Master Thesis

BRAF immunostaining in melanocytic lesions: association with dermoscopic features

submitted by

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under the supervision of

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Graz, 14.07.2021

DECLARATION

I declare on my word of honor that I have written the present work independently, and without external assistance, I have not used sources other than those indicated, and that I have marked the passages taken from the sources used, either literally or in terms of content, as such.

Graz, 14.07.2021 *Kyriakos Orfanidis*

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To my home clinic in Linköping, Sweden, at the Dermatology Department in Östergötland.

Introduction

Melanocytic nevi typically harbor single mutations in components of MAPK pathway known to promote cellular proliferation (Figure 1). Studies show that 80% of common acquired nevi typically have a single *BRAF*^{V600E} mutation (Pollock et al, 2002; Shain et al, 2016). *BRAF*^{V600E} mutation is also reported in 50-66% of melanomas (non-chronically sun damaged melanomas) (Davies et al, 2002; Palmieri et al, 2018). In contrast, Spitz nevi harbor an HRAS mutation or a kinase fusion of ALK, BRAF, ROS1, NTRK1, NTRK3, MET or RET (WHO, 2018) and are *BRAF*^{V600E} mutation negative.

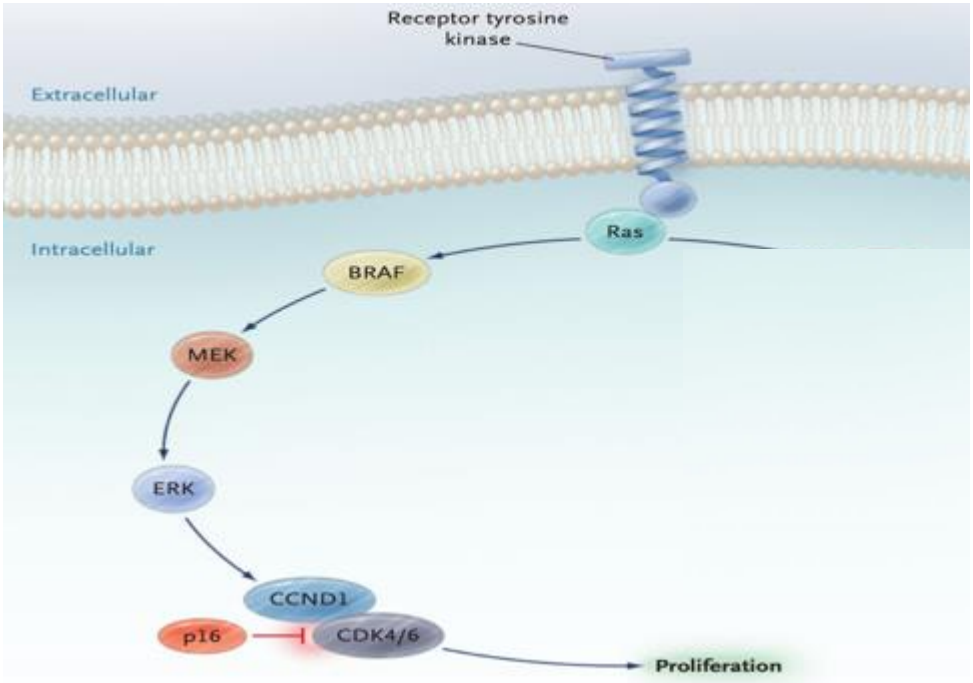
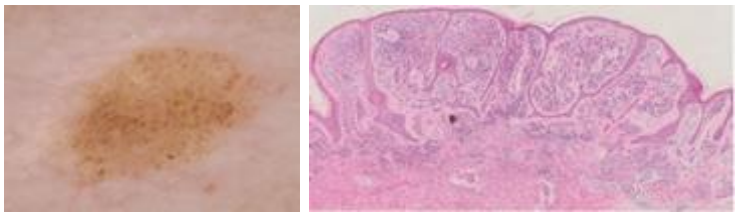
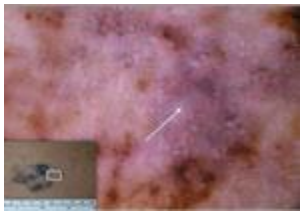


Figure 1. The Mitogen-activated protein (MAP) Kinase. Adapted and modified from Curtin et al, 2005.

BRAF mutation has been linked to specific dermoscopic patterns in common nevi and melanomas (Figure 2). Specifically, it has been associated to the globular pattern and peripheral rim of globules in common nevi (Zalaudek et al, 2011; Tan et al, 2018). In melanomas, *BRAF* mutation has been associated with the dermoscopic features of blue-white veil (Armengot-Carbó et al, 2018) and blue-grey peppering (Pozzobon et al, 2014).



A.



B.

Figure 2. Dermoscopic patterns in nevi and melanomas with BRAF mutations. A. Globular pattern of a common nevus in dermoscopy (left). Histologic correlate of the same nevus in hematoxylin & eosin stained section (right). B. Blue-grey peppering of a melanoma in dermoscopy (insert at left corner: macroscopic image). Adapted from A. Tan et al, 2018 and B. Pozzobon et al, 2014

Immunohistochemistry using a highly sensitive and specific monoclonal antibody to detect the *BRAF*^{V600E} mutation has been developed (Anwar et al, 2016, Orchard et al, 2019). In St John's Dermatopathology Department, this immunomarker has been mainly used in assessing the presence of this mutation in melanoma as it correlates very well with molecular analysis. It has also been used as an adjunct in the diagnosis of complex lesions with spitzoid features (Figure 3); The finding of a positive BRAF^{V600E} immuno-staining would rule out a Spitz nevus. However, occasional lesions within the spectrum of Spitz neoplasms show this mutation, as for example BAP-1 inactivated nevi (Wiesner et al, 2012).

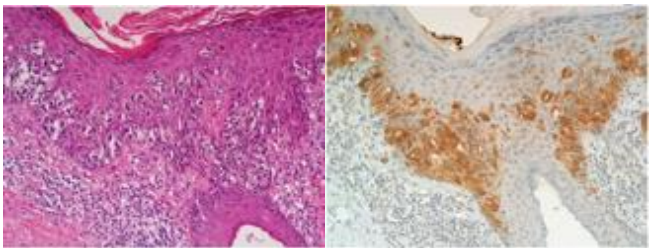


Figure 3. Pagetoid spread of tumour cells within a superficial spreading malignant melanoma. A. Hematoxylin & eosin stained section (left). B. Corresponding BRAF immunostaining (right). Adapted from Orchard et al, 2019.

Objective

The main objective of this study was to examine whether BRAF immunostaining correlates to any dermoscopic features.

Materials & Methods

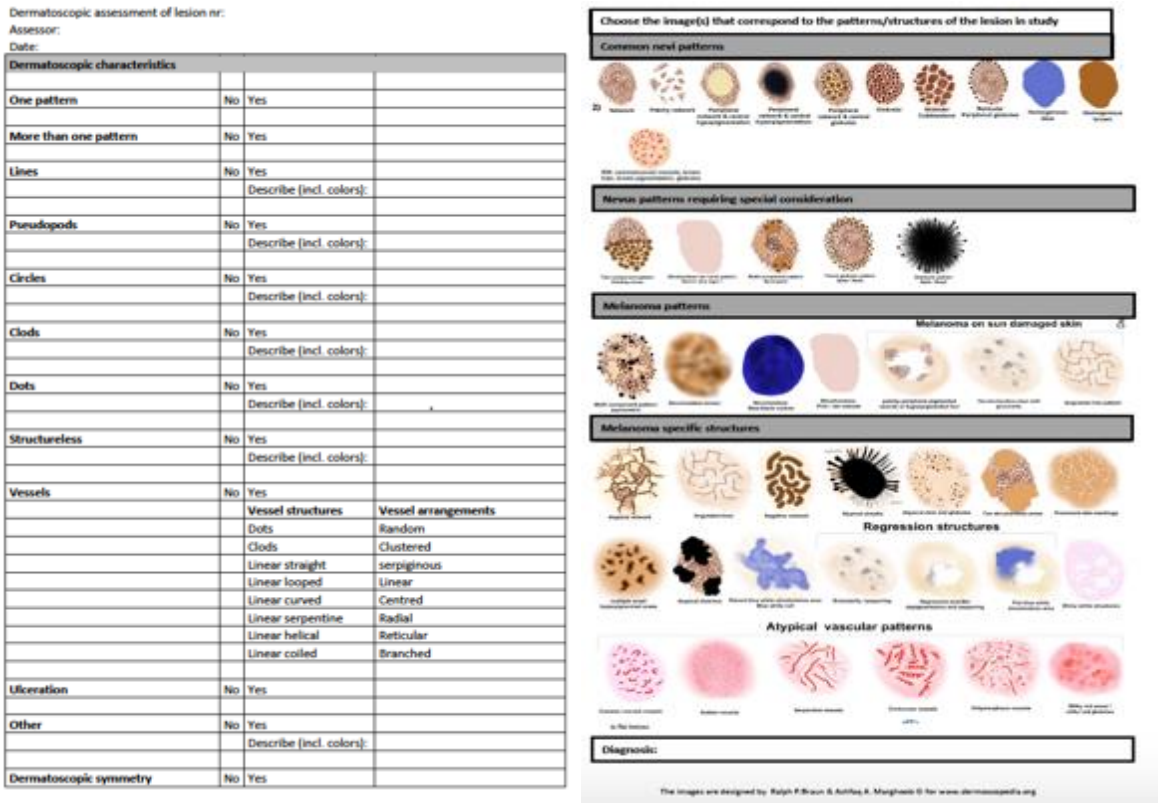
A retrospective, observational study was performed by reviewing the pathology reports of all excised melanocytic lesions (nevi and melanomas) that were BRAF immuno-stained between 1/2018 to 3/2021 in St John's Dermatopathology Department and had available dermoscopic images The patients referred to the Dermatology skin cancer service at Guys and St Thomas' Hospital.

The search of the pathology reports database for 1/2018- 3/2021 using the keyword BRAF generated 4,638 reports. This resulted in 70 lesions that were BRAF immunostained, out of which 44 melanocytic lesions had available dermoscopic images and were, therefore, included in the study (31 nevi, 13 melanomas- mean age 34 years old, 7:3 female to male ratio, 7:4 extremities to trunk ratio). The use of $BRAF^{V600E}$ immunostaining was not consistent among the dermatopathologists and was mainly used to assess the presence of this mutation in melanoma, as it correlates very well with molecular analysis, as well as an adjunct in the diagnosis of complex lesions with spitzoid features.

The used parameters from the pathology reports were: patient age, sex, anatomic location, $BRAF^{V600E}$ immunostaining outcome ($BRAF^{V600E}$ negative and $BRAF^{V600E}$ positive; now on referred to as BRAF negative and BRAF positive respectively), nevus/ melanoma diagnosis. The cases were anonymized and received a random reference number (1-44).

The 44 included samples, 13 melanomas and 31 nevi, had available macroscopic and dermoscopic images. They were independently assessed by 2 assessors (CMS and KO) with experience in dermoscopy and blinded to the histopathological diagnosis and BRAF status using parameters based on the analytic dermoscopic approach and the 2-step algorithm (Kittler et al, 2016; Braun et al, 2019): suspected diagnosis, dermoscopic symmetry, characterization and presence of one or more dermoscopic patterns, lines, pseudopods, circles, clods, dots, structureless areas, vessels, ulceration, common nevus patterns, special consideration nevus patterns, melanoma patterns and melanoma specific structures.

A form was used to assist the dermoscopic assessments, which included images designed by Braun R.P. & Marghoob A.A. © and deposited in dermoscopedia (Figure 4). A consensus was reached in case of initial differences by discussing the dermoscopic features; a process that did not lead to further disagreements. Finally, the dermoscopic features were converted to categorical variables and transferred to a datasheet by KO.



Dermatoscopic assessment of lesion nr:
Assessor:
Date:

Dermatoscopic characteristics			
One pattern	No	Yes	
More than one pattern	No	Yes	
Lines	No	Yes	
		Describe (incl. colors):	
Pseudopods	No	Yes	
		Describe (incl. colors):	
Circles	No	Yes	
		Describe (incl. colors):	
Clods	No	Yes	
		Describe (incl. colors):	
Dots	No	Yes	
		Describe (incl. colors):	
Structureless	No	Yes	
		Describe (incl. colors):	
Vessels	No	Yes	
		Vessel structures	Vessel arrangements
		Dots	Random
		Clods	Clustered
		Linear straight	serpiginous
		Linear looped	Linear
		Linear curved	Centred
		Linear serpentine	Radial
		Linear helical	Reticular
		Linear coiled	Branched
Ulceration	No	Yes	
Other	No	Yes	
		Describe (incl. colors):	
Dermatoscopic symmetry	No	Yes	

Figure 4. Dermoscopy assessment form. Adapted from Orfanidis et al, 2019.

Unpaired t-test and Fisher's exact test were used to determine the significance of difference. Statistically significant differences were indicated as p-values ≤ 0.05. Statistical tests were performed using SPSS (IBM SPSS Statistics, Version 27. Armonk, NY, USA). To determine the effects of different predictors, a logistic regression analysis was performed using SPSS (IBM SPSS Statistics, Version 25. Armonk, NY, USA). In cases of complete separation data patterns, the Firth bias-correction was used (Heinze et al, 2002). The interrater reliability analysis using the Kappa statistic was performed to determine consistency among raters in dermoscopy. A kappa value of 0.81-1.00 indicates almost perfect agreement, 0.61- 0.8 indicates substantial agreement, 0.41-0.60

indicates moderate agreement, 0.21-0.40 indicates fair agreement, 0.00-0.20 slight and <0 poor agreement (Landis & Koch, 1977).

Results

Baseline characteristics

We included 44 melanocytic lesions (31 nevi, 70%; 13 melanomas, 30%) from 44 patients, 30 of which were BRAF negative and 14 BRAF positive (Table 1). The patients' average age was 34 years old, females were mostly represented (31 females, 70%; 13 males, 30%) and most of the lesions were located in the extremities (28 in extremities, 64%; 16 in the trunk, 36%).

The patients with nevi were in their majority females at the average age of 31 years old. The nevi were mostly located on the extremities and were compound. Their histological morphology, as described in table 1, was not common but often classified as of Spitz or dysplastic type.

The patients with primary cutaneous melanomas were also in their majority females at the average age of 40 years old. The melanomas were located both in the extremities and trunk and their type varied from in situ lesions to those invading the subcutaneous tissue, but in their majority, they were invading the reticular dermis with an average Breslow thickness of 1 mm (Table 2).

There were no statistically significant differences for age, sex and anatomic location among the melanoma and nevi groups.

Anatomic location	Histological type/ features	BRAF IHC
compound	Melanocytic nevus with spitzoid features	Positive
compound	Spitz nevus with moderate atypia and traumatisation features	Negative
compound	Dysplastic nevus with moderate to severe atypia	Positive
compound	Dysplastic nevus with moderate to severe atypia	Negative
compound	Lentiginous melanocytic nevus with congenital features	Negative
intradermal	Blue nevus	Negative
compound	Spitz nevus with moderate atypia	Negative
compound	Melanocytic nevus with spitzoid features	Positive
compound	Spitz nevus	Negative
compound	Spitz nevus	Negative
compound	Spitz nevus	Negative
junctional	Spitz nevus with traumatisation features	Negative
compound	Desmoplastic Spitz nevus	Negative
compound	Deep penetrating nevus	Positive
junctional	Spitz nevus	Negative
compound	Spitz nevus with moderate atypia and traumatisation features	Negative
compound	Spitz nevus with focal desmoplasia	Negative
compound	Spitz nevus with focal desmoplasia	Negative
compound	Spitz nevus	Negative
compound	Spitz nevus	Negative
compound	Melanocytic nevus with congenital and traumatisation features	Negative
compound	Spitz nevus with traumatisation features	Negative
junctional	Halo nevus with spitzoid features	Negative
compound	Spitz nevus	Negative
compound	Dysplastic nevus with moderate atypia	Positive
compound	Spitz nevus with traumatisation features	Negative
compound	Dysplastic nevus with moderate atypia	Negative
compound	Combined nevus (Spitz and common)	Negative
compound	Spitz nevus	Negative
compound	BAP-1 inactivated	Positive
junctional	Spitz nevus	Negative

Table 1. Characteristics of the melanocytic nevi in the study.

Stage	Anatomic level	Breslow thickness	Type	Other characteristics	BRAF IHC
T2a	IV	1.8	not specified		Negative
Tis	I		not specified		Positive
T1a	III	0.2	not specified		Positive
T3a	IV	2.4	not specified		Negative
T1b	III	0.8	nevoid		Positive
T2a	IV	1.9	nevoid	associated compound melanocytic nevus	Positive
T3a	V	3	Spitz		Negative
T1b	III	0.8	SSM		Negative
Tis	I		not specified		Positive
T3a	III	2.1	not specified		Negative
Tis	I		not specified	associated compound melanocytic nevus	Positive
T1a	II	0.2	not specified		Positive
Tis	I		not specified	associated compound melanocytic nevus	Positive

Table 2. Characteristics of the cutaneous melanomas in the study.

Since the focus in this study was on distinguishing features among the BRAF immunostained categories, the lesions were grouped in BRAF positive and negative. There were no differences in the patients' age, sex and lesional anatomical location between the BRAF negative and BRAF positive groups (Table 3). However, the proportion of nevi was higher in the BRAF negative than in the BRAF positive group (25/30 vs 6/14, p=0.012).

Clinical & histopathological variables	Category	Total, n=44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)
Age		**33.95±1.8[a]**	**32.7±1.8[a]**	**36.6±4.1[a]**	**0.326[b]**	**-**
Sex	**Male**	**13 (29.5%)**	**10 (33.3%)**	**3 (21.4%)**		**Ref.**
	Female	**31 (70.5%)**	**20 (66.7%)**	**11 (78.6%)**	**0.498[c]**	**0.5 (0.1-2.4)**
Anatomical location	**Trunk**	**16 (36.4%)**	**10 (33.3%)**	**6 (42.9%)**		**Ref.**
	Extremities	**28 (63.6%)**	**20 (66.7%)**	**8 (57.1%)**	**0.738[c]**	**1.5 (0.4-5.5)**
Diagnosis	**Melanoma**	**13 (29.5%)**	**5 (16.7%)**	**8 (57.1%)**		
	Nevus	**31 (70.5%)**	**25 (83.3%)**	**6 (42.9%)**	**0.012[c]**	**6.6 (1.6-27.8)**

Table 3. Baseline characteristics. Associations among BRAF negative and BRAF positive melanocytic tumours.

OR, odds ratio; CI, confidence interval; bold, significance.
[a]Values expressed as the mean and standard error of the mean; [b]Student's t-test; [c]Fisher's exact test

Dermoscopic features

For the dermoscopic analysis, all the 44 lesions, 31 nevi and 13 melanomas, were included (Table 4). Most of the lesions demonstrated dermoscopic asymmetry (n=28, 63.6%), non-spitzoid dermoscopic morphology (n=36, 81.8%), no ulceration (n=0, 0%) and were suspicious for melanomas (n=28, 63.6%). Based on the dermoscopic morphology, the lesions were difficult to be correctly identified as nevi or melanomas (sensitivity=69.2%, specificity=38.7%) and as spitzoid (sensitivity=21.4%, specificity=87.5%). However, it was similarly difficult among the BRAF positive and BRAF negative lesions.

Dermoscopic variables	Category	Total, n=44 (100%)	**BRAF-,n=30 (68.2%)**	**BRAF+,n= 14 (31.8%)**	**p-value**	**OR (95% CI)**	kappa	p-value (agreement)
Dermoscopic symmetry	Absent	28 (63.6%)	18 (60%)	10 (71.4%)		0.6 (0.2-2.4)		
	Present	16 (36.4%)	12 (40%)	4 (28.6%)	0.521	Ref.	0.513	<0.001
Spitzoid dermoscopic morphology	Absent	36 (81.8%)	23 (76.7%)	13 (92.9%)		0.3 (0.03-2.3)		
	Present	8 (18.2%)	7 (23.3%)	1 (7.1%)	0.402	Ref.	0.457	<0.001
Dermoscopic diagnosis	Melanoma	28 (63.6%)	19 (63.3%)	9 (64.3%)		0.96 (0.3-3.6)		
	Nevus	16 (36.4%)	11 (36.7%)	5 (35.7%)	1	Ref.	0.436	0.003
Lines	Absent	17 (38.6%)	12 (40%)	5 (35.7%)		1.2 (0.3-4.5)		
	Present	27 (61.4%)	18 (60%)	9 (64.3%)	1	Ref.	0.436	0.004
Structureless	Absent	7 (15.9%)	5 (16.7%)	2 (14.3%)		1.2 (0.2-7.1)		
	Present	37 (84.1%)	25 (83.3%)	12 (85.7%)	1	Ref.	0.303	0.023
Pseudopods	Absent	39 (88.6%)	25 (83.3%)	14 (100%)				
	Present	5 (11.4%)	5 (16.7%)	0 (0%)	0.161	NA	0.398	<0.001
Circles	Absent	37 (84.1%)	25 (83.3%)	12 (85.7%)		0.8 (0.1-4.9)		
	Present	7 (15.9%)	5 (16.7%)	2 (14.3%)	1	Ref.	0.327	0.003
Clods	Absent	27 (61.4%)	19 (63.3%)	8 (57.1%)		1.3 (0.4-4.7)		
	Present	17 (38.6%)	11 (36.7%)	6 (42.9%)	0.748	Ref.	0.674	<0.001
Dots	Absent	31 (70.5%)	22 (73.3%)	9 (64.3%)		1.5 (0.4-6.0)		
	Present	13 (29.5%)	8 (26.7%)	5 (35.7%)	0.724	Ref.	0.433	0.003
Vessels	Absent	35 (79.5%)	23 (76.7%)	12 (85.7%)		0.5 (0.1-3.1)		
	Present	9 (20.5%)	7 (23.3%)	2 (14.3%)	0.695	Ref.	0.667	<0.001

Table 4. Dermoscopic features. Associations among BRAF negative and BRAF positive melanocytic tumours.

OR, odds ratio; CI, confidence interval; bold, significance.

[c]Fisher's exact test

The majority of the lesions had lines (n=27, 61.4%), of which brown thick reticular (n=9, 20.5%) and brown thin reticular lines (n=6, 13.6%) were presented more often. Structureless areas (n=37, 84.1%) was the most common feature, with brown (n=27, 61.4%) and hypopigmented (n=8, 18.2%) structureless areas being mostly represented.

The dermoscopic features that differentiated significantly among nevi and melanomas were the presence of polymorphous vessels (4/9 in melanomas vs 1/30 in nevi, p=0.022) and of brown structureless areas (11/2 in melanomas vs 16/15 in nevi, p=0.05).

Dermoscopic - BRAF^{V600E} immunohistochemical associations

Two dermoscopic features were associated with BRAF immunostaining: the brown structureless and hypopigmented structureless areas (Table 5, Figure 5A-B).

The odds of brown structureless dermoscopic feature in a BRAF negative melanocytic tumour was 6 times as small the odds of this feature in BRAF positive melanocytic tumours. The odds of hypopigmented structureless dermoscopic feature in BRAF negative melanocytic tumours is 11 times as large the odds of this feature in BRAF positive melanocytic tumours.

Dermoscopic variables	Category	Total,n=44 (100%)	BRAF-,n=30 (68.2%)	BRAF+,n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
Brown structureless	Absent	17 (38.6%)	15 (50%)	2 (14.3%)	**0.044**[c]	6 (1.1-31.5)	0.433	0.002
	Present	27 (61.4%)	15 (50%)	12 (85.7%)		Ref.		
Hypopigmented structureless	Absent	36 (81.8%)	22 (73.3%)	14 (100%)	**0.041**[c]	Ref.	0.831	<0.001
	Present	8 (18.2%)	8 (26.7%)	0 (0%)		11[d] (1.2-1456.8)		

Table 5. Significant dermoscopic - BRAF^{V600E} immunohistochemical associations.

OR, odds ratio; CI, confidence interval; bold, significance.
[c]Fisher's exact test; [d]Firth bias correction

Further analyses within the groups of nevi and melanomas among BRAF negative and BRAF positive melanocytic tumours distinguished the globular cobblestone dermoscopic feature in nevi (Table 6, Figure 5C). The globular cobblestone pattern was found more commonly in BRAF positive than in BRAF negative nevi (2/6 vs 0/25, p<0.001).

Dermoscopic variables	Diagnosis	Category	Total, n=31 (100%)	BRAF-, n=25 (80.6%)	BRAF+, n=6 (19.4%)	p-value	OR (95% CI)	kappa	p-value (agreement)
Globular cobblestone	Nevus	Absent	29 (93.5%)	25 (100%)	4 (66.7%)		28.3[d] (1.9-4179.7)		
		Present	2 (6.5%)	0 (0%)	2 (33.3%)	**0.032**[c]	Ref.	0.656	<0.001

Table 6. Significant dermoscopic - $BRAF^{V600E}$ immunohistochemical associations in nevi and melanomas.

OR, odds ratio; CI, confidence interval; bold, significance.
[c]Fisher's exact test; [d]Firth bias correction

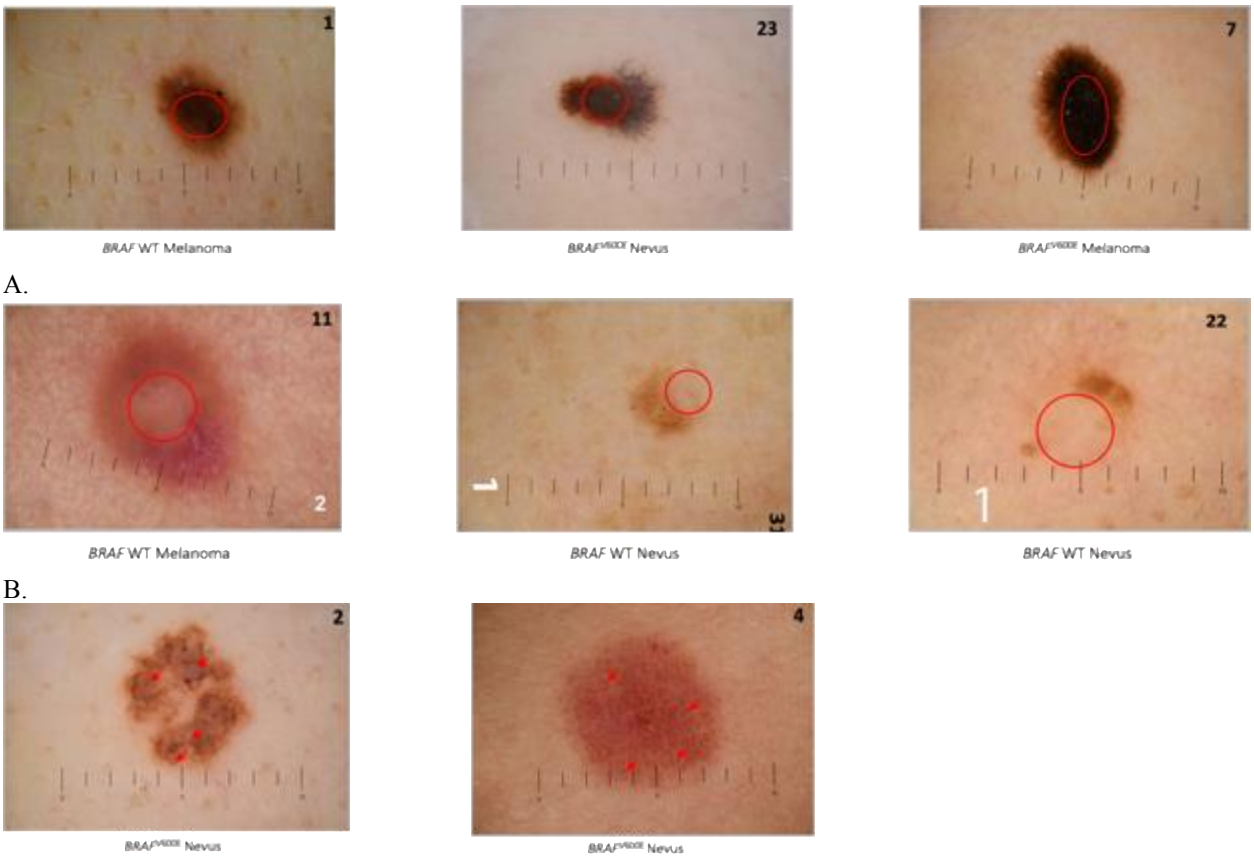


Figure 5. Dermoscopic features associated with BRAF immunostaining. A. Brown structureless areas in melanocytic tumours of our dataset. The red encircled areas depict this feature. B. Hypopigmented structureless areas in melanocytic tumours of our dataset. The red encircled areas depict this feature. C. Globular cobblestone pattern in nevi of our dataset. The red arrows depict the polygonal clods.

BRAF negative prediction model

The hypopigmented (OR:14, 95% CI: 1.4-1887, p=0.021) and brown (OR: 0.17, 95% CI: 0.03-0.74, p= 0.017) structureless areas maintained statistical significance after multivariate analysis and were the most important variables for predicting the BRAF immunostaining status. The highest likelihood of finding BRAF negative melanocytic tumours after immunostaining occurred when the dermoscopic feature of hypopigmented structureless areas is present with or without the simultaneous presence of brown structureless areas (Figure 6).

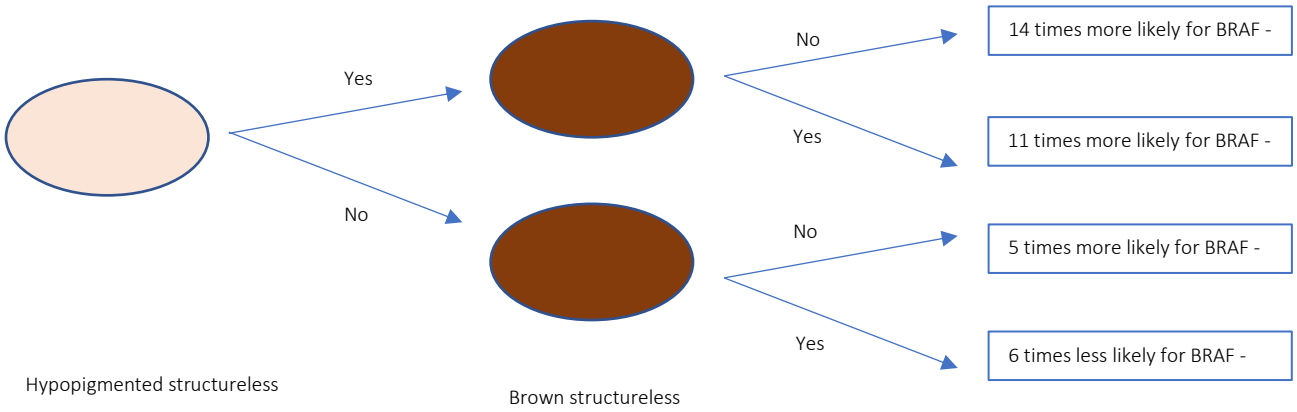


Figure 6. Prediction diagram for BRAF negative melanocytic tumours based on the dermoscopic features of our dataset.

Discussion

In this study, the dermoscopic – $BRAF^{V600E}$ immunohistochemical associations in melanocytic tumours were sought.

In line with previous data (Zalaudek et al, 2011; Tan et al, 2018), we found that the globular cobblestone dermoscopic pattern in nevi is linked to $BRAF^{V600E}$ mutation (Figure 5C). This feature, defined as polygonal clods throughout the lesion, appeared only in 2 out of 6 BRAF positive nevi and in none of BRAF negative nevi. It corresponds histologically to the nests of nevomelanocytes at the dermo-epidermal junction or dermis (Yélamos et al, 2019).

Our results show the association between hypopigmented and brown structureless areas in dermoscopy with $BRAF^{V600E}$ immunostaining of melanocytic tumours. The brown structureless areas (Figure 5A) are defined as hyperpigmented or regularly pigmented areas within a lesion covering at least 10% of the lesion's total surface area that are devoid of any structures and the histological correlate is aggregates of melanin in the stratum corneum or throughout all layers of the epidermis (Braun et al, 2019). The hypopigmented structureless areas (Figure 5B) are defined as areas covering at least 10% of the lesion's total surface area and having a lighter pigment compared with the rest of the lesion, with same or slightly more pigment compared with the surrounding normal skin, that are devoid of any structures (Braun et al, 2019). The histological correlate of this dermoscopic feature has been reported as the flattening of the dermo-epidermal junction and scattering of atypical melanocytes in suprabasal epidermis and as dermal fibrosis (Braun et al, 2019). A search in the literature has not highlighted these two dermoscopic features in association to BRAF status before and the possible implications remain to be elucidated.

The main limitations of our study are: the selection bias for BRAF immunostained melanocytic tumours, the single method to detect BRAF mutation using immunohistochemistry, the availability of dermoscopic images in 44 out of 70 (63%) BRAF immunostained lesions and the fact that the accuracy of the predictive model has not been tested with a new dataset. The power of this study based on the dermoscopic features of brown and hypopigmented structureless areas lies at 61%-

64% indicating that further studies have to be performed on a larger number of melanocytic tumours to investigate these associations.

The strength of this study lies on the selection of melanocytic tumours, irrespective of a nevus or melanoma diagnosis, that were BRAF immunostained. Since many of these lesions were difficult to diagnose based on their dermoscopic and histological morphology, searching for dermoscopic features that associate to BRAF status is an interesting approach to obtain more information for the molecular events taking place in such lesions.

With the broader use of molecular tests on melanocytic lesions, more biological insight is constantly gained that can eventually result in a refinement of the current classification. Therefore, further studies that integrate this information and evaluate the association between dermoscopic, histological and genetic features have to be performed.

References

Anwar, M., Murad, F., Dawson, E., Elmageed, Z., Tsumagari, K., and Kandil, E. (2016). Immunohistochemistry as a reliable method for detection of BRAF-V600E mutation in melanoma: a systematic review and meta-analysis of current published literature. J Surg Res 203, 407–415.

Armengot-Carbó M, Nagore E, García-Casado Z, Botella-Estrada R. (2018) The association between dermoscopic features and BRAF mutational status in cutaneous melanoma: Significance of the blue-white veil. J Am Acad Dermatol. 78(5):920-926.e4.

Braun, R.P., Marghoob, A.A., Lallas, A. (2019). Two-step algorithm. dermoscopedia. Retrieved from https://dermoscopedia.org/w/index.php?title=Two-step_algorithm&oldid=15900.

Braun, R.P., Kerl, K., Yélamos, O. (2019). Structureless areas. dermoscopedia. Retrieved from https://dermoscopedia.org/w/index.php?title=Structureless_areas&oldid=15816.

Curtin, J., Fridlyand, J., Kageshita, T., Patel, H., Busam, K., Kutzner, H., Cho, K.-H., Aiba, S., Bröcker, E.-B., LeBoit, P., et al. (2005). Distinct Sets of Genetic Alterations in Melanoma. New Engl J Medicine 353, 2135–2147.

Davies, H., Bignell, G., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M., Bottomley, W., et al. (2002). Mutations of the BRAF gene in human cancer. Nature 417, 949.

Heinze, G., and Schemper, M. (2002). A solution to the problem of separation in logistic regression. Statistics in Medicine 21, 2409–2419.

Kittler, H., Marghoob, A.A., Argenziano, G., Carrera, C., Curiel-Lewandrowski, C., Hofmann-Wellenhof, R., Malvehy, J., Menzies, S., Puig, S., Rabinovitz, H., et al. (2016). Standardization of terminology in dermoscopy/dermatoscopy: Results of the third consensus conference of the International Society of Dermoscopy. J. Am. Acad. Dermatol. 74, 1093–1106.

Landis, R., and Koch, G. (1977). The Measurement of Observer Agreement for Categorical Data. Biometrics 33, 159.

Orchard, G.E., Wojcik, K., Rickaby, W., Martin, B., Semkova, K., Shams, F., Stefanato, C.M. (2019). Immunohistochemical detection of V600E BRAF mutation is a useful primary screening tool for malignant melanoma. Br J Biomed Sci. 76(2), 77-82.

Orfanidis, K. (2019). Identification and clinical evaluation of senescence-associated markers to distinguish melanocytic nevi from melanomas. (Doctoral dissertation). Linköping: Linköping University Electronic Press.

Palmieri, G., Colombino, M., Casula, M., Manca, A., Mandal., M., Cossu, A., and (IMI), I. (2018). Molecular Pathways in Melanomagenesis: What We Learned from Next-Generation Sequencing Approaches. Current Oncology Reports 20, 86.

Pollock, P., Harper, U., Hansen, K., Yudt, L., Stark, M., Robbins, C., Moses, T., Hostetter, G., Wagner, U., Kakareka, J., et al. (2002). High frequency of BRAF mutations in nevi. Nat Genet 33, ng1054.

Pozzobon FC, Puig-Butillé JA, González-Alvarez T, Carrera C, Aguilera P, Alos L, Badenas C, Grichnik JM, Malvehy J, Puig S. (2014). Dermoscopic criteria associated with BRAF and NRAS mutation status in primary cutaneous melanoma. Br J Dermatol. 171(4):754-9.

Shain, A.H., and Bastian, B.C. (2016). From melanocytes to melanomas. Nat. Rev. Cancer 16, 345–358.

Tan, J., Tom, L., Jagirdar, K., Lambie, D., Schaider, H., Sturm, R., Soyer, H. and Stark, M. (2018), The BRAF and NRAS mutation prevalence in dermoscopic subtypes of acquired naevi reveals constitutive mitogen-activated protein kinase pathway activation. Br J Dermatol, 178: 191-197.

WHO Classification of Skin Tumours, World Health Organization; (2018)

Wiesner, T., Murali, R., Fried, I., Cerroni, L., Busam, K., Kutzner, H., and Bastian, B. (2012). A Distinct Subset of Atypical Spitz Tumors is Characterized by BRAF Mutation and Loss of BAP1 Expression. Am J Surg Pathology 36, 818–830.

Yélamos O, Braun RP, Liopyris K, Wolner ZJ, Kerl K, Gerami P, Marghoob AA. (2018) Dermoscopy and dermatopathology correlates of cutaneous neoplasms. J Am Acad Dermatol. 80(2):341-363.

Zalaudek I, Guelly C, Pellacani G, Hofmann-Wellenhof R, Trajanoski S, Kittler H, Scope A, Marghoob AA, Longo C, Leinweber B, Ferrara G, Saida T, Grichnik JM, Argenziano G, Becker JC. (2011). The dermoscopical and histopathological patterns of nevi correlate with the frequency of BRAF mutations. J Invest Dermatol. 131(2):542-5.

Supplementary tables

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	42 (95.5%)	29 (96.7%)	13 (92.9%)		2.2 (0.1-38.5)		
One pattern	Present	2 (4.5%)	1 (3.3%)	1 (7.1%)	0.54	Ref.	-0.031	0.825
	Absent	2 (4.5%)	1 (3.3%)	1 (7.1%)		0.4 (0.03-7.7)		
More than one pattern	Present	42 (95.5%)	29 (96.7%)	13 (92.9%)	0.54	Ref.	-0.031	0.825
	Absent	17 (38.6%)	12 (40%)	5 (35.7%)		1.2 (0.3-4.5)		
Lines	Present	27 (61.4%)	18 (60%)	9 (64.3%)	1	Ref.	0.436	0.004
	Absent	39 (88.6%)	25 (83.3%)	14 (100%)				
Pseudopods	Present	5 (11.4%)	5 (16.7%)	0 (0%)	0.161	NA	0.398	<0.001
	Absent	37 (84.1%)	25 (83.3%)	12 (85.7%)		0.8 (0.1-4.9)		
Circles	Present	7 (15.9%)	5 (16.7%)	2 (14.3%)	1	Ref.	0.327	0.003
	Absent	27 (61.4%)	19 (63.3%)	8 (57.1%)		1.3 (0.4-4.7)		
Clods	Present	17 (38.6%)	11 (36.7%)	6 (42.9%)	0.748	Ref.	0.674	<0.001
	Absent	31 (70.5%)	22 (73.3%)	9 (64.3%)		1.5 (0.4-6.0)		
Dots	Present	13 (29.5%)	8 (26.7%)	5 (35.7%)	0.724	Ref.	0.433	0.003
	Absent	7 (15.9%)	5 (16.7%)	2 (14.3%)		1.2 (0.2-7.1)		
Structureless	Present	37 (84.1%)	25 (83.3%)	12 (85.7%)	1	Ref.	0.303	0.023
	Absent	35 (79.5%)	23 (76.7%)	12 (85.7%)		0.5 (0.1-3.1)		
Vessels	Present	9 (20.5%)	7 (23.3%)	2 (14.3%)	0.695	Ref.	0.667	<0.001
	Absent	44 (100%)	30 (100%)	14 (100%)				
Ulceration	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	28 (63.6%)	18 (60%)	10 (71.4%)		0.6 (0.2-2.4)		
Dermoscopic symmetry	Present	16 (36.4%)	12 (40%)	4 (28.6%)	0.521	Ref.	0.513	<0.001
	Absent	36 (81.8%)	23 (76.7%)	13 (92.9%)		0.3 (0.03-2.3)		
Spitzoid dermoscopic morphology	Present	8 (18.2%)	7 (23.3%)	1 (7.1%)	0.402	Ref.	0.457	<0.001
	Melanoma	28 (63.6%)	19 (63.3%)	9 (64.3%)		0.96 (0.3-3.6)		
Dermoscopic diagnosis	Nevus	16 (36.4%)	11 (36.7%)	5 (35.7%)	1	Ref.	0.436	0.003

Supplementary table 1. General dermoscopic features

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	35 (79.5%)	23 (76.7%)	12 (85.7%)		0.5 (0.1-3.1)		
Vessels	Present	9 (20.5%)	7 (23.3%)	2 (14.3%)	0.695	Ref.	0.667	<0.001
	Absent	36 (81.8%)	24 (80%)	12 (85.7%)		0.7 (0.1-3.8)		
Vessels dots	Present	8 (18.2%)	6 (20%)	2 (14.3%)	1	Ref.	0.369	0.014
	Absent	42 (95.5%)	28 (93.3%)	14 (100%)				
Vessels clods	Present	2 (4.5%)	2 (6.7%)	0 (0%)	1	NA	NA	NA
	Absent	42 (95.5%)	28 (93.3%)	14 (100%)				
Vessels straight linear	Present	2 (4.5%)	2 (6.7%)	0 (0%)	1	NA	0.656	<0.001
	Absent	41 (93.2%)	28 (93.3%)	13 (92.9%)		1.1 (0.09-13.0)		
Vessels looped linear	Present	3 (6.8%)	2 (6.7%)	1 (7.1%)	1	Ref.	0.788	<0.001
	Absent	41 (93.2%)	29 (96.7%)	12 (85.7%)		4.8 (0.4- 58.5)		
Vessels curved linear	Present	3 (6.8%)	1 (3.3%)	2 (14.3%)	0.234	Ref.	0.307	0.005
	Absent	44 (100%)	30 (100%)	14 (100%)				
Vessels serpentine linear	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	44 (100%)	30 (100%)	14 (100%)				
Vessels helical linear	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	43 (97.7%)	29 (96.7%)	14 (100%)				
Vessels coiled linear	Present	1 (2.3%)	1 (3.3%)	0 (0%)	1	NA	NA	NA
	Absent	39 (88.6%)	27 (90%)	12 (85.7%)		1.5 (0.2-10.2)		
Polymorphous vessels	Present	5 (11.4%)	3 (10%)	2 (14.3%)	0.647	Ref.	0.642	<0.001
	Absent	43 (97.7%)	29 (96.7%)	14 (100%)				
Vessels centred	Present	1 (2.3%)	1 (3.3%)	0 (0%)	1	NA	-0.031	0.825
	Absent	40 (90.9%)	28 (93.3%)	12 (85.7%)		2.3 (0.3-18.6)		
Vessels clustered	Present	4 (9.1%)	2 (6.7%)	2 (14.3%)	0.581	Ref.	0.45	0.003
	Absent	41 (93.2%)	28 (93.3%)	13 (92.9%)		1.1 (0.09-13.0)		
Vessels random	Present	3 (6.8%)	2 (6.7%)	1 (7.1%)	1	Ref.	0.29	0.039
	Absent	43 (97.7%)	29 (96.7%)	14 (100%)				
Vessels reticular	Present	1 (2.3%)	1 (3.3%)	0 (0%)	1	NA	1	<0.001
	Absent	43 (97.7%)	29 (96.7%)	14 (100%)				
Vessels branched	Present	1 (2.3%)	1 (3.3%)	0 (0%)	1	NA	NA	NA
	Absent	44 (100%)	30 (100%)	14 (100%)				
Vessels serpiginous	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	42 (95.5%)	28 (93.3%)	14 (100%)				
Vessels radial	Present	2 (4.5%)	2 (6.7%)	0 (0%)	1	NA	0.656	<0.001

Supplementary table 2. Dermoscopic feature: Vessels

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	7 (15.9%)	5 (16.7%)	2 (14.3%)		1.2 (0.2-7.1)		
Structureless	Present	37 (84.1%)	25 (83.3%)	12 (85.7%)	1	Ref.	0.303	0.023
	Absent	17 (38.6%)	15 (50%)	2 (14.3%)		6 (1.1-31.5)		
Brown structureless	Present	27 (61.4%)	15 (50%)	12 (85.7%)	0.044	Ref.	0.433	0.002
	Absent	41 (93.2%)	27 (90%)	14 (100%)				
Blue structureless	Present	3 (6.8%)	3 (10%)	0 (0%)	0.54	NA	0.788	<0.001
	Absent	39 (88.6%)	27 (90%)	12 (85.7%)		1.5 (0.2-10.2)		
Black Structureless	Present	5 (11.4%)	3 (10%)	2 (14.3%)	0.647	Ref.	0.389	0.006
	Absent	36 (81.8%)	22 (73.3%)	14 (100%)				
Hypopigmented structureless	Present	8 (18.2%)	8 (26.7%)	0 (0%)	0.041	NA	0.831	<0.001
	Absent	39 (88.6%)	26 (86.7%)	13 (92.9%)		0.5 (0.05-4.9)		
Grey Structureless	Present	5 (11.4%)	4 (13.3%)	1 (7.1%)	1	Ref.	0.257	0.011

Supplementary table 3. Dermoscopic feature: Structureless areas

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	17 (38.6%)	12 (40%)	5 (35.7%)		1.2 (0.3-4.5)		
Lines	Present	27 (61.4%)	18 (60%)	9 (64.3%)	1	Ref.	0.436	0.004
	Absent	38 (86.4%)	26 (86.7%)	12 (85.7%)		1.1 (0.2-6.8)		
Brown thin reticular lines	Present	6 (13.6%)	4 (13.3%)	2 (14.3%)	1	Ref.	0.621	<0.001
	Absent	35 (79.5%)	25 (83.3%)	10 (71.4%)		2 (0.4-9.0)		
Brown thick reticular lines	Present	9 (20.5%)	5 (16.7%)	4 (28.6%)	0.434	Ref.	0.781	<0.001
	Absent	39 (88.6%)	26 (86.7%)	13 (92.9%)		0.5 (0.05-4.9)		
Radial lines	Present	5 (11.4%)	4 (13.3%)	1 (7.1%)	1	Ref.	0.402	<0.001
	Absent	39 (88.6%)	27 (90%)	12 (85.7%)		1.5 (0.2-10.2)		
Negative network	Present	5 (11.4%)	3 (10%)	2 (14.3%)	0.647	Ref.	0.294	0.024
	Absent	44 (100%)	30 (100%)	14 (100%)				
Angulated lines	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	44 (100%)	30 (100%)	14 (100%)				
Branched lines	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	35 (79.5%)	22 (73.3%)	13 (92.9%)		0.2 (0.02-1.9)		
White shiny lines/structures	Present	9 (20.5%)	8 (26.7%)	1 (7.1%)	0.233	Ref.	0.761	<0.001

Supplementary table 4. Dermoscopic feature: Lines

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	37 (84.1%)	25 (83.3%)	12 (85.7%)		0.8 (0.1-4.9)		
Circles	Present	7 (15.9%)	5 (16.7%)	2 (14.3%)	1	Ref.	0.327	0.003
	Absent	44 (100%)	30 (100%)	14 (100%)				
Brown circles	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	44 (100%)	30 (100%)	14 (100%)				
Yellow circles	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	37 (84.1%)	25 (83.3%)	12 (85.7%)		0.8 (0.1-4.9)		
White circles	Present	7 (15.9%)	5 (16.7%)	2 (14.3%)	1	Ref.	0.398	<0.001

Supplementary table 4. Dermoscopic feature: Circles

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	27 (61.4%)	19 (63.3%)	8 (57.1%)		1.3 (0.4-4.7)		
Clods	Present	17 (38.6%)	11 (36.7%)	6 (42.9%)	0.748	Ref.	0.674	<0.001
	Absent	27 (61.4%)	19 (63.3%)	8 (57.1%)		1.3 (0.4-4.7)		
Brown clods	Present	17 (38.6%)	11 (36.7%)	6 (42.9%)	0.748	Ref.	0.759	<0.001
	Absent	44 (100%)	30 (100%)	14 (100%)				
Orange clods	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	43 (97.7%)	29 (96.7%)	14 (100%)				
Black clods	Present	1 (2.3%)	1 (3.3%)	0 (0%)	1	NA	NA	NA
	Absent	44 (100%)	30 (100%)	14 (100%)				
Blue/grey clods	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	44 (100%)	30 (100%)	14 (100%)				
White clods	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	44 (100%)	30 (100%)	14 (100%)				
Red clods	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA

Supplementary table 4. Dermoscopic feature: Clods

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	31 (70.5%)	22 (73.3%)	9 (64.3%)		1.5 (0.4-6.0)		
Dots	Present	13 (29.5%)	8 (26.7%)	5 (35.7%)	0.724	Ref.	0.433	0.003
	Absent	32 (72.7%)	23 (76.7%)	9 (64.3%)		1.8 (0.5-7.3)		
Brown dots	Present	12 (27.3%)	7 (23.3%)	5 (35.7%)	0.475	Ref.	0.57	<0.001
	Absent	44 (100%)	30 (100%)	14 (100%)				
Grey dots	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	43 (97.7%)	29 (96.7%)	14 (100%)				
Black dots	Present	1 (2.3%)	1 (3.3%)	0 (0%)	1	NA	NA	NA

Supplementary table 5. Dermoscopic feature: Dots

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	44 (100%)	30 (100%)	14 (100%)				
Globular	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	42 (95.5%)	30 (100%)	12 (85.7%)				
Globular cobblestone	Present	2 (4.5%)	0 (0%)	2 (14.3%)	0.096	NA	0.656	<0.001
	Absent	44 (100%)	30 (100%)	14 (100%)				
Intradermal nevus morphology	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	-0.023	0.877
	Absent	43 (97.7%)	29 (96.7%)	14 (100%)				
Peripheral network & central globules	Present	1 (2.3%)	1 (3.3%)	0 (0%)	1	NA	1	<0.001
	Absent	42 (95.5%)	28 (93.3%)	14 (100%)				
Peripheral network & central hypopigmentation	Present	2 (4.5%)	2 (6.7%)	0 (0%)	1	NA	0.656	<0.001
	Absent	41 (93.2%)	29 (96.7%)	12 (85.7%)		Ref.		
Peripheral network & central hyperpigmentation	Present	3 (6.8%)	1 (3.3%)	2 (14.3%)	0.234	4.8 (0.4-58.5)	0.788	<0.001
	Absent	44 (100%)	30 (100%)	14 (100%)				
Network	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	44 (100%)	30 (100%)	14 (100%)				
Patchy network	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	34 (77.3%)	24 (80%)	10 (71.4%)		1.6 (0.4-6.9)		
Asymmetric multicomponent pattern	Present	10 (22.7%)	6 (20%)	4 (28.6%)	0.701	Ref.	0.192	0.195
	Absent	44 (100%)	30 (100%)	14 (100%)				
Blue-black structureless nodule	Present	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA	NA
	Absent	38 (86.4%)	25 (83.3%)	13 (92.9%)		0.4 (0.04-3.6)		
Pink-tan structureless macule - nodule	Present	6 (13.6%)	5 (16.7%)	1 (7.1%)	0.647	Ref.	0.551	<0.001
	Absent	42 (95.5%)	28 (93.3%)	14 (100%)				
Kissing nevus pattern	Present	2 (4.5%)	2 (6.7%)	0 (0%)	1	NA	NA	NA
	Absent	42 (95.5%)	28 (93.3%)	14 (100%)				
Starburst pattern	Present	2 (4.5%)	2 (6.7%)	0 (0%)	1	NA	1	<0.001

Supplementary table 6. Dermoscopic feature: Nevi and melanoma patterns

Dermoscopic variables	Category	Total, 44 (100%)	BRAF-, n=30 (68.2%)	BRAF+, n=14 (31.8%)	p-value	OR (95% CI)	kappa	p-value (agreement)
	Absent	35 (79.5%)	22 (73.3%)	13 (92.9%)		0.2 (0.02-1.9)		
White shiny lines/structures	Present	9 (20.5%)	8 (26.7%)	1 (7.1%)	0.233	Ref.	0.761	<0.001
	Absent	34 (77.3%)	21 (70%)	13 (92.9%)		0.2 (0.02-1.6)		
Milky red areas	Present	10 (22.7%)	9 (30%)	1 (7.1%)	0.132	Ref.	0.397	<0.001
	Absent	43 (97.7%)	29 (96.7%)	14 (100%)				
Granularity/peppering	Present	1 (2.3%)	1 (3.3%)	0 (0%)	1	NA	0.482	<0.001
	Absent	37 (84.1%)	27 (90%)	10 (71.4%)		3.6 (0.7-19.0)		
Atypical blotches	Present	7 (15.9%)	3 (10%)	4 (28.6%)	0.184	Ref.	0.163	0.177
	Absent	41 (93.2%)	27 (90%)	14 (100%)				
Atypical dots and globules	Present	3 (6.8%)	3 (10%)	0 (0%)	0.54	NA	0.482	<0.001
	Absent	40 (90.9%)	28 (93.3%)	12 (85.7%)		2.3 (0.3-18.6)		
Tan structureless areas	Present	4 (9.1%)	2 (6.7%)	2 (14.3%)	0.581	Ref.	0.257	0.011
	Absent	41 (93.2%)	28 (93.3%)	13 (92.9%)		1.1 (0.09- 13.0)		
Atypical streaks	Present	3 (6.8%)	2 (6.7%)	1 (7.1%)	1	Ref.	0.476	0.002

Supplementary table 7. Dermoscopic feature: Melanoma specific structures