CLINICAL REPORT

Dysplastic vs. Common Naevus-associated vs. *De novo* Melanomas: An Observational Retrospective Study of 1,021 Patients

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The aim of this case-case study was to determine the differences between dysplastic and common naevus-associated melanomas (NAM) and *de novo* melanomas. A total of 1,021 prospectively collected patients with invasive cutaneous melanoma from an oncology referral centre were included in the study. Of these, 75.51% had *de novo* melanomas, 12.93% dysplastic NAM, and 11.56% common NAM. Dysplastic NAM, compared with *de novo* melanomas, were associated with intermittently photo-exposed sites, atypical melanocytic naevi, decreased tumour thickness, and presence of *MC1R* non-synonymous variants. Common NAM presented more frequently on the trunk and were of the superficial spreading type. Comparison of dysplastic with common NAM showed significant difference only with regard to mitoses. Both subtypes of NAM shared less aggressive traits than *de novo* melanomas, albeit with no significant differences in survival after multivariate adjustment. In conclusion, NAM present with less aggressive traits, mostly due to a greater awareness among patients of changing moles than due to their intrinsic biological characteristics.

Key words: cutaneous malignant melanoma; naevus, pigmented; sunburn; pathology; molecular biology; MC1R.

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In Caucasians 5–85% of cutaneous melanomas are clinically or histologically associated with pre-existing melanocytic naevi. These figures are generally lower when the remnants of pre-existing naevi are histologically determined (4.7–50%) (1–3) and higher if based on patients' recall of a clinically evident precursor lesion (42–85%) (4, 5). A recent meta-analysis estimates that 29.1% of melanomas probably arise from a pre-existing naevus (6).

Melanomas that are histologically associated with melanocytic naevi (NAM) may be associated with almost any melanocytic proliferative lesion; however, they are mainly found in conjunction with dysplastic or common acquired naevi and, to a lesser extent, with congenital naevi (7). Current evidence supports the view that NAM, regardless of the type of melanocytic benign lesions, are associated with relatively young age at diagnosis, personal history of sunburns, and high melanocytic naevi count. In addition, NAM have been linked with location (predominantly on the trunk), superficial spreading melanoma subtype, and have less surrounding solar elastosis, thinner Breslow's index, and absence of ulceration (1–3, 8, 9). Nonetheless, many studies report no differences in ulceration (4, 5, 10).

Common acquired naevi differ from dysplastic naevi clinically, histologically and at the molecular level, usually with divergent traits and risks that lead to their formation. It has been suggested that a medical history of high level of cumulative sun exposure plays a role in the appearance of multiple common naevi, while intense sunburns during childhood lead to the development of dysplastic naevi (11–15).

To our knowledge, there are no published studies separately comparing common and dysplastic NAM with *de novo* melanomas. We hypothesize that, given the differences between multiple common naevi and the presence of dysplastic naevi, the melanoma associated with dysplastic and common melanocytic naevi could differ considerably, and similar differences may exist between melanoma associated with naevi and melanomas arising *de novo*.

The aim of this study was to assess the clinical, epidemiological, histopathological and molecular differences between common and dysplastic NAM, and between each of these subsets and *de novo* melanomas.

PATIENTS AND METHODS

A retrospective, observational study was performed using data (collected before the design and development of the present study) from the melanoma database of the Dermatology Department of the Instituto Valenciano de Oncologia (IVO), Valencia, Spain. This database, launched in 2000, has been regularly updated with data from newly diagnosed and follow-up melanoma patients. Clinical, epidemiological, and histological data are collected prospectively from the medical history and physical examination of patients, and the information regarding disease evolution is updated on a daily basis by dermatologists with experience in management of melanoma (16).

The study was approved by the IVO's research ethics board. Informed consent was obtained previously from the participants.

Only incident patients with invasive cutaneous melanoma who had received definitive treatment at our institution between 1

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January 2000 and 31 December 2012 were included in the study. Patients with *in situ* melanomas, mucosal or ocular melanomas, metastatic melanomas with unknown primary tumour, and melanomas associated with other melanocytic lesions (congenital naevi, blue naevi, naevus spilus, etc.), and cases with no information about the presence of remnants of previous melanocytic naevi were excluded from the analysis. We also excluded those patients presenting multiple primary melanomas, since this could bias and modify the survival analyses.

Patients were classified according to whether remnants of pre-existent dysplastic or common melanocytic naevus were observed during pathological examination. Thus, patients were not classified in this group based on clinical criteria (17). Common melanocytic naevi included junctional, compound and intradermal naevi, exhibiting no architectural or cytological dysplastic features. Dysplastic or Clark naevi were defined based on the pathology subgroup of the European Organisation for Research and Treatment of Cancer (EORTC) Malignant Melanoma Cooperative Group diagnostic criteria (18). Gradation of melanocytic dysplasia was not considered in this study. Three groups were defined: group A: melanomas with no associated naevi (i.e. *de novo* melanomas); group B: melanomas associated with dysplastic melanocytic naevi (dysplastic NAM); and group C: melanomas associated with common melanocytic naevi (common NAM).

The following variables were used for comparative analysis:

- *Clinical-epidemiological*: sex, age (<45, 45–64, or ≥65 years), phototype (I–II or III–V), hair colour (black/brown, blonde or red), melanoma location (head/neck, upper extremities, trunk, lower extremities, and acral locations), presence of at least 1 clinically atypical melanocytic naevi, number of melanocytic naevi (<20, 20–50 and >50), past personal lifetime history of severe sunburns (none, 1–5, >5), past personal history of sunburns at the melanoma site.
- *Histopathological*: melanoma subtype (lentigo maligna melanoma (LMM), superficial spreading melanoma (SSM), nodular melanoma (NM), acral lentiginous melanoma (ALM) or other/non-specified (NOS)), Breslow thickness (≤1, 1.01–2, 2.01–4, ≥4.01 mm), number of mitosis/mm^2 (0, ≥1), presence of ulceration, tumour infiltrating lymphocytes (none, non-brisk, brisk), elastosis in the surrounding skin (presence, absence) and sentinel lymph node status (negative, positive, not detected). The same dermatopathologist (VT) reviewed the pathological slides in order to register these variables accordingly. Difficult-to-diagnose cases were discussed thoroughly and confirmed by a multidisciplinary committee including expert melanoma-subspecialized dermatopathologists and dermatologists.
- *Molecular biology*: presence of any non-synonymous red hair colour (RHC) *MC1R* variants (*p.D84E*, *p.D294H*, *p.R142H*, *p.R151C* and *p.R160W*), and presence of *NRAS* and *BRAF* mutations in the primary melanoma. All the molecular alterations were analysed by direct sequencing from samples collected in the Biobank of the Fundación Instituto Valenciano de Oncología. Tumour samples were obtained from formalin-fixed paraffin-embedded (FFPE) tissue by 0.2–0.3 Tru-cut biopsies from pathologically defined areas, as described previously (19).

Statistical analysis

Differences between the distribution of each variable according to the categories were evaluated using Pearson's χ^2 test. Odds ratios (OR) were calculated through univariate and stepwise forward multivariate logistic regression. Logistic regression analyses quantifying the association between the different variables and measures were performed in 3 pairs: common NAM vs. *de novo* melanomas, common NAM vs. dysplastic NAM, and dysplastic NAM vs. *de novo* melanomas. For multivariate analyses, missing values were imputed using a complete case (multiple imputation) model (20), for which 5 iterations were run and combined estimates and standard errors using Rubin's rules. Prior to the development of the model, we tested if the data were randomly missing, using the missing values add-on module in the SPSS statistical package. Survival analyses considering as endpoints separately disease-free survival, overall survival and melanoma-specific survival were performed. Differences in survival probabilities were calculated by the Kaplan–Meier method and the differences evaluated by the log-rank test. Multivariate analyses were performed by forward stepwise Cox regression models to adjust for all selected variables. Proportionality assumption was graphically assessed by log (–log) survival plots. Statistical significance was 2-tail and established for p-value <0.05. All the statistical analyses were performed using the SPSS statistical package for Windows, version 20.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Out of 1,430 patients, 1,021 met the inclusion criteria (Fig. S1[1]). The median age of the patients at diagnosis was 56 years (interquartile range (IQR) 43–68 years). A total of 771 patients (75.5%) presented with *de novo* melanomas, 132 (12.9%) with dysplastic NAM, and 118 (11.6%) with common NAM.

The characteristics of the studied population and results from exploratory χ^2 analyses are shown in **Table I**, and binary logistic regression analyses are shown in **Table II**.

Dysplastic NAM, compared with *de novo* melanomas, were associated with young age (<45 years), location on the trunk and upper extremities, presence of multiple melanocytic naevi (>50), presence of clinically atypical melanocytic naevi, history of mild or intense sunburns at the melanoma site, SSM histological subtype, lower Breslow thickness and mitosis count, and absence of ulceration. Multivariate analyses confirmed associations with location on the trunk (OR 5.8 (95% confidence interval (95% CI) 1.7–19.7)) or upper extremities (OR 4.5 (1.2–16.9)), presence of clinically atypical melanocytic naevi (OR 1.8 (1.0–3.1)), thinner tumours (<1 mm: OR 7.3 (2.6–20.6); 1.01–2 mm: OR 4.3 (1.4–12.9)), and the presence of *MC1R* non-synonymous variants (OR 1.4 (1.1–1.9)). No statistically significant differences were found for presence of *BRAF* and *NRAS* mutations among the 3 subgroups investigated.

Common NAM, compared with *de novo* melanomas, were also associated with young age, location on the trunk, presence of multiple melanocytic naevi (>50), presence of atypical melanocytic naevi, history of intense sunburns at the melanoma site, superficial spreading and nodular histological subtypes, thinner tumours, 1–5 mitoses/mm^2, and absence of ulceration. Multivariate analyses showed that the most significant associations were location on the trunk (OR 2.8 (1.5–5.3)) and SSM subtype (OR 11.9 (2.9–48.6)).

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Table I. Sample description. Univariate (χ^2) analyses results

	(A) No precursor lesion ($n = 771$), n (%)	(B) Dysplastic naevus ($n = 132$), n (%)	(C) Common naevus ($n = 118$), n (%)	Total	p-value for trend[a]
Age group					**0.001 [A–B: 0.004, A–C: 0.007,** B–C: NS**]**
<45 years	179 (23.2)	48 (36.4)	43 (36.4)	270 (26.4)	
45–64 years	331 (42.9)	51 (38.6)	45 (38.1)	427 (41.8)	
≥65 years	261 (33.9)	33 (25.0)	30 (25.4)	324 (31.7)	
Sex					NS [A–B: NS, A–C: NS, B–C: NS]
Male	379 (49.2)	62 (47.0)	63 (53.4)	504 (49.4)	
Female	392 (50.8)	70 (53.0)	55 (46.6)	517 (50.6)	
Fitzpatrick's skin phototype (missing: 14)					NS [A–B: NS, A–C: NS, B–C: NS]
Types I–II	259 (34.2)	48 (36.4)	45 (38.5)	352 (35.0)	
Types III–V	499 (65.8)	84 (63.6)	72 (61.5)	655 (65.0)	
Hair colour (missing: 17)					NS [A–B: NS (0.053), A–C: NS, **B–C: 0.03**]
Black/brown	601 (79.6)	93 (70.5)	90 (76.9)	784 (78.1)	
Blonde	128 (17.0)	31 (23.5)	22 (18.8)	181 (18.0)	
Red	26 (3.4)	8 (6.1)	5 (4.3)	39 (3.9)	
Location (missing: 0)					**<0.001 [A–B: <0.001, A–C: <0.001,** B–C: NS**]**
Head and neck	168 (21.8)	7 (5.3)	14 (11.9)	189 (18.5)	
Upper limb	109 (14.1)	20 (15.2)	17 (14.4)	146 (14.3)	
Trunk	246 (31.9)	76 (57.6)	67 (56.8)	389 (38.1)	
Lower limb	165 (21.4)	26 (19.7)	13 (11.0)	204 (20.0)	
Acral	83 (10.8)	3 (2.3)	7 (5.9)	93 (9.1)	
Presence of multiple melanocytic naevi (missing: 87)					**0.003 [A–B: 0.002, A–C: 0.018,** B–C: NS**]**
<20	529 (75.5)	75 (61.0)	73 (66.4)	677 (72.5)	
20–50	93 (13.3)	22 (17.9)	16 (14.5)	131 (14.0)	
>50	79 (11.3)	26 (21.1)	21 (19.1)	126 (13.5)	
Presence of atypical melanocytic naevi (missing: 29)					**<0.001 [A–B: <0.001, A–C: 0.013, B–C: 0.034]**
No	640 (85.7)	83 (64.3)	89 (76.7)	812 (81.9)	
Yes	107 (14.3)	46 (35.7)	27 (23.3)	180 (18.1)	
Past personal history of intense sunburns (missing: 22)					NS [A–B: NS, A–C: NS, B–C: NS]
None	380 (50.5)	53 (41.1)	50 (42.7)	483 (48.3)	
1–5	239 (31.7)	47 (36.4)	36 (30.8)	322 (32.2)	
>5	134 (17.8)	29 (22.5)	31 (26.5)	100 (10.0)	
Past personal history of sunburns at the melanoma site (missing: 97)					**0.0002 [A–B: 0.017, A–C: 0.018,** B–C: NS**]**
No	264 (37.8)	30 (24.8)	27 (26.0)	321 (34.7)	
Yes	435 (62.2)	91 (75.2)	77 (74.0)	603 (65.3)	
Melanoma histological subtypes (missing: 2)					**<0.001 [A–B: <0.001, A–C: <0.001,** B–C: NS**]**
LMM	101 (13.1)	2 (1.5)	2 (1.7)	105 (10.3)	
SSM	413 (53.6)	114 (86.4)	97 (82.9)	624 (61.2)	
NM	161 (20.9)	14 (10.6)	15 (12.8)	190 (18.6)	
ALM	48 (6.2)	0 (0)	3 (2.6)	51 (5.0)	
Others/not specified	47 (6.1)	2 (1.5)	0 (0)	49 (4.8)	
Staging at diagnosis (missing: 45)					**0.017 [A–B: 0.011,** A–C: NS**,** B–C: NS**]**
Localized	581 (78.3)	103 (90.4)	98 (81.7)	782 (80.1)	
Locoregional	157 (21.2)	11 (9.6)	20 (16.7)	188 (19.3)	
Metastatic	4 (0.5)	0	2 (1.7)	6 (0.6)	
Breslow thickness according to AJCC classification (missing: 142)					**<0.001 [A–B: <0.001, A–C: 0.004, B–C: 0.024]**
≤1.00 mm	269 (40.5)	68 (66.0)	55 (49.5)	392 (44.6)	
1.01–2.00 mm	142 (21.4)	21 (20.4)	33 (29.7)	196 (22.3)	
2.01–4.00 mm	138 (20.8)	10 (9.7)	14 (12.6)	162 (18.4)	
≥4.00 mm	116 (17.4)	4 (3.9)	9 (8.1)	129 (14.7)	
Mitosis (missing: 195)					**<0.001 [A–B: 0.006,** A–C: NS, **B–C: 0.001]**
0 mitoses/mm^2	231 (36.4)	53 (50.5)	23 (26.7)	307 (37.2)	
≥1 mitoses/mm^2	404 (63.6)	52 (49.5)	63 (73.3)	519 (62.8)	
Ulceration (missing: 7)					**<0.001 [A–B: <0.001, A–C 0.019,** B–C: NS**]**
Non-ulcerated	572 (74.7)	121 (92.4)	99 (84.6)	792 (78.1)	
Ulcerated	194 (25.3)	10 (7.6)	18 (15.4)	222 (21.9)	
Inflammatory infiltrate (missing: 300)					NS [A–B: NS, A–C: NS, B–C: NS]
None	318 (58.8)	58 (59.2)	50 (61.0)	426 (59.1)	
Non-brisk	199 (36.8)	37 (37.8)	28 (34.1)	264 (36.6)	
Brisk	24 (4.4)	3 (3.1)	4 (4.9)	31 (4.3)	
Elastosis in the surrounding skin (missing: 581)					NS [A–B: NS, A–C: NS, B–C: NS]
Absent	229 (67.6)	48 (71.6)	28 (82.4)	305 (69.3)	
Present	110 (32.4)	19 (28.4)	6 (17.6)	135 (30.7)	
Selective sentinel lymphatic node biopsy (missing: 510)					NS [A–B: NS, A–C: NS, B–C: NS]
Negative	287 (72.8)	41 (85.4)	53 (76.8)	381 (74.6)	
Positive	85 (21.6)	7 (14.6)	14 (20.3)	106 (20.7)	
Not detected	22 (5.6)	0 (0)	2 (2.9)	24 (4.7)	
BRAF mutations present (missing: 649)					NS [A–B: NS, A–C: NS, B–C: NS]
No	204 (69.9)	33 (71.7)	21 (61.8)	258 (69.4)	
Yes	88 (30.1)	13 (28.3)	13 (38.2)	114 (30.6)	
NRAS mutations present (missing: 651)					NS [A–B: NS, A–C: NS, B–C: NS]
No	257 (87.4)	37 (88.1)	28 (82.4)	322 (87.0)	
Yes	37 (12.6)	5 (11.9)	6 (17.6)	48 (13.0)	
MC1R RHC nonsynonymous variants present (missing: 170)					**0.041 [A–B: 0.017,** A–C: NS, B–C: NS]
No	227 (35.6)	28 (24.1)	29 (29.9)	284 (33.4)	
Yes	411 (64.4)	88 (75.9)	68 (70.1)	567 (66.6)	

[a]p-value comparing between groups: A–B, A–C, B–C. Significant values are shown in bold.
NS: non-significant; LMM: lentigo malignant melanoma; SSM: superficial spreading melanoma; NM: nodular melanoma; ALM: acral lentiginous melanoma.

Table II. Univariate and multivariate binary logistic regression analyses results

	Dysplastic NAM vs. *de novo* melanoma		Common NAM vs. *de novo* melanoma		Common NAM vs. dysplastic NAM	
	Univariate OR (CI 95%)	Multivariate OR (CI 95%)	Univariate OR (CI 95%)	Multivariate OR (CI 95%)	Univariate OR (CI 95%)	Multivariate OR (CI 95%)
Age group						
<45 years	**2.1 (1.3–3.4)**	NS	**2.1 (1.3–3.5)**	NS	1.0 (0.5–1.9)	NS
45–65 years	1.2 (0.8–1.9)	NS	1.2 (0.7–1.9)	NS	1.0 (0.5–1.8)	NS
> 65 years	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Location						
Head and neck	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Upper limb	**4.4 (1.8–10.7)**	**4.5 (1.2–16.9)**	1.9 (0.8–4.0)	NS	0.4 (0.1–1.3)	NS
Trunk	**7.4 (3.3–16.5)**	**5.79 (1.7–9.7)**	**3.3 (1.8–6.0)**	**2.8 (1.5–5.3)**	0.4 (0.2–1.2)	NS
Lower limb	**3.8 (1.6–9.0)**	NS	0.9 (0.4–2.1)	NS	**0.3 (0.1–0.8)**	NS
Acral	0.9 (0.2–3.4)	NS	1.0 (0.9–2.6)	NS	1.2 (0.2–6.0)	NS
Hair colour						
Black/brown	0.5 (0.2–1.1)	NS	0.8 (0.3–2.1)	NS	1.6 (0.5–4.9)	NS
Blonde	0.8 (0.3–1.9)	NS	0.9 (0.3–2.6)	NS	1.1 (0.3–4.0)	NS
Red	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Presence of multiple melanocytic naevi						
<20	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
20–50	1.7 (0.9–2.9)	NS	1.2 (0.7–2.2)	NS	0.7 (0.4–1.5)	NS
>50	**2.3 (1.4–3.9)**	NS	**1.9 (1.1–3.3)**	NS	0.8 (0.4–1.6)	NS
Presence of atypical melanocytic naevi						
No	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Yes	**3.3 (2.2–5.0)**	**1.8 (1.0–3.1)**	**1.8 (1.1–2.9)**	NS	**0.6 (0.3–0.9)**	NS
History of sunburns at the melanoma site						
No	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Yes	**1.8 (1.2–2.9)**	NS	**1.7 (1.1–2.8)**	NS	0.9 (0.5–1.7)	NS
Melanoma histological subtypes						
LMM	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
SSM	**13.9 (3.4–57.4)**	NS	**11.9 (2.9–48.6)**	**10.1 (2.3–44.3)**	0.9 (0.1–6.2)	NS
NM	4.4 (0.9–19.7)	NS	**4.7 (1.1–21.0)**	NS	1.1 (0.1–8.7)	NS
ALM	0 (n/a)	NS	1.7 (0.3–10.8)	NS	0 (n/a)	NS
Others/not specified	2.2 (0.3–15.7)	NS	0 (n/a)	NS	0 (n/a)	NS
Staging at diagnosis						
Localized	Ref.	NS	Ref.	NS	Ref.	NS
Locoregional	**0.4 (0.2–0.8)**	NS	0.8 (0.5–1.3)	NS	1.9 (0.9–4.2)	NS
Metastatic	0 (n/a)	NS	2.9 (0.5–16.4)	NS	0 (n/a)	NS
Breslow thickness according to AJCC classification						
≤1.00 mm	**7.3 (2.6–20.6)**	**6.0 (1.8–20.3)**	**2.6 (1.3–5.5)**	NS	0.4 (0. 1–1.2)	NS
1.01–2.00 mm	**4.3 (1.4–12.9)**	**5.8 (1.7–19.7)**	**3. 0 (1.4–6.5)**	NS	0.7 (0.2–2.6)	NS
2.01–4.00 mm	2.1 (0.6–6.9)	NS	1.3 (0.6–3.1)	NS	0.6 (0.2–2.6)	NS
≥4.00 mm	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Mitoses, mitoses/mm^2						
0	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
≥1	**0.6 (0.4–0.9)**	NS	1.4 (0.8–2.3)	NS	**2.2 (1.2–4.3)**	**2.6 (1.3–5.1)**
Ulceration						
Non-ulcerated	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Ulcerated	**0.2 (0.1–0.5)**	NS	**0.5 (0.3–0.8)**	NS	**2.2 (1.0–5.0)**	NS
MC1R polymorphisms present						
No	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Yes	**1,7 (1.1–2.7)**	**1.4 (1.1–1.9)**	1.3 (0.8–2.1)	NS	0.8 (0.4–1.4)	N.S.

Figures in **bold** show significant associations.
NAM: naevus-associated melanomas; NS: non-significant; Ref.: reference; CI: confidence interval.

Comparison of 2 NAM subtypes showed that common NAM were less frequent on the lower extremities than in patients with clinically atypical melanocytic naevi, but were more frequently associated with the presence of mitoses. Multivariate logistic regression, however, showed that the only statistically significant differences between the 2 subtypes were in terms of mitosis count; common NAM harboured increased mitoses counts (OR 2.6 (1.3–5.1)).

Analyses with a median follow-up of 55 months showed statistically significant differences in disease-free and melanoma-specific mortality survival (**Fig. 1**) between NAM subgroups and *de novo* melanomas. The results showed median disease-free survival of 35.9 months for patients with *de novo* melanoma, 60.4 months for patients with both subtypes of NAM (48.4 months for patients with dysplastic NAM, and 66.8 months for patients with common NAM). Median overall survival was 44.5 months for patients with *de novo* melanoma, 61.7 months for patients with both subtypes of NAM (52.8 months for patients with dysplastic NAM and 72.5 months for patients with common NAM). This was also seen when comparing NAM in general with *de novo* melanomas (**Fig. 2**). However, multivariate adjustment showed that these differences were dependent on other characteristics rather than histological association with a pre-existing naevus (Table SI[1]).

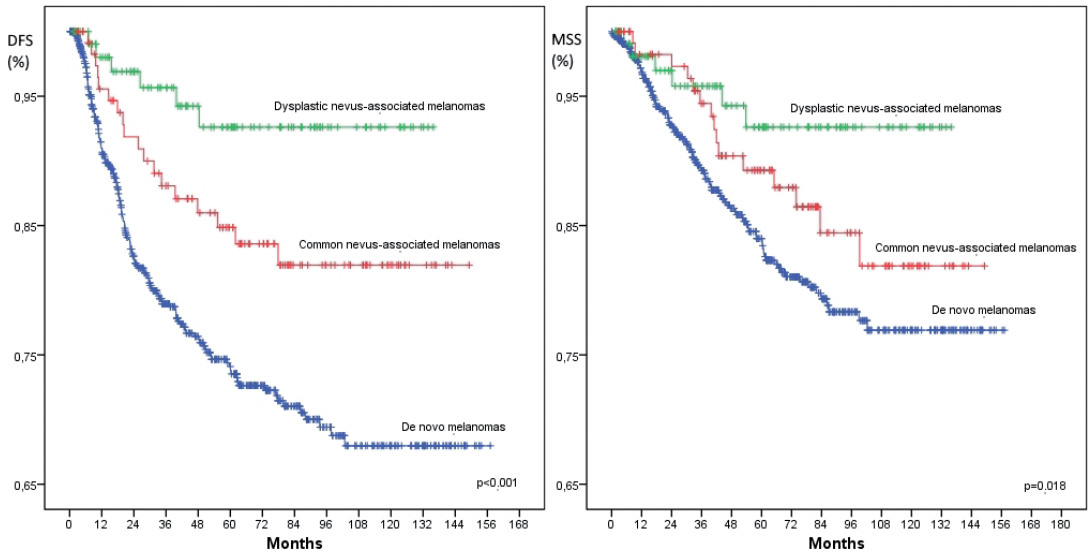

Fig. 1. Disease-free survival (DFS) and melanoma-specific survival (MSS) comparing *de novo* melanomas with naevus-associated melanomas (NAM) subgroups.

DISCUSSION

Some authors have stated that individual melanocytes undergo clonal expansion and senescence to become melanocytic naevi (21–23). However, naevi very infrequently transform into a melanoma (the estimated individual accumulated risk until 80 years of age for melanoma transformation of an individual naevus has been calculated as 0.03% among men and 0.009% among women) (24). Certain genetic mutations are known to be present in both naevi and melanomas, and the process leading to the change from melanocytes to intermediate lesions and, finally, to melanoma involves a number of additional molecular alterations (25, 26). The melanocyte-naevus-melanoma model includes the patients belonging to 1 of the 2 pathways (the "naevogenic" pathway) proposed for the development of melanoma (27, 28). However, this model does not fit certain histological subtypes of melanoma (mainly LMM and ALM), and cannot be applied to all cases in which clinical evidence suggests a NAM.

The subject of naevus-associated melanomas (NAM) is controversial and some questions remain unanswered. The present study, based on a prospective series of 1,021 patients with cutaneous melanoma, is the first to separately assess dysplastic and common NAM together with clinical, histological and molecular factors. Dysplastic NAM, compared with *de novo* melanomas, associate with intermittently sun-exposed sites, such as trunk and upper extremities, the presence of clinically atypical melanocytic naevi, thin tumours, and the presence of *MC1R* non-synonymous variants. Common NAMs, on the other hand, associate with intermittently sun-exposed sites and SSM type. Comparison of dysplastic

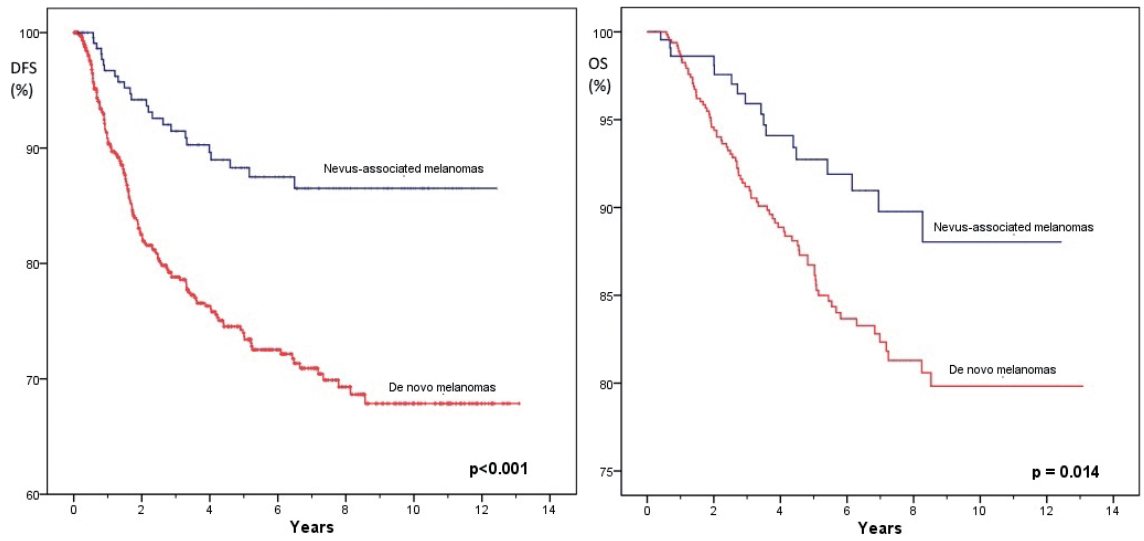

Fig. 2. Disease-free survival (DFS) and overall survival (OS) comparing *de novo* melanomas with naevus-associated melanomas (NAM).

with common NAM showed that the only difference between the 2 was that the latter had an increased number of mitosis.

The proportion of NAM in our study is similar to that in some of the previously published series, although somewhat lower than in a recently published meta-analysis (9, 29–31). Both subtypes of NAM were associated with intermittently sun-exposed areas; in patients with non-synonymous *MC1R* variants, dysplastic NAM were more frequent. These factors highlight the importance of sun exposure reflected through high cumulative exposure and a past personal history of sunburns, which have been identified previously as important in NAM (1–3). NAM present more frequently as SSM and, in general terms, share better prognostic characteristics than *de novo* melanomas, as seen previously (1–3, 8, 9). This study shows that dysplastic NAM characterize tumours with more benign histopathological features than common NAM. However, we did not find differences in survival in multivariate analyses, as indicated previously (9). However, another study showed better survival in NAM vs. *de novo* melanoma (32).

As the previous findings are in accordance with a divergent pathway model, the case for assessment of the type pre-existing melanocytic in NAM is augmented (28, 32). We hypothesize that, although *BRAF* and *NRAS* mutational status was not significant in our analyses, other biological factors may be relevant for those differences (15).

The fact that dysplastic NAM had lower counts of mitoses than common NAM could be an indication of non-compliance with Clark's theory of naevi-melanoma progression (33). It is accepted that the diagnosis of a dysplastic naevus should be considered as a marker for melanoma risk, but there is currently no clear evidence demonstrating that these naevi have a high predisposition to evolve into a melanoma (34). We also consider that the generally more favourable features among NAM can be attributed to a higher awareness of the need for periodic check-ups of moles and pre-existing lesions. This message has been repeated in melanoma screening campaigns, and has probably reached prominently to those patients with dysplastic naevi. Thus, along with the disparate results exhibited by 2 recent studies (35, 36), we believe that future screening and skin cancer awareness campaigns should insist on identification of new lesions, which may present with worse prognostic traits.

All of the patients in the current study were collected prospectively, following homogeneous criteria, from a national reference centre (with a large geographical referral area, and therefore could probably be extrapolated to most Spanish patients with melanoma). However, this study has certain limitations, such as its relatively limited sample, which may have prevented the detection of some associations, its retrospective approach, which implies certain inherent constraints, and the absence of information of the treatment received by patients after metastatic dissemination, which may have influenced the survival figures. Furthermore, molecular analyses were not performed on all of the specimens and were not specifically directed to confirm that the adjacent melanocytic naevi were indeed precursor lesions, given that most, but not all of NAM, correspond to naevi being precursor lesions to melanoma (37). Finally, although histopathological diagnosis was performed by a single expert dermatopathologist, and difficult-to-diagnose cases were discussed and confirmed by a multidisciplinary committee including subspecialized dermatologists and dermatopathologists, a potential classification bias is possible, which could explain the more benign histopathological features observed among dysplastic NAM (38).

In conclusion, previously published epidemiological and histopathological factors were confirmed in our study, and some other features were identified, thanks to separating common and dysplastic NAM into different groups. NAM are more common on intermittently sun-exposed sites. This is a more important factor among dysplastic NAM, which is more frequent among patients carrying at least 1 RHC *MC1R* non-synonymous melanoma-associated variant. More benign histopathological features favouring dysplastic NAM (vs. common NAM) have been seen, and both subtypes of NAM are more frequently SSM, sharing less aggressive traits than *de novo* melanomas, although no significant impact on survival has been observed.

REFERENCES

1. Bevona C, Goggins W, Quinn T, Fullerton J, Tsao H. Cutaneous melanomas associated with nevi. Arch Dermatol 2003; 139: 1620–1624.
2. Carli P, Massi D, Santucci M, Biggeri A, Giannotti B. Cutaneous melanoma histologically associated with a nevus and melanoma de novo have a different profile of risk: results from a case-control study. J Am Acad Dermatol 1999; 40: 549–557.
3. Purdue MP, From L, Armstrong BK, Kricker A, Gallagher RP, McLaughlin JR, et al. Etiologic and other factors predicting nevus-associated cutaneous malignant melanoma. Cancer Epidemiol Biomarkers Prev 2005; 14: 2015–2022.
4. Garcia-Cruz A, Florez A, de la Torre-Fraga C, Cruces Prado M. Observational cross-sectional study comparing Breslow thickness of melanoma arising from naevi and melanoma de novo. Br J Dermatol 2009; 161: 700–702.
5. Weatherhead SC, Haniffa M, Lawrence CM. Melanomas arising from naevi and de novo melanomas – does origin matter? Br J Dermatol 2007; 156: 72–76.
6. Pampena R, Kyrgidis A, Lallas A, Moscarella E, Argenziano G, Longo C. A meta-analysis of nevus-associated melanoma: prevalence and practical implications. J Am Acad Dermatol 2017; 77: 938–945.e4.
7. Massi G, LeBoit PE. Melanoma arising in a pre-existent nevus. In: Massi G, LeBoit PE, editors. Histological diagnosis of nevi and melanoma. 2nd edn. Springer; 2014, p. 619–632.
8. Echeverria B, Botella-Estrada R, Serra-Guillen C, Martorell A, Traves V, Requena C, et al. [Increased risk of developing a second primary cutaneous nevus-associated melanoma in patients previously diagnosed with the disease]. Actas Dermosifiliogr 2010; 101: 710–716.
9. Lin WM, Luo S, Muzikansky A, Lobo AZ, Tanabe KK, Sober AJ, et al. Outcome of patients with de novo versus nevus-

associated melanoma. J Am Acad Dermatol 2015; 72: 54–58.
10. Friedman RJ, Rigel DS, Kopf AW, Lieblich L, Lew R, Harris MN, et al. Favorable prognosis for malignant melanomas associated with acquired melanocytic nevi. Arch Dermatol 1983; 119: 455–462.
11. Carli P, Naldi L, Lovati S, La Vecchia C. The density of melanocytic nevi correlates with constitutional variables and history of sunburns: a prevalence study among Italian schoolchildren. Int J Cancer 2002; 101: 375–379.
12. Chaudru V, Laud K, Avril MF, Miniere A, Chompret A, Bressac-de Paillerets B, et al. Melanocortin-1 receptor (MC1R) gene variants and dysplastic nevi modify penetrance of CDKN2A mutations in French melanoma-prone pedigrees. Cancer Epidemiol Biomarkers Prev 2005; 14: 2384–2390.
13. Gallagher RP, McLean DI, Yang CP, Coldman AJ, Silver HK, Spinelli JJ, et al. Suntan, sunburn, and pigmentation factors and the frequency of acquired melanocytic nevi in children. Similarities to melanoma: the Vancouver Mole Study. Arch Dermatol 1990; 126: 770–776.
14. Harth Y, Friedman-Birnbaum R, Linn S. Influence of cumulative sun exposure on the prevalence of common acquired nevi. J Am Acad Dermatol 1992; 27: 21–24.
15. Roh MR, Eliades P, Gupta S, Tsao H. Genetics of melanocytic nevi. Pigment Cell Melanoma Res 2015; 28: 661–672.
16. Nagore E, Botella-Estrada R, Requena C, Serra-Guillen C, Martorell A, Hueso L, et al. Clinical and epidemiologic profile of melanoma patients according to sun exposure of the tumor site. Actas Dermosifiliogr 2009; 100: 205–211.
17. de Wit PE, van't Hof-Grootenboer B, Ruiter DJ, Bondi R, Brocker EB, Cesarini JP, et al. Validity of the histopathological criteria used for diagnosing dysplastic naevi. An interobserver study by the pathology subgroup of the EORTC Malignant Melanoma Cooperative Group. Eur J Cancer 1993; 29A: 831–839.
18. Duffy K, Grossman D. The dysplastic nevus: from historical perspective to management in the modern era: part I. Historical, histologic, and clinical aspects. J Am Acad Dermatol 2012; 67: e1–16.
19. Garcia-Casado Z, Traves V, Banuls J, Niveiro M, Gimeno-Carpio E, Jimenez-Sanchez AI, et al. BRAF, NRAS and MC1R status in a prospective series of primary cutaneous melanoma. Br J Dermatol 2015; 172: 1128–1131.
20. Jones WM, Williams WJ, Roberts MM, Davies K. Malignant melanoma of the skin: prognostic value of clinical features and the role of treatment in 111 cases. Br J Cancer 1968; 22: 437–451.
21. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature 2005; 436: 720–724.
22. Bennett DC. Human melanocyte senescence and melanoma susceptibility genes. Oncogene 2003; 22: 3063–3069.
23. Robinson WA, Lemon M, Elefanty A, Harrison-Smith M, Markham N, Norris D. Human acquired naevi are clonal. Melanoma Res 1998; 8: 499–503.
24. Tsao H, Bevona C, Goggins W, Quinn T. The transformation rate of moles (melanocytic nevi) into cutaneous melanoma: a population-based estimate. Arch Dermatol 2003; 139: 282–288.
25. Shain AH, Yeh I, Kovalyshyn I, Sriharan A, Talevich E, Gagnon A, et al. The genetic evolution of melanoma from precursor lesions. N Engl J Med 2015; 373: 1926–1936.
26. Shain AH, Bastian BC. From melanocytes to melanomas. Nat Rev Cancer 2016; 16: 345–358.
27. Whiteman DC, Parsons PG, Green AC. p53 expression and risk factors for cutaneous melanoma: a case-control study. Int J Cancer 1998; 77: 843–848.
28. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst 2003; 95: 806–812.
29. Hastrup N, Osterlind A, Drzewiecki KT, Hou-Jensen K. The presence of dysplastic nevus remnants in malignant melanomas. A population-based study of 551 malignant melanomas. Am J Dermatopathol 1991; 13: 378–385.
30. Larsen TE. The classification of primary cutaneous malignant melanoma. A prospective study of 60 cases using Clark's classification. Acta Pathol Microbiol Scand A 1978; 86A: 451–459.
31. Rippey JJ, Whiting DA. Moles and melanomas. Lancet 1977; 2: 137.
32. Cymerman RM, Shao Y, Wang K, Zhang Y, Murzaku EC, Penn LA, et al. De novo vs nevus-associated melanomas: differences in associations with prognostic indicators and survival. J Natl Cancer Inst 2016; 108.
33. Clark WH, Jr, Reimer RR, Greene M, Ainsworth AM, Mastrangelo MJ. Origin of familial malignant melanomas from heritable melanocytic lesions. 'The B-K mole syndrome'. Arch Dermatol 1978; 114: 732–738.
34. Duffy K, Grossman D. The dysplastic nevus: from historical perspective to management in the modern era: part II. Molecular aspects and clinical management. J Am Acad Dermatol 2012; 67: 19e1–2.
35. Haenssle HA, Mograby N, Ngassa A, Buhl T, Emmert S, Schon MP, et al. Association of patient risk factors and frequency of nevus-associated cutaneous melanomas. JAMA Dermatol 2016; 152: 291–298.
36. Geller AC, Mayer JE, Sober AJ, Miller DR, Argenziano G, Johnson TM, et al. Total nevi, atypical nevi, and melanoma thickness: an analysis of 566 patients at 2 US centers. JAMA Dermatol 2016; 152: 413–418.
37. Shitara D, Tell-Marti G, Badenas C, Enokihara MM, Alos L, Larque AB, et al. Mutational status of naevus-associated melanomas. Br J Dermatol 2015; 173: 671–680.
38. Barr RJ, Linden KG, Rubinstein G, Cantos KA. Analysis of heterogeneity of atypia within melanocytic nevi. Arch Dermatol 2003; 139: 289–292.