Abstract. A 23-year-old female with an established diagnosis of neurofibromatosis type 1 (NF1) was found to have two tumours in her trunk. These showed high uptake value in positron-emission/computerized tomography (PET) scans, and were suspected to be malignant peripheral nerve sheath tumours (MPNST). The extirpated tumours proved to be atypical plexiform neurofibromas. Slight to moderate podoplanin expression of the tumour cells was noted in areas of fibrillary growth. Six years following surgery, there is neither evidence of local tumour recurrence nor development of MPNST. Current studies on atypical neurofibroma in NF1 suggest podoplanin expression in subtypes of transformed Schwann cells, resembling schwannoma-like areas in neurofibroma. This marker may be useful in distinguishing different Schwann cell populations in NF1.

Neurofibromatosis type 1 (NF1) is an autosomal-dominantly inherited disorder affecting about 1 in 3,000 living individuals, at birth (1, 2). The hallmark of NF1 is a benign nerve sheath tumour (neurofibroma) that usually develops in large numbers within the skin of the affected individuals. Two types of neurofibromas are differentiated: cutaneous neurofibromas (CNF) and plexiform neurofibroma (PNF). CNF only grow to a maximum diameter of a few centimetres. It is very likely that CNF maintain a benign biological behaviour throughout life (3). PNFs primarily developing within a peripheral nerve may also show a diffuse and invasive growth and have the propensity to de-differentiate into a malignant peripheral nerve sheath tumour (MPNST) (3). Standardized uptake values (SUV) of tumours imaged on positron-emission tomographic (PET) scans are used to differentiate peripheral nerve sheath tumours in NF1 (5-11). However, SUV may be inconclusive in borderline cases (7, 12). Computed-tomography (CT) and magnetic resonance images (MRI) are applied in differentiating peripheral nerve sheath tumours in NF1 (7).

The expression of podoplanin in nerve sheath tumours has been recommended as a diagnostic tool to discriminate between schwannomas and neurofibromas in NF1 (13, 14). This report describes a case with atypical plexiform neurofibromas that exhibited a distinct podoplanin expression and remarkably high SUVs in PET, with a sufficiently long-term follow-up of the clinical course.

Case Report

A 23-year-old female with NF1 was admitted to the outpatient centre for neurofibromatosis patients at the Eppendorf University Hospital for a check-up. She was known to have axillary and inguinal freckling and café-au-lait spots since early childhood. The patient fulfilled the diagnostic criteria for NF1 (15).

On whole-body MRI scans, a large, roundish space-occupying lesion was demarcated in the thorax in close proximity to the ribs of the right side (Figure 1A). The lesion appeared well-demarcated and iso-intense on T2-weighted images (Figure 1B). Further small-cutaneous and sub-cutaneous tumours were also depicted, predominantly in the trunk. None of these cutaneous tumours was suspected to be an MPNST (17). A PET scan was performed in order to better-estimate the tumour biology (5, 6).
PET image acquisition and analysis. PET imaging was performed as detailed elsewhere (9, 12). 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 (18F)2-fluoro-deoxy-D-glucose (18F-FDG), to standardise blood glucose and insulin levels. Sixty minutes after injection of 18F-FDG, whole-body images were acquired from head to foot in a crani-to-caudal direction. PET images were available for review and were displayed in axial, coronal and sagittal planes (Figure 1C). PET images were analysed as previously described (9, 12). Scans were evaluated both qualitatively and semi-quantitatively 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 an SUV$_{\text{max}}$ of 3.5 and above were considered malignant, whereas neurofibromas with SUV$_{\text{max}}$ <2.5 were classified as benign (5). It is recommended to follow-up patients with lesions with SUV$_{\text{max}}$ in the range of 2.5 to 3.5 clinically (6).

PET demonstrated a site of intense FDG uptake in the right thoracic region, corresponding to the tumour identified on MRI (SUV$_{\text{max}}$=4.2). SUV$_{\text{max}}$ of another lesion located in the right thoracic region, corresponding to the tumour identified 2.5 to 3.5 clinically (6).

Surgery. After lateral thoracotomy, the tumour was identified between two ribs and adhering to them, but not to the pleura. Due to the dubious biology of the tumour, it was decided to resect the tumour with sufficiently extended safety margins. A partial resection of the ribs was performed and the tumour was completely excised (Figure 2A). Incision of the tumour showed an encapsulated, firm and oval-shaped tumour, with dense internal structures, without macroscopically evident necrosis (Figure 2B). The wound was closed by primary intention. Healing was uneventful.

Six months following the first intervention, the patient decided to have the other tumour of her trunk, that also had shown increased SUV on PET, extirpated. This nodular tumour was located in the left lumbar region in-side subcutaneous fatty tissues. Recovery from surgery was fast and healing prompt.

Histopathology. Histopathological examination of both resection specimens 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 (Figure 2C).

Immunohistochemistry. Sections of tumour were deparaffinized and heated in the respective manufacturer’s recommended unmasking solution at 95°C for 20 min. Endogenous peroxidases were quenched with 0.3% H$_2$O$_2$ (PBS). Sections were incubated overnight at 4°C using an antibody against podoplanin (clone D2-40, dilution 1:40; Signet, Dedham, MA, USA). Following this incubation, sections were incubated with Envision System$^\text{®}$ (Dako, Cytomation, Glostrup, Denmark) for 30 min at room temperature. Staining each sample without adding anti-human primary antibody was performed as a negative control. Finally, samples were incubated with diaminobenzidine peroxidase substrate to give a brown stain and counterstained with haematoxylin before mounting with coverslips. The intensity of the staining was graded as absent, weak, mild, moderate and strong. Further antibodies were used for immunohistochemical investigation: S-100 protein (Z0311, 1:8000; Dako, Hamburg, Germany), neurofilament (M0762, 1:800, Dako), epithelial membrane antigen [(EMA), M0613, 1:200; Dako], and Ki-67 antigen (RM-9106-S, 1:1000; Lab Vision, Cheshire, UK) were used in a Ventana automatic stainer (Ventana Medical Systems, Tucson, AZ, USA) applying diaminobenzidine (DAB) as a chromogen.

The tumour cells were strongly positive for the S-100 protein. Thickened perineurial cells of the tumour were EMA-positive. Within the tumour, scattered nerve fibres were labelled with a neurofilament antibody. The maximum Ki-67 labelling index reached 14% (28 labelled nuclei out of 201 counted nuclei in a high power field of 0.1 mm$^2$). Slight to moderate podoplanin expression of the tumour cells was noted in areas of fibrillary growth (Figure 2D).

Discussion

High SUV in PET demonstrated the presence of two atypical PNF of a patient with NF1. The SUV for these tumours were in a range diagnostic of malignant peripheral nerve sheath tumour (5, 6). These PNF exhibited focal expression of podoplanin (4). Podoplanin expression is rarely found in neurofibromas but is a constant finding in schwannomas (4). The long-term follow-up of this patient allows for interpretation of the imaging findings on PET and the morphological findings within the tumour tissues. High SUV of NF1-associated peripheral nerve sheath tumours in NF1 depicted in PET, do not identify MPNST in every single case (7). The findings and the clinical course support our previous interpretation of podoplanin as a marker associated with a fibrillary growth pattern, rather than de-differentiation to MPNST (4). This report supports recent studies on the association of podoplanin expression in atypical neurofibromas with a schwannoma-like differentiation in areas of neurofibromas (4, 12).
**PET.** Ferner et al. (5) presented the first study on the application of $^{18}$FDG-PET for the discrimination of benign and malignant nerve sheath tumours in NF1. These authors proposed a scoring system to distinguish between benign and MPNST in NF1, based on SUV (5). They emphasised an overlap of SUV between malignant and benign tumours in a certain range (2.7-3.3). SUV in this range for suspected tumours do not allow-clear distinction of tumour biology. Following this path-finding study (5), others tried to calculate the clinical outcome of patients with NF1 based on the SUV in PET imaging (8-11). One study suggested that SUV in PET may be a predictive marker for clinical outcome in NF1 patients with MPNST (9). These authors used an SUV cut-off $>3$ to predict patients with a significantly shorter survival time. The utility of SUV as a predictive marker for overall survival in NF1-affected individuals with MPNST was not substantiated in a recently published study (6). The mean SUV for PNF was calculated to be 1.5 (SD 1.06), and for MPNST 5.7 (SD 2.6) in a consecutive study on PET/CT characteristics of peripheral nerve sheath tumours in NF1 (6). Out of 80 patients with PNF, 76 were negative on FDG-PET/CT (95%). Five out of 116 tumours were atypical neurofibromas. An interesting finding in the context of the presented case is that these five atypical PNFs had signal intensities different from the expected range for PNF. The rate of atypical neurofibroma positivity in FDG-PET/CT was 2 out of 5 cases, compared to 5% for typical PNF. SUV of these two cases with atypical neurofibroma were higher than in the current case [5.2 and 5.5 (6)]. No MPNST had an SUV below 2.5, but three false-positive scans above SUV=3.5 were recorded in PNF (4.1, 4.8 and 6.4) (6). The SUV of the thoracic site for our case was in the range of the three PNF cases with elevated SUV. Symptomatic neurofibromas with SUV=3.5 should be excised (6). A considerable overlap between MPNST and PNF concerning the increase in size and the change of tumour texture can be an indicator of malignant transformation (6, 17).

Other studies on this subject (7, 10, 11, 16) are discussed elsewhere in detail (12).

**Atypical neurofibroma.** The atypical neurofibroma is characterized by transformed Schwann cells with large, pleomorphic nuclei, distinctive nuclear inclusions smears chromatin. Atypical neurofibromas constitute a part of the lesion and are apportioned between typical neurofibroma cells (3). Mitotic activity is low in atypical neurofibroma in general but may be encountered (3). The Ki-67 labelling index was higher in atypical plexiform neurofibroma compared to normal PNF regions (4). Atypical neurofibromas are benign tumours [World Health Organisation (WHO) grade I]. A tendency for de-differentiation to MPNST has not been proven although the morphological features of these lesions may approach low-grade MPNST, showing increased proliferation index, high cellularity and increased cellular and nuclear atypia (3). It was argued that these tumours match an intermediate stage of peripheral nerve sheath tumours between neurofibroma and MPNST (18). On the other hand, the same group presented results of a later study on clinical follow-up of neurofibromas. The hypothesis that dysplastic features in neurofibromas may represent pre-malignant changes was not substantiated, according to this study (19). We were not able to substantiate the estimation of atypical neurofibromas as a pre-cancerous condition prone to de-differentiate into MPNST (4).

**Podoplanin.** The protein identified by the D2-40 antibody is used to discriminate between lymphatic and blood vessel endothelium in both tumour (20) and normal tissues (21). The D2-40 antigen is termed podoplanin (24). Podoplanin (synonyms: aggrus, TIA-2) is a 38-kDa glycoprotein. It has become evident that the antigen identified by D2-40 is widely expressed in human tissues (22-27). Distinctive differences of podoplanin expression dependent on the type of peripheral nerve sheath tumour appear to be of diagnostic importance (4). Podoplanin expression was suggested as a diagnostic tool to distinguish between schwannomas and neurofibromas (4). The rate of MPNST expressing podoplanin is low: strong podoplanin expression was rarely found in spindle-cell MPNST (12%) (27). It was argued that the differences in podoplanin expression between schwannomas and neurofibromas may be related to distinct genetic anomalies (27). However, this report did not mention the immunoreactivity of podoplanin in schwannomas and neurofibromas with respect to NF1 (27).

The present case report revealed podoplanin expression in an atypical neurofibroma. The expression pattern could be related to known cellular differences of neurofibromas. Podoplanin is expressed in areas of neurofibromas that resemble a schwannoma. 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 increased nuclear pleomorphism, secondary changes, such as perivascular lymphocytic infiltration and the Ki-67 labelling index in an atypical neurofibroma (4, 27).

**Conclusion**

Patients with NF1 are at risk of developing MPNST. Imaging modalities are highly desirable to allow the discrimination of peripheral nerve sheath tumours in NF1. SUV determined by $^{18}$FDG-PET is a well-established diagnostic tool to
investigate MPNST in NF1. MPNST can be defined by PET in many cases. However, overlap of SUV between benign and MPNST interferes with the diagnostic accuracy of PET in individuals with NF1 in some cases.

Subtypes of neurofibromas in NF1 are distinguished with a prevailing descriptive morphology and doubtful biological behaviour. This report described our experience with peripheral nerve sheath tumours in NF1 that exhibited a high SUV typically found in MPNST, indicative of the need for surgical exploration. Final diagnosis of both tumours was atypical neurofibroma. Both tumours exhibited podoplanin expression, a marker predominantly found in schwannomas. This marker may be useful in distinguishing different Schwann cell populations in neurofibromas. Although atypical PNFs are suspected of representing a precursor lesion of MPNST: long-term follow-up revealed no MPNST in this patient. Further studies should be aimed at specifying the podoplanin expression in neurofibromas in NF1.

References
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