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Prognostic significance of detecting micrometastases by tyrosinase RT/PCR in sentinel lymph node biopsies: lessons from 322 consecutive melanoma patients☆

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Abstract

This prospective study was performed to determine the prognostic value of tyrosinase mRNA detection in sentinel lymph nodes (SLN) of melanoma patients. About 847 SLNs from 322 consecutive patients were assessed by histopathology and immunohistochemistry as well as tyrosinase-reverse transcriptase-polymerase chain reaction (RT/PCR) for the presence of micrometastases. The results were correlated with the prognostic parameters employing a multivariate analysis after a median follow-up of 37 months. Histopathological analysis revealed metastases in 34/322 patients (10.6%). Among the 288 patients with histopathologically negative SLN, tyrosinase-mRNA was detected in 39 patients. A relapse of the tumour occurred in 44.1% of the patients with histopathologically positive SLN, in 25.6% with histopathologically negative, but tyrosinase-RT/PCR-positive SLN, and 8.0% with "double-negative" SLN. A multivariate analysis identified tumour thickness, the histopathological SLN status, and the ulceration of the primary tumour as independent prognostic factors. Thus, by assessing tyrosinase mRNA in the SLN of melanoma patients, we identified a subgroup with histopathologically negative, but Tyr-RT-PCR-positive SLN who have a high risk of disease relapse.

Keywords: Sentinel lymph node dissection; Tyrosinase reverse transcriptase/polymerase chain reaction; Malignant melanoma; Microstaging

1. Introduction

Following the inauguration of the sentinel lymph node biopsy (SLNB) approximately a decade ago [1,2], numerous centres worldwide have adopted this dermatosurgical technique as a valuable tool in the diagnostic spectrum influencing pivotal treatment decisions for patients suffering from malignant melanoma [3,4]. Based on several independent studies, the histopathological status of the SLN appears to be an exceedingly important prognostic parameter with regard to the risk of tumour disease progression [3,5]. At the stage of regional metastases, patients' survival time shows the best correlation with the number of metastatically involved lymph nodes [5], while the

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extent of metastatic involvement (i.e. macroscopically versus microscopically detectable metastases) has been identified as the second most relevant prognostic parameter [5,6]. Thus, histology-based analysis of SLN is thought to be of eminent importance for melanoma staging examinations, a notion that is also reflected by the recently published stage classification for cutaneous melanoma refined under the auspices of the American Joint Committee on Cancer (AJCC) [7].

Hitherto, the presence or absence of micrometastases in SLN of melanoma patients has been assessed predominantly by histopathological techniques. Several studies have shown that the sensitivity regarding correct detection of SLN melanoma micrometastases by haematoxylin and eosin (H&E)-based conventional histopathological techniques is 10–15% lower compared with approaches combining serial-sectioning with immunohistochemistry [8–11].

Reverse transcriptase-polymerase chain reaction (RT/PCR)-based diagnostic procedures of SLN have been introduced recently [12–14]. Data mostly relate to mRNA expression of tyrosinase (Tyr), as a melanocytic differentiation antigen, as well as melanoma-associated antigens (MAGE-3, Melan A/MART-1) [12–16]. From these studies, it appears that such RT/PCR analyses yield a significantly higher sensitivity compared with the histopathological and immunohistochemical procedures [12–16]. It has also been demonstrated that the RT/PCR SLN status is an independent prognostic factor with regard to disease relapses [9,12,14]. However, although there is accumulating evidence for higher sensitivity and prognostic value of these RT/PCR techniques compared with histopathological approaches, such molecular diagnostic data are not yet accepted as being reliable for subsequent therapeutic decisions, such as complete lymphadenectomy or adjuvant interferon-α treatment. This is due, at least in part, to the lack of prospective studies performed with large numbers of patients.

To overcome this problem, we have performed a prospective, mono-centric study including more than 300 melanoma patients. We have focused on the SLN Tyr-RT/PCR status in patients whose cut-off melanoma thickness was $\geqslant$0.75 mm. Depending upon the individual circumstances, sonometrically and histometrically assessed tumour thicknesses were both admitted for making the SLNB decision, since there is an excellent correlation between both parameters [17,18]. In most cases, sonometrical assessment of tumour thickness was routinely performed in the pre-operative setting; in the remaining cases, SLNB was performed as a secondary intervention after the melanoma had been excised, histopathologically evaluated and histometrically assessed. Final statistics were based upon the histometrically assessed tumour thicknesses only.

2. Patients and methods

2.1. Patients

From June 1998 to March 2002, we recruited a cohort of 322 consecutive melanoma patients with a tumour thickness according to Breslow of $\geqslant$0.75 mm, assessed either sonometrically by 20 MHz ultrasound or histopathologically in patients, whose tumours were excised prior to the first visit in our department. The patients were recruited prospectively and, after written informed consent, underwent SLNB. Prior to the surgical procedure, the presence of macrometastases had been excluded in all cases by thorough clinical and apparative investigations (including ultrasound check of regional lymph nodes and abdomen, chest X-ray, brain computed tomography or magnetic resonance imaging (MRI), serological tumour markers, i.e. protein S-100B and melanoma inhibitory activity (MIA)).

2.2. Lymphatic mapping and SLNB

On the day of surgery or the day before, the draining lymphatic vessels and the regional lymph nodes were identified by 50–250 MBq. ^{99m}Technetium-labelled human serum albumin (Nanocoll®, Nycomed Mersham Sorin, Saluggia, Italy), which was injected intradermally at 4–6 injection sites in the close vicinity of the tumour. Lymph nodes, as well as draining lymph tracts, were exactly traced by sequential and static lymphoscintigraphy and marked with a waterproof pen on the skin surface. Intraoperative lymphatic mapping was performed using a hand-held gamma probe (C-Trak®, CareWise Medical Products, Morgan Hill, CA, USA), and lymphatics were directly visualised by intradermal injection of patent blue V® (Guerbet, Roissy, France) immediately prior to general or local tumescent anaesthesia. A total of 847 LNs were excised from the melanoma patients and investigated.

A total of 42 lymph nodes derived from 31 non-melanoma patients were also investigated by tyrosinase reverse transcriptase/polymerase chain reaction (Tyr-RT/PCR) as negative controls. This cohort comprised 17 patients with cutaneous lymphoma, 3 patients with squamous cell carcinoma of the skin, 3 patients with Merkel cell carcinoma, 7 patients with chronic venous insufficiency and 1 patient with dermatofibrosarcoma protuberans.

2.3. SLN preparation and histopathological examination

The SLNs were cut *ex vivo* into halves along their longest axis. One half was immediately snap-frozen in liquid nitrogen and stored at −80 °C for subsequent RT/PCR analysis (see below). The corresponding half was fixed in 5% buffered formaldehyde, dehydrated

and embedded in paraffin. Serial-sections were stained with H&E. Immunohistochemistry was performed on rehydrated sections using monoclonal antibodies directed against S100 and HMB-45 (both antibodies purchased from Dako Diagnostika, Hamburg, Germany). For further verification, all SLN found to be negative in the histopathological analysis, but positive by Tyr-RT/PCR were re-evaluated by a complete sectional analysis, including histology and immunohistochemistry, using an additional monoclonal antibody directed against Melan-A (DakoCytomation, Glostrup, Denmark), as described elsewhere in [19].

2.4. Tyrosinase reverse transcriptase/polymerase chain reaction

Tyr-RT/PCR was performed according to published standard procedures with minor modifications [20,21]. Briefly, the SLN tissue was stored and shielded for 3 days frozen at −80 °C in order to wait for the decay of radioactivity (^{99m}Tc, $t_{1/2} = 6$ h). The tissue was weighed and homogenised at 100 mg per 2 ml RNAzol B (WAK Chemie, Bad Soden, Germany) on ice using a Polytron™ tissue dissector (27,000 rpm). Total RNA was isolated by the standard guanidinium isothiocyanate method. Five μg of total mRNA were employed for cDNA synthesis using pd(N)$_6$-primers (first-strand DNA synthesis Kit; cat. no. 27–9261–01, Amersham Biosciences, Little Chalfont Buckinghamshire, UK). The PCR reaction was performed using Tyr-specific primers as well as primers specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an ubiquitously expressed gene for positive control of the tissue integrity, preparation and analytical process. Primers used for Tyr detection were GTCTTTATGCAATGGAACGC (sense) and GCTATCCCAGTAAGTGGACT (anti-sense) leading to a PCR product of 207 bp. Primers used for GAPDH detection were GGTCGGAGTCAACGGATTTG (sense) and ATGAGCCCCAGCC-TTCTCCAT (anti-sense) producing a PCR fragment of 320 bp. PCR cycling conditions were as follows: heating at 94 °C for 5 min, followed by 43 cycles (denaturation at 94 °C for 30 s, anealing at 47 °C for 60 s, and extension at 72 °C for 90 s). Each PCR run was performed using appropriate positive and negative technical control templates for Tyr as well as GAPDH. PCR-products were visualised by electrophoresis in 0.8% agarose gels, dissected and ligated into a pCR® II-TOPO-vector system (cat. no. 45–0640, Invitrogen, Carlsbad, CA, USA) with subsequent heat-shock transformation of *Escherichia coli*. Transformed bacteria were selected from agar plates, grown up in LB-medium and subjected to plasmid preparation (Plasmid-Mini-Kit, cat. no. 12125; Qiagen, Hilden, Germany). Eventually, the identity of the Tyr-RT/PCR product was rigorously verified by a cycle-sequencing step (Thermo Sequenase Primer Cycle Sequencing Kit, cat. no. 25–2438–01, Amersham, Piscataway, NJ, USA) using an ALFexpress DNA sequencer apparatus (Pharmacia Biotech Europe, Freiburg, Germany).

2.5. Consecutive and adjuvant treatment

Patients with positive results from the histopathological SLN examination were recommended to receive a complete dissection of the respective regional lymph basin in a second surgery operation. Melanoma patients with a histologically proven maximum vertical tumour thickness (according to Breslow) $\geqslant 1.5$ mm and/or histopathologically and immunohistochemistically confirmed lymph node metastases were regularly treated by low dose interferon-α (114 of 322 patients). Twenty patients with histologically confirmed lymph node metastases received dacarbazine as well. These adjuvant treatments were initiated according to current protocols as undertaken by the Dermatologic Cooperative Oncology Group (DeCOG).

2.6. Statistical analysis

Survival was analysed by Kaplan–Meier plots. Calculation of relapse-free survival related to the interval from the day of sentinel node biopsy until the appearance of relapse or the latest date of relapse-free follow-up, respectively. Uni- and multivariate survival analyses were performed by Cox regressions addressing the accepted prognostic variables of the primary tumour (i.e. Breslow's tumour thickness, Clark's invasion level, presence of ulceration, anatomic site, number of involved regional lymph node basins) as well as the adjunctive major criteria (age, gender, histopathological status and Tyr-RT/PCR status of the SLN). Comparison of differences between these variables was based upon χ^2 statistics. In all statistical testings, a $P < 0.05$ was defined as significant.

3. Results

3.1. Patients and Tyr-RT/PCR

In a cohort of 322 melanoma patients, a total of 847 LN were removed, with a minimum of 1, median of 2 and maximum of 15 LN, the latter in a patient with a tridirectional lymph drainage. The demographic data of the patients are summarised in Table 1. All LN were analysed by H&E-based histopathology, immunohistochemistry and Tyr-RT/PCR. When 42 LN obtained from 31 non-melanoma patients (control cohort) were analysed, one false positive Tyr-RT/PCR result was found in a patient with a long-standing cutaneous erythrodermic T-cell lymphoma who had been treated with several courses of psoralen and ultraviolet A (PUVA).

Table 1
Clinical and pathological characteristics of melanoma patients ($n = 322$)

Patients' characteristics	N	(%)
Gender		
Male	131	(40.7)
Female	191	(59.3)
Age median (range), years	59.0 (17–90)	
Site of primary tumour		
Trunk	136	(42.2)
Extremity		
Upper	62	(19.3)
Lower	103	(32.0)
Head or neck	21	(6.5)
Breslow's tumour thickness, mm		
Mean ± SD	2.11 ± 2.64	
Median	1.22	
<1.0 mm	137	(42.6)
1.01–2.0 mm	88	(27.3)
2.01–4.0 mm	54	(16.8)
>4.0 mm	39	(12.1)
Not specified	4	(1.2)
Clark's invasion level		
II/III	176	(54.7)
IV	120	(37.3)
V	22	(6.8)
Not specified	4	(1.2)
Ulceration	61	(18.9)
Histological type		
Superficial spreading melanoma	185	(57.5)
Nodular melanoma	63	(19.6)
Lentigo maligna melanoma	17	(5.3)
Acral lentiginous melanoma	17	(5.3)
Unclassified	40	(12.4)
No. of regional lymph node basins involved		
1 basin	272	(84.5)
2 or 3 basins	50	(15.5)

SD, standard deviation.

Based on these data, the specificity of the Tyr-RT/PCR was calculated to be 97.6%.

3.2. Distribution of SLN metastases as detected by histopathology and Tyr-RT/PCR

The results of the histopathological and immunohistochemical evaluation as well as the results of Tyr-RT/PCR are summarised in Table 2. The proportion of melanoma patients with histopathologically (i.e. histology and/or immunohistochemistry) positive SLN was 10.6% (34/322 patients (pts)). Among these 34 patients with a histopathologically positive SLN only 16 cases were found to be concordantly positive in Tyr-RT/PCR.

All SLN found in the initial analysis to be histopathologically negative, but Tyr-RT/PCR-positive were subjected to a thorough histopathological re-evaluation including a complete serial-sectioning of the tissue specimens followed by S100, HMB-45 and Melan-A immunohistochemistry. This procedure led to the retrospective identification of micrometastases in two additional patients (2/41, 5.1%), who were *a posteriori* included into the group of histopathologically positive patients.

3.3. Histopathology, Tyr-RT/PCR results and clinical course

During a median post-surgery follow-up period of 37 months (range 3–62 months), we observed 45 relapses (see Table 2). These comprised 16 patients with satellite and/or cutaneous/subcutaneous in-transit metastases (35.6%), 15 patients with regional lymph node metastases (33.3%) and 14 patients with distant metastases (31.1%). Among the 20 patients with disease relapses, who had been characterised as a subgroup of the histo^{neg}Tyr-RT/PCR^{neg}SLN population of 249 patients, 4 patients (1.6%) developed lymphogenic metastases, namely in the formerly negative regional lymph node basin. Five patients (12.8%) of the histo^{neg}Tyr-RT/PCR^{pos}SLN subgroup of 39 patients developed lymphogenic metastasis *in loco*.

Mean disease-free survival (DFS) of the patient subgroups are summarised in Table 2. The difference with respect to DFS turned out to be highly significant in the log-rank test ($P < 0.0001$). The survival curves are presented by a Kaplan–Meier plot in Fig. 1. Additionally, we assessed the correlation between the SLN status and the Breslow's melanoma thickness (Fig. 2). The difference in DFS between the two histopathologically negative subgroups proved to be highly significant (Student's t-test; $P = 0.003$).

In order to evaluate further the prognostic power of the SLN status, we investigated its correlation with disease-recurrence (Table 3), which was also analysed for correlation with the internationally recognised prognos-

Table 2
Patient groups defined by SLN status ($n = 322$)

Patient group/SLN status	No. of patients	No. of recurrences	DFS (months) [95% CI]
histo^{pos}	34 (10.6%)	15 (44.1%)	39 [31; 47]
histo^{neg} Tyr-RT/PCR^{pos}	39 (12.1%)	10 (25.6%)	47 [40; 54]
histo^{neg} Tyr-RT/PCR^{neg}	249 (77.3%)	20 (8.0%)	58 [57; 60]

pos, positive; neg, negative; DFS, disease-free survival; 95% CI, 95% Confidential Interval, SLN, sentinel lymph node.

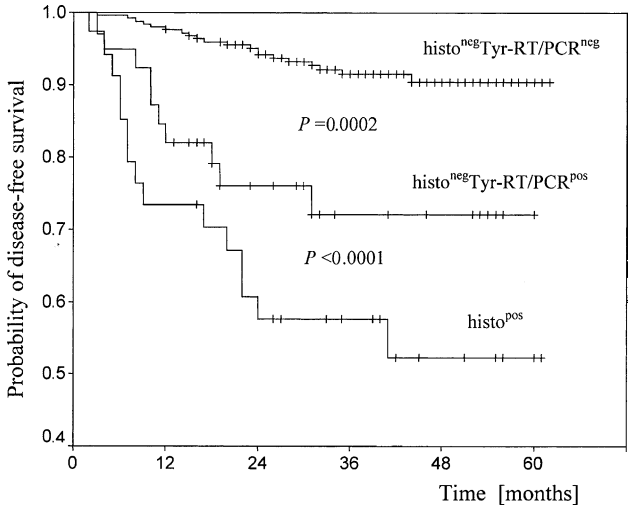

Fig. 1. Probability of disease-free survival (DFS).

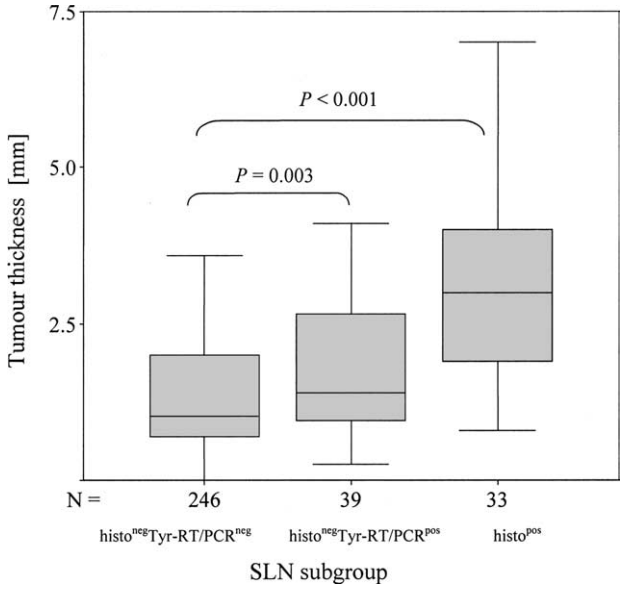

Fig. 2. Correlation between Breslow's tumour thickness, histopathological sentinel lymph node (SLN) and Tyr-reverse transcriptase-polymerase chain reaction (RT/PCR) SLN status.

tic criteria of the primary melanoma. Univariate analysis revealed primary tumour thickness, tumour ulceration, Clark's invasion level and gender as significant prognostic factors. In addition, the histopathological SLN status and the Tyr-RT/PCR SLN status were identified as independent prognostic markers. The patient's age, the anatomical site of the primary tumour and the number of draining regional lymph node basins did not significantly influence recurrence. Performing a multivariate analysis according to the Cox regression model, only the primary tumour thickness, the histological SLN status and tumour ulceration were identified as significant independent predictive variables with regard to disease recurrence (Table 4).

Table 3
Correlation of risk factors with disease recurrence (n = 322)

Risk factor	No. recurrence (n = 277)	Recurrence (n = 45)	P-value
Gender			
Male	106	25	0.034
Female	171	20	
Age (years)			
⩽60	151	20	0.260
>60	126	25	
Primary site			
Head/neck	17	4	
Trunk	123	13	0.143
Extremity	137	28	
No. of regional lymph node basins involved			
1 basin	235	37	0.658
2 or 3 basins	42	8	
Breslow's tumour thickness			
⩽1.0 mm	135	2	
1.01–2.0 mm	81	7	<0.0001
2.01–4.0 mm	36	18	
>4.0 mm	21	18	
Clark's invasion level			
⩽III	169	7	<0.0001
IV/V	104	38	
Ulceration			
Yes	35	26	<0.0001
No	242	19	
SLN histopathology			
Negative	258	30	<0.0001
Positive	19	15	
SLN tyrosinase mRNA expression (Tyr-RT/PCR)			
Negative	238	29	0.001
Positive	39	16	

Table 4
Multivariate analysis of risk factors with disease recurrence (n = 322)

Prognostic factor	HR (95% CI)	P-value
SLN histopathology	2.78 (1.46–5.29)	0.002
Breslow's tumour thickness	2.59 (1.81–3.72)	<0.001
Ulceration of the primary tumour	2.34 (1.16–4.68)	0.017

HR, hazard ratio; 95% CI, 95% Confidence Interval.

4. Discussion

During the last decade, histopathological analysis of the SLN has evolved to a valuable routine procedure in the diagnosis and staging of malignant melanoma [4]. There is a broad consensus with regard to the scintigraphic and surgical aspects of the procedure [22,23]. However, the operational procedures and techniques for detecting potential micrometastases within the dissected SLN are still under debate. It is widely agreed that H&E-based histopathological analysis alone results in an unacceptably high rate of false-negative results. It has been shown that in patients who developed metastases in a regional lymph node basin that was originally

diagnosed negative by histopathological SLN evaluation, re-evaluation by immunohistochemistry revealed SLN micrometastases in 80% of cases [9]. In a retrospective analysis including 143 patients with initially histopathologically negative SLNB, 67% (10/15 patients) of the cases with nodal progression were found to be false-negative due to previous erroneous routine reading of H&E specimens [14]. However, in another study a rate of only 12% false-negative results was reported [10].

Since introduction of the RT/PCR technique into the field of SLN diagnostics, the sensitivity to detect occult micrometastases has increased [12–15], a notion that is confirmed by the results of our present study. In our cohort of 322 consecutive melanoma patients, there was a statistically significant difference regarding disease-free survival and disease-recurrence between patients with Tyr-RT/PCR^{pos} and Tyr-RT/PCR^{neg} SLN. In a former study, Shivers and colleagues have shown that among 91 patients with metastasis-free SLNs, based upon routine histology, 47 patients (51.6%) were positive by Tyr-RT/PCR [14]. These authors described a significant prognostic difference between the patients with a histo^{neg}Tyr-RT/PCR^{pos} SLN and a histo^{neg}Tyr-RT/PCR^{neg} SLN ($P = 0.02$). Moreover, they observed a clear-cut correlation between the vertical thickness of the primary tumour and the rate of positive Tyr-RT/PCR results, whereby a multivariate analysis pointed to the histopathological and Tyr-RT/PCR status of the SLN as prognostic parameters [14]. Along these lines, our current study points to the primary tumour thickness and the state of the SLN as significant interrelated prognostic factors (Fig. 2). In a second, larger series of 233 patients, the same authors detected micrometastases by RT/PCR in 63% of patients with histopathologically and immunohistochemically negative SLN confirming the former results in principle [11]. Similar results were reported in a cohort of 116 patients by Blaheta and colleagues who demonstrated micrometastases by Tyr-RT/PCR in 35.6% of the patients with histologically and immunohistochemically negative SLN [12]. In an accompanying multivariate analysis, it were only the histopathological and Tyr-RT/PCR status of the SLN which reached a significant power as independent prognostic parameters [12]. Bostick and colleagues studied 72 melanoma patients with regard to the SLN expression of tyrosinase, MAGE-3 and MART-1 by RT/PCR [13]. Expression of at least two of these markers was detected in 36% of the patients with histopathologically negative SLN. A significant survival benefit was observed for the subgroup of histopathologically negative SLN patients in cases that had only one positive melanoma marker RNA expression compared with the subgroup of patients who were positive for 2 or more markers. Multivariate analysis confirmed mRNA expression of $\geqslant 2$ melanoma markers as the strongest predictor for disease relapse.

In our study, the rate of positive Tyr-RT/PCR results was 13.5% (39/288 histo^{neg} pts), which is lower than the above-mentioned findings in the studies of Blaheta and colleagues and Shivers and colleagues [12,14]. At least two explanations can be delineated for these apparent discrepancies which are not mutually exclusive. First, a higher proportion of malignant melanomas with a lower Breslow's tumour thickness was included in our study. While the median tumour thickness was 1.22 mm in our study, it reached 2.0 or 2.36 mm in the studies of Blaheta and colleagues and Shivers and colleagues, respectively [12,14]. This constellation would also explain our relatively low rate of 10.6%-positive SLN results by histopathology. Second, there are considerable differences in the preoperative diagnostic procedures. While in most other studies patients with clinically (i.e. palpatory) uninvolved lymph nodes were elected for SLNB, all patients included in our study were examined by high resolution ultrasound diagnostics of the regional lymph node basins, and patients with any suspicion for metastases were excluded from the study. It is well recognised that such ultrasound techniques are significantly more sensitive than palpation alone with regard to the detection of metastases [24,25].

A still unresolved issue of concern in the diagnostic procedure of micrometastases is the postoperative preparation of SLN. In most studies, the SLN is bisected along its longest axis in order to obtain material for both histology/immunohistochemistry and Tyr-RT/PCR. This results in a stochastical allotment of possibly asymmetrically distributed micrometastases and, consecutively, to potentially false-negative outcomes in the subsequent analyses. To overcome this problem, at least in part, van der Velde-Zimmermann and colleagues have proposed a step-wise protocol for SLN analysis, where the decision for a thorough immunohistochemical SLN evaluation in serial-sections depends upon the Tyr-RT/PCR results [26]. In this context, it has also to be mentioned that optimum conditions for histopathological evaluation (i.e. formaldehyde fixation) and gene expression analysis on the mRNA level (shock-freezing of native tissue at −196 °C) are to some extent mutually conflicting. In our study, all 52 LN of the 39 patients which initially were negative by histopathology and immunohistochemistry, but positive by Tyr-RT/PCR were subjected to a second-look evaluation by complete serial-sectioning of the remaining tissue followed by HMB-45 and Melan-A immunohistochemistry. This led to detection of micrometastases in 2 additional patients, who then retrospectively were included in the histo^{pos}Tyr-RT/PCR^{pos}SLN subgroup. These 2 patients stayed disease-free during the follow-up period. In consistent with our results, Blaheta and colleagues detected one patient with micrometastasis in the subsequent serial immunohistochemical re-evaluation when re-examining 33 SLN derived from 21

patients with negative standard histopathology, but positive Tyr-RT/PCR [27].

It is sometimes claimed that Tyr-RT/PCR detection of sentinel lymph node metastasis is fraught with a relatively high false-positive rate, i.e. poor specificity, linked basically to lymph nodal inclusion of melanocytic naevi (in 0.3–22%), nerve structures and even to translocation of tyrosinase mRNA by macrophages [28–30]. Noteworthy, we did not observe a single case of such a melanocytic naevus within a SLN or corresponding control LN within our cohort. In all cases with histological detection of melanoma cells, the differential diagnosis of melanocytic naevus cells was carefully excluded by coexpression of the S100 and HMB-45 antigens, as detected by immunohistochemistry. Taken together, the bias due to such false-positive Tyr-RT/PCR results, if existing at all, seems to be of minor practical importance, given the undoubtable prognostic relevance of a positive Tyr-RT/PCR outcome in a histologically negative SLN.

In conclusion, we present the hitherto largest prospective cohort of cases worldwide addressing the diagnostic and prognostic value of molecular analysis for melanoma SLNB. Our data demonstrate that Tyr-RT/PCR techniques allow detection of micrometastases which were missed by histopathological and immunohistochemical analysis. Indeed, we could identify a subset of histo^{neg}Tyr-RT/PCR^{pos} patients with a significantly unfavourable prognosis in terms of recurrence-free survival. The median follow-up time of 37 months may not yet allow final conclusions with regard to relapse rates. However, our findings confirm and extend results of most recently published studies with smaller cohorts, a higher median thickness of the primary tumour and even shorter follow-up times (i.e. between 12 and 28 months) [12–14]. Thus, we propose that SLN Tyr-RT/PCR analysis may have major prognostic relevance that warrants further multicentre studies with large numbers of patients such as the ongoing Sunbelt melanoma trial. The results of these studies will have to be awaited before clinical consequences (e.g. subsequent LN dissection or adjuvant immunotherapy) can be drawn.

Conflict of interest statement

None declared.

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