Comparison of S100A8 and PRAME as biomarkers for diagnosing melanoma

Josephine Hai[1], Samantha Wong[1], Yueju Li[2], Diana Miglioretti[2], Maxwell Fung[1,3], Maija Kiuru[1,3]

Departments of Dermatology[1] and Pathology and Laboratory Medicine[3] , University of California, Davis, Sacramento, California

Department of Public Health Sciences[2] , University of California, Davis, Davis, California

INTRODUCTION

- Early diagnosis of melanoma is crucial to improved patient survival.
- Some melanomas can be difficult to diagnose from histopathology alone, and inter-observer disagreement among dermatopathologists in 15-35% of cases[1] can delay diagnosis.
- PRAME (PReferentially expressed Antigen in MElanoma) is a tumor-associated antigen found to be overexpressed in human melanomas, making it a helpful tool in differentiating between benign vs. malignant melanocytic lesions.
- PRAME immunohistochemistry (IHC) has been used increasingly in dermatopathology practice, but there is currently no single biomarker or IHC stain that is diagnostic when used alone.
- S100A8 is a calcium-binding protein found to be highly expressed in certain inflammatory conditions and human cancers[2,3]. It was recently found by Kiuru et al. to be expressed by the keratinocyte microenvironment of melanomas but not that of melanocytic nevi[4], suggesting its role as a melanoma biomarker.
- The diagnostic utility of S100A8 IHC when compared to other commonly used melanoma biomarkers, including PRAME, has not yet been assessed.

OBJECTIVE

- The objective of this study was to compare S100A8 immunohistochemistry with PRAME immunohistochemistry in benign and malignant melanocytic tumors to determine the diagnostic utility of S100A8.

METHODS

- A cohort of 252 tumors were previously identified and results of S100A8 IHC stain scoring reported in Kiuru et al.[4]
- For this study, common and dysplastic nevus cases labeled with “irregular growth,” “atypical features,” “residual,” or other modifiers were excluded.
- Melanoma in situ cases that had the modifier “residual” or were “melanoma, at least in situ” were excluded. Melanoma was defined as diagnosis of “melanoma” only.
- In total, 209 cases were included.
- Slides that were previously dual-stained for S100A8 and PRAME were reviewed and consensus PRAME IHC score was assigned by at least two reviewers (two board-certified dermatopathologists), with score indicating the proportion of tumor stained (0=indeterminate; 1= 0%; 2= 1-50%; 3> 50%).
- For S100A8, score indicated the proportion of tumor-associated epidermis stained (0=indeterminate; 1= 0-4%; 2= 5-25%; 3= 26-50%; 4= 51-75%; 5> 75%).
- Frequency tables were generated to summarize proportions of case types and S100A8 and PRAME staining scores.

METHODS

- Receiver operating characteristic (ROC) analysis was used to evaluate diagnostic accuracy of S100A8 and PRAME IHC staining.
- For binary classification, common nevus and dysplastic nevus cases were classified as not having disease, and melanoma in situ and melanoma cases were classified as having disease.
- Adjacent-category logit models were used to evaluate age, sex, and anatomic location of tumor as predictors of staining results.

RESULTS

- Patient demographics, case type, and score frequencies are summarized in Tables 1 and 2.
- The average patient age was 57 years. 53% of total patients were male, 47% female. Mean staining scores for S100A8 and PRAME were 2.07 and 2.08, respectively.
- ROC curve for S100A8 staining score had an AUC of 0.8326, while the curve for PRAME staining score had an AUC of 0.8741. Both AUCs were significantly greater than 0.5, or the AUC for chance (p-value< 0.001), but not significantly different from each other (Figure 1).
- For PRAME, when a positive test was defined as a score of 3 (>50% of tumor stained), the sensitivity was 79.80%, and the specificity was 87.27%.
- For S100A8, when a positive test was defined as a score of 4 or greater (>50% of tumor-associated epidermis stained), the sensitivity was 42.42%, and specificity was 98.18%.

Table 1. Patient demographics and melanocytic lesion characteristics

	Common nevus (n=56)	Dysplastic nevus (n=54)	Melanoma in situ (n=58)	Melanoma (n=41)
Sex of patient				
M	23	28	33	27
F	33	26	25	14
Age of patient				
< 35 yrs old	19	7	1	4
35-70 yrs old	35	40	40	20
>70 yrs old	2	7	17	17
Location of tumor				
Face	5	1	10	7
Scalp, Neck	9	0	5	5
Trunk	29	41	19	10
Upper extremity, including shoulder and hand	5	7	20	10
Lower extremity, including hip and foot	8	5	4	7

Table 2. S100A8 and PRAME staining results

	Common nevi (n=56)	Dysplastic nevi (n=54)	Melanoma in situ (n=58)	Melanoma (n=41)
S100A8 staining score: percentage of tumor-associated epidermis stained				
0-4%	47	42	20	3
5-25%	8	9	11	7
26-50%	0	2	6	10
51%-75%	0	1	14	8
>75%	1	0	7	13
PRAME staining score				
Present, focal	6	18	9	9
Present, diffuse	1	10	44	31
Absent	49	26	5	1

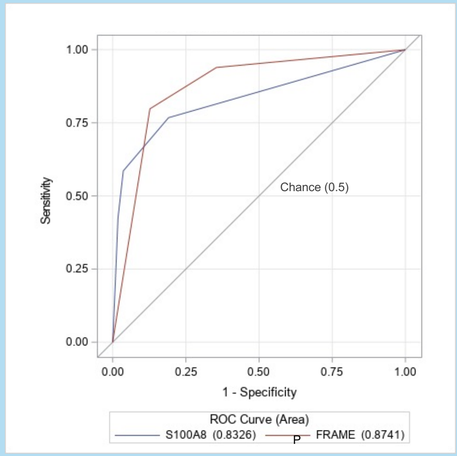

Figure 1: Receiver operating characteristic (ROC) curves comparing diagnostic test accuracy of S100A8 IHC staining to PRAME IHC staining. Both curves are also compared with the curve for chance (AUC=0.5).

Table 1, 2: Frequency tables summarizing case type, patient demographics, S100A8 staining score, and PRAME staining score. For binary classification, common and dysplastic nevus cases were classified as 0 (no disease) and melanoma in situ and melanoma cases were classified as 1(disease).

RESULTS

- When positive for a combined test was defined as S100A8 staining score of 4 or greater *or* PRAME staining score of 3, the sensitivity and specificity was 82.83% and 86.36% respectively.
- The odds of getting the next lowest S100A8 staining score (i.e., 1 vs 2, 2 vs 3 , 3 vs 4, 4 vs 5) were increased when lesions were from female patients, older patients, or from patients’ scalp, neck, trunk, upper extremities, or lower extremities rather than from the face.
- The odds of getting the next lowest PRAME staining score (i.e., 1 vs 2, 2 vs 3) were increased when lesions were from older patients and from patients’ scalp, neck, trunk, upper extremities, or lower extremities than from the face. Patient sex however had no significant effect on PRAME score.

CONCLUSIONS

- S100A8 and PRAME are both useful biomarkers for accurately detecting malignant melanocytic tumors.
- Compared to PRAME, S100A8 IHC staining is less sensitive but more specific.
- When S100A8 is used in conjunction with PRAME, sensitivity increases without significantly changing specificity.
- There is evidence for the diagnostic utility of S100A8 when interpreted alongside other histopathological features and possibly other ancillary tests, like PRAME.
- Patient age, sex, and anatomic location of tumor influence the incidence of cutaneous melanomas[5]; similarly, they can predict IHC staining score.
- This study is limited by the number of cases and participating dermatopathologists at a single institution.
- Further studies in a variety of settings comparing S100A8 to other known melanoma biomarkers may be useful.

REFERENCES

1. Gonzalez ML, Young ED, Bush J, McKenzie K, et al. Histopathologic features of melanoma in difficult-to-diagnose lesions: A case-control study. *J Am Acad Dermatol*. 2017;77(3):543-548. doi:10.1016/j.jaad.2017.03.017.
2. Ryckman C, Vandal K, Rouleau P, Talbot M, Tessier PA. Proinflammatory Activities of S100: Proteins S100A8, S100A9, and S100A8/A9 Induce Neutrophil Chemotaxis and Adhesion. *J Immunol*. 2003;170(6):3233-3242. doi:10.4049/jimmunol.170.6.3233.
3. Gebhardt C, Németh J, Angel P, Hess J. S100A8 and S100A9 in inflammation and cancer. *Biochem Pharmacol*. 2006;72(11):1622-1631. doi:10.1016/j.bcp.2006.05.017.
4. Kiuru M, Kriner MA, Wong S, et al. High-plex spatial RNA profiling reveals cell type-specific biomarker expression during melanoma development [published online ahead of print, 2021 Oct 23]. *J Invest Dermatol*. 2021;S0022-202X(21)02370-8. doi:10.1016/j.jid.2021.06.041
5. Olsen CM, Thompson JF, Pandeya N, Whiteman DC. Evaluation of Sex-Specific Incidence of Melanoma [published correction appears in JAMA Dermatol. 2020 May 1;156(5):604]. *JAMA Dermatol*. 2020;156(5):553-560. doi:10.1001/jamadermatol.2020.0470