Radiación ultravioleta y lesiones melanocíticas. Implicación en prevención y diagnóstico precoz de melanoma

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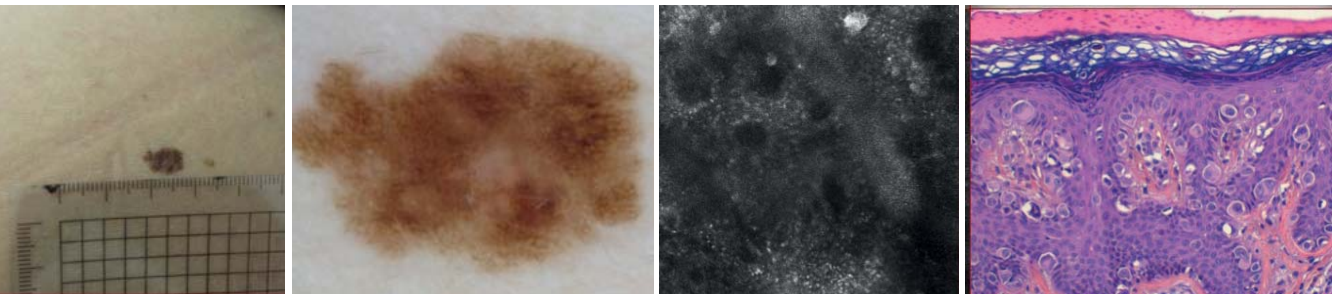

DOCTORAT EN MEDICINA. FACULTAT DE MEDICINA

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Tesis Doctoral Cristina Carrera Álvarez

EFECTOS DE LA RADIACIÓN ULTRAVIOLETA EN LESIONES MELANOCÍTICAS. IMPLICACIONES EN DIAGNÓSTICO PRECOZ Y PREVENCIÓN DE MELANOMA

A mis padres,

Seguiré aprendiendo
como si viviera toda la vida
viviendo como si
muriera mañana

Charles Chaplin

PRESENTACIÓN

Esta tesis doctoral refleja la trayectoria tanto de estudio como investigación iniciada durante los dos años de becas (Premio Final de Residencia Emili Letang y Beca Predoctoral del Instituto de Investigación Biomédica August Pi i Sunyer; IDIBAPS), y consolidada en el actual y continuo avance de la línea de investigación a la cual pertenece; "Melanoma: Imagen, Inmunología y Genética" del IDIBAPS.

Gracias a la oportunidad de formación y trabajo en el equipo multidisciplinar de la Unidad de Melanoma y de las diferentes áreas de sub-especialización del Servicio de Dermatología del Hospital Clínic, los resultados de esta tesis reflejan el amplio abanico de campos de interés que centra la investigación dermatológica en melanoma; la fotobiología y fotocarcinogénesis, la dermatopatología y genética molecular, y las técnicas de diagnóstico no invasivo en dermato-oncología.

La radiación ultravioleta (RUV) juega un papel fundamental en el desarrollo de lesiones melanocíticas adquiridas, benignas y malignas, siendo al igual que la presencia de nevus, los principales factores de riesgo para el desarrollo de melanoma. Asimismo, las nuevas clasificaciones moleculares del melanoma han suscitado un creciente interés por el papel etiopatogénico de la RUV. A pesar de los revolucionarios avances en terapéutica, la prevención continúa siendo la pieza clave del tratamiento del melanoma. Los fotoprotectores han tenido un gran impacto en la prevención del daño en queratinocitos, sin embargo su papel en la protección del daño melanocítico no está totalmente establecido.

La tesis se presenta en formato de tres publicaciones científicas. El primer trabajo es un estudio retrospectivo sobre melanomas de las extremidades, puesto que se consideran un tipo de melanoma estrechamente relacionado con la exposición solar intermitente. Se caracterizaron 36 casos incipientes, tumores clínicamente indistinguibles de lesiones melanocíticas benignas (nevus), aportando nuevas particularidades que mejoran su diagnóstico precoz y abren nuevas hipótesis sobre la etiopatogenia de un tipo de melanoma potencialmente prevenible.

El segundo presenta el diseño de un método de estudio mediante la reproducción in vivo de los efectos agudos de la RUV en la piel y las lesiones melanocíticas benignas (nevus). De forma pionera en la literatura científica, esta innovación ha supuesto un modelo para testar en condiciones reales, el posible papel de la fotoprotección tópica sobre lesiones melanocíticas.

En el tercer trabajo, y en base al modelo publicado, se presenta un estudio prospectivo intervencional que compara los efectos foto-inducidos en lesiones melanocíticas protegidas mediante una barrera física, mediante un fotoprotector en crema o sin protección. Se demuestra por primera vez, que no todos los efectos biológicos de la RUV se pueden evitar mediante una correcta protección local, el fotoprotector en crema puede evitar la mayoría de cambios inducidos, pero no todos, puesto que existen ciertos efectos biológicos en áreas protegidas, y algunos sólo evidentes a nivel molecular.

Se concluye que la interacción de la RUV y las lesiones melanocíticas es compleja, y no solo relacionada con las vías pigmentarias celulares. La prevención primaria mediante una correcta información y actitud de fotoprotección, y la detección precoz de lesiones potencialmente malignas son las principales estrategias que pueden mejorar el pronóstico de melanoma en nuestro medio.

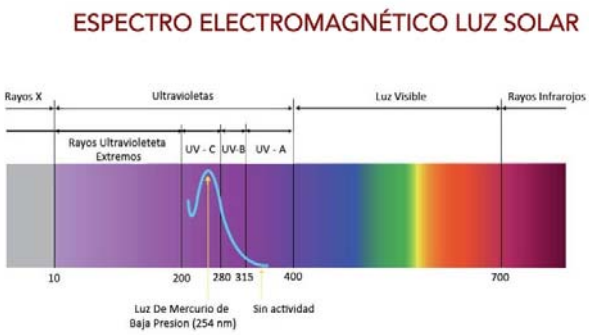
TABLA DE CONTENIDOS

I. RADIACIÓN ULTRAVIOLETA

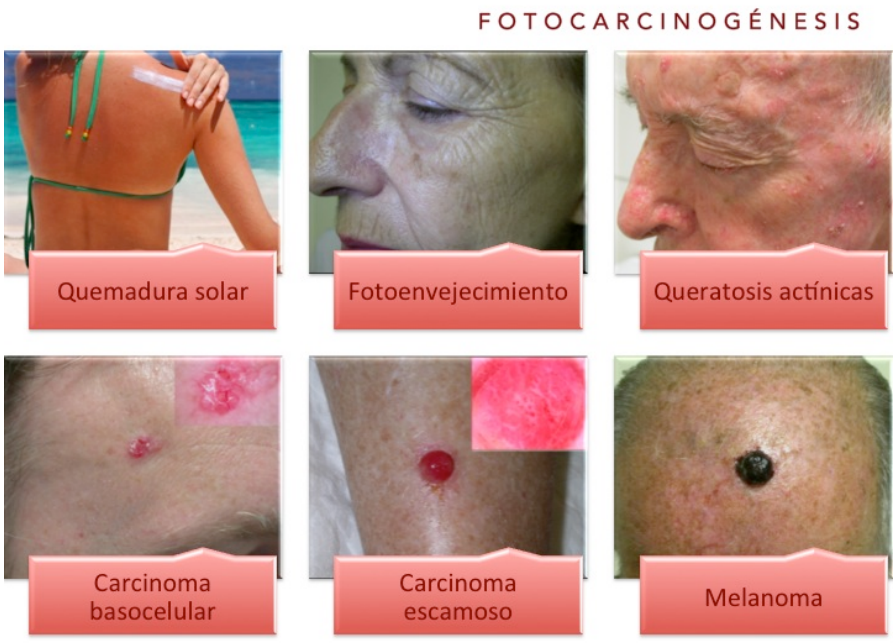
1. EFECTOS BIOLÓGICOS DE LA RADIACIÓN ULTRAVIOLETA

La **radiación ultravioleta (RUV)** es la principal causa del cáncer cutáneo, la neoplasia maligna más frecuente del ser humano. Siendo únicamente el 5% de la radiación emitida por el Sol, provoca sus principales efectos dañinos, y desde el año 2003 ya está incluida dentro de la lista oficial del *National Institute of Environmental Health Sciences* [1] de EEUU como cancerígeno conocido y demostrado.

Tanto de forma aguda como crónica o acumulativa, provoca quemadura solar, inmunosupresión, fotoenvejecimiento cutáneo y ocular, melanoma y otros tipos de cáncer cutáneo.



Debido a la mayor esperanza de vida, y a cambios en los hábitos lúdico-deportivos y estéticos a partir del siglo XX, la carcinogénesis inducida por la RUV se ha convertido en un auténtico problema socio-sanitario, sin olvidar que cerca de 3 millones de personas al año, pierden la visión a causa del daño actínico ocular y las cataratas fotoinducidas[2,3]



Por otro lado, la RUV es necesaria para la vida, y ejerce también efectos beneficiosos mayoritariamente a través de la síntesis de la **vitamina D** en la piel, necesaria para el metabolismo músculo-esquelético, fosfo-cálcico, y también interviene en diversas funciones de homeostasis hormonal e incluso anticancerígenas. Estudios epidemiológicos han demostrado un posible efector protector frente al desarrollo de diferentes tipos de neoplasias, y a su vez, un también un papel pronóstico de la evolución del cáncer[4]. Existen datos contradictorios sobre la necesidad de fotoprotección extrema en los pacientes afectos de melanoma[5]. Como ejemplo, el estudio caso-control de Berwick y col. en el cual la supervivencia de los pacientes de melanoma era mejor en los casos de mayor exposición solar, una vez ajustado el resto de factores pronóstico[6].

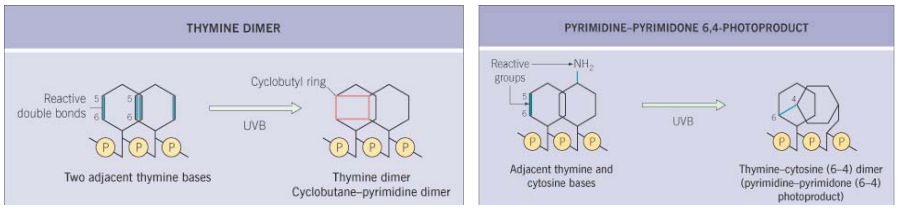
2. FOTOCARCINOGÉNESIS

La RUV es absorbida por diferentes cromóforos en la piel, el ADN es su principal diana y donde provoca sus mayores efectos dañinos, siendo pieza fundamental en la fotocarcinogénesis, melanocítica y queratinocítica[7].

Ejerce estas funciones deletéreas a través de acciones directas e indirectas, agudas y acumulativas, de inmunosupresión, daño directo del ADN celular, y daño indirecto a través del estrés oxidativo y formación de radicales superóxido.

La RUVB supone un 5% de la RUV que atraviesa la capa de ozono, es la de mayor energía y menor longitud de onda (λ 280-320nm), por lo que alcanza la epidermis hasta la unión dermoepidérmica. La RUVA, de menor energía pero mayor longitud de onda, alcanza estructuras hasta la hipodermis (λ 320-400nm) y supone más del 95% de la RUV que recibimos en la superficie terrestre, dependiendo de las condiciones meteorológicas y geográficas puede variar[8,9].

La RUVB se ha considerado la responsable del mayor daño biológico, principalmente a largo-medio plazo de la fotocarcinogénesis, y de forma aguda de la quemadura o eritema solar. Molecularmente se conoce bien su capacidad en generar dímeros de ciclobutano de pirimidina (DCP), y sus fotoproductos (6-4PPs), siendo los principales causantes del daño en el ADN. Los DCP son específicos del daño por RUV, pueden cuantificarse y sub-clasificarse según qué dímero de bases nitrogenadas, y fotoproducto se forme; siendo más característicos de UVB los DCP TT, TC y CT, y los 6-4PP TC y TT.



La RUVA, clásicamente, se había considerado de menor capacidad carcinogénica, más responsable de la inducción de melanina y de la fotoinmunosupresión[10], principalmente a través de la isomerización de la forma trans- a la cis- del ácido urocánico, otro cromófero

cutáneo. Causa principalmente roturas y entrecruzamientos entre proteínas y cadenas simples del ADN, y genera radicales superóxido, responsables del daño oxidativo en la célula. Sin embargo, actualmente, se ha demostrado que la RUVA por sí sola tiene capacidad de producir daño directo genético, generando también DCP (principalmente dímeros TT), por una vía independiente del daño oxidativo[11].

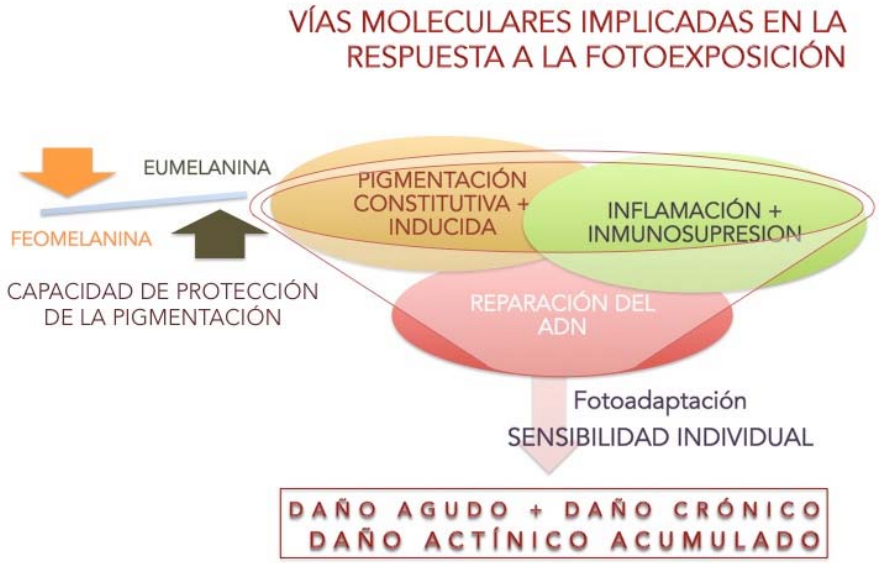
El cáncer cutáneo y el melanoma se incluirían en el modelo de carcinogénesis consistente en iniciación, promoción y progresión tumoral, donde el acúmulo de aberraciones genéticas, sumado a la ineficacia en la eliminación de células anormales, y a factores ambientales favorecedores, explicaría la génesis tumoral.

Probablemente la fotocarcinogénesis de tumores queratinocíticos (o carcinomas) es diferente que la que induce tumores melanocíticos, tanto nevus como melanomas. Se sabe que melanomas y carcinomas exhiben diferentes mutaciones y activación de oncogenes, lo que probablemente traduce diferente tipo de daño del ADN.

La RUV juega un papel fundamental en el desarrollo de las neoplasias melanocíticas, tanto en las benignas (nevus melanocíticos) como en el melanoma[12]. Aunque la fotocarcinogénesis es compleja, y mantiene diferentes cuestiones no resueltas, estudios en modelos animales[13] han demostrado que interviene como factor iniciador, promotor y de progresión en la génesis de tumores melanocíticos[14]. En este sentido, a través de proteínas activadoras del fibroblasto y metaloproteinasas, la degradación de la matriz extracelular puede favorecer la capacidad de migración e invasión de las células neoplásicas *in vitro*[15].

3. FOTOADAPTACIÓN

La sensibilidad a la RUV en el hombre presenta una variabilidad de hasta 1000 veces entre individuos, es decir que ante un mismo tipo y cantidad de RUV los efectos dañinos provocados varían enormemente. Los principales determinantes de esta variabilidad son la capacidad de síntesis de melanina (vías pigmentarias) y los diferentes procesos de inflamación y reparación del daño del ADN, es la llamada foto-adaptación[16,17]. Según las capacidades de cada persona, se alcanza una fotoadaptación y un resultado final del daño fotoinducido, agudo y acumulativo o crónico.



3.1. SÍNTESIS DE MELANINA. VIAS PIGMENTARIAS

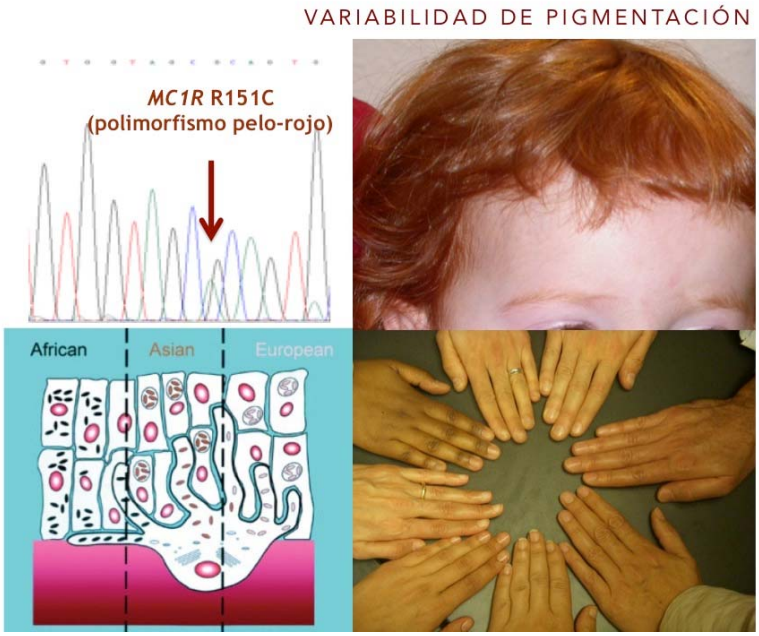
La síntesis de melanina determina el color de cabello, ojos y piel del humano. Según el tipo, cantidad y almacenamiento de melanina, la piel humana tendrá una pigmentación basal (constitutiva) y una pigmentación facultativa, melanina inducida tras la exposición solar. Ambas son diferentes según cada raza e individuo, y están genéticamente determinadas[18]. Cada vez se van describiendo más genes implicados en pigmentación con diferente peso en la susceptibilidad a cáncer y melanoma, aunque la genética de la fotocarcinogénesis no está tan bien aclarada como las vías pigmentarias[19].

Existen múltiples genes implicados en las vías pigmentarias, *MC1R*, *TYR, TYRP, OCA, ASIP*, conociendo se cada vez más y conociéndose mejor los diferentes papeles de cada uno de ellos en determinar el color de ojos, de cabello y de la piel. Variantes genéticas en algunos de ellos implican mayor riesgo a cáncer cutáneo en general y especialmente en melanoma. Globalmente son los llamados "genes modificadores de riesgo" o de "baja-media penetrancia" para el desarrollo de melanoma ya que el incremento de riesgo que supone una variante varía de 1.2 a 10. Explicarían que cada individuo pueda tener una sensibilidad específica al sol, y por tanto el conjunto de ellos puede determinar un mayor o menor riesgo constitutivo al cáncer cutáneo fotoinducido[20–22].

De ellos, el gen mejor caracterizado y más conocido en riesgo a melanoma, es el del receptor de la melanocortina (*MC1R*), receptor en diferentes células del organismo de la hormona melanoestimulante (alfa-MSH)[23]. Codifica una proteína, receptor de membrana acoplada a proteína G-AMPc, de 317–aminoácidos que controla la cantidad relativa de síntesis de los dos tipos de pigmento que producimos:

- la eumelanina: pigmento marrón-negruzco, cromóforo protector del ADN
- la feomelanina: pigmento amarillo-rojizo, sin capacidad de protección, y favorecedor de daño oxidativo

Según la funcionalidad del receptor de alfa-MSH, el melanocito sintetizará en mayor o menor medida eu/feomelanina. Se sabe que la pigmentación final viene determinada precisamente por este índice, existiendo un espectro de color del negro hasta el pelirrojo, determinado entre otros, por las diferentes variantes del gen de *MC1R*[24].



Se sabe que, en la población general, este gen es altamente polimórfico y se conocen más de 65 polimorfismos genéticos que cambian la funcionalidad del receptor respecto a su forma salvaje “wild type”, descritas también como variantes no sinónimas. Según el número y tipo

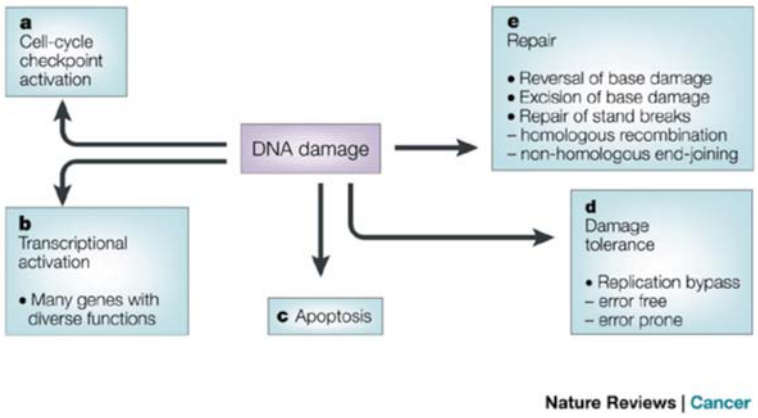
de variante se afectará más o menos la funcionalidad de la proteína y, por tanto, la pigmentación producida[17].

En nuestro medio, el 65% de la población mediterránea presenta alguna variante de la forma salvaje, siendo la V60L la más frecuentemente encontrada[25,26]. Se ha descrito que la presencia de polimorfismos de *MC1R*, en especial los conocidos como "variantes de pelo-rojo" (pD84E, pR151C, pR160W y pD294H), así como la presencia de múltiples variantes, se asocia a un incremento de 2 a 10 veces el riesgo de desarrollar melanoma, así como una edad 10 años menor de debut[22].

3.2. VÍAS DE REPARACIÓN DEL DAÑO DEL ADN

La llamada "inestabilidad genética" (o mutaciones espontáneas) que sufren las células germinales es la responsable de la evolución biológica natural y diversidad de las especies, en cambio, a nivel somático, estas mutaciones provocadas a menudo por agentes exógenos, son generalmente dañinas. La carcinogénesis es una de las consecuencias mejor estudiada y frecuente en el humano. Se estima que a diario cada célula humana sufre una media de 25.000 cambios espontáneos de bases del global de $3x10^9$ que posee su genoma, como consecuencia de las agresiones que recibe. La RUV es la fuente principal de daño que recibe el ADN de las células de la piel.

Sin embargo el organismo tiene diferentes vías intracelulares específica y eficazmente dirigidas a prevenir la carcinogénesis inducida por RUV, mediante identificación y reparación del ADN dañado, o inducción de la apoptosis si la reparación ha sido ineficaz. Son las llamadas vías de reparación del ADN[27]. Una de las vías de regulación celular ante el fotodaño es la llamada activación de los *"checkpoints"* de ciclo celular, tanto en la transición de fase G1 a la S, en la de G2 a la de mitosis, o en la fase de replicación del ADN. Según el tipo de daño del ADN inducido por un carcinógeno, se activarán o detendrá el ciclo celular mediado por una vía diferente[28].



Diversas proteínas están implicadas en la regulación del ciclo celular; como por ejemplo: p53, p21, p14, p16, cuya función sería inducir un estacionamiento o inhibición del ciclo, de forma que haya tiempo suficiente para la reparación de ese daño. En caso contrario, inducen su apoptosis, y así, no permiten que dicha célula se replique y aparezca un clon aberrante.

El caso más evidente de alteración en los mecanismo de identificación y reparación del daño del ADN son los pacientes afectos de Xeroderma Pigmentoso (XP), portadores de diferentes tipos de mutaciones germinales que provocan mayor riesgo de neoplasias ante cualquier agresión carcinogénica, en especial a la luz solar. Así la mayoría de enzimas implicadas en las vías de reparación se denominan con las iniciales XP.

Se conocen distintos mecanismos: 1) Excisión de bases (gen *XRCC1*); 2) Excisión de nucleótidos (genes *XPA-G, y XPV*)[27]; y 3) Reparación de la doble cadena homóloga (gen *XRCC3*)[29]. Según qué polimorfismos y/o mutaciones presenten dichos genes la reparación del daño inducido por RUV será más o menos eficiente[30,31].

También se ha relacionado la expresión de la proteína p16 (inducida por exposición a RUV como mecanismo reparador del daño de ADN) con los diferentes polimorfismos del gen del *MC1R*. De forma que se demuestra una estrecha relación entre síntesis de melanina y reparación del ADN.

1. MELANOMA: EPIDEMIA CRECIENTE DE NUEVOS CASOS

El melanoma constituye la causa de mayor morbimortalidad dermatológica, con una incidencia cada vez mayor, manteniendo un incremento anual del 6%. En los últimos 50 años se ha multiplicado su incidencia en más del cuádruple, pasando de 3-4 casos a 10-15 casos por 100,000 habitantes y año, con especial repercusión entre mujeres jóvenes[32–34]. Estudios a nivel mundial confirman que esta tendencia al alza se mantendrá al menos en las próximas dos décadas, y se espera que la incidencia se doble de nuevo[35].

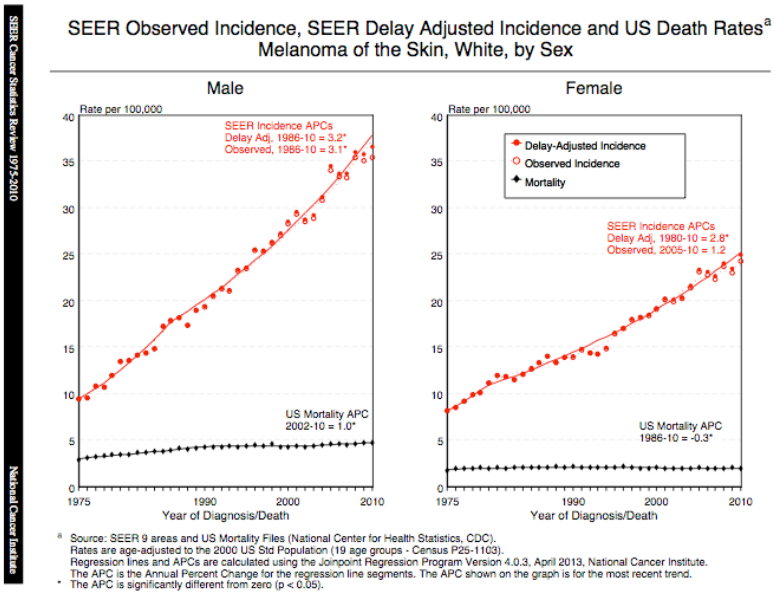


Figura 1. Curvas de evolución de incidencia y mortalidad (nuevos casos/100.000 hab) de melanoma desde 1975 a 2010 según la SEER National Cancer Statistics de EEUU.

Las estimaciones en nuestro medio confirman la misma tendencia en el resto de población caucásica, con el mayor incremento de incidencia que cualquier otro tumor, especialmente en mujeres jóvenes[36,37]. Se estima una incidencia similar a Italia o Francia, con cifras que oscilan entre 10,4-15,7 nuevos casos (hombres y mujeres, respectivamente) por 100.000 habitantes y año. Asimismo, se estima que esta incidencia aumente en los próximos 5 años hasta 12 y 19,7 nuevos casos por 100.000 habitantes y año en hombres y mujeres respectivamente, alcanzando dentro de 10 años valores de 13,9 en el caso de los hombres y 24,9 en las mujeres.

Adicionalmente del interesante papel de la RUV en la inducción de tumores melanocíticos, demostrada en modelos animales y en cultivos celulares[13–15], a nivel epidemiológico esta auténtica y creciente ola de incidencia de MM en poblaciones caucásicas se explica en gran parte por los hábitos de fotoexposición. Múltiples estudios han demostrado el incremento de riesgo de desarrollar MM en relación a la RUV recibida[38,39], principalmente incrementado en la población caucásica expuesta a luz solar de forma intensa e intermitente, en individuos que hayan sufrido quemaduras solares antes de los 15 años de edad, y de forma muy preocupante, en aquellos que usan dispositivos de bronceado artificial[2,40–43].

2. RUV Y MELANOMAGENICIDAD

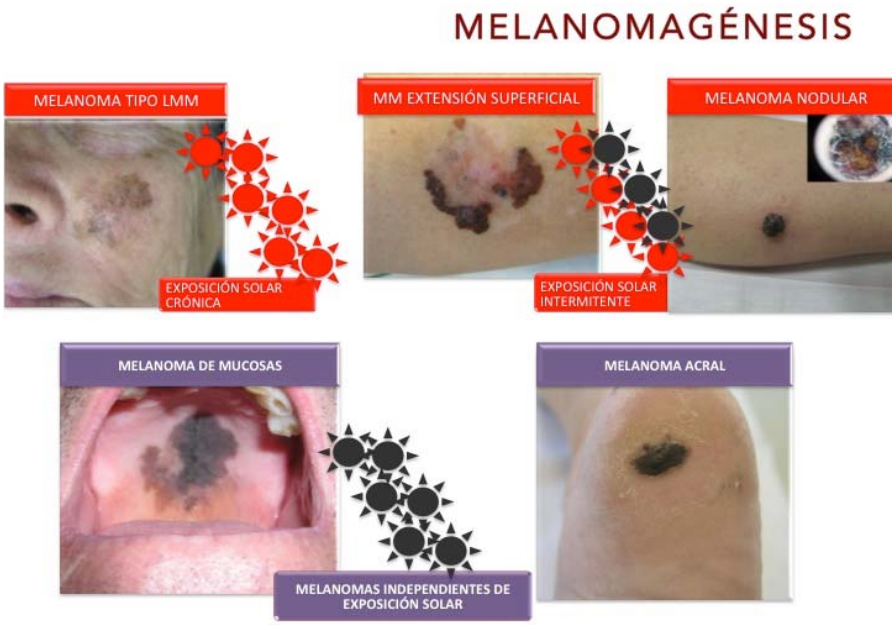
2.1. ETIOPATOGENIA DEL MELANOMA

El melanoma (MM) se puede considerar una enfermedad poligénica multifactorial, en la cual ya se van identificando cada vez más genes de alta susceptibilidad, principalmente implicados en los casos de MM múltiple y familiar (genes supresores tumorales u oncogenes implicados en regulación del ciclo celular *CDKN2A, CDK4*[44–46]), y otros genes menores o modificadores del riesgo (como *MC1R, TYR, ASIP)* en su mayoría implicados en las vías pigmentarias y respuesta cutánea a la RUV.

Así en la etiopatogenia de MM se reconocerán factores endógenos (genéticamente determinados) y un principal factor exógeno modificable, que es la radiación ultravioleta (RUV). El 90% de casos en nuestro medio son MM esporádicos y los principales factores de riesgo implicados son la RUV y la presencia de nevus melanocíticos. Por ello se considera el fenotipo de riesgo definido por la presencia de múltiples nevus melanocíticos y/o el fototipo claro con dificultad para broncearse y tendencia a quemarse[47] y/o color de ojos verdes o azules y/o cabello rubio o pelirrojo. Todos estos factores interaccionan de forma compleja con la RUV recibida, especialmente en los primeros 15 años de vida, se potencian entre sí y podrían explicar las diversas vías patogénicas de los tipos de MM. Así, a pesar que es incuestionable que la RUV es el factor ambiental exógeno de mayor implicación etiopatogénica en la mayoría de MM en caucásicos, la relación entre RUV y melanoma-génesis es compleja.

Green y col. ya en 1992 establecieron las primeras hipótesis sobre la influencia de la localización anatómica y por tanto el tipo de exposición solar, en la diferente respuesta de los melanocitos, y por ello el riesgo y tipo de MM desarrollado puede variar. Así se empezó a diferenciar entre el papel de la exposición solar intermitente intensa, implicada en el MM de tronco y extremidades, de la exposición crónica continua, implicada en el MM de cabeza y cuello[48].

De igual manera, la clasificación clínico-patológica clásica de Clark apoya esta teoría, puesto que el MM tipo de extensión superficial, el 60% en nuestro medio, se asocia al tronco y extremidades, debuta en edades más jóvenes, y se asocia más frecuentemente a la presencia de nevus melanocíticos y exposición solar intermitente. El MM tipo lentigo maligno, se presenta en áreas de daño actínico marcado, en pacientes de edad más avanzada, independientemente de la presencia de nevus. Se asume también que existen una minoría de MM en población caucásica (pero mayoritarios en razas más pigmentadas, y que afectan por igual en el mundo entero, independientemente de la raza) que no encajarían en el modelo de la fotoinducción, como es el MM lentiginoso acral o el de mucosas.



La caracterización molecular de los tumores a nivel somático[49], ha supuesto una auténtica revolución tanto del conocimiento etiopatogénico como en las nuevas terapias del MM metastásico. Molecularmente se pueden clasificar diferentes tipos de tumores (como los que presentan activación de las vías de las MAP-kinasas o los que presentan c-KIT mutado). A su vez, se ha demostrando que existe una clara relación entre el tipo clínico-patológico de MM que se desarrolla, el tipo de RUV recibida en cada área anatómica (intermitente, crónica, o no fotoexpuesto), y el fenotipo del paciente (edad, tendencia a asociar nevus o tendencia a presentar fotoenvejecimiento).

2.2.. MELANOMAS FOTOINDUCIDOS:

Chang y col. estudiaron cada factor de riesgo para MM, en función de la localización anatómica y de la latitud geográfica. En cualquier país, las exposiciones solares vacacionales están más implicadas en el desarrollo de MM de tronco y extremidades, a diferencia del de cabeza y cuello. Las quemaduras solares en la infancia se relacionan con todos los tipos MM. La exposición solar mantenida laboral, al igual que la presencia de signos de daño actínico acumulado (queratosis actínicas) se relaciona principalmente con MM de cabeza y cuello, en

áreas de latitudes bajas. Geográficamente, en latitudes más bajas el daño solar acumulado tiene mayor peso que en latitudes más altas[50].

Ciani y col realizaron un metanálisis sistemático para cuantificar factores de riesgo según el tipo histológico y localización anatómica del MM. Las diferencias en el riesgo relativo que confiere la presencia de múltiples nevus y los distintos patrones de exposición solar, apoyan las teorías de diferentes vías etiopatogénicas del MM; la vía de la nevogenicidad (relacionada con MM de extensión superficial, genes de nevogenicidad, activación de BRAF y exposición solar intermitente) y la vía del daño actínico acumulado (relacionada con MM tipo lentigo maligno, genes de vías pigmentarias, activación de cKit o RAS y exposición solar continua)[51].

Por tanto, los MM que se pueden considerar más claramente relacionados con la RUV (MM fotoinducidos) serían los de tipo extensión superficial en tronco y extremidades, relacionados con la activación de las vías de las MAP-kinasas, y los tipo MM lentigo maligno de cabeza y cuello. El MM tipo lentigo maligno, sería el que clásicamente aparecía en pacientes caucásicos de edad avanzada, lentamente evolutivo, en forma de mácula hiperpigmentada de bordes mal definidos en zonas con marcado daño actínico. Aunque clásicamente se había descrito como el 3º en frecuencia, (10-15 % del total de MM en caucásicos), en las últimas décadas se ha registrado un importante aumento de la incidencia y a edades más tempranas. Los principales factores epidemiológicos causantes de este cambio son el envejecimiento global de la población sumado al cambio en el patrón de fotoexposición con la consiguiente acumulación de radiación solar detectada en sociedades occidentales en las últimas décadas.

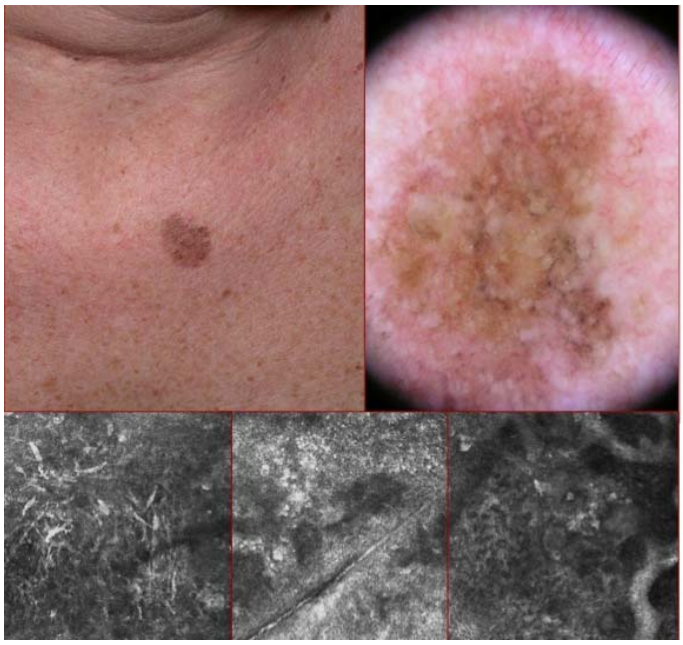


Figura 2. Melanoma fotoinducido en área precordial de paciente de 53 años con marcado daño actínico. Clínica-dermatoscópicamente y mediante confocal compatible con melanoma incipiente tipo lentigo maligno melanoma in situ (confirmado tras biopsia y extirpación quirúrgica).

1. NEVUS MELANOCÍTICOS: MARCADOR DE RIESGO DE MELANOMA

La importancia de la presencia de nevus melanocíticos y en especial cuando son clínica, dermatoscópica o histopatológicamente atípicos, viene determinada por ser **simuladores de MM incipientes** y **marcadores de fenotipo de riesgo**, independientemente del fototipo.

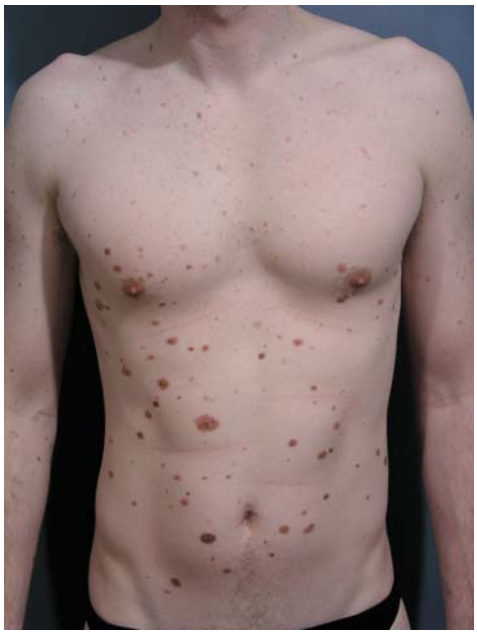


Figura 3. Presencia de múltiples nevus atípicos como **marcador** de fenotipo de individuo de alto riesgo a melanoma.

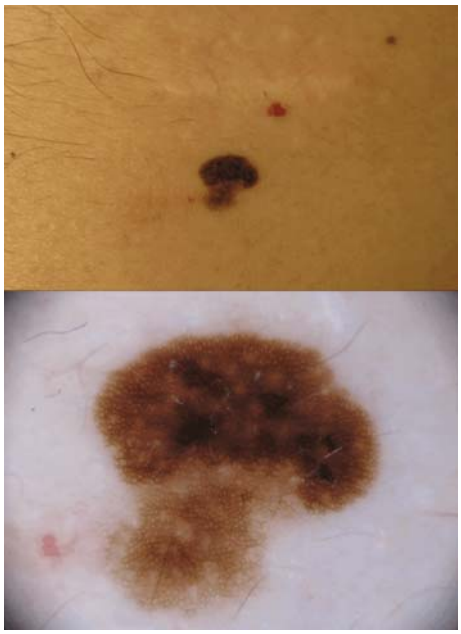


Figura 4. Nevus clínicamente atípico como **simulador** de melanoma incipiente. Dermatoscopia con retículo pigmentado típico pero áreas pigmentadas focales.

En menor medida pueden ser **precursores de MM** desarrollados sobre nevus, pero dicha posibilidad no justifica la extirpación preventiva de nevus[33,52,53]. Tsao y col. estimaron que el riesgo de transformación de un nevus en MM oscilaría entre < 1 de cada 200.000 nevus en menores de 40 años de edad, y 1 de cada 33.000 nevus en varones mayores de 60 años. El riesgo de presentar un melanoma sobre nevus a lo largo de la vida es de 0.03% en hombres y de 0.009% en mujeres, por lo que la probabilidad anual de que al extirpar un nevus evitemos un melanoma, sería en 1 de cada 30.089 nevus en hombres y de cada 39.809 nevus en mujeres[54]. Se demostró así que no está justificada la extirpación de nevus con fines preventivos.

En cambio, la presencia de múltiples nevus cobra especial importancia, tal y como muestra el metanálisis de Gandini y col. Al menos 13 estudios han demostrado un incremento de 10 veces el riesgo de padecer melanoma si se presentan nevus displásicos, [RR 10.1; 95% intervalo de confianza, 5.0–20.3]. En los 15 estudios en los que se estudia como variable continua, se aprecia que el incremento de riesgo también es proporcional al número de nevus atípicos desde 1.6 hasta 10.5 veces el incremento de riesgo[43].

2. RUV Y NEVOGENICIDAD: NEVUS FOTOINDUCIDOS

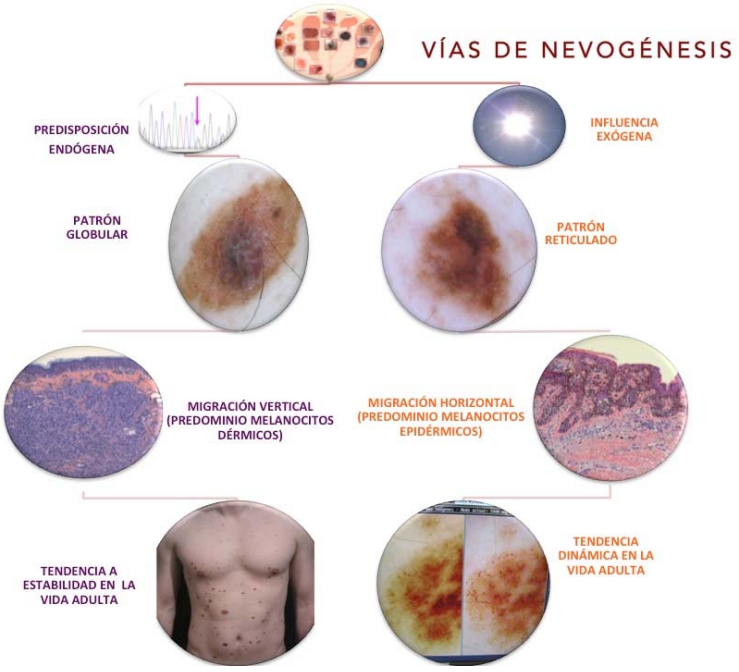
Se estima que la tendencia a presentar múltiples nevus tiene una carga genética de un mínimo del 40%, con herencia autosómica dominante, pero el otro 60% de la tendencia sería la carga ambiental, fundamentalmente la RUV. Así el número y tamaño de los nevus depende de factores endógenos, pero está modulado por la RUV, principalmente en edades tempranas por lo que la RUV jugaría un doble papel en el riesgo a melanoma[55,56].

A pesar de conocer diversos loci implicados en nevogenicidad, como MTAP (9p21), IRF (6p25), PLA2G6 (22q13), el receptor de la vitamina D o PAX3 todavía no se ha definido bien el peso específico en el fenotipo de pacientes con múltiples nevus atípicos[57,58]

Desde el punto de vista epidemiológico se ha demostrado que la exposición solar en edades tempranas, induce un mayor número y tamaño de nevus melanocíticos en la edad adulta y, no sólo se ha podido demostrar en estudios poblacionales, sino también en estudios entre gemelos genéticamente idénticos[56,59,60].

Lee y col. demostraron en un estudio prospectivo que una fotoprotección correcta en edad escolar disminuye el número de nevus en el tronco, y este efecto protector fue más pronunciado entre los niños con tendencia a presentar efélides y mayor sensibilidad al sol[61].

Por otra parte, gracias al uso de las técnicas de imagen no invasivas (dermatoscopia y microscopía confocal *in vivo*) y al seguimiento digital, se ha podido avanzar enormemente en los conocimientos sobre nevogénesis[62–64]. En función de la edad la mayoría de nevus antes de la pubertad presentan patrones globulares, mientras que a partir de la adolescencia suelen predominar los patrones mixtos - con áreas homogéneas y glóbulos en periferia [65]. Gracias al uso de la dermatoscopia digital y de la microscopía confocal se ha podido observar que este tipo de patrón es indicativo de lesión inestable en crecimiento, y el centro de la lesión va adquiriendo o bien patrón reticulado o bien homogéneo hasta que desaparecen los glóbulos en periferia cuando cesa el crecimiento[66]. Así en la edad adulta la mayoría de nevus muestran patrones únicamente reticulados o mixtos con área central homogénea-globular[67]. Zalaudek y col.[68] han demostrado que la presencia de mutaciones de BRAF en nevus puede variar también en función de la edad, al igual que el patrón dermatoscópico de los nevus depende de la edad.



Así, hoy en día se acepta que existirían nevus congénitos o de aparición en primera infancia, independientes de la RUV recibida, que en su mayoría presentan patrones globulares, evolucionando hacia patrones en empedrado/homogéneos, típicos de nevus compuestos,

sin criterio de atipia clínica, dermatoscópica ni histológica, y que pueden involucionar al final de la vida. Estos nevus serían más frecuentemente BRAF mutados.

Un segundo tipo de nevus, serían claramente "fotoinducidos", y sería aquellos adquiridos principalmente a partir de la adolescencia, influenciados por la RUV recibida de forma aguda y acumulativa, que dermatoscópicamente suelen presentar patrones reticulados, que en confocal se traducen por patrones en anillas o en malla, e histológicamente se correlacionan con áreas de hiperplasia lentiginosa de melanocitos, con menor o mayor grado de atipia clínica, dermatoscópica y/o histológica[69,70].

3. ESTUDIOS SOBRE EFECTOS DE LA RUV AGUDA EN NEVUS

Múltiples estudios han descrito los efectos inmediatos producidos por la RUV (natural o artificial) sobre lesiones melanocíticas benignas[71–76]. Se han observado cambios demostrables, clínica, dermatoscópica e histológicamente, en nevus melanocíticos en función de la estación del año que se extirpen, o tras haber recibido fototerapia. Tronnier y col[77] demostraron que una única dosis de radiación UV provoca cambios histopatológicos (incremento del número de melanocitos y nevocitos suprabasales, y mayor índice núcleo/citoplasma en melanocitos), e inmunohistoquímicos (incremento de la expresión de HMB-45). Este aspecto morfológico se ha observado en melanomas in situ, y de hecho los nevus expuestos a radiación UV se consideran simuladores de melanoma[78]. Se ha estudiado mediante inmunohistoquímica la expresión de marcadores de melanogénesis, proliferación celular, y proteínas reguladoras de ciclo celular[79], demostrando que la RUV induce la activación y proliferación de melanocitos, y estimula mecanismos reparadores del daño nuclear generado. También se ha observado una diferente expresión de enzimas colagenasas: metaloproteinasas (MMP-2, y su inhibidor tisular: TIMP), en células névicas y queratinocitos en condiciones fisiológicas. Se ha encontrado, así mismo, que la influencia de la RUV puede potenciar la disminución de TIMP en melanocitos, lo que facilita la progresión de las lesiones melanocíticas hacia la dermis[80,81].

Diversos estudios han observado mediante la cuantificación de dímeros de ciclobutano de pirimidina, el estudio de marcadores de apoptosis (p53, Bcl-2, survivina), y la capacidad de reparación del daño del ADN, que los nevus melanocíticos irradiados, presentan diferente sensibilidad a la RUV comparado con la piel normal o el melanoma[31,82,83] (tabla 1, anexo trabajo II).

Por tanto, existiría una gran variabilidad de respuesta y reparación del daño inducido por la RUV entre individuos, sumada a la compleja interacción entre los diversos factores endógenos que confieren susceptibilidad al desarrollo tanto de nevus como de melanoma. De esta forma, se reconoce que la RUV juega un papel primordial, aunque no bien establecido, como iniciador, promotor y potenciador del riesgo a desarrollar nevus y melanoma[21].

IV. PREVENCIÓN EN MELANOMA

1. ¿ES POSIBLE LA PREVENCIÓN?

El melanoma cumple todos los requisitos necesarios para poder establecer una prevención eficiente. Primero, porque se conoce el principal factor ambiental implicado, la RUV, que es evitable y modificable (prevención primaria). En segundo lugar, porque el MM es visible y cada vez mejor reconocido por el experto, por tanto la prevención secundaria y detección precoz es un deber del dermatólogo. Y, en tercer lugar, porque se puede identificar mediante la historia personal, familiar y el fenotipo, el grupo de población de mayor riesgo endógeno, en quienes se puede diseñar un seguimiento específico dermatológico.

2. ¿NECESIDAD DE PREVENCIÓN?

Desde la declaración del Profesor Ackerman en 1985[84] "Nadie debería morir ya de melanoma", han pasado más de 25 años y el melanoma continua suponiendo más del 80% de las muertes por cáncer cutáneo. Ante la evidente ola creciente de nuevos diagnósticos y a pesar de una mejora en su diagnóstico y tratamiento, la mortalidad por MM no desciende, a diferencia de la mortalidad global por cáncer que tiene una tendencia mundial al descenso, aproximadamente de un 1.5% menor al año[85].

El número de muertes por MM se ha incrementado en la mayoría de las poblaciones caucásicas en las últimas décadas, aunque en menor medida que la tasa de incidencia. Se debe tener presente, que hasta 1 de cada 10 casos incipientes morirá a causa de MM a largo plazo, y puesto que actualmente no podemos predecir qué MM delgados metastatizarán, la conducta correcta es la extirpación de estos tumores de forma precoz. No debemos olvidar, que el MM avanzado o diseminado, a pesar del avance en el conocimiento molecular que ha posibilitado el desarrollo de nuevas terapias diana y una prometedora revolución terapéutica de los últimos 2 años[49,86,87], continua teniendo un pronóstico infausto, con supervivencias medias de 6-12 meses. De hecho, debido a la edad de incidencia en población joven y el porcentaje de muertes que implica, se considera la segunda causa de años de vida potencialmente perdidos y de pérdida de años productivos. Se estima que por cada muerte por MM se pierden 15 años potenciales de vida productiva, lo que alcanza un gasto sanitario anual en EEUU de hasta 40 millones de dólares en morbilidad y de más de 3000 millones de dólares por mortalidad prematura[88–90].

Sin embargo, a partir de los años 90 en algunos países se ha detectado una tendencia a la estabilidad o disminución de mortalidad por MM, atribuible al diagnóstico precoz, y por ello el descenso de mortalidad se espera que evolucione de forma paralela a la disminución del Breslow. Por tanto no hay duda que la prevención es posible y necesaria.

3. PREVENCIÓN PRIMARIA

La fotoprotección se considera el primer paso para una correcta prevención del melanoma. El uso de cremas fotoprotectoras son la medida de protección más aceptada y utilizada en la población general. Green y col. demostraron en 1999[91] que la correcta fotoprotección previene el cáncer cutáneo en Australia. Sin embargo hasta 10 años más tarde no pudieron demostrar que también previene la incidencia de melanoma. En su estudio prospectivo encuentran que una correcta protección solar reduce la incidencia de melanoma, e incluso que mejora el pronóstico, puesto que el Breslow medio de los melanomas en el grupo protegido era menor[92].

A pesar de los resultados del estudio australiano, existen todavía grandes controversias sobre si el uso de cremas de protección aportará un beneficio franco en el riesgo a desarrollar MM. El metanálisis del 2002[93] sólo pudo demostrar una mínima ventaja, y probablemente existen diversos factores a tener en cuenta. La incorrecta aplicación de las cremas puesto que habitualmente no se sigue la pauta recomendada, o sobre todo por existir un sesgo en los estudios epidemiológicos, ya que la población que más se protege es la que más se expone al sol, o el uso de fotoprotectores podría inducir hábitos nocivos como el “bronceado sano” o el incremento de tiempo de exposición[5,94,95].

Probablemente la mejor estrategia en protección solar, es la no exposición, pero dada la imposibilidad de dicha opción, lo correcto sería una combinación de medidas. Una fotoprotección correcta ha de incluir una serie de medidas educacionales y de hábitos correctos para asegurar la optimización de la mejor medida de prevención primaria[96].

NIEVELES DE ACTUACIÓN EN FOTOPROTECCIÓN:

- Evitar / limitar horas de exposición solar y horarios de máxima irradiación. Información adecuada de las condiciones meteorológicas y geográficas que afectan al índice de RUV (UVI).
- Uso de barreras físicas como ropa y sombrero adecuados y gafas con cristal de protección de amplio espectro.
- Fotoprotectores tópicos: correcta información sobre cual, cómo y cuándo se han de aplicar y renovar.
- Fotoprotección sistémica y fotoquimioprofilaxis secundaria; productos tópicos o sistémicos que pueden favorecer la protección y/o reparación del fotodaño.

3.1. FOTOPROTECTORES TÓPICOS:

Los fotoprotectores solares (FPS) están compuestos por moléculas que, gracias a sus características químicas y/o físicas, evitan el eritema y quemadura inducidos por RUV, mediante mecanismos de reflexión, refracción, absorción, y/o trasformación de la energía que llega a la superficie cutánea en forma de fotones.

Un FPS ha de cumplir unos requisitos de seguridad y fotoestabilidad. El ideal debería absorber la RUV en su amplio espectro desde UVA a UVB, ser estable a pesar de recibir la energía de la RUV; no provocar reacciones de fototoxia o fotoalergia, ni ser mutagénico, no debe absorberse, y de forma idónea, debería evitar la formación radicales libres oxígeno y activar la capacidad de reparación del daño del ADN.

CLASIFICACIÓN DE MOLÉCULAS USADAS EN FPS:

- **Químicos/orgánicos:** moléculas con capacidad de absorber y reflejar fotones, transformando su estructura química y convertir la energía en calor. Los más habituales y aprobados por la FDA y la Unión Europea incluyen:

Principalmente UVB:

- Cinamatos (Parsol MCX)
- Salicilatos
- Octocrileno
- Benzofenonas (oxybenzonas)

Principalmente UVA:

- Avobenzona (Parsol 1789)
- Ácido sulfónico de tereftalideno-dialcanfor (Mexoryl® SX)

Amplio espectro :

- Drometizol trisoloxano (Mexoryl® XL)
- Tinosorb M o S

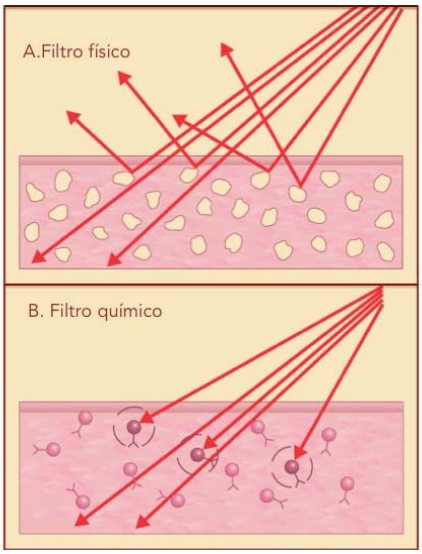


Figura 5. Esquemático del mecanismo de acción de los protectores tópicos.

- **Físicos/inorgánicos:** partículas minerales de mayor tamaño, con capacidad de dispersar, reflejar y absorber la energía fotónica incidente. Permiten evitar prácticamente toda la franja de RUV, y también la visible y parte de la infrarroja. Básicamente se usa el óxido de zinc y el dióxido de titanio micronizados.

Habitualmente, la mayoría de productos comercializados combinan ambos tipos de moléculas, generalmente los químicos son más cosméticos y fáciles de aplicar, pero los físicos son más seguros en cuanto a cubrir la RUVA y mayores porcentajes de energía.

ÍNDICE DE PROTECCIÓN SOLAR.

Desde COLIPA (Federación Europea de Cosmetics, Toiletry and other Perfumery products Association) se consensuó en 2006 que toda la industria farmacosmética debía estandarizar los métodos de medición de capacidad de absorción de la RUV. En la Unión Europea los FPS son considerados productos cosméticos, y para testar su efectividad tanto *in vivo* como *in vitro*, se utilizan las medidas de SPF (Sun Protection Factor), el factor de protección frente UVA (PF-UVA), el ratio entre uno y otro (SPF/PF-UVA) y la longitud de onda crítica.

El SPF es un índice que indica el incremento de tiempo en aparición de eritema sin protección frente al tiempo con protección, es decir mide una proporción del incremento de tiempo que en condiciones ideales de cantidad y homogeneidad en la aplicación de la crema

se podría exponer la piel sin aparición de quemadura solar. De forma que un índice de SPF 20, indica que en vez de aparecer eritema a los 10minutos de exposición, aparecería a los 200 minutos.

SEGURIDAD DE LA FOTOPROTECCIÓN TÓPICA.

Se deben tener en cuenta importantes cuestiones en relación a la eficacia del FPS, por una parte el espectro de RUV que puede abarcar: el eritema solar se sabe que se induce por el espectro de RUVB y la onda corta de la RUVA (320-340nm); la cuestión es cómo medir correctamente la capacidad de absorción y por tanto la protección frente todo el espectro de UVA puede ser más complejo[97].

La segunda cuestión a tener en cuenta es la cantidad y uniformidad de aplicación. Múltiples estudios han revalidado que a pesar de las condiciones reales de aplicación (menos de 2mg/cm2, irregularidad en la aplicación, sudoración, cosmética del producto), a partir de SPF30 se podría considerar que se alcanza una correcta protección[98,99].

Las autoridades sanitarias han de tener en cuenta a la hora de regular el uso de filtros, su seguridad a corto y largo plazo, en cuanto a fotoestabilidad, genotoxicidad, absorción, fotosensibilización La Unión Europea actualmente ha estandarizado y regulado el uso de fotoprotectores sobre todo en cuanto a las técnicas de medición de índices de protección y seguridad. Se han definido las diferentes categorías de protección solar, las recomendaciones de un correcto uso y aplicación, así como la información que debe incluirse en el envase o prospecto. La armonización en medidas debe asegurar un amplio espectro de RUV absorbida, así como en un elevado porcentaje de la misma, permitiendo evitar los efectos agudos, subagudos, crónicos, así como la fotosensibilización y fototoxia. A su vez la autorización reglamentaria de filtros empleados trata de evitar el uso de productos que puedan ser inestables tras la exposición solar, tóxicos para el humano o el medio ambiente, o fotoalergénicos con el uso repetido[100]. También en los últimos años, se ha incrementado el control y la regularización del espectro de luz solar y de energía de radiación que absorben. Por ejemplo, igual que actualmente se sabe que la RUVA también es cancerígena, e incluso puede que con un peso específico importante en el riesgo a melanoma, también está cobrando mayor importancia la radiación infrarroja, puesto que se ha visto que también puede favorecer daño oxidativo celular y foto-envejecimiento[28]. Otros factores que han influido en los estudios de seguridad son la prohibición de experimentación animal para el desarrollo de cosméticos, la sensibilización general por la protección del medio ambiente, y la reciente incorporación de nanopartículas, cada vez más micronizadas,

que pueden mejorar las cualidades cosméticas de los productos, pero también implicar mayor riesgo de absorción.

EFICACIA DE LA FOTOPROTECCIÓN FRENTE CARCINOGÉNESIS

El SPF utiliza el eritema como factor de referencia, y por tanto la quemadura solar. Existen escasos estudios in vivo o in vitro que cuantifiquen y validen la eficacia de los FPS evitando otros efectos biológicos o que tengan en cuenta condiciones reales de utilización en humanos[101].

Bykov y col. cuantificaron la producción de fotoproductos en epidermis con y sin FPS; demostrando una gran variabilidad interindividual sin protección, y evidente disminución con protección, aunque no previsible qué pacientes se protegían más que otros[102]. También se ha estudiado la expresión de p53 en queratinocitos inducida por radiación UV (reflejo indirecto del daño en el ADN) y su disminución al aplicar fotoprotector tópico[99,103].

La fotoprotección y la fotoexposición necesitan un equilibrio para evitar los efectos dañinos de la RUV, permitiendo una correcta activación de la vitamina D, y a su vez que la forma de protección sea segura y completa. Se debe tener en cuenta que los intereses financieros de la industria del turismo y de los cosméticos ejercen una importante peso en la promoción de la fotoexposición, el bronceado y el uso de cremas de protección. Ello favorece que la estrategia de fotoprotección con mayor aceptación por la población sigue siendo el uso de fotoprotectores tópicos, pero a menudo con la intención de obtener "bronceados sanos y seguros".

Hasta la actualidad, no se disponía de estudios in vivo en humanos sobre el posible efecto de los FPS ante los efectos de la RUV como carcinogénico e inductor de tumores melanocíticos.

3.2. FOTOPROTECCIÓN SISTÉMICA. QUIMIOPREVENCIÓN SECUNDARIA.

Existen diversas sustancias administradas de forma oral que pueden tener propiedades de mejorar la capacidad de protección natural contra la RUV, tanto mediante neutralización del daño oxidativo, como favoreciendo los mecanismos de reparación.

Ejemplos de ello serían antioxidantes naturales como los carotenoides: alfa y beta caroteno, licopeno; vitamina C, vitamina E, y ciertos derivados de botánica como los flavonoides o polifenólicos (entre ellos el extracto del helecho Polipodium leucotomus que puede inhibir

los radicales libres de oxígeno entre otros efectos descritos[104,105], las isoflavonas soja, o el te verde).

Existen cada vez más trabajos en la literatura sobre los posibles efectos beneficiosos de todos estos productos, y la combinación de estrategias de fotoprotección y quimioprevención son infinitas[16].

Destaca también la utilización tópica de productos que favorezcan o intervengan en la reparación del daño fotoinducido, como la incorporación de la fotoliasa en las cremas de protección solar. La fotoliasa es una enzima presente en plantas, bacterias, reptiles, anfibios y marsupiales e incluso en placentas de mamíferos que absorbe la luz visible y utiliza la energía para romper el anillo ciclobutano, lo que se conoce como fotorreactivación. Estudios tanto in vivo como in vitro utilizando fotoliasa derivada de las cianobacterias Anacystis nidulans, incorporada a una crema encapsulada en liposomas demuestran reducir tanto la apoptosis como la producción de DCP en un 50%[106,107] y la mejoría del campo de cancerización asociado a las queratosis actínicas (Butille et al, Exp Dermatol 2013, en prensa)

4. PREVENCIÓN SECUNDARIA

4.1. DIAGNÓSTICO PRECOZ DE MELANOMA

La importancia de la detección precoz se hace más evidente al observar las curvas de supervivencia. Únicamente los MM intraepidérmicos, in situ, se puede considerar curados tras el tratamiento quirúrgico correcto. Por el contrario, a los 10 años del diagnóstico los MM microinvasivos o incipientes, de menos de 1 mm de Breslow sobreviven más del 90%, disminuyendo al 80% en cuanto el Breslow está entre 1 y 2 mm (o menos a 1 mm, pero con ulceración o más de 1 mitosis/mm^2), y no llega al 40% de supervivencia en casos localmente avanzados (Breslow superior a 4mm) aún en ausencia de afectación ganglionar o metastásica en el momento del diagnóstico (Estadio IIB/C)[108].

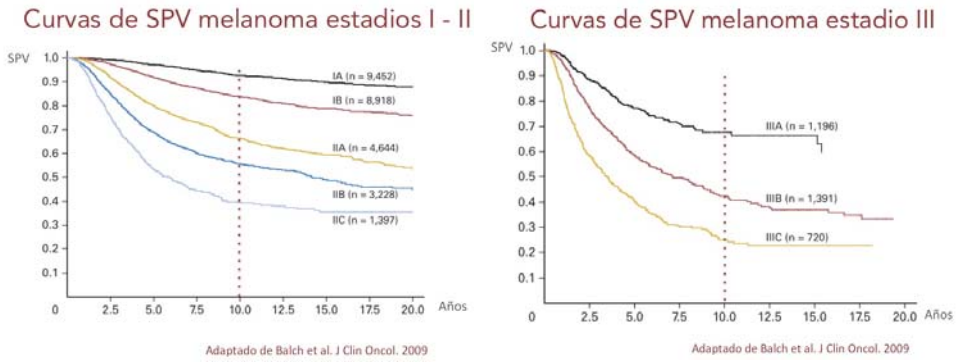


Figura 6. Curvas de supervivencia global de melanoma estadio I-II y estadio III según la JCO 2009.

4.2. DIAGNÓSTICO CLÍNICO Y DERMATOSCÓPICO:

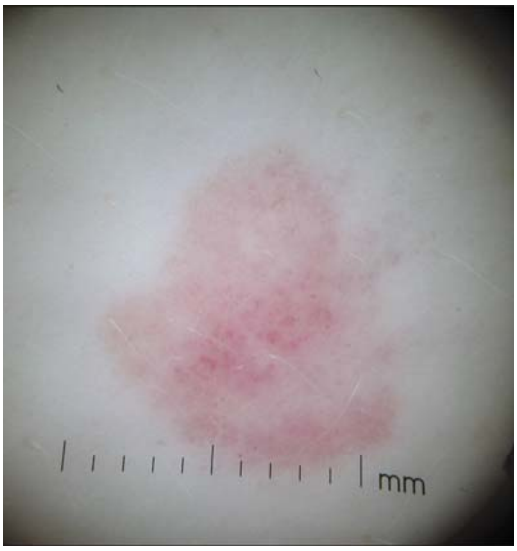
El diagnóstico precoz del melanoma sigue siendo un reto para el dermatólogo y dermatopatólogo. En primer lugar, implica identificar y extirpar MM incipientes, sin incrementar las extirpaciones innecesarias de lesiones benignas. En segundo lugar, la dificultad de controlar y explorar pacientes de alto riesgo de desarrollar MM, que pueden presentar más de 100 o 200 lesiones melanocíticas atípicas, asociadas o no a otras lesiones cutáneas propias del fotoenvejecimiento cutáneo. Y por último, desde el punto de vista dermato-patológico, existen lesiones melanocíticas de diagnóstico difícil, o de comportamiento biológico incierto, en especial bajo la influencia de factores exógenos como puede ser la RUV[78].

La **dermatoscopia** (o microscopía de epiluminiscencia) es una técnica diagnóstica no invasiva, basada en la imagen submacroscópica mediante un sistema óptico que magnifica y evita la refracción de la capa córnea con una luz y filtro de polarización o mediante un medio de inmersión y contacto directo. Permite la observación de estructuras epidérmicas y dérmicas, invisibles para el ojo desnudo, con una correcta correlación microscópica.

La utilidad de la dermatoscopia es incuestionable, siendo una exploración de rutina dermatológica general. Especialmente en el diagnóstico de melanoma, se ha demostrado que mejora la precisión diagnóstica en 3 meta-análisis[109–111], de forma que claramente desciende la relación lesión benigna/melanoma entre las lesiones extirpadas, con una menor tasa global de exéresis, así como una mejor detección de MM simuladores o difíciles. La sensibilidad en el diagnóstico del MM por parte de un experto, únicamente con la clínica

ronda el 70%, mientras que, la dermatoscopia logra una sensibilidad del 92%. También ha demostrado que mejora la precisión diagnóstica de otras lesiones melanocíticas y no melanocíticas.

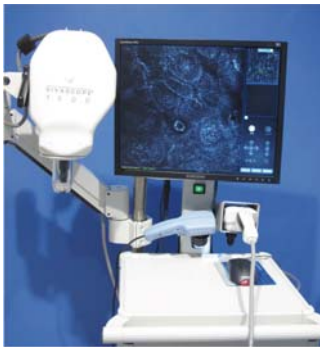
Figura 7. Melanoma in situ sobre nevus en paciente albina. La diferenciación mediante dermatoscopia de los vasos puntiformes (sospecha de MM) de los vasos en coma (típicos de nevus), permitió su detección precoz.



4.3. MICROSOCOPÍA CONFOCAL DE REFLECTANCIA *IN VIVO*

La Microscopía Confocal de Reflectancia *in vivo* (MCR) es una técnica de imagen no invasiva, que se puede considerar hoy en día como un paso intermedio entre la dermatoscopia y la histopatología, pero con la ventaja de ser un microscopio de escaneo de resolución celular que permite obtener imágenes en tiempo real *in vivo*. Utiliza un láser de diodo de baja potencia que emite un haz de luz que será reflejada al atravesar estructuras con diferente índice de refracción, y posteriormente captado por un receptor.

El MRC (VivaScope® 1500; Lucid Inc., NY, USA) compone mosaicos de imágenes de hasta 8 x 8mm alcanzando una profundidad máxima de unas 200 µm con una resolución lateral de 0.5 - 1 µm comparable a la histopatología convencional. Este sistema utiliza un láser de 830nm, trabaja a una potencia inferior a 16 mW y con unas lentes de 30x de inmersión en agua y apertura numérica 0.9, es indoloro e inocuo para la piel.



Actualmente ya se han descrito y validado criterios y terminología para el diagnostico tanto de lesiones melanocíticas como no melanocíticas, así como diversos algoritmos especialmente diseñados para mejorar el diagnóstico de melanoma[112–116].

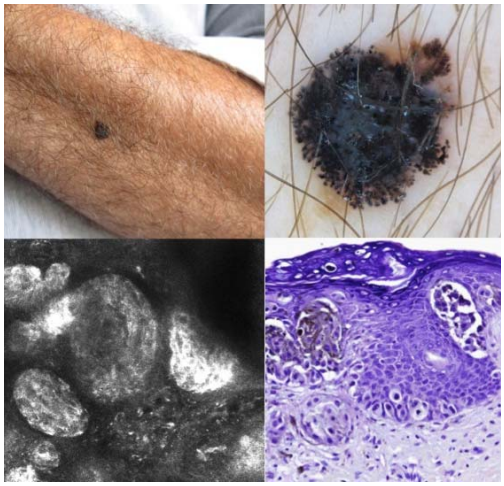


Figura 8. Melanoma de extensión superficial (Breslow 0.8mm) en antebrazo de un tenista de 58 años de edad. Correlación clínica-dermoscópica-confocal-histológica de nidos atípicos intraepidérmicos con células redondas nucleadas.

En los últimos años ha cobrado un creciente interés en sus diferentes aplicaciones clínicas tanto en dermatoncología como también en patología inflamatoria. Permite realizar exploraciones repetidas en diferentes momentos evolutivos, valorando no sólo la arquitectura y citología cutánea, sino también vascularización, infiltrado inflamatorio, o la matriz dérmica papilar.

Destaca su especial utilidad en MM acrómicos, puesto que a pesar de la ausencia de melanina las células de MM son refráctiles y visibles, y en MM faciales tipo LMM. Se ha demostrado que la MCR es capaz de detectar la afectación subclínica mediante la visualización de melanocitos intraepidérmicos atípicos, facilitando el manejo pre y post-terapéutico, así como la detección de recidivas[117–120].

4.4. SEGUIMIENTO DIGITAL. IDENTIFICACIÓN DE POBLACIÓN DE ALTO RIESGO

El seguimiento digital, en especial mediante el denominado método en 2 etapas (mapas corporales totales y dermatoscopia digital)[121], supone la estrategia más eficiente y no invasiva de detectar melanomas incipientes en pacientes de alto riesgo, sin incrementar las extirpaciones innecesarias[122–124]. Numerosos estudios lo han demostrado, se pueden detectar MM in situ o de Breslow menor de 1mm, siendo lesiones en su mayoría indistinguibles de otros nevus de los pacientes, y con índices de extirpaciones de menos de 2

lesiones por paciente durante 96 meses de seguimiento medio, detectando MM en el 8.5% de las lesiones extirpadas y en el 12,5% de los pacientes incluidos.

El meta-análisis de Salerni y col[125] cuantifica el beneficio de este tipo de seguimiento dermatológico, haciendo hincapié en la importancia de realizarlo de forma mantenida en pacientes de alto riesgo, pues uno de cada 8 de ellos desarrollará un nuevo MM durante el seguimiento, y por cada mes más que se realice, un caso más de MM se puede detectar.

Por ello, es esencial identificar adecuadamente pacientes tributarios a realizar seguimiento digital especializado, básicamente aquellos que presenten múltiples nevus con atipia y factores de riesgo asociado; historia personal o familiar de MM, inmunosupresión, genodermatosis con elevado riesgo a cáncer cutáneo, familias y pacientes con elevada susceptibilidad genética conocida.

Resulta de gran importancia detectar asimismo los casos de MM primario múltiple o familiar, o también en casos de MM asociado a otros cánceres como páncreas, carcinoma renal o MM de úvea, ya que podríamos identificar pacientes candidatos a recibir asesoramiento familiar y genético, despistaje de genes de alto riesgo, y poder ofrecerles seguimiento digital para realizar la prevención adecuada[126]. Recientemente se han identificado otros genes de alto riesgo implicados en MM familiar y múltiple aparte de los ya conocidos *CDKN2A* y *CDK4*, como son *MITF* (relacionado con carcinoma renal)[127,128] o *BAP1* (relacionado con mesotelioma y MM de úvea)[129].

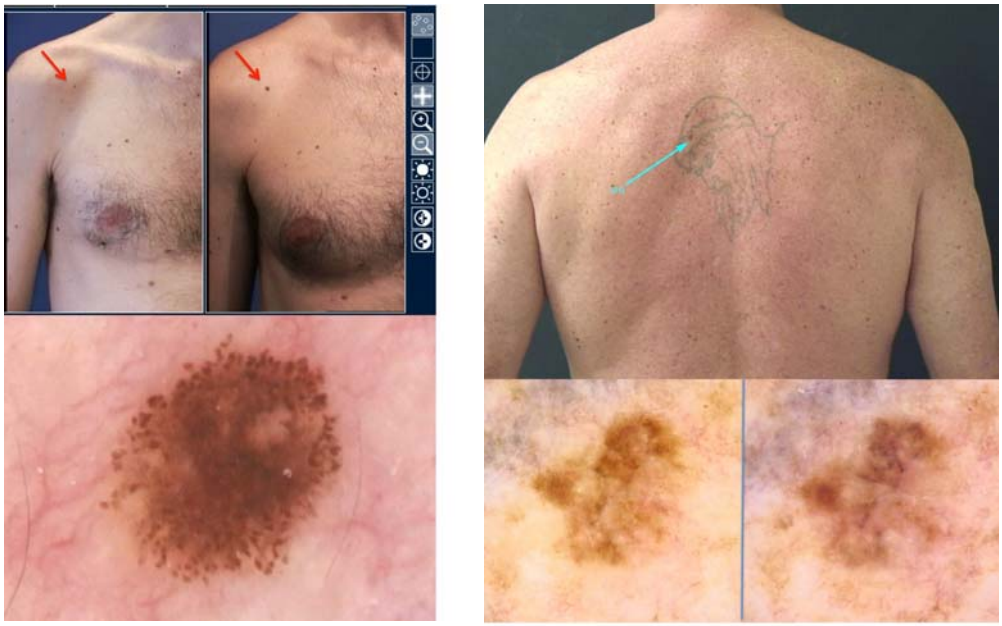


Figura 9. Melanomas incipientes, de menos de 4mm de diámetro (melanomas in situ) detectados en seguimiento digital. Caso 1. Lesión de 3mm detectada por cambios del mapa corporal total, con patrón globular e irregularidad de glóbulos y pseudópodos en periferia. Caso 2. Melanoma detectado por cambios en dermatoscopia digital, mostrando signos de regresión extensa.

HIPÓTESIS

- La prevención primaria y secundaria (detección precoz) del melanoma inducido por radiación ultravioleta es la estrategia más eficiente para mejorar su pronóstico.
- Los melanomas de extremidades pueden considerarse un modelo de desarrollo de melanoma inducido por la exposición a radiación ultravioleta de forma intermitente y suponen una diana preventiva en nuestro medio.
- La radiación ultravioleta provoca un daño biológico en las lesiones melanocíticas cutáneas identificable y cuantificable *in vivo* y *ex vivo*. La aplicación de fotoprotectores tópicos evita el daño inducido por la RUV en la piel y en las lesiones melanocíticas cutáneas.

OBJETIVO PRINCIPAL

- Estudiar los efectos de la radiación ultravioleta en lesiones melanocíticas para optimizar la prevención del melanoma fotoinducido en nuestro medio: fotoprotección y diagnóstico precoz de melanoma incipiente.

SUB-OBJETIVOS

I. Mejorar la detección precoz de lesiones melanocíticas potencialmente malignas: Caracterizar clínica, dermatoscópica, confocal e histopatológicamente melanomas incipientes en extremidades, como modelo de melanomas inducidos por exposición solar intermitente.

II. Desarrollar un modelo in vivo que demuestre y cuantifique los efectos producidos por la RUV en lesiones melanocíticas benignas en condiciones reales.

III. Describir los cambios *in vivo* (clínicos y dermatoscópicos), y *ex vivo* (histológicos y moleculares) inducidos por una irradiación controlada de luz UV en lesiones melanocíticas benignas y piel circundante.

IV. Testar la eficacia de la aplicación de un fotoprotector tópico en evitar los cambios clínicos, dermatoscópicos, histológicos y/o moleculares, inducidos por la RUV en la piel.

MATERIAL Y MÉTODOS

RESULTADOS

PUBLICACIONES

TRABAJO I

RESUMEN TRABAJO I

Early Stages of Melanoma on the Limbs of High-risk Patients: Clinical, Dermoscopic, Reflectance Confocal Microscopy and Histopathological Characterization for Improved Recognition

Cristina Carrera, Josep Palou, Josep Malvehy, Sonia Segura, Paula Aguilera, Gabriel Salerni, Louise Lovatto, Joan A. Puig-Butillé, Llùcia Alós, Susana Puig

Acta Derm Venereol. 2011;91(2):137 – 46. *Factor de impacto: 3.007*

Objetivo

Mejorar la detección precoz de lesiones melanocíticas potencialmente malignas: Caracterizar clínica, dermatoscópica, confocal e histopatológicamente melanomas incipientes en extremidades, como modelo de melanomas inducidos por exposición solar intermitente.

Metodología

Estudio retrospectivo de las características clínicas, dermatoscópicas, de microscopía confocal e histopatológicas de melanomas localizados en extremidades detectados de forma precoz en una Unidad de Melanoma de referencia.

Resultados

Se incluyeron 36 nuevos melanomas detectados en estadio incipiente, 28 de ellos in situ, todos ellos localizados en extremidades. Más del 90% fueron mujeres y localizados en extremidades inferiores, el 50% estaban bajo seguimiento digital por tratarse de pacientes de alto riesgo, congruente a la población de referencia de nuestro Centro, (historia previa de melanoma múltiple 17% y/o melanoma familiar 40%). Ningún tumor era clínicamente sugestivo de melanoma, con diámetro medio de 4.3mm. En base a las características dermatoscópicas, los tumores se clasificaron en 4 categorías; 1. Retículo prominente (n = 16); 2. Retículo delicado (n = 5); 3. Hipopigmentación y vasos puntiformes atípicos (n = 10); 4. Pigmentación marrón clara difusa con refuerzo perifolicular (n = 5). Se complementó el diagnóstico mediante microscopía confocal in vivo en 12 casos, permitiendo en todos ellos la identificación de células atípicas pagetoides intraepidérmicas. Histopatológicamente, el 80% de casos se consideraron melanomas in situ, siendo los 8 restantes microinvasivos con un Breslow medio de 0.5mm± 0.1. Se subclasificaron en aspecto nevoide, lentiginoso, pagetoide o tipo-lentigo maligno, y característicamente la mayoría mostraron grandes células redondeadas y claras, con núcleo grande y atípico, y tendencia a la invasión pagetoide.

Se detectó una asociación significativa entre el tercer grupo de tumores (hipopigmentados con vascularización atípica) y un mayor retraso diagnóstico y aspecto histológico nevoide, y el cuarto grupo con una imagen similar al lentigo maligno

Mediante técnicas diagnósticas de imagen no invasivas (dermatoscopia, dermatoscopia digital comparativa, y microscopía confocal in vivo), se ha podido demostrar la detección precoz de casos incipientes, clínicamente indistinguibles de otros nevus. A pesar de desconocer el potencial biológico de dichos tumores, dadas las características histopatológicas de los mismos, la conducta más apropiada es extirpar correctamente estos tumores.

Acta Derm Venereol 2011; 91: 137–146

INVESTIGATIVE REPORT

Early Stages of Melanoma on the Limbs of High-risk Patients: Clinical, Dermoscopic, Reflectance Confocal Microscopy and Histopathological Characterization for Improved Recognition

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Departments of [1]Dermatology, [2]Pathology and [4]Genetics, Melanoma and Dermatopathology Units, Hospital Clínic de Barcelona, [3]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Spain, CIBER de Enfermedades Raras, Instituto de Salud Carlos III (ISCIII), and [5]Department of Dermatology, Hospital del Mar, Parc de Salut Mar, Barcelona, Spain

Early stages of 36 melanomas on limbs were morphologically characterised. Most occurred in high-risk patients (multiple and/or familial melanoma) attending a referral unit for melanoma and pigmented lesions. None of the tumours was clinically suspicious for melanoma (mean diameter of 4.3 mm). The tumours were classified into four dermoscopic groups: (*i*) prominent network (*n* = 16); (*ii*) delicate network (*n* = 5); (*iii*) hypo-pigmentation with dotted vessels (*n* = 10); and (*iv*) diffuse light pigmentation with perifollicular pigmentation (*n* = 5). Confocal microscopy performed in 12 cases allowed the identification of atypical, single cells within epidermal layers. Histopathology showed marked large atypical cells in a pagetoid spreading pattern in most cases. Significant associations were detected between the third dermoscopic group and naevoid histological appearance and delay in detection, and between the fourth group and lentigo-maligna-like features. Dermoscopy allowed an increase in the suspicious threshold in these difficult melanomas in high-risk patients and enabled the subclassification of early melanomas on the limbs, with a correct confocal and histopathological correlation. Although the biological behaviour of these incipient tumours remains uncertain, the most appropriate treatment seems to be recognition and proper excision. *Key words: atypical mole syndrome; dermoscopy; dermatoscopy; familial melanoma; melanoma; naevus; reflectance confocal microscopy.*

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There is only one effective treatment for malignant melanoma (MM): complete excision of early stage tumours. *In situ* MMs are the only cases with a 100% cure rate after proper surgery, decreasing to 80–85% in thin MM (under 1 mm Breslow). MMs on the limbs are not well characterised in the literature, especially in the early stages, although they appear to be related to different epidemiological settings (e.g. women with intermittent sun-exposure on the lower limbs) (1, 2).

Atypical mole syndrome (AMS), defined by the presence of more than 100 naevi, and/or more than 10 clinical and dermoscopically atypical naevi, and/or previously excised dysplastic naevi, is the most important independent risk marker for developing MM. In addition, naevi are both possible MM precursors and early MM simulators. In fact, the most difficult task in early detection of MM is to differentiate them from the more frequent benign melanocytic lesions. However, systematic excision of atypical naevi has no benefit in preventing MM in these high-risk patients (3, 4).

It is estimated that 10% of cases of MM occur in a familial setting as an autosomal dominant trait. In approximately 50% of these familial multiple melanoma (FamMM) cases a responsible gene can be found, being *CDKN2A* and *CDK4* the two major susceptibility genes most commonly identified. FamMM cases and their relatives, especially when they are affected by AMS and/or are mutation carriers have a very high risk of MM development, even up to 1000 times over general population. To date, no clinical, dermoscopic, or histopathological special feature has been related to tumours in FamMM (5–7). Polymorphisms in *melanocortin 1 receptor (MC1R)* gene, especially the red hair variants (RHV), are considered low susceptibility genes to MM development, increasing the MM risk up to 10 times in respect to wild type (8). We studied the interaction between these low-risk variants among FamMM cases, and found that they can increase the genetic risk in *CDKN2A* (high-risk gene) mutation carriers by up to 14 times and contribute to a less suspicious clinical and dermoscopic appearance of tumours, less colour, and fewer structures (9).

The clinical ABCDE rule fails to recognise MMs that are small (less than 6 mm in diameter) or that exhibit regular shape and homogeneous colour, are symmetrical or undergo unnoticed changes (10, 11). Dermoscopy is now well accepted as a non-invasive technique that improves the accuracy of skin tumour diagnosis (12–14), and is especially useful in the differential diagnosis of MM simulators or hypopigmented MM, avoiding unnecessary excisions (15–17).

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In vivo reflectance-mode laser scanning confocal microscopy (RCM) is a non-invasive imaging technique that allows real-time skin examination at high resolution and thus improves the diagnostic accuracy in MM and other non-melanocytic tumours (18–20).

We performed a retrospective study of 36 very early MMs on limbs. The objectives of this study were: (*i*) to describe the dermoscopic and *in vivo* RCM features in order to improve their future recognition; (*ii*) to correlate these findings with histopathological characteristics of the tumours that could suggest different types of early MM on the limbs in these very early stages.

MATERIALS AND METHODS

A systematic retrospective review of all thin MMs located on limbs diagnosed in a specialised Pigmented Lesion Unit of a referral hospital between 2005 and 2008.

The inclusion criteria were: (*i*) thin MM (< 1 mm Breslow) proven by histopathological examination, located on limbs; (*ii*) clinical, dermoscopic and histopathological data available; and (*iii*) clinically unsuspicious for MM, defined by no clinical ABCD criteria fulfilled.

Complete clinical patient history was recorded, including familial history, previous melanocytic lesions excised and other MM-associated risk factors. Genetic studies were performed when DNA was available. Exons 1alfa, 1beta, 2 and 3, intronic changes IVS2-105 and –34G>T in the *CDKN2A* promoter, and exon 2 from *CDK4* were studied by PCR single-strand conformation polymorphism (PCR-SSCP) analysis and sequencing (7). *MC1R* was studied by direct sequencing (9).

Clinical and dermoscopic images were taken using digital cameras (Olympus Camedia, Canon G7 and/or Nikon Coolpix 4500) and a polarised dermatoscope (DermlitePhoto®; 3 GEN, LLC.Dana Point, CA, USA). In the case of the high-risk patients included in our digital follow-up protocol (21), Mole Max II (Dermamedical Instruments®), able to detect digital clinical and/or dermoscopic changes in a 6-month follow-up, was an additional tool used in the study. Clinical evaluation was based on ABCDE criteria and dermoscopic pattern analysis (22).

Whenever possible, *in vivo* RCM examination was performed with near-infrared reflectance confocal laser scanning microscopes (Vivascope 1500®; Lucid Inc., Henrietta, NY, USA). The instruments and acquisition procedures, as well as the features studied, have been described previously (23).

Conventional haematoxylin-eosin staining and immunohistochemistry (Melan A, HMB45, Ki67) were performed whenever it was considered necessary. Histopathologically, MMs were classified into one of the following groups according to their characteristics:

- Naevoid MM: predominance of nesting pagetoid invasion of the upper layers of epidermis over solitary cells.
- Paget's disease-like MM: characterised by atypical large

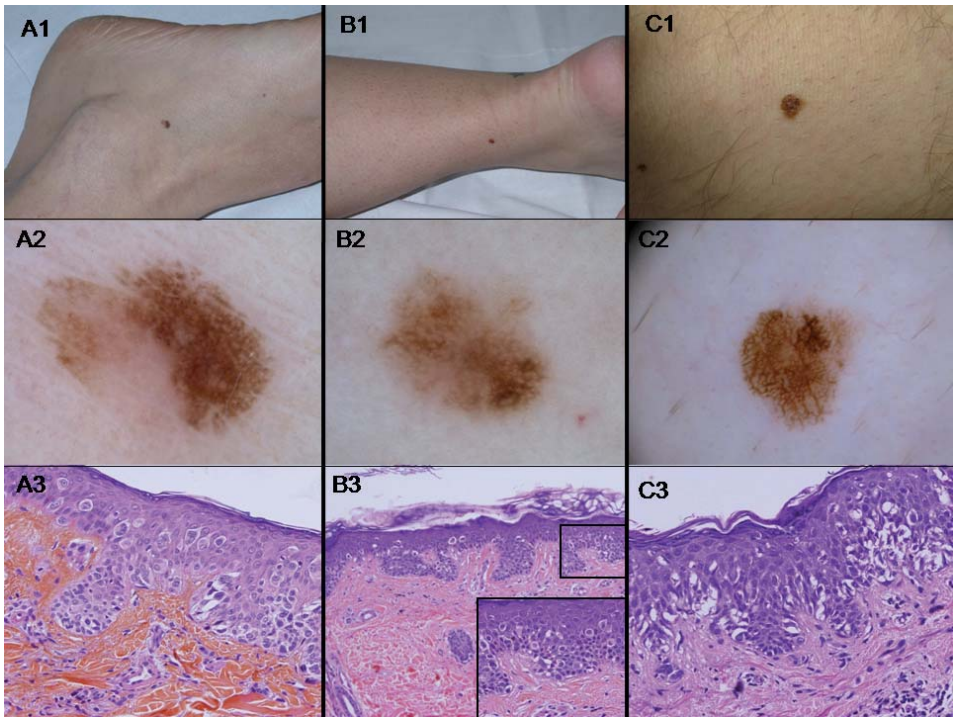

Fig. 1. Dermoscopic group 1: atypical network. Examples of 3 melanomas from group 1. A1, B1, C1: clinical aspect: located on lower limbs, small dark brown lesions, with no malignant criteria A2, B2, C2: dermoscopic images (original magnification × 30). Prominent network pattern, with 2 colours and asymmetrical pigment distribution. Case A is completely asymmetrical in 1 axis. A3, B3, C3: histopathological examination (× 20 (B3) and × 40 (A3, B3 inset and C3)). Proliferation of atypical large melanocytes, both solitary and forming discrete nests, in junctural and intraepidermal layers. These 3 cases were *in situ* malignant melanoma.

epithelioid cells invading the whole epidermis resembling genuine Paget's disease.
- Lentiginous MM: melanocytic hyperplasia, with severe architectural atypia and intraepidermal spreading. Small nests can be found on the bottom of rete ridges.
- Lentigo maligna-type: atypical melanocytic proliferation along a faded dermal-epidermal junction and flattened epidermis, with solitary and small nests invading the upper epidermis and characteristic follicular involvement. It may be associated with marked actinic damage.

Statistical evaluation was carried out using SPSS statistical software package for Windows (version 16.0; SPSS Inc., Chicago, IL, USA). A chi-square test was applied for all category features, and Fischer's exact test was applied if any expected cell value in the 2×2 table was < 5. Each group was compared with the other three. Mean and median values were determined for quantitative variables and compared using the Student's *t*-test.

RESULTS

Patient data

Thirty-six tumours from 35 patients in our high-risk patient-set were reviewed. Tumours were assigned, based on overall appearance in dermoscopic analyses, to 1 of 4 groups (for details see below – Dermoscopic examintion): 1, Prominent network (16 tumours, 46%) (Fig. 1); 2, Delicate network with no specific MM dermoscopic features (5 tumours, 14%) (Fig. 2). Melanomas were detected by changes in digital follow-up; 3, Hypopigmented with atypical vessels (10 tumours, 28%), (Fig. 3); and Group 4, Diffuse light pigmentation and perifollicular pigmentation (5 tumours, 14%), (Fig. 4). Patient clinical characteristics are summarised in Table I.

The most remarkable feature was the predomination of women ($n = 29$) over men ($n = 6$), and the presence of high-risk MM history, since 40% had familial MM history, 49% personal MM history, and 17% had multiple primary MMs (MPM) before the current MM diagnosis. The majority of patients (75%) were affected by atypical mole syndrome. Eighteen had been included in our digital follow-up high-risk surveillance programme, which involves total-body photography mapping and digital dermoscopy of atypical lesions every 6 months, as described previously by our group (21).

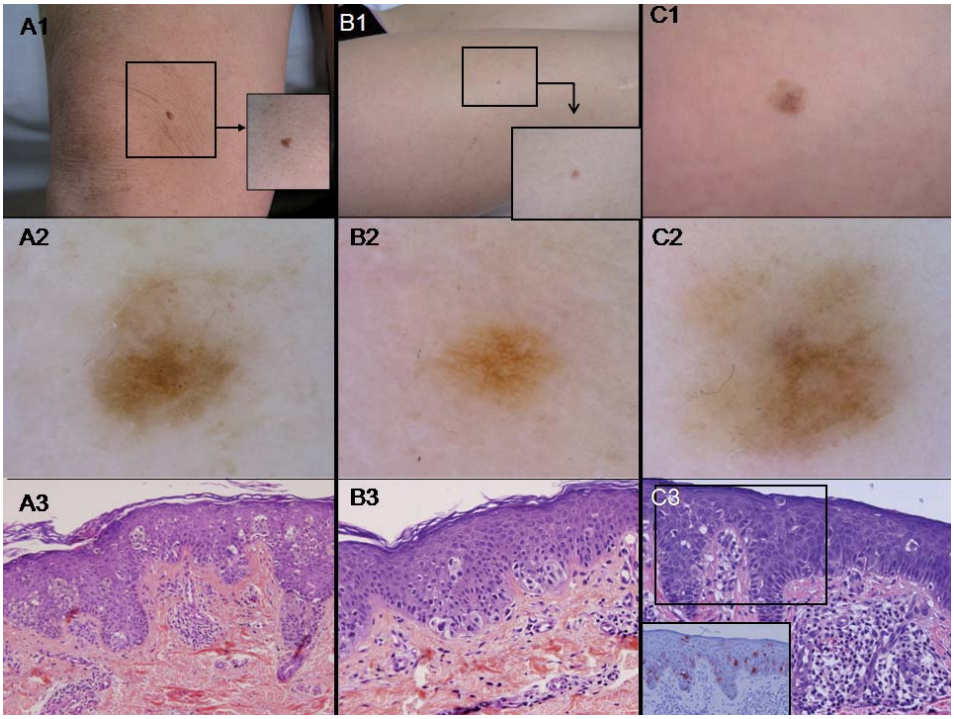

Fig. 2. Dermoscopic group 2: delicate network with changes on digital follow-up. Examples of three melanomas from group 2. A1, B1, C1: clinical aspect: located on lower limbs, the smallest lesions had a completely unremarkable aspect. Case A1 and B1 are mother and daughter, both of them CDKN2A mutation and double-red-hair-variant-MC1R carriers, affected by multiple primary malignant melanoma (MM). A2, B2, C2: dermoscopic images (original magnification ×30). Light-brown very delicate network pattern, with a slight asymmetrical light-brown structureless area in cases A2 and C2 due to a pre-existing naevus. In all cases the lesions were excised due to changes seen in digital follow-up of a very high-risk patient setting. A3, B3, C3 histopathological examination (×20). Proliferation of atypical large melanocytes, both solitary and forming nests, in junctural and intraepidermal layers. All were *in situ* MM. Note the immunohistochemical study in C3 with a more evident pagetoid spreading of Melan-A positive cells.

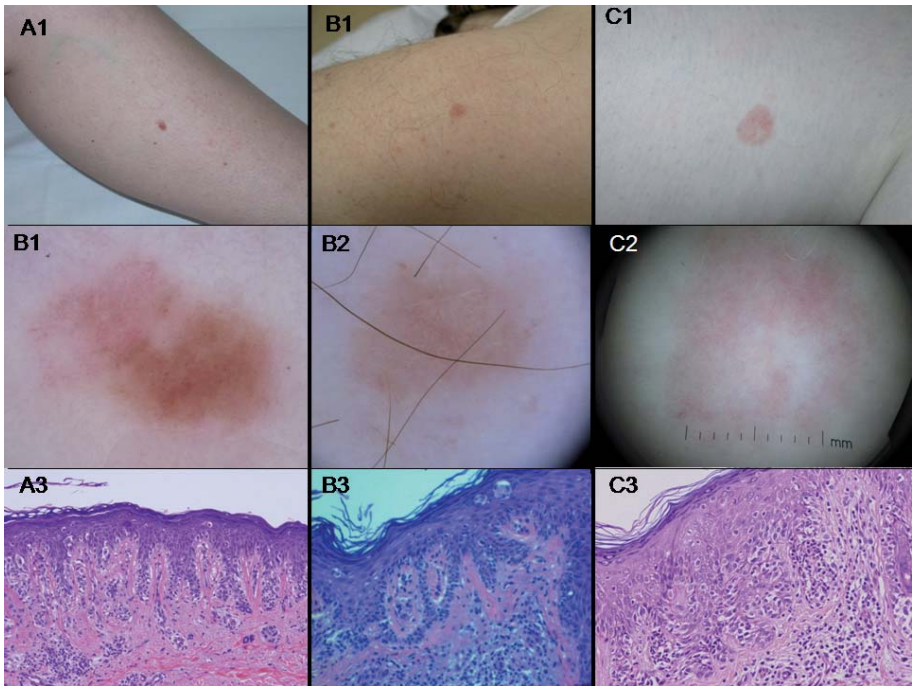

Fig. 3. Dermoscopic group 3: atypical vascular pattern. Examples of three melanomas from group 3. A1, B1, C1: clinical aspect: located on lower limbs, all achromic lesions with erythema. Case C1: albinism type OCA1 in a 34-year-old woman, the largest lesion in the series. A2, B2, C2: dermoscopic images (original magnification × 30). Homogeneous or unspecific pattern, only remarkable by vessels and a light-brown structureless pigmentation. Dotted vessels and whitish linear structures (chrysalides-like) are the only noteworthy features. A3, B3, C3: histopathological examination (× 20). Lentiginous hyperplasia of atypical melanocytes, with mild pagetoid spreading and marked vascular hyperplasia.

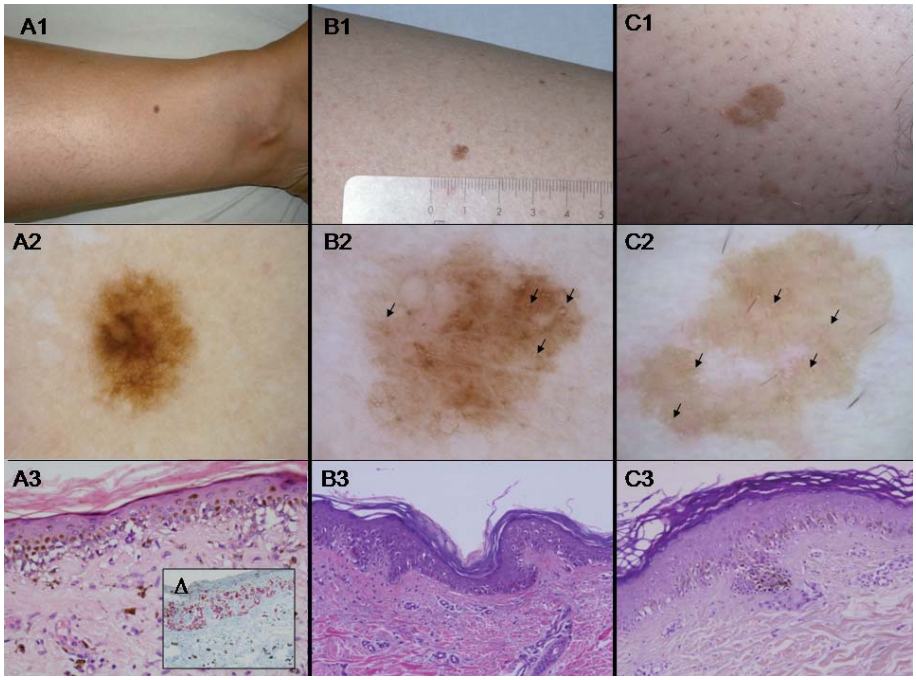

Fig. 4. Dermoscopic group 4: perifollicular pigmentation. Examples of three *in situ* melanomas. A1, B1, C1: clinical aspect: located on lower limbs, the only remarkable feature was irregular borders. A2, B2, C2: dermoscopic images (original magnification × 30). Light-brown structureless pigmentation, with thin and broken pigmented network, and focal hyperpigmentation in case 2. Note some irregular follicular openings (*arrows*). A3, B3, C3: histopathological examination (× 20). Flattened epidermis, with variable elastosis, and proliferation of dendritic melanocytes in both the basal and suprabasal layers. Note the remarkable pagetoid spreading in immunohistochemistry image (Δ) (Melan-A staining).

Table I. *Clinical features of the 35 patients included in this series. Patients were assigned to 1 of 4 groups based on the dermoscopic characteristics of their tumours: 1, "Prominent network"; 2, "Delicate network with no specific MM dermoscopic features"; 3, "Hypopigmented with atypical vessels"; and 4, "Diffuse light pigmentation and perifollicular pigmentation". CDKN2A/CDK4 mutation status was assessed in 21 of the 35 patients. MC1R variants were studied in 20 patients. Multiple malignant melanoma (MM): 2 or more melanomas diagnosed before the present case. Familial MM: 2 or more melanoma cases among first-degree relatives.*

Patient characteristics	Group 1 $n=16$	Group 2 $n=4$	Group 3 $n=10$	Group 4 $n=5$	Total $n=35$
Sex, *n* (%)					
Female	15 (94)	4 (100)	6 (60)	4 (80)	29 (83)
Male	1 (6)	0 (0)	4 (40)	1 (20)	6 (17)
Age (years), mean ± SD	44.7 ± 14.0	40.4 ± 14.7	49 ± 19.3	50 ± 8.3	46 ± 15.4
Atypical mole syndrome, *n* (%)	12 (75)	4 (100)	8 (80)	2 (40)	26 (75)
Previous MM, *n* (%)	7 (44)	3 (75)	6 (60)	1 (25)	17 (49)
Digital follow-up, *n* (%)	8 (50)	4 (100)	4 (40)	2 (40)	18 (51)
Multiple MM, *n* (%)	2 (12)	3 (75)	1 (10)	0 (0)	6 (17)
Familial MM, *n* (%)	6 (37)	4 (100)	2 (20)	2 (40)	14 (40)
Genetic studies performed, *n* (%)	8 (50)	4 (100)	8 (80)	2 (50)	21 (38)
CDKN2A/CDK4 mutation/studied, *n* (%)	4/8 (50)	3/4 (75)	1/7 (14)	0/2 (0)	8/21 (38)
MC1R/studied, *n* (%)					
Any variant	6/8 (75)	3/3 (100)	8/8 (100)	1/1 (100)	18/20 (90)
Red hair variants	4/8 (50)	3/3 (100)	5/8 (62)	1/1 (100)	10/20 (50)
More than one variant	0/8 (0)	2/3 (66)	5/8 (62)	0/1 (0)	7/20 (35)

SD: standard deviation. Note: one patient in group 2 presented with 2 tumours.

Genetic studies. All patients with familial and/or MPM were investigated for major susceptibility MM genes (*CDKN2A*, p14arf, *CDK4*) (as well other patients whose DNA was available**)**. Explicit permission was obtained from all patients tested. Eight of the 21 patients whose *CDKN2A* loci were studied were found to be carriers of known mutations. Six carried the G101W exon 2 mutation (7), the most common in our study population. Polymorphisms in the *MC1R* gene were studied in 20 patients; only 2 of them were wild-type. At least one functional variant was detected in 18 patients, more than one variant in 7, and 13 cases were red hair variant (RHV) carriers and 2 of them had a double RHV polymorphism.

Tumour data

Most of the tumours ($n=33$, 92%) were located on lower limbs, mainly below the knee ($n=28$, 78%). All were less than 6 mm in diameter (except for 2 lesions, 7 and 8 mm in diameter, both lacking pigment, one of them in a patient affected by oculo-cutaneous albinism type 1). The median diameter was 4.3 mm (SD 1.12 mm, range 3–8 mm). On clinical examination none of them fulfilled ABCD criteria for MM suspicion. Only 15 lesions showed mild asymmetry; none presented more than two colours, and borders were slightly irregular in 7 cases.

Dermoscopic examination

Most of the tumours showed two colours and asymmetry in one axis. However, 14 were completely symmetrical and 7 were monochromic. The most frequent overall pattern was reticular pigmented (21 tumours), and no lesion showed a multi-component pattern. An atypical pigmented network was detected in 15 cases, irregular pigment distribution was observed in 20 cases, and atypical vessels in 10 cases. Other worrying, but infrequent, dermoscopic features observed are detailed in Table II.

Based on overall appearance in dermoscopic analyses, tumours were classified into 4 groups (see above):

- Prominent network, characterised by atypical prominent pigmented network with broadened lines and narrow holes.
- Delicate network with no specific MM dermoscopic features.
- Hypopigmented with atypical vessels, with no classical features of MM, but little or no pigment, and dotted vessels and inverse network in several cases.
- Diffuse light pigmentation and perifollicular pigmentation, simulating solar lentigo but with irregular pigmentation of follicule-openings.

Reflectance confocal microscopy (RCM) examination

All the evaluated lesions ($n=12$) were suspicious for melanoma using the second-step algorithm previously described by our group (24). Positive criteria for melanoma were the presence of a pagetoid spread of atypical cells in 8 cases, being roundish in 6 cases, and dendritic in 4 (2 cases showed both cell types) (Fig. 5); the presence of non-edged papillae in eight cases; and the presence of atypical cells in the basal layer in 4 cases and in the dermal papilla in 3.

In the dermis, non-nucleated dermal cells (plump cells) were observed in 4 cases, related to the presence of blue regression (peppering) or melanophages in intense pigmented lesions. Vessels were identified in 2 cases,

Table II. *Clinical and dermoscopic examination of 36 tumours classified by dermoscopic group.*

	Group 1 $n=16$	Group 2 $n=5$	Group 3 $n=10$	Group 4 $n=5$	Total $n=36$
Clinical tumour features					
Site, *n* (%)					
Lower limbs	16 (100)	5 (100)	7 (70)	5 (100)	33 (92)
Upper limbs	0 (0)	0 (0)	3 (30)	0 (0)	3 (7)
In situ malignant melanoma, *n* (%)	13 (72)	5 (100)	5 (50)	5 (100)	28 (78)
Ugly duckling sign, *n* (%)	1 (6)	0 (0)	1 (10)	0 (0)	2 (5)
Size, mm, mean ± SD	4.12 ± 0.9	3.6 ± 0.9	5 ± 1.4	4.4 ± 0.9	4.3 ± 1.12
Clinical asymmetry, *n* (%)	9 (56)	3 (60)	2 (20)	1 (20)	15 (42)
One colour, *n* (%)	4 (25)	2 (40)	7 (70)	3 (60)	16 (45)
Two colours, *n* (%)	12 (75)	3 (60)	3 (30)	2 (40)	20 (56)
Irregular borders, *n* (%)	4 (25)	0 (0)	0 (0)	3 (60)	7 (20)
Dermoscopic tumour features, n (%)					
Asymmetry in one axis, *n* (%)	11 (70)	3 (60)	5 (50)	3 (60)	22 (60)
Only one colour, *n* (%)	0 (0)	2 (40)	4 (40)	1 (20)	7 (20)
Two colours, *n* (%)	13 (72)	3 (60)	5 (50)	4 (80)	25 (69)
More than two colours, *n* (%)	3 (18)	0 (0)	1 (10)	0 (0)	4 (11)
Reticular pattern, *n* (%)	14 (88)	5 (100)	0 (0)	2 (40)	21 (58)
Globular pattern, *n* (%)	1 (6)	0 (0)	1 (10)	0 (0)	2 (5)
Non-specific global pattern, *n* (%)	1 (6)	0 (0)	9 (90)	3 (60)	13 (35)
Atypical network, *n* (%)	14 (88)	0 (0)	1 (10)	0 (0)	15 (42)
Irregular globules, *n* (%)	5 (31)	0 (0)	3 (30)	0 (0)	8 (22)
Radial streaks /pseudopods, *n* (%)	4 (25)	0 (0)	1 (10)	0 (0)	5 (16)
Hyper/hypopigmented irregular areas, *n* (%)	7 (44)	2 (40)	7 (70)	4 (80)	20 (56)
Irregular blotches, *n* (%)	3 (18)	0 (0)	0 (0)	4 (80)	7 (20)
Dotted vessels, *n* (%)	1 (6)	0 (0)	9 (90)	0 (0)	10 (29)
Regression features, *n* (%)	3 (18)	1 (20)	1 (10)	1 (20)	6 (17)
Perifollicular pigmentation, *n* (%)	1 (6)	0 (0)	0 (0)	5 (100)	5 (16)
Negative/inverse network, *n* (%)	0 (0)	0 (0)	3 (30)	0 (0)	3 (8)

with tortuous morphology corresponding to atypical vessels seen under dermoscopy.

Dermoscopic features were the main reason for excision in 31 cases; the remaining 5 cases (dermoscopic group 2) were excised due to minimal changes on digital follow-up in a very-high-risk patient set, despite an unsuspicious clinical and dermoscopic appearance.

Histopathological study

All lesions were evaluated, by 2 independent pathologists (JP and LA).

Twenty-eight tumours (80%) were *in situ* MMs, and the remaining 8 were micro-invasive MMs, Clark II in 5 cases and Clark III in 3 cases. The median Breslow index in these was 0.5 mm. There were only 5 cases

Table III. *Histopathological examination of 36 tumours classified by dermoscopic group. Column headings indicate total numbers and percentages. Note that it was not possible to review the histopathological features of one tumour in group 1 (total of 35 tumours examined), unlike in the clinical/dermoscopic diagnosis (all 36 tumours studied).*

Histopathological features	Group 1 $n=15$	Group 2 $n=5$	Group 3 $n=10$	Group 4 $n=5$	Total $n=35$
Histological classification					
Naevoid malignant melanoma	4 (27)	2 (40)	7 (70)	0 (0)	13 (38)
Pagetoid malignant melanoma	8 (54)	2 (40)	1 (10)	0 (0)	11 (31)
Lentiginous malignant melanoma	3 (20)	1 (20)	2 (20)	0 (0)	6 (17)
Lentigo malignant melanoma-like	0 (0)	0 (0)	0 (0)	5 (100)	5 (14)
Naevus-associated	2 (13)	1 (20)	2 (20)	0 (0)	5 (14)
Marked nest tendency	5 (33)	3 (60)	9 (90)	1 (20)	18 (51)
Marked lentiginous melanocytic hyperplasia	5 (33)	1 (20)	4 (40)	3 (60)	13 (37)
Marked pagetoid spreading	10 (66)	4 (80)	6 (60)	3 (60)	23 (66)
Marked vascular hyperplasia	1 (7)	1 (20)	4 (40)	0 (0)	11 (31)
Marked inflammatory infiltrates	4 (27)	1 (20)	7 (70)	0 (0)	12 (34)
Atypical large cells	6 (40)	2 (40)	2 (20)	1 (20)	11 (31)
Atypical epithelioid-like cells	11 (73)	3 (60)	8 (80)	3 (60)	25 (72)
Histological diagnosis					
Clark I	13 (90)	5 (100)	5 (50)	5 (100)	28 (80)
Clark II	2 (14)	0 (0)	3 (30)	0 (0)	5 (14)
Clark III	1 (7)	0 (0)	2 (20)	0 (0)	2 (6)
Mean Breslow thickness (8 cases), mm	0.41 ± 0.1	–	0.56 ± 0.05	–	0.50 ± 0.1

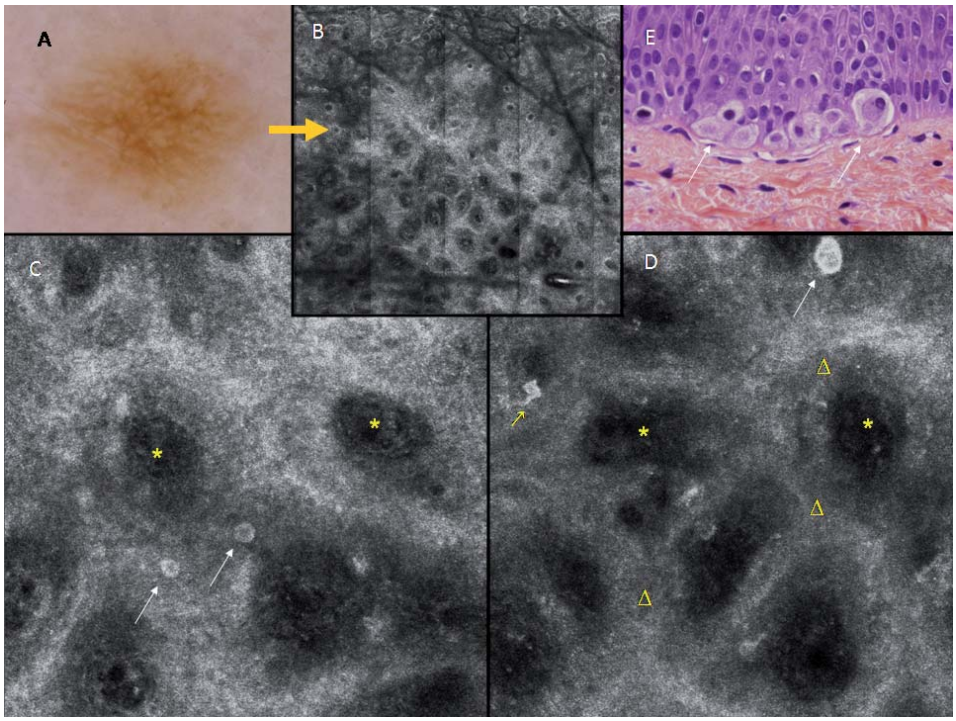

Fig. 5. A: Dermoscopic image of a new pigmented lesion on the knee of a group 2 patient (original magnification × 50). Light-brown, symmetrical pigmented network. B: *In vivo* RCM image sequence in a 4 × 4 mm mosaic: ringed architecture at the dermo-epidermal junction, with (× 30) irregular elongated regular rete ridges with an increased number of refractive cells in the basal layer. C and D: 500 × 500 µm RCM images. Non-edged papillae: dark dermal papillae irregular in size and shape (*), without a demarcated rim of bright cells, separated by interpapillary spaces of different thicknesses (Δ). Scattered atypical junctional nucleated roundish cells at layer (*white arrows*), with a single dendritic cell (*thin yellow arrow*). E: Histopathological examination (original magnification × 200). Atypical large and roundish melanocytes in the dermo-epidermal layer corresponding to the highlighted cells in the RCM and histopathological images.

of MM witn a melanocytic neavus associated in histopathological examination.

Based on the histopathological classification of incipient MMs explained in the Materials and Methods, we were able to divide our cases into groups and to study their possible associations with different dermoscopic groups (Table III). Thirteen cases were classified as naevoid-like MM, with statistically significant associations with marked nesting ($p < 0.001$) and marked vascular hyperplasia ($p < 0.05$). Eleven cases were classified as pagetoid MM-type, with marked pagetoid invasion of the epidermis, and association with very large roundish atypical cells in most cases ($p < 0.03$). Six cases were considered lentiginous MM-type, with this characteristic architecture as the most remarkable feature. And the remaining 5 cases were classified as lentigo-maligna-like MMs. However, not all of these 5 cases showed signs of elastosis.

Defining features of each dermoscopic group

The first group (atypical prominent network) was associated with lesions that were clinically and dermoscopically more pigmented and polychromic ($p < 0.05$). *In vivo* RCM demonstrated that 4 lesions presented striking pagetoid spreading of atypical cells. Histopathologically, group 1 was associated with the most marked pagetoid spreading of atypical solitary cells, so-called Pagetoid-type MM (8 cases, 54% of this group, $p = 0.02$). The diagnosis was *in situ* MM in 13 cases (90%) (Table IV).

The second group (delicate light-brown pigmented network) contained the smallest tumours (mean diameter 3.6 mm), with weak pigmentation, which explains in part the unremarkable aspect of these incipient tumours, and is congruent with *MC1R* variants status. The 3 patients studied had red hair and multiple variants in *MC1R*. Confocal detection of pagetoid cells within the upper epidermis aided the diagnosis in 3 cases. All were *in situ* MMs (Table IV).

The third group (hypopigmented or achromic lesions with atypical vasculature) was the second most frequent pattern, and the only one detected in MM located on upper limbs (30% vs. 0%). The mean size of lesions was slightly larger than the other groups (5 mm ± 1.4 mm), and in two cases the tumour was the reason for consultation because of erythema and pruritus. Most lesions (90%) showed an unspecific overall dermoscopic pattern

Table IV. *Characterisation of each malignant melanoma (MM) subgroup. For each group, the most remarkable features are listed.*

Characteristic features	*n* (%)[a]	Significance (Fisher's exact test)
Group 1 (16 patients, 16 tumours)		
Female	15 (94)	NS
>1 colour clinically	12 (75)	0.04
>1 colour dermoscopically	16 (100)	<0.05
Network global pattern	14 (88)	0.01
Atypical network	14 (88)	0.01
In situ MM	13 (90)	NS
Pagetoid-type MM (histopathological)	8 (54)	0.02
Group 2 (4 patients, 5 tumours)		
Female	4 (100)	NS
Multiple primary MM	3 (75)	<0.01
Familial multiple MM	4 (100)	<0.03
Diameter (mm), median (SD)	3.6 (0.09)	NS (Student's *t*-test)
Network global pattern	5 (100)	<0.01
Diagnosed by changes in digital FU	5 (100)	<0.001
In situ MM	5 (100)	NS
Group 3 (10 patients, 10 tumours)		
Male	4 (40)	0.04
Multiple *MC1R* variants	5 (62)[b]	0.05
Upper limbs	3 (30)	0.02
Only one colour clinically	7 (70)	<0.05
Only one colour dermoscopically	4 (40)	<0.05
Non-specific global pattern	9 (90)	0.01
Dotted vessels	9 (90)	0.01
Inverse negative network	3 (30)	<0.05
Invasive MM	5 (50)	0.03
Naevoid-type MM	7 (70)	0.01
Marked nested tendency	9 (90)	<0.05
Marked vascular hyperplasia	4 (40)	0.05
Marked inflammatory infiltrates	7 (70)	0.01
Group 4 (5 patients, 5 tumours)		
Female	4 (80)	NS
Irregular borders clinically	3 (60)	0.03
Irregular blotches dermoscopically	4 (80)	0.01
Perifollicular pigmentation	5 (100)	<0.001
In situ MM	5 (100)	NS
Lentigo-type MM (histopathological)	5 (100)	<0.001

[a]Unless otherwise indicated. [b]Five cases out of 8 studied.
NS: not significant in Fisher's exact test analysis. FU: follow-up.

($p < 0.001$) and atypical vascularisation, with dotted vessels ($p < 0.001$). In addition 3 cases (30%) presented an inverse network ($p < 0.05$). This group comparing with the other 3, contains the most invasive tumours (*in situ* MM: 50% vs. 88% in the remaining groups, $p < 0.03$; Clark II/III: 50% vs. 9% in the other groups, $p < 0.01$). The third group was statistically associated with the histopathological naevoid MM type ($p = 0.01$), and it was also possible to observe a marked vascular hyperplasia and inflammatory infiltrates (Table IV).

In the fourth group (light-brown structureless and perifollicular pigmentation), a solar lentigo appearance with irregular borders (3 cases) was the most remarkable clinical feature. The dermoscopy criterion for suspicion was pigmentation of the perifollicular openings over a lighter brown structureless pigmentation. These 5 tumours were *in situ* MM with atypical cells invasion of follicles similar to lentigo-maligna-MM but without extensive elastosis (Table IV).

DISCUSSION

Based on this review, mainly dermoscopy, sometimes aided by digital follow-up (DFU) and/or RCM, allowed the excision of 36 early MMs on limbs with unsuspicious clinical aspects.

Our aim was to characterise *in vivo* and *ex vivo* thin MMs on limbs diagnosed over the last 3 years in our unit. A large proportion of the patients in this series belong to a very high-risk MM setting: 49% were affected by previous MPM, 40% had a FamMM syndrome and 75% were affected by AMS. These data are consistent with a population attending a specific pigmented lesions unit in a referral hospital such as ours. The proportion of female patients cannot be explained on the basis of FamMM or MPM (7) and is consistent with the predominant incidence of melanoma on the lower limb in females and on the trunk in males in our general population, as is the case in most countries.

Both primary and secondary prevention strategies are especially important in these families, as the risk of MM may reach 1000 times that in the general population. Early detection of MM without an increase in unnecessary excisions is important in these cases (4–7). To date, the only way to identify this population is through their medical history. However, it would be of great interest to find special clinical, dermoscopic or histopathological features for tumours that form as a result of genetic factors. It has been demonstrated that dermatological surveillance programmes involving total-body photography, digital dermoscopy and *in vivo* RCM are feasible and allow early diagnosis of most *in situ* or micro-invasive MMs, thus avoiding unnecessary excision of benign lesions (optimal ratio benign/malignant) (4, 20, 21, 25). Genetic studies in MM families facilitate the identification of high-risk non-affected individuals who may benefit from specific surveillance programmes. FamMM is a potential pathological candidate for genetic counselling (5–7).

The gender and location of the tumours in this series agree with the well-established higher prevalence of MM on the lower limbs in women (26–28). We also found a higher proportion of male cases among the few upper limb MMs included.

Clinically all lesions were very small and not intensely pigmented, and the clinical "ugly duckling" sign only helped to identify them in only 2 cases. Our series showed that incipient MMs do not usually present the classical malignant appearance and therefore do not fulfil the ABCD criteria. We should, however, assume it is a feasible and useful tool for MM screening among the general population and for use by general practitioners, but not acceptable for use by dermatologists. This clinically unremarkable appearance and the lack of the "ugly duckling" sign in the majority of cases, reminds us that it is important not to clinically pre-select lesions for der-

moscopy (29), especially in high-risk patients. Recently, Zalaudek et al. (30) demonstrated that the time needed for complete skin examination aided by dermoscopy is only one minute longer than for that without, and complete examination with dermoscopy, even in cases with a high naevi count, took approximately 3 min.

In dermoscopic analysis none of our cases showed a multi-component pattern, or marked asymmetry in structure or pigmentation, which are considered clues for recognising MM. This emphasises the importance of finding other dermoscopy features in these early and difficult lesions, such as those we propose in this series, for small, symmetrical and hypopigmented lesions (15–17, 31, 32).

The main open question regards the potential malignant behaviour of these tumours. Obviously, the only way to truly demonstrate the malignant nature of a melanocytic lesion is through the development of metastasis. However, the clinical/dermoscopic and histopathological morphological features of a tumour are usually sufficient to make a diagnosis. As we are now detecting tumours at such an early stage, it is difficult to observe the classical and marked malignant features of more advanced MM. On the other hand, it may be possible that these lesions would never evolve to more invasive MM. Khalifeh et al. (33) reported a series of 11 atypical melanocytic lesions on distal lower limbs, especially on the ankle, which they consider as benign tumours that could be misdiagnosed as MM *in situ*. They concluded that these were benign lesions based on mild cytological atypia, no pagetoid spreading, and no recurrence after a follow-up period of between 4 months and 13 years. These cases showed some similarities to ours, but we found pagetoid invasion in the epidermis in all cases. A benign outcome in such lesions is possible. However; observation of only 11 cases is not sufficient to confirm a benign behaviour.

In agreement with previous studies on RCM in MM, the most frequent features associated with malignancy are the partial or total loss of the honeycomb pattern, pagetoid spreading of roundish or dendritic cells, and irregular or non-edged papillae (24, 34, 35). In our series, despite the unremarkable clinical appearances of the 36 tumours, we were able, based on dermoscopic classification, to establish good correlations between dermoscopic presentation and confocal and histopathological features. In at least two cases in which clinical and dermoscopic features were suggestive of benign lesions or inconclusive, confocal examination according to a 2-step algorithm recently described by our group (24) increased our suspicion and led us to decide on excision instead of follow-up.

Based on our experience, we propose a dermoscopic classification of the early stages of MM on the limbs that could help the further investigation of possible different origins, such as has been proposed in recent observations regarding cutaneous stem cells (36, 37) and MM pathways.

The distribution of *in situ* MMs among the dermoscopic groups was not uniform. Between 90% and 100% of cases in groups 1, 2 and 4 were *in situ* MMs, whereas 50% of MM in group 3 (hypopigmented with atypical vessels) were *in situ* MMs. This may be explained by a delay in diagnosis for more deeply invasive lesions with lesions with greater diameters, which agrees with our observation in a study of MC1R polymorphisms, and which could contribute to a hypopigmented MM aspect with fewer dermoscopic features, thus implying a more difficult early diagnosis (9).

In conclusion, we reviewed 36 cases of very early MMs on the limbs. None of these cases could have been diagnosed by clinical examination alone. Dermoscopy aided by digital follow-up and occasionally by confocal microscopy encouraged us to excise these clinically unsuspicious lesions. The limitation of this retrospective series is that it is not possible to compare these morphological features with those of excised benign lesions, or to confirm the future malignant behaviour of these incipient tumours. Obviously not all thin MMs will disseminate, and not all *in situ* and micro-invasive MMs will become invasive and life-threatening. However, several of the present patients belong to families affected by FamMM, and unfortunately some relatives had died from MM-associated metastasis. Therefore, our aim must be for all MMs in these high-risk patients to be diagnosed at the *in situ* stage. Finally, we can conclude that, despite a banal clinical aspect, melanocytic lesions on the limbs can present some dermoscopic or confocal features that raise suspicion. All of these tumours should be removed or have a short-term follow-up, especially in the case of the very high-risk population attending a referral pigmented lesions unit.

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TRABAJO II

RESUMEN TRABAJO II

Development of a Human in vivo Method to Study the Effect of Ultraviolet Radiation and Sunscreens in Melanocytic Nevi

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Objetivo

Diseño y validación de un modelo humano de estudio intervencional prospectivo de evaluación de los efectos de la radiación ultravioleta (RUV) en lesiones melanocíticas benignas, y, el posible papel de la protección física y tópica en crema en evitar dichos efectos.

Metodología

Selección de lesiones melanocíticas sin criterio de sospecha de malignidad e irradiación de una dosis única y controlada de RUVB (doble de la dosis mínima eritematógena; 2MED). Las lesiones debían ser simétricas al menos un eje para permitir mantener una mitad de la lesión protegida frente a la otra sin proteger, y cada lesión sería su propio control. En un primer grupo se protegían mediante una barrera física opaca, mientras que en un segundo grupo de lesiones se aplicaba un filtro en crema de amplio espectro y alta protección comercializado (conteniendo octocrylene, Parsol 1789, dióxido de titanio, Mexoryl SX®, Mexoryl XL®). Todas las lesiones se caracterizaron clínica y dermatoscópicamente antes de la irradiación, y 7 días después de la misma, momento en el que se extirparon completamente.

Resultados

El modelo permite demostrar cambios in vivo (clínico-dermatoscópicos) en las lesiones a los 7 días post-irradiación, consistentes principalmente en eritema, descamación, cambios de pigmentación, aparición de vasos puntiformes y borramiento del retículo pigmentado. Asimismo esta metodología permite comparar los cambios inducidos por la RUV en las mitades protegidas frente a las no protegidas, de forma que se puede testar la eficacia de la protección tópica. Histopatológicamente y mediante inmunohistoquímica, se pueden comparar ambas mitades de cada lesión a los 7 días de la irradiación, permitiendo diferenciar las zonas protegidas de las no protegidas, principalmente mediante marcadores de proliferación (Ki67) y activación de melanocitos (Melan A y HMB45).

Basándose en experiencias previas, se consigue desarrollar un modelo pionero que compara la protección mediante barrera física frente a un fotoprotector en crema comercializada. En base a los resultados preliminares del modelo, ambas protecciones podrían evitar cambios inducidos por la una dosis de RUV aguda.

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Development of a Human in vivo Method to Study the Effect of Ultraviolet Radiation and Sunscreens in Melanocytic Nevi

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Key Words
Dermoscopy · Immunohistochemistry · Malignant melanoma · Melanocytes · Nevus · Photochemoprevention · Photoprotection · Sun exposure · Sunscreens · Ultraviolet radiation

Abstract
Background: Ultraviolet radiation (UVR) plays an important role in the development of melanocytic lesions. Sunscreens have shown an impact in the prevention of UVR damage; however, their role in melanocytes has not been well established. The aim was to design and validate an in vivo human model to study the influence of UVR and sunscreen protection on nevi. ***Methods:*** A model describing clinical, dermoscopic, histopathological and molecular changes after UVR with or without protection was elaborated. Two UVB minimal erythema doses were irradiated on 4 nonsuspicious nevi from 4 patients; previously one half of each lesion was protected, in 2 cases with a physical opaque material and in the other 2 lesions by applying a high physical and chemical protection sunscreen (containing octocrylene, Parsol 1789, titanium dioxide, Mexoryl SX™, Mexoryl XL™). Lesions were excised 7 days afterwards. ***Results:*** After 7 days, clinical and dermoscopic changes (more pigmentation, erythema, dotted vessels, blurred network) were noted comparing the lesions before and after irradiation, especially when comparing both sides of each nevus (protected and nonprotected). Histopathological and immunohistochemical studies demonstrated marked melanocytic activation on nonprotected areas and a high proliferation index of keratinocytes. Both physical and sunscreen protections seem to avoid these changes. ***Conclusion:*** A useful and secure human model to study the UVR influence, and efficacy of sunscreens, on melanocytic lesions was developed. In vivo and ex vivo differences between irradiated nevus versus irradiated nevus plus sunscreen or physical protection were found.

Introduction

Melanoma (MM) is the human malignancy that has undergone the greatest increase in incidence during the last few decades. Early diagnosis of the in situ stage is still the only way to obtain curative treatment, and the challenge of identifying people at high risk for MM, and knowledge of predisposing and progressing factors are critical to determine and improve prognosis. Ninety percent of MM are considered sporadic, and the main risk factors implicated are ultraviolet radiation (UVR) and the presence of melanocytic nevi [1–5].

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UVR is considered to play an important role in the development of MM and melanocytic benign skin lesions [6–9]. In animal models UVR was shown to be both an initiator and a promoter in the multistep process of the genesis of melanocytic tumors [10]. Multiple epidemiological studies suggest that the risk for MM is increased in Caucasians exposed to intense intermittent sunlight, in individuals with severe sunburns before the age of 15 and in those who use artificial tanning lamps [5]. DNA damage caused by UVR is thought to play a major role in carcinogenesis, in keratinocytes and in melanocytes. UVB radiations at short wavelengths, 290–320 nm, induce damage in the form of cyclobutane pyrimidine dimers and pyrimidine photoproducts. UVA (320–400 nm) causes single-stranded breaks, DNA-protein cross-linking and the generation of free radicals that cause oxidative damage [4, 11–14].

The capacity to repair UVR-induced DNA damage is genetically determined. Intracellular pathways functioning to prevent UV carcinogenesis include those involved in: (a) identification of DNA damage, (b) reparation of UV DNA damage and (c) induction of apoptosis if repair is not efficient. Several proteins involved in the regulation of DNA replication and progression to the cell cycle (such as p53, p16, p14, Bcl2, survivin, CyD1) are involved in the identification of DNA damage, inhibition of cell cycle progression and induction of apoptosis [12–17].

Besides avoiding sun exposure, using a sunscreen is the best and the most accepted photoprotection method in most developed countries, and the preventive effect of using a sunscreen in non-MM skin cancer has often been suggested. However, there is still debate whether it provides adequate protection against MM and nevus induction. UVR-induced p53 has been assessed in keratinocytes as an indirect marker of UVR damage, and its expression has been shown to decrease after sunscreen use [18–22]. Even so, nowadays there is no published study, or in vivo model, that demonstrates the protective effect of sunscreens against cellular and DNA damage in melanocytes and in melanocytic tumors. In 1997, Serre et al. [23] demonstrated the protective effect of sunscreens against the local immunosuppressive reaction after photoexposure. Schiller et al. [24] investigated the stimulation of proopiomelanocortin and melanocyte-stimulating hormone/melanocortin 1 receptor in skin exposed to 2 minimal erythema doses (MED) from a solar simulator. They studied in vitro, by means of suction blister biopsies, the mRNA levels and protein expression for both molecules at different times after UVR. They demonstrated melanocortin 1 receptor stimulation in keratinocytes exposed to UVB, and the absence of this effect after adding a photoprotector.

Nevi are considered potential precursors and simulators as well as risk markers of MM, especially when these lesions are numerous or atypical. Nevi and MM share the same cell origin, the melanocyte, an especially apoptosis-resistant cell. In recent years, different published studies reported that acute UVB irradiation provokes demonstrable and quantifiable changes in melanocytic lesions similar to those found in early MM, as well as that UVR at an early age modifies the number of nevi at the adult age.

In the present study, the two main objectives were to design and validate an in vivo human model to study the clinical, dermoscopic, histological and molecular effects induced by UVR in benign melanocytic lesions and to validate this model for the study of the role of sunscreen protection in avoiding all of these supposed induced changes. To the best of our knowledge, this is the first in vivo model to study a topical sunscreen effect on UVB-irradiated melanocytic nevi.

Methods

To design our in vivo human model, we performed an exhaustive revision of the literature related to UVR effects in melanocytic nevi. All these previous studies are summarized in table 1.

In vivo Model to Demonstrate the Specific UV-Induced Damage in Benign Melanocytic Lesions

Patients

Volunteers, visiting the Pigmentary Lesions Unit in the Dermatology Department of the Hospital Clinic of Barcelona were included after Ethic Committee approval. Criteria for the patient inclusion were: patients with no personal history of MM, who accepted and signed an informed consent form explaining the complete protocol, with strict photoprotection conditions during the study, and the extirpation of the lesion 7 days after UVB irradiation. Patient exclusion criteria were: personal history of skin cancer or photodermatoses, phototoxic/allergic drug intake, active dermatoses, phototherapy or intentional photoexposure in the previous 3 months, or pregnancy.

An exhaustive personal and familial history was collected including familial malignancies, atypical mole syndrome, previous sun exposure and sun protection, artificial sun exposure, childhood sunburns, as well as clinical information about phototype, eyes and hair color, skin photodamage and other skin tumors present on physical examination.

Two melanocytic nevi with a maximum diameter greater than 6 mm and symmetrical in at least one axis, cleared of any suspicion of malignancy or marked atypia by clinical and dermoscopic examination, were selected from 2 patients.

Table 1. Summary of studies about UVB influence on nevi

Authors	Year	Type of radiation	Methods	Main findings
Holman et al. [25]	1983	Seasonal variation	Histological description of nevi excised in summer	Different clinical features in nevi depending on session; more likely to have a junctional component and inflammatory response; possible short-term latency effect of UVR on nevi
Armstrong et al. [26]	1984	Seasonal variation	Clinical and histological description of nevi in summer	
Larsen et al. [27]	1990	Seasonal variation		
Pawlowski et al. [28]	1991	UVR influence	Ultrastructural study	Increased cellular metabolism and mitotic activity
Tronnier et al. [29]	1995	2 MED UVB	Histological changes 7, 14 or 21 days after acutely irradiated nevi	Increased in number of suprabasal and enhanced HMB45 staining melanocytes, but no increased proliferation; after 2 weeks no significant changes were observed
Tronnier and Wolff [30]	1995	2 MED UVB	Histological and ultrastructural findings in nevi 7 days after irradiation	Induced histological changes can simulate melanoma; ultrastructural features suggested an increase in melanocytic metabolic activity
Stanganelli et al. [31]	1996	Seasonal variation	Clinical and dermoscopic differences between winter and summer	Increased prevalence of black dots, pigmentation and prominent network
Stanganelli et al. [32]	1997	Intense sun exposure	Clinical and dermoscopic changes in nevi after 5–13 days of sun exposure	Dermoscopy detects subtle changes; some changes are transient, and massive regression can be induced
Hofmann-Wellenhof et al. [33]	1997	UVB therapy, suberythemal dose	Dermoscopic features in nevi of patients undergoing UVB therapy for a median of 8 weeks	Unprotected nevi became more irregular and darker, protected nevi showed no significant changes
Hofmann-Wellenhof et al. [34]	1998	2 MED (UVB and UVA)	Dermoscopic changes at 3, 7, 14 and 28 days after acute irradiation	Most changes induced at 3 days; transient effects detected at 28 days; some induced changes may suggest melanoma
Tronnier et al. [35]	1997	1 MED UVR	Adhesion molecule expression in irradiated nevi	Upregulation of integrins in suprabasal keratinocytes; possible association with migration of melanocytes into epidermis
Serre et al. [23]	1997	3 MED	Locally UV-induced immunosuppression against topical sensitization; sunscreen prevention	Sunburn can impair contact hypersensitivity, and sunscreen use can avoid this immunosuppressive effect
Böni et al. [36]	1998	4 MED (UVB + UVA)	Removing lesions 7 days after UVR; microdissection and DNA extraction for allelic loss investigation	Acute histological changes after UVR are not followed by allelic loss demonstrated in dysplastic nevi
Rudolph et al. [37]	1998	2 or 4 MED	Immunohistochemical staining of proliferation and repair activity in nevi	UVB induced increased HMB45 reactivity, proliferation and simultaneously compensatory cell cycle regulation by means of p53 and p21
Tronnier et al. [38]	2000	1 MED UVB or mechanical irritant factors	Clinical and histopathological change in nevi	Morphological and transient changes which simulate melanoma, associated with proliferation and repair activity
Krengel et al. [39]	2002	2 MED UVB	Metalloproteinase (MP2, TIMP2, MT1-MMP) expression in irradiated nevi	Different expression between keratinocytes and melanocytes, but none between irradiated and nonirradiated cases
Schiller et al. [24]	2004	2 MED (UVB + UVA)	Suction biopsies and calculated RNA (RT-PCR) and product (IHC) POMC, MSH, IL-10 at 3, 6, 24 h after irradiation; in vitro model	Topical sunscreen prevents upregulation of MSH
Carrera et al. (this study)	2007	2 MED UVB	Physical protection versus sunscreen protection in nevi; 7 days after irradiation, in vivo and ex vivo studies	Clinical and dermoscopic UVB-induced changes were avoided by sunscreen; melanocyte activation and cell proliferation induced were partially avoided by skin protection

MP = Metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; MT1-MMP = membrane type 1 matrix metalloproteinase; IHC = immunohistochemistry; POMC = proopiomelanocortin; MSH = melanocyte-stimulating hormone; IL = interleukin.

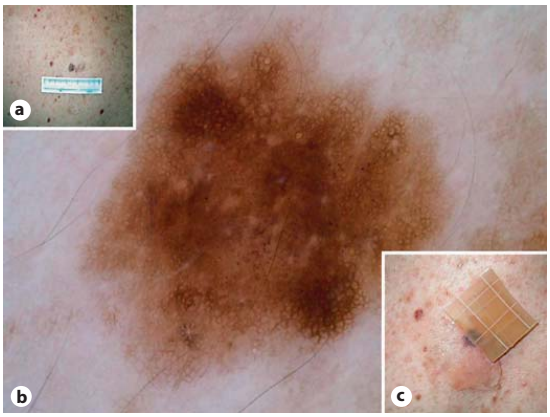

Fig. 1. Methodology: clinical (**a**) and dermoscopic (**b**) images on day 0. **c** Sunscreen application to one half of the lesion while the other half was covered for 30 min.

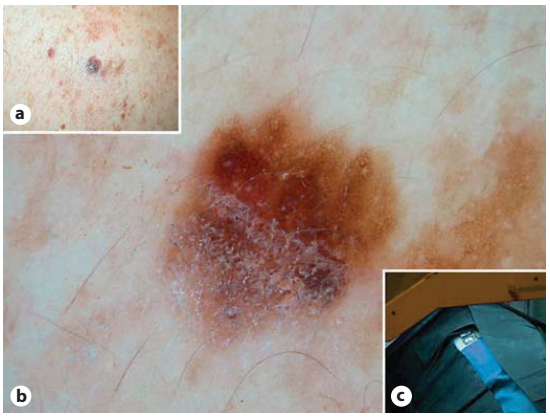

Fig. 2. Methodology: 30 min later, clinical (**a**) and dermoscopic (**b**) images. Note the colored sunscreen on the left side of the lesion. **c** Two-MED UVB irradiation of the whole lesion.

Clinical criteria of the evaluated lesions were: diameter (mm), symmetry, colors, abrupt borders (more or less than 50%), palpable or not.

Dermoscopic criteria evaluated included: symmetry (0/1/2 axis), polychromy (number of colors), global pattern of the lesion (network/globular/homogeneous/starburst/multicomponent/nonspecific), local structures (typical/atypical network, typical/atypical globules and dots, radial streaks, regular or irregular pseudopods, regular or irregular blotches, blue-white veil), regression structures (white/blue-gray, extension less/more than 50% of the surface), typical or atypical vascularization.

Irradiation and Study Schedule

First Day (Day 0). Clinical (ABCD information was collected) and dermoscopic images of all lesions selected were obtained by Dermlitephoto 3Gen* and Nikon Coolpix 4500. The UVB MED was assessed in each patient by means of a Waldmann UV800 lamp by incrementing doses for 10, 20, 30, 40, 60 and 80 s at 2.5 mJ cm^{-2} s^{-1} on a 2-cm^2 area on the back at 20 cm distance from the lamp.

Day 1. MED determination was established after 24 h. Covering one half of each lesion with an opaque object, a unique dose of 2 MED UVB irradiation was applied to the other half of the lesions, using the same conditions as in the MED evaluation.

Day 8. Clinical and dermoscopic images were obtained again 7 days after irradiation. Illumination parameters were the same for all cases evaluated as before irradiation.

Tumor Processing

After the registration of images, 7 days after irradiation, nevi were removed. One half of each lesion was marked with indelible ink at the margin of the biopsy. A 3-mm punch biopsy was performed in each lesion half to obtain a fresh sterile tissue sample to freeze and for further molecular investigations. The remaining lesion was formalin embedded for routine histopathological study.

Immunohistochemistry. Demonstration of different antigenic markers to compare the responses of each nevus half was performed. HMB45 (Biogenex®, the Netherlands; prediluted without pretreatment) and Melan A (Dako, Denmark; 1:50 dilution, pretreatment in pressure cooking with sodium citrate for antigen retrieval) were melanocyte activation and differentiation markers. Bcl2 (Dako, Denmark; 1:50 dilution, pretreatment in pressure cooking with sodium citrate), p53 (Novocastra, Newcastle, UK; 1:50 dilution, pressure cooking with sodium citrate antigen retrieval), $p16^{INK4a}$ (Biocare Medical®, USA; 1:1 dilution, pretreatment with pressure cooking in EDTA) and Ki67 (Biogenex; prediluted, pressure cooking with sodium citrate antigen retrieval) were used as cell cycle regulation and proliferation markers. Slides were then incubated with Envision anti-mouse antibody (Dako, Hamburg, Germany) for 30–60 min and were stained with AEC substrate, and sections were counterstained with hematoxylin before final mounting. Validation of the data was made by consensus with 3 independent investigators (C.C., J.P., D.M.). Positive and negative external controls were included.

In vivo Model for the Study of the Sunscreen Effect after Irradiation of Melanocytic Lesions

Another 2 lesions from 2 different patients were included in this second part of the study. Two nevi were selected after having been cleared of any suspicion of malignancy or of marked atypia, by clinical or epiluminescence criteria. In this model a physical and chemical sun protection (SPF >60, COLIPA method) sunscreen (UVB and UVA spectrum: octocrylene, titanium dioxide, Mexoryl SX™, Mexoryl XL™, Parsol 1789) was applied for 30 min on one half of the nevi, keeping the other half covered with an adhesive waterproof patch during this time. Next, irradiation of the whole lesion with a controlled unique dose of UVB (2 MED) was performed, and the complete protocol was carried out as previously described (fig. 1, 2).

Table 2. In vivo features observed 7 days after irradiation differing from baseline examination of the same lesion

In vivo examination 7 days after UVB	Identified features	Unprotected halves	Protected halves	
		both groups (n = 4)	group 1 (n = 2)	group 2 (n = 2)
Clinical changes	Erythema	1	–	–
	Increased pigmentation	3	–	–
	Scaling	2	–	–
Dermoscopic changes	Increased pigmentation	2	–	–
	Increased localized pigment	2	–	–
	Blurred pigment network	3	–	–
	Nevus dotted vessels	1	1	–
	Adjacent dotted vessels	1	1	–
	Diffuse erythema	1	–	–
	Larger globules and dot size	2	–	–
	Greater globules and dot number	2	–	–
	White regression	2	–	1
	Blue-gray regression	2	–	2

Number of lesions presenting differences: group 1 = physical protection, group 2 = sunscreen protection. Note that most of the features were identified only in the unprotected halves, but in some cases they were also observed in the protected halves.

Results

Four nevi from 4 patients were selected. They were randomized in each group of study as commented in the methodology. Two of them were enrolled in the first group (irradiation vs. irradiation with physical protection), while the other 2 were in the second group (irradiation vs. irradiation with sunscreen). Each lesion was its own control.

Patient gender distribution was uniform in both groups (1:1 in each one). The mean age of patients was 33.25 years, and all of them presented atypical mole syndrome, with more than 100 nevi. All of them had brown hair and brown eye color except for one who was blue-eyed. Fitzpatrick's phototype was II in 3 of them and III in 1. The MED was 50 mJ cm^{-2} in 1 patient, 100 mJ cm^{-2} in 2 and 150 mJ cm^{-2} in the last coinciding with the darkest phototype.

All lesions were located on the trunk. The maximum diameter ranged from 6 to 10 mm.

Basal Examination

Clinical examination revealed brown lesions with abrupt borders in 2 of them, but with no suspicion of malignancy.

Dermoscopic examination demonstrated symmetric lesions (except for 1 one-axis-asymmetric lesion), all showed more than 1 color, and the overall predominant pattern was reticular. Two of them presented globules and dots in the periphery of the lesion (1 from each group), and another had irregular globules distributed along the lesion. The nevus which showed clinical asymmetry and 2 colors was the same that showed dermoscopic asymmetry in one axis and polychromy (4 colors).

One lesion presented dotted vessels. None showed marked atypia or suspicion of MM.

UVB-Induced Effect

In vivo Study

Table 2 summarizes the in vivo features observed 7 days after UVB irradiation. Each irradiated half of the nevus was compared with the other half of the same lesion protected (physically or with a sunscreen), being its own control.

Clinical Features. Most relevant changes were: erythema (1), pigmentation (3) and surface scaling (2). Most of these changes were more evident in adjacent perilesional skin (fig. 3). No clinical change was observed in the protected areas.

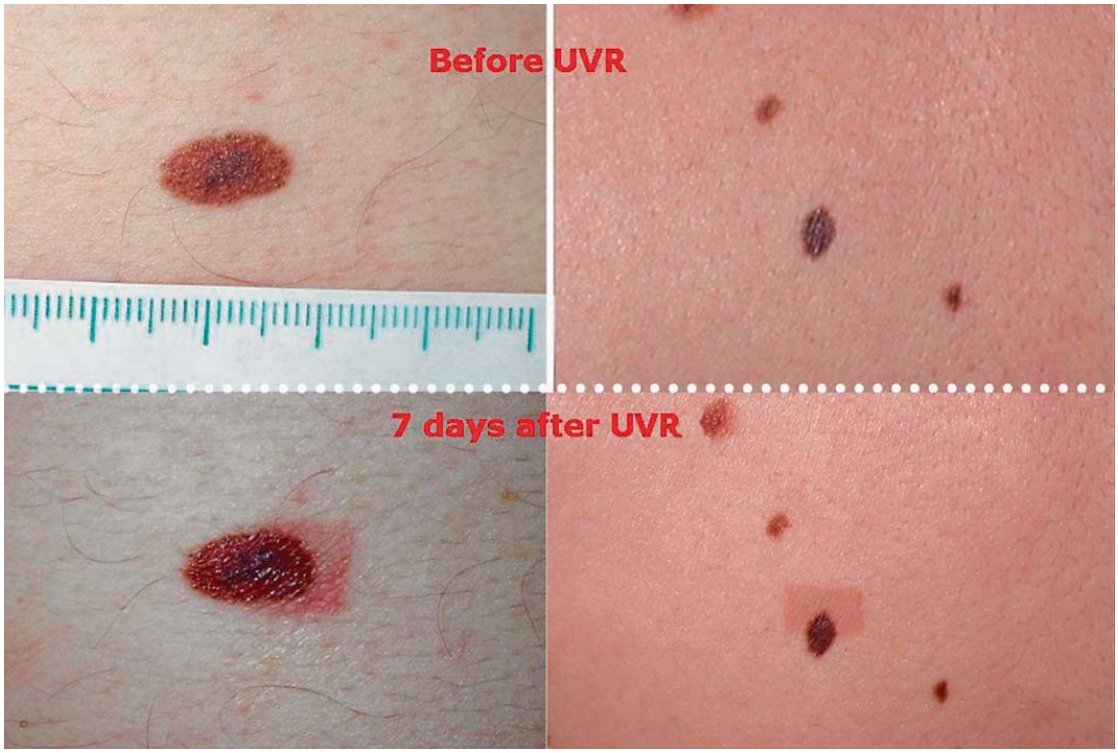

Fig. 3. Clinical aspect before and 7 days after irradiation. Erythema, pigmentation and scale formation are observed on unprotected halves.

Dermoscopic Findings. Diffuse pigmentation (2), localized pigmentation (2), blurred pigment network (3), dotted vessels in nevus (1), dotted vessels in adjacent skin (1), diffuse erythema (1), changes in globules and dot size (2), changes in globules and dot number (2), white regression (2), blue-gray regression (2) (fig. 4, 5). In some cases, dermoscopic changes were observed both in protected and nonprotected areas: in 1 lesion from the first group (physical protection), dotted vessels appeared in the whole lesion in the protected area (both in nevus and perilesional skin) and in the half without protection. Another lesion from group 2 (sunscreen protection) showed white regression in both halves (with and without sunscreen). Two nevi from the second group developed blue regression in the whole lesion, with no differences between protected and nonprotected areas.

Ex vivo Study

Features identified only in one of the halves, and not in the other part of the same lesion, were considered differences between irradiated areas with or without protection, although it is not possible to completely rule out the histological asymmetry characteristic in dysplastic nevi.

Detected histopathological differences were: parakeratotic hyperkeratosis (identified only in the halves without protection, 4 nevi), increase in lentiginous melanocytic hyperplasia (more prominent in the halves irradiated without protection), marked junctional melanocytic hyperplasia in nevus and normal adjacent skin (the latter very prominent in the halves irradiated without protection), epithelial hyperplasia, mild increase in dermal perivascular inflammatory infiltrates, regression, suprabasal melanocyte invasion (notably different in the halves

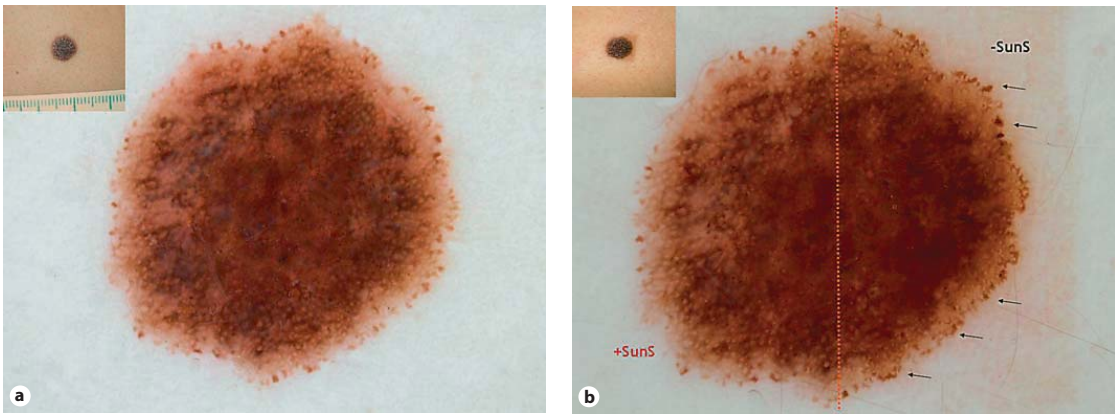

Fig. 4. a Clinical and dermoscopic images before irradiation. Pigment network pattern with peripheral crown globules. **b** Seven days after irradiation. The left side was sunscreen protected. Note more global pigmentation and an increased number and size of crown globules (arrows) on the unprotected side compared with the sunscreen-protected side, and compared with the preirradiation state.

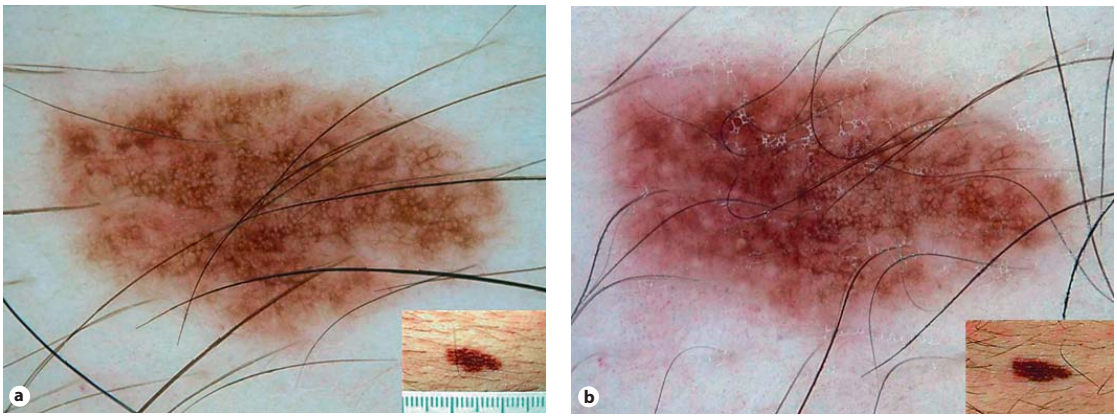

Fig. 5. a Clinical and dermoscopic images before irradiation. Pigment network pattern. **b** Seven days after irradiation. The right side was physically protected. Note the blurred network with multiple dotted vessels in the unprotected area.

irradiated without protection) and prominent elongated dendrites in melanocytes (fig. 6, 7).

Differences in inflammatory infiltrates between irradiated nevi with or without protection were more evident in the first study group (physically protected).

Two lesions were diagnosed as being compound melanocytic nevus of the congenital type, one was diagnosed as a junctional melanocytic nevus with lentiginous melanocytic hyperplasia and moderate architectural atypia, and the other was diagnosed as a compound melanocytic nevus with lentiginous melanocytic hyperplasia and mild architectural atypia.

Immunohistochemical studies showed different staining between keratinocytes and melanocytes. Intensity

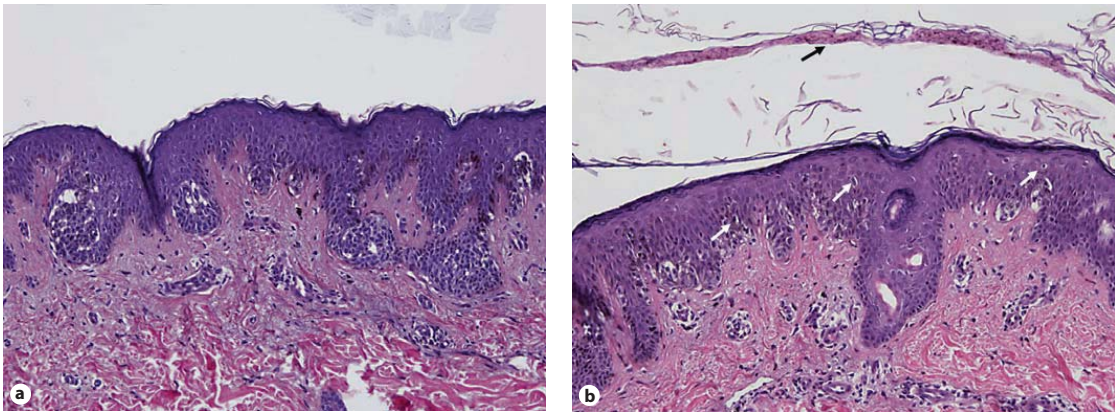

Fig. 6. Hematoxylin-eosin study of the protected area (**a**) and unprotected area (**b**). Parakeratotic scale (arrow), mild inflammatory infiltrates and a higher number of isolated junctional and suprabasal melanocytes (arrows) are shown in unprotected areas (**b**). ×100.

and percentage of staining for each antibody were calculated.

HMB45 and Melan A. Melanocytic markers were intensely positive in the 4 lesions (+++ intensity and more than 80% of nevus cells). Melan A staining was slightly stronger than that of HMB45, probably due to a dermal component (usually HMB45 negative). The main difference between protected and unprotected halves was the adjacent skin, intensely stained with both antibodies in unprotected areas (fig. 7).

p53 Protein. The proapoptotic protein p53 was not expressed in nevus cells, in contrast to keratinocytes from adjacent irradiated skin that showed scattered confluent positive nuclei. In 2 cases a mild increase in the mean number of apoptotic keratinocytes was demonstrated in halves irradiated without protection compared to protected halves (fig. 8).

Marker Ki67. An elevated number of proliferating cells was demonstrated both in nevi and in adjacent skin. All cases showed a notably higher index in irradiated halves compared to those irradiated with protection (fig. 8).

Bcl2 and p16. Antiapoptotic Bcl2 protein and cell cycle regulator p16 protein were similar in both halves of each lesion. All lesions showed positive staining for both antibodies, with different intensity obviously: Bcl2 was very weak and p16 had a very strong staining, and both only in nevus cells. One case was negative for Bcl2.

Table 3 gives a summary of the histopathological examination of each nevus with the additional immunohistochemical stainings.

Discussion

In addition to the important role of UVR in MM pathogenesis, several recent studies have demonstrated the relevance of sun exposure in developing multiple nevi, especially during childhood.

Wachsmuth et al. [8] have just reported the genetic and environmental determinants of nevi by examining teenage twin pairs. They concluded that nevi are mainly genetically determined and modulated by sun exposure. It is emphasized that they were not able to demonstrate a protective effect for either sun protection cream or shirt wearing, and of the 25% of nevus density variation attributable to environmental influences, one third was estimated to be due to sun exposure on hot holidays [8]. Bauer et al. [17] in Germany did not find, in a multivariate analysis, any significant protective effects of sunscreen either, mainly because those who used a sunscreen spent longer periods in sunny climates. As regards Australia, Harrison et al. [18] demonstrated a higher melanocytic nevus count associated with more time spent outdoors and a history of sunburn, while sunscreen use, particularly during winter, appeared to have a protective

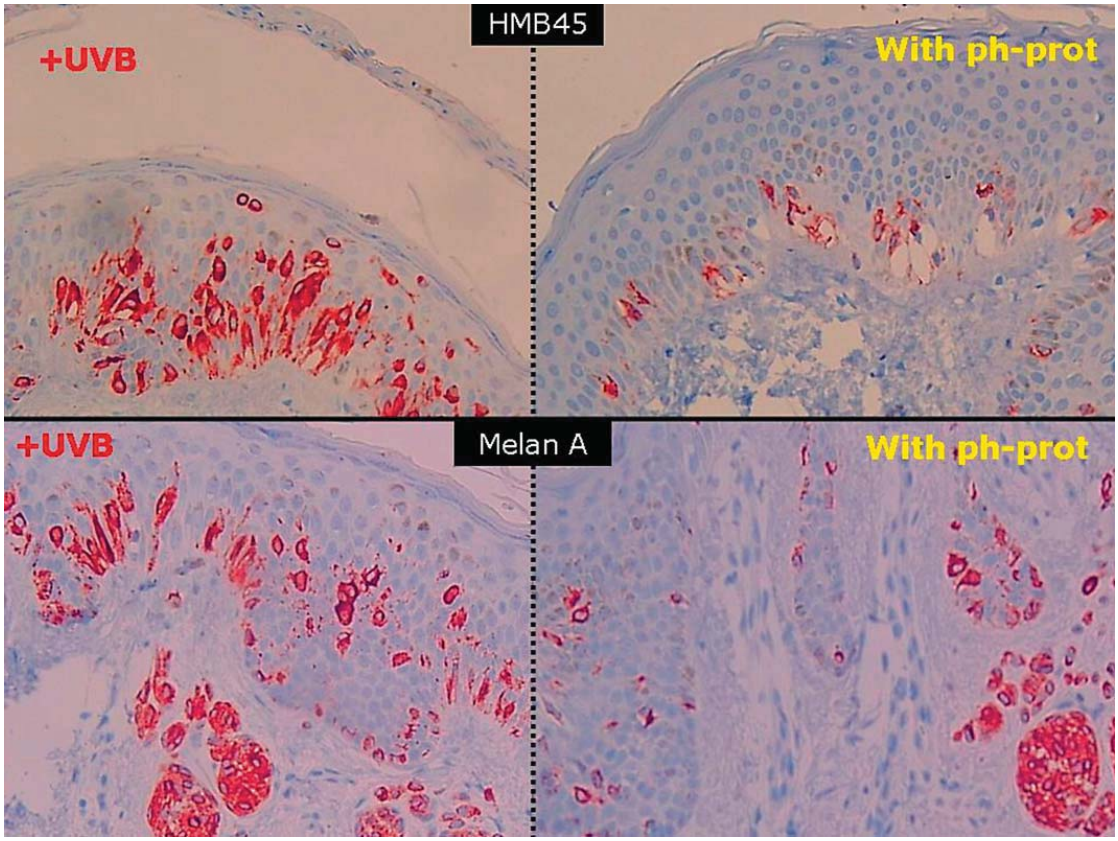

Fig. 7. Immunohistochemical staining with unprotected areas on the left side and physically protected (ph-prot) on the right. Note again parakeratotic scale and more prominent and activated junctional and suprabasal melanocytes at unprotected sites.

effect. Finally, Menzies et al. [6, 9] demonstrated, in an animal model, the role of UVB in the induction of a significant increase in nevi per animal; therefore they reported the active waveband of melanocytic nevus induction being UVB near UVA2. Unfortunately such studies are still lacking in our Spanish or South European population.

Topical photoprotection is supposed to be a suitable tool for avoiding UVR effects, not only in non-MM cancer, but both in melanomagenicity and in nevogenicity. This was a primary objective of our present study. Our model seems to be useful for testing sunscreen photoprotection, and even it would allow comparison of different kinds and brands of topical products. This could be a way to evaluate products by cosmetic companies, although probably with poor feasibility.

Otherwise, it has been known since the 80s that UVR may promote changes in sun-exposed melanocytic nevi. Initial considerations were made by the group of Holman et al. [25] and Armstrong et al. [26], in 1983 and 1 year later, since they pointed out the histological differences in melanocytic nevi depending on seasonal variation. They described an increase in the mitotic index on melanocytes (in the junctional component) and inflammation and regression phenomena in lesions removed during summer compared to winter. Other groups such as Lar-

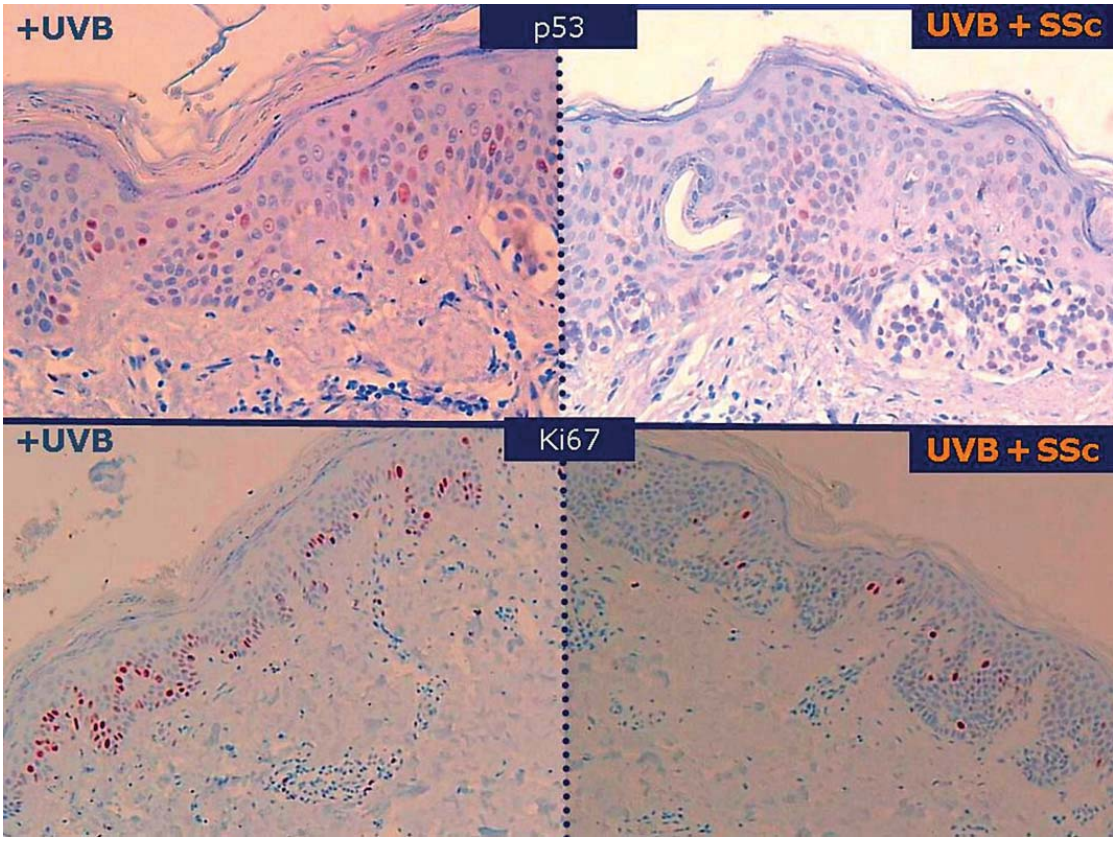

Fig. 8. Immunohistochemical staining, with unprotected areas on the left and sunscreen-protected (SSc) ones on the right, showed more proliferating cells (Ki67+) and p53+ keratinocytes on the unprotected than on the protected side.

sen et al. [27] and Pawlowski et al. [28] again described the seasonal variation in nevi.

The first prospective interventional study of UVR in melanocytic nevi was carried out by Tronnier et al. [29, 30] in 1995. They demonstrated that a single dose of UVR can invoke histopathological changes (increase in the number of melanocytes and nevus cells in the suprabasal stratum, increase in the nuclear and cytoplasmic ratio of melanocytes) and immunohistochemical changes (increase in the expression of HMB45). These changes were observed, such as in our present model, at the examination of lesions 1 week after the UVR of nevi and apparently disappeared in the second and third weeks after irradiation. These histopathological and immunohistochemical changes have been shown to be present in MM in situ, and therefore UV-irradiated nevi have been considered to be simulators of MM. We could demonstrate similar pathological effects in our study, although we did not delay the excision to study the transient effects since all lesions were removed on day 7.

Besides MM diagnosis, several reports have proposed the additional uses of dermoscopy in medicine [40], and the first dermatoscopic observation on the influence of UVR in nevi was performed by Stanganelli et al. [31]. They pointed out the seasonal variations in the dermoscopic (epiluminescence microscopy) appearance of me-

Table 3. Histopathological UVB-induced differences between irradiated (without protection) and irradiated halves with protection in the same lesion

Ex vivo study 7 days after UVB	Parameters differing between both halves of each lesion	Number of lesions with differences	
		group 1 (n = 2)	group 2 (n = 2)
Histological features	Parakeratotic scale	2	2
	Epithelial HPL	2	1
	⇑ adjacent melanocytic HPL	2	2
	⇑ lentiginous melanocytic HPL	1	2
	⇑ inflammatory infiltrates	2	0
	⇑ regression	–	–
	⇑ suprabasal melanocytes	2	1
	⇑ elongated dendrites	1	1
Molecular expression UVB-induced	⇑ intensity of HMB45	–	–
	⇑ HMB45+ nevus cells	1	0
	⇑ adjacent HMB45+ cells	1	2
	⇑ intensity of Melan A	1	1
	⇑ Melan A+ nevus cells	–	–
	⇑ adjacent Melan A+ cells	1	2
	⇑ adjacent p53+ cells	1	1
	⇑ tumoral Ki67+ cells	2	2
	⇑ adjacent Ki67+ cells	2	2

Number of lesions where these differences could be observed: group 1 = physical protection, group 2 = sunscreen protection. HPL = Hyperplasia.

lanocytic lesions, with an increase in atypical features after sun exposure (prominent network, atypical black dots and depigmented areas). In 1997, the acute changes induced by intense sun exposure in 11 Italian divers were studied. The lesions showed variations in the epiluminescence-microscopic findings that were similar to those of early MM (atypical network, atypical dots, regression structures, streaks). However, these changes were transient and persisted for only few weeks [32].

Hofmann's and Tronnier's groups studied the clinical and dermoscopic effects of UVB on nevi in patients treated with artificial UVR suffering from psoriasis and other skin diseases [33] and on intentional 2-MED UVB-irradiated half-nevi [34, 38], similar to our current design. They observed significant modifications in these irradiated lesions compared to nonirradiated nevi. As some of these lesions showed features of MM, the authors suggested that the study of melanocytic nevi should be delayed 1 month after the last UV exposure to avoid overdiagnosis of MM. In contrast to our preliminary results, they did not mention the discordance between the absence of clinical changes and the presence of dermoscopic changes. In this way, we emphasize the importance of this finding, as it indicates the existence of more effects than previously expected (clinical erythema or pigmentation). In our model, thanks to the correlation between clinical, dermatoscopic and histopathological changes, we observed that some cases did not present any in vivo UVB effect (neither clinical nor dermatoscopic); however, they could suffer melanocytic activation and suprabasal cell migration.

Several reports have demonstrated, by means of immunohistochemical staining, the different expression of melanogenesis markers, adhesion molecules such as integrins and collagenasas, and cell cycle regulators and proliferators, depending on UVR damage [35, 37, 39].

We used HMB45 and Melan A antibodies to demonstrate the morphology and location of melanocytic cells, both within the nevi and at the periphery of the lesions, the latter of which had never been mentioned in previous studies. Melan A intensity demonstrated a more UVB-dependent activation, while HMB45 staining did not differ. Both antibodies helped us to observe larger melanocytes, suprabasal cells and elongated dendrites in UVB-irradiated halves. In the adjacent skin, activated junctional melanocytes were very prominent as well. However, unexpectedly, some of these effects could be

observed in the protected halves, both in group 1 (physical barrier) and 2 (sunscreen).

According to previous studies, Ki67 staining showed an increase in the proliferating index in irradiated skin, mainly in keratinocytes but some junctional melanocytes as well. In addition to previous studies, irradiated skin adjacent to the nevus also showed a marked difference between protected and unprotected areas, both with sunscreen and with physical protection. This proliferating stimulation probably translated the keratinocytic necrosis and correlated with parakeratotic scale and epidermal keratinocytic hyperplasia.

p53 showed very light staining in scattered basal keratinocytes, but a mild increase in irradiated areas of 2 cases was observed (one of each group). Bcl2 and p16 did not show any notable differences between the halves of each nevus; it seems that these antibodies are not good targets for studying UVB influence.

Another interesting point would be to demonstrate the molecular genetic changes in irradiated nevi. Böni et al. [36] investigated loss of heterozygosity in DNA extracted from excised nevi, 7 days after 4-MED UVB irradiation. They found several histological features induced by UVB, such as an increase in suprabasal melanocytes, increased nuclear and cytoplasm melanocyte size, and prominent dendrites; however, they could not demonstrate any permanent point mutation or DNA change [36]. Even so, it is expected that some DNA effects occur after acute UVB irradiation, but it is very complicated to detect them. We have now cryopreserved a punch tissue sample from each lesion half studied for further molecular investigations.

In conclusion, we have developed the first in vivo human model that – in addition to studying the UVB effects on melanocytic nevi and adjacent normal skin – allowed us to compare UVB-induced changes in the same lesion, with and without sunscreen application. The preliminary results observed seem to demonstrate a correct protective role of sunscreens avoiding the biological effects of UVR, at least as a physical barrier. However, there were two main unexpected points: (1) it is possible that there are more biological UVR effects than we can normally see, even if there are none detected upon clinical examination; (2) topical photoprotection could not be as secure as nonexposure, since there were some UVB effects observed in protected halves. Since herein we only report our first 4 patients studied in order to establish an experimental model, further studies should be carried out to study the real impact of sunscreens in the protection against UVB-induced changes at clinical, dermoscopic or molecular levels.

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TRABAJO III

RESUMEN TRABAJO III

Impact of Sunscreens on Preventing UVR-Induced Effects in Nevi.
In Vivo Study Comparing Protection Using a Physical Barrier vs Sunscreen

Cristina Carrera, Joan A. Puig-Butillè, Paula Aguilera, Zighereda Ogbah, Josep Palou, Mario Lecha, Josep Malvehy, Susana Puig

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Objetivo

Estudiar la eficacia del uso de fotoprotectores tópicos en evitar los efectos de la radiación ultravioleta (RUV) sobre los nevus melanocíticos para optimizar las estrategias de prevención primaria en melanoma.

Metodología

Estudio prospectivo en 23 nevus melanocíticos, en base al modelo intervencionista de irradiación de nevus con 2MED de UVB, previamente protegidos en una mitad mediante una barrera física o bien mediante la aplicación de un fotoprotector tópico, en crema coloreada con FP50 de amplio espectro, durante 30 minutos previo a la irradiación. Estudio in vivo (clínico-dermatoscópico) y ex vivo (inmuno-histopatológico) a los 7 días de la irradiación.

Resultados

Los principales cambios inducidos tras 7 días de la irradiación fueron: 1) clínicamente la aparición de eritema, pigmentación y descamación superficial; 2) dermatoscópicamente el incremento de glóbulos y puntos de pigmento, aparición de vasos puntiformes, signos de regresión y borramiento del retículo pigmentado. Al comparar los nevus protegidos mediante barrera física opaca o mediante crema fotoprotectora, ambas barreras evitaron la mayoría de cambios, sin embargo en algunas lesiones se demostraron cambios en las áreas protegidas, de ambos grupos.

En más del 30% de los nevus no se evidenció ningún cambio clínico mientras que en un 18% no se objetivó ninguna diferencia dermatoscópica a los 7 días, no obstante, todas las lesiones en el estudio inmuno-histopatológico demostraron algún efecto atribuible a la irradiación. Los cambios histopatológicos evidenciados fueron principalmente la aparición de paraqueratosis e incremento del número y activación de melanocitos epidérmicos.

La única diferencia significativa entre ambos tipos de protección fue una mayor activación de melanocitos y ciertos signos de regresión en las mitades protegidas por crema. No se ha podido encontrar ninguna característica fenotípica que prediga un tipo de respuesta a la RUV.

Por tanto, se concluye que una correcta protección local puede evitar en parte los efectos de la RUVB. Sin embargo hay que tener presentes posibles efectos subclínicos, no siempre visibles in vivo, que pueden aparecer incluso en áreas protegidas. Las cremas de protección solar parecen no proteger de igual manera frente a fenómenos inflamatorios y activación de melanocitos.

STUDY

ONLINE FIRST

Impact of Sunscreens on Preventing UVR-Induced Effects in Nevi

In Vivo Study Comparing Protection Using a Physical Barrier vs Sunscreen

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Importance: Sun damage is the most important environmental factor associated with malignant melanoma. To address the health threat, as well as the economic burden, primary prevention and early detection are crucial.

Objective: To test the efficacy of a topical sunscreen in the prevention of UV-induced effects in nevi.

Design: Prospective study of nevi protected by sunscreen vs a physical barrier.

Setting and Patients: Twenty-three nevi from 20 patients attending a referral hospital.

Intervention: Half of each nevus was protected by either a physical barrier or a sunscreen. Lesions were completely irradiated by a single dose of UV-B.

Main Outcomes and Measures: In vivo examination before and 7 days after irradiation and histopathologic-immunopathologic evaluation after excision on the seventh day.

Results: The most frequent clinical changes after UV radiation were pigmentation, scaling, and erythema; the most frequent dermoscopic changes were increased globules/dots, blurred network, regression, and dotted vessels. Both physical barrier– and sunscreen-protected areas showed some degree of these changes. More than 30% (7) of nevi did not show any change on clinical examination, and 18% (4) had no dermoscopic change. Immunohistopathologic differences between the halves of each nevus were demonstrable even when in vivo examination detected nothing. Parakeratotic scale, increased number and activation of superficial melanocytes, and keratinocyte proliferation were the most remarkable features. The only difference between both barriers was more enhanced melanocytic activation and regression features in the sunscreen group. No phenotypic features were found to predict a specific UV-B response.

Conclusions and Relevance: Both physical barriers and sunscreens can partially prevent UV-B effects on nevi. Subclinical UV radiation effects, not always associated with visible changes, can develop even after protection. Sunscreens are not quite as effective as physical barriers in the prevention of inflammatory UV-B–induced effects.

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SUN DAMAGE IS THE MOST important environmental factor associated with skin cancer, which is the most frequent type of malignant neoplasm in humans. Malignant melanoma (MM) incidence has increased dramatically in the past decades, especially among young women, in part related to the tanning fashion trend.[1-3] Indeed, it poses a significant health threat, since it is the sixth most commonly diagnosed cancer in the United States and its care represents a major economic burden for health insurance systems. Therefore, great effort in primary prevention and early detection of MM is crucial in reducing this expense.[4]

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Ultraviolet radiation (UVR) has been widely demonstrated to be implicated in nevogenesis and melanomagenesis, being the most relevant environmental and exogenous risk factor.[5-9] Recently, for the first time, proper sunscreen use has been demonstrated to reduce the incidence of MM in Australia and the United States.[10]

Previously, this benefit had been proven only for solar erythema, sunburn, actinic keratosis, and squamous cell carcinoma development; however, it is well accepted that basal cell carcinomas and MM seem to follow a more complex pathway and relationship to sun exposure.[11,12] A beneficial effect of sunscreen use in prevention of MM could not be demonstrated or quantified until now, when a recent prospective epidemiologic study[13] documented a reduction in MM incidence among Australian sunscreen users, and with a better prognosis because the sunscreen group presented earlier-stage melanomas compared with nonusers. On the other hand, melanocytic nevi are considered potential MM precursors and simulators, as well as the most important independent phenotypic risk factor for MM development. It has been reported[14] since the 1980s that UVR may promote changes in sun-exposed melanocytic nevi. Seasonal variation[15-17] and the influence of phototherapy have been described in nevi. Several prospective interventional studies[18] on UVR in melanocytic nevi have been carried out since 1995. This issue has been reviewed in depth in a publication[19] in which the present study model was described. Among the most remarkable effects reported using different methodologies, UVR can induce clinical changes, such as increased pigmentation, scale formation, and erythema, as well as dermoscopic changes in pigmentation, such as globules and dots (size and number), regression features (bluish gray granules), blurred pigmented network, and increased vascularity.[16,20,21] At the histopathologic level, the most relevant events are the appearance of parakeratotic hyperplasia; lymphocytic perivascular infiltrates; cell-cycle activation of keratinocytes and melanocytes; activation of melanocytes, consisting of larger nuclear and cytoplasm size of cells; and prominent dendrites in addition to suprabasal location of melanocytes.[22] Some of these studies highlighted the importance of the recognition that acute UV-B irradiation can provoke demonstrable and quantifiable changes in melanocytic lesions similar to those found in early MM.[16]

The first interventional model for in vivo evaluation of the effects of UV-B irradiation on nevi and adjacent skin, depending on the use of a physical barrier or a commercial topical sunscreen, was developed in 2008.[19] With use of this model, in the present study, the main objective was to test the effectiveness of a topical sunscreen in preventing the different UVR effects on nevi. A secondary aim was to evaluate the different types of measurable UVR effects on clinical, dermoscopic, and histopathologic examinations depending on the different phenotype of the patients. To the best of our knowledge, this was the first prospective interventional in vivo study of the efficacy of a topical sunscreen in preventing UV-B–induced damage to melanocytic nevi and surrounding skin.

METHODS

We recruited 20 volunteers with multiple dysplastic nevi attending the Pigmented Lesions Unit in the Dermatology Department of the Hospital Clinic of Barcelona. The study was performed according to the Declaration of Helsinki principles, and our local ethics committee reviewed and approved the interventional protocol. Criteria for patient inclusion were age older than 18 years, a signed informed consent form explaining the complete protocol, and adherence to strict photoprotection conditions before and during the study. Patient exclusion criteria were a history of skin cancer or photodermatoses, phototoxic/allergic drug intake, active dermatoses, phototherapy or intentional photoexposure in the previous 3 months, immunosuppressive treatment, or pregnancy.

According to the previously published model to induce UV-B effects on nevi by a single, double minimal erythematous dose (2 MED) detailed by Carrera et al,[19] in the present study 23 melanocytic nevi were consecutively included in 2 groups. Criteria for selecting nevi were diameter greater than 5 mm, at least 1 axis symmetry, and no suspicion of melanoma. In the first group (n=14) half of each nevus was covered with a physical opaque barrier before irradiation, whereas in the second group (n=9), half of each nevus was protected by a topical sunscreen (2 mg/cm^2) and the remaining half was covered by a patch to avoid diffusion of the cream. The sunscreen (broad-spectrum protection, sun protection factor 50) contained octocrylene, avobenzone (Parsol 1789), titanium dioxide, ecamsul (Mexoryl SX), and Mexoryl XL and was applied 30 minutes prior to UVR. Ultraviolet-B was administered by a lamp (UV800; Waldmann) a 2.5-mJ/cm^2/s dose on a 2-cm^2 skin area that was centered on the nevus and 20 cm from the lamp.

Complete clinical patient history was recorded, including familial history, previous photoexposure, and phenotype. Clinical and dermoscopic images of the 23 nevi included were taken using digital cameras (G7 [Canon, Inc]; and Coolpix 4500 [Nikon Corp]) and a polarized dermoscope (DermliteFoto; 3Gen). Clinical evaluation was based on clinical ABCD-E (asymmetry, irregular borders, multiple colors, diameter >6 mm, and evolution and enlargement), and dermoscopic study on pattern analysis.[23] Each in vivo feature was evaluated before and 7 days after irradiation (baseline time and final time), and features were assessed comparing the unprotected and protected halves of each nevus 7 days after irradiation. All lesions were removed 7 days after UV-B, the protected half of each being labeled. Histopathologic and immunohistochemical studies for human melanoma black-45 antigen (HMB-45monoclonal mouse IgG1; Dako) and for melanoma antigen recognized by T-cells 1 (Melan-A monoclonal antibody A-HU, A103; mouse IgG1; Dako) were performed using standard methods. Semiquantitative scales were established for continuous values for the in vivo and ex vivo evaluations in a blinded manner by 2 of the 3 readers (J.P. and S.P.). In immunostaining, intensity and percentage of positive cells were examined, comparing both halves of each nevus and the adjacent skin. The scale for immunostaining in nevi was as follows: 0, negative or weak (<20% of nevi cells); 1, 20% to 30%; 2, 31% to 50%; 3, 51% to 80%; 4, greater than 80% to 100% of nevi cells. For adjacent surrounding skin, measurements were performed in 0.5-mm intervals (diameter of the ×40 magnification objective): 0, no positive cells; 1, 1% to 2% of keratinocytes; 2, 3% to 5%; 3, 6% to 10%; and 4, 11% to 20%.

Statistical analysis was carried out using commercial software (SPSS, version 18.0; SPSS Inc). The χ^2 test was applied for all category features, and the Fisher exact test was applied if any cell value in the 2×2 table was expected to be less than 5. Mean and median values were assessed for quantitative and semiquantitative variables and compared using a 2-tailed, unpaired *t* test for dependent samples, comparing in vivo lesions before and after UVR and unprotected vs protected halves of the same lesion, as well for independent samples, comparing the effects of physical barrier vs sunscreen. Significance was considered to be $P<.05$.

Table 1. Clinical and Dermoscopic Changes Detected Among 46 Halves of the 23 Irradiated Nevi

Changes 7 Days After UVR	Overall Nevi, No. (%) (N = 23)		Statistical Significance[a]		Group, No. (%)			
					Physical Barrier (n = 14)		Sunscreen (n = 9)	
	Unprotected Halves	Protected Halves	*P* Value	RR (95% CI)	Unprotected Halves	Physically Protected	Unprotected Halves	Sunscreen Protected Halves
Clinical								
Increased erythema	13 (57)	1 (4)	<.001	2.9 (1.7-5.0)	7 (50)[b]	1 (7)	6 (67)[b]	0
Increased pigmentation	14 (61)	1 (4)	<.001	3.2 (1.8-5.6)	5 (36)[b]	0	9 (100)[b]	1 (11)
Scaling	13 (57)	0	<.001	3.3 (1.9-5.5)	7 (50)[b]	0	6 (67)[b]	0
No clinical change[c]	7 (30)	0	0	0	6 (43)	0	1 (11)	0
Dermoscopy								
Increased pigmentation	4 (17)	1 (4)	NS	NS	1 (7)	0	3 (33)	1 (11)
Decreased pigmentation	2 (9)	2 (9)	NS	NS	2 (14)	2 (14)	0	0
Blurred pigment network	12 (52)	1 (4)	<.001	2.8 (1.7-4.6)	5 (36)	1 (7)	7 (78)[a]	0
Increased dotted vessels	7 (30)	2 (9)	<.03	1.8 (1.1-3.0)	6 (43)[b]	2 (14)	1 (11)	0
Increased erythema	10 (43)	6 (26)	NS	NS	7 (50)	5 (36)	3 (33)	1 (11)
Increased size of globules and dots	2 (9)	0	NS	NS	1 (7)	0	1 (11)	0
Increased bluish gray regression	10 (43)	9 (39)	NS	NS	4 (29)	3 (21)	6 (67)	6 (67)
No dermoscopic change[c]	4 (17)		0	0	2 (14)	0	2 (22)	0
No in vivo change detected[c]	2 (9)		0	0	1 (7)	0	1 (11)	0

Abbreviations: NS, not significant; RR, relative risk of appearance of each change in unprotected compared to protected halves; UVR, UV radiation.
[a]Categorical changes between baseline and 7 days after a double minimal erythematous dose of UV-B were analyzed by the χ^2 and Fisher exact tests (unprotected vs protected halves).
[b]Mean differences were significant within the subgroups ($P < .05$).
[c]Values given are for both halves.

RESULTS

PATIENTS

There were no significant differences between the study groups (physical barrier and sunscreen) in characteristics or nevus features. Sex distribution and mean (SD) age were similar in both groups (women, 12 [60%]; age, 36.5 [9.5] years; range, 22-55 years). Most patients had a history of intense sun exposure, inadequate sun protection behavior, and frequent sunburns in infancy and youth. Three patients (15%) were common sunbed users over the past years, and physical examination showed sun-damaged skin in 15 patients (75%). Fourteen patients (70%) had fair skin type (phototype I or II). Mean UV-B MED was 90 (27) mJ/cm^2. As many as 12 patients (60%) presented a very low UV-B MED (≤50 mJ/cm^2), including 3 (50%) of those who experienced sun tolerance and tanning (ie, phototype III or IV). The only significant association found between clinical features was the intense sun-exposure history and higher number of nevi ($P < .02$).

BASAL EXAMINATION OF NEVI

As inclusion criteria required, all lesions were located on the trunk, with a minimum diameter of 5 mm and a maximum diameter of between 6 and 10 mm. All were symmetric on at least 1 axis to permit the division of each into 2 similar halves. Clinical and dermoscopic examination showed variable degrees of atypia, similar to the rest of nevi in such a population attending our unit, but none of the lesions was suspicious for MM. There were no significant differences between nevi characteristics included in the 2 groups (physical vs sunscreen).

IN VIVO UV-B–INDUCED EFFECTS

Comparison Between Baseline Image of the Nevi and 7 Days After UV-B Irradiation

Clinical Features. Clinical and dermoscopic changes in the nevi are reported in **Table 1**. The most relevant changes were the appearance of erythema (13 [57%]), increase in pigmentation (14 [61%]), and presence of surface scaling (13 [57%]) (**Figure 1**). Most of these changes were more evident at the periphery of the lesions. No clinical change was detected in 7 cases (30%).

Dermoscopic Features. Changes observed were the appearance of diffuse pigmentation (4 nevi [17%]) (**Figure 2**), blurring of pigment network (**Figure 3**) (12 [52%]), increase in dotted vessels in the nevus and surrounding skin (7 [30%]), presence of diffuse erythema (10 [43%]), changes in the size of globules and dots (2 [9%]), and increase in regression structures (10 [43%]) (**Figure 4** and **Figure 5**). Only 4 (17%) of the lesions did not show any dermoscopic change. Two lesions (9% of nevi) did not present any change either clinically or dermoscopically 7 days after irradiation.

COMPARISON BETWEEN BOTH HALVES OF EACH NEVUS, 7 DAYS AFTER IRRADIATION

In a comparison between protected halves and unprotected halves, regardless of the type of protection used,

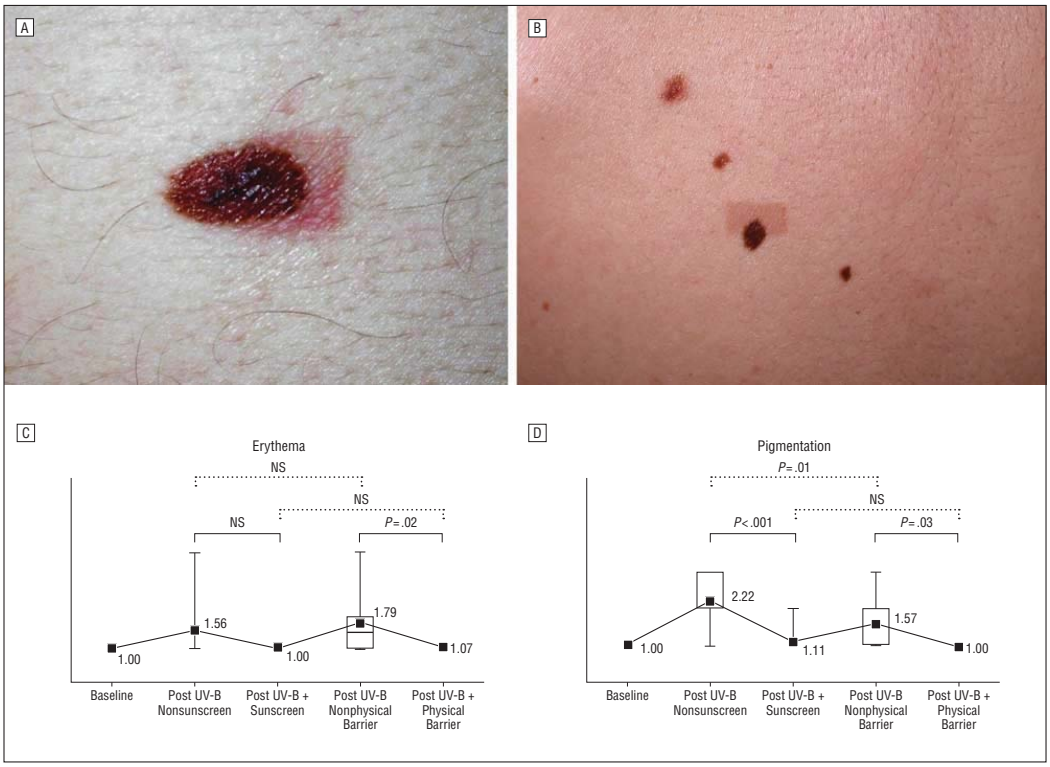

Figure 1. Clinical evaluation. Clinical images demonstrate erythema (A) and pigmentation (B) of unprotected half of lesions. Both physical barrier (A) and sunscreen (B) applied on one-half of each lesion avoided UV-B–visible changes. Semiquantitative scale at baseline and 7 days after UV radiation (C and D) analyzed by *t* test for independent groups (dotted lines; physical barrier vs sunscreen) and for dependent pairs (continuous lines; unprotected vs protected). NS indicates nonsignificant. Bottom and top edges of the boxes in the graphs (C and D) represent the 25th and 75th percentiles, respectively; horizontal line in the box, median value; and limit lines, standard deviation.

all 3 clinically evaluated features were different ($P < .001$). Scale formation was observed only in the unprotected areas; however, changes in pigmentation and erythema could be detected in both protected and unprotected areas, but to a lower, yet statistically significant, degree in the protected halves (Table 1 and Figure 1).

Unexpectedly, all dermoscopic changes evaluated also were observed in protected halves, some of them even with no significant differences when compared with the unprotected (Table 1) halves. In fact, the presence of regression structures in both halves of the nevus appeared in 67% of the sunscreen-protected group and in less than 30% of the physically protected group (21% of the physically protected halves vs 29% of the unprotected halves). The increase in regression was more important in the sunscreen group but for both the protected ($P = .04$) and unprotected ($P = .04$) halves compared with the protected and unprotected halves of the physical barrier group. Other dermoscopic changes observed in the protected halves were an increase in dotted vessels (9%) and blurred network (4%), but these change were significantly less than in the unprotected halves ($P < .001$ and $P = .03$, respectively). Pigmentation changes (increase or decrease) present in 13% of the protected halves were not significantly different compared with the unprotected halves (Table 1).

IN VIVO CHANGES DEPENDING ON PROTECTION

All clinical UV-induced changes were partially prevented by protection in both groups. However, changes in overall pigmentation, vascularity, and regression structures on dermoscopy were not significantly different between the protected and unprotected halves of the nevi. Considering the intensity and semiquantitative scale of these changes regarding the type of protection, blurred network was the only change significantly prevented by the sunscreen barrier ($P < .008$); the increase in dotted vessels was prevented by the physical barrier ($P = .05$).

EX VIVO STUDY OF EXCISED LESIONS

Histopathologic Evaluation

Diagnosis of melanocytic nevus was made in 21 of the 23 cases (91%), 12 of them (53%) with congenital-type

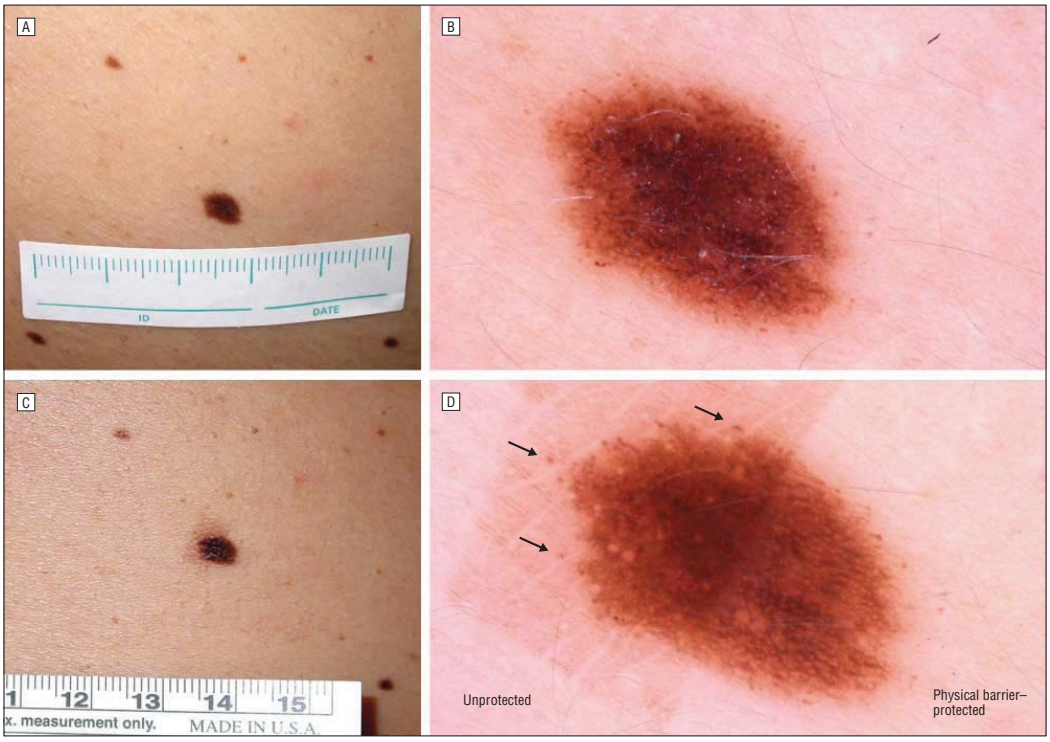

Figure 2. Clinical and dermoscopic evaluation before (A and B) and 7 days after (C and D) UV radiation. Left half of the lesion was unprotected; the right side was protected by a physical barrier. Clinical pigmentation and scale appeared 7 days after UV radiation only on the unprotected left half. Upon dermoscopy, increased pigmentation and peripheral globules (arrows) appeared on the unprotected half. Scales (A and C) indicate millimeters.

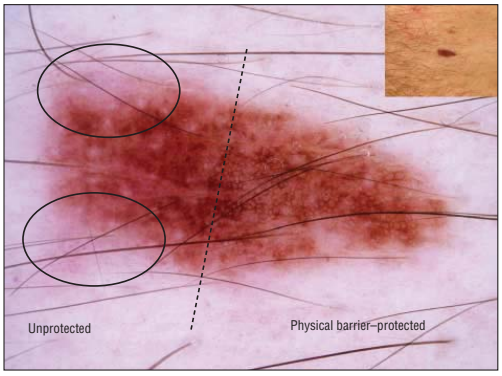

Figure 3. Clinical and dermoscopic evaluation 7 days after UV radiation. The left half of the lesion was unprotected; the right side was protected by a physical barrier. No clinical differences between the halves are visible; however, marked erythema-dotted vessels (circles) and blurred network appeared on the unprotected half.

features (18 compound and junctural nevus), and cases were considered as lentiginous melanocytic hyperplasia without nesting. Two patients decided to delay the removal of their nevi, and further histopathologic evaluation of these 2 cases was not included in the analysis of this series (ex vivo study was performed in 21 cases [9%]: 12 in group 1 and 9 in group 2). Architectural atypia was present in 15 cases (71%) to a variable degree, and in 6 of these (29%), from moderate to marked. Parakeratotic epithelial hyperplasia was observed in almost all lesions (19 [90%]). Inflammatory infiltrate was present in 18 cases (86%), and visible dermal vessels dilatation was observed in 12 cases (57%). Regression signs were observed in 8 cases (38%).

Comparison Between Protected and Unprotected Halves

Histopathologic differences were observed between both halves of each nevus in all cases. Differences were statistically significant in the overall sample (N = 21) and even within each subgroup. **Table 2** summarizes the main differences in unprotected vs protected areas. Features observed to a different degree in the unprotected halves have been assumed to be UVR-induced changes that were prevented by protection: parakeratotic hyperkeratosis (90% of nevi and observed only in the unprotected halves, $P < .001$; odds ratio [OR], 11.5; 95% CI, 3.05-43.23), marked lentiginous melanocytic hyperplasia (both within the nevi and in the surrounding skin (67% of cases, $P < .001$; OR, 4; 95% CI, 2.1-7.6; and 81% of cases, $P < .001$; OR, 6.2; 95% CI, 2.5-15.3, respectively), suprabasal solitary melanocytes (52.4% of cases, $P < .001$;

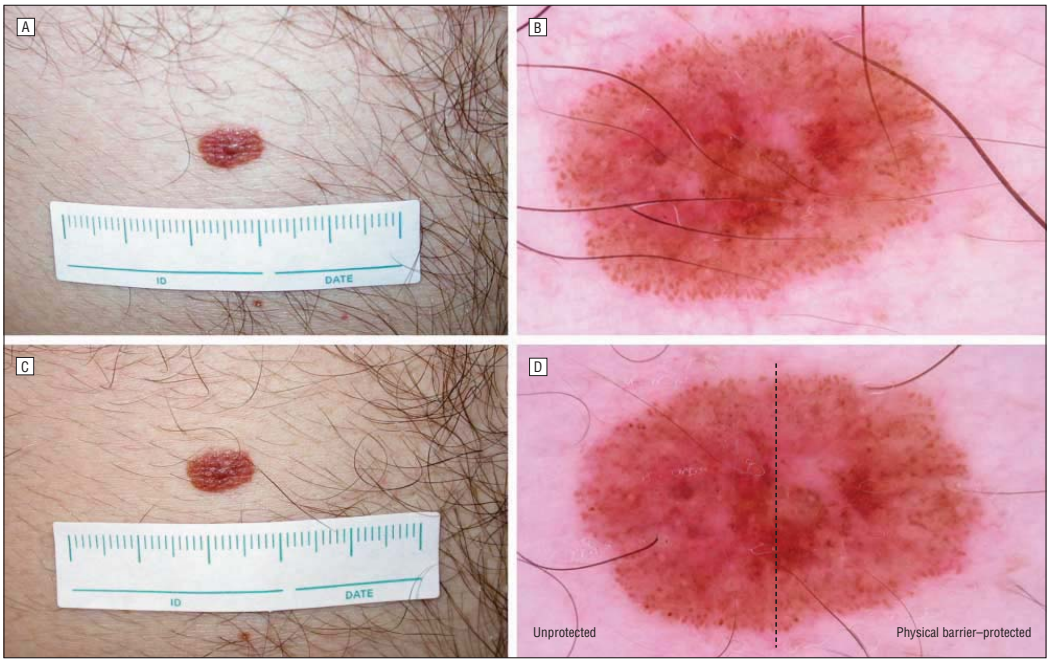

Figure 4. Clinical and dermoscopic evaluation before (A and B) and 7 days after (C and D) UV radiation. The left half of the nevus was unprotected, and the right side was protected by a physical barrier. Despite no clinically visible differences, dermoscopy showed mild erythema and decreased peripheral globules in both halves. Scales (A and C) indicate millimeters.

OR, 3.1 95% CI; 1.9-5.1), and prominent and elongated melanocyte dendrites (52% of cases, $P < .001$; OR, 3.1; 95% CI, 1.9-5.1). Differences in superficial perivascular inflammatory infiltrates and regression features were observed but were not significant between halves of each nevus in any of the groups.

Immunohistochemical staining for melanocytic markers (HMB-45 and Melan-A) were intensely positive in all lesions (marked intensity and >80% of nevus cells) and helped to quantify and demonstrate melanocytic activation (**Figures 6**, **7**, and **8**), more evident in the unprotected halves (Table 2). Regarding the quantification of HMB-45 staining in nevus cells, there was a significant difference depending on the type of protection (Figure 7). The physical barrier group (protected vs unprotected halves) had a greater difference in expression than the sunscreen group, and this also was demonstrated when the intensity of the protected halves was compared ($P < .04$). However, the quantification of staining between unprotected groups was also different, with the expression of HMB-45 being more intense in the physical barrier group ($P < .04$). On the contrary, Melan-A in nevus cells was significantly increased in the unprotected halves, but it also was higher in the halves protected with sunscreen than with physical barrier ($P < .04$) despite it being similar on unprotected halves. Evaluation of adjacent peripheral areas highlighted the differences with both antibodies that were more intensely stained in unprotected areas than protected ones (Figure 7 and Table 2).

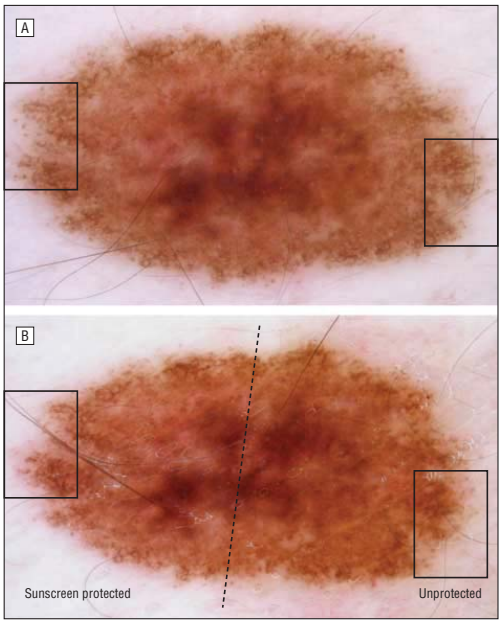

Figure 5. Dermoscopic evaluation before (A) and 7 days after (B) UV radiation. The left half of the lesion was protected by sunscreen, and the right side was unprotected. Mild erythema was present only on surrounding unprotected skin, but a decrease in whole pigmentation and globules (squares) was noted in both sunscreen-protected and unprotected areas.

Table 2. Histopathologic Evaluation; Demonstrated Differences Between Protected and Unprotected Halves of Each Nevus in All Cases[a]

Type of Evaluation	Overall Nevi Unprotected, No. (%) (n = 21)	Group, No. (%)	
		Physical Barrier (n = 12)	Sunscreen (n = 9)
Histopathology			
Parakeratotic scale	19 (90.5)	11 (84.6)[b]	8 (88.9)[b]
↑ Nevus melanocytic HPL	14 (66.7)	8 (66.7)[b]	6 (66.7)[b]
↑ Adjacent melanocytic HPL	17 (81)	9 (75)[b]	8 (88.9)[b]
↑ Suprabasal melanocytes	11 (52.4)	4 (36.4)	7 (63.6)[b]
↑ Melanocytic dendrites	11 (52.4)	4 (36.4)	7 (63.7)[b]
↑ Inflammatory infiltrates	11 (52.4)	6 (50)[b]	5 (55.6)[b]
Immunostaining			
↑ HMB-45+ nevus cells	11 (52.4)	9 (75)[b]	2 (22.2)
↑ HMB-45+ adjacent cells	16 (76.2)	8 (66.7)[b]	8 (88.9)[b]
↑ Melan-A+ nevus cells	13 (61.9)	9 (75)[b]	4 (44.4)
↑ Melan-A+ adjacent cells	19 (90.5)	10 (82.3)[b]	9 (100)[b]

Abbreviations: HMB-45, human melanoma black-45 antigen; HPL, hyperplasia; ↑, increase in; +, positive.

[a]Features identified in unprotected halves were assumed to be those avoided by protection, since they were considered differences between irradiated with or without protection, although it was not possible to rule out the histologic asymmetry characteristic in dysplastic nevi. All differences observed between both halves were significant ($P < .001$) by χ^2 test and Fisher exact correction.

[b]Significant differences between protected and unprotected halves within each subgroup (ie, feature avoided by that specific protection). Despite an increase in activation of immunostaining, there were no significant differences in the sunscreen group, that is, protected halves showed the same activation as unprotected halves.

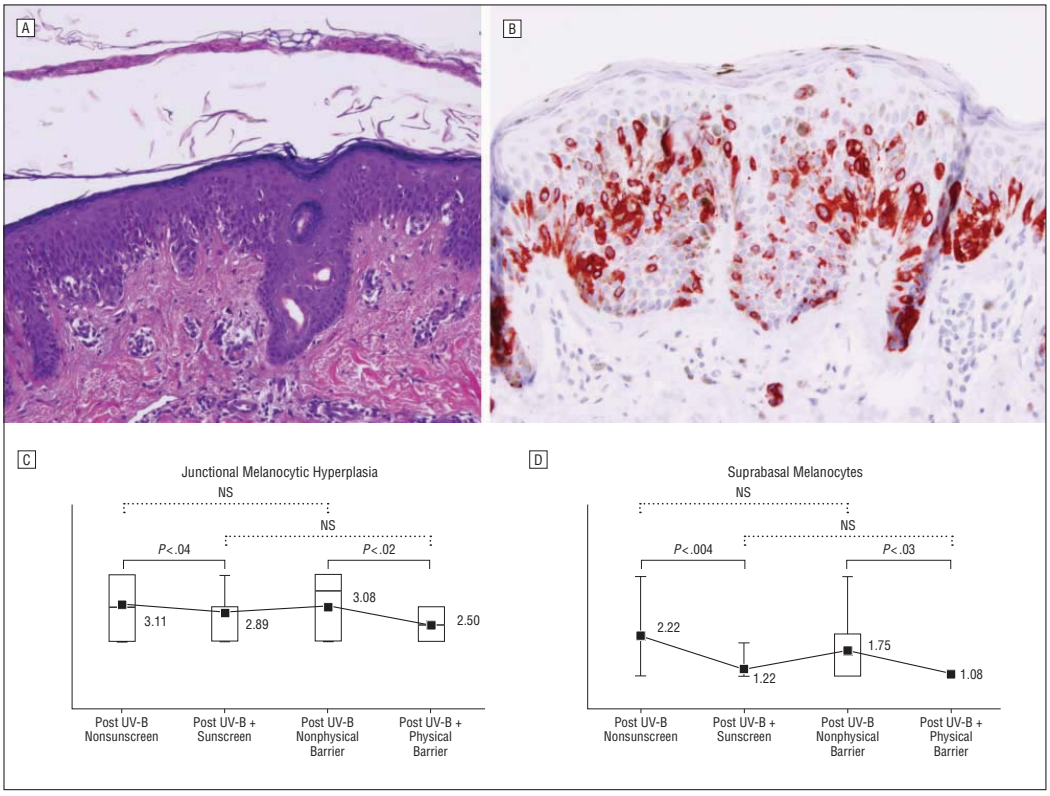

Figure 6. Immunohistopathologic analysis. Image of a hematoxylin-eosin–stained histologic specimen (original magnification ×100) (A) showing parakeratotic scale and junctural melanocytic hyperplasia in unprotected areas. Melan-A immunostaining (B) (original magnification ×100) of the unprotected half enhanced the high index of activated suprabasal melanocytes. Significant differences between protected and unprotected histopathologic features were analyzed (C and D). No significant difference (NS) was detected between the types of protection. See the legend to Figure 1 for an explanation of the graphic elements (C and D).

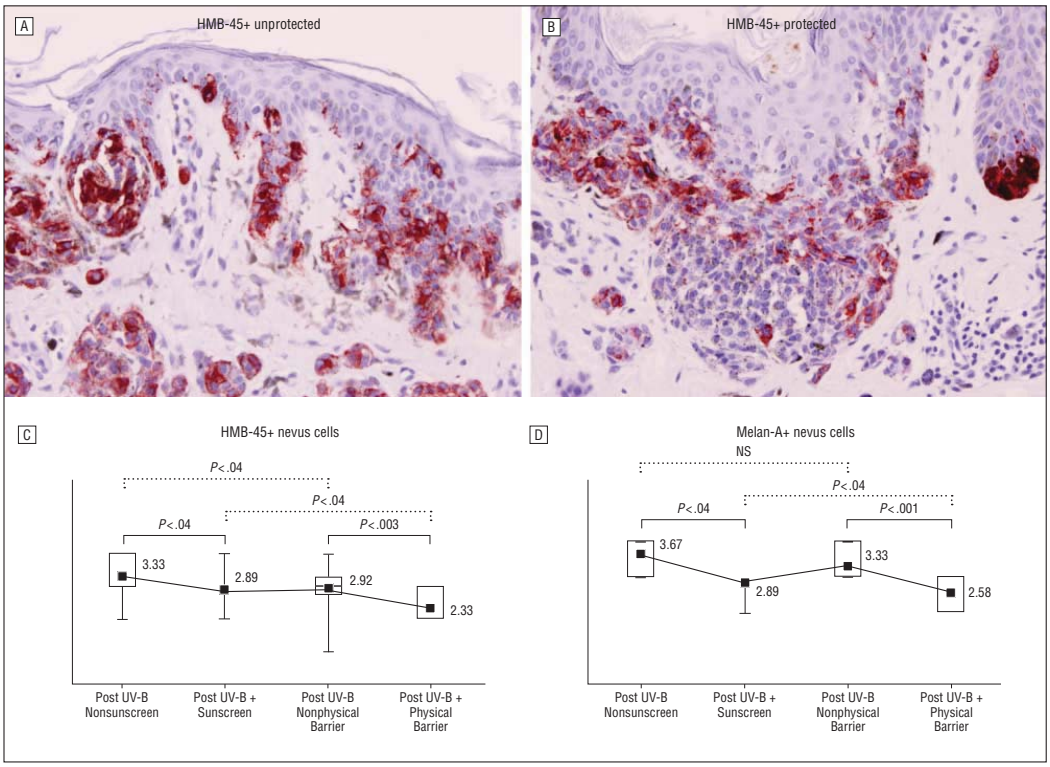

Figure 7. Human melanoma black-45 antigen (HMB-45)–positive nevus cells (original magnification ×100) in unprotected (A) and in sunscreen-protected (B) halves. More intense staining and marked activation of dendritic larger melanocytes were noted in unprotected areas. Significant differences were noted between protected and unprotected halves for intensity of immunohistochemical staining (C and D). Unexpectedly, the intensity of the immunostain in sunscreen-protected areas were higher than physically protected areas ($P < .04$) both for HMB-45 and Melan-A. NS indicates nonsignificant. See the legend to Figure 1 for an explanation of the graphic elements (C and D).

DISCUSSION

The results of the present study demonstrate that both physical barriers and sunscreens are able to decrease or even prevent most of the UV-B–induced biological changes in nevi and surrounding skin. However, some UV-B–induced changes appeared in protected areas, such as regression structures and vessels (erythema and dotted vessels) (Figures 4 and 5). Interestingly, regression structures appeared in 75% of nevi in the sunscreen group but in less than 30% of the lesions in the physical barrier group. Thus, neither sunscreen nor physical barrier prevented inflammation in the protected halves. At the histopathologic level, the main difference between the protected and unprotected halves was the presence of activated melanocytes in the unprotected halves and the adjacent peripheral skin, which was more intensely stained with both antibodies in unprotected areas. Sunscreen seems to be less effective than a physical barrier against melanocyte activation, as evidenced by the intensity and percentage of Melan-A–positive staining (Figure 7). Despite HMB-45 staining being more activated in the sunscreen group, it was slightly higher in both the protected and unprotected halves, so it cannot be used as a measure for comparing the effectiveness of protection type.

Another interesting and previously undescribed fact is the discordance between the different presentations of UVR effects. More than 30% of the nevi did not show any change on clinical examination, and only 18% exhibited changes on dermoscopic examination. However, in an unexpected finding, all cases, even those without in vivo changes, showed some difference between the protected and unprotected halves at the histopathologic level. That is, we observed cases with no visible clinical changes despite the presence of dermoscopic changes (Figure 4), as well some cases (8%) with neither clinical nor dermoscopic changes but with immunopathologic findings related to UVR, such as parakeratotic scale, inflammatory infiltrates, and melanocytic activation. Therefore, the importance of this finding should be emphasized, as it indicates the existence of subclinical biological effects beyond clinical erythema or pigmentation. In our model, with correlation between clinical, dermoscopic, and histopathologic changes, we demonstrated that it is possible, even in the absence of any clinical UV-B effect (erythema or pigmentation changes), that melanocytic nevi could develop some kind of damage.

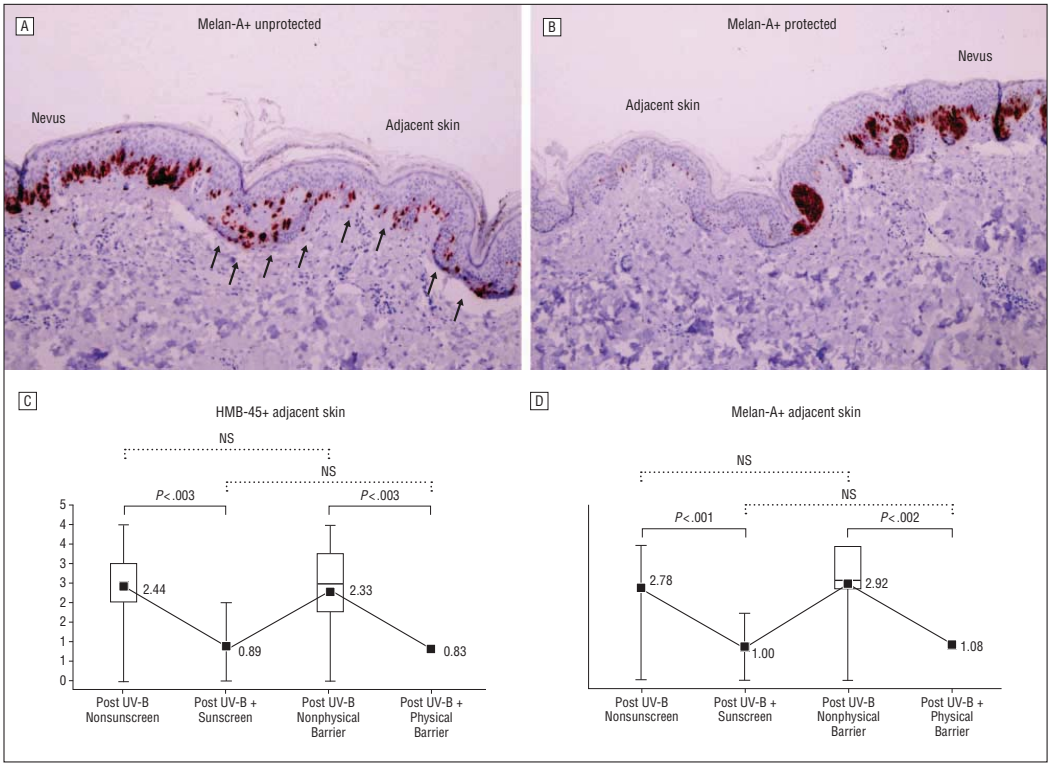

Figure 8. Images of immunohistochemical staining with Melan-A (original magnification ×40). Comparison of unprotected (A) and protected by physical barrier (B) halves of the lesion; note the marked melanocytic activation and hyperplasia in surrounding skin (arrows). Significant differences were noted (C and D) between protected and unprotected positive cells on surrounding adjacent skin with both immunohistochemical staining HMB-45 and Melan-A. No significant differences (NS) were noted between types of protection. HMB-45 indicates human melanoma black-45 antigen. See the legend to Figure 1 for an explanation of the graphic elements (C and D).

Topical sunscreens are supposed to be a suitable tool for preventing UVR effects and are probably the best-accepted method of photoprotection by the general, especially the younger, population.[11] For the first time in dermatology, a prospective study in Australia has demonstrated that proper sunscreen use can prevent MM,[13] but the biological effect of sunscreens in vivo on nevi remains poorly understood. In 1989, Stierner et al[24] demonstrated that UV-B could promote melanocytic activation both in irradiated and in protected skin, and the possible role of keratinocyte interaction seems to play a crucial role. Recent studies[25,26] have shown that both opaque tape and commercial sunscreen can prevent clinical and dermoscopic changes in acquired nevi exposed to repeated equally suberythemogenic UV-B-NB and UV-A-1 radiation. However, since the authors did not find significant differences at the histopathologic level between nevi covered by an opaque barrier or a commercial sunscreen or left unprotected, they proposed that repeated suberythematogenic UVR doses are not a risk factor for the malignant transformation of nevi, since this irradiation does not induce changes at the cellular level. In our model, we tested the more frequent sunbathing dose in summer exposure (twice the erythemogenic dose), and our nevi could be directly compared against themselves (the previous investigators studied different nevi—ones completely covered by a band and others by sunscreen). In contrast to those results, histopathologic differences between both halves were demonstrated in all cases in our study. As previously described,[27,28] chronic sun exposure can promote an increase in melanocyte density and activation in normal skin that can be misdiagnosed as melanocytic proliferations. In view of our findings, it should be taken into account that acute UVR also could interfere with the appropriate surgical margin assessment for melanocytic tumors.

Recently, it has been reported[29] that UV-A response in terms of genotoxicity and cyclobutane pyrimidine dimers induction could be well correlated with phototype and UV-B MED. However, on the basis of the present study, we highlight the importance of further research, taking into account factors in addition to clinical erythema and pigmentation, since inflammation and regression are not well established and could develop even in darker skin phenotypes.

In our experience, no patient phenotype or phototype could be associated with a predictable or specific UVR response. This is a possible bias, since the 20 patients stud-

ied herein are representative of our clinically atypical nevi syndrome and high-risk MM population. Most of them have dark-colored eyes and hair, with phototype II or III but with a very low UV-B MED. Compared with other photosensitive populations, such as those in Northern countries or patients with photodermatosis, our sample presented a very high UVR sensitivity. Up to 60% of patients presented a very low MED ($\leq$50 mJ/cm^2), similar to those reported in Northern European populations or patients with photodermatoses (unpublished data from 65 consecutive photosensitive patients studied in our Photobiology Unit, January 2005 through January 2007). In addition, the MED was significantly lower ($P < .03$, χ^2 test) compared with other control groups studied, such as patients affected by familial multiple MM (mean [SD], 122 [29] mJ/cm^2).[30] Compared with a previous series on irradiated nevi (summarized by Carrera et al[19]), we have observed more intense inflammatory changes other than darkening or other than an increase in the number and size of globules and the network. We cannot find a relationship between which lesions or patients presented a specific change. Another limitation in our interpretation of the results is that it is not clear why the sunscreen-protected nevi showed a higher degree of regression features. Even though this limitation could be a consequence of the small size of the sample, some other explanation may be hypothesized. It could be suggested that physical barriers prevent UV damage and that immunosuppression and sunscreens also may protect against immunosuppression but not against all UV damage inducing inflammation and the presence of regression structures.

In conclusion, we have demonstrated for the first time that the protective role of sunscreen in avoiding UVR-induced effects on nevi is at least similar to a physical barrier under Mediterranean summer weather conditions.

Two findings of the study were the most remarkable. First, neither all patients nor all nevi had the same UVR response after 2 MED UV-B irradiation. Actually, we were not able to distinguish which patients or lesions would be more affected by UVR. Even with no visible changes, histopathologic examination showed some UVR-related effects in all lesions. Thus, UVR provokes effects other than pigmentation and erythema, sometimes not visible in vivo. Second, not all changes after UVR were confined to unprotected areas. Therefore, neither physical nor sunscreen protection could completely prevent the UVR effects. Some local inflammatory effects in addition to erythema and pigmentation changes probably could affect protected areas. There were very weak differences between a physical barrier and sunscreen, not enough to conclude that sunscreen creams are not effective but enough to suggest that sunscreens do not provide the same effect as a physical barrier to prevent 2 MED UV-B irradiation in nevi.

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I. MELANOMA FOTOINDUCIDO. ESTRATEGIAS DE PREVENCIÓN

1. IMPACTO DE LA RUV EN RIESGO GENÉTICO A MELANOMA

El melanoma (MM) de extremidades en poblaciones caucásicas se ha relacionado con una marcada influencia ambiental a través de los hábitos de exposición solar intermitente, confirmándose su asociación a presentarse en mujeres, principalmente en las piernas.

En nuestro medio, según el estudio de la Xarxa de Melanoma Catalano-Balear (pendiente de publicación), 1 de cada 3 melanomas se localizan en extremidades, siendo llamativo que en mujeres, los localizados en extremidades inferiores (28%) prácticamente igualan a los de tronco (29%). Por el contrario, en los hombres, la mitad de casos son en tronco, y la segunda localización sería cabeza y cuello (<12%). Cada vez existen más estudios que implican la RUV artificial en el MM de extremidades, y en especial, el MM de mujeres jóvenes, menores de 40 años[130,131].

Existen dos grandes grupos de genes implicados en la susceptibilidad a MM y ambos estarían modulados o influenciados por la RUV recidiva. Se distinguirían dos "endofenotipos"; los genes relacionados con las vías de pigmentación y daño solar, y por otro los genes implicados en nevogenicidad que predispondrían al desarrollo de lesiones melanocíticas benignas y también a MM[132].

En nuestra serie de 35 pacientes del trabajo I[133] se demuestra esta asociación epidemiológica, con más del 85% mujeres y 90% en extremidades inferiores, y edad media menor de la habitual (media de 46 años vs 52.6 de los datos de la XMCB; 52.6). Esta asociación, justificada por los hábitos de exposición solar, no se pierde a pesar de tratarse de una Unidad de Melanoma de referencia, y por tanto, la población atendida presenta un sesgo inherente al ser de alto riesgo. De hecho, destaca que de los 35 pacientes; la mitad presentaban historia personal de MM, el 40% historia de MM familiar, e incluso un 17% ya habían presentado más de un MM previo al diagnóstico del actual. Ello explica que de los 21 casos estudiados genéticamente, el 38% eran portadores de mutación de alto riesgo en *CDKN2A* (Tabla 2).

Tabla 2: CARACTERIZACIÓN DE PACIENTES DE LA SERIE DE MELANOMAS FOTOINDUCIDOS EN EXTREMIDADES

PACIENTES N(%)		GRUPO1	GRUPO2	GRUPO3	GRUPO4	Total	
		16(46)	4(11)	10(29)	5(14)	35(100)	
CARACTERÍSTICAS POBLACIONALES		16(100)	4(100)	10(100)	5(100)	35(100)	Significancia
Sexo	Mujeres	15(94)	4(100)	**6*(60)**	4(80)	**29(83)**	*p<.04 X 5,1
	Hombres	1(6)	0(0)	**4*(40)**	1(20)	**6(17)**	
Edad media (años)		44.7±14.0	40.4±14.7	49±19.3	50±8.3	**46±15.4**	**Ns
Síndrome de nevus con atipia		12(75)	4(100)	8(80)	2(40)	**26(75)**	Ns
Historia de MM previo		7(44)	3(75)	6(60)	1(25)	**17(49)**	Ns
Seguimiento digital		8(50)	4(100)	4(40)	2(40)	**18(51)**	Ns
MM múltiple previo		2(12)	**3(75)***	1(10)	0(0)	**6(17)**	*p<.01 X10.6
MM Familiar		6(37)	**4(100)***	2(20)	2(40)	**14(40)**	*p<.03 X6.7
Estudio genético		8(50)	4(100)	8(80)	2(50)	**21(38)**	Sifgnificancia
CDKN2A mutado/estudiado		4/8(50)	3/4(75)	1/7(14)	0/2(0)	**8/21(38)**	Ns
MC1R	Alguna variante	6/8(75)	3/3(100)	8/8(100)	1/1(100)	**18/20(90)**	Ns
	Variante pelo-rojo	4/8(50)	3/3(100)	5/8(62)	1/1(100)	**10/20(50)**	Ns
	Múltiples variantes	0/8(0)	2/3(66)	**5/8(62)***	0/1(0)	**7/20(35)**	*p .05 X4.4

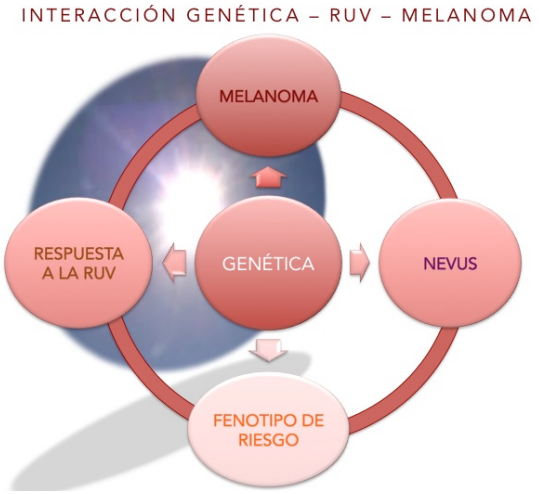
Leyenda: *MC1R*: gen del receptor de melanocortina. *X^2 con corrección de Fisher. **T-Student para medias independientes.

Destaca que el 90% de los pacientes estudiados, presentaba polimorfismos del *MC1R*; siendo en el 50% variantes de pelo rojo, y en el 33% múltiples variantes.

Como ya se había publicado previamente y cuantificado en el meta-análisis del 2010[134], los polimorfismos de *MC1R* incrementan más aún el riesgo de los pacientes ya portadores de mutaciones en *CDKN2A*, es decir la susceptibilidad genética se puede potenciar por vías diferentes. Nuestra serie de MM de extremidades apoyaría la teoría etiopatogénica de que

los factores genéticos y ambientales para el desarrollo de melanoma interactúan y se potencian entre ellos[22].

La utilidad de las medidas de prevención primaria se basa en las evidencias de que la interacción de la RUV y los factores genéticos de cada individuo, no sólo influye en riesgo directo a melanoma fotoinducido, sino que de forma indirecta, modula el fenotipo, el desarrollo de nevus melanocíticos, la presencia de daño actínico acumulado, y estas interacciones pueden potenciarse entre ellas.



Tal y como se hipotetiza en el trabajo reciente del grupo de Nagore y col[135] entre los melanomas fotoinducidos (ni acrales ni mucosas); probablemente existan aparte de las vías de melanomagénesis ya discutidas, de endofenotipos de nevogenicidad y de daño actínico, una tercera vía independiente a la nevogenicidad y al fotodaño, que explicaría el resto de MM fotoinducibles, y que en su serie alcanzaría un 5.5% de casos, se asociaría más frecuentemente a mujeres jóvenes, a melanomas en tronco y extremidades y a aparecer sobre nevus preexistentes.

2. NECESIDAD DE ESTRATEGIAS ESPECÍFICAS EN PREVENCIÓN PRIMARIA

Según la *American Cancer Society* de EEUU se estima que 1 de cada 55 mujeres y 1 de cada 36 hombres serán diagnosticados de MM invasivo a lo largo de su vida[85,136]. De forma muy significativa sigue incrementándose entre mujeres jóvenes, siendo la causa más frecuente de cáncer en mujeres entre 20 y 29 años, y la segunda en población menor de 40 años de edad.

Los recientes datos publicados por de Vries[137] apoyan el hecho de que una correcta fotoprotección laboral y vacacional en Europa reduciría el cáncer cutáneo en un 45% en el 2050. Sin embargo, más del 70% de adultos jóvenes reconocen realizar exposición solar intencionada, sobre todo mujeres jóvenes, siendo cada vez más preocupante la cifra de adolescentes que utilizan dispositivos de bronceado artificial, en países como Australia, Francia, Alemania o países nórdicos se estima que entre 10-35% de la población los utiliza. Más del 85% de todos ellos utilizan fotoprotectores tópicos de forma habitual y refieren conocer las medidas adecuadas de fotoprotección[138].

En población general, se sabe que sigue existiendo una falta de conciencia sobre las dosis que se reciben de RUV, el daño solar y riesgo de cáncer cutáneo que conlleva. La información y educación de hábitos saludables probablemente será por tanto la primera estrategia a revisar. En un estudio reciente a nivel europeo, se detectó que un 5% de la población utiliza fotoprotector sobre sus nevus para evitar el cáncer cutáneo, de ellos más de la mitad referían haber recibido información médica al respecto[138]. Asimismo, se ha calculado que un ciudadano de fototipo claro (estudio realizado en daneses), que pasa 6 días de vacaciones en las Islas Canarias recibe 57 veces su dosis mínima de eritema (MED), lo que llegaría a suponer un 43% de la RUV anual acumulada en tan solo 6 días[139].

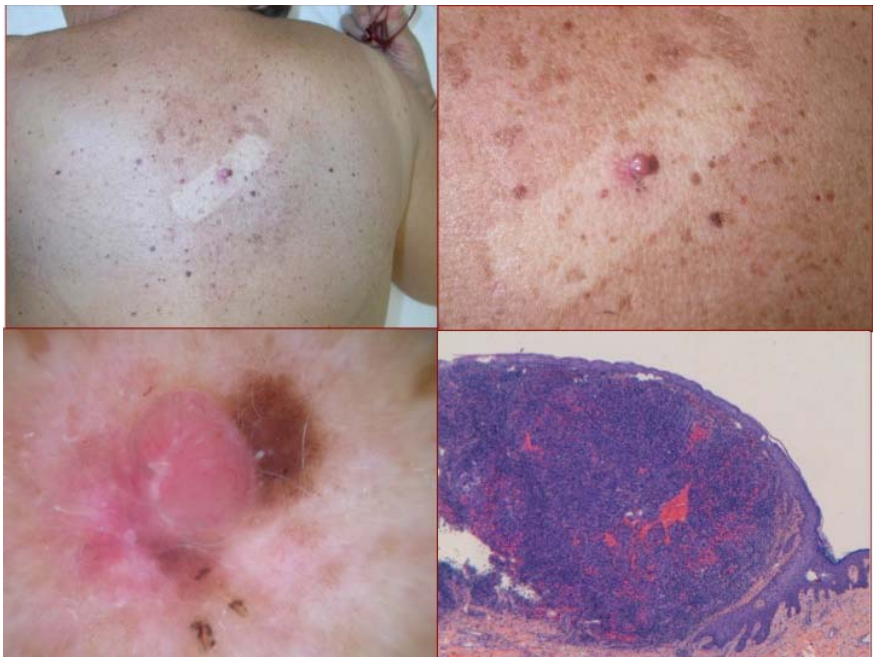


Figura 10. Melanoma de extensión superficial, con nódulo de crecimiento vertical invasivo, desarrollado sobre área de marcado daño actínico. Paciente de 49 años con reciente fotoexposición que refería "proteger del sol el tumor mediante apósito para evitar mayor crecimiento".

En nuestra experiencia, y basándonos en los hábitos y fenotipos recogidos de los pacientes incluidos en el conjunto de nuestros trabajos I y III (melanomas de extremidades de alto riesgo y síndrome de nevus displásico de alto riesgo, respectivamente), demostramos que es muy necesario establecer estrategias específicas de prevención primaria. Siendo todos ellos pacientes de alto riesgo, en su mayoría en seguimiento digital, muchos con historia previa de MM personal y/o familiar, la mayoría reconocían no cumplir las medidas estrictas de fotoprotección, haber utilizado dispositivos de bronceado UVA y haberse quemado en la edad adulta. Incluso, la muestra de población del trabajo III, de edad media joven (36.5 años), el 75% ya presentaban algún signo de daño actínico, y se demostró estadísticamente significativa la asociación entre una intensa exposición solar y mayor número de nevus (p 0.02 t-Student 2.18 IC95 2.36;.18) (Tablas 3-5 en anexo).

Diversos grupos alertan de la importancia de informar, educar y recordar las medidas de prevención en cáncer cutáneo, especialmente en grupos de alto riesgo, y más aún cuanto más tiempo transcurra desde el diagnóstico[140–142]. Se sabe que hoy en día la medida mejor aceptada y utilizada sigue siendo el uso de fotoprotectores tópicos, sin embargo se fracasa en la disminución de horas de fotoexposición.

Un reciente estudio prospectivo en población pediátrica australiana ha demostrado que la implementación de prendas con protección solar y cremas coincide con la disminución significativa del número de nevus en estos niños[143].

3. PREVENCIÓN SECUNDARIA: DETECCIÓN PRECOZ DE MM EN EXTREMIDADES

Tal y como se pretendía en uno de los sub-objetivos de esta tesis, hemos definido ciertas estrategias para un mejor reconocimiento de fases incipientes de melanomas en extremidades.

En base a nuestra experiencia, las técnicas de autoexploración o de mapas corporales para autoexploración en su domicilio no serían útiles en este grupo de pacientes de alto riesgo para detectar lesiones incipientes como preconizan otros autores[144]. Ninguna de los melanomas de nuestra serie en extremidades fue alertado por los pacientes, y solo 2 de los 36 MM cumplían el signo del "patito feo", definido como aquella lesión que clínica o dermatoscópicamente pueda tener un aspecto distintivo respecto al resto de lesiones de un paciente[145,146]. Como ya se ha descrito en trabajos previos, existen MM que no cumplen el ABCD-E clínico clásico, y especialmente de tan pequeño diámetro que pasan desapercibidos, como en nuestra serie de 36 tumores, ya que éste fue el criterio de inclusión requerido.

Seidenari y col[147] demuestran la importancia de la exploración dermatoscópica corporal completa. El despistaje clínico previo puede evitar la identificación de melanomas que ni el paciente ni clínicamente el médico reconocen a simple vista.

La **dermatoscopia** a lo largo de los últimos años ha demostrado su utilidad en la detección de tumores muy incipientes sin apenas semiología típica de malignidad. Diversos trabajos resaltan la ayuda que aporta la dermatoscopia en los llamados "MM incógnito", por simular otras lesiones[148–150].

En base a nuestra observación y detección de lesiones incipientes, se ha podido establecer una clasificación dermatoscópica que ayuda a la detección precoz (tabla 7). Destacan como signos guía:

- RETÍCULO PROMINENTE; como signo único de alerta en la mayoría de casos (grupo 1), constituye un tipo de retículo de líneas gruesas, variante de retículo atípico, y nuestra serie lo describe por primera vez como característico de MM incipientes en extremidades.
- PATRÓN VASCULAR ATÍPICO; a menudo también como signo único de sospecha, importante puesto que las lesiones hipopigmentadas o acrómicas (grupo 3), son las de mayor dificultad y retraso en su diagnóstico. Estas lesiones presentaron una mayor

tendencia a ser melanomas invasores (50%), con Breslow medio superior al resto de melanomas de la serie. El patrón vascular con eritema y/o vasos puntiformes, y la presencia en algunos casos de líneas blancas brillantes cortas ("crisálidas"), fueron los únicos criterios de sospecha.

- PATRÓN LENTIGO MALIGNO-LIKE (grupo 4). Se describe por primera vez otro subtipo de MM en extremidades, no necesariamente asociado a marcado daño actínico crónico. De forma similar al lentigo maligno fotoinducido de áreas faciales, la pigmentación perifolicular dermatoscópica fue la clave diagnóstica. Histopatológicamente se demostraron células atípicas con tendencia a la invasión del embudo folicular, todo ello sugiere un posible diferente sustrato biológico.

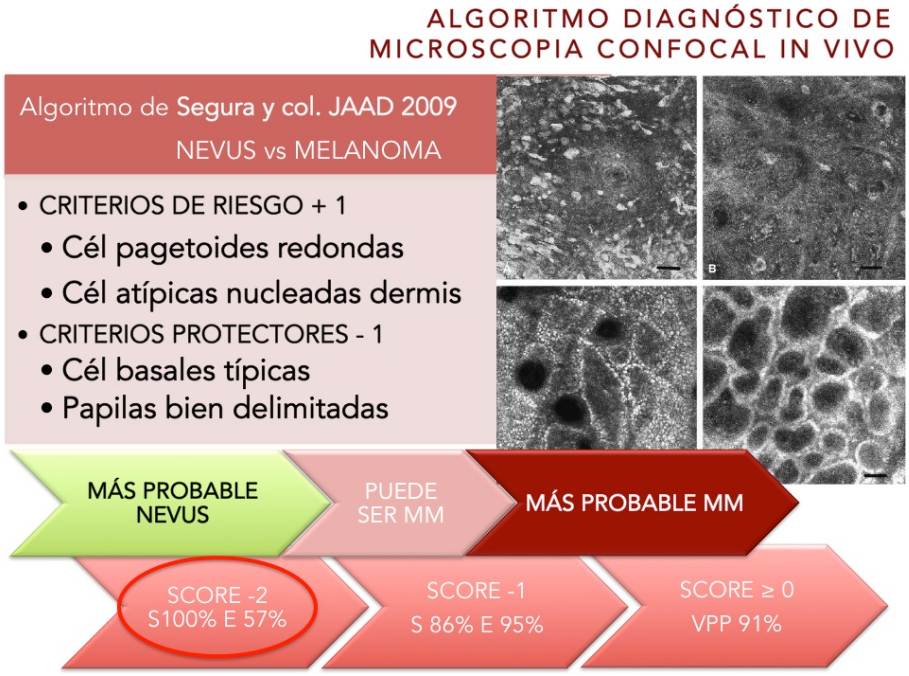


Tabla 6 : CARACTERIZACIÓN CLÍNICA-DERMATOSCÓPICA DE MELANOMAS INCIPIENTES DE EXTREMIDADES

TUMORES N(%)		Grupo1	Grupo2	Grupo3	Grupo4	Total	
		16(44)	5(14)	10(28)	5(14)	36(100)	
CARACTERÍSTICAS CLÍNICAS		16(100)	5(100)	10(100)	5(100)	36(100)	Signific.
Localización	EEII	16(100)	5(100)	**7(70)**	5(100)	**33(92)**	p<.02 X8.5
	EESS	0(0)	0(0)	**3(30)***	0(0)	**3(7)**	
MM in situ		13(72)	5(100)	**5(50)***	5(100)	**28(78)**	p<.05 X8
Signo “patito feo”		1(6)	0(0)	1(10)	0(0)	**2(5)**	Ns
Diámetro medio (mm)		4.12±0.9	3.6±0.9	5±1.4	4.4±0.9	**4.3±1.12**	Ns
Monocromía		4(25)	2(40)	**7(70)***	3(60)	**16(45)**	p<.05 X3.6
2 colores		**12(75)***	3(60)	3(30)	2(40)	**20(56)**	p<.04 X4
CARACTERÍSTICAS DERMATOSCÓPICAS		16(100)	5(100)	10(100)	5(100)	36(100)	Signific.
Monocromía		**0(0)***	2(40)	**4(40)***	1(20)	**7(20)**	p<.05 X8
Dos colores		13(72)	3(60)	5(50)	4(80)	**25(69)**	Ns
Más de 2 colores		3(18)	0(0)	1(10)	0(0)	**4(11)**	Ns
Patrón reticulado		**14(88)***	**5(100)***	0(0)	2(40)	**21(58)**	p<.00 X24
Patrón lobular		1(6)	0(0)	1(10)	0(0)	**2(5)**	Ns
Patrón inespecífico		1(6)	0(0)	**9(90)***	3(60)	**13(35)**	p<.00 X23
Retículo atípico		**14(88)***	0(0)	1(10)	0(0)	**15(42)**	p<.00 X25
Glóbulos irregulares		5(31)	0(0)	3(30)	0(0)	**8(22)**	Ns
Proyecciones periferia		4(25)	0(0)	1(10)	0(0)	**5(16)**	Ns
Pigmento irregular		3(18)	0(0)	0(0)	**4(80)***	**7(20)**	p<.01 X15
Vasos puntiformes		1(6)	0(0)	**9(90)***	0(0)	**10(29)**	p<.00 X27
Signos de regresión		3(18)	1(20)	1(10)	1(20)	**6(17)**	Ns
Pigmento perifolicular		1(6)	0(0)	0(0)	**5(100)***	**5(16)**	p<.001 X29
Retículo invertido		0(0)	0(0)	**3(30)***	0(0)	**3(8)**	p<.05 X8

En esta serie, más de la mitad de los pacientes estaban incluidos en nuestro programa de **seguimiento digital,** método en dos etapas con mapas corporales totales y dermatoscopia digital[121]. En todos ellos, los cambios digitales incrementaron el criterio de sospecha de estas lesiones tan incipientes.

De igual manera que en los estudios prospectivos de la Unidad de Melanoma del Hospital Clínic[123,151,152], (trabajos anexos I y II), así como de grupos europeos, nuestra serie de melanomas incipientes de extremidades muestra que los melanomas detectados mediante dermatoscopia apoyada por programas de seguimiento digital, son indistinguibles de otros nevus, sin criterios específicos de malignidad, y de buen pronóstico, sólo 8 de los 36 casos fueron microinvasivos, con Breslow medio de 0.5mm ±0.1.

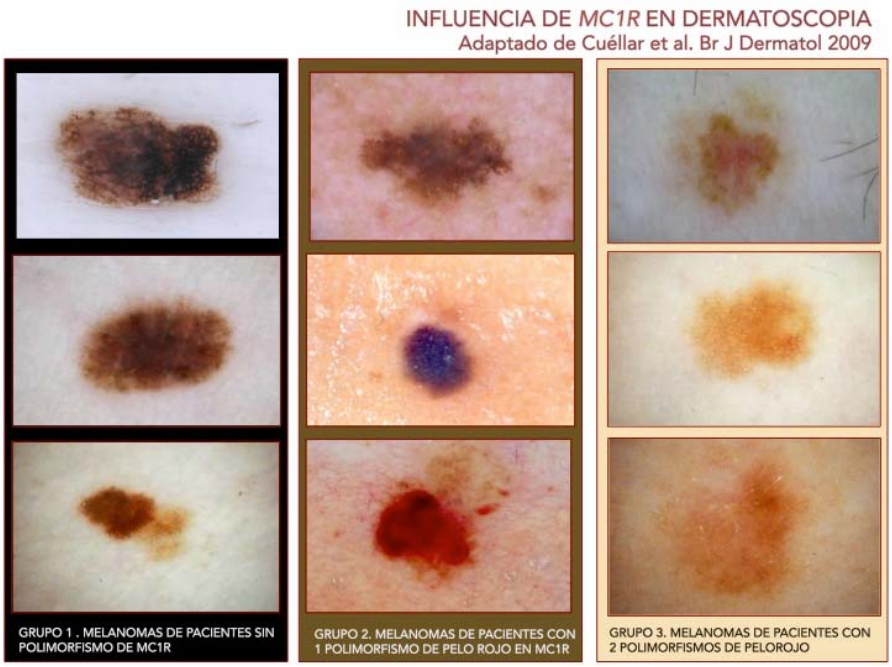
En los cinco casos del grupo número 2, a pesar de no presentar ningún criterio de sospecha dermatoscópica, los cambios observados en seguimiento digital nos incitaron a la exploración mediante microscopía confocal de reflectancia *in vivo*, y su posterior extirpación.

Tanto estos 5 casos planteados en el grupo número 2 de la serie, como en otros 7 sin criterios concluyentes de sospecha dermatoscópica, la **microscopía confocal de reflectancia in vivo** (MRC) nos permitió el diagnóstico de presunción de MM, y por tanto la extirpación de estos tumores incipientes. Gracias a la aplicación del algoritmo diagnóstico desarrollado en la Unidad de Melanoma del Hospital Clínic por Segura y col[116] tal y como se ha demostrado en la publicación original, en lesiones previamente preseleccionadas por sospecha dermatoscópica, permite ahorrar casi la mitad de las biopsias cuando el score final es -2 descartando melanoma con un valor predictivo negativo (VPN) del 100%. En cambio, la observación de criterios ya demostrados y conocidos en MM incipientes, como la presencia de células atípicas redondas intrapidérmicas, la observación de papilas dérmicas mal delimitadas e irregulares, así como la presencia de células atípicas nucleadas basales, permitieron el diagnóstico correcto de estas 12 lesiones exploradas mediante MRC.

4. INFLUENCIA DE GENOTIPO Y RUV EN LA DETECCIÓN PRECOZ

Teniendo en cuenta la complejidad y variabilidad de las vías de pigmentación en el hombre, no es de extrañar que la semiología de los tumores melanocíticos y por tanto su posible detección pueda verse influida por la interacción de la RUV y la genética.

Cuéllar y col[153], ya describieron que los tumores en pacientes portadores de mutación en *CDKN2A* y variantes de pelo-rojo en *MC1R*, presentaban MM hipopigmentados o acrómicos, a menudo sin criterios dermatoscópicos de malignidad, incluso sumando un TDS ("total dermoscopy score" del ABCD de Stolz[154]) dentro del rango de benignidad. Nuestro estudio de MM de extremidades refleja también esta dificultad diagnóstica, independientemente del estatus mutacional de *CDKN2A*, como los tumores de los grupos 2 y 3 (hipopigmentados/acrómicos). Todos los pacientes estudiados tenían algún polimormismo en *MC1R*, y más de dos tercios variantes de pelo-rojo.



Por tanto, se ha de insistir en un estricto seguimiento de portadores de polimorfismos de pelo-rojo en *MC1R,* puesto que además de mayor riesgo inherente a melanoma, también implica mayor dificultad por menor semiología clínica y dermatoscópica[149,155,156].

La microscopía confocal aporta una enorme ventaja pues a pesar de la ausencia de pigmento melánico, los tumores melanocíticos presentan igual semiología gracias a la reflectancia de los melanosomas y células de origen melanocitario.

5. INFLUENCIA DE GENOTIPO Y RUV EN LA CARACTERIZACIÓN DE DISTINTOS SUBTIPOS DE MM

Se han podido establecer unos patrones de concordancia, entre los subgrupos dermatoscópicos propuestos y los patrones histopatológicos de MM incipientes observados habitualmente (clasificación detallada en la metodología del trabajo I de esta tesis) (Tabla 7).

Los patrones histológicos nevoides, con tendencia a la confluencia en nidos se asociaron más al grupo 3 de lesiones hipopigmentadas con patrones vasculares atípicos, mientras que los patrones más pageotides, con abundante invasión epidérmica de células sueltas, o formando hiperplasia lentiginosa en la unión dermoepidérmica, se relacionaba más con los grupos 1 y 2, que mostraban patrones reticulados. El grupo 4, se correlacionaba bien con patrones tipo lentigo maligno o melanosis de Dubreuilh con foliculotropismo, incluso en ausencia de elastosis marcada, lo que podría indicar fases muy incipientes de diferentes tipos de melanomagénesis.

En este sentido, se están dedicando grandes esfuerzos a la posibilidad de inferir de las características fenotípicas (de un paciente o de su tumor), características genéticas moleculares (germinales o somáticas). Sería un gran avance si se llegase a demostrar nuestra hipótesis (meramente basada en nuestra experiencia) de que estos MM de las piernas con grandes células claras pagetoides suelen asociarse a un contexto de MM múltiple o familiar. Recientemente las mutaciones germinales de *BAP1* en MM múltiple y familiar se han relacionado con ciertos tipos de tumores spitzoides[129] por lo que no sería de extrañar que otros cambios germinales se asociaran a una tipología específica de melanoma no demostrada hasta el momento.

Tabla 7. CARACTERIZACIÓN HISTOPATOLÓGICA DE MELANOMAS INICPIENTES EN EXTREMIDADES

CARACTERÍSTICAS HISTOPATOLÓGICAS		Grupo 16(100)	Grupo2 5(100)	Grupo3 10(100)	Grupo4 5(100)	Total (n 34)	Signific.
Clasificación histológica MELANOMA:	Nevoide	5	0	**6***	0	**11**	p<.002 X 26.76
	Pagetoide	**6***	4	1	1	**12**	
	Lentiginoso	2	1	3	0	**6**	
	LMM-like	0	0	0	3	**3**	
Nevus subyacente		2	1	2	0	**5**	Ns
Asimetría arquitectural		12	3	9	4	**28**	Ns
Prolif. en nidos +++		11	5	10	1	**27**	**P<.01 X12**
HM lentiginosa +++		10	0	3	2	**15**	**P<.03 X9**
Prolif. células sueltas +++		14	4	7	3	**28**	Ns
Signos de regresión		1	1	2	0	**4**	Ns
Invasión pagetoide +++		14	4	9	3	**30**	Ns
Cel. grandes atípicas +++		13	5	10	3	**31**	Ns
Cel. epitelioides +++		10	5	8	2	**25**	Ns
Prolif. vascular +++		2	1	**9***	1	**13**	**p<.00 X15.5**
Infiltrado inflamatorio +++		2	2	**8***	0	**12**	p<0.1 X13.5
Elastosis		0	0	1	**3***	**4**	p<.01 X17
Clark I		13	5	**5***	5	**28**	**p .02 X6.1**
Clark II		2	0	3	0	**5**	Nd
Clark III		1	0	2	0	**2**	Nd
Breslow medio (8 casos) mm		(3) 0.41mm	-	**(5) 0.56mm***	-	**(8) 0.50mm**	**p<0.03 T2.0 (-0.02-0.31)**

6. DIFICULTADES HISTOPATOLÓGICAS EN MM INCIPIENTES

Como puede ocurrir en otros casos de melanomas muy incipientes, existe el debate de si estas lesiones deben ser consideradas MM, o nevus con atipia marcada. La discusión sobre si este tipo de tumor adquiriría o no capacidad de metastatizar siempre se puede plantear ante lesiones clínica y dermatoscópicamente incipientes, puesto que histopatológicamente no siempre muestran criterios concluyentes de MM. En 2007 Kalifeh y col. publican una serie de 11 casos en piernas y tobillos, en su mayoría mujeres, (73%), que mostraban atipia arquitectural marcada, pero atipia citológica leve o moderada en el 80% de ellas. Los autores defendían la existencia de estas "lesiones melanocíticas atípicas de tobillo" como lesiones de comportamiento benigno, que no deben clasificarse como melanomas, pero únicamente en base al seguimiento de estos 11 casos sin evidencia de recidiva (4 meses – 13 años)[157]. En nuestra opinión, si bien podría efectivamente existir una apariencia atípica en los nevus de tobillo, como puede ocurrir en otras localizaciones especiales, la evolución de tan solo 11 casos con un seguimiento tan variable no es suficiente tampoco para defender que sean nevus. Nuestros 35 casos mostraban la suficiente atipia arquitectural y citológica con invasión intraepidérmica para haberse considerado MM todos ellos. Partiendo de que la única demostración del potencial maligno de una lesión melanocítica sería la aparición de metástasis, si somos eficientes en la detección precoz de MM, asumiremos que de las lesiones detectadas y extirpadas precozmente, algunas podrían no haber adquirido nunca la capacidad de metastatizar. Por otra parte, en base a nuestra experiencia en seguimiento de pacientes de alto riesgo, algunos de ellos afectos de MM metastásico, en quienes a su vez durante el seguimiento evidenciamos este tipo de tumor atípico, a veces sin criterio suficiente de considerarse MM, consideramos que deben ser lesiones extirpadas, y clasificadas según los criterios que presenten en ese momento. Aunque teniendo en cuenta la epidemiología del MM en las piernas, probablemente sean MM sin tiempo suficiente a desarrollar mayor semiología ya sea clínica, dermatoscópica o a veces tampoco histológica. El avance de técnicas moleculares para caracterizar mejor estos tumores incipientes podrá en un futuro inmediato ayudar a diferenciar casos dudosos.

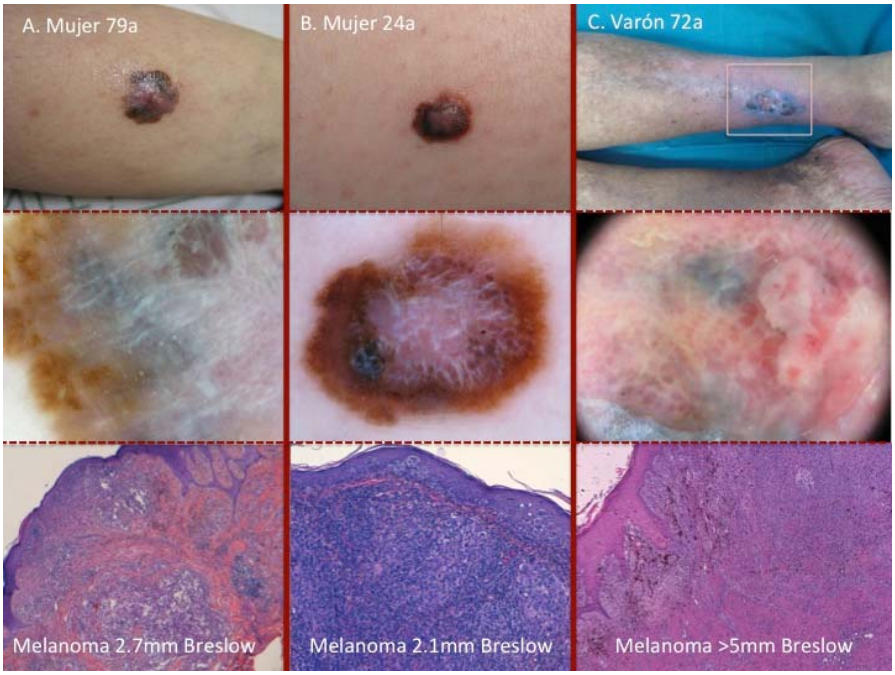


Figura 11. Melanomas en extremidades de diagnóstico tardío en fase invasiva, estadios superiores a IIB de la AJCC con diferente sustrato clínico y dermatoscópico.

A. Fototipo I, y escaso daño actínico. Melanoma que muestra en periferia retículo pigmentado fino marrón claro, y área central con velo azul-blanquecino, estructuras blanquecinas tipo crisálida, y vasos atípicos en zona invasiva. Podría recordar a los melanomas del grupo 2.

B. Paciente sin daño actínico, escasos nevus, y presencia de melanoma con retículo prominente y zona invasiva con retículo invertido, vasos y glóbulos atípicos. Podría corresponder a un melanoma del grupo 1 evolucionado.

C. Marcado daño actínico y dermatoesclerosis bilateral, melanoma mal delimitado, de gran extensión, tipo LMM con áreas desmoplásicas.

II. ESTUDIO IN VIVO DE LOS EFECTOS DE LA RUV AGUDA EN LESIONES MELANOCÍTICAS. PAPEL DE LA FOTOPROTECCIÓN

1. VALIDACIÓN Y UTILIDAD DEL MODELO *IN VIVO* DESARROLLADO

En base a la revisión de la literatura, y tal y como se detalla en el trabajo II de esta tesis (tabla 1 anexo), se consigue desarrollar e implementar (trabajo III) un modelo *in vivo* que permite demostrar cambios *in vivo* (clínico-dermatoscópicos) en nevus y piel adyacente a los 7 días post-irradiación con una dosis única del doble del MED-UVB. Esta metodología de forma pionera, permite mediante un método prospectivo e intervencional, comparar por una parte, los cambios inducidos por la RUV en cada lesión, siendo control de sí mismas a tiempo 0 y tiempo +7 días, y por otra parte, comparar las mitades protegidas ya sea con barrera física o con fotoprotector tópico (FPS), frente a las mitades no protegidas. De esta manera, se ha podido testar la eficacia de la protección tópica.

Histopatológicamente y mediante inmunohistoquímica, se han podido comparar ambas mitades de cada lesión a los 7 días de la irradiación, permitiendo diferenciar las zonas protegidas de las no protegidas, principalmente mediante marcadores de activación de melanocitos (Melan A y HMB45), pero también se han realizado diferentes estudios de proliferación celular (Ki67), apoptosis (Bcl2, survivina, p53), y regulación de ciclo celular (p16), estos últimos no incluidos en la publicación del trabajo III.

Se realizó un estudio comparativo tanto de los 20 pacientes como de sus 26 nevus incluidos en cada grupo (barrera física vs fotoprotector) que fueron asignados al azar. La muestra poblacional fue homogénea y comparable entre sí, y estos pacientes son representativos de la población de riesgo para melanoma que habitualmente se controla en la Unidad de Lesiones Pigmentadas del Servicio de Dermatología. Asimismo, el estudio in vivo comparativo de las lesiones seleccionadas pre-irradiación, también comprobó que los nevus incluidos eran de similares características clínico-dermoscópicas y simétricos en al menos un eje, por tanto comparables.

El método resultó seguro, sin ningún efecto secundario, a excepción de los efectos locales inducidos por la radiación en un área de piel que posteriormente se extirpó, y ninguno de ellos requirió tratamiento.

APLICABILIDAD DEL MODELO DE ESTUDIO: Se dispone de muestras de tejido parafinado y en fresco (criopreservado) de las 46 mitades de los nevus irradiados, de forma que se ha podido extraer ADN y ARN de células névicas para estudios moleculares de expresión y cuantificación de vías de fotoreparación e inflamación (resultados pendientes de análisis).

La utilización de la microscopía confocal in vivo abre una nueva dimensión del mismo modelo in vivo, revalidando el estudio con nuevas lesiones, permitirá la cuantificación de melanocitos suprabasales o de células inflamatorias y melanófagos en diferentes momentos de la fase de daño y reparación inducida por la RUV.

Asimismo repetir el modelo con diferentes fuentes de luz UV o infrarroja, permitirá también realizar controles de seguridad y eficacia de los nuevos fotoprotectores tópicos de amplio espectro. Precisamente en el mismo número de la JAMA de nuestro trabajo III, el grupo de Graz, Austria, publican una carta comentando su experiencia con nuestro modelo, aunque utilizan un simulador solar a dosis de 3MED, y un FPS de SPF 6.5[158]

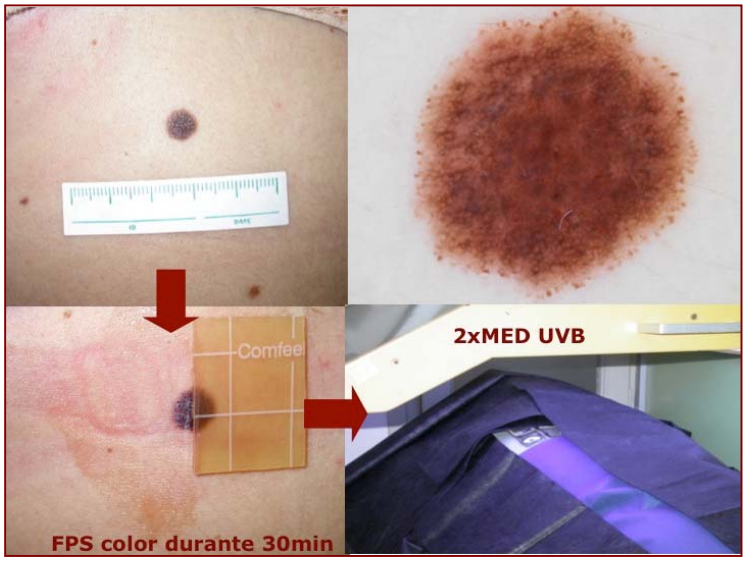


Figura 12. Metodología de irradiación UVB de un nevus tras la aplicación de un FPS tópico en una mitad. Se utilizó un apósito para evitar la difusión de la crema.

2. EFICACIA DE LA PROTECCIÓN TÓPICA

En el estudio comparativo clínico y dermatoscópico de los nevus pre y post RUV, se observaron cambios estadísticamente significativos en las mitades sin protección respecto a las protegidas, por tanto ello permite afirmar que la protección tópica evitó la gran mayoría de efectos inducidos por la RUVB. Sin embargo, interesantes hallazgos obligan a recalcar que no todos los efectos fotoinducidos son evitados por la protección tópica:

2.1. CAMBIOS FOTOINDUCIDOS EN ÁREAS PROTEGIDAS

Todos los cambios clínicos y dermatoscópicos observados a los 7 días de la RUV se pudieron observar también en áreas protegidas, con la excepción de la descamación clínica y el incremento de glóbulos y puntos de pigmento en dermatoscopia. Aunque todos fueron menos frecuentes y de menor intensidad en las zonas protegidas, estadísticamente significativos solo fueron los cambios clínicos, el incremento de vasos puntiformes y borramiento del retículo en dermatoscopia. Destaca principalmente que los signos de inflamación, eritema y regresión dermatoscópica aparecieron en igual medida en áreas tanto protegidas como sin proteger. Por lo tanto, desde el punto de vista clínico, existen evidencias de efectos fotoinducidos no evitados por la protección física ni en crema.

En relación a posibles efectos próximos a la protección, ya en 1989 se pudo demostrar la capacidad de la RUV aguda en estimular la activación y número de melanocitos, tanto en áreas directamente irradiadas, como en las próximas, y especialmente donde había menor densidad de melanocitos de base. Este hallazgo apoyaba la hipótesis de que las exposiciones solares agudas e intermitentes pueden ser más dañinas que la continua, al menos en la génesis del melanoma [159]. En el trabajo del grupo de Viena, comparando los efectos inducidos por PUVA o UVB de banda estrecha en 187 nevus de 38 pacientes sometidos a fototerapia, se demuestran cambios dermatoscópicos tanto en nevus protegidos como sin proteger. Principalmente los cambios son reversibles en el tiempo, y significativamente mayores en los nevus sin proteger que en los cubiertos (incremento en tamaño y pigmentación 67% vs 41%, o aparición de glóbulos en periferia en un 20% vs 5% respectivamente) [160].

El grupo de Graz, con un diseño similar al nuestro pero utilizando un simulador solar de UVB y UVA a dosis de 3MED, observan también cambios dermatoscópicos a los 3 y 7 días postirradiación, sin embargo, sin diferencias significativas entre las mitades protegidas o sin proteger por una FPS. Histopatológicamente tampoco pueden demostrar diferencias entre ambas mitades, excepto en la intensidad de HMB45. En este caso el uso de un FPS de amplio espectro, pero índice bajo, puede ser la causa de la ineficacia de la protección.

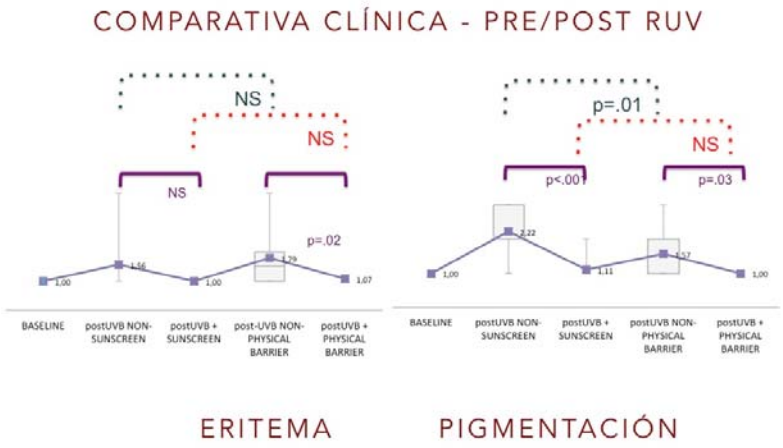
2.2. COMPARACIÓN DE UNA BARRERA FÍSICA FRENTE AL FOTOPROTECTOR TÓPICO

En los cambios demostrables *in vivo* a los 7 días, las diferencias entre ambos tipos de protección, fueron muy sutiles y en escaso número de casos, aunque resultó significativo que la barrera física evitó en mayor medida la aparición de vasos puntiformes, y la crema fotoprotectora evitó en mayor medida el borramiento del retículo.

Sí resultó más llamativo la diferencia entre ambos tipos de protección en los cambios inflamatorios que se observaron al completo en zonas protegidas y sin proteger, puesto que casi el 70% de las lesiones con fotoprotector presentaron signos de regresión, frente a menos de un 30% de las de barrera física.

Entre las diferencias ex vivo valoradas entre mitades protegidas y no protegidas también fue relevante y significativo, que la activación de melanocitos junturales e intraepidérmicos en los nevus (demostrada mediante Melan A y HMB45) fue significativamente mayor en áreas protegidas mediante fotoprotector que con barrera física. Puesto que HMB45 también fue de mayor intensidad en áreas sin proteger, podríamos asumir que es un sesgo por ser diferentes nevus. Sin embargo Melan A en áreas no protegidas mostró una intensidad similar en ambos grupos.

Por tanto, la RUV induce cambios evitables con la aplicación de una protección local (tanto de barrera física como FPS comercial), pero induce otros fenómenos no evitables por una protección local. La eficacia de protección de un FPS parece no ser totalmente comparable a una pantalla física, al menos en relación a fenómenos inflamatorios de regresión, y en cuanto a la activación de melanocitos.



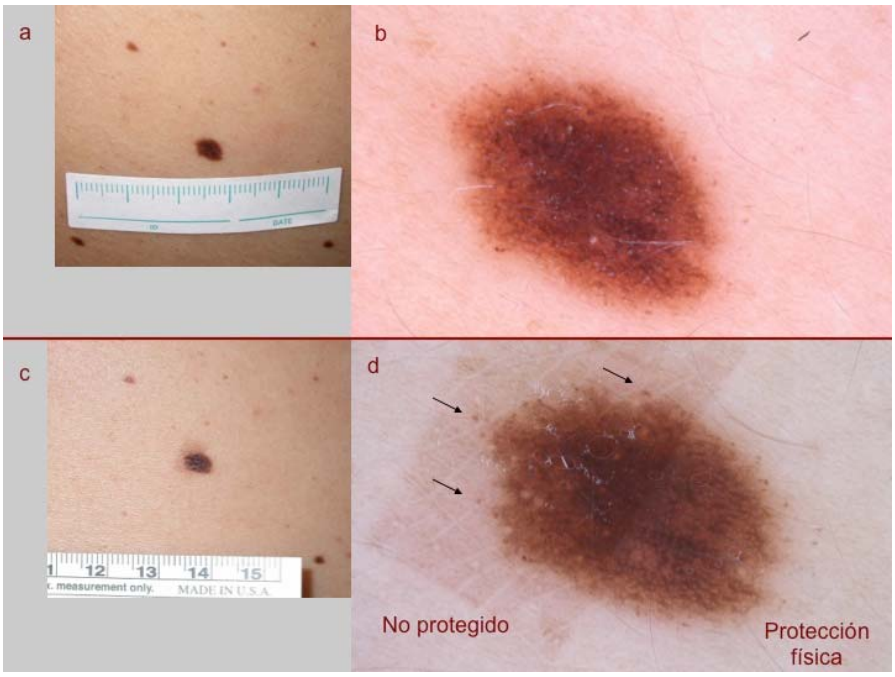
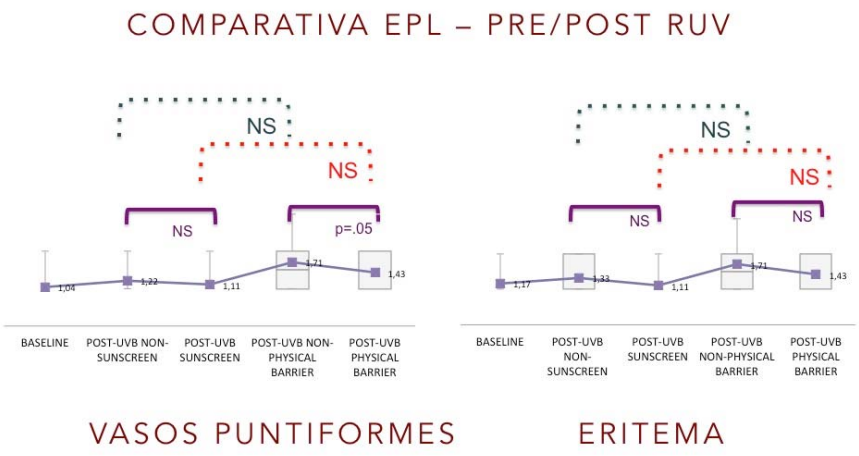


Figura 13. Gráficos y casos ejemplo de cambios clínicos y dermatoscópicos inducidos tras 7 días de la RUV. Comparativa de mitades sin proteger frente protegidas con barrera física o con crema de FPS.



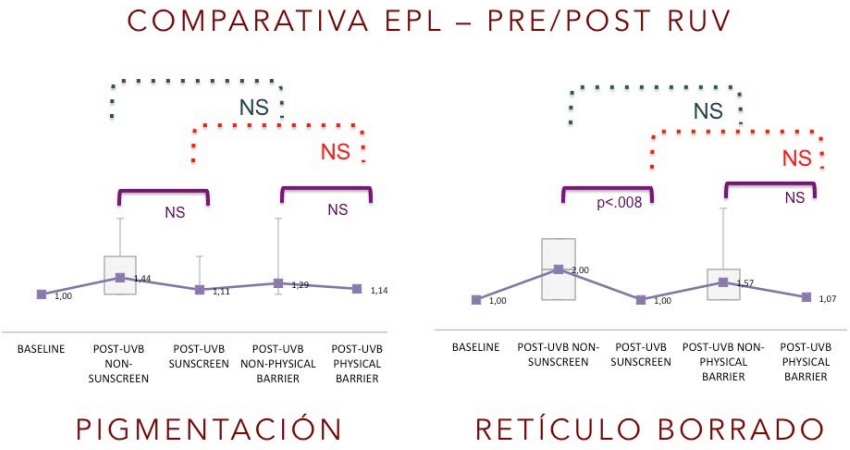


Figura 14. Gráficos de comparativa de cambios dermatoscópicos inducidos tras 7 días de la RUV. Comparativa de medianas de escala semicuantitativa entre mitades sin proteger frente protegidas con barrera física o con crema de FPS. Línea horizontal representa la mediana, los límites horizontales la desviación estándar, y la caja los percentiles 25-75%. NS no significativo estadísticamente.

3. EFECTOS INDUCIDOS POR LA RUV NO PREVISIBLES

3.1. VARIABILIDAD DE EFECTOS FOTOINDUCIDOS.

Los cambios observados en las lesiones post-irradiación fueron heterogéneos, tanto en la distinta respuesta a la RUV en cada paciente, a pesar de ser una dosis controlada y ajustada al MED de cada uno, como en qué lesiones mostraron un tipo concreto de cambio demostrable in vivo y/o ex vivo. Un estudio reciente cuantifica dímeros de ciclobutano de pirimidina como medida de daño genético en melanocitos de piel normal tras recibir RUVA y se demuestra que la pigmentación de eumelanina constitutiva o basal, modula en gran medida el daño que recibe la célula melanocítica, por tanto cabría esperar que los efectos en los nevus también podrían variar según su pigmentación basal[161].

En comparación con estudios previos (resumidos en trabajo II y tabla 1), destacan en nuestra serie, el borramiento del retículo pigmentado, el eritema y vasos puntiformes, y el marcado efecto inflamatorio con fenómenos de regresión aproximadamente en la mitad de los nevus. Sin embargo, a pesar de que histopatológicamente destacó la activación de melanocitos junturales, con migración de células a estratos suprabasales epidérmicos, y elongación de dendritas, en la mayoría de casos, no se observó mayor pigmentación ni crecimiento de las lesiones.

No hemos podido demostrar si esta diferencia tiene una explicación metodológica, biológica por el tipo de nevus, de población, o a que realmente los FPS puedan no evitar todos los efectos inflamatorios que induce la RUV.

Dobrosavljevic y col demuestran en un estudio realizado en 60 nevus de 11 estudiantes (edad media 26.2) la ineficacia del uso de crema de FPS25 en evitar cambios dermatoscópicos durante un mes de exposición solar estival intensa. Observaron cambios dermatoscópicos a los 28 días del cese de exposición tanto en los que usaban crema como en los que no, principalmente incremento de diámetro. Destaca que al igual que en nuestro estudio, describen el borramiento de estructuras en un 20% de nevus protegidos frente a un 60% de los no protegidos, sin ser estadísticamente significativo. Todos los cambios fueron reversibles al cabo de un año,

excepto un 10% de lesiones que mostraron discretas diferencias interpretadas como independientes de la exposición solar, aunque insinúan que quizás los nevus de menor tamaño, en pacientes de bajo riesgo podrían ser los de mayor propensión a cambios. Teniendo en cuenta el diseño de este estudio (tamaño muestra, edad, tipo de FPS, y variabilidad de fotoexposición recibida) hace difícil concluir si la FPS es efectiva[162].

El estudio de Massone, de diseño muy similar al nuestro con aplicación de un FPS, concuerdan con nuestros resultados en cuanto a efectos en áreas protegidas y a activación histopatológica de melanocitos junturales y suprabasales, pero sin embargo no describen fenómenos inflamatorios ni de regresión como en nuestra serie[158].

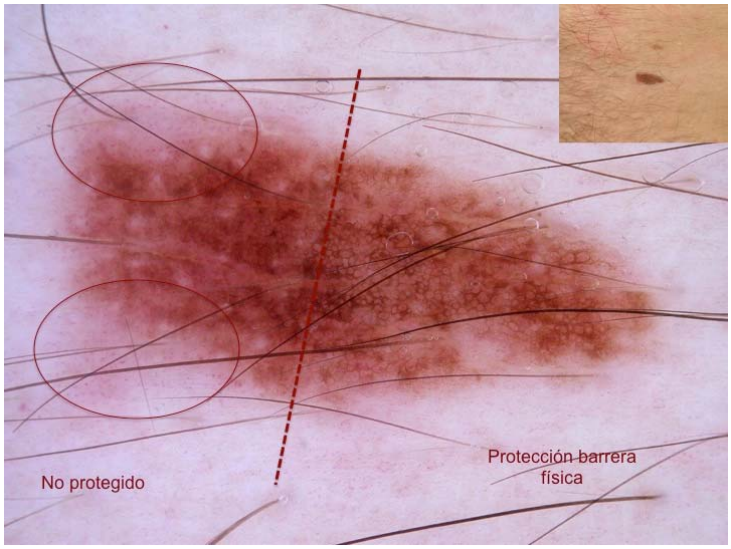


Figura 15. Imagen clínica – dermatoscópica a los 7 días de la RUVB. Comparativa de mitad protegida mediante barrera física (derecha) frente no protegida (izquierda). Borramiento marcado del retículo pigmentado y aparición de vasos puntiformes y eritema.

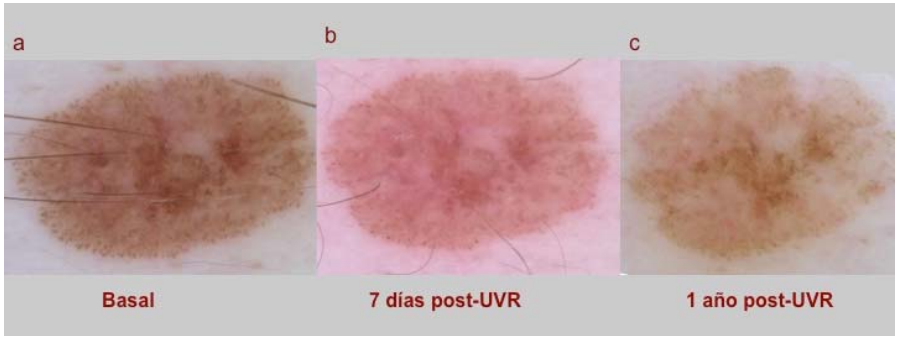


Figura 16. Evolución dermatoscópica de nevus irradiado. Mitad derecha protegida mediante crema FPS. Por deseo del paciente, excluido del estudio, se realizó seguimiento, mostrando marcada regresión e inflamación. Al año se extirpó con diagnóstico histológico de nevus tipo Sutton.

3.2. EFECTOS DE LA RUV NO VISIBLES CLÍNICA-DERMATOSCÓPICAMENTE.

Existe escasa concordancia entre las diferencias *in vivo* (a nivel clínico y dermatoscópico) y los hallazgos *ex vivo,* estudiados por histología e inmunohistoquímica. No todos los efectos biológicos fueron visibles *in vivo*.

En más de un 30% de lesiones no se pudo evidenciar ninguna diferencia clínica tras la irradiación, en un 17% ninguna diferencia dermatoscópica, e incluso en 2 lesiones no mostraron absolutamente ningún efecto in vivo a los 7 días de la irradiación. Sin embargo, el 100% de las lesiones en ese momento, en el cual fueron extirpadas, mostraban algún dato compatible con los efectos fotoinducidos, pues se apreciaban diferencias entre las mitades protegidas y no protegidas. Todos los parámetros inmunohistopatológicos evaluados, con excepción de la melanofagia y signos de regresión histológica, mostraron diferencias entre las áreas sin protección frente a las protegidas. El 90% presentaban paraqueratosis en las áreas sin protección, lo que traduce quemadura solar y necrosis de queratinocitos. Más del 85% infiltrado inflamatorio, más de la mitad vasos dilatados en la dermis superficial, y casi el 40% fenómenos de regresión histológica.

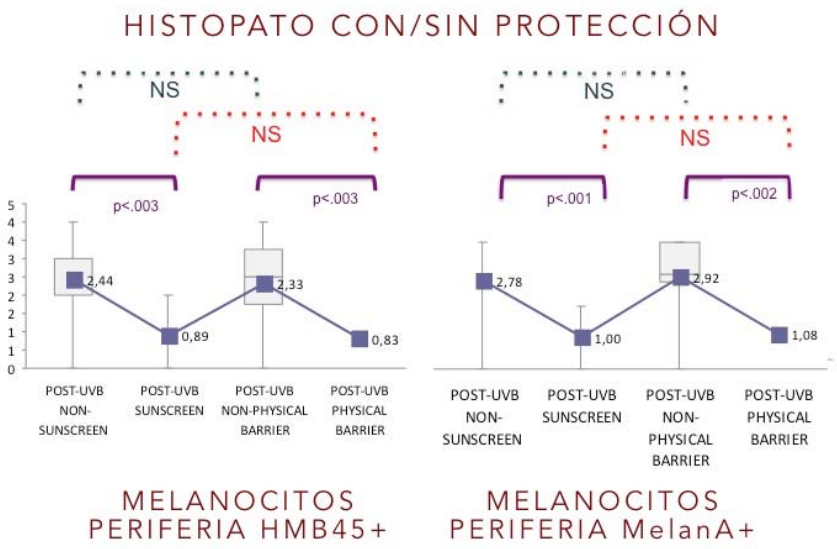


Figura 17. Estudio inmunohistopatológico en piel perilesional. Gráficos de comparativa de mitades sin proteger frente protegidas con barrera física o con crema de FPS.

Por tanto, existen efectos biológicos de la RUV que no son demostrables in vivo, (no provocan eritema, descamación ni cambios de pigmentación ni diferencias dermatoscópicas), a pesar de evidenciar signos de inflamación, necrosis y activación melanocitos.

Un reciente estudio en Italia compara el efecto de la protección física frente a la crema de SPF50, en nevus irradiados de forma repetida durante 1 mes, con dosis suberitematógenas o bien de UVA1 o de UVB banda estrecha. Los efectos con ambos tipo de irradiación, y que también demuestran evitar con ambas protecciones, son incremento de tamaño y de pigmentación y estructuras dermatoscópicas indicativas de crecimiento (proyecciones y glóbulos). En cambio, dado que inmuno-histológicamente no encuentran diferencias entre nevus protegidos o sin proteger, concluyen que las dosis suberitematógenas no provocan daño en los nevus, probablemente gracias a una redistribución melánica que protege del daño del ADN[163].

En nuestra opinión, aparte de las diferencias de tipo y dosis de irradiación con respecto a la nuestra, es complejo asumir que no existan efectos histológicos y más demostrándolos clínica-dermatoscópicamente, pues además comparan lesiones diferentes. Tanto el daño agudo como la reparación del mismo, al cabo de un mes de reiteradas exposiciones puede haber sido ya efectivo, siendo no visibles los fenómenos inflamatorios transitorios.

Por tanto, existen fenómenos biológicos post-RUV (por ejemplo inflamatorios) que pueden activarse a pesar de no producir cambios visibles (bronceado, quemadura solar). De nuevo reflejando la necesidad que existe de mejorar las estrategias de educación y fotoprotección en nuestro medio.

3.3. ESCASA CORRELACIÓN ENTRE SENSIBILIDAD A LA RUV - FENOTIPO - RESPUESTA.

No se pudo encontrar ningún factor predictor de respuesta a la RUV, ni en función del nevus ni de las características fenotípicas de los pacientes.

Incluso después de clasificar los genotipos de los pacientes según sus polimorfismos de *MC1R* o de *XRCC3* (reparador del fotodaño) tampoco se pudo relacionar un tipo de paciente a un tipo de respuesta de RUV.

Se observó que un elevado número de polimorfismos de *MC1R* (75%), incluso un 40% presentaban más de uno, y un 20% presentaban alguna variante de pelo-rojo. Sin embargo, destaca la escasa correlación entre la presencia de estos polimorfismos, la capacidad de bronceado y resistencia al sol que percibe subjetivamente el paciente (fototipo), y la dosis mínima de eritema (MED), medida objetiva de respuesta a la RUV (tablas 3-5 del anexo).

Se observó una tendencia a un menor MED entre los portadores de variantes, un 66% MED<50mJ/cm2, frente a un 40% de los no portadores, pero no resultó significativa, probablemente debido al escaso número de pacientes. El 50% de los pacientes que referían un fototipo III presentaban una MED baja.

4. FOTOCARCINOGÉNESIS MÁS ALLÁ DE LA PIGMENTACIÓN

Diversos estudios ya han demostrado que el papel de *MC1R* en la carcinogénesis no sólo lo ejerce a través de las vías de la pigmentación. Un estudio con ratones albinos, por tanto incapaces de sintetizar melanina, muestras diferente modulación del daño solar según la funcionalidad del *MC1R*, con o sin producción de melanina[164]. *MC1R* juega un papel importante en fotocarcinogénesis también a través de la estimulación de producción de p53 y de p16 ante un daño fotoinducido[165], o a través de la vía de *MITF* (*mitogen transcriptor factor*) la cual está implicada en la proliferación celular tumoral, apoptosis y vías de reparación del DNA[166].

Smith y col., demostraron que según el polimorfismo de *MC1R* podría variar la sensibilidad a la fototerapia. Estudiaron 111 pacientes tributarios de PUVA terapia, y la dosis mínima fototóxica. Encontraron que las variantes V60L y A163G, o la presencia de 2 o más polimorfismos, se asociaba a mayor sensibilidad al PUVA, mientras que la variante de pelo-rojo R151H solo se asoció al fenotipo pelirrojo[167].

También se ha demostrado recientemente que la presencia de ciertos polimorfismos puede conferir riesgo a un tipo de melanoma y no otro[168 169], como la reciente asociación entre la variante R163Q y melanoma tipo lentigo maligno en población mediterránea. Tal vez uno de los resultados más sorprendentes respecto a las variantes del receptor es el hecho de que los pacientes que tienen un receptor salvaje, de desarrollar un melanoma, éste tiene peor pronóstico mostrando la complejidad de la relación entre las variantes del MC1R y el melanoma[170]. Es interesante que cada vez cobra mayor interés las posibles funciones de MC1R independientes de las vías pigmentarias. Esto explicaría el incremento de riesgo de ciertas variantes, a pesar de no presentar fenotipo clásico de riesgo o la presencia de nevus melanocíticos[21,22,171,164].

Probablemente, el daño oxidativo favorecido por la síntesis de la feomelanina ejerce un papel más importante del que se pensaba, e incluso independientemente de la RUV recibida. Mitra y col. recientemente demostraron en modelos murinos, que en ausencia de RUV las variantes de pelo-rojo junto con la activación del oncogén BRAF son suficientes para inducir melanoma, pero si se inhibe totalmente la síntesis de feomelanina (ratones albinos), se bloquea la inducción de MM y expresan menor daño oxidativo celular[172].

Por otra parte se ha sugerido que la RUVA podría tener incluso un mayor papel en la melanomagénesis por la combinación de daño directo del ADN e indirecto del daño oxidativo, que se potenciaría más aún a través de una mayor síntesis de melanina. En un estudio reciente se pudo demostrar que tanto la RUVB como la RUVA inducen DCP de igual manera en melanocitos como queratinocitos. En cambio, el daño oxidativo inducido por UVA (a través de 8-oxo-7,8-dihydroguanine) fue el doble en melanocitos (2.2 veces superior), lo que apoya también las hipótesis de que a mayor síntesis de melanina en el melanocito, mayor estrés oxidativo sufre la célula[173].

III. INFLUENCIA DE LA RUV EN EL DIAGNÓSTICO HISTOPATOLÓGICO DE NEVUS CON ATIPIA vs MM INCIPIENTES.

Los estudios de Tronnier ya alertaban de la posibilidad de dificultad diagnóstica de nevus melanocíticos benignos bajo la influencia de RUV aguda. En ciertos casos con atipia arquitectural y citológica, la presencia de activación de melanocitos basales y suprabasales, sobre todo con positividad para marcadores de inumohistoquímica como Melan A o HMB45, puede suponer un reto diagnóstico a tener en cuenta para evitar sobrediagnósticos de melanomas[78,174].

Estudios previos en muestras de piel fotoexpuesta (márgenes de cirugía de Mohs de carcinomas) estimaron la densidad de melanocitos en la piel normal mediante inmunotinciones de Melan A (MART-1). Se ha demostrado que tanto la densidad de melanocitos, la relación de queratinocitos/melanocito, como la tendencia y confluencia perifolicular, varía según la región anatómica, siendo máxima en región mentón, nariz y mejillas, y mínima en extremidades[175]. También se encontró que esta densidad de melanocitos tiende a disminuir con la edad, y a ser discretamente menor en mujeres. Sin embargo, de forma interesante el trabajo de Hendi y col. no halló diferencias significativas en función de la exposición solar aguda reciente o crónica acumulada, ni al comparar los estados de residencia de los pacientes (Florida frente Minnesota)[176]. Los diversos estudios al respecto, coinciden en que se debe alertar a los dermatopatológicos de que en piel fotodañada se pueden llegar a ver elevado número de melanocitos (media de unos 12 melanocitos/campo de 40x), con la posibilidad de tendencia a la confluencia (raras veces más de 2 o 3 células), y la presencia de alguna célula intraepidérmica pagetoide. Sin embargo, debemos tener en cuenta que estos hallazgos se han descrito en áreas faciales clínicamente no lesionales y siempre en focos aislados, sin coincidir los tres criterios en la misma zona. A diferencia de nuestros hallazgos en nevus irradiados, ninguno en área facial ni con intensa elastosis ni fotodaño, en los cuales sí pudimos apreciar en periferia del nevus una intensa activación de melanocitos Melan A positivos, con tendencia a la confluencia mayor de la descrita en estos trabajos.

Estos datos inmunohistoquímicos deben tenerse en cuenta en la evaluación de lesiones en áreas con daño actínico marcado, en áreas de queratosis actínicas o lentigos actínicos, o para evitar un error diagnóstico en melanomas desmoplásicos, como pudimos demostrar en nuestra serie de casos nasales[177], en los que inicialmente, la mitad no fueron detectados a pesar de ser biopsiados (trabajos anexos III y IV).

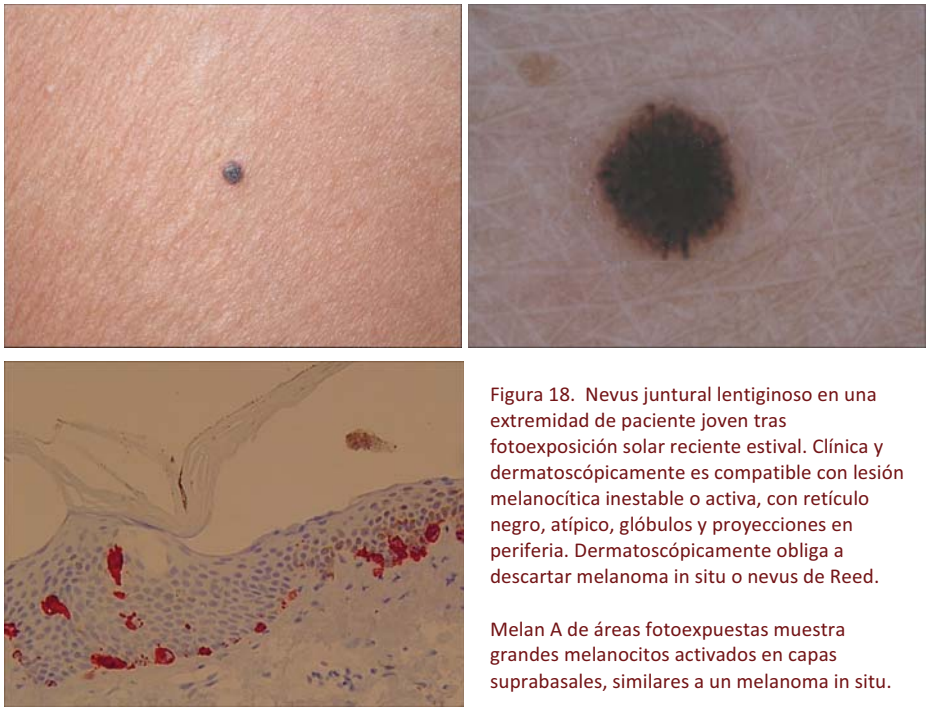


Figura 18. Nevus juntural lentiginoso en una extremidad de paciente joven tras fotoexposición solar reciente estival. Clínica y dermatoscópicamente es compatible con lesión melanocítica inestable o activa, con retículo negro, atípico, glóbulos y proyecciones en periferia. Dermatoscópicamente obliga a descartar melanoma in situ o nevus de Reed.

Melan A de áreas fotoexpuestas muestra grandes melanocitos activados en capas suprabasales, similares a un melanoma in situ.

CONCLUSIONES

1. La dermatoscopia, complementada por el seguimiento digital y la microscopía confocal *in vivo* permiten el diagnóstico de melanomas incipientes en extremidades clínicamente inaparentes.
2. Se han identificado 4 patrones dermatoscópicos de melanomas incipientes en extremidades, con distinto significado biológico.
3. Los melanomas de extremidades que muestran dermatoscópicamente vasos puntiformes tienen mayor riesgo de ser microinvasores y su Breslow medio es superior al resto de tumores.
4. Se ha descrito un nuevo subtipo de melanoma de extremidades que presenta pigmentación perifolicular en dermatoscopia, y foliculotropismo en histología. Este subtipo sería el equivalente del lentigo maligno melanoma, pero en ausencia de daño solar crónico.
5. Se ha desarrollado y validado un método seguro y reproductible para el estudio de los efectos *in vivo* de la RUV en lesiones melanocíticas y en piel perilesional.
6. Los efectos fotoinducidos son heterogéneos, no previsibles según el paciente ni la lesión, destacando los signos de inflamación y regresión no descritos hasta ahora.
7. No todos los efectos biológicos son visibles *in vivo*. Existe una escasa concordancia entre la presencia de cambios clínico-dermatoscópicos y los efectos histológicos e inmunohistoquímicos.
8. El uso del filtro solar tópico evita parcialmente los efectos fotoinducidos.
9. La irradiación aguda de lesiones melanocíticas benignas provoca la activación de melanocitos junturales y fenómenos inflamatorios independientes de la pigmentación.
10. La RUV modula el riesgo genético a desarrollar melanoma y dificulta el diagnóstico de lesiones melanocíticas, siendo por tanto un factor clave de la prevención.

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ANEXO I. TABLAS COMPLEMENTARIAS

Tabla 1: ESTUDIOS SOBRE EFECTOS DE RUV EN NEVUS. Adaptada y actualizada de Carrera et al.(1)

Autores-Referencia	Año	TIPO DE RUV	METODOLOGÍA	PRINCIPALES RESULTADOS / CONCLUSIONES
Holman et al(2)	1983	Variación estacional	Descripción histológica estival	Diferencias clínicas en nevus durante el verano. Marcado compon respuesta inflamatoria. Posiblemente efecto a corto plazo de later cambios.
Amstrong et al	1984	Variación estacional	Descripción clinico-patológica estival	
Larsen et al	1990	Variación estacional		
Pawlowski et al	1991	UVR	Estudio histológico	Incremento metabolismo celular y actividad mitótica
Tronnier et al(3)	1995	2 MED UVB	Estudio histológico a los 7-14-21 días postUVB	Incremento del número de melanocitos HMB45+ suprabasales, pe Cambios no detectables tras 2 semanas.
Tronnier et al (4)	1995	2 MED UVB	Estudio histológico tras 7d postUVB	Cambios inducidos pueden simular MM, con hallazgos compatible actividad metabólica de melanocitos.
Stanganelli et al(5)	1996	Variación estacional	Diferencias clínico-dermoscópicas	Incremento de retículo pigmentado y glóbulos/puntos durante ver
Stanganelli et al(6)	1997	Exposición solar intensa	Diferencias clínico-dermoscópicas 5-13d post-expo solar	Cambios sutiles dermatoscópicos. Mayoría transitorios, aunque pu regresión extensa.
Hofmann et al (7)	1997	Fototerapia UVB (dosis suberitemata)	Diferencias dermoscópicas en nevus tras fototerapia durante una mediana de 8 sem.	Nevus de áreas no protegidas aparecen más pigmentados, irregula áreas protegidas no muestran cambios significativos.
Hofmann et al (8)	1998	2 MED (UVB y UVA)	Cambios dermoscópicos a los 3, 7, 14, 28d postUVR	Mayoría de cambios inducidos a los 3 días, son transitorios, tras 28 Algunos pueden simular MM.
Tronnier et al	1997	1 MED UVR	Estudio histológico de moléculas de adhesión	Sobre-expresión de integrinas en queratinocitos suprabaslaes. Pos migración de melanocitos intraepidérmicos.
Serre et al (23)	1997	3 MED	Inmunosupresión local inducida por RUV. Prevención mediante fotoprotector tópico.	Quemadura solar produce inmunosupresión, y por tanto bloquea hipersensibilidad local. El FPS evitó la inmunosupresión, y por tant reacción local de hipersensibilidad tópica a un alergeno.
Böni et al	1998	4 MED (UVB+UVA)	Microdisección y extracción de ADN tras 7d de irradiación. Estudios de pérdidas alélicas	Cambios histológicos agudos post UVR no provocan pérdidas aléli displásicos.

Autores-Referencia	Año	TIPO DE RUV	METODOLOGÍA	PRINCIPALES RESULTADOS / CONCLUSIONES
Rudolph et al(9)	1998	2MED vs 4 MED UVB	Estudio marcadores de proliferación y reparación celular	RUVB induce incremento de reactividad de HMB45 y proliferación Simultáneamente mecanismo compensatorio de regulación de cic expresión de p53 y p21.
Tronnier et al(10)	2000	1MED UVB vs irritantes mecánicos	Cambios clínicos-patológicos en nevus	Cambios transitorios que pueden simular MM, asociados a activac reparación.
Krengel et al (11)	2002	2 MED UVB	Expresión de metaloproteinasas (MMP2, TIMP2, MT1-MMP) en nevus	Diferente expresión en queratinocitos frente melanocitos, pero no irradiados o no irradiados.
Schiller et al	2004	2 MED (UVB+UVA)	Cuantificación de expresión por RNA (rtPCR) e inmunohistoquímica de POMC, MSH, IL10 a los 3, 6, 24h postRUV	Fotoprotector tópico puede prevenir la sobreregulación de MSH p vitro)
Kilinc et al.(12)	2007	PUVA vs UVBnb durante mediana de 31 sem.	Seguimiento digital clínico-dermoscópico nevus cubiertos vs sin proteger	Efectos similares ambas RUV. Cambios transitorios, mayoría increr pigmentación, mayor porcentaje en nevus no protegidos, pero tar cubiertos. Incremento de glóbulos solo en UVBnb.
Dobrosavlijevic et al.(13)	2009	Exposolar intensa durante media de 10d de verano	Cambios clínico-dermoscópicos a 28d y 365d en usuarios de FPS vs no usuarios.	No diferencias entre los que usaron FPS o no. Incremento de diám postexposición. Mayoría de cambios reversibles, entre ellos borra aumento de pigmentación. Al año no cambios atribuibles a exposi
Manganoni et al. (14)	2012	UVA1 vs UVBnb suberitematógenas (3/sem x 4sem)	Barrera física vs FPS crema en nevus completos. Estudio clínico-dermoscópico e inmuno-histológico	Cambios de tamaño, pigmentación y estructuras dermatoscópicas (proyecciones y glóbulos), sólo en nevus no protegidos. Ambas pro eficaces. No diferencias histológicas en ningún grupo ni protegido
Massone et al. (15)	2013	3MED Simulador solar (UVB-UVA)	FPS crema en mitades de nevus. Estudio clínico-dermoscópico e inmuno-histopatológico a los 3 y 7d	Cambios de eritema y pigmentación, mayores en áreas no protegi detectados en las protegidas. No diferencias significativas entre pr protección. Incremento de HMB45+ significativo mayor en áreas s
Carrera et al(1)(16)	2008-2013	2 MED UVB	Barrera física vs FPS crema en mitades de nevus. Estudio clínico-dermoscópico, inmuno-histopatológico a los 7d postRUVB de nevus.	Efectos heterogéneos, no predecibles: No todos los cambios clínic evitan con protección. Predominio de efectos inflamatorios y regr pigmentación y crecimiento. Diferencias histológicas incluso dond cambios in vivo. Activación de melanocitos Melan A+ y HMB45+ m protegidas, pero mayor en los protegidos con FPS que con barrera

Tabla 3 : CARACTERIZACIÓN PACIENTES DEL ESTUDIO PROSPECTIVO SOBRE EFICACIA DE LA FOTOPROTECCIÓN EN NEVUS IRRADIADOS (TRABAJO III)

% (N)	SEXO		FOTOEXPOSICIÓN Y FOTOPROTECCIÓN PREVIAS					FOTOTIPO		COLOR OJOS		COLOR CABELLO		MED-UVB (mJ/cm^2)		NEVI	POLIMORF MC1R	
	F	M	Leve	Med /Int	No FPS	No RUVA	QS	I-II	>II	MN	AV	MN	RR	≤50	>50	≥100 Nevi	SI	NO
BARR. FÍSICA %(12)	**58.3 (7)**	**41.7 (5)**	**16.7 (2)**	**83.3 (10)**	**83.3 (10)**	**91.7 (11)**	**83.3 (10)**	**58.3 (7)**	**41.7 (5)**	**66.7 (8)**	**33.3 (4)**	**83.3 (10)**	**16.7 (2)**	**58.3 (7)**	**41.7 (5)**	**50 (6)**	**66.7 (8)**	**33.3 (4)**
FPS CREMA %(8)	**62.5 (5)**	**37.5 (3)**	**12.5 (1)**	**87.5 (7)**	**87.5 (7)**	**75 (6)**	**87.5 (7)**	**87.5 (7)**	**12.5 (1)**	**87.5 (7)**	**12.5 (1)**	**100 (8)**	**0 (0)**	**62.5 (5)**	**37.5 (3)**	**75 (6)**	**87.5 (7)**	**12.5 (1)**
TOTAL %(20)	**60 (12)**	**40 (8)**	**15 (3)**	**85 (17)**	**85 (17)**	**85 (17)**	**85 (17)**	**70 (14)**	**30 (6)**	**75 (15)**	**25 (5)**	**90 (18)**	**10 (2)**	**60 (12)**	**40 (8)**	**60 (12)**	**75 (15)**	**25 (5)**

Leyenda: F: femenino, M; masculino. Med/Int: fotoexposición media o intensa. FPS: Fotoprotección solar. RUVA: radiación ultravioleta A artificial. QS: quemaduras solares. MN: marrón o negros. AV: azules o verdes. RR: rubio o rojo. MED: dosis mínima eritema a UVB. Polimrf. *MC1R*: Polimorfismos del gen del receptor de melanocortina.

Tabla 4: CARACTERIZACIÓN GENÉTICA DE PACIENTES DEL ESTUDIO PROSPECTIVO SOBRE EFICACIA DE LA FOTOPROTECCIÓN EN NEVUS IRRADIADOS

GRUPO ESTUDIO NEVUS		POLIMORF. *MC1R*		*MC1R* PELO-ROJO		N° POLIMORF. *MC1R*		POLIMORF. *XRCC3*			*XRCC3-T*	
		SI	NO	SI	NO	1	>1	CT	TT	CC	NO	SI
BARRERA FÍSICA	N	8	4	3	9	5	3	7	2	2	4	9
	%	66.7	33.3	25	75	41.7	25	63.6	18.2	18.2	30.8	69.2
FPS CREMA	N	7	1	1	7	3	4	3	1	4	2	4
	%	87.5	12.5	12.5	87.5	37.5	50	37.5	12.5	50	33.3	66.7
Significancia		n.s.		n.s.		n.s.			n.s.		n.s.	
TOTAL	N	15	5	4	16	8	7	10	3	6	6	13
	%	75	25	20	80	40	35	52.6	15.8	31.6	31.6	68.4

Leyenda: *MC1R*: gen del receptor de melanocortina, Polim: polimorfismo genético. FPS: Fotoprotector tópico. N.s. no significancia

Tabla 5: ESTUDIO DE ASOCIACIÓN ENTRE GENOTIPO Y MED-UVB DEL ESTUDIO PROSPECTIVO SOBRE EFICACIA DE LA FOTOPROTECCIÓN EN NEVUS IRRADIADOS

ASOCIACIÓN GENOTIPO* (*MC1R*) - MED			MED UVB (mJ/cm2)		Total
			≤ 50	>50	
MC1R	POLIMORF	N	10	5	15
		% de polim*MC1R*	66,7	33,3	100
		% de MED	83,3	62,5	75
	NO	N	2	3	5
		% de *wt MC1R*	40	60	100
		% de MED	16,7	37,5	25
Total		N	12	8	20
		% TOTAL	60	40	100

Leyenda: MED: dosis mínima eritema a UVB. MC1R: gen del receptor de melanocortina. Polimorf: polimorfismo genético. Wt: wild type: alelo normal en la población. *p= 0.3, Chi-cuadrado, corrección Fisher

ANEXO II. ASPECTOS ÉTICOS

1. Este proyecto fue evaluado positivamente por el **Comité Ético del Hospital Clínic de Barcelona*** previamente a la inclusión de pacientes.
2. La selección de los pacientes se ha realizado por parte del investigador principal a partir de la historia clínica y entrevista del paciente. Todos los pacientes han sido debidamente informados de los objetivos del estudio, entregándose por escrito la documentación informativa y obteniendo el **Consentimiento informado*** (ANEXOS) a todos los que cumplían los criterios de inclusión en el estudio.
3. Los pacientes incluidos en la parte intervencionista de este estudio, han sido informados correctamente del proceso de cálculo de la dosis mínima eritematógena de UVB, y posteriormente de la irradiación y consecuente extirpación de las lesiones benignas irradiadas.
4. El presente estudio tuvo como únicas muestras biológicas la manipulación de leucocitos y piel humana correspondiente a las piezas quirúrgicas de pacientes con nevus/melanoma del Servicio de Dermatología del HCB. Todas las muestras se procesaron y se custodian de la manera habitual.
5. Los datos clínicos de los sujetos a estudio se han recopilado en una base de datos en formato Microsoft Access y Excel, a las que sólo han tenido acceso los investigadores del proyecto. No se divulgarán los datos personales de los sujetos participantes en el estudio.
6. La participación en este proyecto no ha supuesto ningún riesgo físico adicional para los pacientes. Hasta la fecha no se ha podido demostrar ningún efecto nocivo, acumulativo o inmediato, tras la irradiación de una dosis única del doble del MED, que equivaldría a una exposición solar natural de 10 minutos en las horas centrales de un día estival en una latitud Mediterránea.
7. Los pacientes tienen pleno derecho a denegar su aceptación, sin que ello repercuta en su trato médico.
8. El consentimiento deja clara la opción de salir del estudio en cualquier momento. En el caso que ello se produjera, las muestras serán tratadas según el circuito habitual de muestras asistenciales.
9. No existen implicaciones psicosociales asociadas a la utilización de dichas muestras para finalidad biomédica.
10. No se han ofrecido incentivos de ningún tipo para la participación en el estudio.
11. Los resultados del presente estudio, así como las conclusiones serán utilizados exclusivamente para fines científicos, asistenciales y académicos.
12. No existe ningún conflicto de intereses por parte de los investigadores ni participantes.

CRITERIOS DE INCLUSIÓN DE PACIENTES:

Grupo de estudio MELANOMAS INCIPIENTES DE EXTREMIDADES

Pacientes diagnosticados de melanoma maligno cutáneo en extremidades en la Unidad de Melanoma del Servicio de Dermatología, sin sospecha clínica de melanoma, con imagen clínicodermatoscópica, y laminilla histológica disponibles.

- *Que acepten y firmen el consentimiento informado, conforme permiten el estudio, con fines de investigación, del material de exéresis de su melanoma, y la extracción de sangre para estudio genético.*

Grupo de estudio NEVUS IRRADIADOS

Pacientes sin antecedentes personales de melanoma,

- Que presenten lesiones melanocíticas en el tronco de más de 5mm de diámetro, con diagnóstico claro, mediante exploración clínica y dermatoscópica, de nevus melanocítico benigno sin atipia, y que acepten y firmen el consentimiento informado, conforme:
- Participarán y colaborarán respetando estrictamente las condiciones de fotoexposición y fotoprotección de nuestro estudio.
- Aceptan la extirpación de la/s lesión/es irradiadas, y una extracción de sangre.

CRITERIOS DE EXCLUSIÓN estudio nevus irradiados;

- Antecedentes de cáncer de piel.
- Antecedentes de enfermedades fotoinducidas.
- Medicación con riesgo de fototoxicidad / fotoalergia.
- Presencia de enfermedad cutánea en el momento de irradiación.
- Antecedentes de tratamientos con UV artificial en los tres meses previos.
- Exposición a UV intensa en los tres meses previos al estudio.
- Embarazo.

CONSENTIMIENTOS INFORMADOS

Estudio NEVUS IRRADIADOS

El presente estudio tiene por objetivo valorar la influencia de la radiación ultravioleta en las características clínicas, dermatoscópicas e histológicas de los nevus melanocíticos y la influencia de la aplicación de filtros solares en los mismos.

Los nevus melanocíticos son lesiones cutáneas pigmentadas benignas que pueden alterarse por el efecto de la exposición a la luz solar o artificial.

El estudio se realizará en pacientes voluntarios con nevus melanocíticos sin signos de atipia ni de malignidad. En cada paciente se elegirá 1 o 2 lesiones para su estudio. Las lesiones estudiadas serán expuestas a luz ultravioleta artificial. En un determinado número de lesiones se aplicará un filtro solar antes de la exposición a la luz ultravioleta, para así poder comparar los cambios que tienen lugar en las lesiones no fotoprotegidas y en las fotoprotegidas. Al cabo de 1 semana las lesiones serán extirpadas con anestesia local para su estudio histológico

La exposición a la luz ultravioleta, en dosis única controlada y focalizada sobre la lesión melanocítica, no supone un riesgo valorable para la salud del paciente.

Dado que las lesiones a estudiar son benignas, la extirpación de las mismas no suponen un beneficio terapéutico para el sujeto. El principal beneficio que obtiene el paciente de este estudio es profundizar en el conocimiento de los efectos de las radiaciones ultravioletas sobre los nevus melanocíticos y el efecto protector que tiene la aplicación de los filtros solares en dichas lesiones. Estos conocimientos tienen gran importancia para todas aquellas personas que presenten nevus melanocíticos ya que las radiaciones ultravioletas son el principal factor etiológico externo conocido relacionado con el desarrollo del melanoma y, actualmente existe una controversia importante acerca de la función preventiva que puedan desarrollar los filtros solares.

La participación en el estudio es voluntaria y el paciente puede retirarse del mismo en cualquier momento, sin tener que dar explicaciones y sin que dicha acción repercuta en sus cuidados médicos posteriores.

La información obtenida de cada voluntario es totalmente confidencial.

Presto libremente mi conformidad para participar en el estudio:

Don/Doña ..... DNI

Firma Fecha

Doctor/a ..... DNI

Firma Fecha

CONSENTIMIENTO INFORMADO DE ESTUDIO GENÉTICO SANGUÍNEO

El Servicio de Dermatología y el Servicio de Genética del Hospital Clínic i Provincial de Barcelona pueden realizar un estudio genético de diversas enfermedades cutáneas congénitas.

Para realizar dicho estudio es necesario un análisis genético a partir de una muestra de sangre, que se puede obtener con el mismo procedimiento que se realiza para cualquier analítica general.

El Sr/a. ha recibido adecuada y suficiente información del interés de realizar este estudio, entendiendo que la atención y la asistencia que precise no está en absoluto condicionada o vinculada a la decisión que adopte.

Asimismo, los datos que resulten son de carácter estrictamente reservado y confidencial.

A la vista de los antecedentes expuestos, acepta someterse a dicho estudio genético.

Don/Doña ..... DNI

Firma Fecha

Doctor/a ..... DNI

Firma Fecha

ANEXO III. TRABAJOS ADICIONALES

Trabajos anexos I y II:

Detección de melanoma mediante seguimiento digital en una Unidad de Referencia.

Trabajos anexos III y IV:

Melanomas fotoinducidos en áreas de daño solar crónico: Melanoma desmoplásico.

STUDY

ONLINE FIRST

Melanomas Detected in a Follow-up Program Compared With Melanomas Referred to a Melanoma Unit

Gabriel Salerni, MD; Louise Lovatto, MD; Cristina Carrera, MD; Susana Puig, MD, PhD; Josep Malvehy, MD, PhD

Objective: To compare melanomas diagnosed in patients included in follow-up programs with melanomas diagnosed in patients referred to a melanoma unit.

Design: Retrospective analysis of 215 consecutive melanomas diagnosed between 2007 and 2008.

Setting: Melanoma Unit, Hospital Clinic of Barcelona, Barcelona, Spain.

Patients: The study included 201 patients (105 men and 96 women), 40 of whom were included in a follow-up program in our unit and 161 of whom were referred for evaluation.

Main Outcome Measures: Clinical (ABCD algorithm), dermoscopic (ABCD rule of dermoscopy), and main histologic characteristics were evaluated in both groups.

Results: Most melanomas diagnosed in follow-up did not fulfill some of the ABCD criteria, and only 12.0% fulfilled all 4 ABCD criteria, in contrast with 63.6% of the melanomas referred for evaluation ($P<.001$). The total dermoscopy score was lower in melanomas diagnosed in follow-up (5.04 vs. 6.39, $P<.01$), and 36% were misclassified as benign in this group according to the total dermoscopy score. Seventy percent of melanomas diagnosed in follow-up were in situ; among invasive melanomas, the Breslow index was significantly lower in the group of melanomas diagnosed in follow-up, with a mean (range) of 0.55 (0.25-0.90) mm vs 1.72 (0.25-13.00) mm ($P<.001$).

Conclusions: The inclusion of patients who are at high risk for melanoma in follow-up programs allows the detection of melanomas in early stages, with good prognosis, even in the absence of clinical and dermoscopic features of melanoma. In the general population without specific surveillance, melanoma continues to be diagnosed at more advanced stages

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DERMOSCOPY INCREASES sensitivity in the clinical diagnosis of melanoma from 60% to 90%, with a specificity as high as 95%.[1] This increase in diagnostic accuracy is reflected by a minor excision rate and a decrease in the benign to malignant ratio.[2] Digital dermoscopy monitoring devices allow the follow-up of melanocytic lesions to detect changes over time, offering the double benefit of increasing the possibility that melanoma will not be overlooked with any or few specific criteria of malignancy and minimizing the excision of benign lesions.[3]

The efforts to improve melanoma prognosis have also been focused on the identification and follow-up of individuals with increased risk. Fair-skinned persons, persons who tan with difficulty, blond or red-haired persons, and persons with blue eyes have more risk of developing melanoma than the general population.[4] The presence of many pigmented lesions, including freckles and clinically typical or atypical nevi; intermittent sun exposure and severe sunburns, especially during childhood; and exposure to artificial UV-A radiation have all been associated with an increased risk of melanoma. The history of a previous melanoma is associated with a high risk for the development of a second primary melanoma.[4] Patients with a strong family history of melanoma and atypical mole syndrome (AMS) represent the population of persons who are at major risk of developing melanoma. Hereditary mutations in *CDKN2A* and *CDK4* genes result in a 30% to 90% risk of melanoma throughout life.[5-7]

The aim of our study was to assess the clinical, dermoscopic, and histologic features of melanomas diagnosed in individuals included in follow-up programs in a specialized unit (follow-up melanomas [FUMMs]) and melanomas diagnosed in patients referred to the same unit for evaluation of suspicious lesions (referred melanomas [RMMs]) but not included in a specific follow-up program.

METHODS

We conducted a retrospective analysis of clinical and dermoscopic characteristics of 215 melanomas consecutively excised and diagnosed in our unit over a 2-year period. The study included primary lesions with clinical and dermoscopic pictures of acceptable quality to allow reliable evaluation. Patients who were referred to our unit with a diagnosis of melanoma after excision or biopsy were excluded from the study (n=302), as were melanoma recurrences or cutaneous metastases of prior melanomas (n=9).

One of us (C.C.) collected all melanomas diagnosed between January 2007 and December 2008 that met inclusion criteria for the study from our database; clinical data such as age and sex of the patients and the location and size of the lesions were incorporated along with the clinical and dermoscopic images in a PowerPoint presentation (Microsoft Corp, Redmond, Washington). This collection was presented to 2 dermatologists with experience in dermoscopy (G.S. and L.L.) who performed both clinical and dermoscopic evaluation while blinded to the origin of the lesions (RMM or FUMM), identity of the patients, and histologic features of the lesions. For the clinical evaluation of the lesions, the ABCD clinical criteria for early detection of melanoma[8] were used. The dermoscopic evaluation was performed using the ABCD rule of dermoscopy proposed by Stolz et al,[9] which is based on the evaluation of 4 criteria: asymmetry (A), abrupt borders (B), colors (C), and differential dermoscopic structures (D). The total dermoscopy score (TDS) was calculated in each lesion.

The global pattern classification was made according to the subtypes proposed in the pattern analysis[10] for the evaluation of melanocytic lesions and their differentiation between benign and malignant. These global patterns are reticular, globular, homogeneous, "starburst," parallel, multicomponent, and unspecific.

Our Melanoma Unit, which is composed of a multidisciplinary team, belongs to the Dermatology Department of the Hospital Clinic of Barcelona, Barcelona, Spain. The hospital is a tertiary and high-complexity center that provides service for the public health system in Catalonia (population of about 7 million), where a network of melanoma centers is integrated by main hospitals. Almost 30% of melanomas in Catalonia are attended directly in our unit. Patients who have been examined by primary care physicians and/or area dermatologists are referred according to a specific derivation protocol that includes filling out a referral form and a schedule of visits within 48 hours for the evaluation of highly suspicious lesions or within 2 months for their incorporation in a follow-up program once they have been identified as high-risk individuals. The criteria for inclusion in our follow-up program include moderate to severe AMS, presence of a congenital nevus of medium to giant size, AMS and previous melanoma, familial melanoma, presence of genetic mutations related to melanoma risk, and syndromes associated with melanoma risk.

As standard practice in our unit, at every visit the patients undergo a complete clinical examination with a handheld dermoscope (Dermlite DL100 and Dermlite II Pro Hybrid; 3Gen LLC, Dana Point, California).When necessary, a digital record of atypical lesions is performed with a digital dermoscopic device (MoleMax II; Derma Medical Systems, Vienna, Austria) to assess whether follow-up should be short, medium, or long term, according to the judgment of the evaluator. High-risk patients are included in a follow-up program with total-body photographs and digital dermoscopy, according to the 2-step method previously described,[11] with follow-up visits once or twice a year. In our study, 8 and 32 patients were scheduled for follow-up once and twice a year, respectively. Once a suspicious lesion is identified, a high-resolution dermoscopic photograph (Dermlite Foto; 3Gen LLC) is taken before surgical excision.

Because this was a retrospective study and had no influence on the established clinical treatment of patients, no ethics committee approval was required. Each patient's written consent was obtained for all invasive procedures.

ANALYSIS OF VARIABLES

Analysis of variables included clinical information about the patient (sex, age at diagnosis, personal and familial history of melanoma, nevi count, presence of AMS, skin phototype, hair and eye color, degree of lentiginosis, presence of solar elastosis, and personal history of basal cell carcinoma); clinical information about the lesion (clinical ABCD, clinical stage at diagnosis); dermoscopic features (ABCD rule of dermoscopy, TDS, global pattern); and histologic characteristics (histological subtype, Breslow index, Clark level, association with melanocytic nevus, and presence of ulceration).

STATISTICAL ANALYSIS

The χ^2 test was used to compare qualitative variables, applying Fisher correction when needed because of the small sample size in tables of 2×2, and the *t* test was used to compare means. Differences were considered to be statistically significant at $P \leq .05$.

RESULTS

Of the melanomas diagnosed in 2007 and 2008 in our Melanoma Unit, 215 fulfilled the inclusion criteria of the study, 50 (23.3%) corresponded to clinical suspicious lesions diagnosed in follow-up in our unit (FUMMs), and 165 (76.7%) corresponded to clinically suspicious lesions referred for evaluation (RMMs).

POPULATION

Of the 201 patients (105 men and 96 women) who were diagnosed as having melanoma in the study, 40 were included in the follow-up program in our unit, and 161 were referred for evaluation. The distribution according to sex was homogeneous in both groups. The mean age of the patients with FUMMs was significantly lower (49.9 years vs 61.7 years) than that of the patients with RMMs ($P < .001$).

Twenty-four patients (60%) included in the follow-up were diagnosed as having melanoma before our study began, compared with only 8 of the patients (5.0%) who were referred to our unit ($P < .001$). Atypical mole syndrome was more frequent among patients in follow-up, who also had a higher nevi count ($P < .001$). Three patients with xeroderma pigmentosum were followed up

Table 1. Characteristics of the Study Population

Variable	Follow-up Patients (n=40)	Referred Patients (n=161)	*P* Value[a]
Sex, No. (%)			.86
Male	20 (50)	85 (52.8)	
Female	20 (50)	76 (47.2)	
Age, mean (range), y	49.9 (23-83)	61.7 (23-95)	<.001
Melanoma before the study, No. (%)	24 (60)	8 (5)	<.001
AMS, No. (%)	12 (30)	22 (13.7)	<.001
Nevi count, No. (%)			<.001
<50	7 (18)	99 (61.5)	
50-100	12 (30)	54 (33.5)	
100-200	15 (38)	7 (4.3)	
>200	6 (15)	1 (0.6)	
Skin phototype, No. (%)			.59
I	4 (10)	15 (9.3)	
II	20 (50)	68 (42.2)	
III	16 (40)	78 (48.4)	
IV	0	3 (1.9)	
V	0	0	
VI	0	0	
Eye color, No. (%)			.55
Blue	8 (20)	27 (16.8)	
Green	3 (8)	22 (13.7)	
Brown	29 (72)	112 (69.6)	
Hair color, No. (%)			.64
Red	0	5 (3.1)	
Blonde	7 (18)	23 (14.3)	
Brown	30 (75)	124 (77.0)	
Black	3 (8)	9 (5.6)	
Lentiginosis, No. (%)			.65
No	12 (30)	64 (39.8)	
Mild	18 (45)	63 (39.1)	
Moderate	3 (8)	24 (14.9)	
Severe	7 (18)	10 (6.2)	
Solar elastosis, No. (%)	11 (28)	25 (15.5)	.10
History of basal cell carcinoma, No. (%)	18 (45)	70 (43.5)	.86
Family history, No. (%)			<.001
Melanoma	10 (25)	15 (9.3)	
Melanoma and AMS	3 (8)	1 (0.6)	
AMS	5 (12)	11 (6.8)	

Abbreviation: AMS, atypical mole syndrome.
[a] χ^2 Test.

Table 2. Clinical Characteristics According to ABCD Algorithm

Variable	No. (%)		*P* Value[a]
	Follow-up Melanomas (n=50)	Referred Melanomas (n=165)	
A, asymmetry	35 (70)	142 (86.0)	.18
B, irregular borders	27 (54)	127 (76.9)	.02
C, multiple colors	23 (46)	126 (76.3)	<.001
D, diameter >6 mm	22 (44)	141 (85.4)	<.001
A+B+C+D	6 (12)	105 (63.6)	<.001

[a] χ^2 Test.

Table 3. Dermoscopic Characteristics

Variable	Follow-up Melanomas (n=50)	Referred Melanomas (n=165)	*P* Value[a]
ABCD rule of dermoscopy			
A, asymmetry[b]	2.13	2.48	<.001
B, abrupt borders[b]	0.37	0.53	.003
C, multiple colors[b]	1.55	2.05	<.001
D, dermoscopic structures[b]	1.04	1.34	.002
TDS[b]	5.04 (1.70-6.70)	6.39 (2.10-8.70)	.009
Classification according to TDS, No. (%)			<.001
Benign	18 (36)	11 (6.6)	
Suspicious	7 (14)	11 (6.6)	
Malignant	25 (50)	143 (86.6)	
Dermoscopy global pattern, No. (%)			<.001
Multicomponent	8 (16)	103 (61.8)	
Reticular	32 (64)	32 (19.4)	
Unspecific	8 (16)	24 (15.4)	
Starburst	0	3 (1.8)	
Globular	1 (2)	3 (1.8)	
Parallel	1 (2)	1 (0.6)	

Abbreviation: TDS, total dermoscopy score.
[a] χ^2 Test.
[b] Mean value.

in our unit, and 1 patient with albinism was referred for evaluation. No significant differences between the 2 groups were observed in skin phototype (*P*=.59), hair and eye color (*P*=.64), degree of lentiginosis (*P*=.65), presence of solar elastosis (*P*=.10), or personal history of basal cell carcinoma (*P*=.86). Patients in follow-up had more family histories of melanoma and AMS than patients referred for assessment (*P*<.001). Data related to the study population are summarized in **Table 1**.

CLINICAL EVALUATION

Most of the lesions in both groups were clinically asymmetrical: 142 of the RMMs (86.0%) and 35 of the FUMMs (70%) (*P*=.18). Irregular borders, multiple colors, and a diameter larger than 6 mm were found more frequently in RMMs than in FUMMs; these differences were statistically significant (*P*=.02, *P*<.001, and *P*<.01). Only 12% of the FUMMs (n=6) fulfilled the 4 ABCD criteria, while the number was 63.6% in the RMM group (**Table 2**).

DERMOSCOPIC EVALUATION

The FUMMs were less asymmetrical than the RMMs (*P*<.001), with fewer abrupt borders (*P*=.003) and less variety of colors (*P*<.001), and they displayed fewer dermoscopic structures (*P*=.002). The TDS was significantly lower in the group of FUMMs (mean value, 5.04 vs 6.39) (*P*<.009). Eighteen of the FUMMs (36%) were misclassified as benign according to the TDS value, whereas this misclassification happened in only 11 of the RMMs (6.6%). All these differences were statistically significant (**Table 3**). In the FUMM group, 19 melanomas (38%) were diagnosed because of changes in digital follow-up, since they had no specific criteria for malignancy at the time of excision (**Figure**).

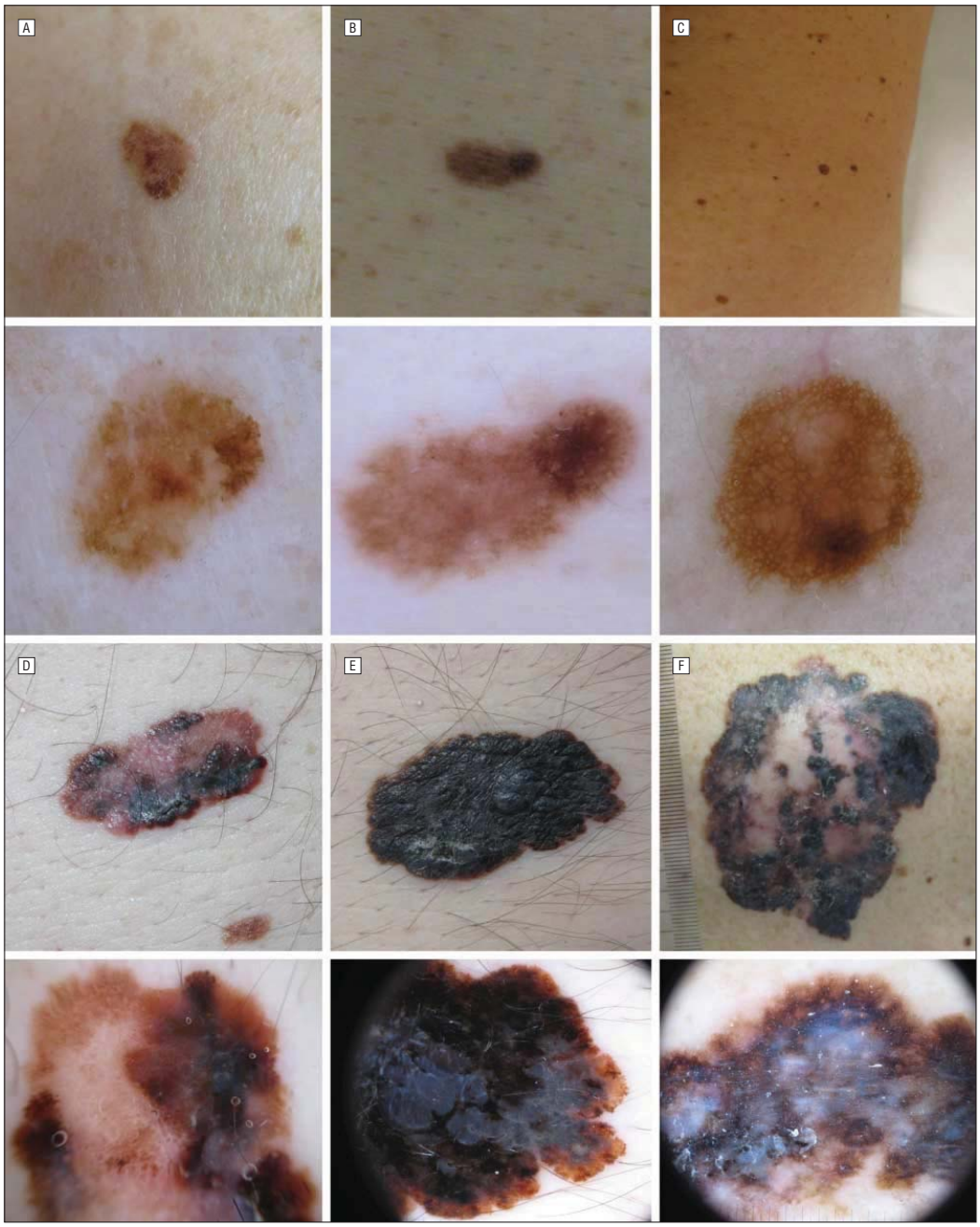

Figure. Examples of melanomas diagnosed in follow-up (A-C) and melanomas referred for evaluation (D-F) with their clinical (top) and dermoscopic (bottom) images. A, In situ melanoma in an 83-year-old man with a history of personal melanoma (total dermoscopy score [TDS], 4.3). B, In situ melanoma arising on a melanocytic nevus in a 48-year-old man (TDS, 2.6). C, In situ melanoma in a 55-year-old man with a history of melanoma (TDS, 4.7). D, Superficial spreading melanoma, Breslow index 0.8 mm, in a 65-year-old man (TDS, 8.7). E, Superficial spreading melanoma, Breslow index 1.8 mm, in a 55-year-old man (TDS, 8.4). F, Superficial spreading melanoma, Breslow index 1.1 mm, in an 84-year-old man (TDS, 8.3).

In the FUMM group, the reticular pattern was the most frequent, observed in 32 melanomas (64%), followed by an unspecific and a multicomponent pattern in 8 cases each (16%) and by a parallel and a globular pattern in 1 case each (2%).The starburst pattern was not observed in the FUMM group. In the RMM group, the most frequent global pattern was the multicomponent pattern, with 102 cases (61.8%), followed by the reticular pattern in 32 cases (19.3%), an unspecific pattern in 24 cases (14.5%), the starburst and the globular pattern in 3 cases each (1.8%), and the parallel pattern in 1 case (0.6%). These differences were statistically significant ($P<.001$).

HISTOLOGIC EVALUATION

Two different dermatopathologists reviewed all the histopathology slides. When there was discordance between the clinical-demoscopic presumptive diagnosis and the histopathologic report, cases were discussed in dermatopathologic conference. A consensus diagnosis was reached in all cases.

Among all melanomas, the most frequent histologic subtype was the superficial spreading type, with 149 cases (69.3%); followed by the lentigo maligna type, with 43 cases (20.0%); the acral lentiginous type, with 18 cases (8.4%); and the nodular type, with 5 cases (3.0%). Except for the nodular melanomas, all of which were RMMs, the distribution of histologic subtypes in both groups was similar, and the differences were not statistically significant ($P=.19$).

Of the FUMMs, 35 (70%) were in situ, while only 46 of the RMMs (27.9%) were in situ. A significantly lower proportion of melanomas in the FUMM group were Clark II or III than in the RMM group, and none of the FUMMs were Clark IV or V ($P<.001$). Among invasive melanomas, the Breslow index was significantly lower in the FUMM group, with a mean (range) of 0.53 (0.25-0.90) mm compared with 1.74 (0.25-13.00) mm in the RMM group ($P<.001$). Histologic ulceration was observed in 23 RMMs (14.1%); none of the FUMMs were ulcerated ($P=.003$). Sixteen of the RMMs (9.7%) and 10 of the FUMMs (20%) developed in association with a preexistent melanocytic nevus ($P=.80$) (**Table 4**).

CLINICAL STAGE AT DIAGNOSIS

The clinical stage of the melanomas was classified according to the American Joint Committee on Cancer staging system.[12] Of the FUMMs, 35 (70%) presented as stage 0 at diagnosis and 15 (30%) as stage IA. Of the RMMs, 46 (27.9%) presented as stage 0 at diagnosis, 62 (37.6%) as stage IA, 21 (12.7%) as stage IB, and 18 (10.9%) as stage II; 14 (8.5%) and 4 (2.4%) presented as stage III and IV, respectively. These differences were statistically significant ($P<.01$).

Table 4. Histologic Characteristics

Variable	Follow-up Melanomas (n=50)	Referred Melanomas (n=165)	*P* Value[a]
Histologic subtype, No. (%)			.19
Superficial spreading	39 (78)	110 (66.6)	
Acral lentiginous	1 (2)	17 (10.3)	
Lentigo maligna	10 (20)	33 (20.0)	
Nodular	0	5 (3.0)	
Breslow index, mean (range), mm	0.54 (0.25-0.90)	1.71 (0.25-13.00)	<.001
Clark level, No. (%)			<.001
I	35 (70)	46 (27.8)	
II	6 (12)	24 (15.4)	
III	9 (18)	44 (26.6)	
IV	0	38 (23.0)	
V	0	13 (7.8)	
Ulceration, No. (%)	0	23 (13.9)	.003
Arising on melanocytic nevus, No. (%)	10 (20)	16 (9.6)	.80

[a] χ^2 Test.

COMMENT

Over the last few decades, efforts in secondary prevention of melanoma have been focused on early recognition and prompt derivation of suspicious lesions. In 1985, the ABCD acronym was designed[8] to provide simple parameters for the detection of suspicious pigmented skin lesions that might require evaluation by a specialist. The sensibility and specificity of these criteria may vary when they are used separately or in combination, and sensitivity decreases as specificity increases.[13] The addition of *E*, for evolution, has substantially improved the ability of clinicians and the general population to detect melanomas at an early stage by recognizing their natural dynamics. The latter criterion is especially important for the diagnosis of nodular melanoma, which frequently, at least initially, is symmetrical, with regular borders and few colors.[14-19] The following EFG acronym has been suggested for the recognition of nodular melanoma: *E* for elevation, *F* for firm, and *G* for growth. Although 35 of 50 of FUMMs were clinically asymmetrical, just 27 of 50 had irregular borders, 23 had multiple colours or 22 a diameter greater than 6 mm, and only 6 fulfilled the 4 ABCD clinical criteria, which raises the question of their usefulness in the recognition of early malignant lesions. In our study, 28 of the lesions in the FUMM group (56%) had a diameter equal to or less than 6 mm, which supports the current main critique of the ABCD clinical system by pointing out that a significant proportion of malignant melanomas may be less than 6 mm in diameter and that they have different aspects and begin as small lesions. No nodular melanoma was diagnosed in patients included in follow-up during the study; this may be explained by the small sample size and the relatively short term of follow-up, which was not sufficient to include the possibility of the occurrence of an early nodular melanoma.

The FUMMs had a lower TDS than the RMMs (5.00 vs 6.42) according to the ABCD rule of dermoscopy proposed by Stolz et al,[9] since they were less asymmetrical, with fewer abrupt edges, fewer colors, and fewer dermoscopic structures. In our study, only 50% of the FUMMs but almost 90% of the RMMS were correctly classified as malignant according to the TDS value, which indi-

cates that melanomas that are difficult to diagnose even with dermoscopy can be detected during follow-up. Furthermore, it should be noted that more than half of FUMMs were small lesions (≤6 mm in diameter); in 2001, Pizzichetta et al[20] reported that the ABCD rule did not seem useful in managing small melanocytic skin lesions, in which specific criteria for melanoma might not yet be present. Whether structured algorithms might be more useful in the assessment of these early lesions is a matter for further analysis.

The multicomponent pattern is defined by the combination of 3 or more distinctive dermoscopic structures within a given lesion.[10] This pattern was observed in more than 60.0% of RMMs but in only 16% of FUMMs; in the latter group, the reticular pattern was the most frequent (64%). If we consider that in the patients under surveillance malignant melanomas are diagnosed in an early phase of tumor progression, when lesions are smaller and display fewer structures and thus have a lower TDS value, it is reasonable to expect that incipient lesions preserve their original structures and that with tumor progression other features of malignant melanomas may appear, elevating the TDS value.

The inclusion of high-risk patients in specific digital programs has proved to be useful as a strategy in early melanoma detection, not only allowing the diagnosis of lesions with a low index of suspicion but also leading to a reduction in the biopsy rates and improving the benign to malignant ratio of the excised lesions.[3,21-24] Most melanomas diagnosed in patients under surveillance in a digital program were in situ; they were thinner among invasive ones; and none were ulcerated. None of the 50 FUMMs required sentinel lymph node biopsy.

In a similar study held in New Zealand, Barker et al[25] compared melanomas referred by general practitioners and those identified in specialist clinics. They found 49% in situ melanomas and a mean Breslow index of 0.57 mm among melanomas detected at plastic surgery or dermatology clinics and 33% in situ melanomas and a mean Breslow index of 1.45 mm among melanomas referred by general practitioners. Recently, in our region, Marcoval et al[26] conducted a study to analyze the changes in incidence of melanoma. They found 30.94% in situ melanomas and a mean Breslow index of 1.86 in the melanomas diagnosed between 1998 and 2006 in another tertiary-level hospital in Catalonia; both values are very similar to those found in the group of melanomas referred to our unit.

Beyond the differences in personal and family history in the risk for melanoma, the mean age of patients diagnosed as having melanoma during follow-up was significantly lower than that of the patients referred to our unit, a finding that could have 2 possible explanations: first, the presence of high risk for melanoma could be associated with the occurrence of melanoma at an earlier age, and second, surveillance aids in the the early diagnosis of melanoma, when the patients are younger and the lesions are diagnosed at initial stages.

Our study does not lack limitations because lesions referred for assessment in our unit were suspicious enough to justify their derivation. Furthermore, an inestimable number of melanomas might not have been referred because they were not clinically or dermoscopically suggestive of melanoma or because they were thin melanomas that were excised without derivation to a referral center. The age of the 2 groups was not equal; it was higher in the RMM group, which could also make a difference in the 2 population groups.

The present study shows the increasing trend in the diagnosis of thin melanomas in our population. This increase is attributable to the early recognition and identification of high-risk individuals. Otherwise, those melanomas will evolve and will be diagnosed as thick and evolved lesions, with positive ABCD clinical criteria.

The inclusion of patients who are high risk for melanoma in follow-up programs allows the detection of melanomas in early stages, with good prognosis, even in the absence of clinical and dermoscopic features of melanoma. In the general population without specific surveillance, melanoma continues to be diagnosed at more advanced stages. Our findings suggest that current efforts in public and medical education might have no substantial effect in this group. Further strategies and educational programs may be needed to improve the early detection of these lesions. We believe that high-risk individuals, whenever proper resources are available, should be referred to melanoma centers or qualified institutions for regular follow-up.

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ORIGINAL ARTICLE

Benefits of total body photography and digital dermatoscopy ("two-step method of digital follow-up") in the early diagnosis of melanoma in patients at high risk for melanoma

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Background: Early detection of melanoma is the best way to improve prognosis. Digital follow-up (DFU) programs of populations at high risk could be an efficient strategy for detecting early melanomas with low morbidity.

Objective: We sought to report the added value of the use of the "two-step method" (digital total body photography and digital dermatoscopy).

Methods: This was an analysis of the surveillance of 618 patients at high risk for melanoma included in our DFU program from 1999 to 2008.

Results: A total of 11,396 lesions were monitored (mean 18.44/patient) during a median follow-up of 96 months (median 10 visits/patient). A total of 1152 lesions, 1.86 per patient, were excised. Almost 70% (798) were lesions previously registered at least twice, whereas 356 (30%) were detected and removed in the same visit. During follow-up, 98 melanomas (8.5% of excised lesions) were diagnosed in 78 patients (12.6%). In all, 53 melanomas were in situ (53.3%), whereas invasive (45) showed a Breslow index of less than 1 mm (median 0.5 mm) and none were ulcerated.

Limitations: Because there are no control groups we cannot determine if the combined use of total body photography and digital dermatoscopy is more beneficial than these techniques used separately.

Conclusion: DFU with total body photography and dermatoscopy in a selected population at high risk demonstrated the early detection of melanomas with a low rate of excisions. Long-term follow-up is required to allow the detection of slow-growing melanomas. Based on our 10-year experience, melanomas can be diagnosed at any time, suggesting that in a population at high risk for melanoma, DFU should be maintained over time. (J Am Acad Dermatol 10.1016/j.jaad.2011.04.008.)

Key words: atypical mole syndrome; dermatoscopy; follow-up; imaging techniques; malignant melanoma; outcome.

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Malignant melanoma (MM) may be clinically and dermatoscopically indistinguishable from melanocytic nevi making early recognition a diagnostic challenge, especially in incipient lesions.[1] Dermatoscopic documentation of melanocytic lesions for the comparison of current and previous images in search of subtle changes over time, known as digital follow-up (DFU), has been shown to be helpful in the diagnosis of early melanomas for which specific criteria for MM may not yet be present.[2]

The use of baseline regional photographs, namely total body photography (TBP), might facilitate the detection of new lesions, and visual changes in pre-existing lesions, by providing a comparative reference point of areas of skin for subsequent examinations.[3] Nevertheless, it has been suggested that a screening strategy focused solely on atypical nevi will likely misdiagnose MM presenting as new lesions or corresponding to lesions not considered adequate for DFU.[4]

CAPSULE SUMMARY

- Digital dermatoscopy follow-up is the most reliable and efficient approach to detect incipient melanoma.
- The combined use of total body photography and digital dermatoscopy (two-step method of digital follow-up) allows the detection of melanomas in early stages with a significant reduction of excisions.
- Long-term follow-up is required to allow the detection of slow-growing melanomas. In a population at high risk, digital follow-up should be maintained over time.

The combined use of TBP and digital dermatoscopy, called the "two-step method" of DFU,[5] has been proposed by our group as an approach for the assessment of individuals at high risk, being potentially more accurate than the two strategies separately.

This study aims to report our 10-year experience at the Melanoma Unit of Hospital Clinic of Barcelona, Spain, using the latter approach in the prospective follow-up of patients at high risk for melanoma included in our specific surveillance program. Our study not only endorses findings from other working groups but also shows new and relevant data derived from the long follow-up period, which is more than twice as long as that reported in previous studies,[6,7] of a cohort of more than 600 individuals with more than 11,000 lesions evaluated.

METHODS

Study population

A total of 629 patients included in the surveillance program with TBP and digital dermatoscopy at the Melanoma Unit of Hospital Clinic of Barcelona, Spain, were followed up between January 1999 and December 2008.

The criteria for patient inclusion in our follow-up program include: moderate to severe atypical mole syndrome (AMS) (defined by >100 nevi and/or >10 clinically atypical according to ABCD criteria, and/or any histologically dysplastic nevi), personal and/or familial history of MM, carriers of high susceptibility for MM gene mutations, and other cancer risk conditions, ie, presence of congenital nevus of medium to giant size, immunosuppression, or genodermatosis (eg, xeroderma pigmentosum, Gorlin-Goltz syndrome) associated or not to AMS.

Patients included in this analysis should have at least two follow-up visits with a minimum of 12 months of surveillance. A total of 11 patients were initially excluded because they did not fulfill these criteria in follow-up.

The study was conducted according to the Declaration of Helsinki and with institutional approval. Patient's written consent was obtained for all invasive procedures.

Examination procedure: Baseline and follow-up registries

In the first visit, a complete clinical history was recorded, including familial history, previous excised melanocytic lesions, and other MM-associated risk factors.

The baseline DFU examination consisted of two steps: the first step, total body mapping, for clinical examination of the patient and total body mapping with digital images; and the second step, digital dermatoscopy, for clinical and dermatoscopic examination in real time of all individual lesions. Digital storage of dermatoscopy images of each lesion showing atypical features was performed. Total body mapping standardized registry was made according to the two-step method of DFU[5] published by our group.

The follow-up examination included: the first step (total body mapping) for comparison of total body images with previous registries to detect any changes in shape, color, or surface eventually occurring in any pigmented skin lesions, and for identification of new lesions, and the second step (digital dermatoscopy follow-up), for dermatoscopic comparison and storage of lesions with atypical features, and for the

Abbreviations used:

AMS: atypical mole syndrome
DFU: digital follow-up
MM: malignant melanoma
TBP: total body photography

clinical and dermatoscopic examination of eventual new lesions not previously registered.

Follow-up visits performing only the second step, digital dermatoscopy follow-up, with no registries of total body mapping were eventually made in the surveillance of selected patients with low or moderate risk, or for monitoring the progress of specific lesions.

Every examination was performed by an expert in dermatoscopy for a total time of 30 to 45 minutes per patient. Images were obtained using a standardized digital system (MoleMax, Derma Instruments, Vienna, Austria). Patients were scheduled for follow-up in 3, 6, or 12 months according to the judgment of the professional who performed the evaluation. Short-term follow-up (3 months) was considered for individual suspicious melanocytic lesions that did not satisfy the dermatoscopic criteria for the diagnosis of melanoma, whereas medium- and long-term follow-up (6 and 12 months) was considered for the surveillance of patients with high or moderate risk, respectively, according to inclusion criteria.

Inclusion criteria for melanocytic lesions to DFU

Melanocytic lesions with atypical clinical or dermatoscopic features were stored on the digital system. Lesions with clear-cut dermatoscopic features of MM (as described in pattern analysis,[8] the ABCD rule of dermatoscopy,[9] or the 7-point checklist[10]) were not registered for follow-up, nor were lesions with definite dermatoscopic features suggestive of benign nevi. Lesions remitted for excision just after our first examinations were excluded from this analysis because they were not part of the follow-up; 16 MMs were detected in 14 patients in the initial visit.

Lesions considered for excision and histopathological study

Any lesion showing the following changes detected by digital dermatoscopy was excised and histopathologically diagnosed: (1) asymmetric enlargement in size; (2) changes in dermatoscopic structures (variation in shape; expansion or decrease of pigment network; variation in the distribution or number of dots/globules; modification of depigmented areas or regression structures; appearance of streaks, scarlike areas, blue-whitish veil, and atypical vessels); (3) increase in the number of colors; (4) regression features affecting more than 50% of the lesion; and (5) focal pigment modifications. All new or not previously registered lesions observed during follow-up and exhibiting atypical features but no criteria for MM were registered and included in follow-up; lesions displaying criteria for MM were removed.

In all, 22 benign lesions were removed because of practical or aesthetic criteria according to either the patient's or physician's judgment. Because they were not suggestive of atypical melanocytic lesion or MM and therefore, not part of the follow-up, they were excluded from the study. All these lesions were confirmed histopathologically as benign lesions.

Histopathology procedure

All lesions removed were step-sectioned and processed for standard histopathological examination. Conventional hematoxylin-eosin staining and immunohistochemistry (Melan A, human melanoma black 45, Ki67) were performed in lesions that were removed, and whenever it was considered necessary by two pathologists. Histology criteria of atypia were reported according to the National Institutes of Health Consensus Conference (1992).

Genetic testing

Genetic studies were performed after informed consent and proper genetic counseling in patients with history of multiple primary and/or familial multiple MM. Exons 1alfa, 1beta, 2, 3; intronic change IVS2-105 and −34G>T at the *CDKN2A* promoter region, and Exon 2 from *CDK4* were studied by PCR-SSCP analysis and sequencing. *MC1R* was studied by direct sequencing as previously reported.[11]

Compliance

Patient's compliance was assessed according to the continuity in the follow-up program. Patients who were excluded from the program and continued with clinical and dermatoscopic examination, left the program, or died were identified.

Statistical analysis

Bivariate analysis was performed to assess differences in patients who were given the diagnosis of melanoma during follow-up and those who were not; the χ^2 test was used for the comparison of qualitative variables, applying Fisher correction according to the sample sizes' need in tables of 2×2

and the Student *t* test was used to compare means of the quantitative variables. Differences were considered to be statistically significant when *P* was less than .05. Multivariable logistic regression analysis was used to obtain the odds ratio using the forward approach, including in the model one by one those variables with *P* less than .2 in the bivariate analysis.

RESULTS

The surveillance program cohort consisted of 618 patients with a mean age of 37 years (mean SD ± 13.3 years) at time of inclusion in the program; 45.5% were men. According to inclusion criteria, the vast majority of the patients (n = 556) had AMS and only 7.1 (n = 44) had less than 50 nevi associated to other high-risk conditions. Of the patients, 277 had a personal history of MM, including 73 with a history of multiple primary MMs, before the beginning of the study; 8 patients with giant congenital melanocytic nevus and 3 patients affected with xeroderma pigmentosum were followed up in our unit. Almost one third of the patients (n = 178) also had a familial history of MM. Descriptive data regarding nevi count, skin phototype, eye and hair color, lentiginosis, and the presence of genetic mutations are shown in Table I.

Patients were followed up for a median of 96 months (range 13-120 months). During 10 years of follow-up, 6149 visits (4155 with TBP and digital dermatoscopy and 1994 with digital dermatoscopy only) were performed. Each patient was evaluated a median of 10 times (range 2-22) during the course of the study, a median of 7 visits (range 2-17) with TBP and digital dermatoscopy, and a median of 3 intermediate visits (range 0-11) only with digital dermatoscopy. During the study, 78,070 body maps (mean 126.3/patient, range 9-410) and 88,283 digital dermatoscopy images (mean 142.9/patient, range 6-726) were stored.

A total of 11,396 lesions were followed up, a mean of 18.44 per patient (1-60). Among those, 1152 lesions, a mean of 1.86 lesions per patient, were excised and remitted for histopathological assessment during the study. In 211 patients no excision was required and in 149 only one lesion was excised in 10 years of follow-up. So, in almost 60% of the cohort, none or only one lesion required excision. In contrast, only 7 patients required 10 or more excisions during surveillance, but they corresponded to patients with personal history of multiple primary MM and familial MM, *CDKN2A* mutations carriers, or patients affected with xeroderma pigmentosum.

Among lesions excised during follow-up, 779 (67.6%) corresponded to lesions previously registered and under surveillance, and 373 (32.4%) corresponded to lesions detected in the visits, which were new or, being already present, were not previously counted for register in DFU. Histopathological diagnosis of melanocytic and nonmelanocytic lesions (initially assumed as melanocytic and thus, registered for DFU) excised in both groups is shown in Fig 1.

During DFU, 98 melanomas (8.5% of excised lesions, benign/MM ratio 10.7:1) were detected in

Table I. Descriptive data of population

Age at inclusion, y	37 (mean SD ± 13.3)
Gender	
Male	281 (45.5%)
Female	337 (54.5%)
Personal history at inclusion	
Melanoma	28 (4.53%)
Melanoma and AMS	245 (39.64%)
AMS	311 (50.32%)
Xeroderma pigmentosum (all with previous MM)	3 (0.5%)
Giant congenital nevus (1 with previous MM)	8 (1.29%)
Others (eg, only familial history of MM, Gorlin-Goltz syndrome)	23 (3.72%)
Nevi count	
<50	44 (7.11%)
50-100	218 (35.30%)
100-200	241 (38.99%)
>200	115 (18.60%)
Phototype	
I	19 (3.1%)
II	249 (40.3%)
III	327 (52.9%)
IV	23 (3.7%)
V	0
VI	0
Eyes color	
Blue	80 (12.9%)
Green	76 (12.3%)
Brown	445 (72.0%)
Black	17 (2.8%)
Hair color	
Red	26 (4.2%)
Blonde	84 (13.6%)
Brown	463 (74.9%)
Black	45 (7.3%)
Lentiginoses	
Mild	209 (33.8%)
Moderate	97 (15.7%)
Severe	72 (11.7%)
No	240 (38.8%)
CDKN2A mutation	39 (11.5% of studied)
MC1R polymorphism	163 (75.1% of studied)
V60L	42
V92M	17
R151C	28

AMS, Atypical mole syndrome; *MM*, malignant melanoma.

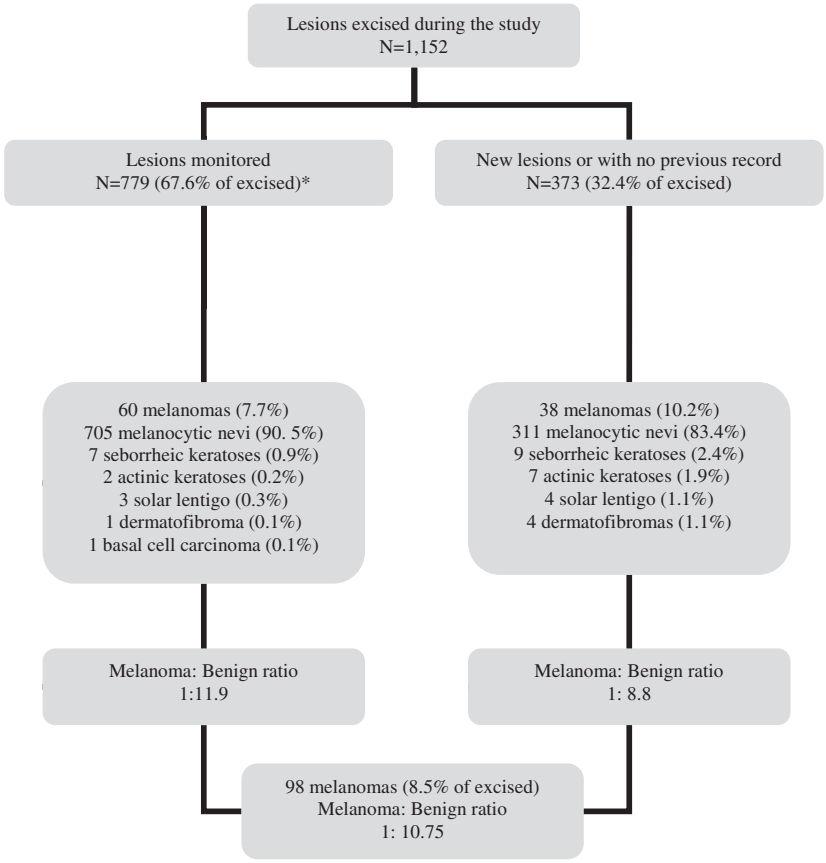

Fig 1. Lesions excised during study. *Corresponded to 6.8% of all monitored lesions.

78 patients; 60 MMs corresponded to monitored lesions (7.7% of registered lesions, benign/MM ratio 11.9:1) (Fig 2) and 38 to lesions with no previous digital record (10.2% of new or unregistered lesions, benign/MM ratio 8.8:1) (Fig 3). MMs detected as a result of changes in digital dermatoscopy required a median of 4 (range 2-15) consecutive controls and a mean follow-up time of 23.9 months (range 1-77 months); of these, 16 arose in a previous nevus, but 44 did not show any evidence of a pre-existing nevus upon histopathology.

Histopathologically, 53 MMs were in situ (53.3%); among invasive MMs, the median Breslow index was 0.5 mm (mean 0.62 mm) and no MM detected during follow-up was thicker than 1 mm or ulcerated, that is, all invasive MMs were staged in IA (American Joint Committee on Cancer 2009).

A total of 1015 melanocytic nevi were excised during the study, almost half with some degree of histologic atypia (18.7% mild, 23.8% moderate, and 6% severe). On histologic examination, 45.4% exhibited regression, inflammatory changes, Sutton phenomenon, or fibrosis that could explain dermatoscopic changes during monitoring.

During follow-up, 78 patients, 12.6% of the cohort, were given the diagnosis of MM. Patients given the diagnosis of MM during DFU were more frequently men ($P = .02$), who were older at the beginning of the study ($P < .001$), with a higher number of lesions monitored ($P < .001$), and a higher number of lesions excised during DFU than those who were not given the diagnosis of MM; no significant differences in length of follow-up between the two groups were observed. History of MM and multiple MM was more frequent among patients given the diagnosis of MM during surveillance ($P < .001$ and $= .003$, respectively), but no significant differences were found regarding the number of MM before the start. No statistically significant differences were found considering the nevi count in the 4 pre-established categories (<50, 50-100, 100-200, and >200), but patients with more than 100 nevi

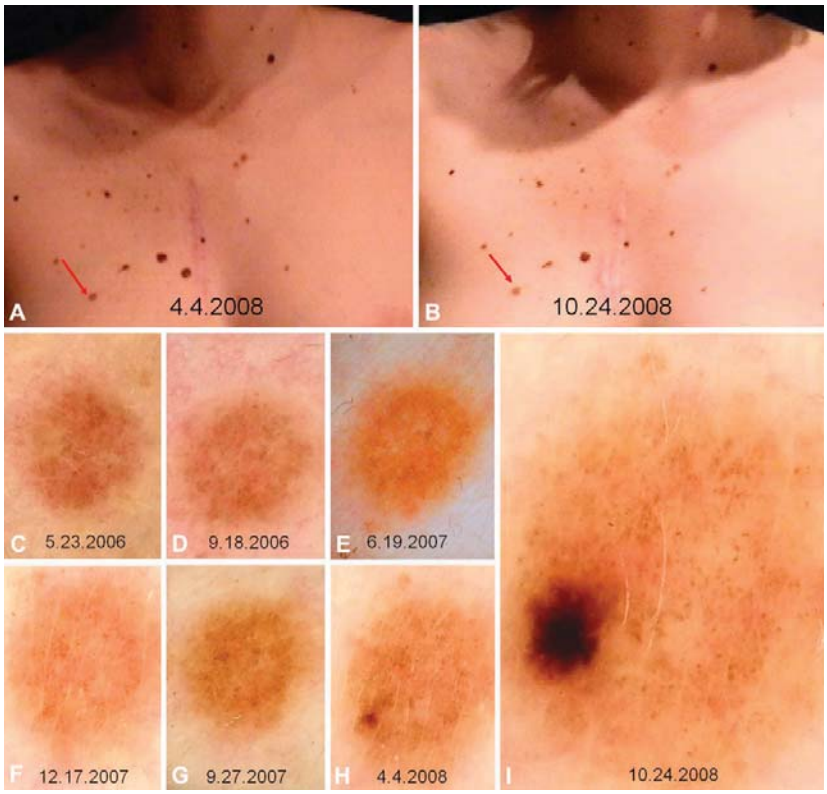

Fig 2. In situ melanoma developed over melanocytic nevus in 23-year-old patient, with personal and familial history of melanoma, given diagnosis as result of changes in digital follow-up. Body mapping images displaying no clinical change (**A** and **B**) and dermatoscopy records in chronological order until excision after 29 months and 7 visits of follow-up (**C** to **I**).

were more frequently given the diagnosis of MM than those with less than 100 nevi (P = .007). As expected, patients with AMS had more MM during follow-up than those without AMS, but differences were not significant (P = .636). No significant differences were found regarding skin phototype, presence and degree of lentiginosis, and presence of *CDKN2A* mutation between the two groups (Table II).

In the multivariable logistic regression analysis (Table III), older age at inclusion and higher number of lesions excised during follow-up were the variables more associated with melanoma diagnosis during DFU (P = .003 and <.001, respectively); male gender, previous melanoma, or the presence of *CDKN2A* mutation were also associated with melanoma during follow-up but differences were not statistically significant. Skin phototype IV and no indication of *CDKN2A* mutation analysis were associated with a lower risk of melanoma during follow-up (P = .033 and <.001, respectively); skin phototype II and III were associated with a lower risk of melanoma than type I, but no statistically significant differences were observed (P = .123 and = .423, respectively).

Regarding DFU compliance, 519 (84.1%) patients continue under surveillance in the follow-up program, 47 (7.6%) were excluded from the program and continue clinical and dermatoscopic examinations in our unit, 38 patients (6.1%) left the program or were referred to dermatologic follow-up at another center, and 14 patients (2.2%) died, 12 because of MM progression, one as a consequence of a heart attack, and one related to Duchenne muscular dystrophy progression.

DISCUSSION

Various strategies have been suggested for MM detection in patients at high risk, such as skin self-examination,[12,13] total cutaneous examination,[14] and the use of TBP[3,15-19] and dermatoscopy.[20,21] It has been well demonstrated that clinical examination is inaccurate for the diagnosis of incipient MM[22]

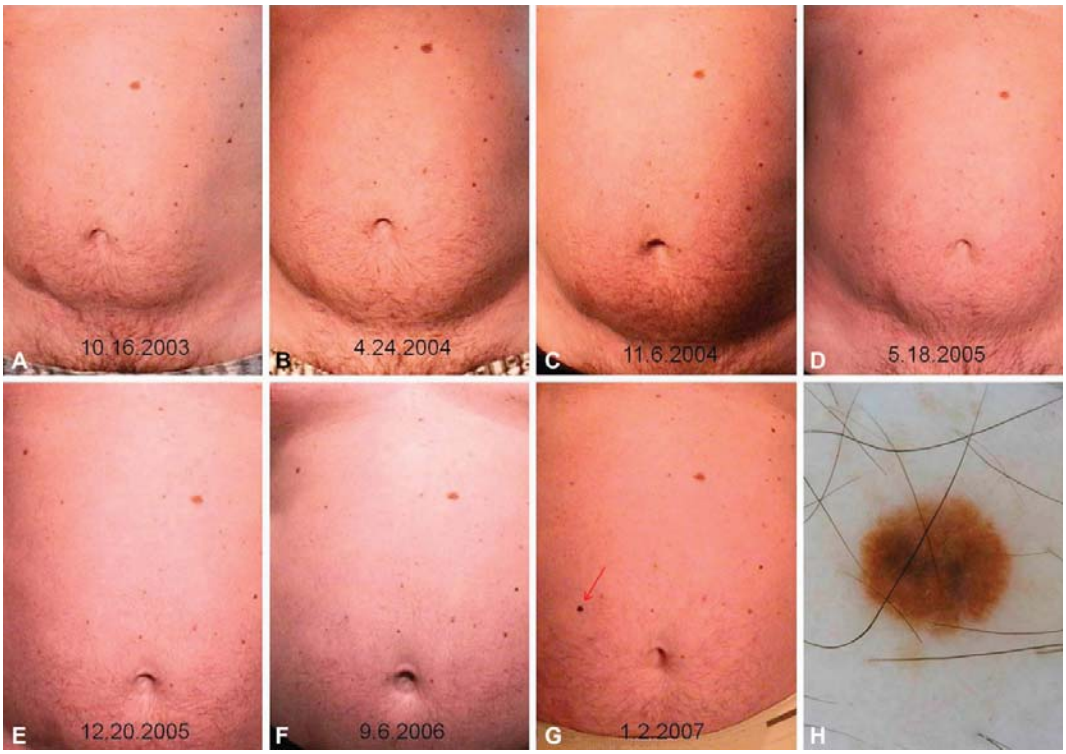

Fig 3. Superficial spreading malignant melanoma, Breslow 0.5 mm, Clark level III, detected as new lesion during total body mapping comparison in abdomen of 48-year-old man, carrier of *CDKN2A* mutation, with history of personal melanoma and familial melanoma and atypical mole syndrome. Body mapping records showing appearance of lesion (**A** to **G**), clinically symmetric and with regular borders. Dermatoscopy image (**H**) showing atypical pigment network, inverted pigment network, and bluish hue.

whereas dermatoscopy has been shown to improve the diagnostic accuracy of nearly all cutaneous tumors including melanoma.[20,21,23]

During the last few years, increasing evidence has accumulated in favor of digital dermatoscopy for the follow-up of atypical melanocytic lesions.[2,6,7,24-30] DFU has proven to be useful in the surveillance of populations at high risk by providing the double benefit of not overlooking MM with few dermatoscopic criteria while minimizing the excision of benign lesions (Table IV).[2]

Because dermatoscopy is not 100% accurate, a certain percentage of suspicious but benign lesions have to be excised to not miss MM. In our study, less than two lesions per patient were excised during a median of 8 years of surveillance with a global MM/benign ratio of 1:10.7 and a MM detection rate of 8.5%, endorsing the fact that DFU is both an efficient and effective strategy for early MM detection in patients at high risk.

The detection of new or clinically changing melanocytic lesions in a population at high risk for melanoma is difficult and almost impossible in patients with a high nevi count unless TBP is available for comparison. Furthermore, it is well known that MM often develops de novo in clinically normal-appearing skin rather than in pre-existing melanocytic nevus.[31]

The two-step method of DFU, routinely used in our unit in the surveillance of patients at high risk for melanoma, consists of the combined performance of TBP and digital dermatoscopy in every visit.[5] We believe that our protocol represents a more complete surveillance approach than those from other working groups, in which DFU is solely focused on digital dermatoscopy of registered lesions. On the other hand, in protocols of digital dermatoscopy in which TBP is performed, body maps are only registered in the first visits, and in subsequent controls body surface is simply compared with overview images.

Table II. Differences between patients who were and were not given diagnosis of malignant melanoma during follow-up

	MM during follow-up						
	No (N = 540)		Yes (N = 78)				
	n	%	n	%	*P* value	OR	(95% CI)
Sex					.020		
Female	304	56.3	33	42.3		1.00	(Reference)
Male	236	43.7	45	57.7		1.76	(1.09-2.84)
Age at inclusion, y					.001		
0-20	51	9.4	5	6.4		1.00	(Reference)
21-40	295	54.6	31	39.7		1.07	(0.40-2.89)
41-60	171	31.7	31	39.7		1.85	(0.68-5.00)
>60	23	4.3	11	14.1		4.88	(1.52-15.66)
AMS					.636		
No	53	9.8	9	11.5		1.00	(Reference)
Yes	487	90.2	69	88.5		0.83	(0.39-1.77)
Previous melanoma					<.001		
No	317	58.7	24	30.8		1.00	(Reference)
Yes	223	41.3	54	69.2		3.20	(1.92-5.33)
Previous multiple melanoma					.003		
No	484	89.6	61	78.2		1.00	(Reference)
Yes	56	10.4	17	21.8		2.41	(1.32-4.41)
No. of melanoma previous to beginning					.070		
1	165	74.3	37	68.5		1.00	(Reference)
2	49	22.1	10	18.5		0.91	(0.42-1.96)
3	5	2.3	3	5.6		2.68	(0.61-11.70)
4	2	0.9	2	3.7		4.46	(0.61-32.69)
5	1	0.5	2	3.7		8.92	(0.79-100.98)
Nevi count					.058		
<50	40	7.4	4	5.1		1.00	(Reference)
50-100	200	37.0	18	23.1		0.90	(0.29-2.80)
100-200	204	37.8	37	47.4		1.81	(0.61-5.37)
>200	96	17.8	19	24.4		1.98	(0.63-6.19)
>100 Nevi					.007		
No	240	44.4	22	28.2		1.00	(Reference)
Yes	300	55.6	56	71.8		2.04	(1.21-3.43)
Phototype					.422		
I	15	2.8	4	5.1		1.00	(Reference)
II	219	40.6	30	38.5		0.51	(0.16-1.65)
III	284	52.6	43	55.1		0.57	(0.18-1.79)
IV	22	4.1	1	1.3		0.17	(0.02-1.68)
Phototype					.966		
I-II	234	43.3	34	43.6		1.00	(Reference)
III-IV	306	56.7	44	56.4		0.99	(0.61-1.60)
Lentigines					.286		
No	214	39.6	26	33.3		1.00	(Reference)
Yes	326	60.4	52	66.7		1.31	(0.80-2.17)
Excised lesions					<.001		
0	211	39.1	0	0.0		—	—
1	135	25.0	14	18.0		1.00	(Reference)
2	70	13.0	14	18.0		1.93	(0.87-4.27)
3	50	9.3	14	18.0		2.70	(1.20-6.06)
4	35	6.5	9	11.5		2.48	(0.99-6.20)
5	15	2.8	5	6.4		3.21	(1.02-10.17)
6	10	1.9	7	9.0		6.75	(2.22-20.52)
≥7	14	2.6	15	19.2		10.33	(4.15-25.74)
CDKN2A					<.001		

Continued

Table II. Cont'd

	MM during follow-up						
	No (N = 540)		Yes (N = 78)				
	n	%	n	%	*P* value	OR	(95% CI)
Negative	239	44.3	61	78.2		1.00	(Reference)
Not performed	272	50.4	7	9.0		0.10	(0.05-0.22)
Positive	29	5.4	10	12.8		1.35	(0.62-2.92)
	Mean	(SD)	Mean	(SD)	*P* value	OR	(95% CI)
Age at inclusion, y	36.2	(12.8)	42.4	(15.5)	<.001	1.03	(1.02-1.05)
No. of controlled lesions	17.6	(8.2)	24.2	(13.0)	<.001	1.07	(1.04-1.09)
No. of excised lesions	1.5	(1.9)	4.3	(3.5)	<.001	1.50	(1.35-1.66)
Length of follow-up, mo	85.3	(29.9)	88.8	(31.0)	.348	1.00	(1.00-1.01)

AMS, Atypical mole syndrome; *CI*, confidence interval; *MM*, malignant melanoma; *OR*, odds ratio.

Table III. Multivariable logistic regression analysis

	OR	(95% CI)	*P* value
Age at inclusion	1.04	(1.01-1.06)	.003
Gender			
Female	1.00	(Reference)	
Male	1.23	(0.68-2.22)	.500
Previous melanoma			
No	1.00	(Reference)	
Yes	1.55	(0.81-2.97)	.181
>100 Nevi			
No	1.00	(Reference)	
Yes	1.37	(0.72-2.60)	.342
No. of lesions excised	1.55	(1.37-1.75)	<.001
Skin phototype			
I	1.00	(Reference)	
II	0.33	(0.08-1.35)	.123
III	0.57	(0.14-2.26)	.423
IV	0.03	(0.00-0.76)	.033
CDKN2A mutation			
No	1.00	(Reference)	
Not performed	0.15	(0.06-0.37)	<.001
Yes	1.39	(0.53-3.68)	.505

CI, Confidence interval; *OR*, odds ratio.

Already in 2007, Fuller et al[4] highlighted that it is unclear in most previous studies whether any MMs were missed because they either presented as new lesions or arose from nevi that were not monitored by dermatoscopy, because the total number of MM occurring in those patients was not reported. In the latter study, only one MM was detected by DFU of 6 MMs detected during a median of 22 months; with a MM/benign lesion ratio of 1:94 and 1:34.4 among lesions with and without previous dermatoscopy record, respectively. In our study, nearly 40% of MMs detected during follow-up corresponded to lesions that were not previously recorded, either because they were newly assessed by TBP or, being already present, they were not atypical, and hence not included for follow-up. MM/benign ratio was, as in the study of Fuller et al,[4] lower among lesions with no previous dermatoscopy record (1:8.8 vs 1:11.9).

The 10-year experience in follow-up of patients at increased risk for MM reported by Haenssle et al[6,7] deserves special attention. As seen in Table IV, general data concerning number of patients, lesions monitored, percentage of lesions excised, malignant/benign ratio, and patients given the diagnosis of MM during the study are remarkably similar to our study. Nevertheless, some differences are clear: first, our median follow-up of 96 months (8 years) is more than twice as long, providing more consistent data in terms of long-term follow-up; and second, unlike their study, we decided not to include lesions excised in the first visit examinations, as they were not part of the follow-up, leaving 16 MMs of the current analysis. Haenssle et al[6,7] found a higher number of MMs in their study (127); if we exclude 40 MMs, which they report to have diagnosed after the first examination, that would leave 87 MMs detected during follow-up, which is more similar to our experience. Another interesting difference is the percentage of MMs detected as a result of dynamic changes during DFU, which is 36.7% (32/87) in their experience but 61.2% (60/98) in ours. No further conclusion can be made because the populations are not equivalent.

Recently, Argenziano et al[32] reported that MM may grow slowly and thus changes can only be seen after long-term follow-up. According to this, we report follow-up as long as 77 months until excision, being almost half of the MM followed up for more than 2 years until showing some significant change in initially featureless lesions. Two findings require special attention; first, 75% of MMs with more than 2 years of follow-up before excision were in situ; and second, almost 65% of MM that required more than 2 years of follow-up showed no pre-existing nevus upon histopathological examination (data not shown). These

Table IV. Comparison of clinical outcomes of our study and those from other working groups

Authors	Lesions-patients, No.	Mean lesions/ patient	Median follow-up, mo	Excisions (%) of lesions registered	Ratio MM/no MM	MM (%) of excisions	Patients given diagnosis of MM during DFU, %
Haenssle et al,[6,7] 2010, Germany	11,137-688	16.18	46	10.9	1:8.5	10.4	11.4
Argenziano et al,[29] 2008, Italy	600-405	1.48	23	9	1:3.4	22.2	3
Fuller et al,[4] 2007, USA	5945-297	20	22	5.4	1:53 PRL 1:95/NPRL 1:34.4	1.9 PRL 1.1/NPRL 2.75	2
Haenssle et al,[25] 2006, Germany	7001-530	13.2	32.2	9.1	1:12	8.3	10
Bauer et al,[26] 2005, Germany (EPL)	2015-196	10.28	25	1.6	1:15.5	6.1	1
Robinson and Nickoloff,[27] 2004, USA	3482-100	34.82	36.2	5.5	1:47.3	2.1	4
Malvehy and Puig,[5] 2002, Barcelona	3170-290	10.93	17.2	1.3	1:4.2	19	2.8
Menzies et al,[30] 2001, Australia	318-245	1.29	3	19.2	1:7.7	11.5	2.9
Kittler et al,[28] 2000, Austria	1862-202	9.21	12.6	4	1:8.4	10.7	4
Current study	11,396-618	18.44	96	10.1	1:10.7 PRL 1:11.9/ NPRL 1:8.8	8.5 PRL 7.7/ NPRL 10.1	12.6

DFU, Digital follow-up; *EPL*, epiluminiscence; *MM*, malignant melanoma; *NPRL*, nonpreviously registered lesions; *PRL*, previously registered lesions.

findings may support the current evidence of the existence of a subgroup of slow-growing MM.

It is well known that the DFU procedure is not only time-consuming but also a technique that requires training, experience, and specific equipment. Chances of success in DFU depend basically on the proper selection of patients.[29] In our study population, with 90% of the patients displaying AMS and almost 45% with previous melanoma, one of 8 developed MM during surveillance, which is more than 1500 times higher than expected in our general population. Not unexpectedly, the percentage of patients given the diagnosis of MM during follow-up increased from 7% among patients with no personal history of MM, to 18% and 23% in patients with one primary MM and multiple primary MM before the inclusion in follow-up, respectively.

The duration of the DFU or the possibility to exclude a patient included in the program after a period with no excisions required have been a matter of debate. According to our results, MM can be diagnosed at any time once a patient is included in the DFU program, and not just at the beginning within the first follow-up examinations. Furthermore, the risk of diagnosing more than one MM during follow-up is relatively high among populations at high risk for melanoma. In light of these findings, maintained surveillance may be required in individuals at high risk.

There is no consensus regarding the most effective melanoma screening strategy in individuals at high risk. Because there are no control groups we cannot convey whether the combined use of TBP and digital dermatoscopy is more beneficial than the TBP, dermatoscopy examination, or DFU separately. Recently, Goodson et al[18] compared their results using TBP and digital dermatoscopy monitoring of nevi in a similar patient population at risk for melanoma and they found that monitoring patients at risk for melanoma using TBP was associated with a lower biopsy rates and lower benign/melanoma ratios than using digital dermatoscopy and facilitated detection of new and changing lesions with a higher MM detection rate during follow-up (4.4% vs 1.9%, respectively). With the use of the two-step method of DFU we achieved a higher melanoma detection rate (8.5%) and a lower nevus:melanoma ratio (9.3 vs 53 with DFU and 22 with TBP). In our study biopsy rate was higher, but this finding may be because of the fact that our median follow-up period is 4 times longer and our population could be considered of higher risk, because incidence of melanoma per patient during follow-up was 6 times higher.

In conclusion, TBP and digital dermatoscopy (two-step method of digital follow-up) in a selected population at high risk for melanoma was shown to allow the detection of melanomas in early stages with a low rate of excisions. This dual modality is useful not only for the detection of MM with few dermatoscopic criteria by DFU of dermatoscopy records, but also for the detection of melanoma either presented as new lesions or arising from nevi that were not monitored by dermatoscopy. Long-term follow-up is required to allow the detection of slow-growing melanomas. Based on our 10-year experience, melanomas can be diagnosed at any time, and not just at the beginning of follow-up, suggesting that in this kind of high-risk population, DFU should be maintained over time.

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ORIGINAL ARTICLE

Desmoplastic melanoma on the nose: electrochemotherapy as an alternative treatment to local advanced disease

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Abstract

Background Desmoplastic malignant melanoma (DMM) is a rare and usually misdiagnosed type of melanoma. Delayed detection at complicated anatomical locations can lead to the necessity of alternative therapies.

Objective Characterization of DMM on the nose, which is the second more frequent type of MM.

Methods Review of case series of eight pathologically proven DMM on the nose from two referral centres with a mean follow-up of 69 ± 40.5 months.

Results According to a single centre experience, there is a more than 70-fold increased risk of having a DMM on the nose compared with a non-DMM ($P < 0.0005$, CI99% 16.3–317.3). Clinical and pathological misdiagnoses were frequent, only three of the eight cases were properly diagnosed and treated and indeed they did not experience relapses. Due to non-clinical suspicion and superficial biopsies, three cases were initially pathologically misdiagnosed as basal cell carcinomas and a nevus respectively. Atypical vessels and remnants of pigment on dermoscopy are indicative findings even in non-pigmented cases. Although not significant, the mean disease-free survival differed between cases with a correct initial management (four cases, 66.7 ± 57.3 months) in contrast to improper (four cases, 16.25 ± 18.9 months). Electrochemotherapy achieved a complete local control of disease in two cases unsuitable for surgery.

Conclusions Use of dermoscopy and correctly selected biopsy of lesions on the face is mandatory to improve early diagnosis of DMM. Improper management of challenging cases implies a more complicated therapy and loco-regional invasion risk. Electrochemotherapy could be a promising therapy in local advanced tumours.

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Conflict of interests

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Introduction

Desmoplastic malignant melanoma (DMM) is a very rare variant of spindle cell melanoma[1] characterized by a heterogeneous clinical–pathological appearance and frequent misdiagnosis.[2] Recent reviews have found a median Breslow thicker than 4 mm and a high tendency of local recurrence. However, it remains uncertain whether it has a relatively better outcome than classic melanoma (MM) adjusted for Breslow thickness[3–6] Recently, the first report on dermoscopic features of DMM emphasized the importance of avoiding misdiagnosis of hypopigmented and clinically unsuspicious scar-like lesions. Frequently, the delayed detection and the tendency of peri-neural invasion make the surgical resection of DMM difficult. Electrochemotherapy is an effective therapy for local tumours that are unsuitable for surgery. It has mainly been applied in cutaneous or subcutaneous metastasis of breast cancer, head and neck cancer and melanoma.[7–11] The application of intense and brief electric pulses to a tissue leads to the transient permeation of cell membranes to otherwise non-per-

Table 1 Desmoplastic melanomas on the nose. Description of case series: clinical, ermoscopical and histopathological details and clinical outcome.

Case number	Gender /Age (years)	Clinical suspicion at onset	Pigment	Dermoscopy	Site on nose	Type of initial biopsy	Initial histopathological diagnosis	First management	Time to recurrence (months)	Type of relapse	Secondary management	Updated AJCC stage – DFS	Overall survival (months)
1	F 74	Solar lentigo	+ Ill-defined polychromic lesion	Irregular rhomboidal structures and follicular occluding. Irregular linear vessels. Whitish scar-like areas (chrysalis-like)	Nasal Tip and Septum	Not performed	Not performed	Cryotherapy (multiple sessions)	60	DMM mixed with LMM (Breslow 0.6 mm, Clark III)	Wide margins excision	IA 35 m	95
2	F 88	Lentigo maligna	+ Variegated pigmentation and ground of erythema	Irregular perifollicular pigmentation. Whitish scar-like areas (chrysalis-like) and 'rosettes' figures. Polymorphous lineal and dotted vessel	Nasal Tip	Punch biopsy	DMM mixed with LMM (Breslow 1.55 mm, Clark IV)	Wide margins excision	–	No recurrence	–	IB 40 m	40
3	M 74	Lentigo maligna	+ Ill-defined polychromic lesion	Irregular perifollicular pigmentation and rhomboidal structures. Erythema and whitish scar-like areas (chrysalis-like)	Nasal Ala	Punch biopsy	DMM mixed with LMM (Breslow 1.4 mm, Clark IV)	Wide margins excision	–	No recurrence	–	IB 56 m	56
4	F 68	Dermal nevus	+ Mild pigmented plaque	–	Nasal Ala	Shaving biopsy	Pure DMM variant, (Clark III, no measurable Breslow)	Wide excision margins	–	No recurrence	–	IB 150 m	150
5	F 67	Dermal nevus	+ Mild pigmented papule	Diffuse light-brown ground with pigmentation within the hair follicle	Nasal Tip	Shaving biopsy	DMM mixed with LMM (Clark III no measurable Breslow)	Simple excision (no wide margins due to patient decision)	41	Amelanotic DMM on scar (dermoscopic atypical linear vessels and whitish scar -like areas)	Wide excision margins and negative SLN biopsy Lymphadenectomy (cervical and submaxillary regions 24 m later)	IIIC 30 m	84
6	M 69	Basal cell carcinoma	No	--	Nasal Back	Punch biopsy	Infiltrating basal cell carcinoma*	Simple excision and free graft reconstruction	24	Amelanotic pure DMM on graft (Breslow >4 mm and Clark IV with marked neurotropism) and lymph node metastasis	Electrochemotherapy (two cycles in 6 weeks) of nasal tumour Lymphadenectomy of nodal disease	IIIC 0 m	63
7	M 78	Basal cell carcinoma	No	Erythematous unspecific plaque	Nasal Back	Punch biopsy	Pure DMM variant Clark IV Breslow > 2 mm with marked neurotropism	Electrochemotherapy (one cycle) of nasal tumour	–	No recurrence	–	IIB 21 m	21 (died due to lung cancer)
8	M 73	Solar lentigo	+	–	Nasal Back	Shaving biopsy	Melanocytic nevus*	Cryotherapy (multiple session in the 2 years after primary biopsy)	24	Pure DMM with marked neurotropism (Clark V infiltrating margins, infraorbitary nerve canal, soft tissue in eye orbitary ground and erosion of bone	Multiple local relapses despite full-thickness skin graft surgery. Progression after Taxol+Avastin. Ipilimumab systemic immunotherapy (anti-CTLA4 immunotherapy, endovenous 3 mg/ kg protocol)	IIIC 0 m	43

Legend; Cases 1–3 were collected from Hospices Civils de Lyon, Centre Hospitalier Lyon-Sud, Pierre-Bénite, France. Cases 4–8 were collected from Hospital Clinic de Barcelona, Barcelona, Spain. DMM, desmoplastic malignant melanoma; M, male; F, female; Y, years; m, months.

*Means 'initial misdiagnosis at histopathological evaluation' as primary biopsies were reviewed when recurred and DMM was confirmed in these.

meable molecules, such as endovenous Bleomycin, administered before the local electroporation of tumoral lesions.

Herein is presented the challenging diagnosis and management of a series of DMM located on a complicated anatomical area such as the nose, and for the first time, we report the possible promising use of electrochemotherapy for local control of primary tumours in locally advanced cases.

Materials and methods

Eight cases of histopathologically confirmed DMM on the nose were retrospectively reviewed, five from the Dermatology Department, Hospital Clínic Barcelona, Spain, and three from the Dermatology Department, Centre Hospitalier Lyon-Sud, Pierre Bénite, France.

Exhaustive study included demographics and the patient's medical record. Particular attention was focused on initial misdiagnosis and delayed detection, definite histopathological evaluation and treatment, highlighting margins of surgical excision and outcome.

Clinical information and clinical images when available were evaluated for their primary presentation. Dermoscopic images (DermliteFoto®; 3 GEN, LLC.Dana Point, CA, USA) were assessed for the presence or absence of melanocytic features, as well as vessels and other non-specific MM structures.

Cases were histopathologically confirmed and reviewed by three pathologists (A.G.-H., Ll.A., J.P.). Special attention was given to histopathological subtypes – pure and mixed DMM – based on the degree of desmoplasia present in the tumour, as described by Busam *et al.*[12] The presence of an associated epidermal non-desmoplastic component and neurotropism was specifically evaluated by means of conventional haematoxylin–eosin and immunostaining with S100, HMB45 and Melan A (MART-1) proteins.

In addition, a retrospective analysis of frequency of DMM on the nose compared with other MM registered in the database was performed in the Barcelona referral centre. This register includes 3994 consecutive primary MM tumours collected since 1988 with the specific anatomical location clearly defined.

Statistical evaluation was carried out using the SPSS statistical software package for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). Descriptive frequencies of all features were studied for the eight cases. Mean follow-up time and disease-free survival was assessed and patients with initial proper handling were compared with those with initial misdiagnosis. In addition, frequencies of anatomical presentation were studied within the 3994 registered cases in the Barcelona database. P-values were calculated based on Chi-square test and Fisher's exact test for categorical variables, and Kaplan–Meier survival test was performed to disease-free survival time depending on DMM histological type and initial management.

Results

Table 1 summarizes the clinical, dermoscopic and histopathological findings of the eight cases with histologically proven DMM on the nose. There were four men and four women, with a mean age of 73.87 ± 6.79 years, range from 67 to 88 (CI 95% 68.2–79.5).

Clinical presentation

Note that only two cases (cases 2 and 3) presented suspicious features of melanoma at baseline time. Initial clinical diagnosis was of melanocytic nevus in two cases, solar lentigo in another

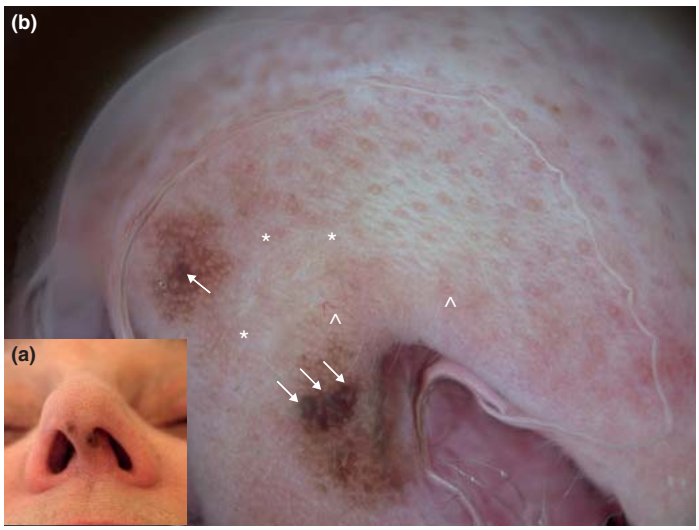

Figure 1 (a) History of cryotherapy on the tip of the nose for clinical 'solar lentigo' in a 74-year-old female. At the time of consultation, dermoscopy (b) showed a multiple colour ill-defined tumour, irregular rhomboidal structures and follicular occluding (arrows), irregular linear vessels (^) and whitish scar-like areas resembling chrysalis (*).Desmoplastic malignant melanoma combined with lentigo maligna, Breslow 0.6 mm, Clark III.

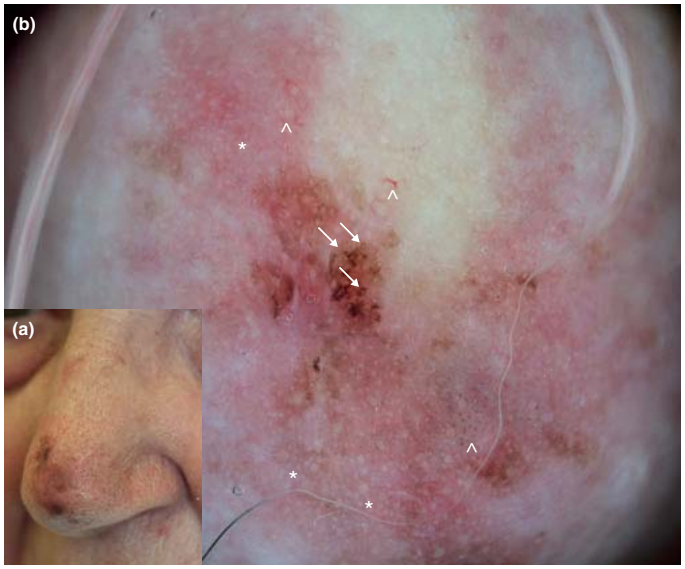

Figure 2 Clinical (a) and dermoscopy (b) in a 88-year-old female (b) demonstrating variegated pigmentation and ground of erythema, with irregular perifollicular pigmentation (arrows), whitish scar-like areas as chrysalis and 'rosettes' figures (*), and polymorphous lineal and dotted vessels (^).Desmoplastic malignant melanoma combined with lentigo maligna, Breslow 1.55 mm, Clark IV.

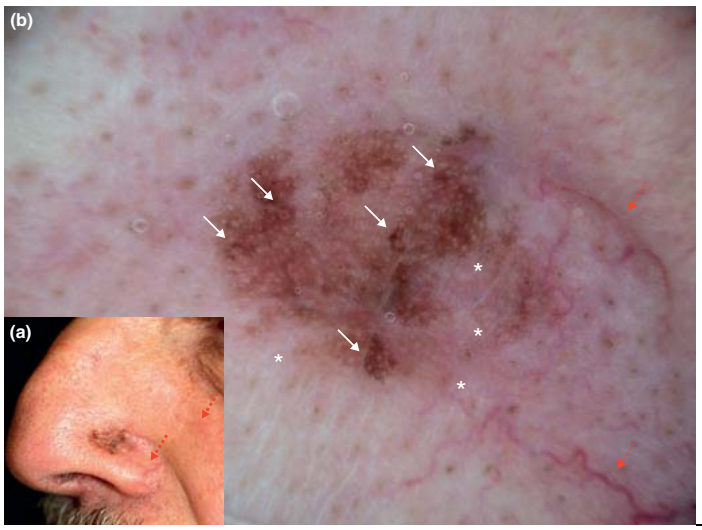

Figure 3 Clinical (a) and dermoscopy (b) in a 74-year-old male showing ill-shaped and multiple colour lesion, irregular and perifollicular pigmentation and rhomboidal structures (arrows), erythema and whitish scar-like areas (chrysalis-like) (*). Note the dilated broad telangiectasias typical of couperosis (red dotted arrow) on this anatomical region. Desmoplastic malignant melanoma combined with lentigo maligna Breslow 1.4 mm, Clark IV.

two and basal cell carcinoma in the remaining two. Cases with dermoscopic evaluation were properly biopsied and DMM could be confirmed at the first handling (Figs 1–4). All local relapses were non-pigmented and mainly presented as infiltrated scar-like tumours (Fig. 4d). Interestingly, the two cases with completely amelanotic tumours (cases 6 and 7) had the most delayed diagnosis and were not suitable for surgery (Figs 5 and 6).

Treatment

Wide margin excision was attempted in all cases after definite confirmation. Unluckily, three cases were considered non-surgical candidates when they were referred to our Unit (detailed in table).

Cases 6 and 7 presented a complete impairment of the anatomical nasal region, with an indurate and painful subcutaneous and ill-defined tumoral infiltration. Electrochemotherapy over

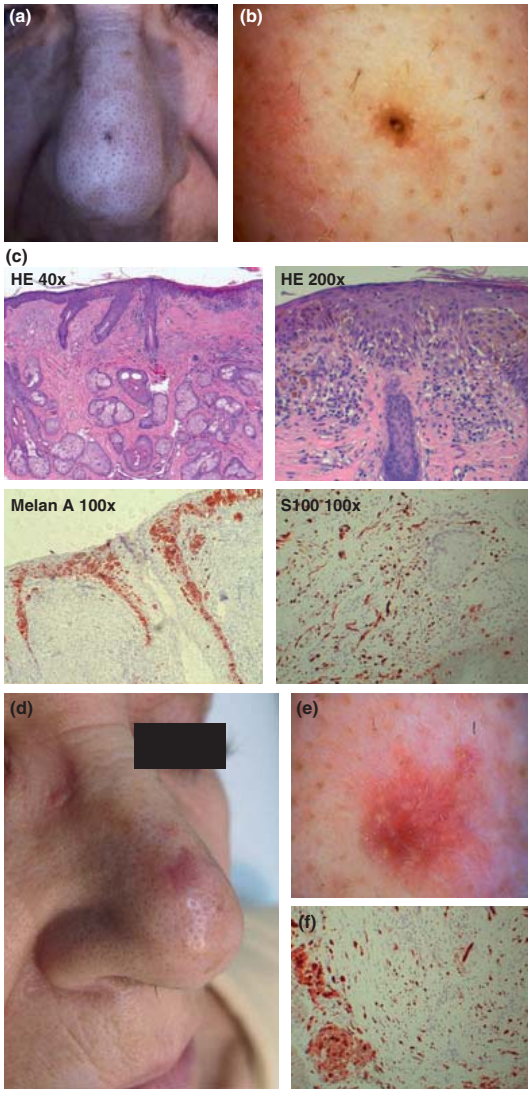

Figure 4 a-b Clinical suspicion of nevus (a) in a 67-year-old female and dermoscopy showing pigmentation within the hair follicle (b).Figure 4c Complete excision confirming a desmoplastic malignant melanoma combined with lentigo maligna. Note Melan A-positive cells on the junctural component, whereas spindle cells in the reticular dermis were only S100 positive. Figure 4d–f Patient refused a wide excision because of cosmetic impairment and 3 years later (d) an infiltrated erythematous lesion appeared on the scar. On dermoscopy (e), atypical linear vessels and whitish scar-like area, (f) S100-positive atypical spindle cells infiltrating the reticular dermis confirmed local relapse.

the nasal area was applied to attempt the relief of symptoms and control tumour growth. Similar to literature described elsewhere, under general anaesthesia, an endovenous bolus of bleomycin (30 mg vial) was administered and between 28 and 48 electric pulses were applied over the cutaneous and subcutaneous areas affected (detailed in table and Figs 5 and 6). The treatment and postsurgical period was well tolerated, they were hospitalized just for 1 day and required non-opioid analgesia. There were no immediate or long-term complications.

In case 6, because of the partial response, a second session of electrochemotherapy was applied 6 weeks later with a progressive reduction in tumour burden and decrease in local pain. Nodal relapse in the cervical and submandibular area presented 8 months later. The two patients treated by ECT showed complete cutaneous response of their tumours. Six months after two sessions in case 6 and just one in case 7, punch biopsies of the treated areas (Figs 5 and 6) were negative for DMM in both cases.

Case 8 presented a broad and invasive tumour with peri and intraneural infiltration of the infraorbitary nerve canal, soft tissue in eye orbitary ground and erosion of bone (Fig. 7). Treatment with Taxol Avastin was not effective, and he is currently undergoing systemic anti-CTLA4 immunotherapy (endovenous ipilimumab 3 mg/kg protocol). He remains with no evidence of distant disease and stabilization of non-surgical stage IIIC melanoma after 43 months of follow-up.

Outcome

The range of follow-up was 21–150 months, with a mean of 69 ± SD 40.5 months (CI 95% 35.6–108.6). The disease-free survival (DFS) time ranged from 21 to 150 months, with a mean of 41.5 ± 47.8.

Local recurrence presented in four of eight cases, all of them with inadequate management at onset of disease ($P < 0.001$). Although not statistically significant, the disease-free survival (DFS) differed between cases with a correct initial handling (four cases, mean DFS 66.7 ± 57.3 months) in contrast to those with improper diagnosis and treatment at tumour onset (four cases, mean DFS 16.25 ± 18.9 months) ($P = 0.14$).

Three of our eight cases presented non-surgical disease when they were referred to our Unit. Two of them (cases 6 and 8) had been previously submitted to reconstructive surgery 2 years earlier using full-thickness skin graft. The surgical treatment was applied for a pathological misdiagnosis of 'basal cell carcinoma' in case 6 and for the relapse over the scar of a supposed 'nevus' in case 8. Histopathological revision confirmed that both were pure DMM, with Breslow greater than 4 mm and Clark level V, with all margins affected.

Risk to develop desmoplastic MM on the nose

Based on one centre's database register (Melanoma Unit of Hospital Clinic de Barcelona) only 16 of 3994 MM were located on

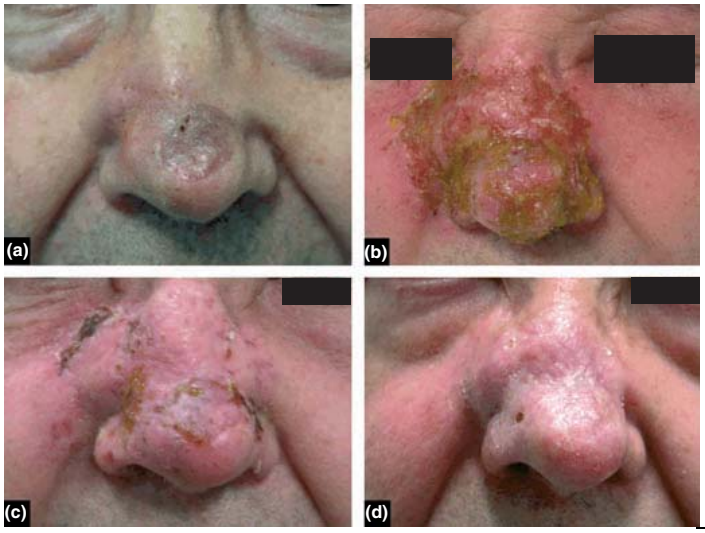

Figure 5 Recurrent desmoplastic melanoma in a 68-year-old male. (a) Erythematous indurated plaque affecting the whole nasal pyramid and the medial cheek. (b) Under general anaesthesia electrochemotherapy (ECT) was performed and 48 electroporation shocks were discharged including the surrounding normal-appearing skin. B, Severe oedema and crust formation followed the first ECT session with rapid healing. (c) The procedure was well tolerated, with no relevant side effects with a progressive reduction in tumour burden. (d) Complete clinical response was achieved in 6 months. Multiple punch biopsy of the scar tissue was negative for melanoma.

the nose (excluding mucosal melanomas) since 1984. Five of 16 melanomas on the nose (31%) were found to be desmoplastic, in contrast to 25 DMM of 3978 melanomas at other sites (less than 0.6%). Thus, according to our single centre experience, this is a more than 70-fold increased risk of having a DMM on the nose (odds ratio 72.07; $P < 0.0005$, CI 99% 16.3–317.3)

Discussion

Desmoplastic malignant melanoma has been considered a challenging diagnosis for clinicians but also pathologists since its first report in 1971 by Conley.[13] To date, the origin of the desmoplastic component with this neurotropism and infiltrative behaviour is intriguing[4,14,15] In our series, three of eight presented clear-cut neurotropic features, especially notable in case number 8, in which DMM invaded orbital ground through the infraorbital nerve canal (Fig. 7).

Desmoplastic malignant melanoma is usually hypopigmented or amelanotic with a scar-like appearance, which makes its clinical recognition more difficult and frequently leads to the incorrect management of incipient cases. Dermoscopy and confocal reflectance microscopy of both pigmented and non-pigmented lesions, especially of sun damaged skin on the face and scalp, could help in the detection and differentiation of these difficult cases that simulate basal cell carcinomas and solar lentigo. In our cases, in addition to the features described by Debarbieux *et al.*[16] from the group of Lyon, we have observed whitish structures such as 'rosettes' figures. This feature, seen exclusively under polarized dermoscopy, had been previously described to be specific to squamous cell carcinoma and actinic keratosis.[17] According to a recent observation by Liebman, rosettes could also be observed in melanoma and basal cell carcinomas and hypothetically they are attributable to an optical effect in adnexal openings either narrowed by fibroplasia or filled with keratin.[18]

Histopathologically DMM can also be easily misdiagnosed as a scar or a non-melanocytic tumour such as neural crest-derived tumours, as the sparse cells are often negative for melanocytic differentiation upon immunohistochemical staining (Melan A, HMB45 or even S100). Histological diagnosis can be even more challenging under circumstances where biopsy specimens are small or superficial, or if there is no clinical suspicion, or re-excision scars. This is particularly true when the overlying junctional component is absent or the spindle cells lack melanin pigment, such reasons could explain the three misdiagnosed cases included in this study.

All our cases were clear-cut DMM by means of haematoxylin–eosin and conventional immunohistochemical staining even the two cases that were misdiagnosed by pathologists from other centres as BCC and melanocytic nevus respectively (original material was reviewed by us).

Desmoplastic malignant melanoma is considered combined or mixed when desmoplastic and classic components are greater than 10% of the lesion.[12] It is well known that a local recurrence of any kind of MM may present desmoplastic findings.[6] It is unclear whether the desmoplastic appearance is present in the MM from the start of its natural history as a DMM or if it is a consequence of the prior incorrect treatment in the cases of combined DMM-lentigo maligna type. Due to the increasing cosmetic concern of the population, it is very important for aesthetic clinicians offering laser and other therapies to be aware of

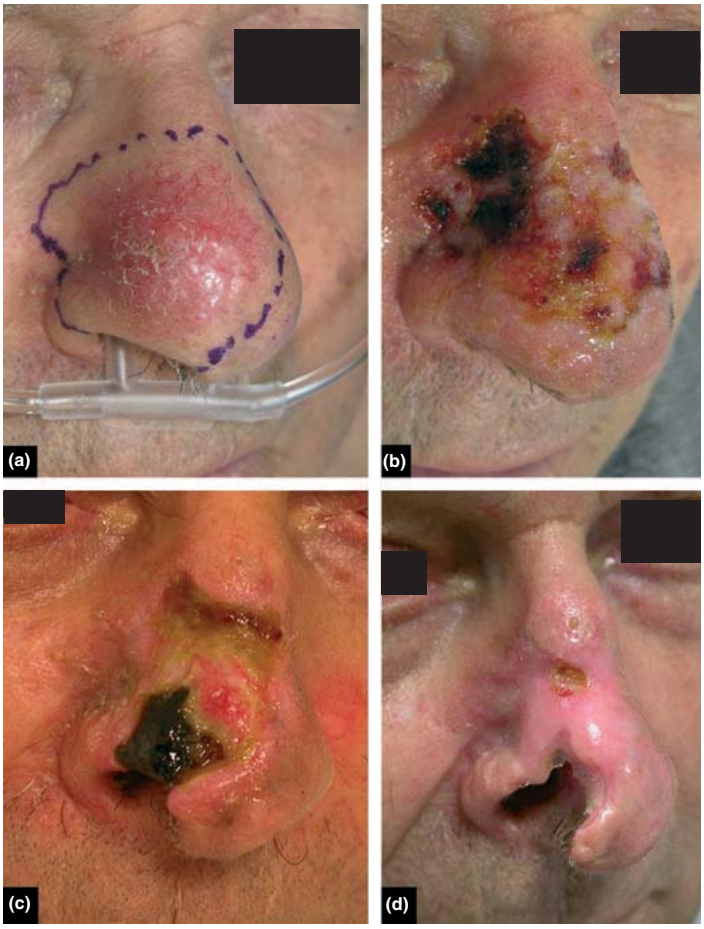

Figure 6 Desmoplastic melanoma on the nose in a 78-year-old male. (a) Infiltrated erythematous plaque occupying the tip, right ala, dorsum and lateral sidewall of the nose. A partial biopsy was consistent with desmoplastic melanoma. (b) Under general anaesthesia, a total of 29 electrochemotherapy (ECT) electric pulses were applied throughout the nasal pyramid. A severe oedema–erythema with crust formation followed the first ECT session with intense inflammation. (c) necrosis and finally (d) partial loss of the right nasal ala. 6 months after performing ECT, the patient presented no evidence of neither clinical nor pathological recurrence.

the possibility of occurrence of DMM after the incorrect treatment of misdiagnosed melanomas as 'solar lentigos'.

Desmoplastic malignant melanoma is rare, but curiously on the nose it is the second most frequent MM type, the most frequent being lentigo maligna melanoma.[19] Based on our single tertiary referral centre database, a MM on the nose has a greater than 70-fold risk of being desmoplastic than on any other anatomical location. Twenty per cent of our cases were located on the nasal region, from a total of 30 DMM (60% of them on the head).

The anatomical limitations of the nasal pyramid makes the management guidelines of MM of the nose poorly defined, and excision margins are often narrower than for MM occurring elsewhere. Surgical reconstruction of defects on the nose requires special considerations.[20] Moreover, in the cases of DMM where the infiltrating behaviour can be even more aggressive, and both clinical and histopathological margins are more difficult to assure.

In our series, four of our eight cases were incorrectly managed and suffered delayed diagnoses before they were referred to a Pigmented Lesions Unit. In three of them, surgery could not achieve complete tumour removal at the moment of evaluation and other therapies had to be considered.

Electrochemotherapy (ECT) is an emerging therapeutic technique that has been applied to date only to non-surgical cutaneous or subcutaneous metastatic melanomas, rather than primary melanoma as we present herein. Although ECT is considered a new effective local tumour ablation modality in the treatment of solid cancers, this combination of electroporation with the administration of cytotoxic drugs was first described in 1991.[21,22] In recent years, ECT with bleomycin or cisplatin has

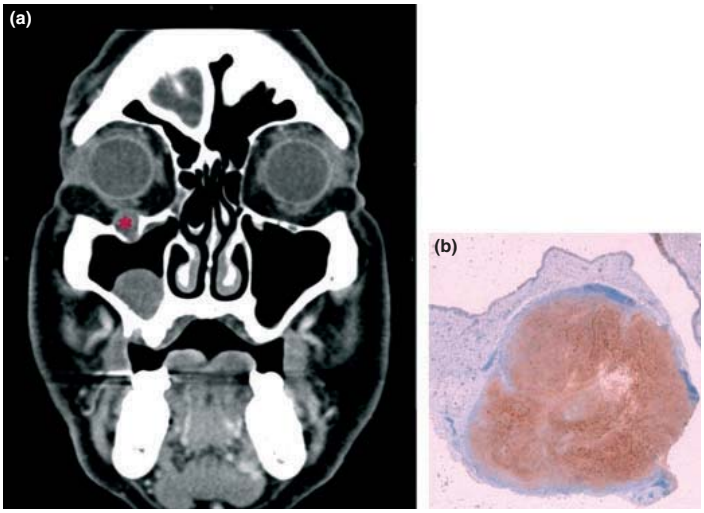

Figure 7 (a) Computerized tomography scan demonstrating the recurrence of desmoplastic malignant melanoma (DMM) infiltrating the infraorbitary nerve canal (*) with bone erosion of orbitary ground in a 73-year-old male patient. Despite multiple surgical attempts, recurrence of this DMM affects cutaneous, soft tissue and bone structures of skull base through peri and intraneural invasions. (b) Immunohistochemical stain for S100 protein demonstrating the presence of DMM infiltrating the infraorbitary nerve.

been proven to be effective on different types of tumours, specially melanoma,[23–28] head and neck cancer and breast cancer.

A multicentre project has been developed (European Standard Operating Procedure of Electrochemotherapy (ESOPE)) to standardize the protocol for treatment and to validate the overall clinical results.[8]As ECT has a demonstrated good safety profile, it is becoming a promising and effective treatment in the palliative management of non-surgical recurrent disease with overall objective response rates of approximately 80–90%.[7–10,28,29]

In the two patients presented in this series, ECT was proposed to control local disease when surgical planning of primary tumour was not able to assure wide enough margins. In another case, wide excision including the removal of the orbital floor and the implant of a titanium plaque to substitute the bone was not able to avoid local relapse, reinforcing the idea that ECT may be a good option at least to be considered when DMM affects structures other than the skin. Both cases achieved complete control of local disease demonstrated by repeated biopsies with no secondary systemic effects. It has been postulated that ECT could enhance the cytotoxic effect of bleomycin and the electric pulses could cause an additional ischaemic reaction of neoplastic vessels.

In conclusion, DMM on the nose region constitutes a challenging diagnosis, and due to the diagnostic delay, added to the anatomical characteristics and the infiltrating behaviour of this type of MM, it is often a therapeutic challenge too. It remains unclear whether prior inadequate ablative treatments, such as cryotherapy, could induce melanoma progression and this desmoplastic reaction in some lentigo maligna melanomas on sun damaged skin.

Our responsibility as dermatologists includes educating patients and physicians about the difficult early diagnosis of melanoma on sun damaged skin, and specially the DMM type and highlight the mandatory biopsy in many cases.[30,31]

In our experience, dermoscopy and other non-invasive diagnostic tools such as confocal microscopy could help to resolve clinical suspicions, to rule out other tumours such as BCC, to recognize some areas of classic lentigo maligna melanoma and finally to select the more appropriate area for partial biopsy if necessary. Electrochemotherapy could be an effective alternative therapy for very invasive tumours that are not suitable for surgery. In our two cases with extended and local infiltrating DMM on the nose, it was a well-tolerated and less morbid way to control local disease. Further studies and longer follow-up periods are needed to recommend this second line therapy.

Acknowledgement

We are indebted to our patients and their families who are the main reason for our studies; to nurses from the Melanoma Unit of Hospital Clínic of Barcelona, Daniel Gabriel, Pablo Iglesias and Maria Eugenia Moliner for helping collect data of patients; and to Helena Kruyer for helping with the English editing and correction of the manuscript.

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STUDY

ONLINE FIRST

Clinical and Dermoscopic Characteristics of Desmoplastic Melanomas

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Objective: To describe and analyze the clinical and dermoscopic characteristics of desmoplastic melanoma (DM) as a function of pathologic subtype and phenotypic traits.

Design: Retrospective case series.

Setting: Eight high-risk dermatology clinics.

Patients: Patients with DM confirmed by histopathologic analysis whose records included a high-quality dermoscopic image.

Main Outcome Measures: Clinical, dermoscopic, and histopathologic features of DM.

Results: A total of 37 DM cases were identified. The majority of patients had fair skin, few nevi, and no history of melanoma. Lentigo maligna was the most frequent subtype of melanoma associated with DM. The most frequent clinical presentation of DM was a palpable and/or indurated lesion located on sun-exposed skin. Forty-three percent of cases were classified as pure DM, and 57% as mixed DM. Pure DM lesions were thicker than mixed DM lesions (4.10 vs 2.83 mm) ($P=.22$) and were less likely to have an associated epidermal non-DM component (63% vs 100%) ($P=.004$). Dermoscopically, DMs had at least 1 melanoma-specific structure, the most frequent being atypical vascular structures. Peppering was more frequently seen in pure DM (44% in pure DM vs 24% in mixed DM) ($P=.29$). In contrast, crystalline structures, polymorphous vessels, and vascular blush were more commonly seen in mixed DM.

Conclusions: Though DM can be difficult to diagnose based on clinical morphologic characteristics alone, dermoscopy has proved to be a useful aid during the evaluation of clinically equivocal lesions or those lesions with a benign appearance. The most common dermoscopic clues observed in DMs included atypical vascular structures, peppering, and occasionally other melanoma-specific structures.

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DESMOPLASTIC MELANOMA (DM) is a rare variant of melanoma, making up less than 4% of all melanomas.[1,2] The overall incidence rate of DM is 2.0 per million, with a peak of 15.2 per million for persons 80 years or older.[3] Typically, DMs are diagnosed later in life than non-DMs. In addition, it is not uncommon for the definitive diagnosis of DM to be delayed because of difficulties in its initial clinical recognition.[4]

Often DM manifests features commonly associated with benign lesions. Based on primary clinical morphologic characteristics alone, it may be difficult for clinicians to recognize DM as a malignant neoplasm. However, dermoscopy may provide clues prompting a biopsy in these otherwise clinically benign-appearing lesions. Although dermoscopy has been shown to increase diagnostic accuracy for most melanoma subtypes, there are limited data regarding the role of dermoscopy in the diagnosis of DM.[5,6] In fact, to our knowledge, there has been only 1 retrospective study that described the dermoscopic features of 6 cases of DM.[7]

Dermoscopy may be a useful aid during the evaluation of DM, and together with a clinical history and examination, it can guide the clinician toward performing a biopsy. The aim of the present retrospective study is to describe and analyze the most common phenotypic traits associated with DM and to evaluate its most frequent clinical and dermoscopic characteristics.

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Table 1. Demographic and Clinical Characteristics of Patients With Desmoplastic Melanomas

Demographic and Clinical Characteristics	Desmoplastic Melanomas[a]			*P* Value
	Pure (n = 16)	Mixed (n = 21)	All (n = 37)	
Age, mean (range), y	67 (48-88)	70 (30-89)	69 (30-89)	.50[b]
Sex				
Male	9 (56)	13 (62)	22 (60)	.75[c]
Female	7 (44)	8 (38)	15 (41)	
Nevi				
Multiple nevi	3 (25)	3 (20)	6 (22)	>.99[c]
Few nevi	9 (75)	12 (80)	21 (78)	
Missing data	4 (25)	6 (29)	10 (27)	
Family history of melanoma				
Yes	2 (17)	1 (6)	3 (10)	.35[c]
No	10 (83)	17 (94)	27 (90)	
Missing data	4 (25)	3 (14)	7 (19)	
Personal history of melanoma				
Yes	3 (20)	6 (29)	9 (25)	.71[c]
No	12 (80)	15 (71)	27 (75)	
Missing data	1 (6)	0	1 (3)	
Personal history of nonmelanoma skin cancer				
Yes	3 (23)	8 (62)	11 (42)	.11[c]
No	10 (77)	5 (39)	15 (58)	
Missing data	3 (19)	8 (38)	11 (30)	

Abbreviation: DM, desmoplastic melanoma.
[a] Unless otherwise indicated, data are reported as number (percentage) of lesions.
[b] Based on the *t* test.
[c] Based on the Fisher exact test.

METHODS

Cases of patients diagnosed with DM were retrospectively selected from 8 melanoma centers in New York, New York (Memorial Sloan-Kettering Cancer Center [MSKCC]), Barcelona, Spain (Hospital Clinic of Barcelona), Lyon, France (Hospitalier Lyon Sud), Graz, Austria (Medical University of Graz), Reggio Emilia, Italy (Arcispedale Santa Maria Nuova), Sydney, Australia (Royal Prince Alfred Hospital), Melbourne, Australia (Alfred Hospital), and Zurich, Switzerland (University Hospital). Cases were included only if there was a confirmed pathologic diagnosis of DM and a high-quality dermoscopic image available. Whenever possible, clinical images were also evaluated. The study was approved by the institutional review board at MSKCC.

All lesions were confirmed to be a DM by histopathologic analysis. In addition, cases were further categorized into 2 histopathologic subtypes (pure DM [pDM] and mixed DM [mDM]) based on the degree of desmoplasia present in the tumor, as described by Busam et al.[8] Specifically, pDMs were defined as having more than 90% desmoplasia, and mDMs had less than 90% desmoplasia. The presence of an associated epidermal non-DM component was also recorded. Neurotropism was evaluated by means of conventional hematoxylin-eosin and, when available, with S100 stain.

Demographic, clinical, and histopathologic information was obtained from the patients' medical records. Patients were assessed for the number of nevi, skin type, personal and family history of melanoma, nonmelanoma skin cancers, and history of chronic sun damage. When clinical images were available, the lesions were evaluated for their primary morphologic characteristics (macule, papule, plaque, nodule), colors (skin color, pink, red, blue, white, brown, and black), borders, and anatomic location.

Dermoscopic images were assessed for the presence or absence of melanocytic structures, including pigment network (gridlike network consisting of pigmented lines and hypopigmented "holes"), aggregated globules (3 or more clustered, well-demarcated, round to oval symmetric structures larger than 0.1 mm; may be brown, black, and blue), streaks (radial projections at the periphery of the lesion extending from the tumor toward the surrounding normal skin; may present as pseudopods or radially streaming structures), and negative network (serpiginous interconnecting hypopigmented lines that surround irregularly shaped pigmented structures resembling elongated, curvilinear globules).[9]

In addition, vascular structures and the presence or absence of melanoma-specific structures were evaluated, including atypical network (increased variability in the width of the network lines, their color and distribution; hole sizes of increased variability), negative network, streaks, atypical dots and/or globules (multiple globules irregularly distributed within the lesion or asymmetrically located off center or focally at the periphery; not associated with the pigmented network), off-center blotch (off-center homogeneous areas of pigment that obscure visualization of any other structures; may be dark brown to black), peripheral tan structureless areas (structureless areas located at the periphery of the lesion larger than 10% of a lesion area), blue-white veil (confluent blue pigmentation with an overlying white "ground glass" haze), regression structures (ie, scarlike depigmentation lighter than the surrounding skin and appearing shiny white under polarized dermoscopy; and/or peppering, which consists of tiny blue-gray granules giving the appearance of a blue-white veil), crystalline structures (shiny, white linear streaks that are often oriented parallel or orthogonal to each other), and atypical vascular structures (including dotted vessels consisting of red dots of 0.01 to 0.02 mm, serpentine vessels consisting of linear irregular or undulating short vessels, polymorphous vessels consisting of a combination of 2 or more vessel morphologic characteristics, corkscrew vessels consisting of coiled or tortuous vessels, milky-red globules and/or vascular blush consisting of ill-defined globules with a milky-red color and ill-defined areas of milky-red color).[9]

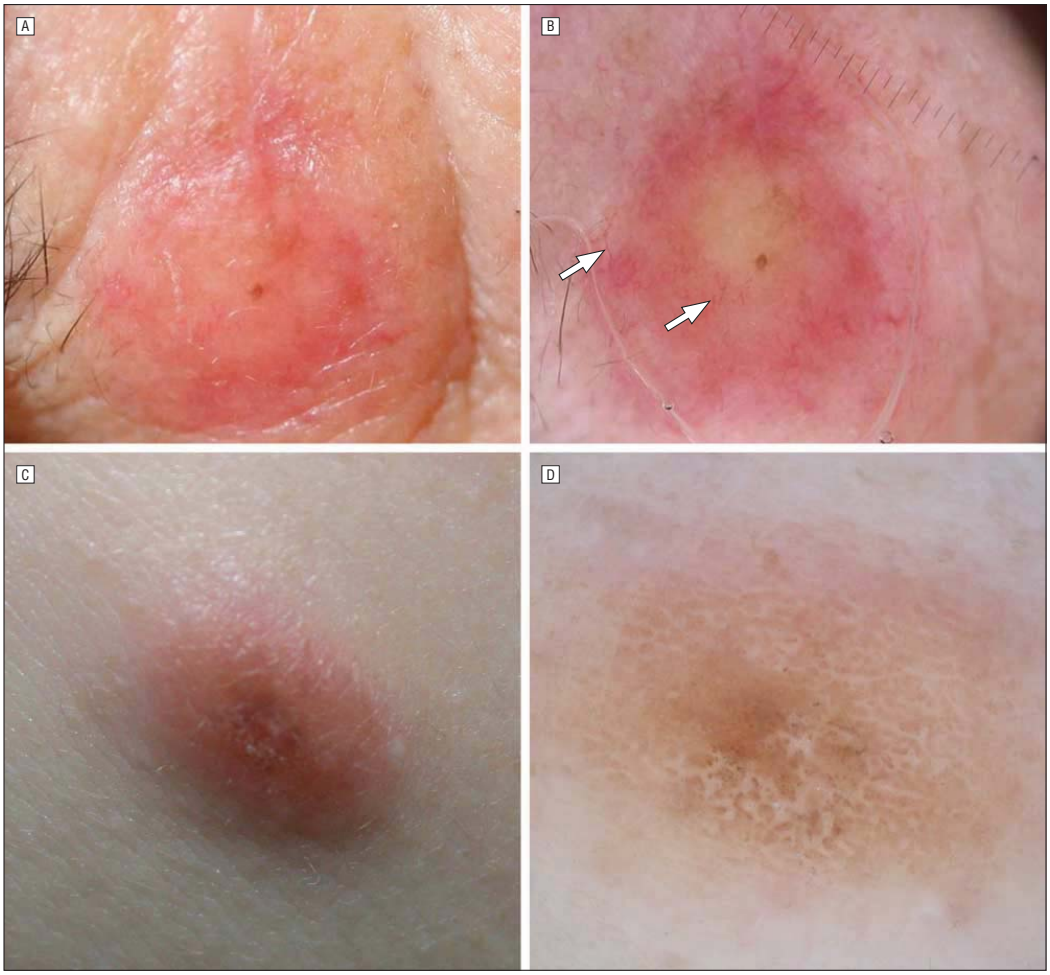

Figure 1. Pure and mixed desmoplastic melanomas (DMs) may be indistinguishable and may present with features that are commonly associated with benign lesions. A and B, Pure DM with a Breslow thickness of 7.2 mm on the glabella of an 81-year-old man. A, Clinical image reveals an irregular and ill-defined pink nodule. B, Under dermoscopy, atypical vascular structures are seen, including serpentine vessels (arrows) and vascular blush. C and D, A 2-mm mixed DM located on the back of a 30-year-old woman. A, Clinical image demonstrates a pink nodule. B, Under dermoscopy, a negative network is seen.

Finally, the clinical diagnosis, previous treatments, and follow-up status were recorded for each case. Presence or absence of local recurrence or metastases was documented. Descriptive frequencies were calculated for all cases and for each category (pDM and mDM). *P* values were calculated based on the Fisher exact or *t* test.

RESULTS

A total of 37 cases of DM that met the inclusion criteria were identified. Of these, 22 patients (60%) were men, and 15 (41%) were women, with an average age of 69 years (age range, 30-89 years). All patients had fair skin, and more than half of patients (78%; n=21) had few nevi (fewer than 20 nevi). Only 3 patients (10%) had a family history of melanoma, and 9 patients (25%) had a history of melanoma (**Table 1**). Although 42% patients had a history of nonmelanoma skin cancer (NMSC) (n=11), patients with a pDM were less likely to have a history of NMSC than patients with mDM (23% [n=3] vs 62% [n=8]) (*P*=.11).

Eighty-nine percent of DM lesions developed on sun-exposed areas (n = 33). Clinically, most DMs presented as palpable and/or indurated lesions (87% [n = 27]) with irregular and ill-defined borders (64% [n = 23]) (**Figures 1**, **2**, and **3**) (eFigures 1-4; http://www.jamaderm.com). The most common primary morphologic characteristics were plaques (30% [n = 11]), macules (22% [n = 8]), papules (16% [n = 6]), or nodules (16% [n = 6]). Overall, DMs had at least 2 colors (58% [n = 21]), with pink/red and brown being the most common (**Table 2**; Figures 1, 2, and 3) (eFigures 1-4).

The prebiopsy clinical diagnoses and reasons for performing a biopsy were available for 11 cases. The prebi-

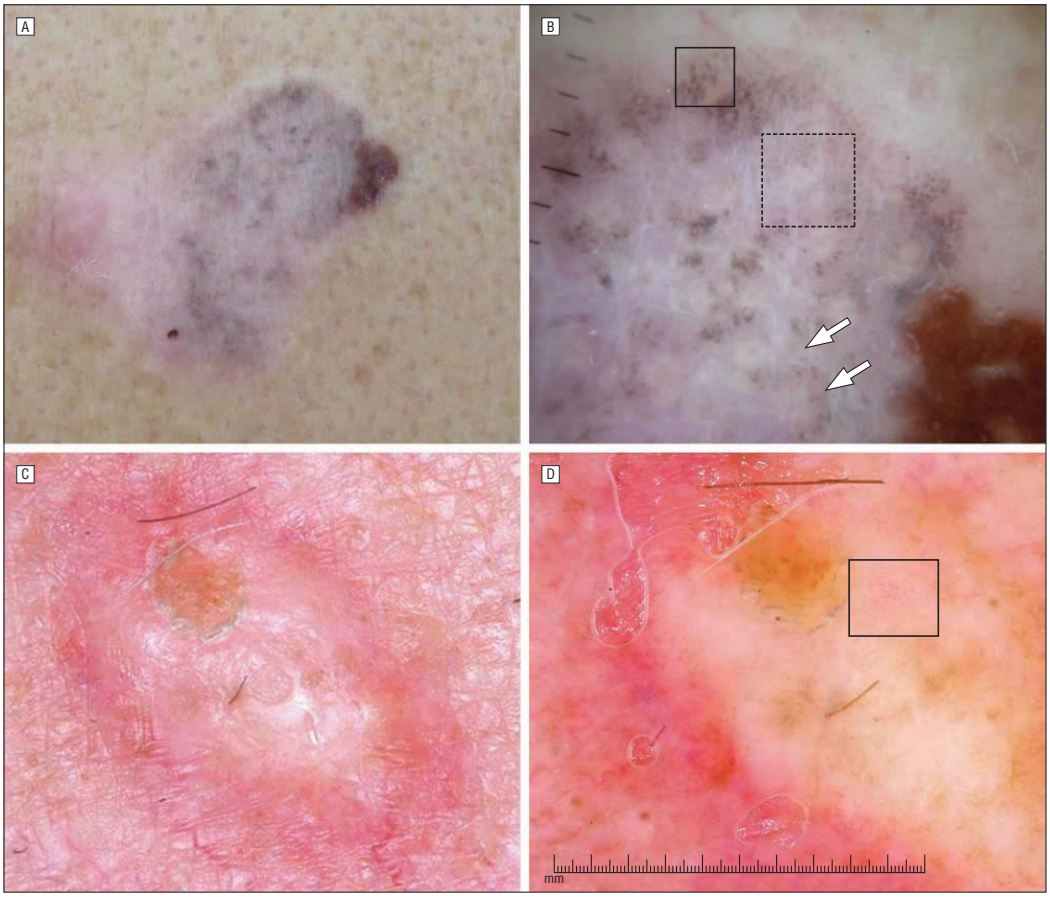

Figure 2. Pure desmoplastic melanomas (pDMs) can present as pigmented or amelanotic lesions. Although some pDMs lack an associated epidermal non-DM component, they usually reveal at least 1 atypical vascular structure. A, The clinical image of a 3.25-mm pDM on the back of a 65-year-old man. B, Dermoscopy revealed regression structures including scarlike areas and peppering (dashed square), which is the most frequent regression structure observed in pDMs. In addition, atypical globules (solid square) and crystalline structures (white arrows) were observed. C, Clinical image of an amelanotic, indurated, and ill-defined nodule located on the chest of a 67-year-old man. D, Under dermoscopy, subtle dotted vessels are seen (square). A biopsy was performed, revealing a 6.1-mm pDM with no associated epidermal component.

opsy clinical diagnoses reported included melanoma (n = 3), basal cell carcinoma (n = 3), melanoma vs dysplastic nevus (n = 1), basal cell carcinoma vs lichen planus–like keratosis (n = 1), squamous cell carcinoma (n = 1), seborrheic keratosis (n = 1), and cyst (n = 1). Reasons for suspecting a malignant neoplasm included the presence of melanoma-specific structures noted under dermoscopy, new lesion found on total body skin examination, and/or the lesion identified as an outlier. In addition, for at least 16 of the 37 patients, the diagnosis of a malignant neoplasm was not entertained, and the lesion underwent treatment with cryotherapy (n = 8), laser (n = 1), intralesional steroids (n = 1), or other treatment not specified (n = 6) prior to the final biopsy that disclosed the diagnosis of DM. These lesions were 8 pDMs and 8 mDMs, located on the head and neck (44% [n = 7]), lower extremities including soles (25% [n = 4]), and trunk (19% [n = 3]); less frequently on the back (6% [n = 1]) or upper extremities (6% [n = 1]). Clinically, these lesions appeared as macules and/or plaques with irregular and ill-defined borders, with 1 color in 44% of the cases (n = 7), mainly pink or light brown. The rest of the lesions had 2 or more colors, in particular pink and light brown. Dermoscopically, the predominant structures observed in these lesions were vascular blush (56% [n = 9]), polymorphous vessels (38% [n = 6]), peppering (38% [n = 6]) and asymmetric perifollicular hyperpigmentation (38% [n = 6]) (Figure 1C and D).

Sixteen of the 37 cases were classified as pDM (43%), and 21 were classified as mDM (57%). Even though there were no statistically significant differences between pDM and mDM by anatomic location, it was observed that pDMs were more frequent than mDMs on the trunk (31% [n = 5] vs 19.0% [n = 4]) and lower extremities (6% [n = 1] vs 0% [n = 0]) (P = .84). In addition, none of the pDMs developed on sun-protected sites, whereas 4 of the

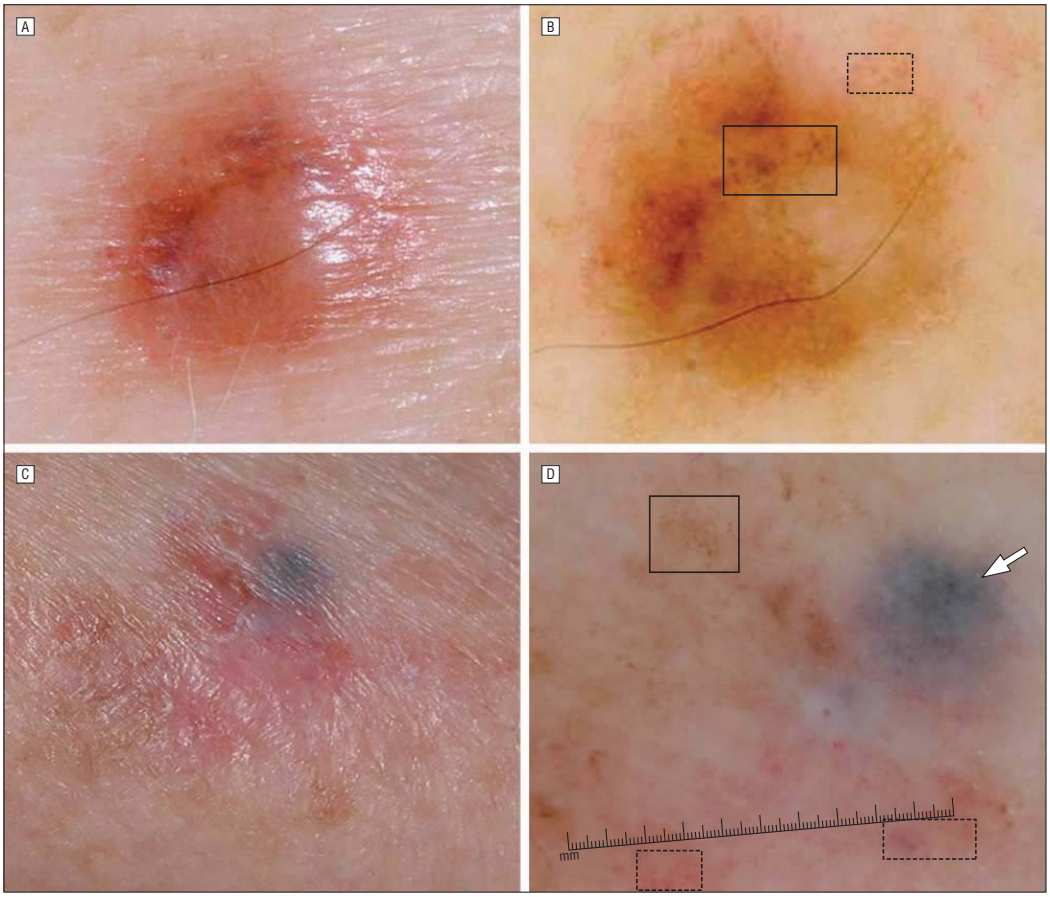

Figure 3. Mixed desmoplastic melanomas (mDMs) can reveal a greater variety of melanoma-specific structures under dermoscopy, which may facilitate their detection. A, Clinical image of a 1.2-mm mDM on the trunk of a 67-year-old man that presented as a pink and brown papule. B, Dermoscopy revealed atypical globules (solid square) and dotted vessels (dashed square). C, A 1.6-mm mDM that presented clinically as an erythematous lesion with a focal bluish discoloration on the arm of an 81-year-old woman. Palpation of the lesion revealed a firm component. D, Dermoscopy demonstrated atypical dots/globules (solid square), polymorphous vessels (dashed squares), off-center blotch, and blue-white veil (arrow).

21 mDMs developed on sun-protected sites, including 2 lesions on the sole and 2 on the lower back (Table 2).

Dermoscopically, 16 of the 37 lesions (43%) revealed melanocytic structures including globules (44% [n = 7]), pigment network (38% [n = 6]), pseudonetwork (25% [n = 4]), and negative network (6% [n = 1]) (**Table 3**; Figures 1D, 2B, and 3B). None of the lesions presented homogeneous blue pigmentation or streaks. While 57% of the DMs lacked any of the aforementioned melanocytic structures [n = 21], they all revealed at least one melanoma-specific structure. The most frequent melanoma-specific structures observed were atypical vascular structures (81% [n = 30]), followed by regression structures (ie, peppering and scarlike areas), blue-white veil, atypical globules, and atypical network (Figures 1B and D, 2B and D, and 3B and D) (eFigures 1-4). Peppering (also known as granularity) was the most frequent regression structure observed and was more frequent in pDM than mDM (44% [n = 7] vs 24% [n = 5]) (Figure 2B). It was noted that within the group of mDMs, there was a greater variety of other melanoma-specific structures that were not manifested in the pDMs, including off-center blotch, negative network, and peripheral tan structureless areas. In addition, features associated with lentigo maligna (LM), including annular granular pattern (24% [n = 9]) and polygonal lines (11% [n = 4]), were also observed.

Fifteen of the dermoscopic images of lesions were acquired using polarized dermoscopy (PD), and 19 using non-PD. For the 3 remaining lesions, it was unknown what device was used to acquire the images. Of the 15 lesions evaluated with PD, 80% revealed crystalline structures (n = 12) (Table 3; Figure 2B) (eFigures 1 and 3). It was observed that crystalline structures, polymorphous vessels, and vascular blush were more commonly seen in mDM than in pDM.

Vascular structures were observed in 81% of the lesions (n = 30), in particular atypical vessels and/or vas-

Table 2. Clinical Characteristics of Desmoplastic Melanomas

Clinical Features	Desmoplastic Melanomas, No. (%)			*P* Value
	Pure (n = 16)	Mixed (n = 21)	All (n = 37)	
Sun-exposed areas	16 (100)	17 (81)	33 (89)	.12[a]
Trunk	5 (31)	4 (19)	9 (24)	
Head and neck	8 (50)	10 (48)	18 (49)	
Upper extremities	2 (13)	3 (14)	5 (14)	
Lower extremity	1 (6)	0	1 (3)	
Sun-protected areas	0	4 (19)	4 (11)	
Sole (acral)	0	2 (10)	2 (5)	
Lower back	0	2 (10)	2 (5)	
Primary morphologic characteristic				
Plaque	2 (13)	9 (43)	11 (30)	.29
Macule	5 (31)	3 (14)	8 (22)	
Papule	2 (13)	4 (19)	6 (16)	
Nodule	3 (19)	3 (14)	6 (16)	
Combination	3 (19)	2 (10)	5 (14)	
Borders				
Irregular and ill-defined	10 (67)	13 (62)	23 (64)	.77
Regular	5 (33)	8 (38)	13 (36)	
Colors				
Single color	5 (33)	10 (48)	15 (42)	.39
Multiple colors	10 (67)	11 (52)	21 (58)	
Palpable and/or indurated lesion				
Yes	10 (91)	17 (85)	27 (87)	.64
No	1 (9)	3 (15)	4 (13)	

[a] *P* value for the difference between sun-exposed and sun-protected areas (based on Fisher exact test).

cular blush. When both groups of DM were compared, pDMs were less likely to present with polymorphous vessels (31% [n = 4] vs 53% [n = 9]) and vascular blush (54% [n = 7] vs 77% [n = 13]) than were mDMs (Table 3).

Overall, pDMs tended to be thicker tumors than mDMs (4.10 vs 2.83 mm) (*P* = .22) and were less likely to have an associated epidermal non-DM component (63% [n = 10] vs 100% [n = 20]) (*P* = .004). There was 1 mDM for which this information was unavailable. The most commonly associated epidermal non-DM component was LM (53% [n = 16]) followed by superficial spreading melanoma (SSM) (10% [n = 3]) (**Table 4**).

Once the diagnosis of DM was confirmed, the most common treatment for the DM was surgical (97% [n = 31]), including wide local excision (94% [n = 30]) or staged excision (3% [n = 1]). Two patients with pDM received adjuvant radiation therapy. The average length of follow-up for the 37 patients was 30 months (range, 1-96 months). Two patients with mDM (10%) presented with local recurrence, one after 24 months and the other after 40 months (Breslow thickness, 4 mm and not available, respectively). The patient with longer follow-up also developed metastases to left submaxillary lymph nodes 54 months after the initial diagnosis. In addition, another patient with mDM (Breslow thickness, 10 mm) developed bilateral inguinal lymph node metastases 20 months after the initial diagnosis. Two patients with pDM developed metastases (13%), one at 17 months, and the other at 9 months after the initial diagnosis (Breslow thicknesses, 12 mm and 6.1 mm, respectively). The patient with the 12-mm pDM developed metastasis to lymph nodes, and the patient with the 6.1-mm pDM developed metastasis to lymph nodes, liver, and lungs.

COMMENT

Desmoplastic melanoma is a relatively rare entity that often presents with features that are more commonly associated with benign lesions. Thus, based on clinical morphologic characteristics alone, DM can prove to be a challenging diagnosis. Even under histopathologic examination, DM can be confused with other entities such as scars, dermatofibromas, and desmoplastic nevus.[4,10,11] Ferrara et al[11] describe the dermoscopic patterns of 3 cases of desmoplastic nevus that all appeared clinically as small, flesh-colored papules characterized by a subtle light-brown network overlying a pinkish erythematous background. All nevi were devoid of asymmetry or melanoma-specific patterns. Based on its nondescript appearance and the difficulties in the initial clinical recognition, it is not surprising that DM is seldom suspected in its early stages.[4] In addition, it is not uncommon for these lesions to be diagnosed as benign entities and get treated as such. In fact, in our study at least 10 cases (27%) had received previous treatments including cryotherapy, laser, and intralesional steroids, before the definitive diagnosis of DM was confirmed by biopsy.

To our knowledge, this is the largest series describing the clinical and dermoscopic characteristics of DM. In addition, this study attempts to describe the most common phenotypic traits present in patients with DM. Overall, our patients with DM had fair skin, and most of them demonstrated actinic damage, few nevi, and a negative personal and family history of melanoma (Table 1). Dermoscopically, our study demonstrates that while 57% of the DMs (n = 21) lacked melanocytic pigmented struc-

Table 3. Dermoscopic Characteristics of DMs

Dermoscopic Feature	DMs, No. (%)			*P* Value[a]
	Pure (n = 16)	Mixed (n = 21)	All (n = 37)	
Melanocytic structures present	7 (44)	9 (43)	16 (43)	>.99
Globules (atypical)	4 (57)	3 (33)	7 (44)	.44
Pigment network (typical or atypical)	2 (29)	4 (44)	6 (38)	.68
Negative network	0	1 (11)	1 (6)	>.99
Homogeneous blue pigmentation	0	0	0	NA
Streaks	0	0	0	NA
Pseudonetwork (facial skin)	2 (29)	2 (22)	4 (25)	>.99
Melanoma-specific structures present	16 (100)	21 (100)	37 (100)	NA
Atypical vascular structures	13 (81)	17 (81)	30 (81)	>.99
Peppering	7 (44)	5 (24)	12 (32)	.29
Crystalline structures[b]	3 (19)	9 (43)	12 (32)	.17
Annular granular pattern	4 (25)	5 (24)	9 (24)	>.99
Blue-white veil	2 (13)	5 (24)	7 (19)	.67
Atypical globules	4 (25)	3 (14)	7 (19)	.44
Atypical network	2 (13)	3 (14)	5 (14)	>.99
Scarlike areas	2 (13)	1 (5)	3 (8)	.57
Off-center blotch	0	3 (14)	3 (8)	.24
Peripheral tan structureless areas	0	1 (5)	1 (3)	>.99
Negative network	0	1 (5)	1 (3)	>.99
Streaks	0	0	0	NA
Polygonal lines	2 (13)	2 (10)	4 (11)	>.99
Follicular obliteration	0	1 (5)	1 (3)	>.99
Vascular structures present	13 (81)	17 (81)	30 (81)	>.99
Dotted vessels	2 (40)	4 (67)	6 (55)	NA
Serpentine vessels (linear irregular)	2 (40)	2 (33)	4 (36)	NA
Coiled vessels	1 (20)	0	1 (9)	NA
Vascular blush/milky-red areas	7 (54)	13 (77)	20 (67)	.33
Polymorphous vessels (>2 types) present	4 (31)	9 (53)	13 (43)	.32

Abbreviations: DM, desmoplastic melanoma; NA, not applicable.
[a] Based on the Fisher exact test.
[b] Crystalline structures can only be seen with polarized dermoscopy (PD) (n = 15). Four pure DMs and 11 mixed DMs were evaluated with PD. Crystalline structures were present in 12 DMs (80%), specifically in 3 pure DMs (75%) and 9 mixed DMs (82%).

tures (ie, globules, pigment network, and pseudonetwork on facial skin), all cases of DM revealed at least 1 melanoma-specific structure, in particular atypical vascular structures, peppering, blue-white veil, atypical globules, crystalline structures, and atypical network (Figures 1B and D, 2B and D, and 3B and D) (eFigures 1-4). Furthermore, dermoscopic features of LM such as annular granular pattern and polygonal lines were seen in one-third of the cases. In fact, 83% of our cases had an associated epidermal non-DM (n = 30), with LM being the most common type, followed by SSM. Thus, we are of the opinion that since DMs can be associated with LM or SSM, all of these lesions should be palpated to rule out a dermal component of DM (Figure 3D).[10,12]

Previous studies have reported that DM lesions tend to grow slowly and are more frequently located on the head and neck, extremities, and trunk of fair-skinned elderly men. The male to female ratio has been reported to be 2:1, and the mean age at diagnosis is 66 years, which is older than the 60 years found for non-DM lesions.[2-4,13,14] Our data reflect a similar male to female ratio (2:1) and average age. In addition, we also found that DM has a predilection for sun-exposed areas (89% [n = 33]), in particular for the head and neck (49% [n = 18]). Only 4 DMs of the mixed type were found on sun-protected areas. Thus, our results are in accordance with other studies that have reported a link between DM, chronic actinic damage, and LM.[2,3] We speculate that mDM is more likely to have risk factors and clinical and phenotypic characteristics similar to those of LM. In contrast to DM, desmoplastic nevus appears to be associated with younger age and development on non–sun-exposed areas. Thus, patient age and anatomic location seem to be important criteria in the differential diagnosis between benign and malignant desmoplastic neoplasia.[11]

The clinical and dermoscopic characteristics of DMs were described as a function of the pathological subclassification (pDM vs mDM), which is based on the extent of desmoplasia present in the invasive component. This classification appears to have prognostic and perhaps even therapeutic implications.[15,16] Evidence suggests that pDMs are less likely to have regional lymph node involvement and are associated with a more favorable outcome. In contrast, mDMs have been associated with more locoregional recurrences.[15]

From a clinical perspective, pDMs and mDMs may be indistinguishable. Both types of DM can present as firm and indurated lesions with irregular and ill-defined borders (Figure 1A and C). While there are limited data regarding the role of dermoscopy in the diagnosis of DM,[5,6] the present study supports its use during the evaluation of skin lesions because it provides additional information that will prompt the clinician to perform a proper

Table 4. Histopathologic Characteristics of Desmoplastic Melanomas

Histopathologic Characteristics	Desmoplastic Melanomas[a] Pure (n = 16)	Mixed (n = 21)	All (n = 37)	*P* Value
Breslow depth, mean (range), mm	4.10 (0.5-12.0)	2.83 (0.5-10.0)	3.38 (0.5-12.0)	.22[b]
Mitotic index, mean (range), mm	1.1 (0-2)	1.9 (0-8)	1.6 (0-8)	.19[b]
Associated epidermal component				
Yes	10 (63)	20 (100)	30 (83)	.004[c]
No	6 (38)	0	6 (17)	
Missing data	0	1 (5)	1 (3)	
Melanoma subtype				.66[c]
Lentigo maligna	6 (60)	10 (50)	16 (53)	
Superficial spreading melanoma	1 (10)	2 (10)	3 (10)	
ALM	0	1 (5)	1 (3)	
Not specified	2 (20)	7 (35)	9 (30)	
Other associated pathologic condition (Spitz nevus)	1 (10)	0	1 (3)	
Ulceration				
Present	0	1 (5)	1 (3)	.65[c]
Absent	7 (44)	12 (57)	19 (51)	
Missing data	9 (56)	8 (38)	17 (46)	
Regression				
Present	1 (6)	5 (24)	6 (16)	.35[c]
Absent	4 (25)	6 (29)	10 (27)	
Missing data	11 (69)	10 (48)	21 (57)	
Lymphovascular invasion				
Present	1 (6)	2 (10)	3 (8)	.68[c]
Absent	4 (25)	10 (48)	14 (38)	
Missing data	11 (69)	9 (43)	20 (54)	
Perineural invasion				
Present	5 (31)	4 (19)	9 (24)	.37[c]
Absent	3 (19)	7 (33)	10 (27)	
Missing data	8 (50)	10 (48)	18 (49)	

Abbreviation: ALM, acral lentiginous melanoma.
[a] Unless otherwise indicated, data are reported as number (percentage) of lesions.
[b] Based on the *t* test.
[c] Based on the Fisher exact test.

biopsy. One retrospective study performed by Debarbieux et al[7] describes the dermoscopic features of 6 DM cases. The authors reported that 3 lesions lacked melanocytic criteria but presented with ulceration, vascular structures, and regression structures including scarlike areas, peppering, and/or blue-white veil. The other 3 lesions presented with melanocytic features such as irregular pigment network or pseudonetwork in addition to white scarlike areas, peppering (2 lesions), blue-white veil (2 lesions), and vascular structures (2 lesions). These 3 lesions presented in association with a LM or SSM. Vascular structures were noted in 5 of the 6 lesions and consisted of serpentine vessels (also known as linear irregular vessels) and/or milky red areas. In accordance with Debarbieux et al,[7] we found that 81% of DMs revealed vascular structures (n = 30), particularly atypical vessels and/or vascular blush. When DMs were separated into histopathologic subtypes, we observed that pDMs tended to present with monomorphous vessels (38% [n = 6] vs 35% [n = 5]), whereas mDMs tended to present with a polymorphous pattern (31% [n = 4] vs 53% [n = 9]) and vascular blush (54% [n = 7] vs 77% [n = 13]) (Table 3).

Although 38% of the pDMs in the present study lacked an associated epidermal non-DM component (n = 6), all of these cases revealed at least 1 atypical vascular structure (Figures 1B and 2D) (eFigures 1-4). Therefore, it may be hypothesized that since mDM lesions can reveal a greater variety of melanoma-specific structures under dermoscopy (eg, crystalline structures, polymorphous vessels, vascular blush, off-centered blotch, negative network, and peripheral tan structureless areas), they may be easier to detect than pDM lesions (Figures 1D and 3B and D). This in turn may result in greater delays in diagnosis and greater Breslow thickness at diagnosis for pDMs (4.01 mm vs 2.83 mm). We suggest that the most important indicator prompting the dermatologist to perform a biopsy to rule out a melanoma may be the presence of dermoscopic structures associated with the epidermal non-DM component. In cases in which there is no epidermal component, the presence of atypical vascular structures may lead to a heightened suspicion for malignant neoplasm (Figures 1B and 2D and eFigures 2 and 4).

Limitations of our study are that it is a retrospective study of cases that included high-quality dermoscopic images but did not always include clinical and demographic information. The histopathologic diagnosis of each DM lesion was based on the official pathology diagnosis from the corresponding high-risk dermatology center, and a second pathologist did not confirm the diagnosis of DM. Although dermoscopic differences between pDM and

mDM were observed, the power of the study did not reach statistical significance owing to the small number of cases. However, we believe that it is still important to highlight these differences, although future larger databases will be required to validate our findings.

In conclusion, this study demonstrates that dermoscopy is a useful aid during the evaluation of clinically equivocal lesions or those with a benign appearance. Although DM can be difficult to diagnose based on clinical morphologic characteristics alone, clinical information and risk factors for DM need to be considered when evaluating indurated and firm lesions on sun-damaged skin, including male sex, older age, chronic sun exposure, and the presence of an associated LM. In addition, all lesions suggestive of LM should be palpated and evaluated with dermoscopy. Palpation can lead to the detection of a subcutaneous nodule, which may be another clue for the diagnosis of DM. Dermoscopy can provide additional clues that might heighten the clinical suspicion for DM and may guide the clinician to perform a biopsy on these otherwise benign-appearing lesions. Common dermoscopic structures in DM include atypical vascular structures, peppering, or other melanoma-specific structures.

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