Title

Factors associated with use of immunohistochemical markers in the histopathological diagnosis of cutaneous melanocytic lesions

Permalink

https://escholarship.org/uc/item/4f05x74d

Journal

Journal of Cutaneous Pathology, 47(10)

ISSN

0303-6987

Authors

May, Caitlin J
Piepkorn, Michael W
Knezevich, Stevan R
et al.

Publication Date

2020-10-01

DOI

10.1111/cup.13736

Peer reviewed

Factors associated with use of immunohistochemical markers in the histopathological diagnosis of cutaneous melanocytic lesions

Caitlin J. May[1,2], Michael W. Piepkorn[1,2], Stevan R. Knezevich[3], David E. Elder[4], Raymond L. Barnhill[5], Annie C. Lee[6], Martiniano J. Flores[7], Kathleen F. Kerr[8], Lisa M. Reisch[8], Joann G. Elmore[6,9]

[1]Dermatopathology Northwest, Bellevue, Washington

[2]Division of Dermatology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington

[3]Pathology Associates, Clovis, California

[4]Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

[5]Department of Translational Research, Institut Curie, Paris Sciences and Lettres Research University; Faculty of Medicine, University of Paris Descartes, Paris, France

[6]Division of General Internal Medicine and Health Services Research, UCLA David Geffen School of Medicine, Los Angeles, California

[7]Edwards Lifesciences, Irvine, California

[8]Department of Biostatistics, University of Washington School of Public Health, Seattle, WA

[9]Division of Dermatology, UCLA David Geffen School of Medicine, Los Angeles, California

Abstract

Background: Melanocytic tumors are often challenging and constitute almost one in four skin biopsies. Immunohistochemical (IHC) studies may assist diagnosis; however, indications for their use are not standardized.

Methods: A test set of 240 skin biopsies of melanocytic tumors was examined by 187 pathologists from 10 US states, interpreting 48 cases in Phase I and either 36 or 48 cases in Phase II. Participant and diagnosis characteristics were compared between those who reported they would have ordered, or who would have not ordered IHC on individual cases. Intraobserver

Correspondence Caitlin J. May, MD, Dermatopathology Northwest 2330 130th Ave NE Bellevue, WA 98005 c.may@dermpathnw.com.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

analysis examined consistency in the intent to order when pathologists interpreted the same cases on two occasions.

Results: Of 187 participants interpreting 48 cases each, 21 (11%) did not request IHC tests for any case, 85 (45%) requested testing for 1 to 6 cases, and 81 (43%) requested testing for 6 cases. Of 240 cases, 229 had at least one participant requesting testing. Only 2 out of 240 cases had more than 50% of participants requesting testing. Increased utilization of testing was associated with younger age of pathologist, board-certification in dermatopathology, low confidence in diagnosis, and lesions in intermediate MPATH-Dx classes 2 to 4. The median intraobserver concordance for requesting tests among 72 participants interpreting the same 48 cases in Phases I and II was 81% (IQR 73%−90%) and the median Kappa statistic was 0.20 (IQR 0.00, 0.39).

Conclusion: Substantial variability exists among pathologists in utilizing IHC.

Keywords

1 | INTRODUCTION

The skin biopsy rate has increased over time, to the point where approximately 1 in 10 older adults have a skin biopsy each year.[1] Melanocytic tumors constitute a substantial portion (nearly 1 in 4) of pathologists' skin biopsy caseloads.[2] Unfortunately, the histopathologic diagnosis of some melanocytic tumors can be quite challenging, and use of immunohistochemical markers (IHC) varies because of limitations in technology and the lack of universally agreed upon morphologic criteria. Our research group and others have observed poor agreement among pathologists in distinguishing benign melanocytic nevi from in situ and invasive melanoma.[3–7]

Immunohistochemical (IHC) markers potentially provide useful information that is not readily available by review of hematoxylin and eosin (H&E) stained slides alone.[8,9] While there are IHC staining patterns that lend support to either benign or malignant patterns, guidelines are currently lacking that would identify those skin biopsy cases that could benefit most by such testing. Therefore, to better under-stand current practices of IHC testing, we sought to determine the case, pathologist, and interpretative factors associated with utilization of IHC for melanocytic tumors.

2 | MATERIALS AND METHODS

2.1 | Participants

Pathologists from 10 US states participated in the study. Eligibility required completion of residencies and/or fellowships, interpretation of cutaneous melanocytic tumors in their own clinical practice within the previous year, and the expectation to interpret cutaneous melanocytic tumors in the subsequent 2 years. A baseline survey gathered demographic and clinical practice characteristics among participants. This study has been performed according to the Declaration of Helsinki. Study activities were approved by the institutional review board, and all participating pathologists signed an informed consent form.

2.2 | Data collection

240 test cases from shave, punch, and excisional skin biopsies of melanocytic tumors were selected using a stratification method based on patient age and documentation of the original diagnosis. Independent reviews of an H&E-stained glass slide for each case by three experienced dermatopathologists with specialized expertise in cutaneous melanocytic proliferations preceded a modified Delphi approach— a systematic, interactive approach of structuring group communication using a facilitator—to establish a reference diagnosis for each.[10,11] The reference diagnoses were then assigned to one of the five diagnostic classes in the Melanocytic Pathology Assessment Tool and Hierarchy for Diagnosis (MPATH-Dx),[12] which is a classification system that maps the diverse diagnostic terminology used by pathologists into five classes with stratified perceptions regarding lesion risk and corresponding treatment suggestions (see Table S1).

The cases in this study were distributed across the MPATH-Dx classes using the updated AJCC eighth Staging Manual[6,13] as follows: 10% (n = 25) in class I, 15% (n = 36) in class II, 25% (n = 60) in class III, 24% (n = 70) in class IV, and 25% (n = 46) in class V. Five sets of 48 cases, each comprising a full spectrum of MPATH-Dx classes, were assembled for interpretation by study participants across two phases.

In phase 1 of the study, all participating pathologists interpreted 48 cases in glass slide format. Patients' age, sex, biopsy type, and anatomic location of the biopsy site were provided for each case. Phase 2 occurred for each participant after a washout period of approximately 8 months. Participants agreeing to participate in a substudy in Phase 2 were randomized to either glass or digital whole-slide imaging format and interpreted a subset of 36 of their original 48 cases. Participants who declined to participate in the substudy interpreted the same set of 48 cases that they interpreted in Phase 1 using the same glass slides.

Participants provided their diagnoses and corresponding treatment suggestions for each case on a standardized online form. This information was then mapped by our computer programmer to one of the five MPATH-Dx classes (see Appendix).[12] For each case interpreted, pathologists also indicated whether they would order immunohistochemical (IHC) tests as ancillary measures to make a definitive diagnosis. This prompt was near the end of the histopathology form:

"Special Considerations: Would you need additional information to make a definitive diagnosis in real clinical practice?

- No

- Yes, I would need to know if it is a partial sampling of a larger lesion, and may not be representative of the larger lesion

- Yes, to make a definitive diagnosis, I would need to know additional clinical history:

  – Lesional Diameter

  – History of Change

- I would order special stains or ancillary tests in this case (check all that apply)."

Specific IHC tests available as choices were: BAP1, BRAF, cKit, HMB45, Ki-67, MART-1, MITF, p16, S100, SOX10, Tyrosinase, and Vimentin.

2.3 | Statistical analyses

Data were analyzed and presented at the pathologist level (eg, participants' demographic and clinical characteristics, $N = 187$) and interpretation level (eg, $N = 8976$ interpretations in Phase I). Pathologists' diagnoses for each case interpreted were mapped to one of the MPATH-Dx diagnostic classes. We compared participant characteristics between pathologists based on their frequency of requesting IHC testing. At the interpretation level, we examined differences in how physicians responded to particular cases and made comparisons based on whether or not an IHC test was requested. We used Fisher's exact test for all participant-level and interpretation-level comparisons.

We analyzed intraobserver concordance for IHC testing using interpretations from the $N = 72$ participants who interpreted the same 48 cases in phases I and II in glass slide format. We defined a concordant interpretation as one where a participant either requested an IHC test for a particular case in both phases, or one where a participant did not request an IHC test in both phases. We examined overall concordance as well as Cohen's Kappa for each participant and summarized these across participants. We also calculated these statistics separately for board-certified dermatopathologists and other pathologists and compared the distribution between the two groups of participants.

3 | RESULTS

Of the 187 participants who each interpreted 48 cases in phase I, 21 (11%) did not order any IHC tests, 85 (43%) ordered tests for 1 to 6 of their cases, and 81 (41%) ordered tests for 6 or more of their cases (Figure 1). The distribution of the specific IHC tests requested is shown in Table 1. IHC testing was identified for 1584 (18%) out of 8976 Phase I interpretations. The most commonly requested tests among these 1584 interpretations were MART-1 (1073 total interpretations), HMB45 (586 total interpretations), and Ki-67 (567 total interpretations). These three tests also tended to be ordered together. Ki-67 was requested 6% of the time overall, but 50% of the time when HMB45 was requested and 28% of the time when MART-1 was requested. HMB45 was requested 7% of the time overall, but 52% of the time when Ki-67 was requested and 33% of the time when MART-1 was requested. MART-1 was requested 12% of the time overall, but 60% of the time when HMB45 was requested and 53% of the time when Ki-67 was requested. In addition, p16 tended to be requested when Ki-67 was requested: p16 was requested just 2% of the time overall, but 25% of the time when Ki-67 was requested.

We did not find any differences in IHC test frequency between the five test sets comprising the full 240 study sample.

3.1 | Participant and Interpretation Level Comparisons

Participants who requested more IHC tests in phase I tended to be younger, board-certified in dermatopathology, and more likely to find melanocytic proliferations more challenging to interpret than other pathologies (Table 2). Participants were more likely to request an IHC test for a particular case if they were not confident in their diagnosis, they found the case difficult to interpret, their most advanced diagnosis for the case was borderline between two diagnoses, and/or they reported that they would have requested a second opinion (Table 3). Figure 2 shows participants' requests for IHC testing with reference to the MPATH-Dx classification of their interpretation of the case.

3.2 | Concordance results for IHC testing on the same cases interpreted twice

For the 72 participants who interpreted the same cases in Phases I and II, the median intraobserver concordance was 81% (IQR 73%–90%). The median Kappa statistic was 0.20 (IQR 0.00–0.37). Low Kappa statistics largely reflect that requesting IHC testing was uncommon so a large degree of concordance is expected by chance. In particular, 9 of 72 participants never indicated IHC testing in one of Phase I or Phase II, necessarily yielding a Kappa of 0. Intraobserver concordance was similar for board-certified and non-board-certified participants. Among 24 board-certified participants, the median concordance was 81% (IQR 68%−89%) and among 48 non-board-certified participants, the median concordance was 83% (IQR 74%−90%). Kappa statistics were higher for board-certified participants (median 0.26, IQR 0.19–0.43) than non-board-certified participants (median 0.13, IQR 0.00–0.32), largely reflecting that non-board-certified participants ordered IHC testing substantially less often (Table 2).

4 | DISCUSSION

Immunohistochemical studies are considered important ancillary tools in dermatopathology and are increasingly used in clinical practice. IHC studies have been reported to change the H&E diagnosis in approximately 11% of cases, and melanocytic proliferations constitute approximately 23% of IHC used in dermatopathology.[14] The prevalence of IHC testing for the diagnosis of melanoma has been increasing over time.[15] Although the exact reasons for this trend are unclear, it may be due, at least in part, to pathologists' desire to improve their accuracy and assure patient safety. In addition, concerns about malpractice liability are reflected in the observation that most pathologists show assurance behaviors to varying degrees.[16,17] Financial incentives are currently an unquantified motivation for IHC utilization. Although the use of these studies is becoming more common, prior to our study there had been no data summarizing the case and pathologist characteristics associated with the utilization of immunohistochemical studies for the interpretation of melanocytic tumors and no data on reproducibility of use.

Our study shows inconsistency and substantial disagreement among pathologists in IHC utilization for melanocytic tumors. Approximately half of the participants requested IHC tests for six or more of their assigned 48 cases. In addition, while 229 of the 240 cases had at least one participant requesting IHC testing, only two of the 240 cases had >50% of interpreting pathologists requesting IHC testing. There was disagreement among

pathologists on whether to order IHC tests for the majority of cases. For example, Figure 3 depicts two cases, both classified by expert consensus reference panel as invasive melanoma but having very different IHC requests among the participating pathologists. The case shown in Figure 3A prompted 4 (11%) of the 36 interpreting pathologists in phase I to request IHC, while the case in Figure 3B had 18 (46%) of the 39 interpreting pathologists desiring such studies. While these cases are certainly different histopathologically, the disparity of features may not be obvious enough to explain the large difference in testing frequency.

Our study also finds that the cases associated with IHC requests tend to be in the middle of the diagnostic spectrum (eg, MPATH-Dx classes 2–4), coinciding with the increased diagnostic challenges that these types of cases impose. Participants were also more likely to order a test if they believed a case to be difficult or borderline between two diagnoses; if they were younger; or if they were board-certified in dermatopathology. We surmise that this is because of a number of factors. As the availability of tests has increased, pathologists are progressively trained (directly or indirectly) to order more tests, on the other hand, providers that trained in an earlier era with less testing availability remain less likely to order these tests. The increased usage of tests among dermatopathology board-certified providers may be related to more overall experience with diagnosing melanocytic tumors, more familiarity with different tests and an increased likelihood of seeing more diagnostically complex cases on a more regular basis for which these tests serve as important diagnostic tools.

While the use of immunohistochemistry in anatomic pathology has been shown to be an overall cost-effective method to assist in making histopathologic diagnoses,[18] there are few guidelines for the use of IHC in pathology to date, warranting a clear need for the development of such guidelines in clinical practice. Moreover, within dermatopathology in general, and in the interpretation of melanocytic tumors specifically, these guidelines do not yet exist. It should also be emphasized that no single IHC reagent or panel of markers has thus been validated as having objective utility in the diagnostic interpretation of melanocytic tumors.[14,19] However, particular combinations of agents such as p16-Ki-67-HMB45, for example, may provide additional important information but require more detailed study.[19]

No study is without limitations. In comparison to usual practice, this study used more cases in the middle of the diagnostic spectrum (eg, MPATH-Dx classes 2–4). Participants may have shown a greater frequency of IHC utilization in our study compared to clinical practice. Moreover, both the intraobserver and interobserver concordance in IHC testing may be different, and is probably lower than, concordance for a set of cases more typical of clinical practice. Further, the use of a testing situation to gather these data, with limited but standardized clinical history and no ability to obtain a second opinion, could mean that participants were more likely to order a test for a given case compared to actual diagnostic scenarios. No data were available on pathologists' practice type or setting, which might influence ordering of IHC in the United States. Our data may also underestimate testing frequency due to continued increases in use and availability of IHC since the time of our data collection. The strengths of our study include a large test set, a full spectrum of diagnostic cases, and a large number of participating pathologists who are actively interpreting skin biopsies as part of their own clinical practices in the United States.

In conclusion, for the majority of melanocytic tumors across the diagnostic spectrum there is notable disagreement between pathologists as to whether or not they would utilize IHC. There is also a low rate of concordance for individual pathologists on whether they would employ the same test(s) when independently interpreting the same skin biopsy case a second time. These findings suggest the need for a closer examination of the diagnostic utility of IHC testing along the spectrum of melanocytic tumor diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

REFERENCES

1. Welch HG, Woloshin S, Schwartz LM. Skin biopsy rates and incidence of melanoma: population based ecological study. BMJ. 2005;331 (7515):481–481. [PubMed: 16081427]

2. Lott JP, Boudreau DM, Barnhill RL, et al. Population-based analysis of histologically confirmed melanocytic proliferations using natural language processing. JAMA Dermatol. 2018;154(1):24–29. [PubMed: 29094145]

3. Cook MG. Editorial. Diagnostic discord with melanoma. J Pathol.1997;182(3):247–249. [PubMed: 9349225]

4. Corona R, Mele A, Amini M, et al. Interobserver variability on the histopathologic diagnosis of cutaneous melanoma and other pigmented skin lesions. J Clin Oncol. 1996;14(4):1218–1223. [PubMed: 8648377]

5. Farmer ER, Gonin R, Hanna MP. Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. Hum Pathol. 1996;27(6):528–531. [PubMed: 8666360]

6. Elmore JG, Barnhill RL, Elder DE, et al. Pathologists' diagnosis of invasive melanoma and melanocytic proliferations: observer accuracy and reproducibility study. BMJ. 2017;357:j2813. [PubMed: 28659278]

7. Lott JP, Elmore JG, Zhao GA, et al. Evaluation of the melanocytic pathology assessment tool and hierarchy for diagnosis (MPATH-dx) classification scheme for diagnosis of cutaneous melanocytic neoplasms: Results from the international melanoma pathology study group. J Am Acad Dermatol. 2016;75(2):356–363. [PubMed: 27189823]

8. Idikio HA. Immunohistochemistry in diagnostic surgical pathology: contributions of protein life-cycle, use of evidence-based methods and data normalization on interpretation of immunohistochemical stains. Int J Clin Exp Pathol. 2009;3(2):169–176. [PubMed: 20126585]

9. Palit A, Inamadar AC. Immunohistochemistry: relevance in dermatology. Indian J Dermatol. 2011;56(6):629–640. [PubMed: 22345760]

10. Carney PA, Reisch LM, Piepkorn MW, et al. Achieving consensus for the histopathologic diagnosis of melanocytic lesions: use of the modified Delphi method. J Cutan Pathol. 2016;43(10):830–837. [PubMed: 27247109]

11. Dalkey NCBB, Cochran N. The Delphi method III, use of self-ratings to improve group estimates. RAND Corp. 1969.

12. Piepkorn MW, Barnhill RL, Elder DE, et al. The MPATH-dx reporting schema for melanocytic proliferations and melanoma. J Am Acad Dermatol. 2014;70(1):131–141. [PubMed: 24176521]

13. Elmore JG, Elder DE, Barnhill RL, et al. Concordance and reproducibility of melanoma staging according to the 7th vs 8th edition of the AJCC cancer staging manual. JAMA Netw Open. 2018;1(1): e180083.

14. Naert KA, Trotter MJ. Utilization and utility of immunohistochemistry in dermatopathology. Am J Dermatopathol. 2013;35(1):74–77. [PubMed: 22722468]

15. Kim RH, Meehan SA. Immunostain use in the diagnosis of melanomas referred to a tertiary medical center: a 15-year retrospective review (2001–2015). J Cutan Pathol. 2017;44(3):221–227. [PubMed: 27873341]

16. Titus LJ, Reisch LM, Tosteson ANA, et al. Malpractice concerns, defensive medicine, and the histopathology diagnosis of melanocytic skin lesions. Am J Clin Pathol. 2018;150(4):338–345. [PubMed: 30007278]

17. Carney PA, Frederick PD, Reisch LM, et al. How concerns and experiences with medical malpractice affect dermatopathologists' perceptions of their diagnostic practices when interpreting cutaneous melanocytic lesions. J Am Acad Dermatol. 2016;74(2):317.e3248–324. e3248.

18. Raab SS. The cost-effectiveness of immunohistochemistry. Arch Pathol Lab Med. 2000;124(8):1185–1191. [PubMed: 10923081]

19. Uguen A, Talagas M, Costa S, et al. A p16-Ki-67-HMB45 immunohistochemistry scoring system as an ancillary diagnostic tool in the diagnosis of melanoma. Diagn Pathol. 2015;10:195–195. [PubMed: 26503349]
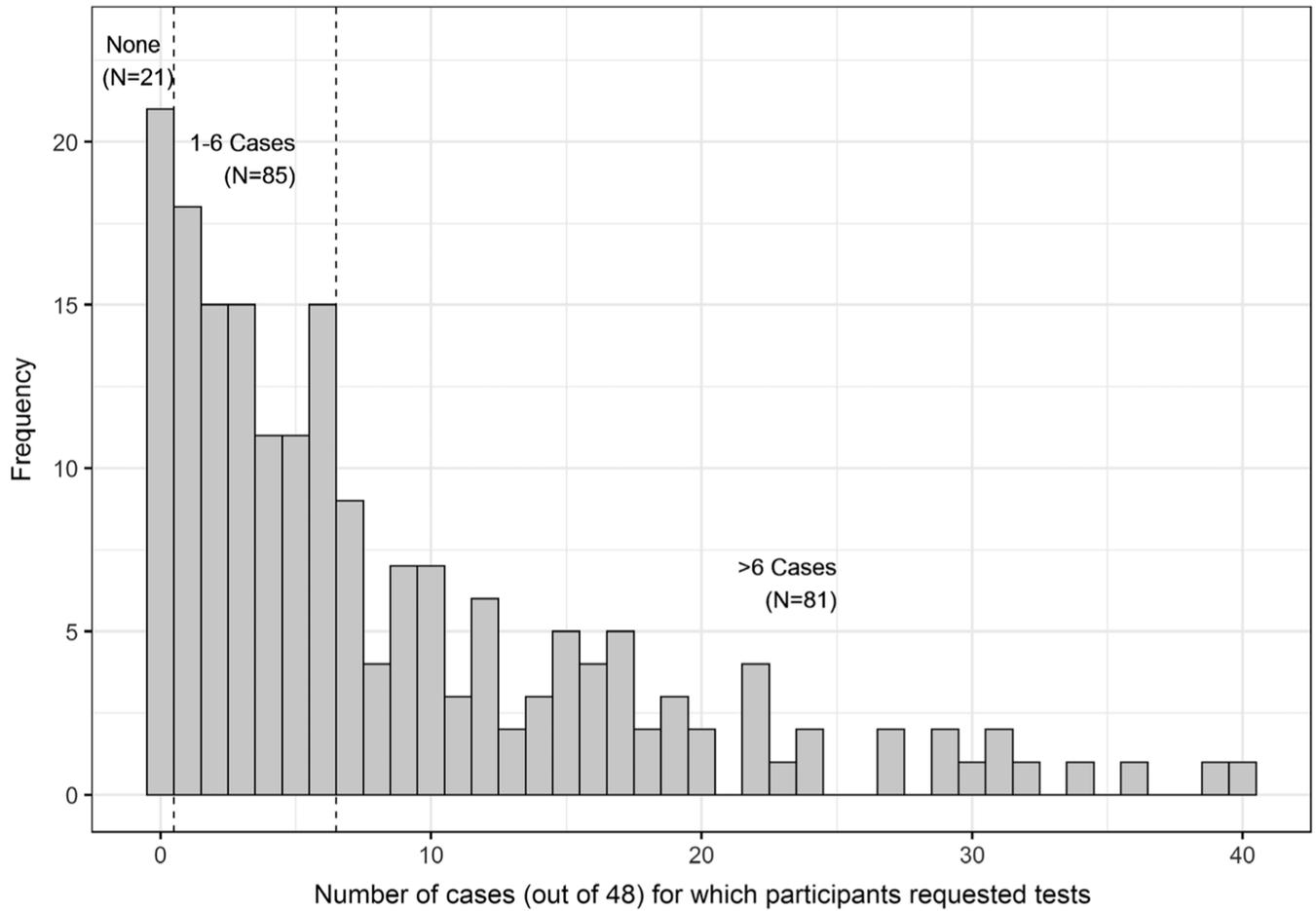
FIGURE 1.
Frequency distribution of number of skin biopsy cases (out of 48) for which participants requested IHC testing in phase I
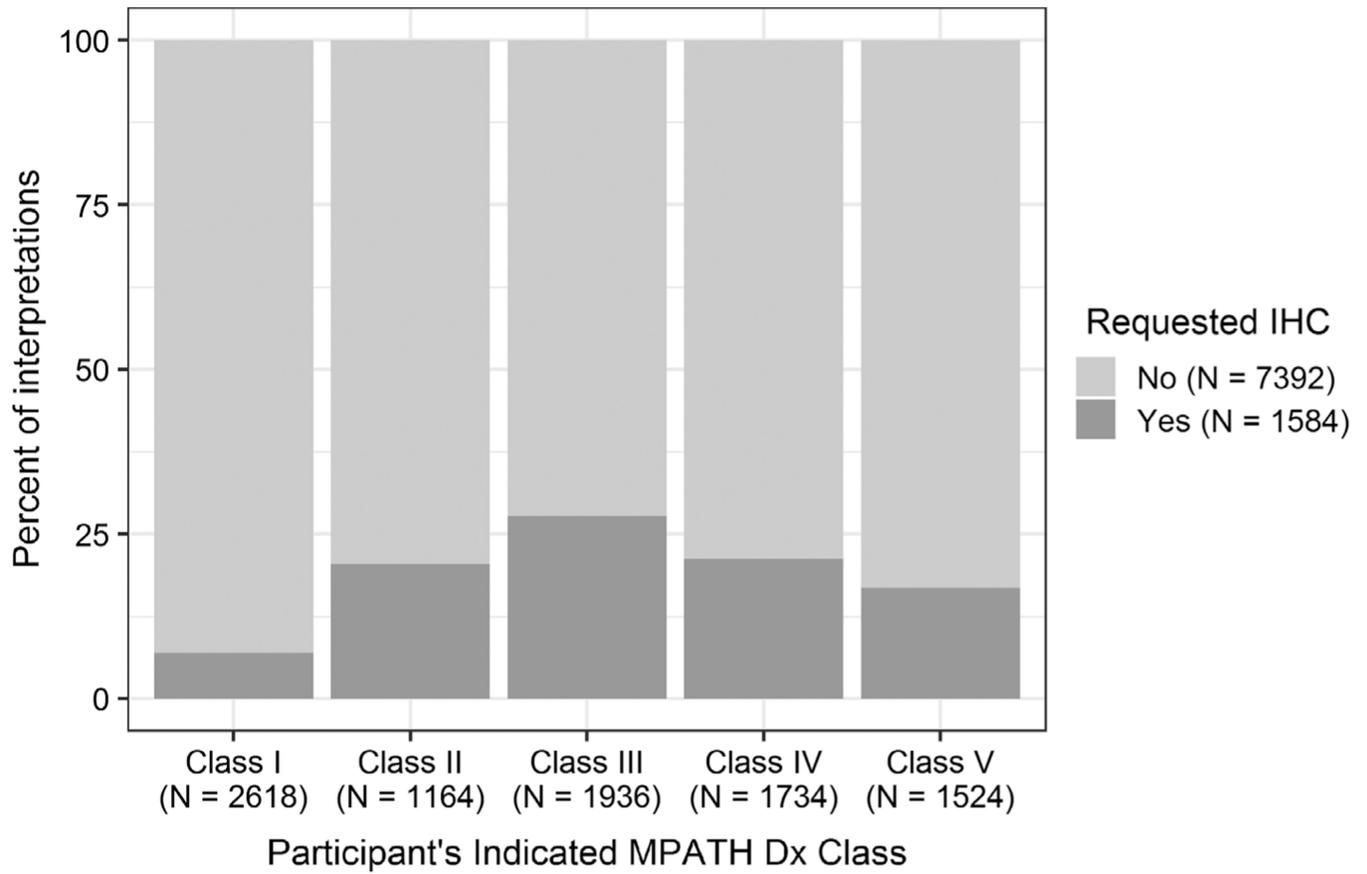
FIGURE 2.
Percentage of interpretations where IHC tests were requested, by MPATH-Dx class of the participants' interpretations
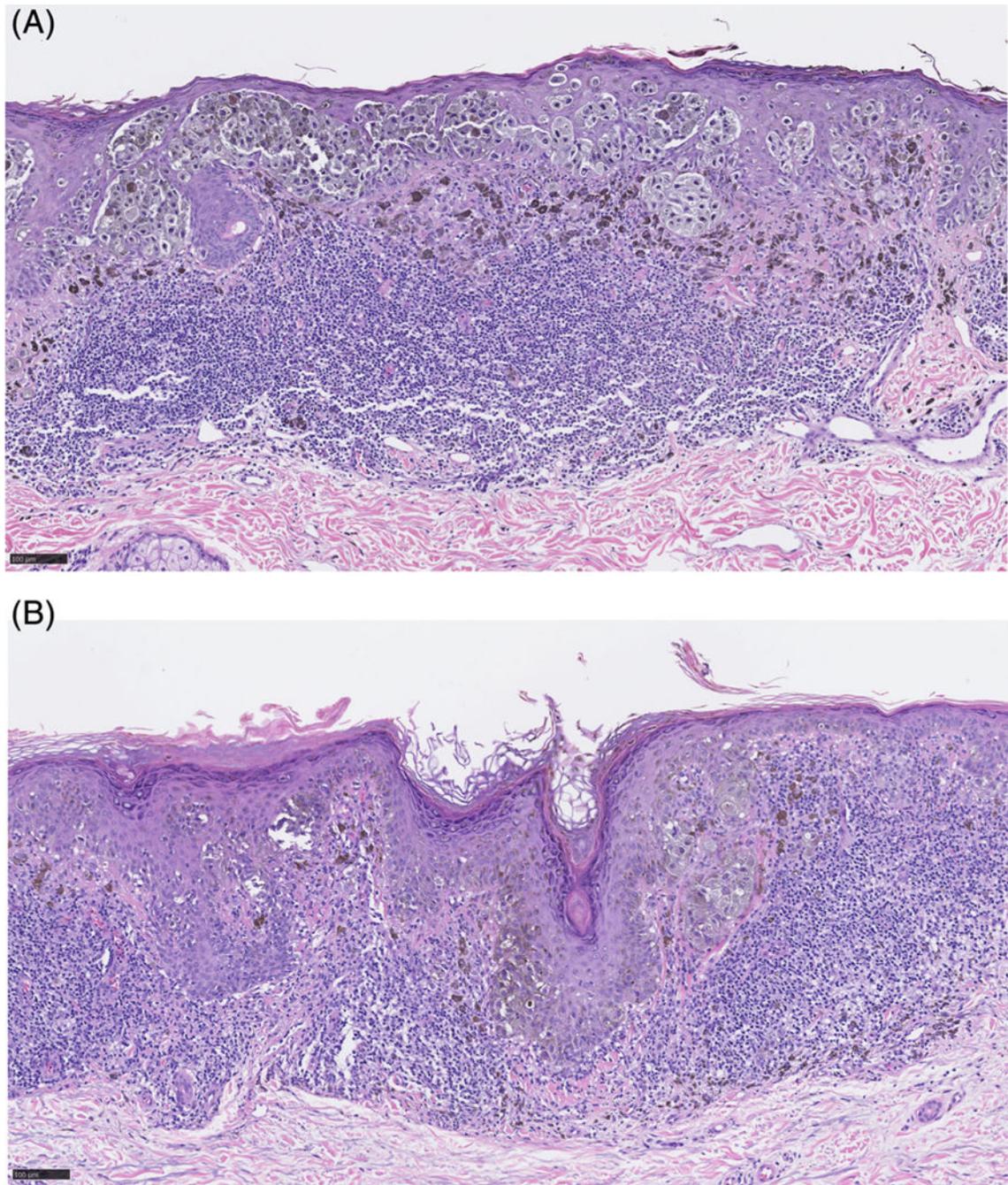
FIGURE 3.
Comparison of staining patterns between two cases, both classified by expert reference consensus as invasive melanoma. Case A (Top; H&E ×25) was interpreted by 36 pathologists in phase I, 4 (11%) of which requested at least one IHC test. Case B (Bottom; H&E ×25) was interpreted by 39 pathologists in phase I, 18 (46%) of which requested at least one IHC test

TABLE 1

Among the 8976 interpretations from 187 pathologists independently interpreting 48 cases each in phase I, IHC testing was requested in 1584 (18%) interpretation, with the type of IHC test shown here

Test	Number (%) of interpretations[a]
MART-1	1073 (67.7%)
HMB45	586 (37.0%)
Ki-67	567 (35.8%)
S100	299 (18.9%)
MITF	282 (17.8%)
p16	168 (10.6%)
SOX10	94 (5.9%)
BRAF	27 (1.7%)
Tyrosinase	21 (1.3%)
Vimentin	6 (0.4%)
BAP1	5 (0.3%)
cKit	2 (0.13%)

[a] Percent calculated out of 1584 interpretations with at least one IHC test requested (phase I). Multiple tests can be ordered per interpretation so percentages do not sum to 100%.

TABLE 2

Characteristics of pathologists (N = 187) stratified by whether or not they ordered IHC testing for none of the cases, for 1 to 6 cases or for >6 cases (phase I, each pathologist interpreted 48 total cases)

Pathologists' characteristic	All pathologists (N = 187)	How many of the pathologists' cases had IHC testing requested			P value*
		None (N = 21)	1–6 cases (N = 85)	>6 cases (N = 81)	
Participant age (y)					0.0656
<50	87	6 (6.9%)	36 (41.4%)	45 (51.7%)	
50 to <60	63	7 (11.2%)	34 (54.0%)	22 (34.9%)	
60	37	8 (21.6%)	15 (40.5%)	14 (37.8%)	
Participant gender					0.9318
Male	114	12 (10.5%)	52 (45.6%)	50 (43.9%)	
Female	73	9 (12.3%)	33 (45.2%)	31 (42.5%)	
Affiliated with academic medical center					0.4137
No	134	15 (11.1%)	65 (48.5%)	54 (40.3%)	
Yes, adjunct	34	5 (14.7%)	14 (41.1%)	15 (44.1%)	
Yes, primary appointment	19	1 (5.3%)	6 (31.6%)	12 (63.2%)	
Certified dermatopathologist					0.0109
No	115	16 (13.9%)	59 (51.3%)	40 (34.8%)	
Yes	72	5 (6.9%)	26 (36.1%)	41 (56.9%)	
What percent of usual caseload are melanocytic skin lesions?					0.6302
<10%	79	9 (11.3%)	39 (49.4%)	31 (39.2%)	
10%	108	12 (11.1%)	46 (42.6%)	50 (46.3%)	
Interpreting melanocytic skin lesions makes me more nervous than other pathologies					0.0497
Disagree	58	11 (18.9%)	21 (36.2%)	26 (44.9%)	
Agree	129	10 (7.8%)	64 (49.6%)	55 (42.6%)	
Have you ever been named in a medical malpractice suit?					0.0724
No	126	10 (7.9%)	54 (42.9%)	62 (49.2%)	
Yes, related to melanocytic skin lesions	10	1 (10.0%)	6 (60.0%)	3 (30.0%)	
Yes, related to other pathology	51	10 (19.6%)	25 (49.0%)	16 (31.4%)	

*
P value from Fisher's exact test.

TABLE 3

Pathologists' ratings of a case stratified by whether or not an IHC test was requested ($N = 8976$ independent interpretations, phase I data)

Pathologis s rating of case	All ($N = 8976$)	IHC test ordered		P value
		No ($N = 7392$)	Yes ($N = 1584$)	
Participant assigned a rating of "Not Confident" for this case				<0.0001
No	7240 (100%)	6213 (85.8%)	1027 (14.1%)	
Yes	1735 (100%)	1178 (67.9%)	557 (32.1%)	
Participant assigned a rating of "Difficult" for this case				<0.0001
No	5898 (100%)	5276 (89.5%)	622 (10.5%)	
Yes	3073 (100%)	2112 (68.7%)	961 (37.3%)	
Participant considered most advanced diagnosis for this case to be borderline				<0.0001
No	6964 (100%)	6142 (88.2%)	822 (11.8%)	
Yes	2007 (100%)	1245 (62.0%)	762 (38.0%)	
Participant would have asked for a second opinion on the case for diagnostic reasons				<0.0001
No	5077 (100%)	4610 (90.8%)	467 (9.2%)	
Yes	3899 (100%)	2782 (71.5%)	1117 (28.6%)	