ORIGINAL ARTICLE

Detection of mitotic figures in thin melanomas—Immunohistochemistry does not replace the careful search for mitotic figures in hematoxylin-eosin stain

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Background: The mitotic rate is an important prognostic criterion in patients with thin melanoma ≤ 1 mm.

Objective: The aim of this study was to investigate the reproducibility of the mitotic rate in thin melanoma in hematoxylin-eosin (H&E) stain and compare it with the detection of mitotic figures by immunohistochemistry.

Methods: The number of mitoses stated in the routine diagnostic report in 190 pT1 melanomas was compared with the number gained from re-evaluation of H&E sections and the number detected after staining with the mitotic marker, phosphohistone H3 (PHH3). Two different approaches were used for choosing the "hot spot" for evaluation (dermal vs epidermal/dermal).

Results: Comparing routine H&E-stained slides with re-evaluation slides, the number of mitotic figures was slightly variable. However, findings did not result in a change of the tumor stage. In 34% of the tumors with dermal mitotic figures on H&E, mitoses could not be found in the corresponding PHH3 slide anymore. In 4% of the cases, stage relevant mitoses could only be found by PHH3 immunohistochemistry.

Limitation: This is a single center study.

Conclusion: Immunohistochemical staining for mitotic figures does not replace a careful evaluation of H&E-stained slides. Immunohistochemical detection of mitosis is only an additional tool; the time-saving effect is therefore negligible. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2015.07.007.)

Key words: hot spot; immunohistochemistry; mitosis; mitotic rate; phosphohistone H3; pT1; thin melanoma.

INTRODUCTION

Tumor thickness is the most powerful independent prognostic criterion in primary melanoma. In thin melanoma, the mitotic rate was considered one major prognostic indicator by the American Joint Committee on Cancer (AJCC) guidelines.[1-4] The presence of ≥1 mitosis in the dermal tumor part results in a shift to pT1b.[4,5] Several studies have found that patients with melanoma less than 1 mm with additional risk factors, such as mitoses, might benefit from sentinel lymph node biopsy.[6-9] The evaluation of mitotic figures is still a matter of debate. In reviewing pathology sections, it may be difficult to unequivocally identify a mitotic figure in a melanocyte versus another cell in the dermis. Moreover, high interobserver variability has been reported.[10-12] The antibody phosphohistone H3 (PHH3, ser10) has been identified as a reliable and sensitive marker to

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detect mitotic figures in every stage.[13-15] To differentiate mitoses in melanocytes from mitoses in other cells with certainty, double staining for Melan A and PHH3 are helpful.[11] The aim of our study was to evaluate the reproducibility of the mitotic count in hematoxylin-eosin (H&E)-stained slides and to determine the benefit of immunohistochemical staining with PHH3 in the subgroup of pT1 melanomas. Moreover, we looked into whether the extension of the "hot spot" to the epidermal tumor part has an influence on the number of stage-relevant mitoses.

CAPSULE SUMMARY

- Mitotic rate is a staging criterion for pT1 melanomas.
- There is high reproducibility of dermal mitotic rate on H&E-stained sections.
- One-third of the mitoses noted on routine review are no longer detected in PHH3. Mitotic rate in pT1 melanoma should be evaluated with H&E.
- PHH3 staining is better reserved for special cases.

PATIENTS AND METHODS

All anonymized clinical and histologic data were stored in a database. All examinations were performed in a blinded fashion. Our study was approved by the local ethics commission.

Mitotic figures in hematoxylin-eosin—reproducibility in re-evaluation

We examined the reproducibility of the mitotic count given in the official reports on 150 pT1 melanomas (first cohort, see below), which have been diagnosed by a board-certified dermatopathologist from our department. The mitotic rate was re-evaluated by a different investigator, using the existing Hematoxylin-eosin (H&E) slides from routine diagnostics only. In both settings, the evaluation of mitotic figures was performed, according to the proposals of the AJCC and the recommendations of Garbe et al[16] in the "mitotic hot spot" of the dermal component of the melanoma (Fig 1).

Comparison of the mitotic rate from routine diagnostics (H&E) and from pHH3 staining

Immunohistochemical staining was carried out using the PHH3 antibody (PhosphoSer10, polyclonal, Biocare Medical, Concord, CA; dilution 1:400) in accordance with the manufacturer's protocol. As a detection system, we used a BenchMark GX immunostainer and ultraView Universal Alkaline Phosphatase Red Detection Kit (both Ventana Medical Systems, Tucson, AZ). Like in daily routine, a new cut from the paraffin block has to be prepared for PHH3 staining that is directly adjacent to the H&E sections from routine histology. Before starting the evaluation, we checked the slides for sufficient representative tumor material. The number of mitotic figures given in official reports (H&E) was compared with the one calculated with PHH3.

One hundred fifty pT1 melanomas (first cohort). In 147 of 150 melanomas, enough representative tumor material was present. These cases were evaluated for PHH3 in the same way as already described. Nine of the 11 cases with mitoses found with H&E could no longer be found with PHH3. To confirm this observation, the cohort was extended by 40 additional pT1b melanomas.

Forty further pT1b melanomas (second cohort). The mitoses were examined in the same way as described above and compared with the number of mitotic figures from routine diagnostics (H&E) and those identified with PHH3.

How does the extension of the hot spot to the epidermal part of the tumor influence the mitotic rate?

In a second approach, the first cohort (150 pT1 melanomas) was evaluated again by choosing a hot spot encompassing epidermal and dermal tumor components. The mitotic figures were examined in the same way.

Evaluation of the time score

We measured the time from putting the first slide under the microscope until the screening of all the existing slides was accomplished. The time for documenting and organizing the slides was not included.

Statistical analyses

Statistical analyses were performed using SPSS for Windows. The χ^2; test, the Mann-Whitney U test, and the Jonckheere-Terpstra test were used to calculate correlations between patient and tumor characteristics. The paired t test was used to calculate the correlation between the mitotic indices of H&E and anti-PHH3. To measure interrater observer agreement, we used Cohen's kappa statistics. The significance level was determined at $P < .05$.

RESULTS

Patient and tumor characteristics: relation to the mitotic rate

We found a significant correlation between the number of dermal mitoses and Breslow thickness