Aus der Klinik für Dermatologie, Venerologie und
Allergologie des Helios Klinikums Hildesheim

„Reevaluation etablierter und neuerer Kriterien zur Differenzierung von Spitz Nävi und malignen Melanomen”

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Reevaluation of established and new criteria in differential diagnosis of Spitz nevus and Melanoma

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Abstract:

The histopathologic differentiation between Spitz nevus and melanoma is of particular interest in routine diagnostic procedures of melanocytic tumors. Atypical Spitz nevi are sometimes difficult to distinguish from melanoma. There is still no single criterion that ensures a distinction of melanoma and atypical Spitz nevus.

The aim of this study was to reevaluate established and new criteria to differentiate Spitz nevus from melanoma more reliably.

We analyzed 25 melanomas with a Breslow index ≥ 1 mm and 18 classical compound Spitz nevi concerning their histopathologic, immunohistochemical and molecular genetic characteristics. Moreover, clinical follow-up data for five years were collected.

We found statistically significant differences between Spitz nevus and melanoma for the following features: pagetoid spread, atypia, maturation, elastosis, Kamino bodies, p16 expression and the staining pattern of HMB45. BRAF was positive in 7/21 melanomas and in 1/14 Spitz nevi. Fluorescence in situ hybridization confirmed the histopathologic diagnosis in 36/37 cases.

The established clinical, histopathologic and immunohistochemical criteria to differentiate Spitz nevus and melanoma could be reproduced in our collective. Especially the expression of p16, BRAF analysis and fluorescence in situ hybridization proved to be helpful tools to improve the differentiation of Spitz nevus and melanoma in our study. Nevertheless, there is- until now- no reliable histopathologic and immunohistochemical parameter which can discriminate Spitz nevus and melanoma with absolute certainty.

1 Introduction

Histopathologic evaluation of melanocytic neoplasms often allows a certain distinction between benign and malignant tumors. Within the large group of melanocytic tumors, there are spitzoid melanocytic neoplasms composed of large epitheloid and/or spindle-shaped melanocytes whose analysis sometimes becomes very challenging for dermatopathologists due to conflicting morphological features [73]. The spectrum of spitzoid melanocytic neoplasms encompasses benign Spitz nevi, spitzoid melanomas and atypical Spitz tumors (ASTs) [26,44,72]. The latter show features of benign Spitz nevus and spitzoid melanoma simultaneously and their clinical behavior is not always reliably predictable, which leaves uncertainties for clinicians and patients [26,44,72,73].

The aim of many studies was to find criteria that enable the assignment of spitzoid lesions to the correct and distinct diagnosis and thus keep the uncertain group as small as possible [1,4,11,19,21,28]. Evaluation of genetic aberrations has proven to be valuable in the characterization of spitzoid melanocytic neoplasms [73]. In the last few years striking papers on this topic have been published. In the focus were TERT promotor mutations [34,73], BAP1 [52,73,76], NTRK1 [71,73], ROS1 [71,73], ALK [71,73], BRAF [12,17,23,25,29,36,52,54,57,59,67,73-75] and RET [71,73] mutations. Techniques such as fluorescence in situ hybridisation (FISH), comparative genomic hybridization (CGH) and next generation sequencing were used [52,71,76]. However, due to the high costs and the low availability in daily routine, these complex methods have been reserved for very challenging spitzoid lesions. Thus we focussed in our study on clinical features, histomorphologic criteria, immunohistochemical staining patterns and results of the multiprobe melanoma FISH assay, which is widely

distributed for commercial purposes [18,22,45,58]. To reevaluate these diagnostic criteria, tumors (18 Spitz nevi and 25 melanomas) with a clear diagnosis and long-term follow-up were considered.

2 Patients / Material and Methods

2.1 Patients' characteristics

We investigated 18 classical compound Spitz nevi (SN) and 25 melanomas (MM) with a tumor thickness ≥ 1 mm diagnosed in our hospital in 2004-2006. Only tumors with enough residual tissue and SN with a distinct dermal component were considered. Tumors with ulceration were excluded. The clinical data of the melanoma patients were collected by a retrieval of the German registry for skin cancer. Missing clinical information and particularly the clinical data of the SN patients were obtained through retrospective note review and communication with clinicians and patients. The data of the patients were stored in a database. We obtained a signed consensus form of all melanoma patients and the approval of the ethics committee (Medical University Hannover, Germany).

2.2 Tissue samples

All samples used in this study were formalin-fixed paraffin-embedded tissues retrieved from the archives of the Dermatopathology section of the Department of Dermatology, HELIOS-Klinikum Hildesheim.

2.3 Histopathologic features

The histopathologic features were evaluated by two independent investigators (A. R. and C. M.). We focussed on tumor silhouette (asymmetry/symmetry), lack of sharp circumscription (absent, present to one side, present to both sides), cytologic features (atypia defined as pleomorphism, hyperchromasia and prominent nucleoli of melanoma cells: absent/moderate/present; for Spitz nevi shape of the tumor cells: epitheloid/spindle), maturation respectively nuclei of melanocytes become smaller with progressive descent into the dermis (present/absent), pagetoid spread (present/focal/absent), infiltration depth (mean in mm), pigmentation (present/absent), Kamino bodies (present/absent) and solar elastosis (present/absent). In addition, the presence of tumor-infiltrating lymphocytes (TIL) and their distribution (focal, band-like, intratumoral lymphocytes) were evaluated (see figure 1). The evaluation of all parameters occurred double-blinded.

2.4 Mitotic rate

The mitotic activity was counted in H&E stained slides under light microscope (microscope: Leica DM 2000) according to the recommendation of the AJCC 2009 classification [3] and as described by Ottmann et al. [48]. We used the H&E slides from routine diagnostic, no serial sections were performed. Additionally, immunohistochemistry was performed using the Phospho-Histone H3 Antibody (pHH3) and it was evaluated in the same way, also stated in number per mm2. The Phospho-Histone H3 Antibody (pHH3) is described as a valuable diagnostic adjunct in the evalution of melanocytic tumors [24].

Furthermore, we examined all tumors for the presence of deep mitoses (mitoses located in the lower third of the tumor) and marginal mitoses (mitoses located close to the lateral borders of the tumor) by using the the Phospho-Histone H3 Antibody.

2.5 Immunohistochemistry

Immunohistochemical stainings were performed on paraffin-embedded sections using the commercially prepared antibodies listed in table 1. Preparation of the tissue sections and staining was performed according to the manufacturers' instructions using a fully automated staining machine (BenchMark® GX, Ventana, Mannheim). The chromogen used was Ultraview Universal Alkaline Phosphatase Red Detection Kit (Ventana Medical Systems, Tucson, AZ, USA). For BRAF, the OptiView DAB IHC Detection Kit (Ventana, Mannheim) was used.

After a training period, all slides were reviewed in a blind fashion by one person (A. R.) twice.

For Melan A, HMB45 and p16 the following parameters were evaluated:

(i) Staining distribution pattern

- p16 and Melan A: diffuse, checkerboard or single cells (see figure 2)
- HMB45: diffuse versus loss of HMB45 staining intensity towards deeper tumor parts (see figure 3)

(ii) H-Score: analysis of immunohistochemical staining was performed using a semi-quantitative method, which incorporates both the number of staining cells and the intracellular staining intensity [40]. The intensity of staining was characterized as no staining (0), weak but detectable (1), distinct (2) or very strong (3), see figure 4.

The H-Score was calculated by H= Σi pi (*I*i +1), where "pi" is the percentage of stained cells in each intensity category and "*I*i" the intensity score (0, 1, 2 or 3). The analysis was performed two times at intervals of four weeks by one investigator (A. R.). Both results were summed up and divided by two. If the H-Score of the first and the second counting differed more than 10%, a second investigator (C. M.) performed another counting and all results were averaged.

For BRAF-V600E, tumors were examined to either positive or negative staining. Because only few cells have been stained positive in Spitz nevi and melanomas for BCL-6, we abandoned the H-Score for evaluation of BCL-6. Instead, any nuclear staining was regarded as positive.

2.6 Fluorescence in situ Hybridisation (FISH)

FISH analysis was performed in all cases using the Vysis Melanoma FISH Probe Kit from Abbott Molecular Inc (Des Plaines, IL, USA), applied exactly according to the manufacturers' recommendations. Criteria for FISH positivity were those published by Gaiser et al. [18].

2.7 Statistical analysis

Data were analyzed with the software package IBM SPSS Statistics, Version 21. For continuous variables descriptive statistics like mean, standard deviation, median, minimum and maximum were calculated. For this type of data nonparametric Mann-Whitney Test and Kruskal-Wallis Test were applied for group comparisons. Analyses of categorical variables were performed by means of cross tabulation and Pearson Chi-Square Test or Fisher's Exact Test.

All tests were two-sided. In all cases a p-value less than 0.05 was considered as statistically significant.

3 Results

3.1 Clinical features

We received clinical features and follow-up data of all patients.

The mean follow-up time for patients with melanoma was 71.44 months (range 60-88, median 68), for patients with Spitz nevus 74.83 months (range 62-85, median 77).

In the Spitz nevus group 13 of 18 (72%) patients were female, in the melanoma group 10/25 (40%). The mean age at the time of diagnosis for Spitz nevus was 31 years (range 11-66 years, median 31), for melanoma 67 years (range 30-83 years, median 73).

The diameter of the lesion could be evaluated in 16 melanomas and 2 Spitz nevi. 14 melanomas (88%) showed a diameter of 10mm or larger, the other two melanomas had, as well as both Spitz nevi, a diameter between 5 and 9mm.

In 4 patients (16%) with melanoma, metastases or local recurrence could be detected. All 4 patients died because of their melanoma. Another patient died during follow-up due to other reasons. In none of the Spitz nevus patients recurrence of the disease was detected.

Table 2 shows the patients' characteristics in detail.

3.2 Histopathologic features and mitoses

Of the 25 melanomas, 12 (48%) were classified as superficial spreading melanoma, 6 (24%) as nodular melanoma, 4 (16%) as secondary nodular superficial spreading melanoma and 3 (12%) were not classified. The mean infiltration depth in the melanoma group was higher (2.03 mm; SD 1.78, median 1.5 mm, range 1.0-

10.0 mm) than in the group of Spitz nevi (0.94 mm; SD 0.53, median 0.83 mm, range 0.4-2.5 mm). 50% (9/18) of the Spitz nevi showed predominating epitheloid-shaped cells, in 11% (2/18) the cell morphology was spindle-shaped and in 39% (7/18) nearly equal distribution of epitheloid and spindle-shaped cells was present.

One melanoma reached into the subcutaneous fat (Clark Level V), all other melanomas and Spitz nevi showed Clark Level III or IV. Deep mitoses were present in 3/18 Spitz nevi and 16/25 melanomas. Marginal mitoses were observed in 2/18 Spitz nevi and in 14/25 melanomas. All tumors presenting marginal mitoses showed simultaneously deep mitoses.

The results of the evaluation of histopathologic features and the mitotic rates of both groups are detailed in table 3. The histopathologic features with significant differences between Spitz nevus and melanoma are presented in figure 5.

3.3 Immunohistochemistry

3.3.1 p16

(i) The H-Score for p16 was significantly (p=0.001) higher in Spitz nevi (mean ± SD; min-max: 258.21 ± 63.09; 104.5-340), than in melanomas (mean ± SD; min-max: 183.58 ± 85.29; 100-372.5).

(ii) We found no significant differences between the staining patterns as described in section 2.5.

3.3.2 Melan A

(i) No statistically significant difference was found for the Melan A H-Score (p=0.848).

(ii) All Spitz nevi and melanomas showed a diffuse staining pattern for Melan A.

3.3.3 HMB45

(i) The H-Score for HMB45 (mean ± SD; min-max: 222.68 ± 77.50; 102.5-360) was significantly (p=0.028) higher in melanomas than in Spitz nevi (mean ± SD; min-max: 172.34 ± 90.59; 100-347).

(ii) For HMB45, we focussed on the stratified HMB45 staining pattern with fewer positive cells or a negative reaction in the deeper portion. Spitz nevi showed more often a stratified staining pattern (47.06%) than melanomas (4.17%), which is statistically significant (p=0.001).

3.3.4 BRAF

16 Spitz nevi and 21 melanomas were analyzed for BRAF-V600E mutation by immunohistochemistry. Melanomas (7/21; 31%) were significantly ($p=0.047$) more often positive than Spitz nevi (1/16; 6.25%).

3.3.5 BCL-6

1000 cells were enumerated in a representative area and within these 1000 cells, we evaluated the positive stained cells. If fewer than 1000 cells were present, as many cells as possible were evaluated. Only nuclear staining was regarded as positive.
In 50% of the melanomas (12/24), a very few cells showed positivity for BCL-6 (0.5% at most). The other melanomas (12/24; 50%) were negative for BCL-6.
In Spitz nevi, 6/17 (35%) were negative for BCL-6, 11/17 (65%) showed at maximum 2% positive stained cells. Therefore no statistically significant difference between Spitz nevi and melanomas could be found.

3.4 FISH Analysis

22 of 25 melanomas and 15 of 18 Spitz nevi could be analyzed. All melanomas (22/22) revealed a FISH positive result whereas all Spitz nevi except one were FISH negative. We received therefore a statistically significant difference ($p<0.001$). All melanomas showed positivity in at least two criteria whereas the FISH positive Spitz nevus was positive in only one criterion (MYB amplification ≥ 2.5).
Table 4 gives an overview of the FISH results.

The FISH-positive Spitz nevus in our study showed the following criteria: 11-year-old female patient, localisation on the lower limbs, presence of Kamino bodies, good maturation, asymmetry, focal pagetoid spread, no pigmentation, no solar elastosis, epitheloid-shaped cells, intratumoral lymphocytes, no mitoses in HE and pHH3, a strong and diffuse staining for p16, only few cells positive for HMB45 and a long-time follow-up (76 months) without any sign of recurrence. BRAF was negative.

4 Discussion

Despite many new developments, spitzoid tumors often remain a diagnostic challenge for the (dermato-) pathologist. Although many spitzoid tumors can be diagnosed by histology and immunohistochemistry, few problematic cases are left, which need further, often complex and expensive diagnostics. It is of major interest to identify tumors with increased risk of recurrence, risk for metastasis and risk for death. Therefore, many criteria have been investigated in the past and research on this topic is still ongoing.

Clinical data can partially provide helpful information, but there is no criterion that is exclusively found in either Spitz nevus or melanoma.

Spitz nevi commonly arise in childhood [26], but few studies reported their occurrence also in adults [9,55], which is also shown in our study with a comparatively high mean age of 31 years. Melanomas in contrast are a rare event in children [50], anyhow it is important to select these seldom cases. Regarding the risk stratification, Spatz et al. [62], Barnhill et al. [5] and Paradela et al. [50] found a higher risk for fatal outcome of a certain spitzoid tumor in patients older than 10 years. In our study, all of the Spitz nevi patients were older than 10 years, but in none of the patients, recurrence of the disease could be detected.

Concerning the gender distribution, we found, like some other authors [6,9,10,15,20] a clear tendency for Spitz nevi in female patients, whereas other studies showed an almost equal distribution [19,55,68].

Spitz nevi are most often localized on the (lower) extremities [15,19,20,55,68], which could also be found in our collective. Even if melanomas most often (in approximately

35% of the cases) occur on the trunk [19,46,50], we found 28% melanomas localized on the lower limbs, which is in agreement with Paradela et al.[50] and Nagore et al [46]. Thus age, gender and localisation can give some hints, but are de facto no reliable criteria in the differentiation of Spitz nevus and melanoma.

It is well known that Spitz nevi are mostly symmetric and well described tumors, show maturation and present Kamino bodies. Pagetoid spread is quite unusual with the exception of Spitz Nevi in childhood where pagetoid spread is occasionally present [9]. Melanomas are more likely to be asymmetric and lack sharp lateral margins, exhibit pagetoid spreading and atypical melanocytes (see table 6). All of these criteria were reproduced in our study and they could significantly distinguish between Spitz nevus and melanoma. Nevertheless, in some of our studied Spitz nevi, atypical features like asymmetry, pagetoid spread, lack of maturation or atypia of melanocytes could be found, but only to a minor degree and thus not sufficient enough to classify them as “atypical Spitz nevi”. Except the presence of Kamino bodies, there is no criterion that could be exclusively found in either Spitz nevus or melanoma. Kamino bodies (coalescent eosinophilic globules composed of basement membrane components) are often described as helpful tool to differentiate between Spitz nevus and melanoma [31,35,51,69]. The authors in [6,55,68] found Kamino bodies in Spitz nevi in 11-34%, we could even find them in 44%. In [31,35], the authors mentioned that Kamino bodies were never or extremely rarely found in melanomas.

Our results support the opinion that the presence of Kamino bodies is one of the most reliable histopathologic criteria to classify a spitzoid lesion as Spitz nevus.

Another point of interest in our study was the evaluation of the mitotic activity and the observation of deep and marginal mitoses in Spitz nevi and melanomas. Cerroni et

al. [8] analysed a large number of “melanocytic tumors of uncertain malignant potential" (MELTUMP) and pointed out, that in the group of the MELTUMP with an unfavorable clinical behavior mitoses and also deeply located mitoses were statistically more often present than in the group of MELTUMP with a favorable behavior. In our study, we observed a significantly higher mitotic rate in melanomas than in Spitz nevi.

In addition, deep and marginal mitoses were significantly more often present in melanomas than in Spitz nevi. Interestingly, with regard to the clinical behavior, within the small group of the four metastasizing melanoma patients in our study, three of them did not present deep or marginal mitoses. In contrast, in 14 melanomas and two Spitz nevi with no evidence of metastases during follow-up time deep and marginal mitoses were found.

Spatz et al.[62] developed a grading system for risk stratification in atypical spitzoid tumors and found a higher risk for developing metastases in tumors with six or more mitoses.

Contrary to this study, we did not find a significant difference between Spitz nevi and melanomas when regarding a cutoff at six mitoses in HE staining. Interestingly, a cutoff at one mitoses in contrast could significantly distinguish between Spitz nevi and melanomas in our collective.

Immunohistochemical analysis of markers, such as HMB45 and p16 can be useful to distinguish between benign and malignant melanocytic tumors [1,19,21,60].

Spitz nevi often, but not exclusively show maturation with loss of HMB45 staining at the base of the lesion [26,39,53]. In contrast, melanomas usually retain HMB45 staining throughout the lesion [26,39,53]. In our study, we could reproduce these patterns allowing a reliable distinction between Spitz nevi and melanomas in many

cases. We additionally scored HMB45 with the H-Score and pointed out a significant higher value for melanomas compared to Spitz nevi. Despite this, evaluating the H-Score is time-consuming and thus no realistic option for daily routine.

Several studies have been focusing on p16, a cyclin-dependent kinase inhibitor, in the distinction of Spitz nevus and melanoma [1,21,32]. The protein p16 is encoded by the CDKN2A locus, which maps to chromosome 9p21 [7]. Mutations in the CDKN2A locus can account for tumorigenesis and are often found in familiar melanoma [7]. Former studies found a loss of p16 immunostaining in 22-100% of their studied melanomas [1,21,28,32,42,61] whereas Spitz nevi, as well as other benign nevi, commonly retain expression of p16 [1,21,32]. In our melanoma group, only 1 of 25 melanomas showed a complete loss of p16 immunostaining, which was not found in the Spitz nevi group. An obvious partial lost was observed in 22 melanomas, whereas this was only seen in two of the 17 studied Spitz nevi. All other Spitz nevi showed a diffuse and intense p16 staining, which is reflected in the high p16 H-Score. Our results suggest p16 to be valuable in the evaluation of spitzoid neoplasms.

However, in the literature, the usefulness of p16 in the evaluation of melanocytic tumors is regarded as controversial [73]. Mason et al. [38] compared 18 Spitz nevi and 19 spitzoid melanomas and found no significant difference in p16 staining. Uguen et al.[66] proposed a valuable triple p16-HMB45-Ki67 scoring system, because none of the single parameters was able to discriminate all of their studied melanomas from all of the studied nevi. Other authors [1,21,32] in contrast could identify obvious differences when comparing Spitz nevi and melanomas.

Regarding the risk for metastasis and death, the four melanoma patients in our study, who died because of their metastasizing melanoma, showed a low to

intermediate p16 H-Score, but there was no obvious difference in p16 staining compared to the other melanoma patients, who did not develop metastases.

In addition, we evaluated the staining distribution pattern for p16 in Spitz nevi and melanomas, but neither for Spitz nevus nor for melanoma we could identify a certain staining pattern. In contrast, Al Dhaybi et al. [1] found a checkerboard pattern for p16 in 5 of 18 studied Spitz nevi whereas their studied melanomas were all negative for p16. Therefore no staining distribution pattern could be identified.

Alonso et al. [2] revealed, that BCL-6 positivity is a prognostic indicator in melanomas. Thus, we expected that BCL-6 expression would be a promising criterion for differentiating Spitz nevi and melanomas. However, we found no differences in nuclear expression of BCL-6 when comparing Spitz nevi and melanomas. Yet it has to be taken into account that the number of the tumor samples in our study was quite small compared to Alonso et al., who examined 165 melanomas and this is a major limitation of our study.

Fluorescence in situ hybridisation can be another helpful tool in equivocal melanocytic tumors. Yet in spitzoid neoplasms, the usefulness of the standard melanoma FISH test targeting RREB (6p25), MYB (6q23), CCND1(11q13) and centromere 6, is regarded as controversial due to low sensitivity and specifity [18,47] and occurrence of false positive results due to tetraploidy in Spitz nevi [30]. Requena et al. investigated five Spitz nevi and eight spitzoid melanomas and did not find any FISH positive Spitz nevus in their study [56]. Martin et al. [37] in contrast identified in 19 of 51 (37%) Spitz nevi chromosomal changes leading to a “FISH-positive” result. Recently, Ferrara and De Vanna [16] proposed to implement a new FISH algorithm (standard 4-probe test followed by either C-MYC or

CDKN2A/centromere 9) to improve the sensitivity and specificity in spitzoid neoplasms.

In our study, we regarded tumors with a clear diagnosis (Spitz nevi versus melanoma). For this purpose, the sensitivity for detecting melanomas in FISH was convincing, all melanomas were FISH positive, as in the studies of DeMarchis et al. [13] and Martin et al. [37]. All Spitz nevi, except of one, were negative. This case showed several „benign“ criteria such as young age of the patient at the time of diagnosis, presence of Kamino bodies, good maturation, a high p16 H-Score and a long-time follow-up (76 months) without any sign of recurrence. This latter shows, that any FISH positive result has to be placed in the proper clinicopathological context.

Another point of interest was the evaluation of BRAF mutation. Mutational analyses in melanoma have tremendously increased during the recent years. BRAF, a member of the rat sarcoma (RAS) mitogen-activated protein kinase (MAPK) pathway, regulates cell growth and proliferation. Mutations of BRAF have been reported in 29-75% of melanomas [33,36,41,57,59,65,75] and also in more than 80% of benign melanocytic nevi [17,54,57]. In contrast, BRAF mutation in Spitz nevi is a rare event [14,17,23,29,43,49,52,64,75], see also table 7.

The BRAF-positive Spitz nevus in our study showed no melanoma typical features except asymmetric architecture and missing Kamino bodies. FISH was negative and p16 H-Score was high in this case. Although a few Spitz nevi harbour a BRAF mutation, it is, in consideration of our study as well as other studies a useful additional tool in the differentiation of Spitz nevi and melanomas.

In conclusion, until now, there are no reliable histopathologic and immunohistochemical parameters for discriminating Spitz nevi and melanomas, and when atypical Spitz tumors are considered, this becomes remarkably difficult without the aide of clinical evolution of disease and application of molecular genetic techniques in specialised laboratories.

We recommend a stepwise approach to examine spitzoid tumors. First, the tumors should be checked for maturation, atypia and presence of Kamino bodies.

In benign lesions, a prominent pagetoid spread is quite unusual and the circumscription is often sharp.

If further information is needed, immunohistochemical stainings should be added. A high p16 level, negativity for BRAF and a low HMB45 expression argues for a Spitz nevus. If the diagnosis is still unclear, a FISH-analysis should be performed. If possible, a probe for either C-MYC or CDKN2A/centromere 9 should be included.

Nevertheless, in some cases the diagnosis cannot be made, even if all the mentioned criteria are considered. In this situation, additional diagnostics such as comparative genomic hybridisation or application of TERT-p mutational assays may represent further helpful components. Anyhow, some spitzoid tumors may still remain undiagnosable and their clinical behavior cannot be predicted with absolute certainty.

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Tables

Figures

Table 1

Protein	Clone	Company	Dilution
HMB45	HMB45	DAKO, Germany	1:100
Melan A	A103	DAKO, Germany	1:100
p16	E6H4	MTM Laboratories AG, Germany	Company-prepared
BCL-6	GI191E/A8	Zytomed Systems, Germany	1:400
PhosphoSer10	N/A	Zytomed Systems,Germany	1:400
BRAF V600E	VE1	DCS Innovative Diagnostik-Systeme, Germany	1:200

Table 2

	SN (n=18)	Melanoma (n=25)	Statistical significance
Age (in years) at diagnosis			**p<0.001**
Mean	31.05	67.28	
Range	11-66	30-83	
Sex			p=0.255
Male	5/18 (28%)	15/25 (60%)	
Female	13/18 (72%)	10/25 (40%)	
Localisation			p=0.283
Head/neck	0/18 (0%)	3/25 (12%)	
Trunk	6/18 (33.33%)	9/25 (36%)	
Upper extremities	3/18 (16.67%)	6/25 (24%)	
Lower extremities	9/18 (50%)	7/25 (28%)	
Follow-up months			p=0.146
Mean	74.83	71.44	
Range	62-85	60-88	
Follow up			
NOD	18/18 (100%)	20/25 (80%)	p=0.619
DOD	0/18 (0%)	4/25 (16%)	p=0.099
SLNB			
Performed	0/18 (0%)	20/25 (80%) 18: negative 2: Micrometastasis	
Not performed	18/18 (100%)	5/25 (20%)	

DOD, Dead of disease; *NED,* no evidence of disease; *SLNB,* sentinel lymph node biopsy.

Table 3

	SN (n=18)	MM (n=25)	Analysis (univariate)
Infiltration depth			
Mean (in mm)	0.94	2.03	**p<0.001**
Pagetoid spread			**p<0.001**
Absent	12/18 (67%)	1/25 (4%)	
Focal	5/18 (28%)	8/25 (32%)	
Present	1/18 (5%)	16/25 (64%)	
Atypia			**p<0.001**
Absent	9/18 (50%)	0/25	
Moderate	8/18 (44%)	0/25	
Significant	1/18 (6%)	25/25 (100%)	
Cell morphology (only SN)			
Epitheloid	9/18 (50%)	-	
Spindle	2/18 (11%)	-	
Both	8/18 (39%)	-	
Maturation			
Present	15/18 (83%)	0/25	**p<0.001**
Kamino Bodies			
Present	8/18 (44%)	0/25	**p<0.001**
Solar elastosis			
Present	0	10/25 (40%)	**p<0.001**
Mitotic rate			
Mean, range (HE)	0.33 (0-2)	2.54 (0-16)	**p<0.001**
Mean, range (pHH3)	0.52 (0-7)	7.48 (0-55)	**p<0.001**
>1 mitosis in HE	5/18 (27.8%)	21/25 (84%)	**p<0.001**
>1 mitosis in pHH3	6/18 (33.3%)	21/25 (84%)	**p<0.001**
>6 mitoses in HE	0/18 (0%)	2/25 (8%)	p=0.219
>6 mitoses in pHH3	1/18 (6.3%)	9/25 (36%)	**p=0.020**
Deep mitoses	3/18 (16.7%)	16/25 (64%)	p=0.163
Marginal mitoses	2/18 (11.1%)	14/25 (56%)	**p=0.021**
Asymmetry			
Present	6/18 (33%)	19/25 (76%)	**p=0.005**
Inflammatory infiltrate			
Present	14 (78%)	25/25 (100%)	**p=0.013**
Localisation of the tumor-infiltrating lymphocytes (TIL) (multiple answers are permitted)			
Focal	2/18 (14%)	12/25 (48%)	**p=0.011**
Band-like	8/18 (57%)	8/25 (32%)	p=0.405
Intratumoral lymphocytes	10/18 (71%)	14/25 (64%)	p=0.977
Circumscription	n=13	n=22	**p=0.021**
Absent	0/13	7/22 (32%)	
Present to one side	4/13 (31%)	9/22 (41%)	
Present to both sides	9/13 (69%)	6/22 (27%)	
Pigmentation			
Present	14/18 (78%)	23/25 (92%)	p=0.184
H-Score			
p16 (mean)	258.21	183.58	**p=0.001**
HMB45 (mean)	172.34	222.68	**p=0.028**
Melan A (mean)	288.26	298.47	p=0.848
BRAF	n=16	n=21	
Positive staining	1/16 (6.3%)	7/21 (33%)	**p=0.047**
BCL-6	n=17	n=24	
Positive staining	11/17 (64.7%)	12/24 (50%)	p=0.350
FISH	n=15	n=22	
Positive	1/15 (6.7%)	22/22 (100%)	**p<0.001**

Table 4

	MM (n=22)	SN (n=15)
FISH result positive	22 (100%)	1 (7%)
% loss of MYB ≥31	19 (86%)	0 (0%)
MYB ≥2.5 amplification (average)	6 (27%)	1 (7%)
RREB1 % nuclei with signal ≠2 ≥ 63%	18 (81%)	0 (0%)
CCND1 ≥2.5 amplification (average)	21 (95%)	0 (0%)

MYB (located at 6q23), *RREB1* (located at 6p25), *CCND1* (located at 11q13)

Table 5

	Subjects (sorted by the number of positive criteria, SN 1-18 and MM 19-43)																																										
Criteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
FISH result	□	□	x	□	x	■	□	x	□	□	□	□	□	□	□	□	□	□	■	■	■	■	■	■	■	■	■	■	■	■	■	■	x	■	■	■	■	■	■	x	■	■	x
No maturation	□	□	□	□	□	□	■	■	□	□	■	□	□	□	□	□	□	□	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Atypia	□	□	▒	□	▒	▒	□	▒	▒	□	□	□	■	▒	□	□	▒	□	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
>1 mitosis in HE	■	■	■	■	□	□	□	□	□	□	□	□	■	□	□	□	□	□	■	■	■	■	■	■	■	■	■	■	■	■	■	■	□	■	□	■	■	■	■	□	■	■	□
>6 mitoses in HE	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■
Marginal mitoses	□	□	■	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	■	■	■	■	□	□	■	□	□	■	■	□	■	□	□	■	■	■	□	□	□	■	□	■	■
BRAF positive	□	x	□	x	□	□	□	x	□	□	□	■	□	□	x	□	□	□	■	□	□	■	■	□	□	x	□	■	x	■	■	□	x	□	■	□	□	□	□	□	x	□	x
Solar elastosis	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	■	■	□	□	□	■	□	■	□	□	□	□	■	■	■	□	□	□	□	□	■	□	□	■
Asymmetry	■	□	□	■	■	■	□	□	□	■	□	■	□	□	□	□	□	□	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	□	■	■	■	■	■	■	□	□	□
Pagetoid spread	□	■	▒	□	▒	▒	□	□	▒	□	□	□	□	□	▒	□	□	□	■	■	■	■	▒	■	■	■	▒	▒	■	■	■	■	■	▒	■	■	▒	▒	■	■	▒	▒	□
No Kamino Bodies	■	■	□	■	□	□	■	□	□	■	■	■	□	□	■	■	□	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Inflammatory infiltrate	■	■	■	■	■	■	■	■	■	■	■	□	■	■	□	■	□	□	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

■ criterion found
□ criterion not found
▒ moderate atypia / focal pagetoid spread
x criterion not evaluable

Table 6

	Our study		Crotty et al. [10]		Peters et al. [51]		Walsh et al. [69]		Haupt et al. [27]		Weedon and Little [70]		Requena et al. [55]		Verardino et al. [68]		Spatz et al. [63]		Berlingeri-Ramos et al. [6]		Paradela et al. [50]	
	MM	SN	MM	SN	MM	SN	MM	SN	MM	SN	MM	SN	MM	SN	MM	SN	MM	SN	MM	SN	MM	SN
Maturation	**0%** (0/25)	**83%** (15/18)	**9%** (1/11)	**27%** (4/11)	MM= SN	SN= MM	**20%** (2/10)	**50%** (6/12)	-	-	-	**25%** (53/211)	-	**72%** (251/349)	-	**81%** (13/20)	**2%** (1/60)	-	-	**91%** (118/130)	-	-
Kamino Bodies	**0%** (0/25)	**44%** (8/18)	-	-	**26%** (5/19)	**52%** (17/33)	**0%** (0/10)	**67%** (8/12)	-	-	-	-	-	**34%** (117/349)	-	**25%** (8/32)	-	-	-	**11%** (14/130)	-	-
Pagetoid spread	**focal 32%** (8/25) **present 64%** (16/25)	**focal 28%** (5/18) present 5% (1/18)	-	-	**58%** (11/19)	**33%** (11/33)	**27%** (3/11)	**17%** (2/12)	**96%**	**38%**	-	-	-	**focal13%** (47/349)	-	**68%** (21/31)	-	-		-	-	-
Circum-Scription	**one side 41%** (9/22), **both sides 27%** (6/22)	**one side 31%** (4/13), **both sides 69%** (9/13)	-	-	**47%** (9/19)	**78%** (26/33)	**73%** (8/11)	**83%** (10/12)	-	-	-	-	-	-	-	**81%** (26/32)	**28%** (17/60)	-	-	**91%** (118/130)	-	-
Asymmetry	**76%** (19/25)	**33%** (6/18)	**50%** (4/8)	**11%** (1/9)	MM= SN	SN= MM	**73%** (8/11)	**8%** (1/11)	-	-	-	-			-	**22%** (7/32)	**62%** (37/60)	-	-	**16%** (21/130)	-	-
Mitoses detectable	**84%** (21/25)	**28%** (5/18)	**91%** (10/11)	**27%** (3/11)	**95%** (18/19)	**58%** (19/33)	**90%** (9/10)	**42%** (5/12)	-	-	-	**58%** (122/211)	-	**23%** (79/349)	-	**34%** (11/32)	-	-	-	-	**91%** (32/35)	-
Inflam-matory infiltrate	**100%** (25/25)	**78%** (14/18)	-	-	MM= SN	SN= MM	**82%** (9/11)	**75%** (9/12)	-	-	-	**69%** (146/211)	-	**70%** (243/349)	-	**75%** (24/32)	**49%** (29/60)	-	-	-	**100 %** (33/33)	-

Table 7

References	BRAF positive in SN
Our study	6.3% (1/16)
Saldanha et al. [57]	0% (0/26)
Mihic-Probst et al. [43]	0% (0/20)
Yazdi et al. [75]	0% (0/69)
Palmedo et al. [49]	0% (0/21)
Takata et al. [64]	0% (0/12)
Indsto et al. [29]	4,5% (1/22)
Piris et al. [52]	6% (1/17)
Emley et al. [14]	16.6% (1/6)
Fullen et al. [17]	21% (10/48)

Zusammenfassung

Einleitung

Seit der Erstbeschreibung des Spitz Nävus als sogenanntes “juveniles malignes Melanom” durch die amerikanische Pathologin Sophie Spitz im Jahr 1948 [46] haben sich zahlreiche Studien mit der Evaluation diagnostischer Kriterien zur korrekten Einordnung dieser Entität befasst. Die in der Erstbeschreibung gewählte Bezeichnung weist auf die histomorphologische Nähe des Spitz Nävus zum Melanom hin. Melanozytäre Tumoren, die mikroskopisch Veränderungen gleich oder ähnlich denen eines Spitz Nävus zeigen (Spitz Nävi oder Spitz Nävus-ähnliche Melanome), werden als spitzoide Tumoren zusammengefasst.

Dabei wurde eine Vielzahl an histologischen Kriterien, immunhistochemischen Markern und molekularbiologischen Techniken beschrieben, die in zahlreichen Fällen eine Diagnosestellung ermöglichen, jedoch fehlen bis heute zuverlässige Kriterien, die auch in schwierigen Fällen die Einordnung der Dignität eines spitzoiden Tumors ermöglichen.

Neben dem klassischen benignen Spitz Nävus und dem malignen Melanom gibt es eine Vielzahl an atypischen spitzoiden melanozytären Tumoren (AST), welche sowohl eine Reihe an Benignitätskriterien aufweisen, als auch Merkmale eines malignen Tumors zeigen und somit den Dermatohistopathologen nicht selten vor eine schwere Aufgabe stellen.

Für den Patienten und dessen korrekter Behandlung ist es jedoch von großer Bedeutung, die Dignität des spitzoiden melanozytären Tumors korrekt einzuordnen.

Wir haben in unserer Studie 25 maligne Melanome mit einer Tumordicke nach Breslow ≥ 1 mm und 18 klassische Compound Spitz Nävi auf bereits etablierte und

neuere histologische, immunhistochemische und molekulargenetische Kriterien untersucht und deren Wertigkeit analysiert. Histologisch untersuchten wir die Tumoren auf das Vorhandensein von Tumorzellausreifung (Maturation), Kamino Bodies (eosinophile Globuli), Zellatypien, solarer Elastose, Pigmentierung und auf eine pagetoide Ausbreitung von Tumorzellen in der Epidermis. Betrachtet wurde zudem die Begrenzung des Tumors, die Gesamtarchitektur sowie die Infiltrationstiefe. Überdies analysierten wir die Mitoserate aller Tumoren, zum einen in den bereits vorhandenen HE-gefärbten Präparaten, zum anderen mit dem immunhistochemischen Mitosemarker pHH3.

Immunhistochemisch untersuchten wir die Tumoren auf die Expressionsintensität und das Expressionsmuster für die Marker p16, HMB45, Melan A und BCL-6.

Da in den letzten Jahren die Durchführung von Mutationsanalysen in der Melanomdiagnostik enorm an Bedeutung zugenommen hat, prüften wir die Tumoren zudem auf das Vorliegen einer BRAF-Mutation.

Ergänzend hierzu wurden klinische Daten der 43 Patienten über einen Zeitraum von mindestens fünf Jahren retrospektiv erhoben.

Diskussion

Die Diagnostik von spitzoiden melanozytären Tumoren gehört zu einer der anspruchvollsten Aufgaben eines Dermatohistopathologen. Viele Kriterien wurden in der Vergangenheit etabliert, jedoch gibt es bis dato kein einziges Kriterium, welches eine eindeutige Zuordnung eines spitzoiden Tumors zu der Entität Spitz Nävus oder Melanom zulässt. Von großem Interesse ist hierbei die Untersuchung von Kriterien, welche zu einer Aufdeckung von Tumoren mit erhöhtem Risiko für die Ausbildung von Rezidiven, Metastasen und Todesfällen führen.

Klinische Daten wie das Patientenalter, das Geschlecht des Patienten oder die Lokalisation des Tumors können in der Diagnostik spitzoider Tumoren erste Hinweise geben, reichen aber zu einer exakten Diagnosestellung nicht aus.

Während der Spitz Nävus vor allem im Kindes- und jungen Erwachsenenalter auftritt [3,19,21], manifestiert sich das Melanom vermehrt im Erwachsenenalter [4,21]. Jedoch gibt es Ausnahmen und auch in unserer Studie zeigte sich für den Spitz Nävus ein relativ hohes Durchschnittsalter mit 31 Jahren.

Bezüglich der Geschlechterverteilung bei Spitz Nävi zeigten einige der Studien, dass Spitz Nävi wie auch in unserer Untersuchung vermehrt bei Frauen auftreten [3,7,12,16]. Daneben gibt es jedoch auch einige Autoren, die eine gleichmäßige Geschlechterverteilung der Spitz Nävi finden konnten [15,40,52].

Spitz Nävi sind am häufigsten im Bereich der unteren Extremitäten lokalisiert [12,16,40,52], dies konnten wir auch in unserem Kollektiv zeigen. Bezüglich der Lokalisation im Kopf-Hals-Bereich zeigt sich jedoch ein Unterschied zwischen unserer Studie und den Angaben in der Literatur. In unserem Kollektiv ist kein Spitz Nävus in diesem Bereich lokalisiert, in anderen Studien finden sich bis zu 22 % der Spitz Nävi im Kopf-Hals-Bereich [3,12,15,17,40]. Spitz Nävi im Kopf-Hals-Bereich

finden sich jedoch vor allem bei sehr jungen Patienten [35]. Das verhältnismäßig hohe Durchschnittsalter (31 Jahre) der Patienten mit Spitz Nävus in unserem Kollektiv mag ein Grund dafür sein, dass in unserer Studie kein Spitz Nävus in diesem Bereich lokalisiert war.

Histologisch wird der typische Spitz Nävus in der Literatur als symmetrisch aufgebauter, scharf begrenzter Tumor mit epitheloidzelliger oder spindelzelliger Zytomorphologie [3,40,52] beschrieben. Typisch für den Spitz Nävus ist das Auftreten von sog. Kamino-Bodies (PAS-positive homogene globuläre Ablagerungen) sowie eine Ausreifung der Melanozyten zur Tiefe [40,52]. Eine pagetoide Ausbreitung von Melanozyten in die Epidermis findet man bei dem Spitz Nävus gelegentlich bei jüngeren Patienten [7], für den Spitz Nävus im Erwachsenenalter ist dies eher ungewöhnlich [7,11].

Bei Melanomen hingegen zeigt sich häufig eine asymmetrische Gesamtarchitektur mit einer unscharfen Begrenzung der lateralen intraepidermalen melanozytären Komponente [42,53]. Es imponieren in Nestern und einzeln gelegene atypische Melanozyten mit pleomorphen Kernen. Weiterhin fällt eine transepidermale Migration sowie eine fehlende Ausreifung der Melanozyten in der tieferen Dermis auf [8,20,37,53]. Daneben lassen sich Mitosen, insbesondere an der Tumorbasis, beobachten [8,37,53].

Statistisch signifikante Unterschiede bezüglich der histologischen Kriterien ergaben sich in unserer Studie für die pagetoide Ausbreitung, Atypie, Maturation, solare Elastose und Kamino Bodies. Bis auf das Auftreten von Kamino Bodies konnten wir in unserer Studie jedoch kein histologisches Kriterium finden, welches ausschließlich im Spitz Nävus oder im Melanom zu finden war. In unserer Studie zeigten 44 % der

Spitz Nävi Kamino Bodies, während in anderen Studien [3,40,52] zwischen 11 und 34 % der untersuchten Spitz Nävi Kamino Bodies aufwiesen.

In Zusammenschau mit anderen Studien [24,28,37,53] gehört das Auftreten von Kamino Bodies zu den sichersten histologischen Unterscheidungsmerkmalen zwischen Spitz Nävus und Melanom.

Wir untersuchten in unserer Studie die Spitz Nävi und Melanome auf das Vorhandensein von Mitosen. Hierbei wurde neben der Mitoserate auch das Vorhandensein von basisnahen und randständigen Mitosen evaluiert. In einer Studie von Cerroni et al. [6], in welcher eine große Anzahl an “Melanocytic tumors of uncertain malignant potential" (MELTUMP) untersucht wurde, konnte gezeigt werden, dass jene Tumoren mit einer ungünstigen Prognose statistisch häufiger Mitosen sowie tiefliegende Mitosen aufwiesen als jene Tumoren mit einer für den Patienten günstigen Prognose. In unserer Studie zeigte sich eine signifikant höhere Mitoserate bei den Melanomen im Vergleich zu den Spitz Nävi und auch tiefliegende Mitosen waren deutlich häufiger in Melanomen vorzufinden. Interessanterweise zeigte sich aber - in Hinblick auf die Prognose - in der kleinen Gruppe der vier metastasierenden Melanome in unserer Studie, dass drei der vier Melanome keine tiefliegenden Mitosen aufwiesen. Dahingegen zeigten sich tiefliegende Mitosen in 14 Melanomen und in zwei Spitz Nävi mit einer gänzlich unauffälligen Nachsorge.

Die Diagnostik spitzoider Tumoren muss sehr häufig durch die Hinzunahme der Immunhistochemie ergänzt werden.

In unserer Studie wie auch in anderen Studien [1,15,17,45] haben sich hierfür die immunhistochemischen Marker HMB45 und p16 als hilfreich erwiesen.

Der monoklonale Antikörper HMB45 zeigt in benignen Nävi, wie auch dem Spitz Nävus meist eine kräftige Reaktion in der junktionalen Komponente bei Negativität

der dermalen Komponenten [19,32,39]. Melanome hingegen weisen meist eine kräftige Anfärbung auch der dermalen Komponenten auf [19,32,39]. Dies zeigte sich auch in unserem Kollektiv mit einem signifikanten Unterschied zwischen den Spitz Nävi und Melanomen.

Die unterschiedliche Expression des Proteins p16 in Melanomen und Spitz Nävi wurde vielfach untersucht [1,17,22,25]. p16 wird vor allem in gutartigen Nävi und in in-situ Melanomen exprimiert, jedoch auch mit meist geringerer Frequenz und Intensität in invasiven Melanomen [1,17,25]. Der Verlust von p16 wurde insbesondere in Melanomen mit negativen Prognosefaktoren wie hoher Tumordicke, Ulzeration, Gefäßeinbrüchen, erhöhter Mitoserate, Lymphknotenmetastasen und fortgeschrittenem Tumorstadium beschrieben [2,22,25,36,47]. Wir werteten p16 mittels H-Score aus, welcher die Intensität und die Anzahl der gefärbten Zellen berücksichtigt. Der H-Score wurde erstmals von McCarty beschrieben [33]. Es zeigte sich in unserem Kollektiv für p16 ein signifikanter Unterschied zwischen den Spitz Nävi und Melanomen; die Spitz Nävi wiesen einen deutlich höheren H-Score als die Melanome auf. Ein charakteristisches Färbemuster für Spitz Nävi, wie von Al Dhaybi et al. [1] beschrieben, zeigte sich in unserem Kollektiv nicht. In der Literatur wird die Nützlichkeit des Markers p16 in der Differenzierung zwischen Spitz Nävi und Melanomen kontrovers diskutiert [55].

Während einige Studien [1,17,25] deutliche Unterschiede in der p16 Färbung zwischen Spitz Nävi und Melanomen detektieren konnten, zeigten andere Autoren [31,50], dass p16 in der Evaluation spitzoider Tumore nicht wesentlich weiterführend ist.

Zu den in Melanomen bisher kaum erforschten immunhistochemischen Markern gehört der BCL-6 Antikörper. Dieser wird regelmäßig in der Diagnostik von

Lymphomen eingesetzt [5,10]. Alonso et al. konnten 2004 in ihrer Studie zeigen, dass die Wahrscheinlichkeit, an einem Melanom zu versterben, bei einem Verlust von p16 oder einem positiven Nachweis von BCL-6 um etwa das Achtfache erhöht war [2]. Es existiert bislang keine Studie, in der BCL-6 zur Differenzierung zwischen Melanomen und Spitz Nävi untersucht wurde. Es zeigten sich in unserem Kollektiv insgesamt sehr wenig positiv gefärbte Zellen sowohl bei den Spitz Nävi als auch bei den Melanomen. Statistisch ergab sich kein Unterschied in der Expression von BCL-6 zwischen Spitz Nävi und Melanomen, so dass aus unserer Sicht BCL-6 in der Diagnostik spitzoider Tumoren kein zuverlässiges Kriterium darstellt.

Weiterhin hilfreich in der Diagnostik spitzoider Tumoren und bereits in vielen Kliniken routinemäßig durchgeführt ist die Fluoreszenz in situ Hybridisierung (FISH). In unserer Studie erreichten wir eine hohe Sensitivität und Spezifität der untersuchten Tumoren. Alle Melanome waren FISH-positiv, die Spitz Nävi waren mit einer Ausnahme FISH-negativ. Andere Studien zeigen einen variablen Anteil an FISH-positiven Spitz Nävi, von 0 % [41] bis 37 % [30] der untersuchten Spitz Nävi reichend, so dass jüngst Ferrara und De Vanna [13] einen neuen FISH Algorithmus in der Diagnostik spitzoider Tumoren vorgeschlagen haben, um die Sensitivität und Spezifität zu erhöhen. Hierzu sollte der bereits erhältliche Vier-Sonden-Test um eine Sonde für C-MYC oder CDKN2A/Centromer 9 ergänzt werden.

Mutationsanalysen haben in den letzten Jahren in der Melanomdiagnostik enorm an Bedeutung zugenommen. Im Fokus der in den letzten Jahren veröffentlichten Arbeiten standen TERT [27], BAP1 [38,58], NTRK1 [54], ROS1 [54], ALK [54], BRAF [9,14,18,23,38,43,51,56] und RET [54] Mutationen.

In der täglichen Routine wird in der Diagnostik des fortgeschrittenen Melanoms die BRAF Mutationsanalyse regelmäßig durchgeführt. Daher untersuchten wir 21

Melanome und 16 Spitz Nävi immunhistochemisch auf das Vorhandensein einer BRAF Mutation. Es zeigte sich ein signifikanter Unterschied zwischen den beiden Entitäten. Während nur ein Spitz Nävus BRAF positiv war (6,25%), zeigten sich bei den Melanomen sieben Tumoren (33%) BRAF positiv.

Auch andere Studien [23,38,43,48,57] untermauern unser Resultat, dass eine BRAF Mutation in Spitz Nävi selten auftritt, wohingegen bei den Melanomen zwischen 29-75% der Tumoren eine BRAF Mutation aufweisen [26,29,34,43,44,49,57]. Daher ist die BRAF Mutationsanalyse zur Differenzierung zwischen Spitz Nävus und Melanom ein weiteres hilfreiches Kriterium.

Schlussfolgernd aus unseren Ergebnissen empfehlen wir für die Diagnostik spitzoider melanozytärer Tumoren ein schrittweises Vorgehen.

Zunächst sollte der zu untersuchende Tumor auf die histologischen Kriterien untersucht werden. Hierbei sollte das Augenmerk insbesondere auf Kamino Bodies, Maturation, Atypie der Tumorzellen und pagetoides Aufsteigen von Melanozyten in die Epidermis gelegt werden.

Bei unklaren Befunden in der Histologie sollte im zweiten Schritt die Immunhistochemie ergänzt werden. Hier haben sich p16 und HMB45 als hilfreich erwiesen. Ein hoher p16 H-Score und eine geringe HMB45 Expression sprechen eher für den Spitz Nävus. Auch eine BRAF Mutation ist in schwer zu diagnostizierbaren Tumoren zu empfehlen; der Nachweis einer BRAF Mutation spricht eher für das Melanom.

Für schwierig einzuordnende Tumoren ist weiterhin die Fluoreszenz in situ Hybridierung eine ergänzende diagnostische Methode. Wenn möglich, sollte die Untersuchung eine zusätzliche Sonde für C-MYC oder CDKN2A/Centromer 9 beinhalten.

Dennoch wird es auch weiterhin einzelne spitzoide melanozytäre Tumoren geben, deren exakte Diagnosestellung äußerst schwierig bleibt. Die Methode der Microarray-basierten komparativen genomischen Hybridisierung oder auch die Anwendung von TERT-p Mutationsanalysen könnte in Einzelfällen hilfreich sein, wenngleich es spitzoide Tumoren gibt, deren Dignität vermutlich nie mit ausreichender Sicherheit bestimmt werden kann.

Kurzzusammenfassung

Wir haben in unserer Studie 25 Melanome mit einer Tumordicke ≥ 1 mm und 18 klassische Compound Spitz Nävi auf histologische, immunhistochemische und molekulargenetische Kriterien untersucht, die zu einer Differenzierung zwischen den Entitäten beitragen. Als hilfreich haben sich in unserer Studie histologische Kriterien wie pagetoide Ausbreitung, Atypie, Maturation, solare Elastose und Kamino Bodies erwiesen.

Immunhistochemisch untersuchten wir die Tumoren auf die Expressionsintensität (H-Score) und das Expressionsmuster für die Marker p16, HMB45, Melan A und BCL-6. Zudem wurden die Tumoren auf das Vorliegen einer BRAF (V600E) Mutation immunhistochemisch untersucht. Hierbei zeigte sich ein hoher p16 Score bei niedrigem HMB45 Score als hilfreiches Unterscheidungsmerkmal. Die mitotische Aktivität, dargestellt mit dem Mitosemarker pHH3, war bei den Spitz Nävi geringer, zudem zeigten diese signifikant seltener eine BRAF Mutation. Die BCL-6 Expression erwies sich als nicht hilfreich zur Differenzierung zwischen Spitz Nävus und Melanom.

Zudem zeigten die Ergebnisse der Fluoreszenz in situ Hybridisation (Vysis Melanoma FISH Probe Kit von Abbott Molecular Inc) in unserem Kollektiv eine hohe Sensitivität und Spezifität.

Unsere Arbeit zeigt einen Vergleich aller heute in der täglichen Routine anwendbaren Parameter zur Differenzierung von Spitz Nävi und Melanomen an einem Kollektiv.

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Erklärung nach § 2 Abs. 2 Nrn. 6 und 7

Ich erkläre, dass ich die der Medizinischen Hochschule Hannover zur Promotion eingereichte Dissertation mit dem Titel

„Reevaluation etablierter und neuerer Kriterien zur Differenzierung von Spitz Nävi und malignen Melanomen"

in der Abteilung für Dermatologie, Venerologie und Allergologie des Helios Klinikums Hildesheim unter Betreuung von Herrn Prof. Dr. med. Michael Tronnier und Frau PD Dr. med. Christina Mitteldorf ohne sonstige Hilfe durchgeführt und bei der Abfassung der Dissertation keine anderen als die dort aufgeführten Hilfsmittel benutzt habe.
Die Gelegenheit zum vorliegenden Promotionsverfahren ist mir nicht kommerziell vermittelt worden. Insbesondere habe ich keine Organisation eingeschaltet, die gegen Entgelt Betreuerinnen und Betreuer für die Anfertigung von Dissertationen sucht oder die mir obliegenden Pflichten hinsichtlich der Prüfungsleistungen für mich ganz oder teilweise erledigt.
Ich habe diese Dissertation bisher an keiner in- oder ausländischen Hochschule zur Promotion eingereicht. Weiterhin versichere ich, dass ich den beantragten Titel bisher noch nicht erworben habe.
Ergebnisse der Dissertation wurden in dem Journal „Archives of Dermatological Research" veröffentlicht.

Hannover, den 07.11.2018

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Alexandra Ritter

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