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CLINICAL–PATIENT STUDIES

Histopathology and clinical outcome of NF1-associated vs. sporadic malignant peripheral nerve sheath tumors

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Abstract The differences in the clinical course and histopathology of sporadic and neurofibromatosis type 1 (NF1)-associated malignant peripheral nerve sheath tumors (MPNST) were investigated retrospectively. The collective comprised 38 NF1 patients and 14 sporadic patients. NF1 patients were significantly younger at diagnosis ($p < 0.001$) and had a significantly shorter survival time than sporadic patients (median survival 17 months vs. 42 months, Breslow $p < 0.05$). The time interval to local recurrence and metastatic spread was also significantly shorter in NF1 patients (9.4 months vs. 30.0 months, $p < 0.01$; 9.1 months vs. 33.2 months, $p < 0.001$, respectively). In patients with the original histopathological data available (22 NF1 patients, 14 sporadic cases), NF1-associated MPNST showed a significantly higher cellularity compared to sporadic tumors ($p < 0.001$) whereas sporadic MPNST featured a significantly higher pleomorphism ($p< 0.01$). Most importantly, while histopathological variables correlated with French Fédération Nationale des Centres de Lutte Contre le Cancer grading in sporadic MPNST, this was not the case for NF1-associated tumors. The differences between NF1-associated and sporadic MPNST in regard to the clinical course and histopathology may reflect some fundamental differences in biology and pathomechanism of the two tumor groups. Our findings indicate the necessity for a separate grading scheme which takes into account the genetic background in NF1 patients.

Keywords Malignant peripheral nerve sheath tumor · Neurofibromatosis · Prognosis · Histopathology

Introduction

Malignant peripheral nerve sheath tumors (MPNST) are rare neoplasms with an incidence of 0.001% in the general population [1]. Because of their invasive growth and metastatic potential as well as their limited sensitivity to radio- and chemotherapy [2, 3], these neoplasms remain incurable in most cases. More than half of the MPNST occur in patients with neurofibromatosis type 1 (NF1). The life time risk for NF1 patients to develop MPNST was reported to be as high

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as 8–12% [4], which makes this tumor the most significant factor for the reduced life expectation in these patients.

Neurofibromatosis type 1 is an autosomal genetic disease associated mostly with multiple benign tumors. The hallmarks of NF1 are peripheral neurofibromas and café au lait spots while other neurological and neuropsychological deficits are also common. Approximately 30% of NF1 patients develop plexiform neurofibromas which differ fundamentally from cutaneous neurofibromas in terms of size, location, growth pattern and timing of tumor development. Most importantly, plexiform neurofibromas have a high risk of malignant transformation into MPNST.

As defined by the World Health Organization (WHO), MPNST refers to any malignant tumor that arises from the nerve sheath or shows features of nerve sheath differentiation [5]. The tumor cells of MPNST are likely to be derived from Schwann cells, although the origin of the tumor cells has not been established so far with certainty. Though NF1-associated MPNST mostly develop as malignant progression of pre-existing plexiform neurofibromas [2], the tumor cells often do not show the morphological and immunohistochemical characteristics of Schwann cells such as S100 expression [6, 7], which however may suggest dedifferentiation of the involved Schwann cells. In regard to sporadic MPNST a progression from neurofibroma has not been demonstrated so far, thus, the tumor may develop ''de novo'' as malignancy. Concerning the grading of MPNST, the WHO classification of tumors of the nervous system solely states that these tumors correspond to grade III or IV [5]. Two other classification schemes which are listed in the recent WHO classification of soft tissue tumors [8] define the grading of MPNST in more detail. The classification of the United States National Cancer Institute (NCI) and the French Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) differentiate three tumor grades which are diagnosed according to tumor differentiation, mitotic activity and extent of necrosis [9, 10].

However, histopathological diagnosis of MPNST remains a challenge because of (1) the rarity of this tumor entity, (2) its histomorphology which may range from tumors resembling atypical neurofibromas to highly dedifferentiated sarcoma, (3) the lack of specific immunohistochemical markers and (4) because of sampling errors at operation (small biopsy, incomplete excision, etc.). The latter pose a problem particularly in deeply located plexiform tumors of the head, neck and trunk. As a consequence of these diagnostic problems no firm association between histological grade and survival has yet been established.

NF1 mutations are not only found in NF1-associated MPNST but also in the majority of patients with sporadic MPNST [7]. In addition, other genes like the *CDKN2A*, *KIP 1* and *TP53* may show mutations or inactivation of their products p16, p27 and p53 in MPNST [11–13]. Further, over-expression of epidermal growth factor has been described [14]. Recent gene expression profiling studies did not reveal significantly different molecular signatures between NF1-associated and sporadic MPNST [15, 16].

However, clinical experience showed that NF1-associated tumors occur significantly earlier in life than sporadic cases and that patients with NF1-associated MPNST may have a shorter survival than patients with sporadic MPNST [4]. In the present study, these two tumor groups were compared systematically.

Patients, materials and methods

Patients

The subjects included in this study comprised a total of 52 patients, 14 patients with sporadic MPNST (8 women, 6 men, median age at diagnosis 60.6 years) and 38 NF1 patients (16 women, 22 men, median age at diagnosis 26.5 years). From the 14 sporadic and 22 out of the 38 NF1 patients, tumor tissue was available for histopathological re-evaluation by some of the authors. All NF1 patients included in this study met the NIH criteria for NF1 [17, 18]. For all sporadic patients, NF1 was excluded based on their medical history and thorough clinical examination. The study was approved by the institutional review boards of the participating institutions and informed consent was obtained from all patients. Twenty-eight out of the 38 NF1–MPNST patients presented a consecutive subset of the more than 4,000 NF1 patients who received medical care at our NF-center in Hamburg, Germany, from 1991 to 2003. The other ten NF1–MPNST patients were referred to our NF-center with the suspect of NF1 and MPNST.

All 14 sporadic MPNST patients were diagnosed and underwent surgery at the Surgical Department of the University Medical Center Hamburg-Eppendorf, Hamburg, Germany. Clinical diagnosis and surgery were performed by one of the authors (MP). NF1 was excluded for these patients by VFM. These patients were included in the study consecutively.

Though we are not aware of any obvious bias in patient recruitment, given the small sample size, we cannot exclude the possibility of various biases.

Clinical and histopathological examination

Clinical characteristics of the patients determined were symptoms and age at diagnosis of MPNST, tumor location, radical surgery, tumor size, post-operative radio- or chemotherapy, disease-free interval, time to local recurrence and/or metastatic spread, overall survival and histopathological classification of the tumors according to the FNCLCC scheme. All patients were examined for tumor recurrences clinically and by means of MRI and/or CT at intervals of 6–12 months. Histologically tumor differentiation, cellularity, pleomorphism, mitotic count in ten high power fields (hpf) and extent of necroses were recorded as separate variables.

Statistical analysis

Survival distributions of patients with NF1-associated and sporadic MPNST were computed using the Kaplan–Meier method (Breslow test for significance level) and according to the Wilcoxon (Gehan) test. Time was censored at the last follow-up if death by tumor was not observed. Correlations were calculated two tailed according to the non-parametric Kendall-tau-*b* test. All two-tailed values of $p < 0.05$ were considered statistically significant. The following variables with ordinal scale were included in the computation: numbers and scores for mitotic activity in ten hpf (scores 1–3: 0–9; 10–19; ≥20), scores for pleomorphism (1–3), cellularity (1–3) and extent of necrosis (1–3: no; <50%; ≥50%) and the resultant FNCLCC grading (1–3); gender (f/m), occurrence of local recurrence, metastasis or death (no/yes), radiation (no/yes), tumor location (1, axial; 2, peripheral), tumor size (<5; 5–10; >10 cm), neurological deficits (no/yes), pain (constant, periodic, none), radical surgery (0–2). The following parameters were interval scaled data (age in years, all other times in months): age at diagnosis, time to local recurrence, time to metastasis and survival (Table 1). The Cox proportional hazards regression model (inclusion method) was used to analyze the effect of potential prognostic factors on survival. Variables were removed from the final model if they were not significant at the level of 0.05. All two-tailed values of $p < 0.05$ were considered statistically significant.

The SSPS for Windows software release 11.5 was used for all calculations.

Table 1 Patient characteristics

	NF1-associated MPNST	Sporadic MPNST
Age at diagnosis	Years	
Mean	29.6	54.6
Median	26.5	60.6
Range	10.7–66.4	24.4–76.1
Age range	*n*	
<20 years	6	0
20–30 years	18	2
31–40 years	8	3
>40 years	6	9
Gender	*n*	
Male	22	6
Female	16	8
Death	*n*	
Alive	13	5
Dead	25	9
Tumor location	*n*	
Axial	27	0
Peripheral	11	0
Missing	0	14
Tumor size (cm)	*n*	
<5	4	0
5–10	13	0
>5	21	0
Missing	0	14
Metastases	*n*	
Present	24	8
Absent	14	6
Resection quality	*n*	
R0	8	0
R1	19	0
R2	11	0
Missing	0	14
Neurological deficits	*n*	
Present	13	0
Absent	25	0
Missing	0	14
Pain	*n*	
Constant	27	0
Periodic	6	0
None	5	0
Missing	0	14

Results

Clinical features

Tumor location was available only for NF1 patients; in this group 27 tumors were located axially and in 11 cases in the periphery. Men and women were nearly equally affected by MPNST, but men developed local recurrences and metastases earlier ($p < 0.05$ for both variables) and

suffered more frequently from metastatic spread than women ($p < 0.02$). Significantly more men than women died due to the existing MPNST (23 vs. 11, $p < 0.01$).

Overall, MPNST led to a more severe course in NF1 patients than in patients with sporadic MPNST. NF1 patients were significantly younger at age of diagnosis ($p < 0.001$), developed local recurrences and metastases at an earlier stage ($p < 0.01$ and $p < 0.001$, respectively) and showed a significantly shorter survival in Kaplan–Meier survival statistics (Breslow test $p = 0.0323$; median survival 17 months in NF1-associated cases vs. 42 months in sporadic cases, Fig. 1). Calculation of survival using the Wilcoxon (Gehan) test resulted in a comparable p-value ($p = 0.0231$). The difference in survival was marked during the first 10 years after diagnosis (Fig. 1).

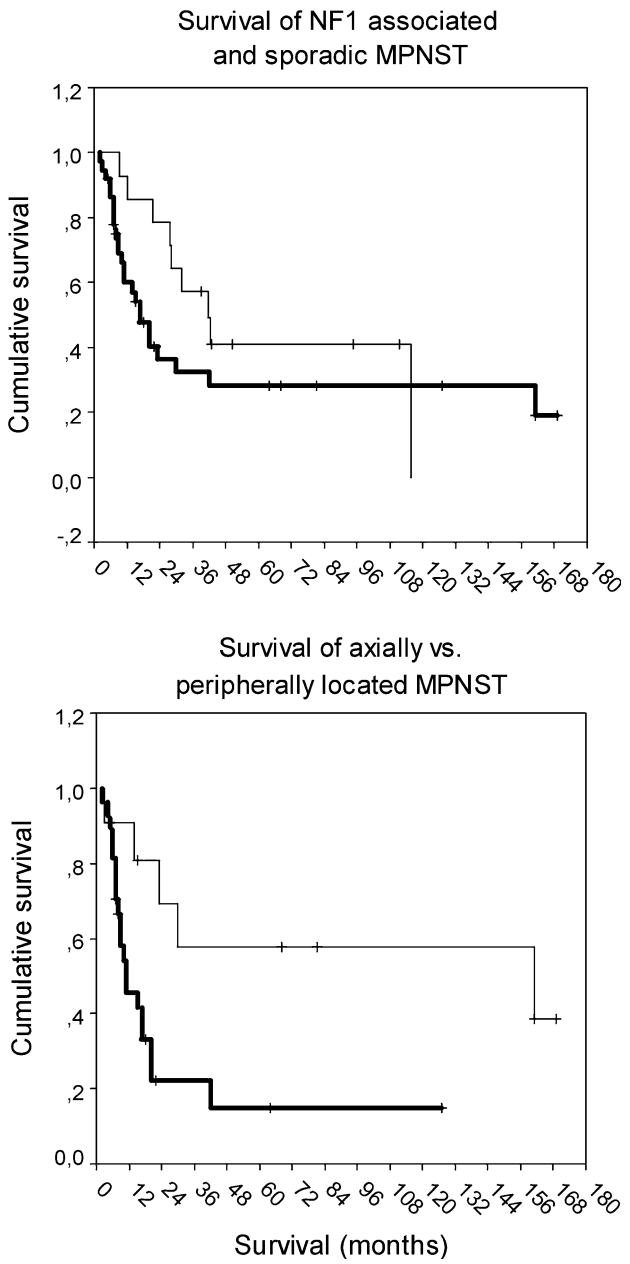

Fig. 1 Patient survival *curves* for 52 patients (18 alive) for NF1-associated vs. sporadic MPNST (Breslow test, 4.58; $p = 0.0323$) and axially vs. peripherally tumor location (Breslow test, 4.96; $p = 0.0259$). *Broad lines* delineate NF1-associated MPNST and axially located MPNST, respectively

Analysis of all 52 patients revealed that those patients who developed local recurrence late in the course of the disease suffered from metastases later ($p < 0.001$), had a longer period of survival ($p < 0.001$) and died less frequently ($p < 0.05$). Development of metastases was associated with large primary tumors ($p < 0.05$) and patients with metastatic spread died more often due to the tumor ($p < 0.001$). Peripherally located tumors were found to recur later ($p < 0.05$) and were associated with longer survival periods (Breslow $p = 0.026$).

Cox regression analysis identified time to local recurrence ($p < 0.02$) and time to occurrence of metastases ($p < 0.02$) as the only independent prognostic variables.

Histopathology

Only the parameters pleomorphism and mitotic count were found to correlate with grading ($p < 0.05$ and $p < 0.02$, respectively) in the entire samples. High cellularity was associated with young age at diagnosis ($p < 0.01$), short time interval to metastases ($p < 0.02$) and short survival ($p < 0.02$) but no significant correlation was found with grading.

Very interestingly, separate analyses on NF1-associated and sporadic cases revealed that cellularity and mitotic scores correlated with grading only in sporadic cases. Furthermore, mitotic activity correlated with local recurrence of tumors and period to death in sporadic patients ($p < 0.05$ for both comparisons) but not in patients with NF1, though there were more NF1 than sporadic cases analyzed. As a matter of fact, none of the histological parameters in NF1 patients was found to correlate with FNCLCC grading or to have a prognostic significance for survival, interval to local recurrence, formation of metastases or metastatic spread.

Comparison of NF1 and sporadic MPNST demonstrated, that NF1-associated tumors had a significantly higher cell density ($p < 0.001$) whereas sporadic tumors were characterized by a marked pleomorphism as compared to NF1 tumors ($p < 0.01$).

Discussion

The most significant finding in our present study was that FNCLCC grading did not correlate with prognosis

or outcome in NF1-associated MPNST. In contrast, FNCLCC grading parameters were useful for the evaluation of sporadic MPNST. Previous evaluation of the prognostic power of grading in MPNST yielded contradictory results. A study comparing FNCLCC and NCI grading [19] found the FNCLCC system to be slightly better suited for predicting development of metastases and tumor mortality. However, only 29 MPNST were included in the 410 soft tissue sarcomas investigated in that study. In another series, of 32 NF1-associated and 103 sporadic MPNST grading emerged as prognostic factor only in univariate but not in multivariate analysis [20]. However, the grading scheme used in that study was not conform with either NCI, FNCLCC or WHO classifications. However, in a sample of 23 NF1-associated MPNST tumor grading according to FNCLCC was found to be of prognostic value ($p = 0.003$) upon multivariate analysis [21]. Thus, the best set of parameters for grading MPNST still needs to be determined.

In our series strongest correlation of individual parameters and FNCLCC grade was observed for mitotic activity ($p < 0.02$) and pleomorphism ($p < 0.05$). The extent of necrosis showed no significant correlation with FNCLCC grade ($p = 0.188$). Our results demonstrate that the biological behavior of NF1-associated MPNST is underestimated in the present grading systems. A new grading scheme for MPNST should take into account the genetic background of NF1 patients.

In addition, we found that cellularity and mitotic scores correlated with grading only in sporadic MPNST but not in NF1-associated ones. Interestingly, the mitotic score itself was higher in NF1-associated than in the sporadic MPNST. In addition, NF1-associated tumors were characterized by an increased cellularity ($p < 0.001$) whereas sporadic tumors showed a higher pleomorphism ($p < 0.01$). These observations may reflect biological differences between the two tumor groups.

Other findings in our study confirmed data from previous studies: NF1 patients develop MPNST at younger age than non-NF1 patients ($p < 0.001$); NF1 was associated with survival in univariate survival statistics ($p = 0.0231$) but was no independent prognostic factor in Cox regression analysis; male patients had a worse prognosis [22]: peripheral location of MPNST was associated with a significantly later development of local recurrences and with longer overall survival in NF1 patients ($p = 0.026$) [23]; size of the primary tumor correlated with development of metastases ($p < 0.05$) and metastatic tumor spread was associated with short survival ($p = 0.001$) [20]. We did not find significant correlation of tumor size and survival in Cox regression analysis.

Several factors may be responsible of the clinical and histopathological differences observed in NF1-associated and sporadic MPNST. Plexiform neurofibromas certainly play a key role for the understanding, since they often present early in childhood in NF1 patients and may reach large sizes. Frequently, the histology of MPNST is seen within a plexiform neurofibroma. The tumors are present for a long time and thus the chance for further genetic hits increases. Further, NF1-associated MPNST arise in an environment where all other cells also carry at least a *NF1* mutation in one allele and thus may react differently form normal cells to proliferative stimuli such as growth factors. This again, may particularly apply to plexiform neurofibromas in which tumor cells grow in a compartment enclosed by connective tissue (epineurium) whereas in dermal and diffuse tumors growth factors, cytokines, etc. may diffuse into the neighboring tissue more easily. In addition, the affected genes may be different in sporadic and NF1-associated MPNST. This is supported by the observation, that NF1-associated MPNST often arise from progression of low-grade nerve sheath tumors whereas sporadic MPNST arise ''de novo.''

In conclusion, the present grading systems are not suitable for predicting development and outcome of NF1-associated MPNST. We suggest setting up a multi center study to include large cohorts of patients with sporadic and NF1-associated MPNST. Parameters to be assessed may include detailed and consistent clinical data, whole body MRI for evaluation of tumor load, extent of tumor resections to estimate sampling error, consistent histopathological evaluation by a board of experts in this field and a detailed molecular analysis of the tumors.

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