4-scale-scoring system differentiated a range from ‘no uptake’ to ‘high uptake’. Whereas the biology of neurofibroma in NF1 is poorly understood and the metabolism of these tumours certainly is controlled by a number of substances that may interfere with PET imaging in each individual, the accuracy of the diagnosis would probably have been improved using SUV. However, this report provided a valuable hint to point to the overlap of benign peripheral nerve sheath tumours, cutaneous and plexiform neurofibroma in PET.
In the patient of the present study, the PNF of the right upper limb was demarcated only on PET/CT. All other cutaneous neurofibromas did not show any signal on PET/CT and were histologically inconspicuous lesions.

Bredella et al. (10) retrospectively investigated FDG-PET scans of 45 patients with 50 lesions. Semiquantitative analysis of FDG uptake was performed in 41 lesions of 36 NF1 patients. Out of 50 lesions, 41 were correctly diagnosed as either benign or malignant, leaving one patient with MPNST misdiagnosed as having a benign tumour and 8 lesions that were incorrectly diagnosed as malignant. This high rate of false-positive diagnosis was reduced from 8 to 2 when 11C methionine PET scans were performed additionally. The SUV of FDG-PET for MPNST ranged from 3.8 to 13.0 (mean 8.5±0.63, standard error of the mean (SEM)) and for benign peripheral nerve sheath tumours from 0 to 5.3 (mean 1.5±0.37 SEM). These differences proved to be statistically significant (p=0.001, Student’s t-test). This study included 3 patients with atypical neurofibromas that were not specified further for their SUV (10).

**Atypical neurofibroma.** The term 'atypical neurofibroma' is used to describe neurofibroma cells with large, pleomorphic nuclei, distinctive nuclear inclusions, smeary chromatin, and inconspicuous nuclei. These cells do not comprise the entire lesion and are situated in between typical neurofibroma cells (3). Mitotic activity is low in atypical neurofibroma but may be encountered (3). Atypical neurofibromas are benign tumours according to the World Health Organisation (WHO) and correspond to grade I. A propensity to dedifferentiation to MPNST has not been proven although the lesions may resemble low-grade MPNST featuring increased proliferation index, highcellularity and increased cellular and nuclear atypia (3). Valerye-Allanore et al. hypothesised that these tumours represent an intermediate form between neurofibroma and MPNST (18). However, the same group recently reported on a clinical follow up of neurofibromas and their distinct genetic anomalies and the different tumour environment (27). In this report the immunoreactivity of podoplanin in schwannomas and neurofibromas was not detailed with respect to the diagnosis of NF1.

The present case report revealed that an atypical neurofibroma expresses podoplanin. It is suggested that some tumourous nerve sheath cells in atypical neurofibromas possess characteristics of Schwann cells typically found in schwannomas. In line with this assumption are the increased nuclear pleomorphism, the secondary changes such as perivascular lymphocytic infiltration and the Ki-67 labelling index in an atypical neurofibroma. The Ki-67 index is high with regard to a pleomorphic neurofibroma but in the expected range of a schwannaoma.

**Conclusion**

Patients affected by NF1 are at risk of developing MPNST. SUV determined by 18FDG-PET is a well established diagnostic tool to clinically differentiate benign and malignant peripheral nerve sheath tumours in NF1. However, overlaps of SUV between benign and malignant peripheral nerve sheath tumours render therapeutic decisions difficult in some cases. There are subtypes of neurofibromas in NF1 with a prevailing descriptive morphology and doubtful biological behaviour. This report detailed the experience about a peripheral nerve sheath tumour in NF1 that showed a high SUV indicative of surgical exploration. The extirpated tumour was diagnosed an atypical neurofibroma and showed podoplanin expression, a marker predominantly found in schwannomas. Hence, this marker may be useful in distinguishing different Schwann cell populations in neurofibromas. Further studies should be aimed to differentiate neurofibromas in NF1 for podoplanin expression.

**References**


8 Benz MR, Czemin J, Dry SM, Tap WD, Allen-Auerbach MS, Elshoff D, Phelps ME, Weber WA and Eilber PC: Quantitative F18-fluorodeoxyglucose positron emission tomography accurately characterizes peripheral nerve sheath tumors as malignant or benign. Cancer (e-pub. 18.11.09)