UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA

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ASSOCIAÇÃO DOS PARÂMETROS HISTOPATOLÓGICOS (ÍNDICE DE BRESLOW MODIFICADO E DIMENSÃO MACROSCÓPICA) E IMUNOISTOQUÍMICOS (KI-67 E PRAME) COM O RESULTADO DO LINFONODO SENTINELA E SOBREVIDA EM CASOS DE MELANOMA CUTÂNEO

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para obtenção do título de Doutor em Patologia.

Orientador: Prof. Dr. José Cândido Caldeira Xavier-Júnior
Coorientadora: Profa. Dra. Mariângela Esther Alencar Marques

Botucatu
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1. Estadiamento de tumores. 2. Melanoma. 3. Pele - tumores. 4. Patologia.

Palavras-chave: Estadiamento de neoplasias; Melanoma; Neoplasias da pele; Patologia.

IMPACTOS POTENCIAIS DESTA PESQUISA

IMPACTO CIENTÍFICO

Esta pesquisa contribui significativamente para a área de dermatopatologia, trazendo novos conhecimentos relacionados a fatores prognósticos no melanoma cutâneo. A introdução de novas maneiras de mensuração do tumor (medida de Breslow modificada e DOI), além de certos marcadores imunoistoquímicos (Ki-67 e PRAME) pode ajudar a prever melhor o prognóstico desta neoplasia agressiva. Os resultados podem servir como base para futuras pesquisas acadêmicas, potencialmente levando ao desenvolvimento de novas abordagens para avaliação prognóstica e tratamento do melanoma cutâneo.

IMPACTO SOCIAL

Ao identificar marcadores que podem prever com maior precisão a sobrevida e o risco de metástases dos casos de melanoma cutâneo, os profissionais da saúde poderão fornecer um tratamento mais personalizado e eficaz. Isso pode resultar em melhores desfechos clínicos e mais qualidade de vida. Além disso, o desenvolvimento da tese de doutorado culmina com a formação de um pesquisador que já atua em universidade pública e poderá criar novos projetos de pesquisa relacionados em sua universidade, inclusive atuando na formação de recursos humanos na área.

IMPACTO ECONÔMICO

A melhoria da precisão na avaliação prognóstica no melanoma cutâneo pode reduzir a necessidade de procedimentos mais caros e complexos, resultando em economia para os sistemas de saúde pública e privada. Trata-se de projeto de base morfológica, baseado em técnica acessíveis, que pode ser reproduzido facilmente em outros centros de pesquisa e cujos resultados são aplicáveis à rotina diagnóstica.

POTENTIAL IMPACTS OF THIS RESEARCH

SCIENTIFIC IMPACT

This research significantly contributes to dermatopathology by introducing knowledge of prognostic factors in cutaneous melanoma. Introducing ways to measure the tumor (modified Breslow thickness and DOI) and immunohistochemistry markers (Ki-67 and PRAME) can help predict the outcomes of this aggressive neoplasm. The results will serve as the basis for future academic research, potentially leading to new approaches for prognostic evaluation and treatment of cutaneous melanoma.

SOCIAL IMPACT

By identifying markers that can accurately predict survival and the risk of metastasis in cutaneous melanoma, healthcare professionals can provide personalized and effective treatment. This advance can lead to significantly better clinical outcomes and a substantial enhancement in the quality of life for patients. Furthermore, the development of the doctoral thesis culminates in the training of a researcher who already works at a public university and will be able to create new related research projects at the university, including working on the training of human resources in the area.

ECONOMIC IMPACT

Improving the accuracy of prognostic evaluation in cutaneous melanoma can reduce the need for more expensive and complex procedures, resulting in savings for public and private healthcare systems. This is a morphological-based project based on accessible techniques that can be easily reproduced in other research centers and whose results apply to routine diagnosis.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Botucatu

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE MARCEL ARAKAKI ASATO, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA, DA FACULDADE DE MEDICINA - CÂMPUS DE BOTUCATU.

Aos 19 dias do mês de julho do ano de 2024, às 08:30 horas, por meio de Videoconferência, realizou-se a defesa de TESE DE DOUTORADO de MARCEL ARAKAKI ASATO, intitulada **ASSOCIAÇÃO DOS PARÂMETROS HISTOPATOLÓGICOS (ÍNDICE DE BRESLOW MODIFICADO E DIMENSÃO MACROSCÓPICA) E IMUNOISTOQUÍMICOS (KI-67 E PRAME) COM O RESULTADO DO LINFONODO SENTINELA E SOBREVIDA EM CASOS DE MELANOMA CUTÂNEO**. A Comissão Examinadora foi constituida pelos seguintes membros: Prof. Dr. JOSÉ CÂNDIDO CALDEIRA XAVIER JÚNIOR (Orientador(a) - Participação Virtual) do(a) Instituto de Patologia de Araçatuba, Prof. Dr. GILLES LANDMAN (Participação Virtual) do(a) Depto. de Patologia / EPM/São Paulo - Unifesp, Profa. Dra. MIRIAN NACAGAMI SOTTO (Participação Virtual) do(a) Depto. de Patologia / FM/São Paulo - USP. Após a exposição pelo doutorando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final: aprovado . Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Prof. Dr. JOSÉ CÂNDIDO CALDEIRA XAVIER JÚNIOR

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“Para resolver um problema desta natureza, o segredo é raciocinar de trás para a frente. É uma proeza da maior utilidade, mas as pessoas não costumam praticá-la.”

(Sherlock Holmes, de Arthur Conan Doyle)

RESUMO

Introdução: O melanoma cutâneo é um dos cânceres de pele com pior prognóstico, frequentemente evoluindo com metástases e alta mortalidade. Os principais subtipos histológicos são o extensivo superficial (baixo dano solar cumulativo), nodular, lentigo maligno (alto dano solar cumulativo) e o lentiginoso acral, sendo este considerado o subtipo de pior prognóstico. Para avaliação prognóstica do melanoma cutâneo a medida micrométrica de Breslow é considerado o fator mais importante, entretanto, possui limitações. O tamanho macroscópico não é um parâmetro considerado para estadiamento no melanoma cutâneo, embora a dimensão do tumor seja considerada no melanoma uveal e carcinoma de células escamosas. Vários marcadores imunoistoquímicos são utilizados para auxílio no diagnóstico do melanoma, porém não há marcadores consolidados para avaliação prognóstica. **Objetivo**: Correlacionar a extensão macroscópica do tumor, a profundidade de invasão medida a partir da membrana basal (Breslow modificado), o DOI ("depth of invasion"), índice de proliferação celular medido pelo Ki-67, padrões de marcação nuclear do Ki-67 e a expressão do PRAME, com o resultado do linfonodo sentinela e a sobrevida em pacientes com melanoma cutâneo. **Métodos:** Estudo transversal retrospectivo baseado na análise de dados de prontuário eletrônico (tamanho macroscópico, resultado do linfonodo sentinela e sobrevida), avaliação de lâminas coradas em Hematoxilina e Eosina (Breslow modificado e DOI), imunoistoquímica para Ki-67 e PRAME, e digitalização de lâminas e análise do padrão de marcação do Ki-67 por dois dermatopatologistas. **Resultados**: O tamanho macroscópico demonstrou correlação significativa apenas com o resultado do linfonodo sentinela em análise univariada. O Breslow modificado mostrou-se capaz de predizer a sobrevida específica e resultado do linfonodo sentinela, assim como a medida de Breslow convencional. O DOI foi capaz de predizer a sobrevida específica do melanoma acral apenas em análise univariada. Elevada expressão de PRAME e Ki-67 estão associadas a pior sobrevida geral em análise univariada. Em análise multivariada, entretanto, apenas a medida de Breslow foi significativa. A análise dos padrões de expressão nuclear do Ki-67 demonstrou uma elevada concordância interobservador (coeficiente de concordância de Linn: 0.9783) com maior frequencia de três padrões específicos no grupo de pacientes que morreram por melanoma. **Conclusão**: O Breslow modificado é capaz

de predizer o prognóstico no melanoma cutâneo assim como a medida de Breslow convencional. O tamanho macroscópico e o DOI possuem correlação significativa com desfechos (linfonodo sentinela e sobrevida, respectivamente) no melanoma cutâneo. Elevada expressão do PRAME e do Ki-67 estão associadas a pior sobrevida geral. Além disso, alguns padrões de marcação do Ki-67 estão associados a pior desfecho. Entretanto, em análise multivariada, a medida de Breslow continua sendo o principal fator para avaliação prognóstica no melanoma cutâneo.

Palavras-chave: Melanoma, patologia, estadiamento de neoplasias, neoplasias da pele

ABSTRACT

Introduction: Cutaneous melanoma is one of the skin cancers with the worst prognosis, often developing metastases and high mortality. The main histological subtypes are superficial spreading (low cumulative solar damage), nodular, lentigo maligna (high cumulative solar damage) and acral lentiginous, which is considered the subtype with the worst prognosis. For the prognostic assessment of cutaneous melanoma, the Breslow micrometric measurement is considered the most important factor, however, it has limitations. Macroscopic size is not a parameter considered for staging in cutaneous melanoma, although tumor size is considered in uveal melanoma and squamous cell carcinoma. Several immunohistochemical markers are used to aid in the diagnosis of melanoma, but there are no consolidated markers for prognostic assessment. **Objective**: To correlate the macroscopic extension of the tumor, the depth of invasion measured from the basement membrane (modified Breslow), the DOI ("depth of invasion"), cell proliferation index measured by Ki-67, Ki-67 nuclear labeling patterns and PRAME expression, with sentinel lymph node outcome and survival in patients with cutaneous melanoma. **Methods**: Retrospective cross-sectional study based on analysis of electronic medical record data (macroscopic size, sentinel lymph node results and survival), evaluation of slides stained with Hematoxylin and Eosin (modified Breslow and DOI), immunohistochemistry for Ki-67 and PRAME, and digitalization of slides and analysis of the Ki-67 staining pattern by two dermatopathologists. **Results**: Macroscopic size demonstrated a significant correlation only with the sentinel lymph node result in univariate analysis. The modified Breslow was able to predict specific survival and sentinel lymph node outcome, as well as the conventional Breslow measurement. DOI was able to predict acral melanoma-specific survival only in univariate analysis. High PRAME and Ki-67 expression are associated with worse overall survival in univariate analysis. In multivariate analysis, however, only the Breslow measurement was significant. Analysis of Ki-67 nuclear expression patterns demonstrated high interobserver agreement (Linn's coefficient of agreement: 0.9783) with a greater frequency of three specific patterns in the group of patients who died from melanoma. **Conclusion:** The modified Breslow is capable of predicting the prognosis in cutaneous melanoma as well as the conventional Breslow measurement. Macroscopic size and DOI have a significant correlation with outcomes

(sentinel lymph node and survival, respectively) in cutaneous melanoma. High expression of PRAME and Ki-67 are associated with worse overall survival. Furthermore, some Ki-67 staining patterns are associated with a worse outcome. However, in multivariate analysis, the Breslow measurement remains the main factor for prognostic assessment in cutaneous melanoma.

Keywords: Melanoma, pathology, neoplasm staging, skin neoplasms

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1 INTRODUÇÃO

1.1 Melanoma cutâneo

O melanoma cutâneo é uma neoplasia que surge de melanócitos e representa um dos tipos de câncer de pele mais perigosos já que frequentemente leva a metástases para órgãos distantes. (1)

A incidência de melanoma tem aumentado nas últimas décadas em populações brancas, provavelmente relacionada ao comportamento recreacional e exposição solar. As maiores incidências foram reportadas na Austrália e Nova Zelândia com até 60 casos por 100.000 habitantes por ano. (2) No Brasil a incidência estimada é de 4,13 casos por 100.000 habitantes por ano. No ano de 2020 foram estimados 1.120 óbitos em homens e 803 óbitos em mulheres. (3, 4)

Apesar do aumento da incidência, as taxas de mortalidade permaneceram estáveis desde a década de 1980, refletindo as melhorias na detecção precoce de melanomas finos em um estágio potencialmente curável. (2)

O melanoma surge como consequência de uma complexa interação de fatores ambientais e constitucionais. Estudos moleculares indicam que o melanoma é uma doença heterogênea com vários subtipos biológicos distintos possivelmente surgindo por diferentes vias. (2)

Fatores de risco para melanoma incluem, dentre outros:

- Exposição à radiação ultravioleta natural e bronzeamento artificial. Tanto a radiação UV-A, que é mais abundante na luz solar natural, quanto a UV-B estão relacionadas ao aumento do risco de desenvolvimento do melanoma. (5) A radiação UV-A provoca formação de radicais livres que causam dano oxidativo ao DNA, sendo um potencial mutagênico. Já a UV-B penetra na camada basal da epiderme levando à formação de dímeros de pirimidina no DNA. O reparo incorreto dessas alterações no DNA leva a mutações que alteram o funcionamento celular. Indivíduos expostos de maneira intermitente à luz solar intensa também têm maior risco de desenvolver melanoma. (6)

- Pele clara. A incidência de câncer de pele é menor em indivíduos de pele escura do que naqueles de pele clara. A melanina cutânea é o fator protetor mais importante, já que além de absorver a radiação UV, tem atividade antioxidante. (7)

- Múltiplos nevos melanocíticos. (8)

- Nevo displásico. A presença de nevo displásico está associado a um aumento de 1,5 a 10x no risco de melanoma, sendo que quanto maior o número de nevos displásicos mais elevado é o risco. (9)

- Sardas. (9)

- Imunossupressão. Sabe-se que há um aumento da incidência de melanoma em indivíduos transplantados. (10) Indivíduos infectados pelo vírus HIV possuem um risco 2,6x maior do que indivíduos não infectados, além de apresentar um pior prognóstico. (11)

- História familiar de melanoma. Cerca de 8 a 14% dos pacientes possuem história familiar de melanoma, sendo que o principal gene identificado relacionado a melanoma familiar é o *CDKN2A*. Mutações em outros genes predisponentes (*CDK4, BAP1, TERT, POT1, ACD, TERF2IP* e *MITF*) são mais raros. (2, 12, 13)

Os melanomas são mais comuns entre os 30 e 60 anos de idade, com uma frequência ligeiramente maior em homens do que em mulheres. Ocorrem em qualquer parte da pele, e podem atingir as mucosas. (14)

O mnemônico ABCDE pode ajudar a identificar um melanoma. A letra A vem de **a**ssimetria, já que no melanoma há perda da simetria (normalmente encontrada nos nevos melanocíticos). A letra B refere-se às **b**ordas da lesão, que são irregulares no melanoma. A letra C corresponde à **c**oloração heterogênea, ao contrário dos nevos melanocíticos que são uniformemente pigmentados. A letra D refere-se ao **d**iâmetro superior a 6mm e a letra E significa **e**xpansão em superfície ou modificação do aspecto da lesão. (14)

Podem ser classificados em diversos tipos com características epidemiológicas, histológicas e prognósticos diferentes. (14)

O melanoma extensivo superficial corresponde a 70% dos melanomas, ocorrendo principalmente no tronco, dorso em homens e membros inferiores nas mulheres. Apresenta-se como lesão levemente elevada, com margens irregulares e coloração variada. (14) Aproximadamente 50% possuem mutações no gene *BRAF* e 15-25% mutações no gene *NRAS*. Estes tendem a ser mais espessos e apresentar uma taxa mitótica mais elevada. (15) No exame histopatológico observam-se células neoplásicas isoladas ao longo da junção dermoepidérmica ou formando ninhos, frequentemente com invasão pagetóide da epiderme e podendo exibir invasão da derme. (16)

O melanoma nodular corresponde a 15-30% dos casos, ocorre mais comumente na quinta década de vida, é agressivo e pode crescer rapidamente em poucas semanas. É uma lesão nodular ou polipoide de coloração negro-azulada a vermelha, ocorrendo principalmente no tronco de homens e perna de mulheres, frequentemente confundidas com hemangioma ou granuloma piogênico. (14, 15) Aproximadamente 50% possuem mutações no gene *BRAF* e 20% no gene *NRAS*. (15) Na histopatologia predomina o componente invasor e a epiderme adjacente à lesão apresenta no máximo 2 cones epiteliais acometidos. (16)

O melanoma lentiginoso acral é a forma mais comum em negros e asiáticos, mais comumente na sexta década de vida, principalmente nas regiões palmoplantares e falanges distais. (14) Este melanoma comumente possui mutação do gene *kit*. (15) À histologia a epiderme é hiperplásica com proliferação de melanócitos na camada basal, melanocitose pagetoide e prolongamentos dendríticos pigmentados por entre os queratinócitos. (16)

O lentigo maligno é um tipo de melanoma in situ mais comumente encontrado na face de indivíduos idosos com pele danificada pelo sol. Cresce por um longo período antes de se tornar invasivo, podendo se tornar bastante extenso à apresentação mesmo sendo restrito à epiderme. Quando ocorre invasão (lentigo maligno melanoma), comporta-se da mesma maneira que um melanoma invasivo *de novo.* Ao exame histopatológico observa-se atrofia e retificação da epiderme, elastose solar e proliferação de melanócitos na camada basal da epiderme. (15)

Melanoma é um diagnóstico histológico. Lesões pigmentadas e não pigmentadas clinicamente suspeitas para melanoma devem ser excisadas e submetidas a exame histopatológico. (15) Algumas características associam-se à índole maligna e são úteis para o diagnóstico de melanoma:

- Assimetria horizontal e vertical: Lesões com silhueta assimétrica, margens laterais opostas com diferentes morfologias, distribuição irregular do pigmento melânico, distribuição irregular dos ninhos na junção dermoepidérmica e uma epiderme com diferentes espessuras, por vezes mais fina ou ulcerada são características que podem favorecer melanoma. O infiltrado linfocitário é geralmente assimétrico nos melanomas enquanto nos nevos é uniformemente distribuído. (16, 17)

- Padrão lentiginoso extenso: consiste de melanócitos alinhados individualmente em áreas da epiderme.

- Falta de circunscrição: circunscrição é definida pela presença de ninhos de melanócitos nos limites da lesão. A falta de circunscrição ocorre na maioria dos melanomas extensivos superficiais. O melanoma nodular é exceção, já que se apresenta como uma lesão nitidamente bem circunscrita.

- Ninhos de melanócitos grandes, confluentes e irregulares.

- Perda da maturação dos melanócitos: maturação é um fenômeno que consiste na diminuição do tamanho dos melanócitos nas porções mais profundas da lesão. Os melanócitos também perdem a pigmentação e se tornam mais alongados. A maturação é característica dos nevos melanocíticos e geralmente ausente nos melanomas.

- Consumo da epiderme: adelgaçamento de parte da epiderme com perda dos cones epiteliais, provavelmente associado a ulceração incipiente.

- Disseminação pagetoide: presença de melanócitos neoplásicos intraepidérmicos acima da camada basal.

- Atipias citológicas: presença de melanócitos grandes (epitelióides) e fusiformes, pleomorfismo nuclear com variação do diâmetro do núcleo e da distribuição da cromatina, hipercromasia e nucléolo eosinofílico proeminente podem indicar uma natureza maligna.

- Elevado número de mitoses: encontro de número signficiativo de mitoses, mitoses agrupadas ou mitoses na porção mais profunda da lesão são sugestivas de melanoma.

- Necrose tumoral

- Sinais de dano solar: qualquer lesão melanocítica juncional ou composta que se desenvolve em pele severamente danificada pelo sol, caracterizado por elastose solar acentuada, deve ser considerado um provável melanoma. (16, 17, 18)

O laudo de melanoma relata as feições que permitiram o estabelecimento do diagnóstico assim como as características que são importantes para o prognóstico e conduta. O prognóstico é dependente principalmente da espessura do tumor. (18) Wallace H. Clark, em 1969, percebendo a relação entre a profundidade de invasão do melanoma e o prognóstico, desenvolveu um sistema baseado em níveis de envolvimento anatômico cutâneo, classificando os melanomas de acordo com a

profundidade máxima de invasão da lesão nos níveis da derme ou tecido subcutâneo, sendo o nível I: *in situ*; nível II: invasão superficial da derme; nível III: lesão preenche a derme papilar; nível IV: há invasão da derme reticular; nível V: alcança o tecido subcutâneo. (16, 19, 20, 21)

1.2 Medida de Breslow

A profundidade de invasão como fator prognóstico também foi reportada por Alexander Breslow em 1970, que demonstrou uma correlação da espessura do melanoma com risco de recorrência e metástase. Particularmente ele encontrou que melanomas com espessura menor do que 0,76 mm não evoluíram com recorrência ou metástases. (19, 21)

Estudos posteriores, mostraram que o nível de Clark, embora ainda justificadamente adotado, possui um valor preditivo baixo, é menos reproduzível e é mais operador-dependente quando comparado com a medida de Breslow. (19)

A medida micrométrica de Breslow tornou-se o fator mais importante para prognóstico e conduta, sendo utilizado amplamente e está incluído nos sistemas de estadiamento da AJCC (American Joint Committee on Cancer). A medida de Breslow define o estadiamento patológico, tamanho das margens da excisão e aplicabilidade da biópsia de linfonodo sentinela. (22, 23)

A medida de Breslow é mensurada utilizando-se um micrômetro ocular, escala de Vernier do microscópio, uma régua micrométrica sobre a lâmina ou programas de computador para mensuração de imagens digitalizadas. A medida é então realizada desde a porção mais superficial da camada granulosa (ou da camada espinhosa em sua ausência) até a porção mais profunda do tumor (Figuras 1 e 2). (17) Se a lesão é ulcerada, a medida começa desde a base da úlcera. Nestes casos a espessura poderá estar subestimada pois não se está levando em conta a quantidade de tumor perdida devido à ulceração (Figura 3). De acordo com a oitava edição da AJCC a mensuração deve ser arredondada para o décimo de milímetro (0,1mm) mais próximo, e não mais para o centésimo de milímetro como utilizado previamente, devido à imprecisão e impraticidade desta última medida. (24, 25) Convencionou-se ainda arredondar os valores na casa dos centésimos para baixo aqueles que terminam de 1 a 4 e para cima aqueles que terminam de 5 a 9 (Por exemplo, um melanoma medindo 0,75 mm de espessura seria arredondado para 0,8

mm. E aqueles que medem entre 0,95 mm e 1,04 mm são arredondados para 1,0mm). (25, 26)

Existem algumas particularidades na mensuração do Breslow como: a espessura apenas pode ser avaliada de forma acurada em cortes perpendiculares à superfície da epiderme; (26) caso haja extensão perianexial do melanoma e este represente o único foco de invasão, a espessura de Breslow deve ser mensurada a partir do centro da estrutura anexial que teve origem; (27) a espessura de Breslow não pode ser determinada em casos de ressecção superficial da lesão apenas. (26) A mensuração pode ser difícil na pele acral devido à epiderme extensamente espessada. (24) Portanto, percebe-se que a medida de Breslow possui algumas limitações. Quando há presença de ulceração, a medida começa deste a base da úlcera já que não há camada granulosa; e, quando há ausência da camada granulosa como ocorre na região ungueal, a mensuração pode ser desafiadora (28). Além disso a medida de Breslow não leva em conta as variações de espessura da epiderme normal nos diferentes sítios anatômicos, podendo mostrar diferenças mesmo quando o componente invasivo possui espessura semelhante, já que inclui a espessura total da epiderme. Sabe-se que, por exemplo, a pele acral possui uma epiderme mais espessa enquanto algumas áreas da face como a pele da região posterior da orelha possuem uma epiderme mais fina. (29, 30) Soma-se o fato de que para o subtipo de melanoma acral lentiginoso o valor da medida de Breslow para prognóstico ainda é discutido. (31)

Figura 1. Espessura de Breslow. A mensuração é realizada utilizando-se de um micrômetro ocular desde a camada granulosa até a célula tumoral mais profunda.

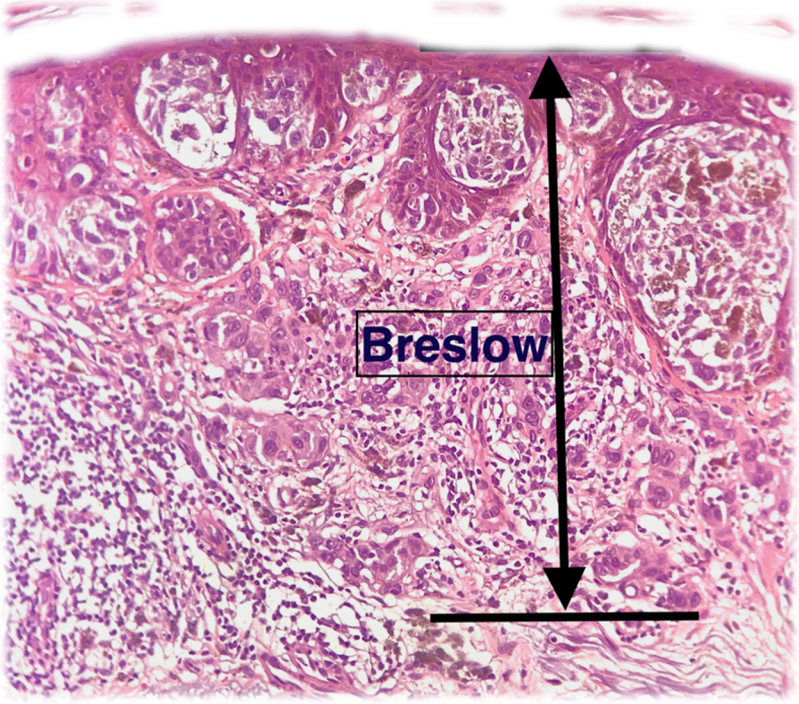


Figura 2. Espessura de Breslow. Neste exemplo a medida de Breslow é de 0,8mm.

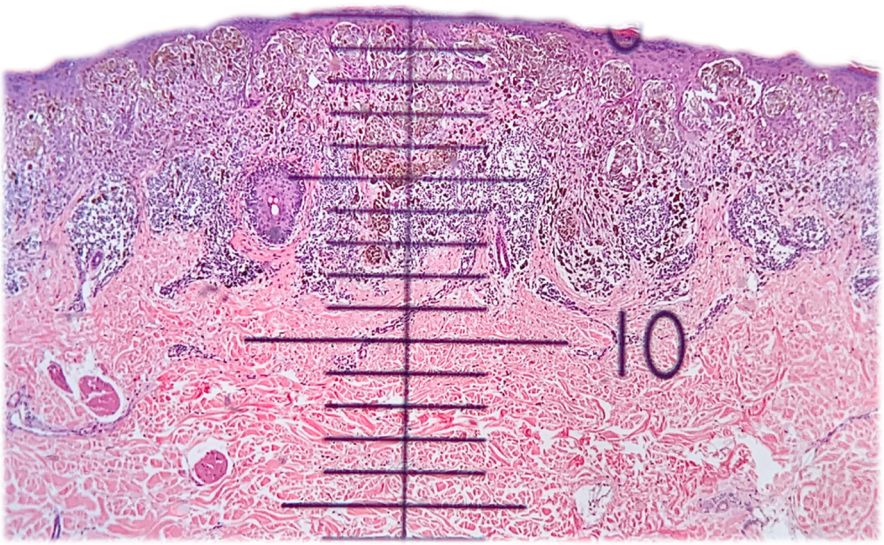
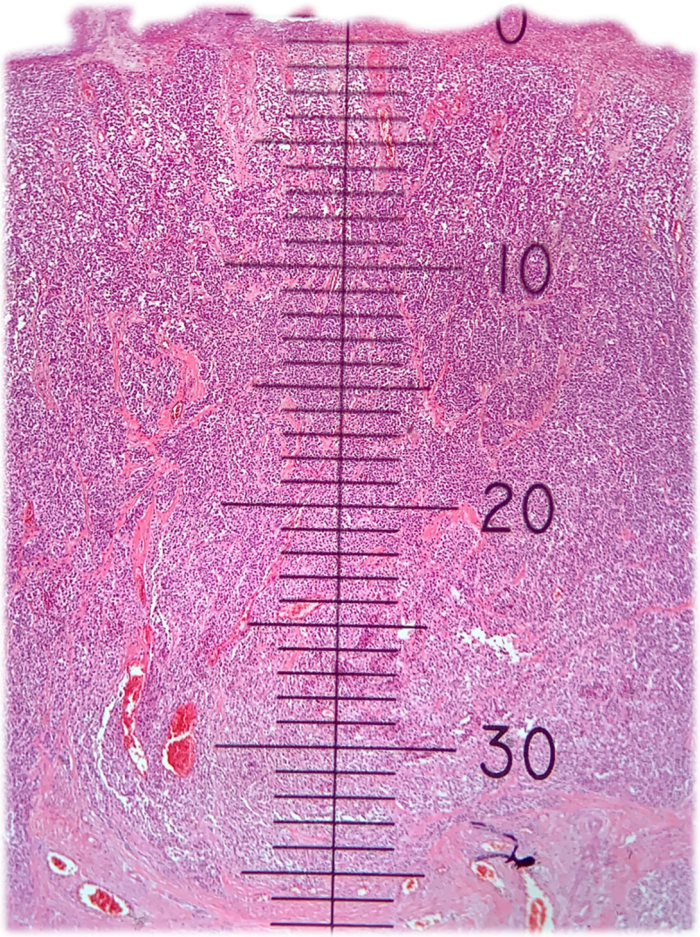


Figura 3. Espessura de Breslow em melanoma com ulceração. Mensura-se à partir da base da úlcera. Neste exemplo a espessura de Breslow é de 3,6mm.



1.3 Estadiamento patológico (Tamanho)

A oitava edição do Manual de Estadiamento do Câncer da AJCC (2017) estabelece a seguinte divisão da categoria T, de T1 a T4 de acordo com a espessura do tumor (22):

- TX: espessura do tumor primário não pode ser mensurada (por exemplo, diagnóstico por curetagem).

- T0: Sem evidência de tumor primário (por exemplo, melanoma que sofreu regressão total)

- Tis: Melanoma *in situ*.

- T1: Menor ou igual a 1,0 mm, sendo T1a < 0,8mm sem ulceração e T1b < 0,8mm com ulceração ou 0,8 mm a 1,0 mm (com ou sem ulceração).

- T2: > 1,0 mm a 2,0 mm, sendo T2a sem ulceração e T2b com ulceração.

- T3: > 2,0 mm a 4,0 mm, sendo T3a sem ulceração e T3b com ulceração.

- T4: > 4,0 mm, sendo T4a sem ulceração e T4b com ulceração. (27, 25)

A sobrevida aproximada em 5 anos é de 95 - 100% para melanomas *in situ* e melanomas finos (espessura menor ou igual a 1,0 mm) e varia de 50 - 96% para melanomas com mais de 1,0 mm de espessura sendo: 80 - 96% para melanomas de 1,1 mm a 2,0mm; 60 – 75% para melanomas de 2,1 mm a 4,0 mm; e 50% para melanomas com mais de 4,0 mm de espessura. (19, 32)

A ampliação de margens como parte do tratamento do melanoma cutâneo deve se basear na profundidade de Breslow. Para melanomas *in situ* a ampliação recomendada é de 0,5 – 1,0cm. Em melanomas com espessura menor ou igual a 1,0 mm (T1) a ampliação deve ser de 1,0cm. Para melanomas T2 (maior que 1,0 mm até 2,0mm), a ampliação pode ser de 1,0 cm a 2,0 cm devendo-se levar em consideração a localização do tumor, aspectos funcionais e cosméticos. E uma ampliação de margens de 2,0 cm é recomendada para tumores com espessura maior do que 2,0 mm (T3 e T4). (33)

Alguns estudos mostram que melanomas finos localizados na cabeça e pescoço exibem uma taxa maior de recorrência e metástases, possivelmente em consequência das margens cirúrgicas mais estreitas pela localização anatômica, por motivos estéticos e funcionais. Outra explicação plausível é de que a pele da cabeça e pescoço possui uma derme reticular mais fina, frequentemente danificada pela radiação solar. Por isso um Breslow fino poderia estar associado a um nível anatômico de invasão profundo. (34, 35, 36)

1.4 Linfonodo sentinela e outras características prognósticas

Os linfonodos regionais são os sítios mais comuns de metástases iniciais em pacientes com melanoma. A biópsia de linfonodo sentinela é uma técnica para detectar micrometástases linfonodais que contribui para determinar o prognóstico, adequar o tratamento e possui uma morbidade e efeitos adversos muito menores do que uma linfadenectomia eletiva. A biópsia de linfonodo sentinela deve ser discutida e recomendada / oferecida nos casos de melanoma com pelo menos 1,0mm de

espessura ou nos casos com menos de 1,0mm com características adversas (presença de ulceração, invasão linfovascular e/ou índice mitótico elevado principalmente em pacientes com menos de 40 anos). Melanomas com a espessura de Breslow maior ou igual a 1,0mm possuem um risco de aproximadamente 10% de invasão linfonodal, enquanto que em melanomas mais finos o risco é em torno de 5%. A dissecção linfonodal completa em pacientes com linfonodo sentinela positivo tradicionalmente tem sido recomendada, porém os estudos não demonstram uma diferença significativa na sobrevida dos pacientes submetidos ao procedimento. (33, 37)

Outras características importantes para o prognóstico incluem:

- Ulceração: a presença de ulceração, mesmo mínima tem impacto prognóstico. Além disso, a extensão da ulceração de um melanoma se correlaciona tanto com o status do linfonodo sentinela quanto à sobrevida geral. (38)

- Índice mitótico: é definido como o número de mitoses por milímetro quadrado no componente dérmico. Um índice mitótico elevado está fortemente associado a pior prognóstico, independente da espessura do tumor, ainda que esse parâmetro tenha sido excluído da última edição do AJCC para estadiamento (22)

- Invasão linfovascular: a presença de invasão está associada a maior risco de metástase para linfonodo sentinela assim como é um preditor de pior sobrevida geral em pacientes com evidência de regressão do tumor primário. (39)

- Infiltrado linfocitário: a presença de um infiltrado linfocitário tumoral denso (TIL "*tumor-infiltrating lymphocytes*", em que os linfócitos infiltram e rompem os ninhos tumorais e estão em contato direto com as células tumorais), está associado com um prognóstico favorável. (40)

- Necrose: presença de necrose tumoral está associado a uma maior espessura do melanoma, ulceração, invasão vascular, maior índice proliferativo e uma sobrevida geral reduzida. (41)

- Subtipo histológico: melanoma extensivo superficial possui características prognósticas melhores do que os subtipos lentiginoso acral e nodular. (42)

- Características clínicas como idade, sexo e localização do tumor. (18)

1.5 Tamanho macroscópico

De acordo com o conceito de progressão tumoral aplicado para melanomas, a primeira fase de crescimento é a radial, caracterizada por aumento do tumor em sua

periferia. A fase seguinte é a de crescimento vertical, em que o tumor adquire potencial metastático, caracterizado por uma nova população de células mitoticamente ativas que tendem a crescer como um nódulo expansivo intradérmico como um balão. (43, 44, 45, 46)

O tamanho macroscópico é um parâmetro utilizado para estadiamento do carcinoma de células escamosas e no melanoma uveal, porém não no melanoma cutâneo (47, 48), estando sua relevância ainda indeterminada neste caso. Raros estudos demonstraram correlação entre a dimensão macroscópica tumoral e a sobrevida (49, 50). Um estudo demonstrou através de análise multivariada, que a dimensão tumoral é um preditor independente de sobrevida específica do melanoma, reforçando a importância da informação quanto ao tamanho do tumor no laudo anatomopatológico. (49) Outro estudo comparou o valor prognóstico da maior largura microscópica do melanoma com a largura macroscópica, mostrando que ambas as mensurações estão associadas a prognóstico, embora a dimensão microscópica tenha sido superior. (50)

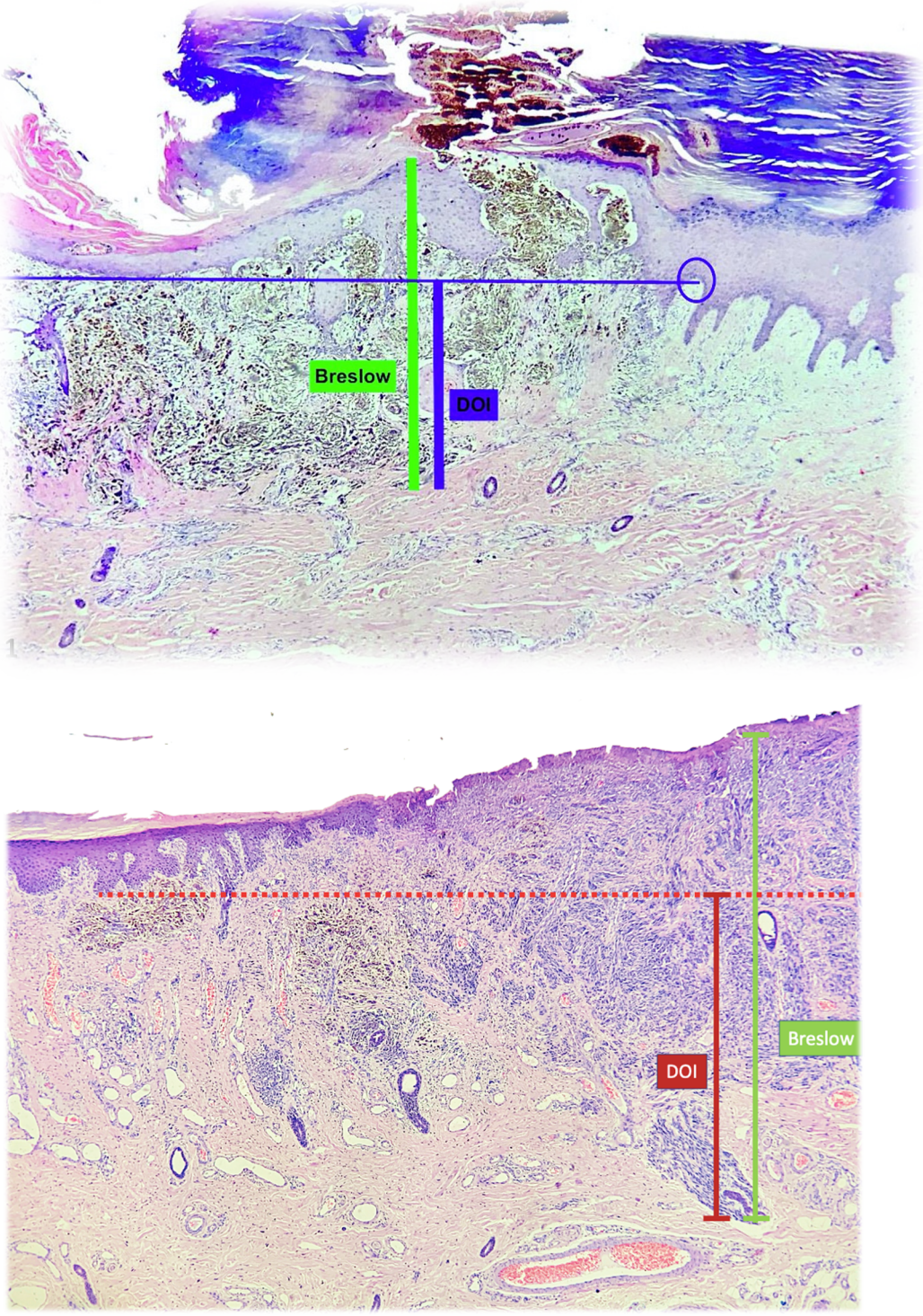
1.6 DOI (Depth Of Invasion)

O DOI (“Depth of Invasion”) ou “profundidade de invasão” é uma medida utilizada para estadiamento e prognóstico do carcinoma de células escamosas (CEC) da cavidade oral e foi incluído na oitava edição da American Joint Committee on Cancer (AJCC), enquanto a espessura tumoral (“tumor thickness”), uma mensuração com semelhanças à medida de Breslow, foi excluída. (51, 52) Para o CEC oral, um estudo demonstrou que a espessura tumoral não está associada a sobrevida específica do CEC oral, enquanto um aumento do DOI teve uma correlação significativa com redução da sobrevida específica. Outro estudo que comparou a espessura tumoral com o DOI encontrou que ambos estão independentemente associados com risco de metástase linfonodal. (52)

Existem diferenças na mensuração do DOI e da espessura tumoral. O DOI é medido da membrana basal normal adjacente até a porção mais profunda do tumor, em milímetros. (Figura 4) Já a espessura tumoral, assim como a medida de Breslow é mensurada do ponto mais alto da superfície tumoral até a porção mais profunda do tumor. No DOI, caso a membrana basal esteja comprometida ou ausente, uma linha hipotética é desenhada na superfície, permitindo a mensuração. (51, 52, 53). O DOI independe, portanto, da espessura do epitélio, da presença de ulceração ou

papilomatose, fatores estes que podem dificultar a medida de Breslow no melanoma cutâneo. Não há estudos, entretanto, avaliando a aplicabilidade do DOI em melanomas cutâneos e comparando com a medida de Breslow.

Figura 4. Comparação da medida de Breslow com DOI em pele não ulcerada (imagem superior) e ulcerada (imagem inferior), considerando a possível aplicação do DOI no melanoma cutâneo.



1.7 Estudo imunoistoquímico

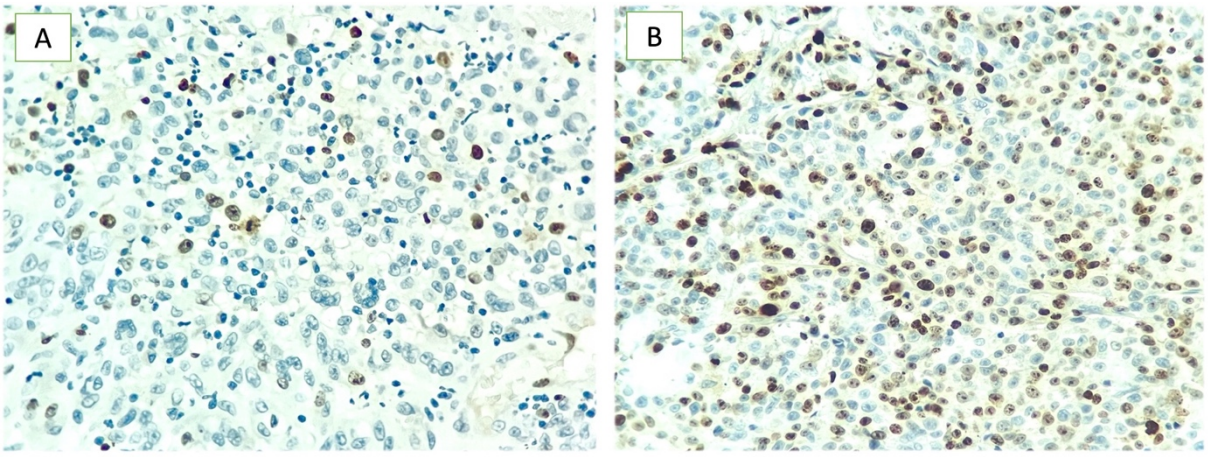
Os marcadores imunoistoquímicos são utilizados de forma complementar no estudo histopatológico, de diversas neoplasias, tanto para auxiliar no diagnóstico quanto no prognóstico. Vários marcadores são rotineiramente utilizados para o diagnóstico do melanoma, porém, até o momento não há marcadores imunoistoquímicos consolidados para avaliação prognóstica dos melanomas cutâneos que estejam incorporados nos protocolos de estadiamento (22, 54)

O Ki-67 é uma proteína encontrada no núcleo de células em proliferação, codificada pelo gene *MK167*, e é expressa nas fases G1, S, G2 e M do ciclo celular, mas não na G0. O antígeno Ki-67 pode reagir com seu anticorpo monoclonal MIB-1, tornando-se, portanto, um importante marcador de proliferação celular para a graduação de diversos tipos de câncer. (55, 56)

Vários estudos mostram uma correlação entre a expressão do Ki-67/MIB-1 e um pior prognóstico no câncer de ovário, câncer de mama, carcinoma de células escamosas do esôfago, câncer de pulmão, carcinoma do ducto e vesícula biliar, câncer cervical, dentre outros. (56)

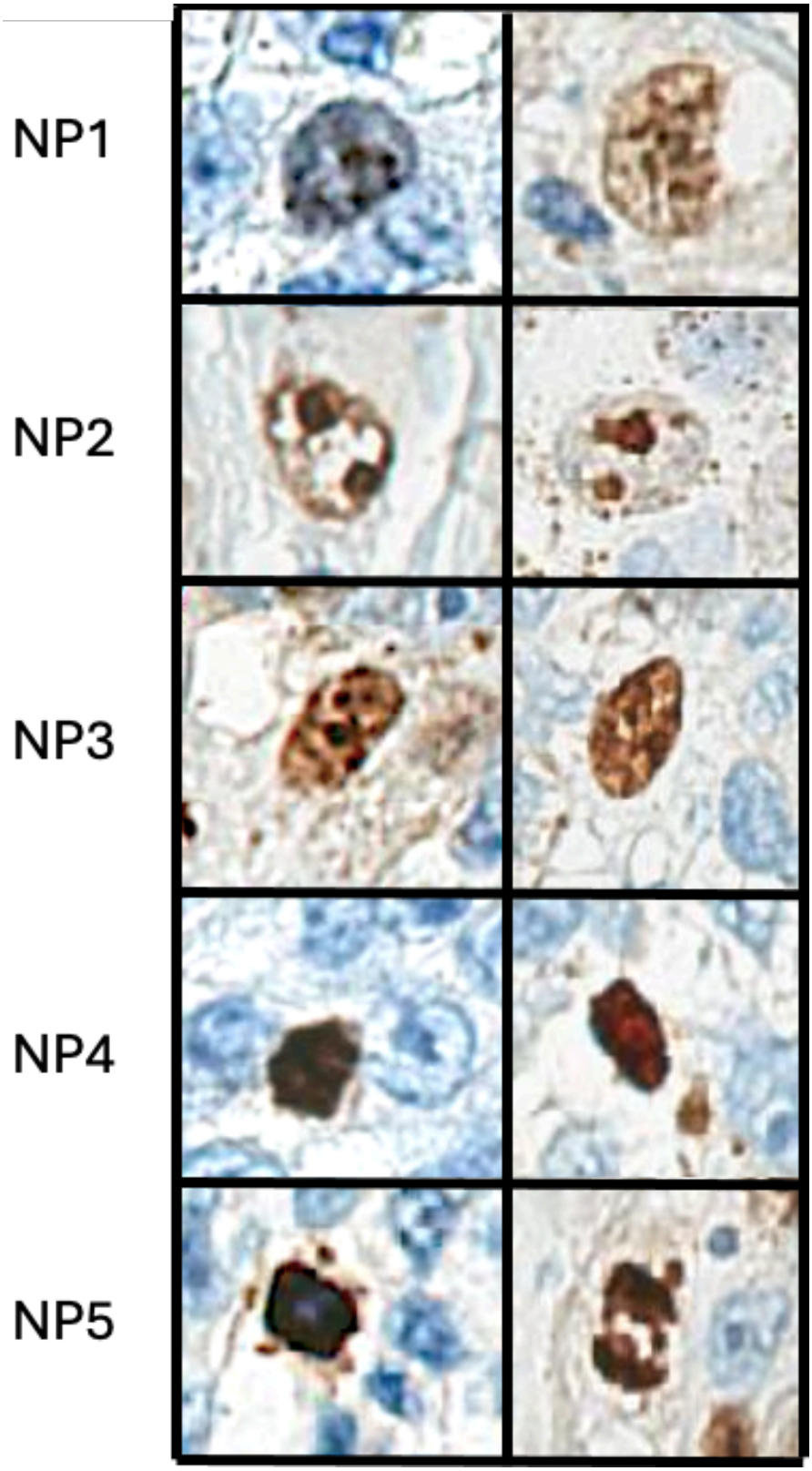
O índice do Ki-67 também é um fator prognóstico significativo no melanoma, sendo que o aumento de sua expressão está relacionado à diminuição da sobrevida. (57) Um trabalho mostrou que uma contagem mitótica maior ou igual a 1 por milímetro quadrado e expressão do Ki-67 em 20% ou mais das células tumorais é um fator prognóstico independente para metástases em melanomas finos, com uma taxa de metástase em 10 anos de cerca de 39%. (58) Um estudo demonstrou índices do Ki-67 significativamente mais elevados nos pacientes com metástases em linfonodo sentinela. (59) Outro estudo mostrou que o MIB-1 não é um fator prognóstico independente, porém está associado a parâmetros clinicopatológicos preditivos de pior evolução clínica (maior espessura e maior estádio). (60) Índice elevado do Ki-67 também foi associado a uma maior medida de Breslow. (61) (Figura 5)

Figura 5. Exemplo de índice do Ki-67 baixo (A) e elevado (B).



Além disso, verificou-se que o Ki-67 possui um padrão de marcação distinto durante os vários estágios do ciclo de proliferação celular (55, 62). (Figura 6) Localiza-se na região ao redor do cromossomo no começo da mitose; na forma de centenas de focos homogeneamente distribuídos na fase G1 inicial; um ou dois nucléolos na fase G1 tardia; grânulos grosseiros densos na fase S; grânulos e nódulos irregulares no nucleoplasma na fase G2. (63, 64) Um estudo de Dias et al., mostrou diferenças significativas no padrão de expressão do Ki-67 entre lesões epiteliais benignas e lesões epiteliais malignas ou pré-malignas. (64) Não há, entretanto, estudos que avaliaram o padrão de marcação do Ki-67 no melanoma cutâneo até o momento.

Figura 6. Exemplos de padrões de marcação nuclear do Ki-67 de acordo com a fase do ciclo celular, sendo NP1 (G1 inicial), NP2 (G1 tardio), NP3 (fase S), NP4 (G2) e NP5 (mitose)



O PRAME (*PReferentially expressed Antigen in MElanoma*), gene correspondente aos cDNAs 5E10 e Hi2 foi descrito pela primeira vez por Ikeda et al., em 1997. Trata-se de um antígeno tumoral identificado através da análise da especificidade de clones tumorais reativos de células T derivados de pacientes com melanoma cutâneo metastático em que as células do melanoma perderam a expressão de várias moléculas HLA. (65) Codificado em uma região de 12 quilobases no cromossomo 22, o PRAME atua como um correpressor da sinalização ligante-dependente do receptor do ácido retinoico que auxilia na inibição da diferenciação celular e apoptose (66, 67, 68) É expresso em alguns tecidos normais como testículos, adrenais, ovário e endométrio. (65) Nas neoplasias, além do melanoma cutâneo, o PRAME também é expresso no melanoma ocular (69), carcinoma da mama (70), carcinoma renal (71), carcinoma ovariano (72), sarcoma sinovial (73), carcinoma não de pequenas células pulmonar, dentre outros. (74, 68)

A alta frequência de expressão do PRAME (cerca de 90%) nos melanomas convencionais o tornou útil para diferenciar de nevos melanocíticos que em sua maioria são negativos para o PRAME (quando positivos, geralmente são focais, ao contrário dos melanomas que apresentam marcação difusa), e também na distinção dos melanomas metastáticos para linfonodo com os nevos melanocíticos nodais. Além da utilidade no diagnóstico, o PRAME é membro da família dos antígenos do câncer testicular (CTA) tornando-o um possível alvo na imunoterapia. (74, 75).

O PRAME foi identificado como um possível biomarcador para avaliação de prognóstico e risco de metástases em diversos tumores. (74, 68) O aumento da expressão do PRAME em estágios mais avançados da doença e a associação com maior risco de metástase sugere que o PRAME desempenha um papel na aquisição de várias características do câncer incluindo a capacidade de invasão e metástases. Demonstrou-se que o PRAME facilita a motilidade celular aumentando o comportamento invasivo do câncer de mama triplo negativo in vitro. (68) Foi evidenciado que uma alta expressão do PRAME se associa com um pior prognóstico clínico no câncer de mama aumentando-se a taxa de metástases a distância e menor sobrevida total. (70) No osteosarcoma, a alta expressão do PRAME se associa com pior prognóstico e maior risco de metástases pulmonares. (76) Em carcinomas espinocelulares da cabeça e pescoço, a intensidade de expressão do PRAME esteve diretamente relacionada ao grau da neoplasia, ao tamanho do tumor, metástases

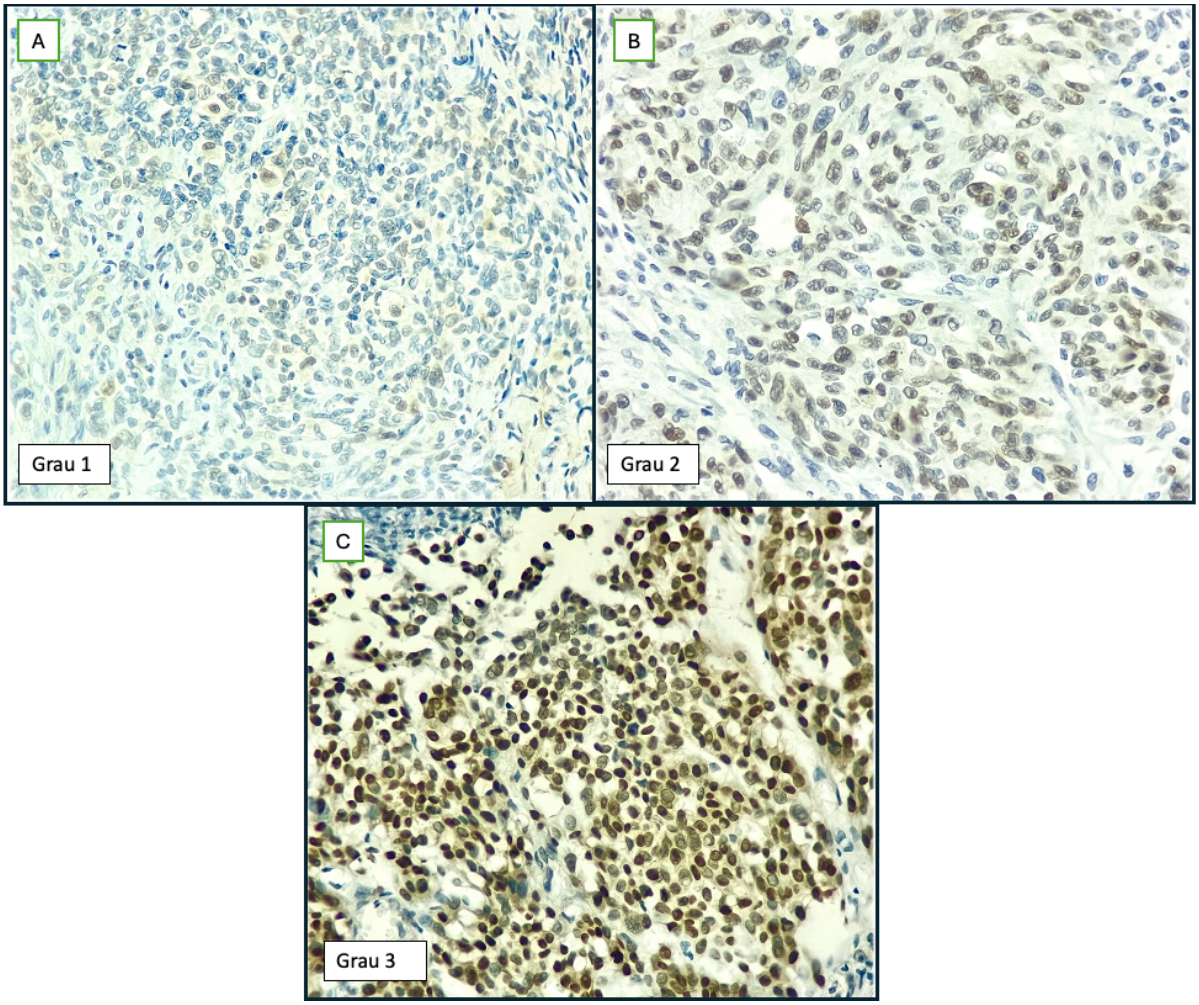
linfonodais e progressão da doença. (77). No melanoma uveal a expressão do PRAME foi fortemente associada ao risco de metástases e a um maior diâmetro do tumor (69).

Apesar de existirem vários estudos sobre o uso do PRAME para o diagnóstico de melanoma, raros estudos focaram na relação entre PRAME e prognóstico do melanoma. Os escassos estudos existentes não demonstraram correlação entre a expressão do PRAME com a sobrevida geral ou específica do melanoma cutâneo. (61, 78, 79). Quanto ao risco de metástases linfonodais, um estudo com melanomas do tipo lentiginoso acral mostrou uma maior expressão do PRAME naqueles casos com metástases em linfonodo do que naqueles livres de metástase. (80)

Para avaliação do PRAME no melanoma cutâneo a maioria dos estudos adota a estratificação utilizada por Lezcano et al., que leva em conta a extensão da expressão do PRAME em toda a área tumoral, sendo: 0= negativo; 1= entre 1% e 25% de células neoplásicas positivas; 2= entre 26% e 50%; 3= entre 51% e 75%; 4= entre 76% e 100%. Os estudos iniciais evidenciaram que os melanomas cutâneos exibem expressão difusa (grau 4) na maioria dos casos (mais de 80%). Entretanto estudos posteriores demonstram uma maior variabilidade da extensão de expressão do PRAME. (74, 81, 61)

Outro estudo adota ainda a intensidade da marcação do PRAME, sendo classificado em graus: 0= negativa; 1= fraca; 2= moderada; 3= forte (Figura 7). E um escore combinado consistindo na soma do grau de intensidade com a classificação da extensão da marcação, variando então de 0 a 7. (82). Um trabalho também demonstrou uma correlação entre o índice do Ki-67 mais elevado e uma maior expressão do PRAME. (61)

Figura 7. Graus de intensidade do PRAME: A = grau 1 (fraca), B = grau 2 (moderada), C = grau 3 (forte)



2 RESULTADOS

Os resultados serão apresentados na forma de artigos:

- Artigo 1

ASATO M. A. *et al.* Macroscopic tumor dimension, sentinel lymph node outcome, and survival analysis among cutaneous melanoma**. International Journal of Dermatolology,** v. 63, n. 6, p. 765-772, jun. 2024. DOI: 10.1111/ijd.17024. Epub em 13 jan. 2024. PMID: 38217520.

- Artigo 2

ASATO, M. A. *et al.* Proposal for the applicability of modified Breslow (measured from the basal membrane) as a predictor of survival and sentinel lymph node outcome in patients with cutaneous melanoma. **Anais Brasileiros de Dermatologia**, v. 99, n. 3, p. 398-406, maio/jun. 2024. DOI: 10.1016/j.abd.2023.09.002. Epub em 19 fev. 2024. PMID: 38378363; PMCID: PMC11074618.

- Artigo 3

ASATO, M. A. *et al.* Depth of invasion analysis to predict acral melanoma outcomes. **Annals of Diagnostic Pathology**, v. 71, ago. 2024, p. 152305. DOI: 10.1016/j.anndiagpath.2024.152305. Epub em 16 abr. 2024. PMID: 38640808.

- Artigo 4

ASATO, M. A. *et al.* The utility of PRAME and Ki-67 as prognostic markers for cutaneous melanoma.

Submetido na revista “The American Journal of Dermatopathology” (número AJD-D-24-00315). O artigo foi redigido seguindo as normas do periódico (https://edmgr.ovid.com/ajd/accounts/ifauth.htm).

- Artigo 5

ASATO, M. A. *et al.* The Ki-67 staining pattern as a cutaneous melanoma prognostic marker.

Submetido na revista “Pathobiology” (número PAT-2024-7-17). O artigo foi redigido seguindo as normas do periódico (https://karger.com/pat/pages/guidelines).

International Journal of Dermatology

Report

Macroscopic tumor dimension, sentinel lymph node outcome, and survival analysis among cutaneous melanoma

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doi: 10.1111/ijd.17024

Abstract

Background Cutaneous melanoma is characterized by a high risk of metastasis to distant organs and a substantial mortality rate. For planning treatment and assessing outcomes, the Breslow micrometric measurement is critical. The tumor macroscopic dimension is not considered a prognostic parameter in cutaneous melanoma, although there are studies showing that tumor size is an independent prognostic factor for melanoma-specific survival. Therefore, this study aimed to evaluate the macroscopic dimension of melanoma and other known prognostic factors (i.e., Breslow index, mitoses, regression, and ulceration) as predictors of sentinel lymph node outcome and survival outcome.

Methods We performed a retrospective cross-sectional study of 227 melanoma lesions subjected to sentinel lymph node biopsy at two Brazilian referral centers.

Results On univariate analysis, there was a statistically significant correlation between the largest macroscopic tumor dimension and the sentinel lymph node result ($P = 0.001$); however, on multivariate analysis considering all evaluated parameters, there was no significant difference between the sentinel lymph node result and the tumor macroscopic dimension ($P = 0.2689$). Regarding melanoma-specific survival, the macroscopic dimension showed no significant correlation ($P = 0.4632$) in contrast to Breslow's dimension ($P < 0.0001$).

Conclusion The Breslow thickness was the only significant factor related to both the sentinel lymph node outcome and melanoma specific survival among the evaluated variables.

Introduction

Cutaneous melanoma represents only 1% of skin tumors but is considered the most dangerous skin cancer. It frequently metastasizes, with high morbidity and mortality (more than 80% of skin cancer deaths).[1–4] Its incidence has increased in recent decades in Caucasian populations, probably related to recreational behavior associated with sun exposure.[5]

According to Globocan, in 2020, there were more than 320,000 new cases of melanoma worldwide, and more than 57,000 associated deaths. The highest incidences and deaths occurred in North America, Europe, Australia, and New Zealand. In the latter two countries, the standardized incidence rate was 35.8 cases per 100,000 inhabitants in 2020.[6] In Brazil, there is an estimated incidence rate of 2.01 for men and 1.78 for women per 100,000 inhabitants.[7]

Breslow's micrometric measurement is the most critical factor for prognostic assessment and management of invasive melanomas.[8] It has been widely used worldwide and included in the American Joint Committee on Cancer (AJCC) staging systems.[9] This system defines pathological staging (T), excision margin size, and sentinel lymph node applicability.[9,10] Other features for prognostic evaluation include the presence or absence of ulceration, lymphovascular invasion, lymphocytic infiltrate, regression, satellites, mitotic index, and histological subtype.[11–18]

The tumor dimension is a parameter for staging squamous cell carcinoma and uveal melanoma, but not for cutaneous

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melanoma.[19,20] Thus, the relevance of the macroscopic dimension of the tumor remains undetermined.[21–23] For example, there is no reference to the tumor's macroscopic size in the current protocol of the College of American Pathologists.[24]

Because there are only a few studies on this topic, our objective was to investigate a possible correlation between the macroscopic dimension, sentinel lymph node (SNL) outcomes and melanoma specific survival (MSS), in association with other known prognostic factors (i.e., the Breslow index, mitoses, regression, and ulceration). To our knowledge, the present study is the first in Latin America with this objective.

Materials and methods

This retrospective cross-sectional study analyzed formalin-fixed paraffin-embedded skin specimens collected from primary melanoma lesions and lymph node excisions performed at two Brazilian referral centers from 2005 to 2021. The study was approved by the committee of ethics in research (CAAE 52618721.0.1001.5411 and CAAE 52618721.0.3001.5434). From pathological reports stored in the digital systems of the participating institutions, we recorded clinical data (i.e., sex, age, location) and pathological information (i.e., macroscopic width, measured with a precision of up to 0.1 cm on the largest axis parallel to the epidermis, histological type, Breslow thickness in millimeters, sentinel lymph node result, and the presence or absence of ulceration, mitosis $\geq$1, and regression). Survival data were obtained from digital medical records to analyze outcomes. In relation to sentinel lymph node biopsy, it followed the guidelines of the Brazilian Group of Melanoma, which recommends the procedure for patients with cutaneous melanoma stage pT2 and pT3.[25] Lymph node biopsies were sectioned entirely every 3 mm and, if H&E (Hematoxylin and Eosin) staining was negative for metastasis, immunohistochemical analysis was performed with HMB45 and Melan-A on 5 slices from different paraffin block levels. Exclusion criteria included lack of macroscopic size or Breslow thickness in the pathology report, secondary melanoma, and thin melanomas (i.e., <1.0 mm in thickness).

We performed a descriptive analysis calculating mean, standard deviation (SD), minimum, maximum, and median values for quantitative variables and frequencies for categorized variables. Data were expressed as absolute (n) and relative (%) values.

Comparisons between means for tumor macroscopic size and Breslow thickness were made for lymph nodes, ulcerations, mitoses, and regression outcomes using a gamma-distribution adjustment followed by Wald's multiple comparison test. A logistic regression model was applied, considering the positive/negative result as the response variable and the others as explanatory variables to assess the risk or protective factors.

Cox proportional hazards modeling was applied to access MSS, adjusting for the largest dimension and Breslow thickness. This same model was used for each subtype, considering only those with the highest frequencies. Associations of survival with sex, lymph node status, ulceration, mitosis and regression were also evaluated. The significance level was set in all tests at 5% or the corresponding P-value. All analyses were performed using SAS for Windows v.9.4.

The study was approved by the Committee of Ethics in Research (CAAE 52618721.0.1001.5411 and CAAE 52618721.0.3001.5434).

Results

We included 227 cases of melanoma diagnosed from 2005 to 2021 that underwent sentinel lymph node biopsy. There were 107 (47.14%) women and 120 (52.86%) men. The mean age was 63.98 years (range: 21–94 years; SD: 15.25 years; median: 65 years). The tumor measurement in its largest axis ranged from 0.4 to 10.0 cm, with a mean of 2.83 cm (SD: 1.88 cm; median of 2.4 cm). Breslow's micrometric measurement ranged from 1.0 to 30.0 mm with a mean of 6.58 mm (SD: 6.24 mm; median of 4.5 mm). Melanomas on the limbs predominated, with 149 cases (corresponding to 65.53%), followed by the trunk (21.14%; 48 cases), head and neck (11.01%; 25 cases), and genital region (2.2%; 5 cases). Regarding histological subtype, 65 cases (30.09%) were superficial spreading melanoma, 71 (32.87%) were nodular melanomas, 75 (34.72%) were acral lentiginous melanoma, 2 (0.93%) were lentigo maligna melanoma, 2 (0.93%) were desmoplastic melanoma, 1 (0.46%) was impossible to classify and 11 cases were missing.

Of the 227 cases, 140 (61.67%) had metastasis to sentinel lymph nodes, and 87 (38.33%) were free of metastasis. There was ulceration in 62.24% (122 with ulceration; 74 without ulceration; 31 not reported), mitosis in 94.85% (184 with mitosis, 10 without mitosis, and 33 not reported), and regression in 11.98% (20 with regression, 147 without regression, and 60 not reported) (Table 1). Satellites were absent in all cases.

There was a significant correlation between the largest macroscopic tumor dimension and the sentinel lymph node result ($P = 0.001$, $n = 227$) on univariate analysis. The mean macroscopic dimension for those with negative lymph nodes was 2.36 cm (SD: 1.51 cm), and for positive lymph nodes, the value was 3.12 cm (SD: 2.02 cm). The Breslow thickness strongly correlates with the sentinel lymph node result ($P < 0.0001$, $n = 227$). The mean thickness was 3.68 mm (SD: 4.13 mm) for those with negative lymph nodes and 8.38 mm (SD: 6.66 mm) for those with positive sentinel lymph nodes. There was also a significant correlation between the tumor macroscopic dimension and the Breslow thickness with the presence of ulceration ($P = 0.0047$ and $P < 0.0001$, respectively; $n = 196$) and between the Breslow thickness and regression ($P = 0.0011$, $n = 167$). There was a significant correlation between the macroscopic dimension and the Breslow thickness with the

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Table 1 Descriptive analysis of clinicopathologic characteristics of the cases

Variables	*N* (%)
Sex	
Female	107 (47.14)
Male	120 (52.86)
Age (years)	
Mean	63.98
Median	65
Breslow thickness (mm)	
Mean	6.58
Median	4.5
Location, *N* (%)	
Limbs	149 (65.53)
Trunk	48 (21.14)
Head and neck	25 (11.01)
Genital	5 (2.2)
Histological subtype, *N* (%)	
Superficial spreading melanoma	65 (30.09)
Nodular melanoma	71 (32.87)
Acral lentiginous melanoma	75 (34.72)
Lentigo maligna melanoma	2 (0.93)
Desmoplastic melanoma	2 (0.93)
Not classified	1 (0.46)
Missing	11
Sentinel lymph node, *N* (%)	
Positive for metastasis	140 (61.67)
Negative for metastasis	87 (38.33)
Ulceration, *N* (%)	
Present	122 (53.74)
Absent	74 (32.59)
Missing	31 (13.65)
Mitosis, *N* (%)	
Present	184 (81.05)
Absent	10 (4.4)
Missing	33 (14.53)
Regression, *N* (%)	
Present	20 (8.81)
Absent	147 (64.75)
Not reported	60 (26.43)

Table 2 Univariate analyses of means for tumor macroscopic size and Breslow thickness were made for lymph node, ulceration, mitosis, and regression outcomes

	Sentinel lymph node— for metastasis			Sentinel lymph node+ for metastasis			
	N	Mean	SD	*N*	Mean	SD	*P*-value
Largest macroscopic size (cm)	87	2.36	1.51	140	3.12	2.02	0.001
Breslow thickness (mm)	87	3.68	4.13	140	8.38	6.66	<0.0001
	Ulceration absent			**Ulceration present**			
	N	Mean	SD	*N*	Mean	SD	
Largest macroscopic size (cm)	74	2.42	1.72	122	3.11	1.88	0.0047
Breslow thickness (mm)	74	3.45	3.79	122	8.45	6.7	<0.0001
	Mitosis absent			**Mitosis present**			
	N	Mean	SD	*N*	Mean	SD	
Largest macroscopic size (cm)	10	1.82	0.75	184	2.89	1.88	0.0265
Breslow thickness (mm)	10	3.48	4.24	184	6.84	6.27	0.0217
	Regression absent			**Regression present**			
	N	Mean	SD	*N*	Mean	SD	
Largest macroscopic size (cm)	147	2.82	1.87	20	2.4	1.44	0.2802
Breslow thickness (mm)	147	6.49	6.24	20	3.19	2.59	0.0011

−, negative; +, positive; *N*, number; SD, standard deviation.

presence of mitosis ($P = 0.0265$ and $P = 0.0217$, respectively; $n = 194$) and no significant correlation between the tumor macroscopic dimension and the presence of regression ($P = 0.2802$, $n = 167$) (Table 2).

On multivariate analysis, however, considering all evaluated parameters, there was no significant correlation between the sentinel lymph node result and the tumor macroscopic dimension ($P = 0.2689$; odds ratio [OR]: 1.428, $n = 227$), the presence of ulceration ($P = 0.1823$; OR: 1.752, $n = 196$), the presence of mitosis ($P = 0.9844$; OR: 1.017, $n = 194$), and the presence of regression ($P = 0.8122$; OR: 1.143, $n = 167$). Only the Breslow thickness remained statistically significant ($P = 0.0023$; OR 1.324, $n = 227$) (Table 3).

Regarding melanoma-specific survival, the macroscopic dimension showed no significant correlation ($P = 0.4632$; hazard ratio [HR]: 0.837, $n = 227$). In contrast, Breslow depth had a statistically significant association with survival outcomes ($P < 0.0001$; HR: 1.13, $n = 227$). Both the sentinel lymph node status and the presence of regression were also significantly associated with melanoma-specific survival ($P < 0.0001$; HR: 11.553 and $P = 0.0076$; HR: 3.385, respectively; $n = 227$). Other factors, such as gender, ulceration, and mitosis, did not demonstrate significant correlations with the survival outcome (Table 4).

Comparing melanoma subtypes using the Wilcoxon test with adjustment for multiple comparisons, no statistically significant differences on survival were observed between acral and

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superficial spreading subtypes ($\chi^2 = 0.6626$, raw $P = 0.4156$, Sidak-adjusted $P = 0.8005$), or between acral and nodular subtypes ($\chi^2 = 1.3160$, raw $P = 0.2513$, Sidak-adjusted $P = 0.5803$). However, the comparison between superficial spreading and nodular subtypes showed a statistically significant difference with a raw *P*-value of 0.0417, which was not maintained after Sidak adjustment for multiple comparisons ($P = 0.1199$) (Figure 1).

In regard to the other variables, there was a statistically significant correlation between histological subtypes and the following factors: lymph node status ($P = 0.0044$, $n = 211$), presence of ulceration ($P < 0.0001$, $n = 185$), presence of mitoses ($P = 0.0425$, $n = 186$), and presence of regression ($P = 0.0002$, $n = 159$). The incidence of positive lymph nodes was notably higher in acral melanomas (70.7%) and nodular melanomas (69%) as compared to superficial spreading melanomas (46.2%). The prevalence of ulceration was highest in nodular melanomas (75.4%), followed by acral melanomas (72.7%) and then superficial spreading melanomas (35.2%). The occurrence of mitoses was most frequent in nodular melanomas (100%), then in acral melanomas (95.6%), and superficial spreading melanomas

Table 3 Adjustment of the logistic regression model for a positive result due to other outcomes

Variables	OR	95% CI		*P*-value
Female × male	0.868	0.408	1.848	0.714
Ulceration vs. no ulceration	1.752	0.769	3.991	0.1823
Mitosis vs. no mitosis	1.017	0.195	5.291	0.9844
Regression vs. no regression	1.143	0.379	3.45	0.8122
Age	1.001	0.975	1.026	0.9687
Largest macroscopic measurement in cm	1.428	0.759	2.684	0.2689
Breslow thickness (mm)	1.324	1.105	1.587	0.0023

OR, odds ratio; CI, confidence interval.

Table 4 Cox model adjustment for the survival data analysis

Parameter	dF	Parameter estimate	Standard error	χ^2	$Pr > \chi^2$	Hazard ratio (HR)	95% HR confidence limits	
Sex (male)	1	0.42765	0.31023	1.9003	0.168	1.534	0.835	2.817
Positive SNL	1	2.44692	0.56795	18.5615	<0.0001	11.553	3.795	35.17
Ulceration present	1	0.11249	0.35404	0.101	0.7507	1.119	0.559	2.24
Mitosis present	1	0.55844	1.02452	0.2971	0.5857	1.748	0.235	13.02
Regression present	1	1.21935	0.45697	7.1199	0.0076	3.385	1.382	8.29
Macroscopic size	1	−0.17851	0.24333	0.5382	0.4632	0.837	0.519	1.348
Breslow	1	0.12245	0.02852	18.435	<0.0001	1.13	1.069	1.195

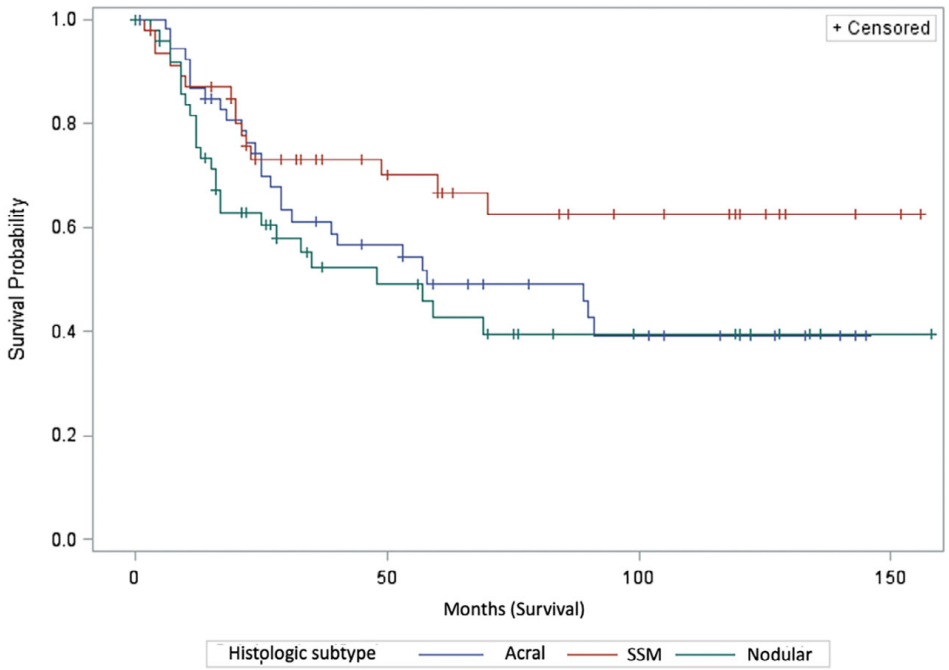


Figure 1 Kaplan–Meier analysis of survival probabilities over time for the main three histological subtypes of melanoma. SSM, superficial spreading melanoma

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(90.6%). The presence of regression was more common in superficial spreading melanomas (26.5%), followed by nodular melanomas (8.2%) and acral melanomas (1.6%). There was no statistically significant correlation between subtype and gender.

Discussion

Univariate analysis showed a correlation between a tumor's macroscopic dimensions and lymph node outcome; however, this finding was not confirmed in the multivariate analyses. Of all parameters (i.e., macroscopic dimension, Breslow thickness, presence of ulceration, mitosis, and regression), only the Breslow thickness remained statistically significant and a risk factor for the lymph node result outcome in the logistic regression.

According to the tumor progression concept applied to melanomas, the first step is the radial growth phase, characterized by the enlargement of the tumor at its periphery, forming a patch or a plaque lesion in the skin.[26] The next step is the vertical growth phase, in which the tumor acquires metastatic potential, characterized by a new population of mitotically active cells that tend to grow as an expanding intradermal nodule like a balloon.[26–29] According to this paradigm, we hypothesized that the tumor macroscopic size would be influenced by its vertical growth phase and thickness, explaining why it is not an independent risk factor for predicting the sentinel lymph node result (as is the Breslow measurement).

Breslow thickness is a standard parameter for assessing prognosis and is a significantly correlated independent risk factor for positive sentinel lymph nodes.[30,31] Our study revealed a statistically significant correlation between Breslow depth and both survival outcome and sentinel lymph node status. This finding is consistent with the literature, as exemplified by the study conducted by Gershenwald et al.,[32] which involved 612 patients and showed that the frequency of positive sentinel lymph nodes increases as tumor thickness increases, ranging from 4.8% in patients with tumors ≤1.50 mm to 34.4% in patients with tumors thicker than 4.0 mm. Also Balch et al.[33] demonstrated that the two most potent independent characteristics of the primary melanoma among all prognostic variables were tumor thickness and ulceration. In an attempt to enhance the Breslow thickness, Rashed et al.[34,35] proposed a new type of measurement called the Breslow density, determining the density of malignant melanoma cells at the point of Breslow thickness measurement. The study showed that both measurements, although strongly correlated, are independent prognostic factors. However, relative measurement difficulty may limit its adoption into routine practice.

Few studies analyzed the correlation between the tumor size and Breslow thickness in malignant melanoma. Moreno-Ramírez et al.[22] performed a cross-sectional study with 1,610 patients and recorded only a moderate association ($0.30 \leq r \leq 0.70$) between tumor size and Breslow's thickness ($r = 0.42$), depending on the anatomic location, sex, and age. Crocetti et al.[23] studied 2,071 melanomas and demonstrated a correlation between the tumor size and the Breslow thickness ($P < 0.001$), although this association was weak ($r = 0.39$). In the present study, the Pearson's correlation coefficient for the relationship between the largest macroscopic size and the Breslow thickness resulted in only a moderate correlation ($r = 0.58$; $P < 0.0001$). The substantial number of nodular cases (which have no radial growth phase) may have influenced our results.

In terms of melanoma-specific survival, our study revealed no significant association between the macroscopic dimension and patient survival outcomes. In contrast, Ma et al.[21] studied 28,593 patients and performed a multivariate analysis with MSS as the primary endpoint, adjusting for factors including regional metastasis. They found that the tumor size is a critical, independent predictor of MSS, reinforcing the importance of the pathology report regarding tumor size. Bamford et al.,[36] in a study comparing the prognostic value of the microscopically measured invasive width with the macroscopic width of cutaneous melanoma, showed that both were significantly associated with prognosis, although the microscopic measurement was superior. It is essential to highlight that these studies analyzed all sizes of melanoma, including thin melanomas (<1.0 mm) that were excluded from the present study.

We also analyzed the relationship between the sentinel lymph node result and ulceration, mitosis, and regression. We found no significant correlation between the presence of ulceration and positive lymph node status ($P = 0.1823$) in multivariate analysis. This finding agrees with Cadili et al.,[30] who studied 348 patients with malignant melanoma and showed no significant correlation ($P = 0.09$). In contrast, Nguyen et al. studied 112 patients; multivariate analysis revealed that ulceration independently predicted sentinel lymph node positivity ($P < 0.01$).[34] A total of 215 patients by Mraz-Gernhard et al. showed that ulceration was present more than twice as often in patients with a positive sentinel lymph node than in those with a negative sentinel lymph node ($P < 0.001$).[37] It is possible that histological subtype and tumor staging explain these conflicting results.

Our study showed no significant correlation between presence of mitosis and lymph node metastasis on multivariate analysis ($P = 0.9844$), in agreement with Nguyen et al.,[38] who found no predictive value between a high mitotic rate and lymph node involvement. Mraz-Gernhard et al.[37] found that a high mitotic rate (>5 mitoses/mm^2) was almost twice as frequent among those with a positive sentinel lymph node result than a negative result ($P = 0.03$). Sondak et al.[39] studied 419 patients and demonstrated that mitoses per square millimeter were an independent predictor of positive sentinel lymph nodes in addition to Breslow depth. In the present study, we did not evaluate mitosis as a quantitative variable. Because we divided the cases into those with and without mitosis, we could compare them regarding the number of mitoses. In the seventh edition of AJCC Melanoma Staging (2010),[40] mitosis ≥1/mm^2 was used as a primary criterion for categorizing T1 melanoma as T1b, for

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which sentinel lymph node biopsy should be selectively recommended. However, in the sixth (2002),[41] and the current eighth edition (2017),[42] the mitotic rate is not used for staging. Our study agrees with the AJCC eight edition[43] because we did not find an influence of mitosis in the sentinel lymph node results.

Our series did not reveal a significant correlation between regression and the lymph node result ($P = 0.8122$), following the study of Mraz-Gernhard et al.[37] In a study with 403 patients from Letca et al.,[44] there was no significant correlation between regression and sentinel lymph node metastasis ($P = 0.6$). Aivazian et al.[45] studied 8,693 patients, and multivariable analysis revealed that regression with tumor-infiltrating lymphocytes independently predicted a negative sentinel lymph node biopsy. A systematic review of 10,098 patients Ribero et al.[46] found that patients with regression had a lower likelihood to have sentinel lymph node-positivity than patients without regression. Our investigation demonstrated a statistically significant correlation between the presence of regression and poorer melanoma-specific survival. Contrasting with our findings, Aivazian et al.,[45] observed that patients presenting with both tumor regression and tumor infiltrating lymphocytes (TILs) exhibited the highest rates of 10-year overall survival, melanoma-specific survival, and recurrence-free survival. However they noted that in Stage III melanoma patients, presence of regression predicted lower survival outcomes.

Our study identified a significant association between sentinel lymph node status and melanoma-specific survival, which aligns with the existing literature. For instance, the study conducted by Chen et al.[47] reported that patients with a negative sentinel lymph node exhibited a markedly higher five-year overall survival rate and MSS rate compared to patients with positive sentinel lymph node biopsy. The presence of ulceration and mitosis are established in the literature as predictors of MSS, and their presence confers a worse prognosis[48] while the prognostic relevance of regression remains undetermined.[44]

There was a significant correlation between Breslow thickness and the presence of ulceration ($P < 0.0001$). This result agrees with Bønnelykke-Behrndtz et al.,[49] who found that cleft formation (which is associated with ulceration) was significantly correlated with increased Breslow thickness ($P < 0.0001$); there was also a significant correlation between Breslow thickness and absence of regression ($P = 0.0011$), which is following Aivazian et al.,[45] who demonstrated that regression was significantly associated with a smaller Breslow thickness ($P < 0.0001$).

In relation to survival related to different histological melanoma subtypes, our study did not demonstrate statistically significant differences in survival among the three most common subtypes encountered (superficial spreading melanoma, nodular melanoma and acral melanoma). This contrasts with the findings of Giavina-Bianchi et al.,[50] who examined 565 cases of invasive melanoma and analyzed disease-specific survival according to histological type and the Breslow Index. In their study, univariate analysis showed significant differences in survival between the subtypes; however in multivariate analysis with adjusted hazard ratios, they found no significant correlation between histological type and survival. Similarly, the study conducted by Buja et al.,[51] identified significant differences in five-year survival rates among the different histological subtypes, with the nodular subtype exhibiting the poorest prognosis.

This study has some limitations that should be considered. It was a retrospective study from Brazil, and the frequency of acral and nodular melanomas may be different from other countries. As a reference center in the Brazilian countryside, our institution receives a high number of advanced melanoma cases, reflecting the region's socioeconomic challenges that often lead to delayed diagnoses. It is important to highlight that the Brazilian population demonstrates considerable diversity with substantial genetic admixture, which may account for distinctive findings compared to previous studies conducted in Northern Hemisphere populations. Furthermore, the impact of formalin fixation on the macroscopic size was not evaluated. It is accepted that the specimen size can reduce about 16–18% of its width after surgical excision and formalin fixation, underestimating the in vivo diameter.[52] Beside the above, the cases were quite advanced since they presented high Breslow thickness, a high rate of ulceration and a high rate of positive sentinel lymph node. It is also the first study that directly correlates macroscopic melanoma size and sentinel lymph node result using univariate and multivariate analysis, especially considering the Latin American population.

Although there was a significant correlation between the tumor size and the sentinel lymph node result in the univariate analysis, the multivariate analysis did not confirm this finding. Breslow thickness was the only significant factor related to both the sentinel lymph node outcome and MSS among the evaluated variables. More studies are necessary to understand the role of tumor size in outcome prediction, including studies considering the in vivo diameter.

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References

1 Ferreira FR, Nascimento LFC. Mortality due to cutaneous melanoma in south region of Brazil: a spatial approach. *An Bras Dermatol*. 2016;**91**(4):437–41.
2 Saginala K, Barsouk A, Aluru JS, Rawla P, Barsouk A. Epidemiology of Melanoma. *Med Sci*. 2021;**9**(4):63.
3 Urban K, Mehrmal S, Uppal P, Giesey RL, Delost GR. The global burden of skin cancer: a longitudinal analysis from the Global Burden of Disease Study, 1990–2017. *JAAD Int*. 2021;**2**:98–108.

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4 American Cancer Society. Cancer Facts & Figures 2022. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2022/2022-cancer-facts-and-figures.pdf. Accessed July 18 2022.
5 Nikolaou V, Stratigos AJ. Emerging trends in the epidemiology of melanoma. *Br J Dermatol*. 2014;**170**(1):11–9.
6 The Global Cancer Observatory. Melanoma of skin. Globocan; 2020. https://gco.iarc.fr/today/data/factsheets/cancers/16-Melanoma-of-skin-fact-sheet.pdf. Accessed July 18 2022.
7 Instituto Nacional de Câncer José Alencar Gomes da Silva. Estimativa 2020: incidência de câncer no. Brazil: INCA; 2019. https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/estimativa-2020-incidencia-de-cancer-no-brasil.pdf
8 Ge L, Vilain RE, Lo S, Aivazian K, Scolyer RA, Thompson JF. Breslow thickness measurements of melanomas around American Joint Committee on cancer staging cut-off points: imprecision and terminal digit bias have important implications for staging and patient management. *Ann Surg Oncol*. 2016;**23**(8):2658–63.
9 Keung E, Gershenwald J. The eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system: implications for melanoma treatment and care. *Expert Rev Anticancer Ther*. 2018;**18**(8):775–84.
10 Swetter SM, Tsao H, Bichakjian CK, Curiel-Lewandrowski C, Elder DE, Gershenwald JE, et al. Guidelines of care for the management of primary cutaneous melanoma. *J Am Acad Dermatol*. 2018;**80**(1):208–50.
11 Sarpa HG, Reinke K, Shaikh L, Leong SPL, Miller JR, Sagebiel RW, et al. Prognostic significance of extent of ulceration in primary cutaneous melanoma. *Am J Surg Pathol*. 2006;**30**(11):1396–400.
12 Egger ME, Gilbert JE, Burton AL, McMasters KM, Callender GG, Quillo AR, et al. Lymphovascular invasion as a prognostic factor in melanoma. *Am Surg*. 2011;**77**(8):992–7.
13 Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, McCarthy SW, et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol*. 2012;**30**(21):2678–83.
14 Ocanha-Xavier JP, Xavier-Junior JCC, Marques MEA. Melanoma: clinical, evolutive and histopathological characteristics of a series of 136 cases. *An Bras Dermatol*. 2018;**93**(3):373–6.
15 Azzola MF, Shaw HM, Thompson JF, Soong SJ, Scolyer RA, Watson GF, et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: an analysis of 3661 patients from a single center. *Cancer*. 2003;**97**(6):1488–98.
16 Garbe C. Partial histological tumor regression in primary melanoma as protective factor for lymph node micrometastasis. *JAMA Dermatol*. 2015;**151**(12):1291–2.
17 Antonialli AZ, Bertolli E, Macedo MP, Pinto CAL, Calsavara VF, Duprat Neto JP. How does the mitotic index impact patients with T1 melanoma? Comparison between the 7th and 8th edition of the American Joint Committee on Cancer melanoma staging system. *An Bras Dermatol*. 2020;**95**(6):691–5.
18 Niebling MG, Haydu LE, Lo SN, Rawson RV, Lamboo LGE, Stollman JT, et al. The prognostic significance of microsatellites in cutaneous melanoma. *Mod Pathol*. 2020;**33**(7):1369–79.
19 Shields CL, Kaliki S, Furuta M, Fulco E, Alarcon C, Shields JA. American Joint Committee on cancer classification of uveal melanoma (anatomic stage) predicts prognosis in 7,731 patients: the 2013 zimmerman lecture. *Ophthalmology*. 2015;**122**(6):1180–6.
20 Cheng J, Yan S. Prognostic variables in high-risk cutaneous squamous cell carcinoma: a review. *J Cutan Pathol*. 2016;**43**(11):994–1004.
21 Ma Q, Suo H, Zhu L, Qian Y, Sun X, Li Q, et al. Prognostic significance of tumor size for primary invasive cutaneous melanoma: a population-based study, 2004-2016. *Cancer Med*. 2020;**9**(13):4561–71.
22 Moreno-Ramírez D, Ojeda-Vila T, Ríos-Martín JJ, Nieto-García A, Ferrándiz L. Association between tumor size and Breslow's thickness in malignant melanoma: a cross-sectional, multicenter study. *Melanoma Res*. 2015;**25**(5):450–2.
23 Crocetti E, Fancelli L, Caldarella A, Buzzoni C. Thickness and diameter in melanoma: is there a relation? *Tumori*. 2016;**102**(1):e1–3.
24 College of American Pathologists. Protocol for the examination of specimens from patients with melanoma of the skin. https://documents.cap.org/protocols/Skin.Melanoma_4.3.0.2.REL_CAPCP.pdf. Accessed July 18 2022.
25 Grupo Brasileiro de Melanoma. Recomendações para o Tratamento do Melanoma cutâneo. 2nd ed. 2023. https://gbm.org.br/wp-content/uploads/2023/05/Cartilha_Recomendacoes_GBM_maio23.pdf
26 Clark WH Jr, Elder DE, Guerry D, Epstein MN, Greene MH, Van Horn M. A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. *Hum Pathol*. 1984;**15**(12):1147–65.
27 McDermott NC, Hayes DP, al-Sader MH, Hogan JM, Walsh CB, Kay EW, et al. Identification of vertical growth phase in malignant melanoma. A study of interobserver agreement. *Am J Clin Pathol*. 1998;**110**(6):753–7.
28 Elder DE. Melanoma progression. *Pathology*. 2016;**48**(2):147–54.
29 Laga AC, Murphy GF. Cellular heterogeneity in vertical growth phase melanoma. *Arch Pathol Lab Med*. 2010;**134**(12):1750–7.
30 Cadili A, Dabbs K. Predictors of sentinel lymph node metastasis in melanoma. *Can J Surg*. 2010;**53**(1):32–6.
31 Laga AC, Haefner HK, Granter SR. Melanocytic lesions of the vulva. In: Crum CP, Lee KR, Nucci MR, et al., editors. *Diagnostic obstetric and gynecologic pathology*. Philadelphia, PA: Elsevier Saunders; 2006. p. 181–96.
32 Gershenwald JE, Thompson W, Mansfield PF, Lee JE, Colome MI, Tseng CH, et al. Multi-institutional melanoma lymphatic mapping experience: the prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients. *J Clin Oncol*. 1999;**17**(3):976–83.
33 Balch CM, Soong SJ, Gershenwald JE, Thompson JF, Reintgen DS, Cascinelli N, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol*. 2001;**19**(16):3622–34.
34 Rashed H, Flatman K, Bamford M, Teo KW, Saldanha G. Breslow density is a novel prognostic feature in cutaneous malignant melanoma. *Histopathology*. 2016;**70**(2):264–72.
35 Saldanha G, Yarrow J, Pancholi J, Flatman K, Teo KW, Elsheik S, et al. Breslow density is a novel prognostic feature that adds value to melanoma staging. *Am J Surg Pathol*. 2018;**42**(6):715–25.
36 Bamford M, Udensi L, Khanna A, O'Riordan M, Saldanha G. Comparison of the prognostic value of microscopically measured invasive width versus macroscopic width in cutaneous melanoma shows the superiority of microscopic

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invasive width measurement. *J Cutan Pathol*. 2022;**49**(6):536–42.
37 Mraz-Gernhard SM, Sagebiel RW, Kashani-Sabet M, Miller JR, Leong SPL. Prediction of sentinel lymph node micrometastasis by histological features in primary cutaneous malignant melanoma. *Arch Dermatol*. 1998;**134**(8):983–7.
38 Nguyen CL, McClay EF, Cole DJ, O'Brien PH, Gillanders WE, Metcalf JS, et al. Melanoma thickness and histology predict sentinel lymph node status. *Am J Surg*. 2001;**181**(1):8–11.
39 Sondak VK, Taylor JMG, Sabel MS, Wang Y, Lowe L, Grover AC, et al. Mitotic rate and younger age are predictors of sentinel lymph node positivity: lessons learned from the generation of a probabilistic model. *Ann Surg Oncol*. 2004;**11**(3):247–58.
40 Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. *AJCC cancer staging manual*. 7th ed. New York, NY: Springer; 2010.
41 Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, et al. *AJCC cancer staging manual*. 6th ed. New York, NY: Springer; 2002.
42 Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al. *AJCC cancer staging manual*. 8th ed. Chicago, Il: Springer; 2017.
43 Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009;**27**(36):6199–206.
44 Letca AF, Ungureanu L, Şenilă SC, Grigore LE, Pop Ş, Fechete O, et al. Regression and sentinel lymph node status in melanoma progression. *Med Sci Monit*. 2018;**24**:1359–65.
45 Aivazian K, Ahmed T, El Sharouni MA, Stretch JR, Saw RPM, Spillane AJ, et al. Histological regression in melanoma: impact on sentinel lymph node status and survival. *Mod Pathol*. 2021;**34**(11):1999–2008.
46 Ribero S, Gualano MR, Osella-Abate S, Scaioli G, Bert F, Sanlorenzo M, et al. Association of histologic regression in primary melanoma with sentinel lymph node status: a systematic review and meta-analysis. *JAMA Dermatol*. 2015;**151**(12):1301–7.
47 Chen J, Xu Y, Zhou Y, Wang Y, Zhu H, Shi Y. Prognostic role of sentinel lymph node biopsy for patients with cutaneous melanoma: a retrospective study of surveillance, epidemiology, and end-result population-based data. *Oncotarget*. 2016;**7**(29):45671–7.
48 Thompson JF, Soong SJ, Balch CM, Gershenwald JE, Ding S, Coit DG, et al. Prognostic significance of mitotic rate in localized primary cutaneous melanoma: an analysis of patients in the multi-institutional American Joint Committee on Cancer melanoma staging database. *J Clin Oncol*. 2011;**29**(16):2199–205.
49 Bønnelykke-Behrndtz ML, Schmidt H, Christensen IJ, Damsgaard TE, Møller HJ, Bastholt L, et al. Prognostic stratification of ulcerated melanoma: not only the extent matters. *Am J Clin Pathol*. 2014;**142**(6):845–56.
50 Giavina-Bianchi MH, Festa-Neto C, Sanches JA, Teixeira ML, Waldvogel BC. Worse survival of invasive melanoma patients in men and "de novo" lesions. *An Bras Dermatol*. 2020;**95**:158–64.
51 Buja A, Bardin A, Damiani G, Zorzi M, De Toni C, Fusinato R, et al. Prognosis for cutaneous melanoma by clinical and pathological profile: a population-based study. *Front Oncol*. 2021;**11**:737399.
52 Dauendorffer JN, Bastuji-Garin S, Guéro S, Brousse N, Fraitag S. Shrinkage of skin excision specimens: formalin fixation is not the culprit. *Br J Dermatol*. 2009;**160**(4):810–4.

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ORIGINAL ARTICLE

Proposal for the applicability of modified Breslow (measured from the basal membrane) as a predictor of survival and sentinel lymph node outcome in patients with cutaneous melanoma☆

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KEYWORDS
Melanoma;
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Abstract
Background: Cutaneous melanoma is a neoplasm with a high mortality rate and risk of metastases to distant organs. The Breslow micrometric measurement is considered the most important factor for evaluating prognosis and management, measured from the granular layer to the deepest portion of the neoplasm. Despite its widespread use, the Breslow thickness measurement has some inaccuracies, such as not considering variations in the thickness of the epidermis in different body locations or when there is ulceration.
Objective: To evaluate the applicability of a modified Breslow measurement, measured from the basal membrane instead of from the granular layer, in an attempt to predict sentinel lymph node examination outcome and survival of patients with melanoma.
Methods: A retrospective and cross-sectional analysis was carried out based on the evaluation of slides stained with hematoxylin & eosin from 275 cases of melanoma that underwent sentinel lymph node biopsy from 2008 to 2021 at a reference center in Brazil.

☆ Study conducted at the Hospital Amaral Carvalho, Jaú, Brazil and Universidade Estadual Paulista, Botucatu, Brazil.

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Results: Analysis of the Cox model to evaluate the impact of the Breslow measurement and the modified Breslow measurement on survival showed that both methods are statistically significant. Logistic regression revealed a significant association between both measurements and the presence of metastasis in sentinel lymph nodes.

Conclusion: Measuring melanoma depth from the basal membrane (modified Breslow measurement) is capable of predicting survival time and sentinel lymph node outcome, as well as the conventional Breslow measurement.

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Introduction

Cutaneous melanoma is a neoplasm that arises from melanocytes and, at an advanced stage, often leads to metastases to distant organs.[1] The incidence of melanoma has increased in recent decades in light-skinned populations, probably related to recreational behavior and sun exposure. It is believed to arise as a consequence of a complex interaction of environmental and constitutional factors.[2]

The depth of invasion as a prognostic factor was reported by Alexander Breslow in 1970, who demonstrated a correlation between melanoma thickness and risk of recurrence and metastasis.[3,4] The Breslow micrometric measurement has become the most important factor for prognosis and conduct, is widely used and represents the main factor in staging systems, including that of the American Joint Committee on Cancer.[5] The Breslow measurement is obtained using a calibrated ocular micrometer to measure, from the most superficial portion of the granular layer to the deepest portion of the tumor.[6,7] However, the Breslow measurement has some limitations. For instance, when ulceration is present, the Breslow measurement may be underestimated due to the amount of tumor loss that is not taken into account; and, in the absence of the granular layer, as it occurs in the nail region, the measurement can be challenging.[8] Furthermore, the Breslow measurement does not take into account variations in the thickness of the normal epidermis in different anatomical sites and may show differences even when the invasive component has a similar thickness, as it includes the total thickness of the epidermis.

The depth of invasion is also a prognostic factor for squamous cell carcinoma of the cervix, for instance. In this tumor, however, the depth of tumor invasion is measured from the base of the epithelium, eliminating the influence of the thickness of the epithelium or the presence of ulceration on the final measurement.[9,10]

The aim of this study was to evaluate the applicability of the modified Breslow measurement, measured from the basal membrane instead of the granular layer, in an attempt to predict the sentinel lymph node outcome and the survival of patients with cutaneous melanoma compared to the classic Breslow measurement (Fig. 1). Additionally, the aim is to evaluate the relationship between sentinel lymph node status (positive or negative for metastases), presence of ulceration, and survival time; and the relationships between anatomical site and histopathological subtype with survival and sentinel lymph node outcome.

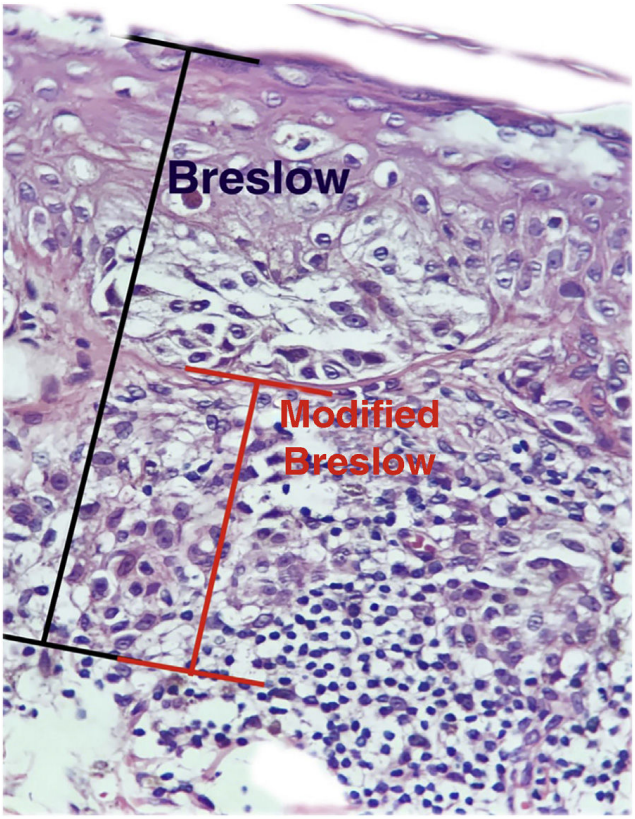


Figure 1 Breslow measurement (in black) compared to the modified Breslow (in red).

Materials and methods

This was a retrospective and cross-sectional study that analyzed slides stained with hematoxylin & eosin obtained from formalin-fixed, paraffin-embedded skin samples collected from primary melanoma lesions at Hospital Amaral Carvalho from 2008 to 2021. Clinical and histopathological information (gender, age, location, sentinel lymph node outcome, survival time, and presence or absence of ulceration) were collected from pathology reports stored in the participating institution digital systems. Regarding the topography of the lesion, the cases were divided into: areas not exposed to the sun (anterior chest, posterior chest, abdomen, genital region, and proximal portion of the limbs), areas exposed to the sun (head, neck, and distal portion of the limbs, except the acral region), and acral region (palms of hands, soles of feet and fingers/toes). The microscopic analysis (histopathological type; Breslow measurement; modified Breslow measurement) was performed by two pathologists.

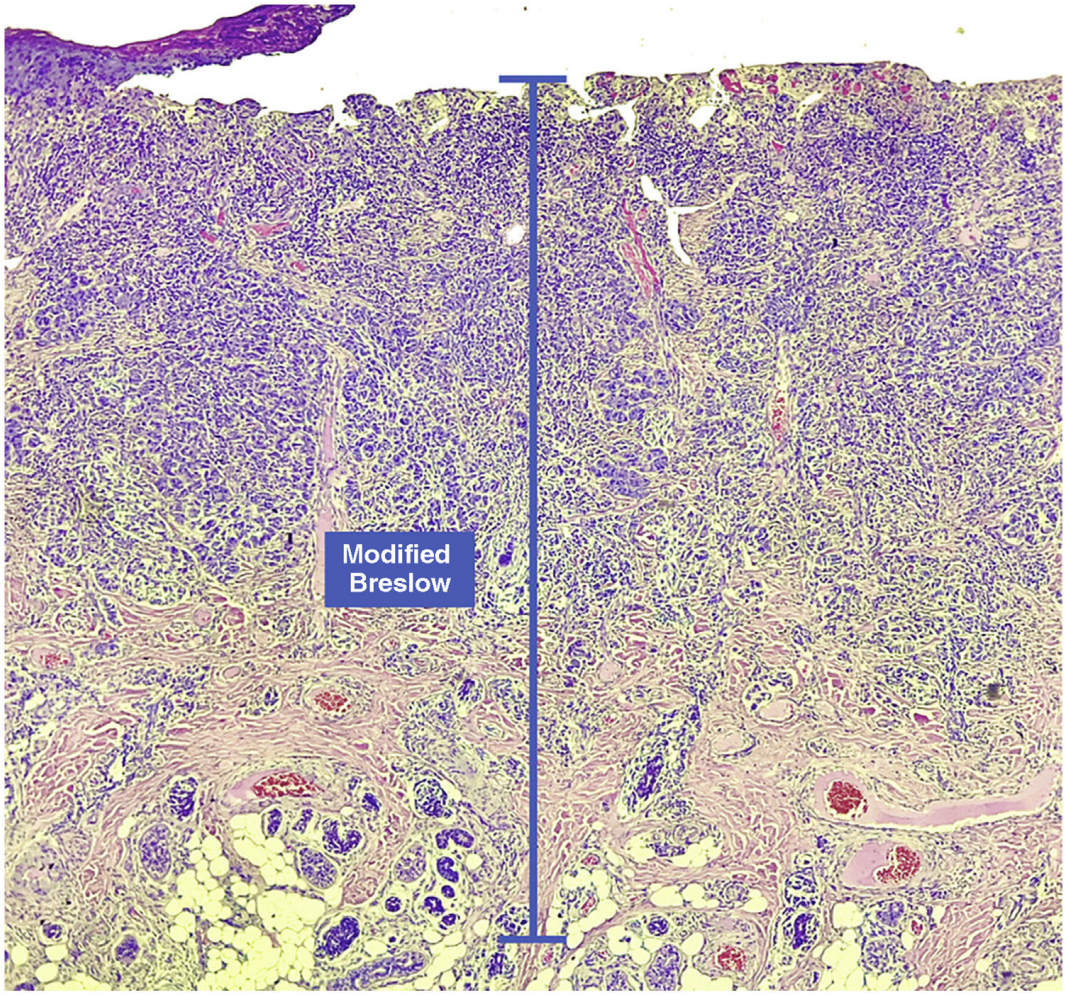


Figure 2 In cases with extensive ulceration, the modified Breslow measurement is taken from the base of the ulcer, as with the conventional Breslow measurement.

The Breslow measurement was measured in the conventional way, from the granular layer to the deepest portion of the neoplasm, while the modified Breslow measurement was measured from the basal membrane to the deepest portion of the tumor, disregarding the ''*in situ*'' component. In ulcerated cases in which there was an intact epidermis overlying the area of deeper invasion, there was no influence of ulceration in relation to the Breslow measurement. In those cases with extensive ulceration, both measurements were taken from the base of the ulcer (Fig. 2). The exclusion criteria included: *in situ* or thin melanomas (less than 1.0 mm thick), metastases, diagnostic disagreement between pathologists, and those with missing paraffin blocks or with scarce tissue in the paraffin block.

After the data were obtained, a descriptive analysis was initially carried out with the calculation of mean, standard deviation, minimum, maximum, and median values for the quantitative variables and frequencies and percentages for the categorized variables. Considering survival in months, Kaplan-Meier curves were obtained followed by the log-rank test for the variables of interest. In the case of variables with more than two categories, the curves were compared using the Sidak test. Risk factors for survival considering continuous variables were obtained by adjusting the Cox model. For the lymph node as a response variable, a logistic regression model was adjusted considering the Breslow and modified Breslow values as explanatory variables. Associations between categorized variables were assessed using the Chi-Square test. In all tests, the significance level was set at 5% or the corresponding p-value, and all analyses were performed using the SAS program for Windows, v.9.4.

The study was approved by the Research Ethics Committee (REC) of Hospital Amaral Carvalho (CAAE 52618721.0.3001.5434).

Results

A total of 275 cases of melanoma diagnosed from 2008 to 2021 and which underwent sentinel lymph node biopsy were analyzed. Of these, a total of 141 (51.27%) were men 134 (48.73%) were women and the age median was 63 years (14-87 years). The micrometric Breslow measurement median was 3.8 mm (1.0–27.5 mm), while the modified Breslow measurement median was 3.7 mm (0.5–26.5 mm). The median survival time was two years (0 to 13 years; Table 1).

Of the 275 cases, a total of 138 (50.18%) showed metastasis for the sentinel lymph node. Regarding the anatomical site of the lesion, 100 cases (36.36%) were located in areas not exposed to the sun, 84 cases (30.54%) in areas exposed to the sun, 78 cases (28.36%) in the acral region and 13 (4.72%) in unspecified areas. Regarding the histopatholog-

Table 1 Descriptive analysis of the clinicopathological characteristics of cases studied.

Variables	
Sex, n (%)	
Female	134 (48.73)
Male	141 (51.27)
Age (years)	
Median (min.–max.)	63 (14–87)
Breslow measurement (mm)	
Median (min.–max.)	3.8 (1.0–27.5)
Modified Breslow (mm)	
Median (min.–max.)	3.7 (0.5–26.5)
Survival	
In months– Median (min.–max.)	34 (0–158)
In years– Median (min.–max.)	2 (0–13)
Sentinel lymph node, n (%)	
Positive for metastasis	138 (50.18)
Negative for metastasis	137 (49.82)
Anatomical site, n (%)	
Non-exposed areas	100 (36.36)
Exposed areas	84 (30.54)
Acral	78 (28.36)
Not specified	13 (4.72)
Histopathological subtype, n (%)	
Superficial spreading melanoma	101 (36.72)
Nodular melanoma	95 (34.54)
Acral lentiginous melanoma	75 (27.27)
Lentigo maligna melanoma	1 (0.36)
Others	3 (1.09)
Ulceration, n (%)	
Present	144 (59.02)
Absent	100 (40.98)

ical subtype, 101 cases (36.72%) were superficial spreading melanomas, 95 cases (34.54%) were nodular melanomas, 75 cases (27.27%) were acral melanomas, one case (0. 36%) was lentigo maligna melanoma and three cases (1.09%) were other rare or unclassifiable subtypes. Of the 244 cases with information on ulceration, 144 (59.02%) had ulceration (Table 1).

The results of the Cox model analysis to evaluate the impact of different methods used to measure melanoma thickness (Breslow measurement and modified Breslow measurement) on survival showed that both methods were statistically significant. For the conventional Breslow measurement, a chi-square value of 59.40 was obtained; a p-value < 0.0001; HR of 1.121. For the modified Breslow measurement, a chi-square value of 57.66 was obtained; a p-value < 0.0001; HR of 1.119 (Table 2). The logistic regression disclosed a significant association between both measurements (conventional Breslow measurement and modified Breslow measurement) and the presence of metastasis in the sentinel lymph nodes. The Breslow measurement showed an Odds Ratio (OR) of 1.189 (95%CI 1.111–1.271; p-value < 0.0001). Likewise, the modified Breslow measurement showed an OR of 1.19 (95%CI 1.112–1.273; p-value < 0.0001; Table 3).

The analysis of survival time in relation to sentinel lymph node status (positive or negative for metastasis) was performed using the Log-Rank (Chi-Square 39.49; DF 1, $p < 0.0001$), Wilcoxon (Chi-Square 40.54; DF 1, $p < 0.0001$) and -2Log(LR; Chi-Square 48.24; DF 1, $p < 0.0001$; Fig. 3) tests. Likewise, in the survival analysis in relation to the presence of ulceration, the Log-Rank (Chi-Square 11.56; DF 1, $p < 0.0007$), Wilcoxon (Chi-Square 12.61; DF 1, $p < 0.0004$) and -2Log (LR; Chi-Square 12.92; DF 1, $p < 0.0003$; Fig. 4) tests were performed. Moreover, the presence of ulceration showed a significant association with metastases in the sentinel lymph nodes ($p = 0.0019$).

The survival analysis in relation to the anatomical site did not show any significant results (Fig. 5). The Log-Rank Test obtained a Chi-Square of 2.9495 with DF 2, and a p-value of 0.2288. When adjusting for multiple comparisons using the Sidak Test, none of the pairwise comparisons between anatomical sites reached statistical significance. The comparison between sites exposed to the sun (E) and non-exposed (NE) obtained a p-value of 0.6668 (Chi-Square 1.0447). The comparison between sun-exposed (E) and acral sites resulted in a p-value of 0.2427 (Chi-square 2.9014). Lastly, the comparison between non-exposed (NE) and acral sites resulted in a p-value of 0.9014 (Chi-Square 0.3791). Regarding the anatomical site and its association with sentinel lymph node metastasis, the analysis did not demonstrate any significant differences either ($p = 0.1217$).

When considering survival in relation to the melanoma histopathological subtypes, the Log-Rank test detected significant differences in the survival rate between different subtypes (Chi-Square 13.11; DF 4 and p-value 0.0107; Fig. 6).

Table 2 Survival analysis using the COX model.

Parameter	DF	Parameter estimate	Standard error	Chi-square	> Chi-square	HR	95% CI of HR	
Breslow	1	0.11437	0.01484	59.4019	<0.0001	1.121	1.089	1.154
Modified Breslow	1	0.11255	0.01482	57.6687	<0.0001	1.119	1.087	1.152

Table 3 Logistic regression adjustment for sentinel lymph node outcome for Breslow and modified Breslow.

Variable	OR	95% CI		p-value
Breslow (mm)	1.189	1.111	1.271	<0.0001
Modified Breslow (mm)	1.19	1.112	1.273	<0.0001

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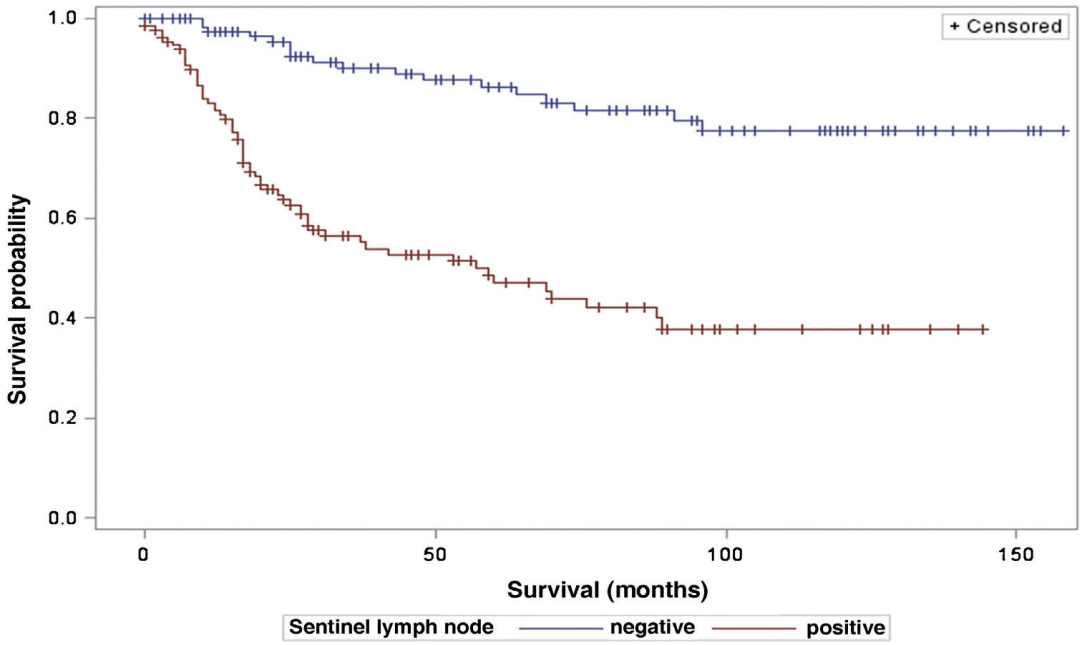


Figure 3 Analysis of survival time in relation to sentinel lymph node status.

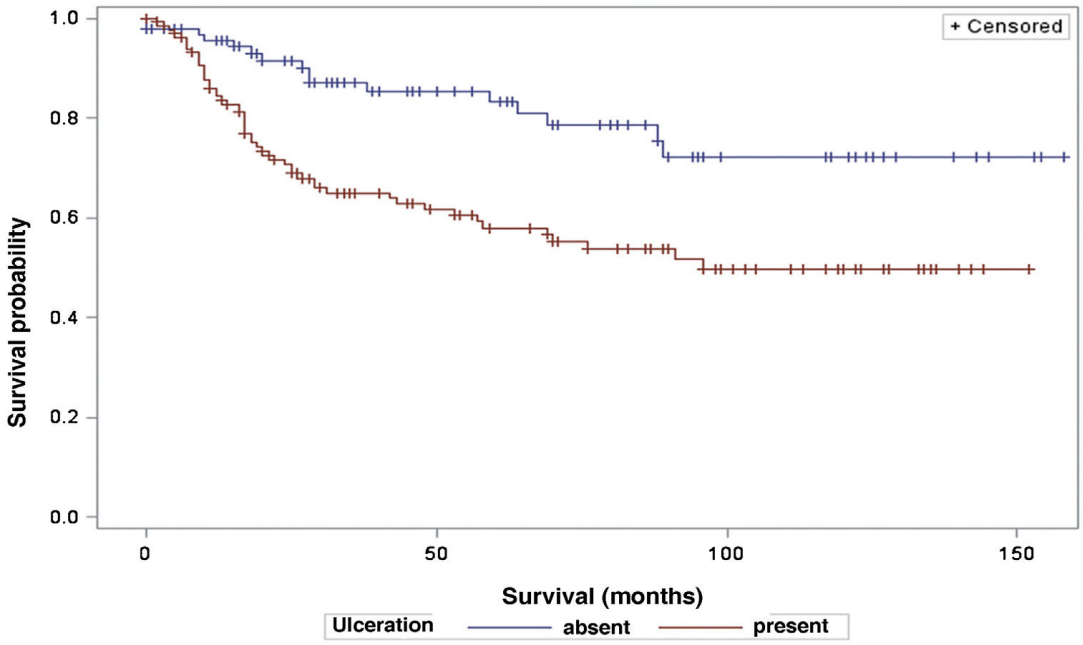


Figure 4 Analysis of survival time in relation to the presence or absence of ulceration.

Regarding metastases in the sentinel lymph node, there was no statistically significant association between the histopathological subtype ($p = 0.0735$) and the status of the sentinel lymph node.

Discussion

The results of this study showed that the modified Breslow measurement (measured from the basal membrane instead of the granular layer) was able to predict survival time and sentinel lymph node outcome, as well as the conventional Breslow measurement.

The Breslow measure has some limitations. When the lesion is ulcerated, the measurement starts from the base of the ulcer. In these cases, the thickness may be underestimated, as the amount of tumor lost due to ulceration is not taken into account. The current parameters for melanoma staging also do not take into account the thickness of the epidermis when obtaining the Breslow measure. It is known that, for instance, the acral skin has a thicker epidermis, while some areas of the face, such as the skin behind the ear, have a thinner epidermis.[11,12] In this context, cases of lentigo maligna melanoma, of which the epidermis is generally atrophic, may show a lower Breslow measurement than cases of acral melanomas (since the epidermis

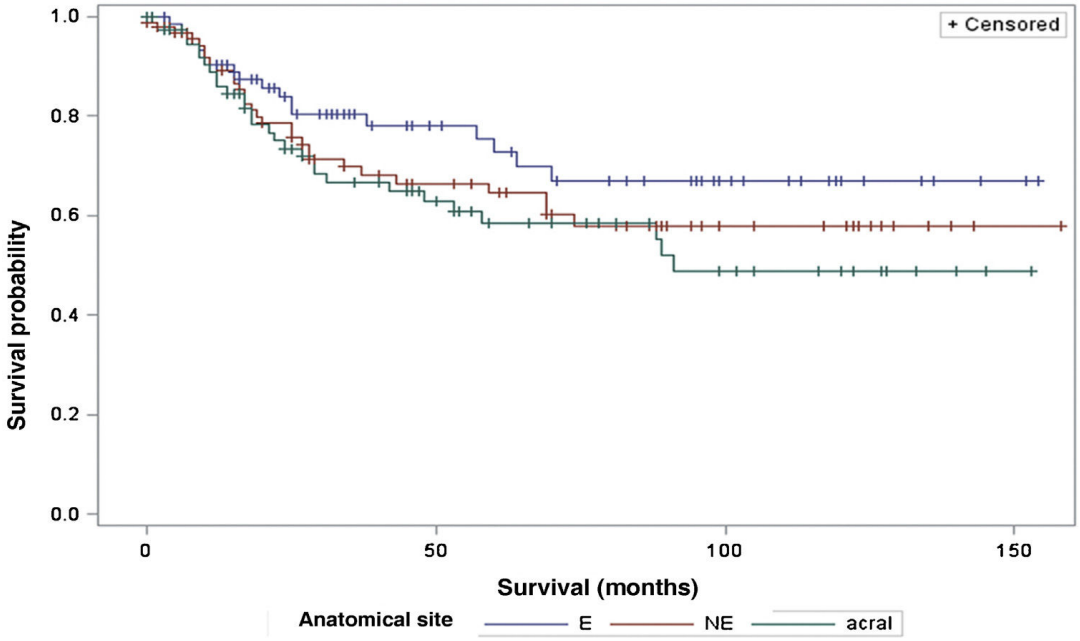


Figure 5 Analysis of survival time in relation to the anatomical site (E, Exposed Areas; NE, Non-exposed Areas).

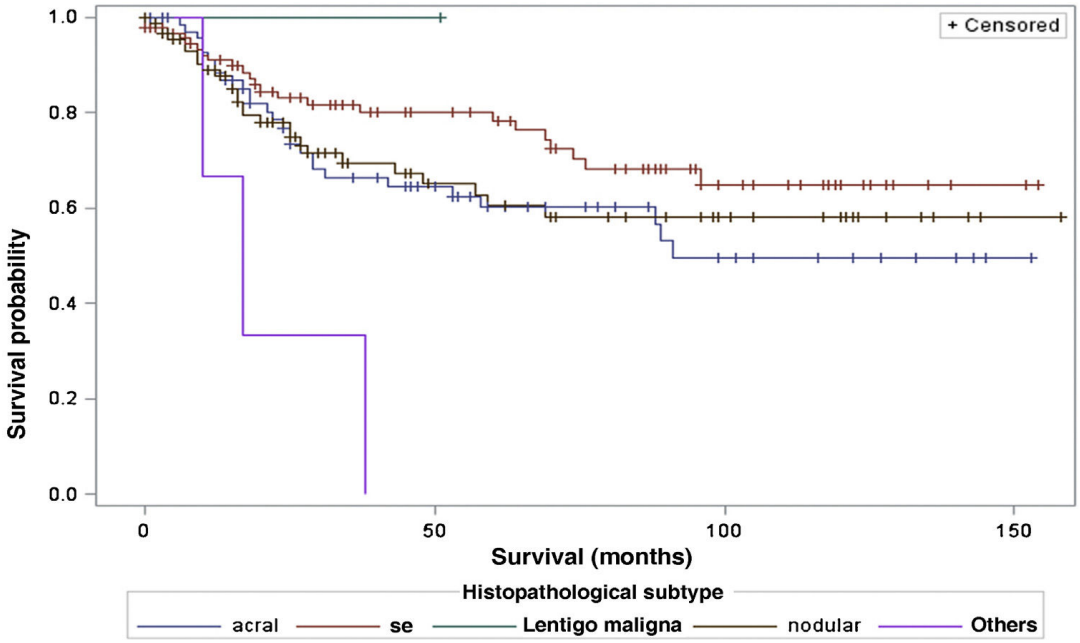


Figure 6 Analysis of survival time in relation to histopathological subtype.

of the acral region is thick) even though both show involvement of similar strata in the dermis (Fig. 7). Moreover, the Breslow measurement can only be accurately evaluated in sections perpendicular to the epidermis surface; if there is periadnexal extension of the melanoma and this represents the only focus of invasion, the best methodology for this measurement becomes questionable.[13,14] The modified Breslow measurement, proposed in the present study, is not affected by variations in epidermis thickness in different anatomical sites, nor by the presence or absence of ulceration (Fig. 7). This approach may show greater reliability, undergoing fewer variations and demonstrating good reproducibility between pathologists.

The results also corroborate that the sentinel lymph node status has a significant effect on the survival of patients with melanoma, and the presence of metastasis in the sentinel lymph node is associated with reduced survival time, in line with other studies such as that of Tejera-Vaquerizo et al., who analyzed 4249 cases of thin melanomas and found that the sentinel lymph node status is the most important prognostic factor for melanoma-specific survival.[15] Jafari et al., in a study with 1111 patients, showed that those who did not have sentinel lymph node metastases had longer disease-free survival than those who had them. Additionally, in patients with intermediate-thickness melanoma (1.0 to 4.0 mm), better overall survival was found in those with a

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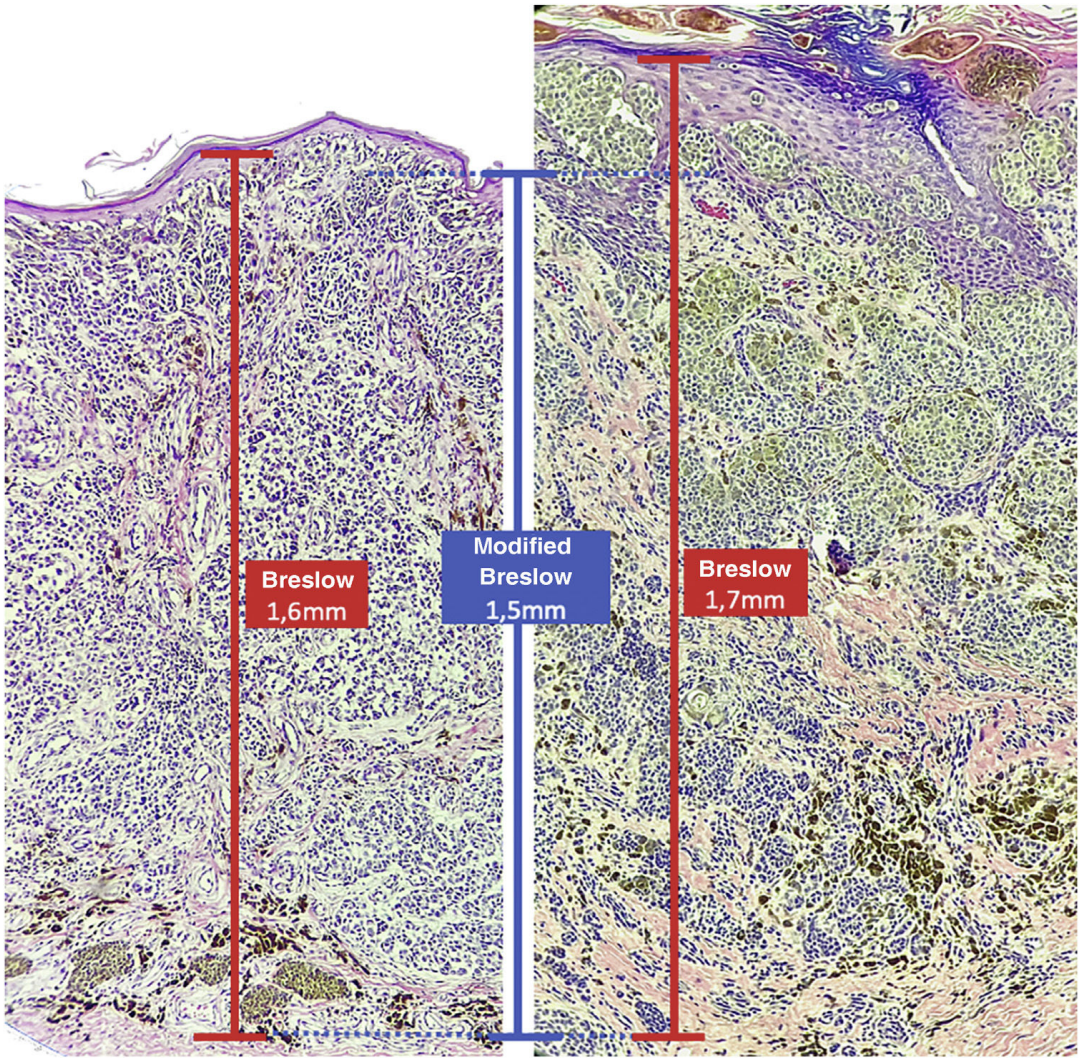


Figure 7 Both cases show an invasive component with similar thickness (1.5 mm, coinciding with the modified Breslow measurement). However, the conventional Breslow measurement is 1.6 mm on the left (thin epidermis) and 1.7 mm on the right (thick epidermis).

metastasis-negative sentinel lymph node.[16] Another study, however, showed that sentinel node status is an independent prognostic factor for disease-free survival, but not for overall survival in a multivariate analysis of 309 cases of melanoma.[17] Lemos et al., in a study with 43 patients with thick melanoma (> 4 mm), did not demonstrate the statistical significance of sentinel lymph node status in overall survival.[18]

Moreover, ulceration had a significant impact on patient survival, indicating lower survival in those patients with ulceration. These results are in agreement with other studies, such as that by Sarpa et al., with 235 patients, which showed a significant correlation between the extent of ulceration and overall survival as well as sentinel lymph node status.[19] The study by Hout et al., which showed that both the presence and extent of ulceration are independent predictors of survival.[20]

Regarding the anatomical site of the melanoma, the present study did not demonstrate a significant impact on patient survival, nor any association with the sentinel lymph node outcome. These results are in disagreement with other studies, such as that by Callender et al., with 2500 patients, which demonstrated that the anatomical site is an independent predictor for sentinel lymph node status, as well as survival.[21] The study by Howard et al., demonstrated that sites with intermittent or chronic sun exposure had better survival compared to sites rarely exposed to the sun.[22]

Regarding the histopathological subtype, there were significant statistical differences in the survival rate between different histopathological subtypes (Kaplan-Meier curve). There was no statistically significant association between histopathological subtype and sentinel lymph node status. The study by Buja et al. showed that the histopathological subtype is an independent risk factor for death, with the nodular subtype being the one with the worst melanoma-specific survival.[23] Sharouni et al., showed in a study with 48,361 patients that the nodular and acral lentiginous subtypes have worse survival than the superficial spreading and lentigo maligna melanoma subtypes.[24] The work of Robsahm et al., on the other hand, did not demonstrate histopathological subtype as an independent predictor of melanoma-specific survival.[25]

One weakness of the present study is its retrospective design, the limited number of total cases, the number of

ulcerated cases, and the high frequency of advanced-stage cases. Due to a characteristic of the service profile, many cases of acral melanoma and nodular melanoma were also observed, compared to other studies; as well as just one case of lentigo maligna melanoma. Nonetheless, it is the first study to propose the possibility of adapting the way the Breslow measurement is obtained. Thus, the present study demonstrated that the modified Breslow measurement, that is, measured from the basal membrane instead of the granular layer, is capable of predicting prognosis, as well as the conventional Breslow measurement. However, more studies are needed to validate this method.

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Authors' contributions

Marcel Arakaki Asato: Data collection; statistical analysis; drafting and editing of the manuscript; collection, analysis and interpretation of data;; critical review of the literature.

Francisco Alves Moares Neto: Critical review of important intellectual content.

Marcelo Padovani de Toledo Moraes: Critical review of important intellectual content.

Juliana Polizel Ocanha-Xavier: Critical review of important intellectual content.

Luiz Carlos Takita: Critical review of important intellectual content.

Mariangela Esther Alencar Marques: Critical review of important intellectual content.

José Cândido Caldeira Xavier-Júnior: Design and planning of the study; effective participation in research orientation; critical review of the literature; critical review of important intellectual content; approval of the final version of the manuscript.

Conflicts of interest

None declared.

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References

1. Mirea MA, Eckensperger S, Hengstschläger M, Mikula M. Insights into differentiation of melanocytes from human stem cells and their Relevance for melanoma treatment. Cancers (Basel). 2020;12:2508.
2. Nikolaou V, Stratigos AJ. Emerging trends in the epidemiology of melanoma. Br J Dermatol. 2014;170:11–9.
3. Roncati L, Vergari B, Del Gaudio A. The' All-or-None Law' applied to the vertical growth phase of cutaneous malignant melanoma. Chonnam Med J. 2017;53:234–5.
4. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg. 1970;172:902–8.
5. Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al. AJCC Cancer Staging Manual. 8 ed. Chicago: Springer; 2017.
6. Tejera-Vaquerizo A, Fernández-Figueras MT, Santos-Briz A, Ríos-Martín JJ, Monteagudo C, Fernández-Flores A, et al. Protocol for the histologic diagnosis of cutaneous melanoma: consensus statement of the Spanish society of pathology and the Spanish academy of dermatology and venereology (AEDV) for the National cutaneous melanoma registry. Actas Dermosifiliogr (Engle Ed). 2021;112:32–43.
7. Gershenwald JE, Scolyer RA, Hess KR, Sondak VK, Long GV, Ross MI, et al. Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017;67:472–92.
8. Tan KB, Moncrieff M, Thompson JF, McCarthy SW, Shaw HM, Quinn MJ, et al. Subungual melanoma: a study of 124 cases highlighting features of early lesions, potential pitfalls in diagnosis, and guidelines for histologic reporting. Am J Surg Pathol. 2007;31:1902–12.
9. CAP – College of American Pathologists [Internet]. Protocol for the Examination of Resection Specimens from Patients with Primary Carcinoma of the Uterine Cervix; 2023 Dec [citado em 2023 Jul 04]. Disponível em: https://documents.cap.org/documents/Cervix_5.1.1.0.REL_CAPCP.pdf.
10. Salvo G, Odetto D, Saez Perrotta MC, Noll F, Perrotta M, Pareja R, et al. Measurement of tumor size in early cervical cancer: an ever-evolving paradigm. Int J of Gynecol Cancer. 2020;30:1215–23.
11. Chopra K, Calva D, Sosin M, Tadisina KK, Banda A, De La Cruz C, et al. A comprehensive examination of topographic thickness of skin in the human face. Aesthe Surg J. 2015;35:1007–13.
12. Oltulu P, Ince B, Kokbudak N, Findik S, Kilinc F. Measurement of epidermis, dermis, and total skin thicknesses from six different body regions with a new ethical histometric technique. Turkish Journal of Plastic Surgery. 2018;26:56–61.
13. Scolyer RA, Judge MJ, Evans A, Frishberg DP, Prieto VG, Thompson JF, et al. Data set for pathology reporting of cutaneous invasive melanoma. Am J Surg Pathol. 2013;37:1797–814.
14. Scolyer RA, Rawson RV, Gershenwald JE, Ferguson PM, Prieto VG. Melanoma pathology reporting and staging. Mod Pathol. 2019;33:15–24.
15. Tejera-Vaquerizo A, Ribero S, Puig S, Boada A, Paradela S, Moreno-Ramírez D, et al. Survival analysis and sentinel lymph node status in thin cutaneous melanoma: a multicenter observational study. Cancer Med. 2019;8:4235–44.
16. Seyed Jafari SM, Jäckle P, Michel A, Angermeier S, Hunger R, Shafighi M. Prognostic value of sentinel lymph node biopsy in melanomas of different Breslow's thickness. Swiss Med Wkly. 2016;146:w14358.
17. Roka F, Kittler H, Cauzig P, Hoeller C, Hinterhuber G, Wolff K, et al. Sentinel node status in melanoma patients is not predicitive for overall survival upon multivariate analysis. Br J Cancer. 2005;92:662–7.
18. Lemos M, Vieira R, Brinca A, Figueiredo A. Biópsia do gânglio sentinela em melanomas espessos: estudo clínico retrospectivo num único centro. Portuguese Society of Dermatology and Venereology; 2016. p. 74.
19. Grande Sarpa H, Reinke K, Shaikh L, Leong SPL, Miller JR, Sagebiel RW, et al. Prognostic significance of extent of ulceration in primary cutaneous melanoma. Am J Surg Pathol. 2006;30:1396–400.

20. In' t Hout FEM, Haydu LE, Murali R, Bonenkamp JJ, et al. Prognostic importance of the extent of ulceration in patients with clinically localized cutaneous melanoma. Ann Surg. 2012;255:1165–70.
21. Callender GG, Egger ME, Burton AL, Scoggins CR, Ross MI, Stromberg AJ, et al. Prognostic implications of anatomic location of primary cutaneous melanoma of 1 mm or thicker. Am J Surg. 2011;202:659–65.
22. Howard MD, Wee E, Wolfe R, McLean CA, Kelly JW, Pan Y. Anatomic location of primary melanoma: survival differences and sun exposure. J Am Acad Dermatol. 2019;81:500–9.
23. Buja A, Bardin A, Damiani G, Zorzi M, De Toni C, Fusinato R, et al. Prognosis for cutaneous melanoma by clinical and pathological profile: a population-based study. Front Oncol. 2021;11:737399.
24. El Sharouni MA, van Diest PJ, Witkamp AJ, Sigurdsson V, van Gils CH. Subtyping cutaneous melanoma matters. JNCI Cancer Spectr. 2020;4:pkaa097.
25. Robsahm TE, Helsing P, Nilssen Y, Vos L, Rizvi SMH, Akslen LA, et al. High mortality due to cutaneous melanoma in Norway: a study of prognostic factors in a nationwide cancer registry. Clin Epidemiol. 2018;10:537–48.

2.3 Artigo 3

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Depth of invasion analysis to predict acral melanoma outcomes

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ABSTRACT

Background: Acral melanoma is a subtype with worse outcomes. The Breslow micrometric measurement is the most critical parameter in planning treatment and predicting outcomes. However, for acral lentiginous melanoma, the value of the Breslow thickness is a matter of debate. Depth of Invasion (DOI) is a well-established measure for staging oral squamous cell carcinoma.
Objective: This study compared DOI and Breslow thickness for predicting acral melanoma outcomes.
Methods: We performed a retrospective cross-sectional study of 71 acral melanoma lesions subjected to sentinel lymph node biopsy at one Brazilian referral center.
Results: Cox model univariate analysis showed that both DOI and Breslow thickness predicted melanoma specific survival (HR 1.12; $p = 0.0255$ and HR 1.144; $p = 0.0006$, respectively), although Kaplan Meier curve was only significant for Breslow ($\chi^2 = 5.792$; $p = 0.0161$) and not for DOI ($\chi^2 = 0.2556$; $p = 0.6132$). Sentinel lymph node status and presence or absence of ulceration also predicted specific survival in patients with acral melanoma ($\chi^2 = 6.3514$; $p = 0.0117$ and $\chi^2 = 4.2793$; $p = 0.0386$, respectively). Multivariate analysis, however, demonstrated that Breslow depth was the only independent parameter for predicting acral melanoma specific survival (HR 1.144; $p = 0.0006$).
Conclusion: Even though Breslow thickness remains the main predictor for survival in acral melanoma, it is not a perfect parameter. The introduction of DOI in this context opens new perspectives for predicting acral melanoma outcomes.

1. Introduction

Cutaneous melanoma constitutes only 1 % of cutaneous tumor cases; nevertheless, it is among the deadliest forms of skin cancer due to high tendency to metastasize, resulting in significant morbidity and mortality. Cutaneous melanoma is responsible for over 80 % of deaths due to skin cancer [1-4]. Its occurrence has risen among Caucasian population in recent years, likely linked to leisure activities involving exposure to the sun [5].

There are four major histologic subtypes of cutaneous melanoma: superficial spreading, nodular, lentigo maligna, and acral lentiginous [6]. Reed first described acral melanoma in 1976 [7]. It exhibits a distinct clinical, demographic, and genetic profile and outcomes compared with melanomas from other cutaneous sites [8]. It is the least common of the four, representing about 1 %–36 % of all melanoma cases, depending on the patient's ethnic group (it is the most common subtype in African and Asian patients). It typically occurs on the soles, palms, and nail beds [9,10].

Acral lentiginous melanoma is characterized by marked acanthosis, elongation of rete ridges, and a lentiginous proliferation of atypical melanocytes in the epidermis [7]. It is also considered a subtype with worse outcomes [9].

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The measurement of tumor thickness, also known as Breslow's depth, plays a crucial role in the prognosis and treatment of invasive melanomas. [11] This method has been incorporated into the staging systems established by the American Joint Committee on Cancer. [12] It specifies the pathological staging, determines the size of excision margins, and identifies when the sentinel lymph node biopsy is appropriate. [12,13] But for acral lentiginous melanoma, the value of the tumor thickness for assessing prognosis is still discussed. [14] The Breslow thickness in acral skin may be overestimated because of extensive epidermal hyperplasia [15]. Also, when there is ulceration, the thickness may be underestimated due to the amount of tumor loss, which is not considered. One study demonstrated that the Breslow thickness has prognostic value, but there was no association between tumor thickness and survival in patients with acral melanoma with a Breslow thickness >2 mm. [14]

Depth of invasion (DOI) is a measure for staging oral squamous cell carcinoma (SCC), and it was included in the eighth edition of the American Joint Committee on Cancer (2017). Although DOI and tumor thickness have been used interchangeably, some differences exist. DOI is measured from the adjacent normal basement membrane to the deepest point of the tumor in millimeters. The tumor thickness is measured from the highest point of the tumor surface and the deepest point of the tumor [16,17], similar to the Breslow thickness measurement. When the basement membrane is damaged, a hypothetical line is reconstructed at the surface, allowing the measurement [17,18] (Fig. 1).

This study aimed to compare DOI with Breslow thickness for staging and predicting outcomes in acral melanoma. To our knowledge, this is the first study to make such a comparison.

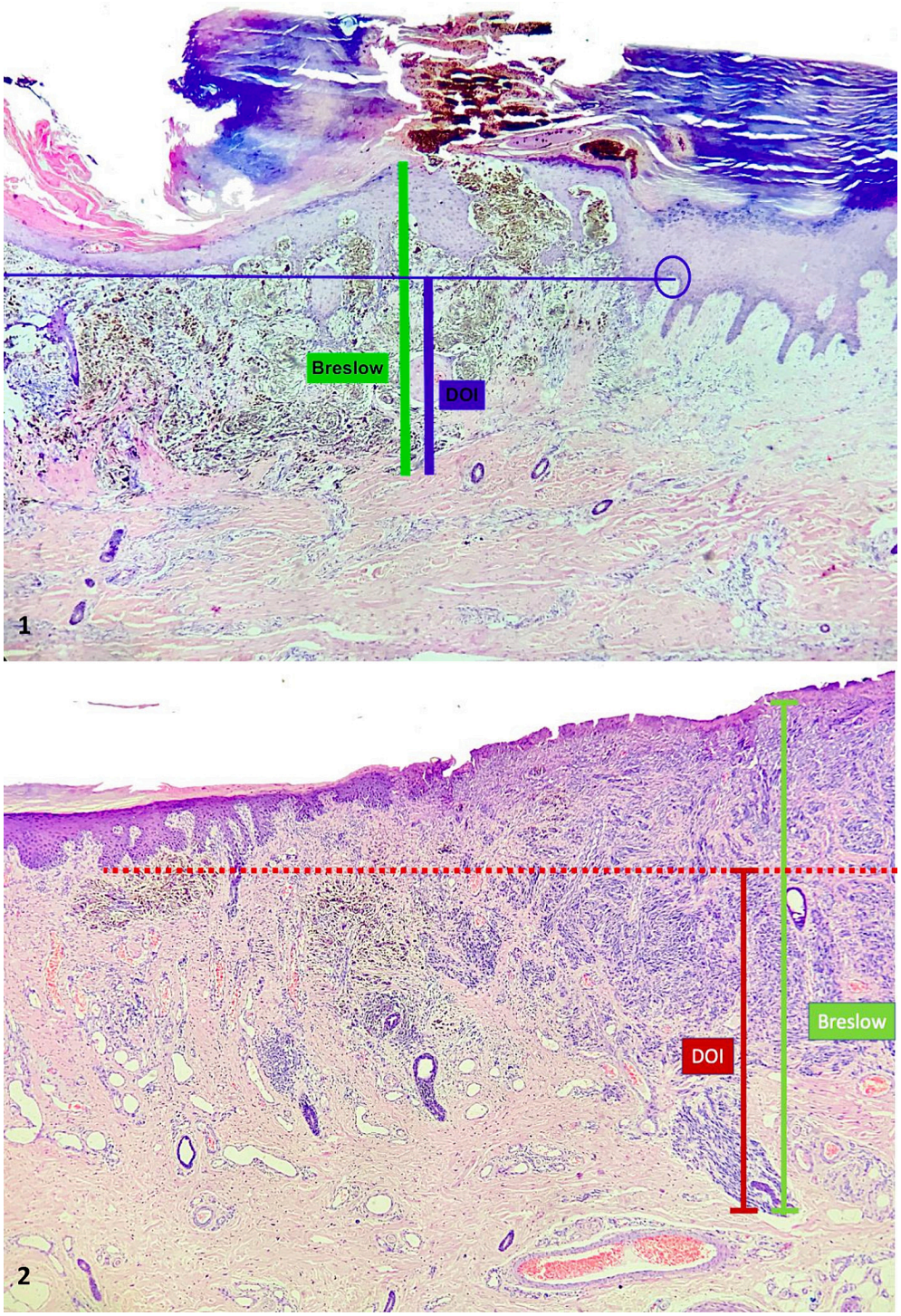


Fig. 1. Breslow and DOI measurement comparisons in a non-ulcerated lesion (1) and an ulcerated lesion (2).

2. Materials and methods

In this retrospective and cross-sectional analysis, slides stained with hematoxylin and eosin from formalin-fixed, paraffin-embedded skin samples taken from primary melanoma lesions at Amaral Carvalho Hospital between 2008 and 2021 were examined. The study documented clinical information (gender, age, sentinel lymph node outcomes and survival) from the pathological records maintained in the digital archives of the involved institution. Pathological analysis (Breslow thickness; DOI; presence or absence of ulceration) was assessed by two dermatopathologists. Exclusion criteria were melanoma in situ, metastases, diagnostic disagreements, histologic subtypes other than acral lentiginous melanoma, absence of post-diagnosis follow-up, missing paraffin blocks and thin melanomas (<1.0 mm in thickness) since these are not routinely indicated for sentinel lymph node biopsy and they have good prognosis.

A descriptive analysis was initially performed by calculating the quantitative variables' mean, standard deviation, minimum, maximum, and median values and frequencies and percentages for categorized variables. The log-rank test evaluated survival time by drawing Kaplan-Meier curves stratified by the variables of interest. For continuous variables, survival was assessed using the Cox model. A logistic regression model was used, considering the positive/negative lymph node results as the response variable and the Breslow/DOI as explanatory variables to assess risk factors. A logistic regression model was also used to compare the areas under the ROC curve (AUC). For multivariate analysis, considering survival time as the outcome and other variables (gender, lymph node status, ulceration, age, Breslow thickness, and DOI) as explanatory variables, a Cox proportional hazards model was fitted using a stepwise method to select the most significant explanatory variables in relation to the outcome. Following the adjustment, a correction was made for confounding factors.

For all tests, a significance threshold of 5 % or the corresponding *p*-value was established. The analyses were conducted using SAS for Windows version 9.4 and SPSS for Windows version 21.

This research received approval from the research ethics committee (CAAE 52618721.0.1001.5411 and CAAE 52618721.0.3001.5434). Patient consent not applicable.

3. Results

Seventy-one cases of melanoma were subjected to sentinel lymph node biopsy from 2008 to 2021 and fulfilled the inclusion criteria. Of these, 31 (43.66 %) were women, and 40 (56.34 %) were men. The mean age was 68.52 years (range 37–87 years; standard deviation [SD] 13.28 years; median 71 years). The Breslow's micrometric measurement ranged from 1.1 mm to 27.5 mm with a mean of 5.33 mm (SD 4.1 mm; median 4.5 mm), while the DOI ranged from 0 to 20.0 mm with a mean of 2.15 mm (SD 2.94 mm; median 1.2 mm). The mean survival time was four years (range 0–12 years; median three years). Of the 71 cases, 41 (57.75 %) had metastasis to the sentinel lymph node, and 30 (42.25 %) were metastasis-free. Forty-nine cases (69.01 %) had ulceration, and 22 cases (30.98 %) had no ulceration (Table 1).

The Cox model analysis showed that Breslow thickness and DOI (measured in mm) predicted melanoma-specific survival. The DOI had a hazard ratio (HR) of 1.12 (95 % confidence interval [CI]: 1.014–1.237, $p = 0.0255$), while the Breslow thickness had an HR of 1.144 (95 % CI: 1.059–1.236, $p = 0.0006$). (Table 2).

The comparison of sentinel lymph node status (positive vs. negative) on melanoma-specific survival is presented in Fig. 2. The log-rank and Wilcoxon tests for equality over strata yielded significant results ($p = 0.0117$ and $p = 0.0111$, respectively), suggesting differences in survival outcomes between patients with positive and negative sentinel lymph node status. The −2Log(LR) test also showed a significant result ($p = 0.0047$) (Table 3). These findings suggest that the sentinel lymph node status is an essential prognostic factor for survival outcomes in patients with melanoma. The log-rank and Wilcoxon tests for equality over strata were also used to compare survival outcomes between patients with and without ulceration (Fig. 2). Both tests yielded significant results, with *p*-values of 0.0386 and 0.0201, respectively. The −2Log(LR) test also showed a significant result, with a p-value of 0.0104 (Table 3). These findings suggest that ulceration may be an essential prognostic factor for specific survival outcomes in patients with acral melanoma.

ROC analysis assessed the predictive accuracy of Breslow thickness and DOI in millimeters on the sentinel lymph node result. The AUC for Breslow thickness was 0.653 (95 % confidence interval [CI]: 0.524–0.781, $p = 0.030$). The AUC for DOI was 0.608 (95 % CI: 0.475–0.741, $p = 0.124$). The standard model used for the analysis had an AUC of 0.066 for Breslow thickness and 0.068 for DOI. There was no significant difference between the AUCs ($p = 0.5179$). ROC analysis was also used to compare the Breslow thickness and DOI in millimeters for predicting specific survival. The AUC for Breslow thickness was 0.690 (95 % confidence interval [CI]: 0.555–0.825, $p = 0.010$) while the AUC for DOI was 0.626 (95 % CI: 0.488–0.763, $p = 0.087$). There was no significant difference between the AUCs ($p = 0.4025$). The cutoff point for Breslow thickness was 4.65 mm and for DOI was 1.25 mm. Using these cutoff points, Kaplan Meier curve was significant for Breslow ($p = 0.0161$) and it was not significant for DOI ($p = 0.6132$). (Table 3; Fig. 3) In the multivariate analysis, an initial Cox proportional hazards model was constructed incorporating gender, lymph node status, ulceration, age, Breslow thickness, and DOI as explanatory variables. This model did not reveal any statistically significant predictors. Subsequently, a stepwise method was applied, which identified Breslow thickness as the only statistically significant independent predictor of survival, with a Chi-Square value of 11.6638, a hazard ratio (HR) of 1.144, and a *p*-value of 0.0006. Further adjustment of the model to account for potential confounders, namely gender and age, maintained the significance of the Breslow thickness (Chi-Square = 11.6592, HR = 1.15, $p = 0.0006$), solidifying its role as an independent prognostic factor. (Table 4).

Table 1
Descriptive analysis of clinicopathologic characteristics of the cases.

Variables	
Sex, no. (%)	
Female	31 (43.66)
Male	40 (56.34)
Age (y)	
Mean	68.52
Median	71
Breslow thickness (mm)	
Mean	5.33
Median	4.5
DOI (mm)	
Mean	2.15
Median	1.2
Survival time (years)	
Mean	4
Median	3
Sentinel lymph node, no. (%)	
Positive for metastasis	41 (55.75)
Negative for metastasis	30 (42.25)
Ulceration, no. (%)	
Present	49 (69.01)
Absent	22 (30.98)

Table 2
COX model analysis for specific survival outcome.

Parameters	p-Value	HR	95 % CI	
Breslow thickness (mm)	0.0006	1.144	1.059	1.236
DOI (mm)	0.0255	1.12	1.014	1.237

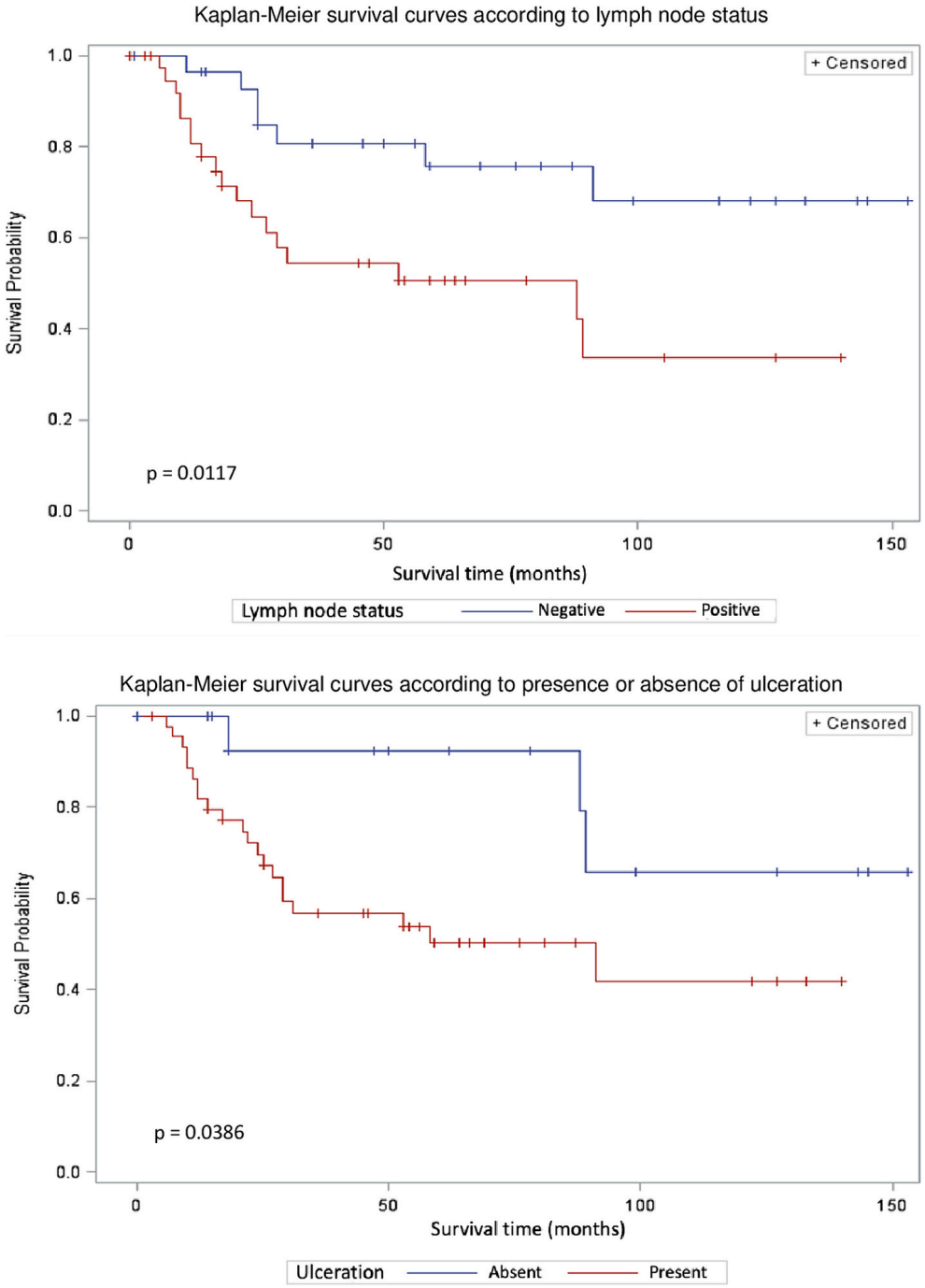


Fig. 2. Kaplan-Meier survival curves for sentinel lymph node status and presence of ulceration.

Table 3
Test of equality over strata for specific survival outcome: lymph node status, presence of ulceration, Breslow depth and DOI.

Log-rank test	Chi-square	p-Value
Lymph node status	6.3514	0.0117
Presence of ulceration	4.2793	0.0386
Breslow depth	5.792	0.0161
DOI	0.2556	0.6132

4. Discussion

The present study demonstrated in univariate analysis that DOI, like Breslow thickness, significantly predicts acral melanoma outcomes. DOI is a well-established oral SCC staging and outcomes prediction technique [16]. DOI is included in the oral SCC American Joint Committee on Cancer (eighth edition), while tumor thickness, similar to Breslow, was excluded [17]. Unlike Breslow, DOI is measured from the normal basement membrane adjacent to the neoplasm to the tumor's deepest portion, regardless of the epidermis thickness or ulceration, suggesting it is an advantageous and reproducible method for evaluating acral melanomas. Lee et al. found that tumor thickness was not associated with disease-specific survival in oral SCC, whereas an increase in DOI was significantly linked to a disease-specific survival reduction [16]. Another investigation compared tumor thickness with DOI and found that both were independently associated with the risk of lymph node metastasis [17]. Aside from that, among cutaneous SCC, tumor thickness measured in a method analogous to Breslow, was independently associated with recurrence-free survival in a multivariate analysis [19].

While the Breslow micrometric measurement is widely accepted for determining melanoma thickness and staging, its significance in acral melanoma is debated [9]. We showed that Breslow micrometric measurement significantly impacts acral melanoma survival, both in univariate analysis and multivariate analysis. Other studies, including Phan

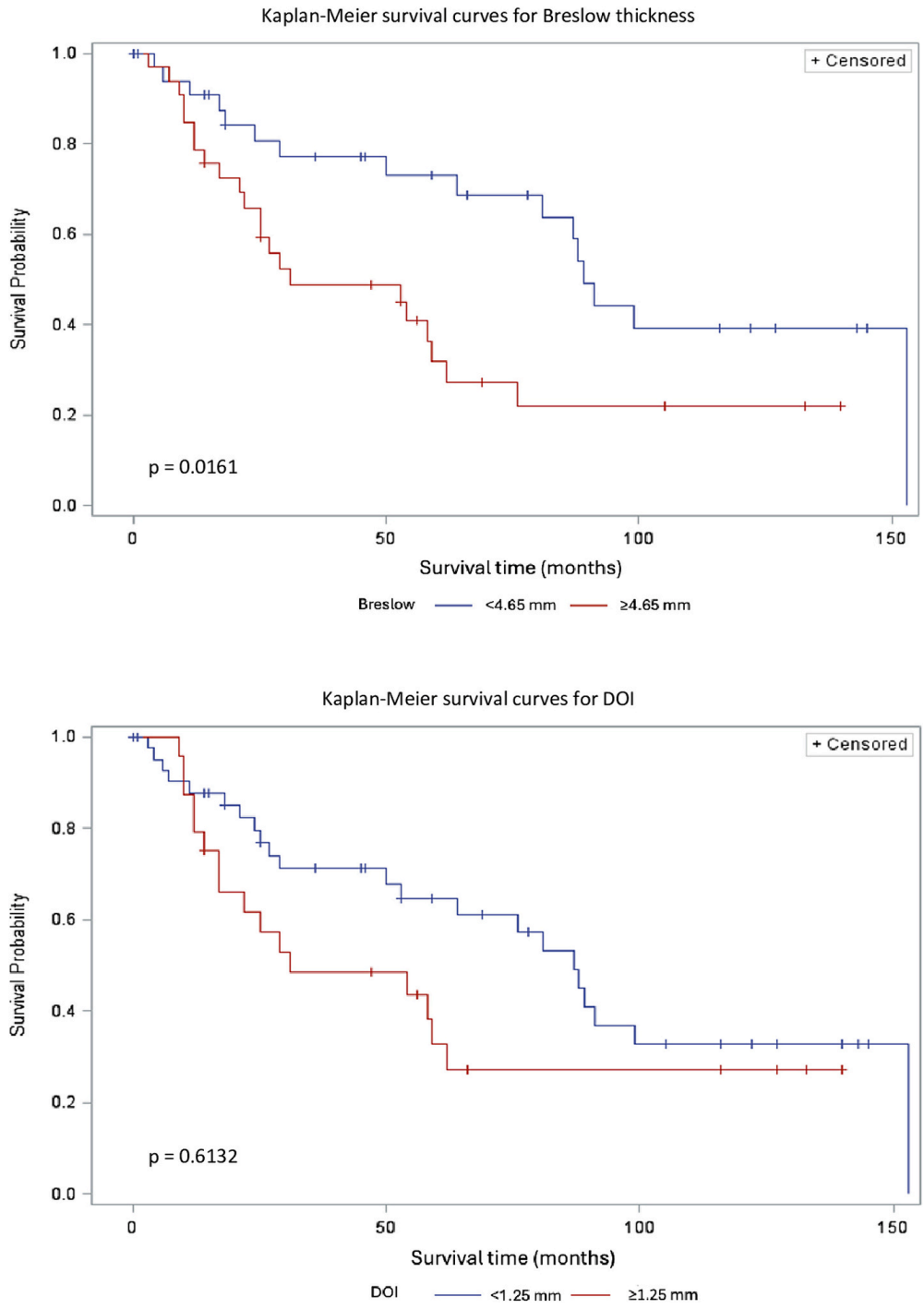


Fig. 3. Kaplan-Meier survival curves for Breslow thickness and DOI.

Table 4
Cox proportional hazards model for survival analysis with stepwise method.

Parameter	p-Value	HR	95 % CI	
Breslow	0.3341	1.077	0.926	1.252
DOI	0.6858	1.038	0.866	1.245
Positive sentinel lymph node	0.0994	2.174	0.863	5.477
Presence of ulceration	0.2509	2.038	0.605	6.872
Gender (male)	0.9058	1.052	0.454	2.44
Age	0.4091	0.986	0.954	1.02
(Stepwise method) Breslow	0.0006	1.144	1.059	1.235

et al. (121 patients), did not find a significant and independent relationship between the Breslow index and acral melanoma outcome [20]. Wei et al. studied 853 patients and showed that melanoma-specific survival was significantly different between T1–T3 stage acral melanoma patients but not between stages T3 and T4 (corresponding to thickness > 2 mm) [14].

The Breslow measurement has some limitations, including failure to consider variations in normal epidermal thickness across different anatomical sites and the potential for overestimating acral locations due to thickened epidermis [9,20,21]. Furthermore, measuring the Breslow thickness in the ungual region can be less accurate, among other reasons due to the lack of a granular layer [22]. Because DOI helps assess oral SCC, DOI might provide a more reproducible index in acral melanomas, including when they are ulcerated, a common feature that can affect outcomes [11]. These findings suggest that DOI can predict outcomes because it indicates only the true invasive component regardless of epidermal thickness or possible papillomatosis.

Differences in survival were observed between patients with positive and negative sentinel lymph node status. These findings align with previous studies, such as Nunes et al., who found an association between metastases in the sentinel lymph node and reduced overall survival [23].

Bello et al. also showed that increased Breslow thickness played a significant role in disease-specific survival; sentinel lymph node status was the most critical prognostic factor for survival in patients who underwent sentinel lymph node biopsy [6]. Similarly, Egger et al. concluded that the sentinel lymph node status is the most critical prognostic predictor in lentiginous acral melanoma [9].

Ulceration in patients with acral melanoma was associated with worse survival outcomes in univariate analysis. This finding aligns with Asgari et al., who demonstrated that ulceration was associated with worse overall and melanoma-specific survival than patients without ulceration [24]. Conversely, Egger et al. investigated 85 patients and showed that ulceration was not an independent risk factor for survival or recurrence [9]. Although ulceration is essential for staging considering current guidelines [12,25] (as in oral SCC), if the applicability of DOI is confirmed in future studies, the presence of ulceration will be de-emphasized.

The cases in our study had a higher Breslow thickness (mean of 5.33 mm) than another study of acral melanoma in Caucasians, in which the Breslow thickness mean was 2.04 mm [7]. However, similar findings have been reported in studies on acral melanoma in Brazilians, such as those by Nunes et al., Maia et al., and Souza et al., who reported mean Breslow thicknesses of 8.3 mm, 4.83 mm, and 5.09 mm, respectively, corroborating our findings [23,26,27]. Moreover, the high presence of ulceration in our study (64.78 % of cases) was higher than in other studies, such as Souza et al. and Susok et al., who reported ulceration rates of 42.22 % and 33.3 %, respectively [27,28]. However, our findings were lower than Maia et al., who reported an 82.9 % ulceration rate in Brazilians with lentiginous acral melanoma [26].

Although our study yielded significant results, there are some limitations. Our study was retrospective, and the sample size was limited to Brazilian patients from a single cancer center. Furthermore, we did not include in situ or thin melanomas, although they usually have good outcomes. Nevertheless, the novelty of this morphological-based study can open new study frontiers to understand acral melanomas, possibly influencing the staging system and treatments. Even though Breslow remains as the main predictor in melanoma, it is not a perfect parameter. Our results suggested DOI as a potential complementary marker that could lead to novel insights for predicting outcomes in acral melanoma. Further research will be necessary to validate the utility of DOI in assessing acral melanoma outcomes in other services with bigger population.

CRediT authorship contribution statement

Marcel Arakaki Asato: Writing – original draft, Investigation, Formal analysis, Data curation. **Francisco Alves Moares-Neto:** Writing – review & editing, Formal analysis. **Marcelo Padovani de Toledo Moraes:** Writing – review & editing, Formal analysis. **Juliana Polizel Ocanha-Xavier:** Writing – review & editing, Conceptualization. **Luiz Carlos Takita:** Writing – review & editing, Formal analysis. **Mariangela Esther Alencar Marques:** Writing – review & editing, Formal analysis. **José Cândido Caldeira Xavier-Júnior:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Ethics statement

The committees of ethics in research at the Faculdade de Medicina – UNESP/Botucatu – SP (CAAE 52618721.0.1001.5411) and the Amaral Carvalho Hospital (CAAE 52618721.0.3001.5434) approved the study. Patient consent not applicable.

References

[1] Ferreira FR, Nascimento LFC. Mortality due to cutaneous melanoma in south region of Brazil: a spatial approach. An Bras Dermatol 2016;91(4):437–41.
[2] Saginala K, Barsouk A, Aluru JS, Rawla P, Barsouk A. Epidemiology of melanoma. Med Sci 2021;9(4):63.
[3] Urban K, Mehrmal S, Uppal P, Giesey RL, Delost GR. The global burden of skin cancer: A longitudinal analysis from the global burden of disease study, 1990–2017. JAAD Int 2021;2:98–108.
[4] American Cancer Society. Cancer facts & figures 2022. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2022/2022-cancer-facts-and-figures.pdf.
[5] Nikolaou V, Stratigos AJ. Emerging trends in the epidemiology of melanoma. Br J Dermatol 2014;170(1):11–9.
[6] Bello DM, Chou JF, Panageas KS, Brady MS, Coit DG, Carvajal RD, et al. Prognosis of Acral melanoma: a series of 281 patients. Ann Surg Oncol [Internet] 2013;20(11):3618–25. Jul 10.
[7] Kuchelmeister C, Schaumburg-Lever G, Garbe C. Acral cutaneous melanoma in caucasians: clinical features, histopathology and prognosis in 112 patients. Br J Dermatol 2000 Aug;143(2):275–80.
[8] Wei X, Wu D, Li H, Zhang R, Chen Y, Yao H, et al. The clinicopathological and survival profiles comparison across primary sites in acral melanoma. Ann Surg Oncol 2020;27(9):3478–85. Apr 6.
[9] Egger ME, McMasters KM, Callender GG, Quillo AR, Martin RCG, Stromberg AJ, et al. Unique prognostic factors in acral lentiginous melanoma. Am J Surg 2012 Dec;204(6):874–80.
[10] Bradford PT, Goldstein AM, ML McMaster, Tucker MA. Acral lentiginous melanoma. Arch Derm 2009;145(4). Apr 1.
[11] Ge L, Vilain RE, Lo S, Aivazian K, Scolyer RA, Thompson JF. Breslow thickness measurements of melanomas around American Joint Committee on cancer staging cut-off points: imprecision and terminal digit bias have important implications for staging and patient management. Ann Surg Oncol 2016;23(8):2658–63. Apr 13.
[12] Amin Mahul B. AJCC cancer staging manual. 8 ed. Chicago: Springer; 2017.
[13] Swetter SM, Tsao H, Bichakjian CK, Curiel-Lewandrowski C, Elder DE, Gershenwald JE, et al. Guidelines of care for the management of primary cutaneous melanoma. J Am Acad Dermatol [Internet] 2019 Jan;80(1):208–50.
[14] Wei X, Chen Y, Yao H, Wu D, Li H, Zhang R, et al. Prognostic impact of Breslow thickness in acral melanoma: a retrospective analysis. J Am Acad Dermatol 2022 Dec;87(6):1287–94.
[15] Tejera-Vaquerizo A, Fernández-Figueras MT, Santos-Briz A, Ríos-Martín JJ, Monteagudo C, Fernández-Flores A, et al. Protocolo de diagnóstico histológico para muestras de pacientes con melanoma cutáneo. Documento de consenso de la SEAP y la AEDV para el Registro Nacional de Melanoma. Actas Dermo-Sifiliograficas [Internet] 2021;112(1):32–43. Jan 1 [cited 2021 Nov 23].
[16] Lee YJ, Kwon TG, Kim JW, Lee ST, Hong SH, Choi SY. Evaluation of depth of invasion and tumor thickness as a prognostic factor for early-stage oral squamous cell carcinoma: a retrospective study. Diagnostics 2021;12(1):20. Dec 23.
[17] Salama AM, Valero C, Katabi N, Khimraj A, Yuan A, Zanoni DK, et al. Depth of invasion versus tumour thickness in early oral tongue squamous cell carcinoma: which measurement is the most practical and predictive of outcome? Histopathology 2020;79(3):325–37. Dec 14.
[18] Ghazi N, Ghazi A, Shafiee S, Fayyazi M. Importance of depth of invasion in patients with oral squamous cell carcinoma: A review article. J Orofac Sci 2018;10(1):3–6.
[19] Yildiz P, Aung PP, Milton DR, Hruska C, Ivan D, Nagarajan P, et al. Measurement of tumor thickness in cutaneous squamous cell carcinomas: do the different methods provide better prognostic data? Am J Dermatopathol 2020 May;42(5):337–42.
[20] Phan A, Touzet S, Dalle S, Ronger-Savlé S, Balme B, Thomas L. Acral lentiginous melanoma: histopathological prognostic features of 121 cases. Br J Dermatol 2007 Aug;157(2):311–8.
[21] Feibleman CE, Stoll H, Maize JC. Melanomas of the palm, sole, and nailbed: a clinicopathologic study. Cancer 1980;46(11):2492–504. Dec 1.
[22] Tan KB, Moncrieff M, Thompson JF, McCarthy SW, Shaw HM, Quinn MJ, et al. Subungual melanoma: a study of 124 cases highlighting features of early lesions, potential pitfalls in diagnosis, and guidelines for histologic reporting. Am J Surg Pathol 2007 Dec;31(12):1902–12.
[23] Nunes LF, Quintella Mendes GL, Koifman RJ. Acral melanoma. Melanoma Res 2018 Jul:1.
[24] Asgari MM, Shen L, Sokil MM, Yeh I, Jorgenson E. Prognostic factors and survival in acral lentiginous melanoma. Br J Dermatol 2017;177(2):428–35. Jul 28.

[25] College of American Pathologists. Protocol for the examination of specimens from patients with melanoma of the skin. Available at: https://documents.cap.org/protocols/Skin.Melanoma_4.3.0.2.REL_CAPCP.pdf.
[26] Maia M, Russo C, Ferrari N, Ribeiro MCS de A. Melanoma acrolentiginoso: um desafio ao diagnóstico precoce. An Bras Dermatol 2003 Oct;78(5):553–60.
[27] Souza B de C e, DHM Silva, Miyashiro D, Kakizaki P, NYS Valente. Clinicopathological analysis of acral melanoma in a single center: a study of 45 cases. Revista da Associação Médica Brasileira [Internet] 2020 Oct;66(10):1391–5 [cited 2022 Apr 19].
[28] Susok L, Gambichler T. Caucasians with acral lentiginous melanoma have the same outcome as patients with stage- and limb-matched superficial spreading melanoma. J Cancer Res Clin Oncol 2022 Feb;148(2):497–502.

2.4 Artigo 4

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Title: The utility of PRAME and Ki-67 as prognostic markers for cutaneous melanoma

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Data access statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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Title: The utility of PRAME and Ki-67 as prognostic markers for cutaneous melanoma

ABSTRACT

Cutaneous melanoma can lead to metastasis and it is associated with high mortality. Currently, there are no widely accepted immunohistochemistry markers for melanoma prognosis in routine staging. PRAME is a possible biomarker for prognosis in several non-cutaneous neoplasms. Ki-67 is a cell proliferation marker correlated with poor outcomes in many cancers. This study assessed PRAME and Ki-67 as potential prognostic markers for sentinel lymph node outcomes and survival among melanoma patients. This is a retrospective study analyzing cutaneous melanoma cases from a Brazilian cancer center (2005 – 2021). All cases were tested using immunohistochemistry to evaluate PRAME expression and Ki-67 index. Descriptive analysis, Spearman's correlations, means comparison, Kaplan-Meier analysis, chi-square, and Cox models were performed. In univariate analysis of 123 cutaneous melanoma cases, high extent ($p = 0.0267$) and elevated intensity ($p = 0.043$) of PRAME were associated with decreased overall survival. The Ki-67 index was associated with overall survival ($p = 0.05$) and sentinel lymph node status ($p=0.0403$), with a positive correlation between the markers ($p = 0.0004$) and between Ki-67 and Breslow thickness ($p = 0.0001$). However, in multivariate analysis, only Breslow thickness significantly influenced overall survival ($p = 0.0003$). Then, the present results can suggest elevated PRAME and Ki-67 expression are associated with poor overall survival in cutaneous melanoma; however, in multivariate analysis, only the Breslow thickness had a significant influence. These findings highlight the potential of PRAME and Ki-67 as prognostic markers, opening frontiers that could improve strategies for treating cutaneous melanoma.

Keywords: Melanoma, pathology, neoplasm staging, skin neoplasms, immunohistochemistry

INTRODUCTION

Cutaneous melanoma is a public health concern because of its frequency of metastasis to distant organs[1] and its increased incidence in several countries. The estimated incidence of cutaneous melanoma in Australia was 69 cases per 100,000 inhabitants in 2023.[2] In comparison, Brazil had 4.13 estimated cases per 100,000 per year, according to the Brazilian National Cancer Institute.[3]

For predicting melanoma outcomes, the Breslow micrometric thickness is an essential parameter; it is used worldwide and included in the American Joint Committee on Cancer staging system.[4] Other characteristics used to predict outcomes of cutaneous melanoma include presence or absence of ulceration,[5] lymphovascular invasion,[6] lymphocytic infiltrate,[7] regression,[8] satellitosis,[9] mitotic index,[10] and histologic subtype.[11]

Many immunohistochemistry (IHC) stains are used to evaluate challenging melanocytic lesions; however, currently, there are no universally accepted markers for prognostic evaluation of cutaneous melanoma in staging protocols.[12]

Recently, a high frequency of PReferentially expressed Antigen in MElanoma (PRAME) expression in conventional melanoma was described regarding applicability for microscopic diagnosis.[13] PRAME was identified as a possible biomarker for prognostic evaluation and metastasis risk in tumors such as breast cancer,[14,15] osteosarcoma,[16] squamous cell carcinoma of the head and neck,[17] and uveal melanoma.[18] The high expression of PRAME in more advanced disease stages and its association with a higher risk of metastasis suggest that PRAME participates in the acquisition of cancer characteristics, including its capacity for invasion and metastasis.[19]

The antibody Ki-67 is a protein found in the nucleus of proliferating cells. It is an essential marker of cell proliferation and has been correlated with poor outcomes in many types of cancer.[20,21] The Ki-67 index is also a significant prognostic factor in melanoma, with increased expression related to decreased survival.[22]

The evaluation of PRAME is used for the diagnosis of melanocytic neoplasms and prognostic evaluations in other non-melanocytic neoplasms. Nevertheless, few studies correlate the PRAME expression with the outcomes and lymph node metastasis risk in cutaneous melanoma. Therefore, the present study evaluated the correlation between PRAME and Ki-67 expression with sentinel lymph node (SNL) biopsy results and specific and overall survival among patients with cutaneous melanoma.

MATERIALS AND METHODS

This retrospective cross-sectional study analyzed formalin-fixed paraffin-embedded skin specimens collected from primary melanoma lesions and lymph-node excisions performed at a Brazilian referral cancer center, from 2005 to 2021. From pathological reports stored in the institution's digital systems, we recorded clinical data. The slides were stained with hematoxylin and eosin (H&E), and two dermatopathologists assessed pathological information. Immunohistochemical analysis was performed on the Benchmark GX automatic staining system (Ventana Roche), with the application of the UltraView DAB kit using an anti-PRAME antibody (Abcam, clone EPR 20330, dilution 1:500) and Ki-67 (Dako, clone MIB-1, prediluted). To ensure consistency of PRAME staining conditions, external controls were used in all cases (testis tissue). For PRAME, we calculated the intensity of the staining (grade 0 = negative, grade 1 = weak, grade 2 = moderate, grade 3 = strong), and the extension

based on the percentage of stained cells throughout the lesion (grade 0 = negative, grade 1 = 1%–25%, grade 2 = 26%–50%, grade 3 = 51%–75%, and grade 4 = 76%–100%). A combined score was also performed, consisting of the sum of the staining intensity with the extension grades ranging from 0 to 7.[23.] A variable consisting of the multiplication of the Ki-67 index and the combined score was also created. We also divided samples into diffuse staining (more than 75% of positive cells) and non-diffuse staining (75% or fewer positive cells). For assessing the labeling of the sebaceous glands was evaluated for the internal control of PRAME. Exclusion criteria included dubious lesions on H&E, absence of SLN results, negative cases with negative internal or external controls, and thin melanomas (i.e., less than 1.0 mm in thickness, as they are not routinely subjected to SLN biopsy).

A descriptive analysis was performed to calculate means, standard deviations (SD), minimum, maximum, and median values for quantitative variables, and frequencies and percentages for characterized variables. Spearman's correlations were used to assess the strength and direction of the associations between variables, and were calculated for PRAME, Ki-67, age, survival time (in months), and Breslow thickness (in mm). Mean comparisons for Ki-67 index, PRAME percentage, PRAME staining extension, the combined score and the “Ki-67 x combined score” multiplication were stratified by sex, anatomical site, histological subtype and SLN status using a Poisson distribution adjustment or by adjusting for a negative binomial distribution followed by Wald's multiple comparison test. Receiver operating characteristic (ROC) curves for Ki-67 concerning survival were generated, while Kaplan-Meier survival analyses were conducted for the determined cutoff point. For Ki-67, mean tests were performed considering subtype and SLN status. For PRAME classification, chi-square associations with SLN status were computed. Correlations among Ki-67, PRAME,

survival time, and the combined score were also calculated. For overall survival, Cox proportional hazards models were adjusted for the explanatory variables: gender, anatomical site, lymph node status, PRAME intensity, extension, combined score, Ki-67 index, “Ki-67 index X combined score”, and Breslow depth.

A 5% significance level was defined for all analyses, or the corresponding p-value was used. Chi-square tests were performed to evaluate associations between categorical variables. All analyses were performed using SAS for Windows, version 9.4, and SPSS for Windows, version 21.

The Committee of Ethics in Research approved the study (CAAE 52618721.0.1001.5411 and CAAE 52618721.0.3001.5434). Patient consent not applicable.

RESULTS

We considered 123 invasive cutaneous melanomas. There were 71 (57.72%) men and 52 (42.28%) women. The median age was 65 years (14 to 87 years). Fifty-seven cases (46.34%) affected non-exposed areas; 34 cases (27.64%) occurred in acral skin; 25 cases (20.33%) affected exposed areas; and for seven cases (5.69%), the anatomical site was not specified. The mean Breslow thickness was 6.4 mm (SD 5.9) with a median of 4.4 mm (1.0 to 27.5 mm). Regarding the subtype, there were 54 nodular melanomas (43.9%), 41 superficial spreading melanomas (33.3%), 27 acral melanomas (21.9%), and one spindle cell melanoma (0.8%); 64 cases (52.03%) had metastasis to SLN, while 59 were free of metastasis (47.97%). Regarding the pathological staging, 24 cases (19.5%) were pT2, 32 cases (26.0%) were pT3, and 67

cases (54.5%) were pT4. The overall survival mean was 38.2 months (SD 34.8), and the median was 25.0 (0 to 142 months).

PRAME showed positive staining (regardless of intensity or extension) in 107 cases (86.9%) and was negative in 16 cases (13.01%). Nevertheless, only 38 (30.89%) had more than 75% (grade 4) of positive cells; 14 cases (11.38%) had between 51% to 75% (grade 3); 28 (22.76%) had between 26% to 50% (grade 2); 27 (21.95%) had between 1% to 25% (grade 1). Regarding the intensity of PRAME staining, 31 cases (25.2%) were strong (grade 3), 27 (21.95%) were moderate (grade 2), and 49 (39.84%) were weak (Table 1; Figure 1).

There was difference regarding overall survival between cases with diffuse staining of PRAME and non-diffuse staining ($\chi 2$ 4.9101; $p = 0.0267$) (Table 2; Figure 2). While the non-diffuse staining group exhibited a mean survival of 43.04 months (SD = 36.12/ median: 28 months, range: 2 to 142 months), the group with diffuse staining of PRAME exhibited a mean survival of 26.25 months (SD = 28.68, median: 16.5 months; range: 0 to 140 months). This finding was supported by Spearman's correlation between PRAME staining extension and patient survival (correlation coefficient = -0.25387 with $p = 0.0072$), suggesting that a greater extent of PRAME expression is associated with reduced survival. Otherwise, the log-rank test comparing melanoma-specific survival resulted in a $\chi 2$ of 2.3155 ($p = 0.1281$), suggesting no statistically significant differences between these groups (Table 2, Figure 2).

The log-rank test to assess the overall survival across different levels of PRAME intensity staining (categorized as 0, 1, 2, and 3) resulted in a $\chi 2$ of 8.12, $p = 0.043$. Intense staining for PRAME had a worse overall survival than negative cases (Table 2, Figure 3). For melanoma-specific survival and PRAME intensity, the log-rank

test did not demonstrate statistically significant differences between the groups ($\chi 2$ 4.9604, p=0.1747) (Table 2, Figure 2).

Regarding the SLN result and PRAME expression, there were no significant differences between “diffuse PRAME staining” and “non-diffuse PRAME staining” (p = 0.6316). The group with negative SLN (59 cases) had a PRAME expression mean of 46.53% (SD 34.87, median: 50%; range: 0 to 100%), while the group with positive SLN (64 cases) had a PRAME expression mean of 47.75% (SD = 32.81, median: 50%; range: 0 to 100%).

In the analysis of the combined score (intensity grade plus extension grade), Spearman’s correlation coefficient demonstrated a negative correlation with survival (r = -0.2674, p = 0.0046), indicating that higher values of the combined score correlate with decreased survival. There was no significant correlation between the combined score and SLN status (p = 0.1205).

Comparing the means for PRAME percentage of expression with the three anatomical sites (exposed areas, non-exposed areas, and acral), the statistical analysis yielded a p-value of < 0.0001. The mean PRAME expression in exposed areas was 38.24% (SD = 34.38, median: 40%; range: 0 to 100%). For non-exposed areas, the mean expression was 45% (SD = 35.15, median: 40%; range: 0 to 100%). For acral skin, the mean expression of PRAME was 62.06% (SD = 27.15, median: 67.5%; range 0 to 100%).

In the comparative analysis of PRAME expression across the main melanoma subtypes, the distribution between non-diffuse and diffuse staining cases demonstrated significative differences (p = 0.026). Diffuse staining was identified in 14

(51.8%) acral melanomas, nine (21.9%) superficial spreading melanomas, and 15 (27.7%) nodular ones. The comparison of means of the combined score revealed significant differences among the three main histological subtypes (p = 0.0283). The mean combined score for the acral subtype was 4.81 (SD 1.9; median 5; range 0-7). For the nodular subtype the mean score was 3.83 (SD 2.43; median 4; range 0-7). And the mean score for the superficial spreading subtype was 3.22 (SD 2.15; median 3; range 0-7). Additionally, there were significant differences in the “Ki-67 X combined score” multiplication among the histological subtypes (p=0.0005). The mean value for this multiplication in the acral subtype was 117.96 (SD 79.85; median 100; range 0-280). For the nodular subtype, the mean was 118.7 (SD 112.69; median 90; range 0-420). For the superficial spreading subtype, the mean was 57 (SD 75.24; median 30; range 0-360). Regarding PRAME extension (%), no significant differences were found among the three subtypes (p = 0.1721). Similarly, there were no significant differences in PRAME intensity levels among the histological subtypes (p = 0.0766).

The Ki-67 had a mean index of 22.9% (SD = 15.4%, median: 20%; range: 1% to 60%). Using the ROC curve, a cutoff point of 22.5% was obtained for Ki-67. The log-rank test to compare the overall survival between cases with Ki-67 expression of less than or equal to 22.5% and more than 22.5% resulted in a $\chi 2$ of 3.8236 and p = 0.05, indicating a significant difference in survival between the groups. For melanoma-specific survival, the log-rank test resulted in a $\chi 2$ of 3.0154. The corresponding p-values (raw and adjusted by the Sidak method) were p = 0.0825, suggesting no significant differences in disease-specific survival between groups with Ki-67 expression less or equal to 22.5% and those with more than 22.5% (Table 2; Figures 2 and 3). The Cox model to evaluate the relationship between the multiplication of “Ki-

67 index and PRAME combined score" and melanoma-specific survival resulted in a χ2 of 3.7781 and a p-value of 0.0519.

Regarding SLN results, there was a difference between groups regarding the Ki-67 index (p = 0.0403). For patients with negative SNL, the Ki-67 mean index was 19.86% (SD = 14.22, median: 15%; range: 1% to 50%). For patients with positive SNL, the Ki-67 mean was 25.7% (SD = 16,11, median: 20%; range 5% to 60%).

In relation to the comparison of Ki-67 means among the three main histological subtypes, statistically significant differences were observed (p=0.0015). The acral subtype exhibited a mean of 24.07% (SD 13.52; median 20; range 5-50). The nodular subtype showed a mean of 27.5% (SD 16.39; median 30; range 5-60). In contrast, the superficial spreading subtype presented a mean of 16.39% (SD 13.26; median 15; range 1-60). Grouping the acral and nodular melanomas together and comparing them with the other histological subtypes revealed no significant differences regarding the SNL outcome (p = 0.0647) or melanoma specific survival (p = 0.0628).

Spearman correlation coefficient demonstrated a positive correlation between Ki-67 with PRAME intensity (r = 0.31451, p = 0.0004), percentage (r = 0.23142, p = 0.01), extension grade (r = 0.21284, p = 0.0181) and the combined score (r = 0.27433, p = 0.0021). The test demonstrated a significant positive correlation between Ki-67 and Breslow thickness (r = 0.39412, p = 0.0001). There was no significant correlation between Breslow thickness with PRAME intensity (r = 0.15433, p = 0.0883), extension (r = 0.12097, p = 0.1826), or combined score (r = 0.15220, p = 0.0927).

In the multivariate analysis using the Cox analysis, among several variables, only the Breslow depth had a statistically significant influence on overall survival ($p = 0.0003$) (Table 3).

DISCUSSION

In the studied population, high extent and elevated intensity of PRAME expression were associated with decreased overall survival. However, these parameters were not statistically associated with specific survival, SLN status, or Breslow thickness. Notably, PRAME expression demonstrated a relationship with the anatomic location and the histological subtype; the Ki-67 index was associated with overall survival and SLN status. Furthermore, there was a correlation between both markers and between Ki-67 and Breslow thickness. Upon multivariate analysis, only Breslow thickness significantly influenced overall survival.

Several studies related PRAME to outcomes of various neoplasms. Field et al. studied uveal melanoma and found that PRAME expression was associated with metastasis and a larger tumor diameter.[18] Tan et al. showed that in osteosarcoma, high PRAME expression was associated with poor outcomes and lung metastasis.[16] Doolan et al. reported 103 cases of breast cancer and showed that PRAME mRNA expression correlated with shortened disease-free survival and overall survival.[15] Szczepanski et al. found that, in squamous cell carcinoma of the head and neck, the intensity of PRAME staining correlated with the tumor grade, with high expression being related to poorly differentiated tumors and low expression being related to well-differentiated tumors. PRAME expression levels were correlated with lymph node metastasis.[17]

Although there are many studies about using PRAME immunohistochemistry for cutaneous melanoma diagnosis, few focused on PRAME and cutaneous melanoma prognosis. Our study revealed that melanomas with diffuse PRAME expression (> 75%) had a significantly worse overall survival than melanomas with non-diffuse expression. In comparison, Parra et al. analyzed 102 melanomas in The Cancer Genomic Atlas and did not identify a significant correlation between PRAME mRNA expression and melanoma overall survival; the same study analyzed 111 melanomas and did not show a significant correlation between higher PRAME expression and disease specific-survival, in agreement with our study.[24] Gassenmaier et al. studied 70 thin melanoma cases and did not find significant differences in overall survival between patients with diffuse (> 75% staining) and non-diffuse PRAME expression ($p = 0.22$).[25] Lo Bello et al. studied 137 cutaneous melanomas (with Breslow > 1 mm) and did not identify a prognostic significance of diffuse PRAME expression for relapse/progression-free survival or overall survival.[26] For these reasons, the prognostic value of PRAME for cutaneous melanoma is debated.

Studies of other neoplasms showed that PRAME expression was associated with metastasis, including lymph node metastasis.[16,17,18] Hu et al. studied 75 acral lentiginous melanoma and reported that 82.6% of the cases with lymph node involvement expressed PRAME, compared with 57.6% of those without, although this difference did not reach statistical significance ($p = 0.051$).[27] The present study did not demonstrate significative differences in the SLN status between the groups with diffuse and non-diffuse PRAME expression.

In the present study, PRAME expression was higher in acral skin than in other anatomical locations. We also observed more cases with diffuse PRAME expression

among acral lentiginous melanoma subtypes than in superficial spreading and nodular subtypes. The “Ki-67 and PRAME combined score” multiplication showed significantly higher values in acral and nodular melanomas, compared to superficial spreading subtype. In agreement with our data, Lezcano et al. showed that the histologic subtype with more 4+ (diffuse) labeling cases was acral melanoma (94.4% of the cases), followed by superficial spreading melanomas (92.5%), nodular melanomas (90%), lentigo maligna melanoma (88.6%) and desmoplastic melanoma (35%).[13] Rawson et al. also demonstrated a higher percentage of PRAME-positive cells in acral melanoma (mean: 76%; median: 83%) than in nodular melanoma (mean: 60%; median: 69%), lentigo maligna melanoma (mean: 60%; median: 64%), superficial spreading melanoma (mean: 51%; median 51%), and desmoplastic melanoma (mean: 50%; median: 49%).[28] Given that acral melanoma is known for its poorer outcomes, we hypothesize that higher PRAME expression maybe linked to more aggressive behavior.

In the present study, higher Ki-67 (> 22.5%) was associated with poor overall survival but not specific survival. Furthermore, cases with positive SNL were associated with a higher Ki-67 index. These results partially agree with Parra et al., who showed that a higher Ki-67 was associated with shorter overall and disease-specific survival.[24] Henrique et al. studied 84 patients and showed in univariate analysis that a high Ki-67 index (> 14%) displayed significantly worse outcomes, although when stratified for tumor thickness, no survival differences were found.[29] Udovicic-Gagula et al. analyzed the expression of Ki-67 in 55 primary cutaneous melanoma and observed a statistically significant higher value of Ki-67 expression in patients with positive lymph nodes [30], in agreement with our study. Also the Ki-67 index revealed higher values in nodular and acral subtypes, in comparison with superficial spreading melanoma.

Although there was no correlation between Ki-67 and specific survival, the Ki-67 index can be considered an established prognostic marker.

Our study also demonstrated a correlation between Ki-67 and PRAME expression, in agreement with Parra et al., who showed that higher PRAME expression correlated with a higher Ki-67 index.[24] Also in agreement with other studies, elevated Ki-67 index was associated with higher Breslow thickness, but no association was found between PRAME expression and tumor thickness.[24,29.30]

Lezcano et al. studied 155 primary cutaneous melanomas and found diffuse PRAME expression (grade 4) in 83.2% of the cases.[13] This frequency was higher than we found in the present study, in which PRAME diffuse expression occurred in only 30.89% of the cases. Rawson et al. had a similar result, with 35% of the melanomas showing diffuse staining (grade 4).[28] Parra et al. demonstrated diffuse staining in only 26.1% of the cases.[24] Gassenmaier et al. found that 58.6% of melanomas showed diffuse PRAME staining (score 4+).[25] One hypothesis for these discrepant results is that PRAME can exhibit heterogeneous staining within the lesion, complicating the categorization regarding the extent of staining, particularly in more extensive lesions, as in our study, which included many large advanced-stage specimens. Another possibility involves the case selection, because in the present study we analyzed only melanoma confirmed cases and did not include comparisons with benign melanocytic lesions.

The present study's limitations include a retrospective nature and the population limited to Brazilian patients in a single cancer center, which had many advanced cases with many acral and nodular ones. Furthermore, our study population did not include patients with in situ or thin melanomas, and none of these sections were

Ki-67 double-stained with Melan-A. The adopted nomenclature (diffuse x non-diffuse) differs somewhat from studies in which only cases with more than 75% of positive cell were considered “positive.” It is also critical to highlight that PRAME was performed only in primary lesions and not in the positive lymph node cases. The lack of a standardized protocol for PRAME expression assessment could also lead to variability, making direct comparisons with other studies challenging.

In conclusion, this study revealed an association between higher PRAME expression and lower overall survival in melanoma patients, in univariate analysis. Elevated Ki-67 index was associated with lower overall survival and positive SLN. Acral and nodular melanoma subtypes are associated with higher Ki-67 index and “Ki-67 X PRAME combined score”. Multivariate analysis indicated that only the Breslow thickness influenced overall survival. Ki-67 and PRAME did not demonstrate to be independent prognostic parameters, in the analyzed sample. More studies with larger sample sizes are necessary to evaluate PRAME expression's role in predicting cutaneous melanoma outcomes in different populations. These results suggest a potential association between higher PRAME expression and melanoma survival, opening frontiers that could improve clinical practice and therapeutic strategies in cutaneous melanoma.

REFERENCES

1. Mirea MA, Eckensperger S, Hengstschläger M, et al. Insights into Differentiation of Melanocytes from Human Stem Cells and Their Relevance for Melanoma Treatment. Cancers (Basel). 2020;12(9):2508.

2- Australian Institute of Health and Welfare. Cancer data in Australia. Cat. no. CAN 122.[Updated Aug 2023] Avalailable at: https://www.aihw.gov.au/reports/cancer/cancer-data-in-australia [Accessed Jan 2024];

3- Estimativa 2023: incidência de câncer no Brasil / Instituto Nacional de Câncer (Brasil) – Rio de Janeiro: INCA, 2022. Available at: https://www.inca.gov.br/publicacoes/livros/estimativa-2023-incidencia-de-cancer-no-brasil [Accessed Jan 2024];

4- Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al.: AJCC Cancer Staging Manual (8th edition). Chicago, Il: Springer; 2017.

5- Sarpa HG, Reinke KA, Shaikh L, et al. Prognostic Significance of Extent of Ulceration in Primary Cutaneous Melanoma. Am J Surg Pathol. 2006;30(11):1396-400.

6- Egger ME, Gilbert JE, Burton AL, et al. Lymphovascular Invasion as a Prognostic Factor in Melanoma. Am Surg. 2011;77(8):992-7.

7- Azimi F, Scolyer RA, Rumcheva P, et al. Tumor-Infiltrating Lymphocyte Grade Is an Independent Predictor of Sentinel Lymph Node Status and Survival in Patients With Cutaneous Melanoma. J Clin Oncol. 2012;30(21):2678-83.

8- Aung PP, Nagarajan P, Prieto VG. Regression in primary cutaneous melanoma: etiopathogenesis and clinical significance. Lab Invest. 2017;97(6):657-668.

9- Niebling MG, Haydu LE, Lo S, et al. The prognostic significance of microsatellites in cutaneous melanoma. Mod Pathol. 2020;33(7):1369-1379.

10- Thompson JF, Soong SJ, Balch CM, et al. Prognostic Significance of Mitotic Rate in Localized Primary Cutaneous Melanoma: An Analysis of Patients in the Multi-Institutional American Joint Committee on Cancer Melanoma Staging Database. J Clin Oncol. 2011;29(16):2199-205.

11- Ocanha-Xavier JP, Xavier-Júnior JCC, Marques MEA. Melanoma: clinical, evolutive and histopathological characteristics of a series of 136 cases. An Bras Dermatol. 2018;93(3):373-376.

12- Ferringer T. Immunohistochemistry in Dermatopathology. Arch Pathol Lab Med. 2015;139(1):83-105.

13- Lezcano C, Jungbluth AA, Nehal KS, et al. PRAME Expression in Melanocytic Tumors. Am J Surg Pathol. 2018;42(11):1456-1465.

14- Al-Khadairi G, Decock J. Cancer Testis Antigens and Immunotherapy: Where Do We Stand in the Targeting of PRAME? Cancers (Basel). 2019;11(7):984.

15- Doolan P, Clynes M, Kennedy S, et al. Prevalence and prognostic and predictive relevance of PRAME in breast cancer. Breast Cancer Res Treat. 2008;109(2):359-65.

16- Tan P, Zou C, Yong B, et al. Expression and prognostic relevance of PRAME in primary osteosarcoma. Biochem Biophys Res Commun. 2012;419(4):801-8.

17- Szczepański MJ, DeLeo AB, Łuczak MW, et al. PRAME expression in head and neck cancer correlates with markers of poor prognosis and might help in selecting candidates for retinoid chemoprevention in pre-malignant lesions. Oral Oncol. 2013;49(2):144-51.

18- Field MG, Decatur CL, Kurtenbach S, et al. PRAME as an Independent Biomarker for Metastasis in Uveal Melanoma. Clin Cancer Res. 2016;22(5):1234-42.

19- Al-Khadairi G, Naik A, Thomas R, Al-Sulaiti B, Rizly S, Decock J. PRAME promotes epithelial-to-mesenchymal transition in triple negative breast cancer. J Transl Med. 2019;17(1):9.

20- Sun X, Kaufman PD. Ki-67: more than a proliferation marker. Chromosoma. 2018;127(2):175-186.

21- Pan D, Wei K, Ling Y, et al. The Prognostic Role of Ki-67/MIB-1 in Cervical Cancer: A Systematic Review with Meta-Analysis. Med Sci Monit. 2015;21:882-9.

22- Nielsen PS, Riber-Hansen R, Jensen T, et al. Proliferation indices of phosphohistone H3 and Ki67: strong prognostic markers in a consecutive cohort with stage I/II melanoma. Mod Pathol. 2013;26(3):404-13.

23- Hornick JL, Plaza JA, Mentzel T, et al. PRAME Expression Is a Useful Tool in the Diagnosis of Primary and Metastatic Dedifferentiated and Undifferentiated Melanoma. Am J Surg Pathol. 2023;47(12):1390-1397.

24- Parra O, Ma W, Li Z, et al. PRAME expression in cutaneous melanoma does not correlate with disease-specific survival. J Cutan Pathol. 2023;50(10):903-912.

25- Gassenmaier M, Hahn M, Metzler G, et al. Diffuse PRAME Expression Is Highly Specific for Thin Melanomas in the Distinction from Severely Dysplastic Nevi but Does Not Distinguish Metastasizing from Non-Metastasizing Thin Melanomas. Cancers (Basel). 2021;13(15):3864.

26- Bello G, Pini GM, Giagnacovo M, et al. PRAME expression in 137 primary cutaneous melanomas and comparison with 38 related metastases. Pathol Res Pract. 2023;251:154915.

27- Hu J, Cai X, Lv J, et al. Preferentially expressed antigen in melanoma immunohistochemistry as an adjunct for differential diagnosis in acral lentiginous melanoma and acral nevi. Hum Pathol. 2022;120:9-17.

28- Rawson RV, Shteinman ER, Ansar S, et al. Diagnostic utility of PRAME, p53 and 5-hmC immunostaining for distinguishing melanomas from naevi, neurofibromas, scars and other histological mimics. Pathology. 2022;54(7):863-873.

29- Henrique R, Azevedo R, Bento MJ, et al. Prognostic value of Ki-67 expression in localized cutaneous malignant melanoma. J Am Acad Dermatol. 2000;43(6):991-1000.

30- Udovičić-Gagula D, Ahmovic A, Bilalović N, et al. Expression of Ki-67 and Estrogen Receptor Beta in Primary Cutaneous Melanoma as a Potential Indicator of Regional Lymph Node Positivity. Appl Immunohistochem Mol Morphol. 2019;27(1):27-32.

Legends:

Figure 1: Examples of different patterns of PRAME intensity, increasing staining from (A) to (C): A) weak, B) moderate, and C) strong.

Figure 2: Kaplan-Meier curves of PRAME extension with overall survival (A) and specific survival (B); PRAME intensity with overall survival (C); specific survival (D). And Kaplan-Meier curves of Ki-67 index with overall survival (E) and specific survival (F).

Figure 3: Examples of low Ki-67 index (A) and high Ki-67 index (B).

Table 1. Descriptive analysis of clinicopathologic characteristics of the cases

Variables	
Sex, No. (%)	
Female	52 (42.2)
Male	71 (57.7)
Age (y)	
Mean	61.5
Median (range)	65 (14–87)
Breslow thickness (mm)	
Mean	6.4 (SD 5.9)
Median (range)	4.4 (1–27.5)
Location, No (%)	
Non-exposed areas	57 (46.34)
Exposed areas	25 (20.33)

Acral skin	34 (27.64)
Not informed	7 (5.69)
Histological subtype, No. (%)	
Superficial spreading melanoma	41 (33.3)
Nodular melanoma	54 (43.9)
Acral lentiginous melanoma	27 (21.9)
Spindle cell melanoma	1 (0.8)
Sentinel lymph node, No. (%)	
Positive for metastasis	64 (52.03)
Negative for metastasis	59 (47.97)
Survival (months)	
Mean	38.1 (SD 34.8)
Median (range)	25 (0–142)
PRAME extension grade, No. (%)	
Grade 4 (>75%)	38 (30.89)
Grade 3 (51% to 75%)	14 (11.38)
Grade 2 (26% to 50%)	28 (22.76)
Grade 1 (1% to 25%)	27 (21.95)
Grade 0 (negative)	16 (13.01)
PRAME intensity grade, No. (%)	
Grade 3 (Strong)	31 (25.2)
Grade 2 (Moderate)	27 (21.95)
Grade 1 (Weak)	49 (39.84)
Grade 0 (Negative)	16 (13.01)

Ki-67 index	
Mean	22.9 (SD 15.4)
Median (range)	20 (1%–60%)

Table 2. Test of equality over strata: PRAME extension, PRAME intensity, overall survival, and melanoma-specific survival

Logrank Test	$\chi 2$	Pr > $\chi 2$
PRAME extension and overall survival	4.9101	0.0267
PRAME extension and melanoma-specific survival	2.3155	0.1281
PRAME intensity and overall survival	8.1243	0.0435
PRAME intensity and melanoma-specific survival	4.9604	0.1747
Ki-67 index and overall survival	3.8236	0.0505
Ki-67 index and melanoma-specific survival	3.0154	0.0825

Table 3. Multivariate analysis using the Cox proportional, among several variables for melanoma overall survival

Parameter	Pr > χ2	Hazard Ratio	95% HR Confidence Limits	
Location: exposed area	0.7211	1.341	0.268	6.703
Location: non-exposed	0.462	1.769	0.387	8.084
Location: acral	0.4813	1.751	0.368	8.326
Location: not informed	-	1	-	-
Positive SLN	0.1958	1.639	0.775	3.465
Negative SLN	-	1	-	-
Female	0.8042	1.086	0.565	2.09
Male	-	1	-	-
PRAME intensity: grade 0	0.9811	1.047	0.024	45.54
PRAME intensity: grade 1	0.9488	1.078	0.109	10.62
PRAME intensity: grade 2	0.6551	0.724	0.175	2.987
PRAME intensity: grade 3	-	1	-	-
PRAME extension (%)	0.7966	0.995	0.956	1.035

PRAME combined score	0.6409	1.253	0.486	3.23
Ki-67 index	0.3082	1.011	0.99	1.032
Breslow thickness	0.0003	1.094	1.042	1.149

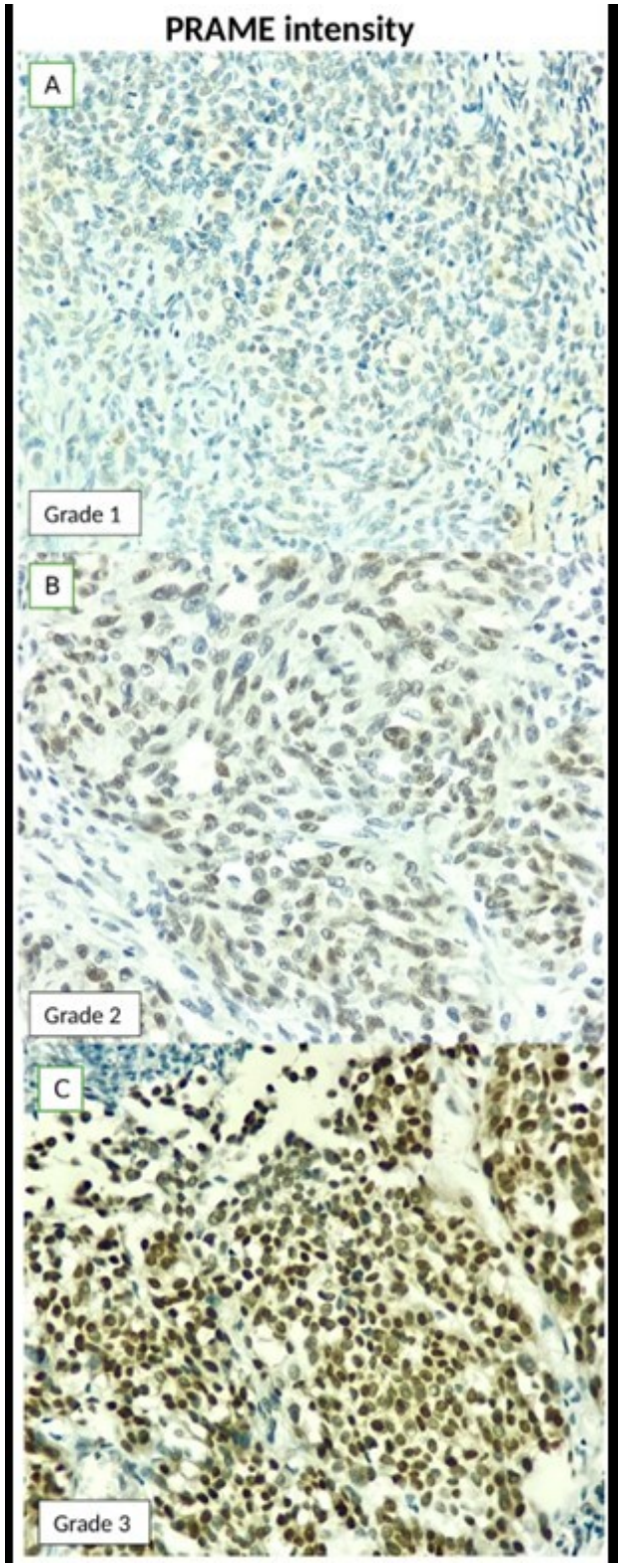


Figure 1: Examples of different patterns of PRAME intensity, increasing staining from (A) to (C): A) weak, B) moderate, and C) strong.

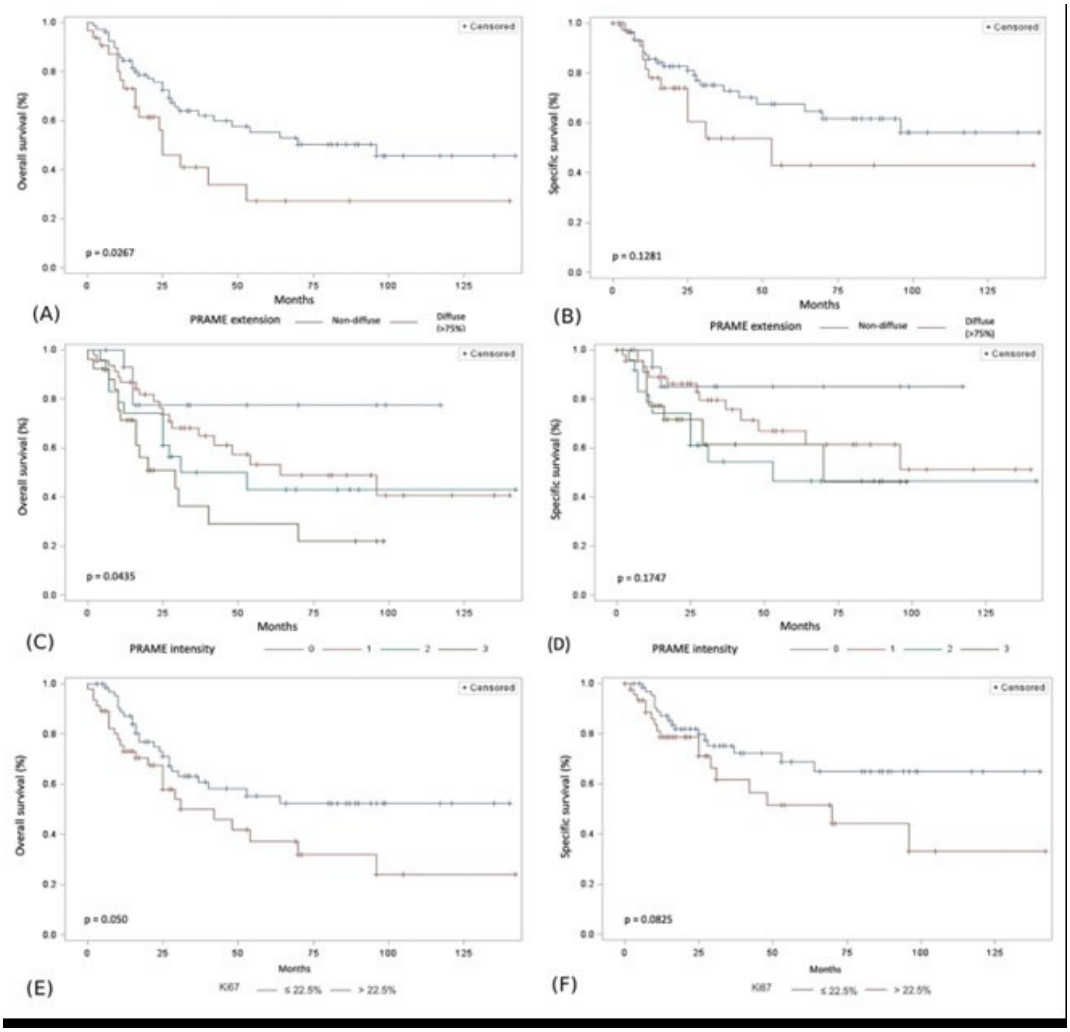


Figure 2: Kaplan-Meier curves of PRAME extension with overall survival (A) and specific survival (B); PRAME intensity with overall survival (C); specific survival (D). And Kaplan-Meier curves of Ki-67 index with overall survival (E) and specific survival (F).

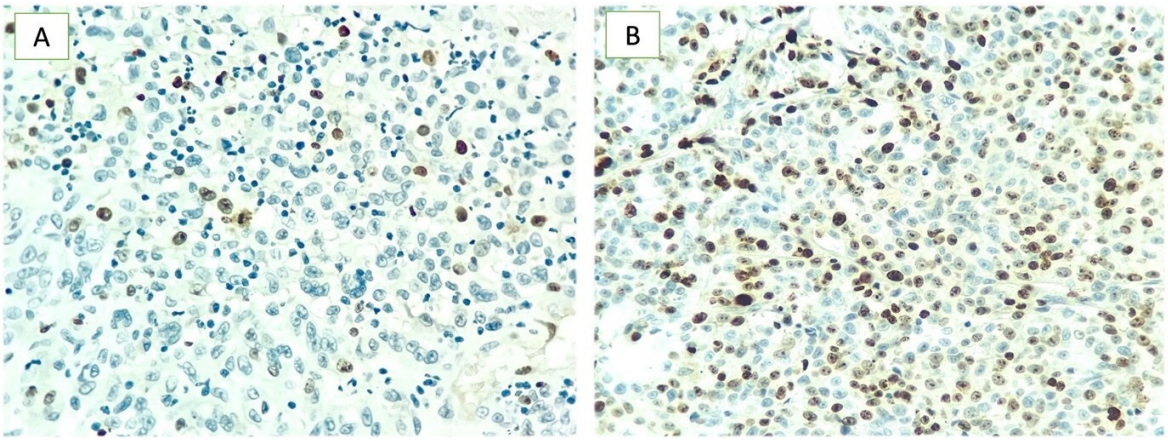


Figure 3: Examples of low Ki-67 index (A) and high Ki-67 index (B).

2.5 Artigo 5

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Research Article

The Ki-67 staining pattern as a cutaneous melanoma prognostic marker

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Short Title: ki-67 and cutaneous melanoma

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Abstract

Introduction: Cutaneous melanoma is an aggressive skin cancer. The Ki-67 is a proliferation marker and a rapid tool for proliferation rate analysis. Previous data indicated that its staining pattern is distinct during the several stages of the cell cycle. No studies have evaluated these patterns in cutaneous melanoma as a prognostic marker. **Methods:** This retrospective study analyzes Ki-67 immunostaining patterns among acral melanoma cases. Two pathologists classified staining patterns, and analyses were performed to assess interobserver concordance and compare patterns between patients alive and those who died of melanoma. **Results:** The study analyzed 5,676 Ki-67 positive nuclei. Lin's Concordance Correlation Coefficient was reported at 0.9783, reflecting a substantial level of agreement. Means comparison between the two groups revealed differences in patterns NP1 ($p = 0.0014$), NP4 ($p = 0.038$), NP5 ($p = 0.0193$), and the total number of stained nuclei ($p = 0.0258$). There were no significant differences between the NP5 pattern and conventional mitosis counting in both groups. **Conclusion:** Our analysis highlights the importance of Ki-67 staining patterns as a potential prognostic marker among cutaneous melanomas. High Ki-67 positive nuclei was associated with worse outcomes (death), particularly in staining patterns NP1, NP4, and NP5.

Introduction

Cutaneous melanoma is an aggressive skin cancer. [1,2] Breslow depth is considered the most critical factor for assessing outcomes, and it was included in the American Joint Committee on Cancer (AJCC) staging system. [3,4] Cutaneous melanoma can be classified into four primary histologic subtypes: superficial spreading, nodular, lentigo maligna (melanoma in chronically sun-exposed skin), and acral lentiginous [5]. Acral cutaneous melanoma (ACM), first described by Reed in 1976 [6], is the least common, with a higher prevalence in African and Asian populations and typically found on palms, soles, and nail beds [7,8]. Notably, ACM is associated with poorer outcomes when compared with the other melanoma subtypes. [7]

Immunohistochemical markers complement the histopathological analysis for the diagnosis and outcomes of many types of cancer. However, until now, no consolidated markers for cutaneous melanoma have been incorporated into staging protocols. [9,10] Some studies have shown that Ki-67 is a prognostic factor in cutaneous melanoma. A High Ki-67 index is associated with the poorest outcomes. [11-15]

The Ki-67 is a cell proliferation marker, with nuclear expression in the cell cycle's G1, S, G2, and mitotic phases, but it is absent in G0 cells. Therefore, it is a simple, consolidated, and rapid tool for determining tumors' proliferation rates. [16]

Previous studies suggested Ki-67 has distinct intracellular localization patterns during the several stages of the cell proliferation cycle. [17,18] It localizes to the peri chromosomal layer at the beginning of mitosis, to hundreds of distinct foci homogeneously distributed in the early G1 phase, to one or two nucleoli in the late G1 phase, to larger dense granules throughout the nucleus in the S phase, and granules and irregular nodules in the nucleoplasm in G2 phase. [19,20] Dias et al., in 2021, created a classification based on the morphological patterns of Ki-67 immunostaining and cell cycle phases. Based on the analysis of epithelial tumors, they also found differences in the pattern of Ki-67 expression between benign epithelial lesions and malignant or pre-malignant epithelial lesions. [20]

To date, no studies have evaluated the pattern of Ki-67 expression in cutaneous melanoma. This study aims to analyze the interobserver concordance of Ki-67 expression pattern classification in ACM and to compare the different types of patterns between two groups of patients: those who are alive (Al) and those who died of melanoma (De).

Methods

We conducted a retrospective cross-sectional study that analyzed formalin-fixed paraffin-embedded skin specimens collected from ACM, all classified as the same stage, from Amaral Carvalho Hospital, a Brazilian referral cancer center. Ventana Roche performed immunohistochemical (IHC) analysis on the Benchmark GX automatic staining system, applying the UltraView DAB kit using Ki-67

(Dako, clone MIB-1, prediluted). The IHC slides were scanned using Aperio CSTM (Leica Biosystems, USA) at 40x magnification. At first, a mean Ki-67 index was estimated considering the whole lesion (general Ki-67). Aside from that, a one-square-millimeter section was demarcated in hot spots for a detailed examination of each sample. This area size was chosen to be aligned with AJCC mitosis counting. [10] The slides were evaluated by two pathologists, independently identifying Ki-67 positive nuclei within the designated area with a 40x zoom, utilizing a colored circle scheme based on Dias' classification, as follows: blue circle for NP1 (randomly Ki-67 immunopositive fine or coarse granules, corresponding to early G1 phase), green circle for NP2 (Ki-67 stained in one or two well defined and centralized nodules, corresponding to late G1 phase), orange circle for NP3 (Ki-67 seen as granules or nodules occupying most of the nucleus, corresponding to S phase), brown circle for NP4 (nucleoplasm with intense and homogeneous Ki-67 staining, corresponding to G2 phase) and red circle for NP5 (empty central area with peripheral Ki-67 or exclusively on the chromosomes, corresponding to mitosis) as shown in figures 1 and 2. Then, the numbers of stained nuclei were counted for each pattern in all cases. Conventional mitosis counting was also performed. Outcome measures were correlated with survival data extracted from electronic health records, leading to the categorization of the cases into two groups: those who were alive and those who died of melanoma.

This study defined the inclusion criteria as cases with invasive ACM at the same advanced stage from 2008 to 2020. The exclusion criteria included instances where the immunohistochemical reaction failed or performed poorly or where survival data were absent. Ungual melanomas were also excluded. To compare two distinct groups (Al – alive and De- melanoma-related death)—we selected patients with the same histological subtype, same stage, and similar time of diagnosis. We opted for the ACM subtype and stage T4 because those are the cases with poor outcomes. We then selected an equal number of cases for each group, totaling seven for the "Al" group and 12 for the “De” group. All seven individuals from the "Al" group were selected, and seven from the “De” group were chosen randomly. Regarding the analyzed nuclei, overlapping or blurred nuclei were not considered.

To assess the agreement between measurements, a Bland-Altman graphical analysis was conducted to calculate the bias between measurements, Lin's Concordance Correlation Coefficient, and the corresponding plot. This analysis was performed using the STATA software for Windows, version 21. An average of each evaluator's data was computed. A comparison of means for each nuclear staining pattern was conducted, utilizing a negative binomial distribution adjustment.

A significance threshold of 5% or the corresponding p-value was established for all tests. The analyses were conducted using SAS for Windows version 9.4. and SPSS for Windows version 21.

This research received approval from the research ethics committee (CAAE 52618721.0.1001.5411 and CAAE 52618721.0.3001.5434).

Results

In this analysis, a total of 5,676 Ki-67 positive nuclei from 14 cases were evaluated, with 7 cases in the “AI” group and 7 cases in the “De” group. In both the “AI” and “De” groups, 4 cases (57.15%) were male, while 3 cases (42.86%) were female. The mean age of the “AI” group was 65 years, whereas in the “De” group, the average age was 73.85 years. Regarding Breslow thickness, the mean Breslow thickness in the “AI” group was 7.2mm (SD 1.6; median 7.4mm; range 4.3-9.3), while in the “De” group, the mean Breslow thickness was 9.1mm (SD 2.08; median 8.1mm; range 7-13). In both groups, considering the general Ki-67 for the whole lesion, some cases showed an essential range from 5% to 60% of positive cells. In the “AI,” the mean Ki-67 index was 27,1%, while in the “De” group, it was 28,5%. In both groups, there were four ulcerated cases and three non-ulcerated ones. Considering the application of the computer program in the hot spot, patients from group “AI” exhibited an average of 1688 Ki-67positive nuclei, in contrast to the “De” group, who presented with 3987 positive nuclei (Table 1). Additionally, a significant difference was noted in the total number of positive nuclei ($p = 0.0258$), with the “AI” group having a mean of 241.21 (SD: 184.47, range: 51–493.5) and the “De” group having a mean of 569.57 (SD: 258.45, range: 130–855) (Table 2). Lin's Concordance Correlation Coefficient of Absolute Agreement was reported at 0.9783, reflecting a substantial level of agreement among pathologists. [21] The Bland-Altman analysis revealed a bias of 6.36 with a 95% confidence interval from -26.61 to 39.32 (SD 57.10) as shown in figure 3.

A comparative analysis of means between the two groups revealed statistically significant differences in nuclear staining patterns NP1 ($p = 0.0014$), NP4 ($p = 0.038$), and NP5 ($p = 0.0193$). Specifically, the “AI” group had a mean of 50 nuclei classified as NP1 (SD: 30.68, range: 18–98). The “De” had a mean NP1 of 164 (SD: 71.56 range: 31–241). For the NP4 pattern, the “AI” group's mean was 16 nuclei (SD: 30.74 range: 2–85.5), whereas the “De” group had an NP4 mean of 56.14 (SD: 51.72 range: 25-170) As for NP5, the mean for the “AI” group was 3.86 (SD: 5.01 range: 1-15), compared to the “De” group, which had a mean NP5 of 14.86 (SD: 15.08 range: 1-45). No significant differences were found for NP2 and NP3 patterns ($p = 0.4322$ and $p = 0.051$, respectively).

A comparison of means was also performed to compare the staining pattern NP5 and conventional mitosis counting in the “AI” group and “De”. In the “AI” group, the NP5 pattern had a mean of 3.86 nuclei (SD: 5, median 3, range: 1-15), while the mitosis counting had a mean of 7,14 nuclei (SD: 7.1, median 5, range: 2-22). There were no statistically significant differences between the two counting methods, with p-value of 0.2103. For the “De” group, the NP5 pattern had a mean of 14.86 nuclei (SD: 15, median 8, range: 1-45), while the mitosis counting had a mean of 15.86 nuclei (SD: 14, median 9, range: 3-45). There were also no statistically significant differences between the two counting methods, with p-value of 0.8863.

Discussion

Our analysis highlights the importance of Ki-67 staining patterns as a prognostic indicator among advanced ACM. Lin's Concordance Correlation Coefficient was high, in agreement with Dias et al., which showed a moderate diagnostic interobserver agreement in their study (k = 0.523). Significant differences in nuclear staining patterns were observed between both analyzed groups. Higher counts of Ki-67 positive nuclei were associated with worse outcomes (death), particularly in staining patterns NP1 (early G1 phase), NP4 (G2 phase), and NP5 (mitosis), which may indicate more aggressive tumor behavior. Dias et al. showed that malignant or pre-malignant epithelial lesions predominated NP4 nuclei, while benign lesions predominated NP1 and NP2 nuclei. (20) Our study, however, did not encounter a pattern that was predominant in “Al” (alive) patients with ACM. NP1, NP4, and NP5 patterns were more frequent in the “De” (melanoma-related death) group. Loddo et al. studied cell-cycle-phase progression in 182 patients with invasive breast cancer. They found that increased tumor grade is associated with an increased proportion of cells engaged in the cell division cycle (G1 – S and G2 – M regulators). [22]

The similar variability of Ki-67, considering the whole lesion for both groups, also reflected the heterogeneous nature of cellular proliferation within melanoma cases. Cases exhibited markedly different Ki-67 indices, indicating a spectrum ranging from high to low proliferative activity, even in the “De” group. Previous studies showed a high proliferation index is associated with a worse prognosis. [11-13] Indeed, until 2015, mitosis was a criterion for staging in the seventh edition of AJCC Melanoma Staging. [22] However, based on these data, we can hypothesize that its predominant patterns are more important than the general Ki-67. Moreover, the similar rate of general Ki-67 and the difference in the number of positive cells in the hot spot of both groups can indicate the last methodology as a more reliable way to predict ACM prognosis based on this marker.

The majority of neoplasms exhibit a dysregulated cell cycle, due to alterations in the regulators of CDK function. Additionally, many oncogenes contribute to this dysregulation of the cell cycle. [23] The identification of the cell cycle phase could play an important role in therapeutic strategies, given that a variety of anti-cancer agents, including those used for melanoma, not only affect the cell cycle but are also dependent on specific phases to be effective. Bortezomib, for example, induces G1 and G2 arrest and causes apoptosis of G2 phase cells. [24,25] CDK4/6 inhibitors block progression out of the G1 phase. Gemcitabine acts during the S phase. [26] Flavopiridol acts in the G1 and G2 phases, causing cell cycle arrest and preventing cells from entering the M-phase. Paclitaxel is more active in M-phase. [27]

Furthermore, certain drugs target highly proliferative cells more effectively than those in slow cycling. [24] Our study showed that most classified cells were situated before mitosis, and the

time required for cells to reach mitosis after initial Ki-67 expression can vary based on the cell cycle speed. This finding may explain the possible failure of more active drugs in the M-phase. It can also corroborate the idea of mitotic count as a suboptimal parameter to predict outcomes. Considering the importance of the cell cycle and the action of drugs on it, classifying the nuclear patterns of Ki-67 to determine the phase of tumor cells could provide a personalized patient report plan to guide oncologists' conduct and unlock new possibilities for treatment optimization. However, more studies are necessary for standardization and to establish a protocol for clinical application.

Previous studies established Ki-67 as a prognostic marker in cutaneous melanoma, but until now, no studies have investigated and compared the specific staining patterns. Our study has some limitations. It is retrospective, examines a concise number of cases (although a total number of 5,676 nuclei), and was restricted to the ACM subtype in the advanced stage. Another weakness of the study is the notable bias value of 6.35 regarding observers' analysis, although Lin's Concordance Correlation Coefficient indicated a high level of agreement. The high bias may be attributable to interpersonal subjectivity in pattern assessment. Furthermore, the absence of pre-selection of nuclei for analysis could contribute to the variability, although it made the analyses closer to the routine practice scenario (real world). Nevertheless, to our knowledge, it is the first study with this specific design concerning melanoma outcomes.

These findings highlighted the importance of Ki-67 and the applicability of nuclear staining patterns to predict outcomes among advanced ACM cases. Further studies with more significant cases are needed to elucidate the differences in Ki-67 staining patterns across various cancer types and their correlation with patient outcomes. The presented results open new perspectives to create computer algorithms to help pathologists analyze ACM cases considering their prognosis.

Statements

Acknowledgement (optional)

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Statement of Ethics

This study protocol was reviewed and approved by ethics committees for research at the Faculdade de Medicina—UNESP/Botucatu—SP (CAAE 52618721.0.1001.5411) and the Amaral Carvalho Hospital (CAAE 52618721.0.3001.5434). The need for informed consent was waived by the

research ethics committee from Faculdade de Medicina—UNESP/Botucatu—SP and Amaral Carvalho Hospital.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

MAA: Investigation, Data curation, Formal analysis, Writing-original draft. ICJ: Investigation, Data curation, Formal analysis, Writing – Review & Editing. NSCO: Conceptualization, Review & Editing. FAMN: Formal analyses and Review & Editing. MEAM: Review & Editing. JPOX: Conceptualization, Review & Editing. JCCXJ: Conceptualization, Methodology, Investigation, Writing – Review & Editing, Supervision.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1. Saginala K, Barsouk A, Aluru JS, Rawla P, Barsouk A. Epidemiology of Melanoma. Med Sci. 2021;9(4):63.
2. Mirea MA, Eckensperger S, Hengstschläger M, Mikula M. Insights into Differentiation of Melanocytes from Human Stem Cells and Their Relevance for Melanoma Treatment. Cancers (Basel). 2020;12(9):2508.
3. Keung E, Gershenwald J. The eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system: implications for melanoma treatment and care. Expert Rev Anticancer Ther. 2018;18(8):775-784.
4. Swetter SM, Tsao H, Bichakjian CK, Curiel-Lewandrowski C, Elder DE, Gershenwald JE, et al.: Guidelines of care for the management of primary cutaneous melanoma. J Am Acad Dermatol. 2018; 80(1):208-250.
5. Bello DM, Chou JF, Panageas KS, Brady MS, Coit DG, Carvajal RD, et al. Prognosis of Acral Melanoma: A Series of 281 Patients. Ann Surg Oncol. 2013;20(11):3618–25.
6. Kuchelmeister C, Schaumburg-Lever G, Garbe C. Acral cutaneous melanoma in caucasians: clinical features, histopathology and prognosis in 112 patients. Br J Dermatol. 2000;143(2):275–80.
7. Egger ME, McMasters KM, Callender GG, Quillo AR, Martin RCG, Stromberg AJ, et al. Unique prognostic factors in acral lentiginous melanoma. Am J Surg. 2012;204(6):874–80.
8. Bradford PT, Goldstein AM, McMaster ML, Tucker MA. Acral Lentiginous Melanoma. Arch Derm. 2009;145(4).
9. Ferringer T. Immunohistochemistry in dermatopathology. Arch Pathol Lab Med. 2015;139(1):83-105.
10. AMIN MB. AJCC Cancer Staging Manual. 8 ed. Chicago: Springer, 2017.
11. Nielsen PS, Riber-Hansen R, Jensen TO, Schmidt H, Steiniche T. Proliferation indices of phosphohistone H3 and Ki67: strong prognostic markers in a consecutive cohort with stage I/II melanoma. Mod Pathol. 2013;26(3):404-13.
12. Gimotty PA, Van Belle P, Elder DE, Murry T, Montone KT, Xu X, et al. Biologic and prognostic significance of dermal Ki67 expression, mitoses, and tumorigenicity in thin invasive cutaneous melanoma. J Clin Oncol. 2005;23(31):8048-56.
13. Hazan C, Melzer K, Panageas KS, Li E, Kamino H, Kopf A, et al. Evaluation of the proliferation marker MIB-1 in the prognosis of cutaneous malignant melanoma. Cancer. 2002;95(3):634-40.

14. Parra O, Ma W, Li Z, Coffing BN, Linos K, LeBlanc RE, et al. PRAME expression in cutaneous melanoma does not correlate with disease-specific survival. Journal of Cutaneous Pathology. 2023;50(10):903-912
15. Udovicic-Gagula D, Ahmovic A, Bilalovic N, Doric M. Expression of Ki-67 and Estrogen Receptor Beta in Primary Cutaneous Melanoma as a Potential Indicator of Regional Lymph Node Positivity. Appl Immunohistochem Mol Morphol 2019; 27(1):27-32.
16. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol. 1984;133(4):1710-5.
17. Sun X, Kaufman PD. Ki-67: more than a proliferation marker. Chromosoma. 2018;127(2):175-186.
18. Verheijen R, Kuijpers HJ, van Driel R, Beck JL, van Dierendonck JH, Brakenhoff GJ, et al. Ki-67 detects a nuclear matrix-associated proliferation-related antigen. II. Localization in mitotic cells and association with chromosomes. J Cell Sci. 1989;92(Pt 4):531-40.
19. Matheson TD, Kaufman PD. The p150N domain of chromatin assembly factor-1 regulates Ki-67 accumulation on the mitotic perichromosomal layer. Mol Biol Cell. 2017;28(1):21-29.
20. Dias EP, Oliveira NSC, Serra-Campos AO, da Silva AKF, da Silva LE, Cunha KS. A novel evaluation method for Ki-67 immunostaining in paraffin-embedded tissues. Virchows Arch. 2021;479(1):121-131.
21. McBride G.B. A Proposal for Strength-of-Agreement Criteria for Lin's Concordance Correlation Coefficient. NIWA (National Institute of Water & Atmospheric Research Ltd, New Zealand). Prepared for Ministry of Health. May 2005.
22. Macro- Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC Cancer Staging Manual (7th edition). New York, NY: Springer; 2010.
23. Carnero, A. Targeting the cell cycle for cancer therapy. Br J Cancer, 2002; 87(2), 129–133.
24. Haass NK, Gabrielli B. Cell cycle-tailored targeting of metastatic melanoma: Challenges and opportunities. Exp Dermatol. 2017;26(7):649-655.
25. Beaumont KA, Hill DS, Daignault SM, Lui GYL, Sharp DM, Gabrielli B, et al. Cell Cycle Phase-Specific Drug Resistance as an Escape Mechanism of Melanoma Cells. J Invest Dermatol. 2016;136(7):1479-1489.
26. Gross SM, Mohammadi F, Sanchez-Aguila C, Zhan PJ, Liby TA, Dane MA, et al. Analysis and modeling of cancer drug responses using cell cycle phase-specific rate effects. Nat Commun. 2023;14(1):3450.
27. Schwartz GK, Shah MA. Targeting the cell cycle: a new approach to cancer therapy. J Clin Oncol. 2005;23(36):9408-21.

Figure Legends

Fig. 1. Examples of each Ki-67 staining pattern, based on Dias' classification.

Fig. 2. Examples of the one-square-millimeter demarcated area and the classification of Ki-67 staining patterns through colored circles.

Fig. 3. Bland-Altman agreement plot of the analysis of the nuclear patterns between two groups (alive patients and patients with acral melanoma-related death, n = 14) performed by two pathologists. On the abscissa (X) axis are the means of positive nuclei of each case performed by the two evaluators, and on the ordinate (Y) axis is the difference between positive nuclei counts.

Table 1. Ki-67 index and quantification of Ki-67 staining pattern across the 14 cases

Alive group	Ki-67 index	Ulceration	NP1	NP2	NP3	NP4	NP5	Total
Case 1	20%	Absent	56 (29.3%)	46 (24.0%)	81 (42.4%)	5 (2.6%)	3 (1.6%)	191
Case 2	5%	Present	18 (35.2%)	5 (9.8%)	11 (21.5%)	2 (3.9%)	15 (29.4%)	51
Case 3	5%	Absent	20 (37.7%)	9 (16.9%)	19 (35.8%)	2 (3.7%)	3 (5.6%)	53
Case 4	60%	Present	65 (23.9%)	81 (29.8%)	112 (41.3%)	10 (3.6%)	3 (1.1%)	271
Case 5	50%	Absent	24 (5.0%)	331 (68.9%)	121 (25.2%)	3 (0.6%)	1 (0.2%)	480
Case 6	10%	Present	72 (48.3%)	23 (15.4%)	48 (32.2%)	5 (3.3%)	1 (0.6%)	149
Case 7	40%	Present	98 (19.8%)	38 (7.6%)	271 (54.8%)	86 (17.4%)	1 (0.2%)	494
Total (%)	27.1%		353 (20,8%)	533 (31,5%)	663 (39,2%)	113 (6,6%)	27 (1,59%)	1689
De* group	Ki-67 index	Ulceration	NP1 (%)	NP2 (%)	NP3 (%)	NP4 (%)	NP5 (%)	Total
Case 1	10%	Present	191 (38.5%)	55 (11.0%)	208 (41.9%)	38 (7.6%)	4 (0.8%)	496
Case 2	5%	Present	136 (35.5%)	76 (19.8%)	101 (26.3%)	25 (6.5%)	45 (11.7%)	383
Case 3	40%	Present	226 (27.0%)	141 (16.8%)	392 (46.8%)	61 (7.2%)	16 (1.9%)	836

Case 4	30%	Present	132 (22.0%)	175 (29.2%)	258 (43.0%)	26 (4.3%)	8 (1.3%)	599
Case 5	5%	Absent	31 (23.8%)	18 (13.8%)	50 (38.4%)	30 (23.0%)	1 (0.7%)	130
Case 6	60%	Absent	241 (28.1%)	120 (14.0%)	316 (36.9%)	170 (19.8%)	8 (0.9%)	855
Case 7	50%	Absent	191 (27.7%)	216 (31.3%)	216 (31.3%)	43 (6.2%)	22 (3.1%)	688
Total (%)	28.6%		1148 (28,7%)	801 (20%)	1541 (38,65 %)	393 (9,8%)	104 (2,6%)	3987

*De- melanoma-related death

Table 2. Means comparison of Ki-67 staining patterns between the two groups

Alive group					Deceased group				
	Total (%)	Mean	SD	Median (min-max)	Total (%)	Mean	SD	Median (min-max)	p-value
NP1	353 (20.9%)	50.43	30.68	56 (18-98)	1148 (28.8%)	164	71.56	191 (31-241)	**0.0014**
NP2	533 (31.6%)	76.14	115.28	38 (5-331)	801 (20.1%)	114.43	69.56	120 (18-216)	0.4322
NP3	663 (39.3%)	94.71	88.67	81 (11-271)	1541 (38.7%)	220.14	117.94	216 (50-392)	0.051
NP4	112.5 (6.6%)	16.07	30.74	5 (2-85.5)	393 (9.8%)	56.14	51.72	38 (25-170)	**0.038**
NP5	27 (1.6%)	3.86	5.01	3 (1-15)	104 (2.6%)	14.86	15.08	8 (1-45)	**0.0193**
Total	1688.5 (100.0%)	241.21	184.47	191 (51-493.5)	3987 (100.0%)	569.57	258.45	599 (130-855)	**0.0258**

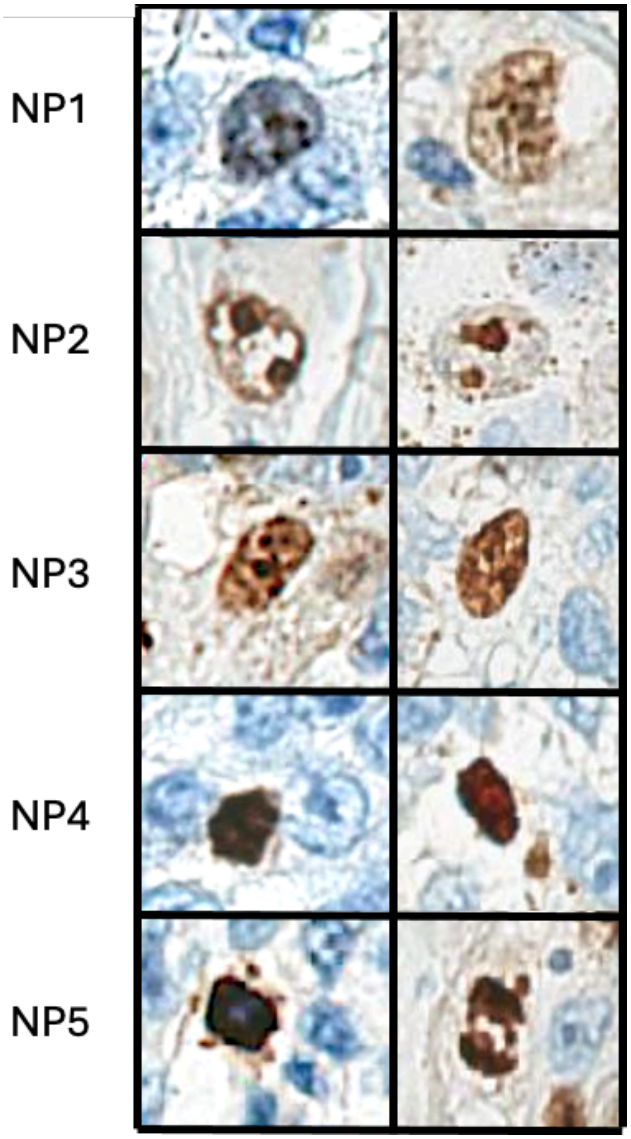


Figure 1. Examples of each Ki-67 staining pattern, based on Dias' classification.

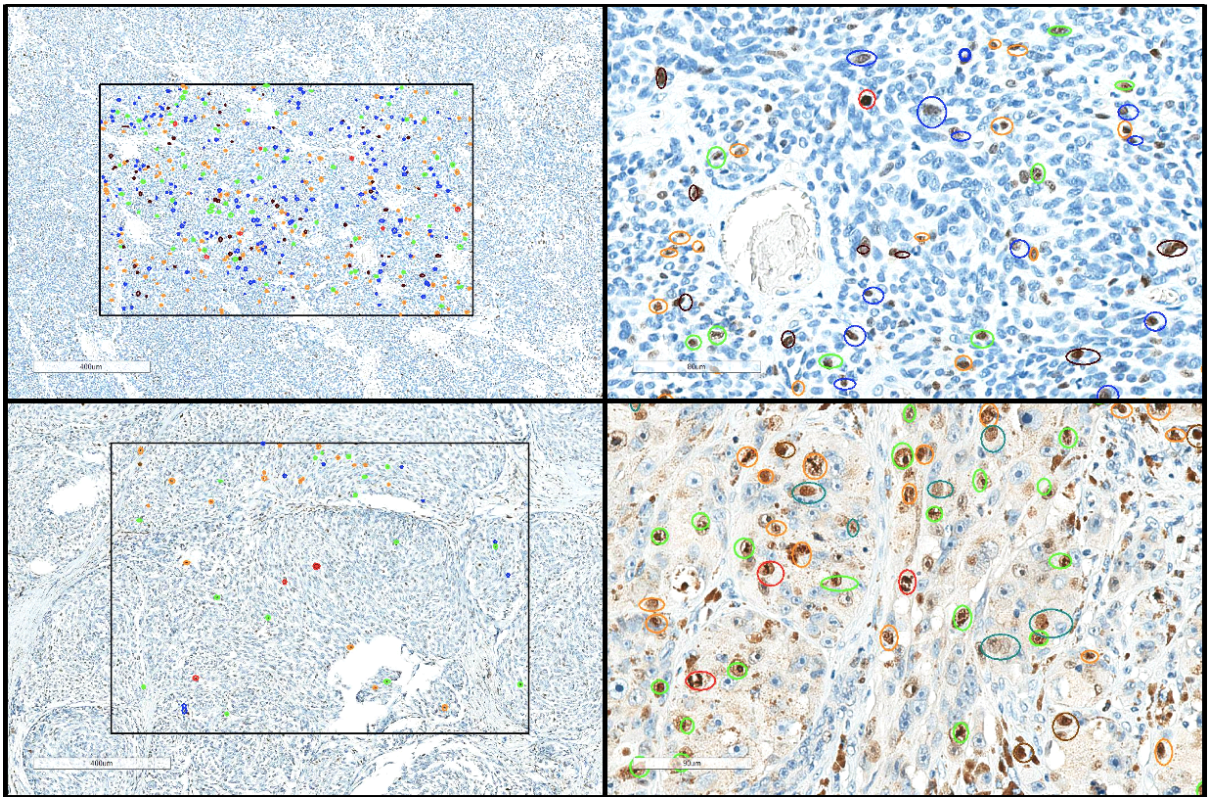


Figure 2. Examples of the one-square-millimeter demarcated area and the classification of Ki-67 staining patterns through colored circles.

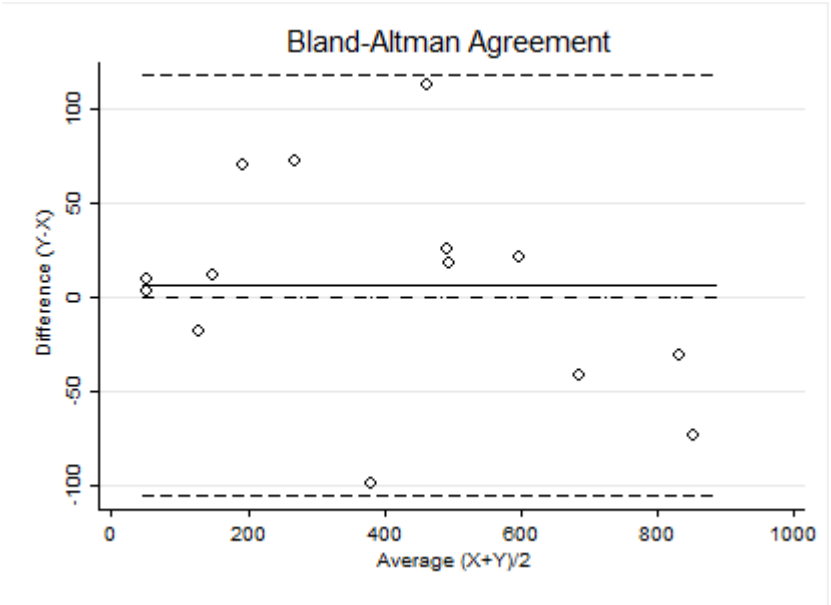


Figure 3. Bland-Altman agreement plot of the analysis of the nuclear patterns between two groups (alive patients and patients with acral melanoma-related death, n = 14) performed by two pathologists. On the abscissa (X) axis are the means of positive nuclei of each case performed by the two evaluators, and on the ordinate (Y) axis is the difference between positive nuclei counts.

3 CONCLUSÃO

- A dimensão macroscópica do melanoma cutâneo não apresentou correlação estatisticamente significativa com a sobrevida-específica.
- Medida de Breslow, status do linfonodo sentinela e presença de regressão tiveram associação significativa com a sobrevida melanoma-específica.
- Em relação ao linfonodo sentinela, houve correlação com o tamanho macroscópico apenas na análise univariada. Na análise multivariada, entretanto, apenas a medida de Breslow foi capaz de predizer o resultado do linfonodo sentinela.
- A medida de Breslow modificada, mensurada a partir da membrana basal, ao invés da camada granulosa, foi capaz de predizer a sobrevida e resultado do linfonodo sentinela assim como a medida de Breslow convencional.
- O DOI (Depth Of Invasion - profundidade de invasão), medido a partir da membrana basal da epiderme normal adjacente, utilizado no carcinoma de células escamosas oral, foi capaz de predizer a sobrevida específica do melanoma acral, em análise univariada. Análise multivariada, entretanto, demonstrou que apenas a medida de Breslow foi fator independente.
- O DOI, ao contrário da medida de Breslow, não obteve significância estatística para predizer o resultado do linfonodo sentinela no melanoma acral.
- Forte intensidade de marcação do PRAME, bem como elevada extensão do PRAME e também elevado índice do Ki-67 estão associados a redução da sobrevida geral. Em análise multivariada, entretanto, apenas a medida de Breslow teve influência significativa.
- Elevado índice do Ki-67 também está associado a metástase em linfonodo sentinela. Além disso, foi demonstrada uma correlação significativa entre a expressão do Ki-67 e PRAME.
- Os subtipos histológicos acral e nodular estão associados a um índice do Ki-67 mais elevado, bem como maiores valores do escore combinado do PRAME e da multiplicação “Ki-67 x escore combinado do PRAME”.
- Elevado índice do Ki-67, particularmente exibindo os padrões de marcação nuclear NP1 (G1 inicial), NP4 (G2) e NP5 (mitose) estão associadas a pior desfecho (óbito).

4 PERSPECTIVAS

- Inclusão de novas formas de mensuração do tumor para avaliação prognóstica do melanoma cutâneo

Espera-se que novos métodos de mensuração do tumor, além da medida de Breslow convencional, como a medida de Breslow modificada, o DOI (Depth Of Invasion), bem como novos métodos a serem desenvolvidos, possam ser utilizados nos protocolos de estadiamento, com a intenção de aprimorar ou complementar a avaliação prognóstica do melanoma cutâneo.

- Incorporação de marcadores imunoistoquímicos para avaliação prognóstica

Abre-se um caminho para mais investigações sobre a expressão do Ki-67 e PRAME em relação ao prognóstico no melanoma cutâneo. Considerando a possibilidade de que a expressão elevada do Ki-67 e PRAME esteja associada a desfechos clínicos desfavoráveis, esses marcadores podem ser incorporados nos protocolos de estadiamento. Isso pode melhorar a capacidade de predição da agressividade do melanoma. Além disso, a partir de um melhor entendimento do comportamento das células tumorais em relação ao ciclo celular, torna-se possível o desenvolvimento e a escolha de terapias mais personalizadas, baseadas nos perfis de expressão do Ki-67 e PRAME.

- Digitalização de lâminas e análise digital

Os padrões de marcação nuclear do Ki-67 podem ser utilizados futuramente para avaliação prognóstica e decisão terapêutica. A análise desses padrões poderá ser realizada tanto por patologistas treinados, dada a elevada concordância interobservador, quanto por ferramentas de inteligência artificial, reduzindo o tempo de avaliação e a variabilidade interobservador.

REFERÊNCIAS

1- MIREA, M. *et al.* Insights into Differentiation of Melanocytes from Human Stem Cells and Their Relevance for Melanoma Treatment. **Cancers**, [S.L.], v. 12, n. 9, p. 2508, 2020.

2- NIKOLAOU, V.; STRATIGOS, A.J.. Emerging trends in the epidemiology of melanoma. **British Journal of Dermatology**, [S.L.], v. 170, n. 1, p. 11-19, 2014.

3- INSTITUTO NACIONAL DO CÂNCER. **Estimativa 2023: incidência de câncer no Brasil:** Disponível em: <https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/estimativa-2023.pdf> Acesso em: abr. 2024.

4- MENDES, G. L. Q. **Melanoma no Brasil: Tendência Temporal de Mortalidade com Modelagem idade-período-coorte, e Análise de sobrevida em coorte hospitalar.** 2014. 134f. Tese (Doutorado) – Escola Nacional de Saúde Pública Sergio Arouca. Rio De Janeiro. 2014.

5- IARC – International Agency for Research on Cancer. **Exposure to artificial UV radiation and skin cancer**. Lyon (France); 2005. Disponível em: <https://publications.iarc.fr/_publications/media/download/4033/c6dbb6ef039134a92cce28b4bfc7ce5d21ad9a8f.pdf> Acesso em: abr. 2024.

6- MULLIKEN, J. S.; RUSSAK, J. E.; RIGEL, D. S.. The Effect of Sunscreen on Melanoma Risk. **Dermatologic Clinics**, [S.L.], v. 30, n. 3, p. 369-376, 2012.

7- LABERGE, Greggory S. et al. Recent Advances in Studies of Skin Color and Skin Cancer. **Yale Journal of Biology and Medicine**, [S.L.], v. 93, p. 69-80, 2020.

8- HOLLY, E. A. *et al.* Number of melanocytic nevi as a major risk factor for malignant melanoma. **Journal of The American Academy Of Dermatology**, [S.L.], v. 17, n. 3, p. 459-468, 1987.

9- CARR, S.; SMITH, C.; WERNBERG, J.. Epidemiology and Risk Factors of Melanoma. **Surgical Clinics of North America**, [S.L.], v. 100, n. 1, p. 1-12, 2020.

10- BREWER, J. D.. Melanoma in immunosuppressed patients. **Expert Review of Dermatology**, [S.L.], v. 5, n. 3, p. 247-250, 2010.

11- FACCIOLÀ, A. *et al.* Malignant melanoma in HIV: epidemiology, pathogenesis, and management. **Dermatologic Therapy**, [S.L.], v. 33, n. 1, 2019.

12- CARVALHO, C. A. *et al.* Melanoma hereditário: prevalência de fatores de risco em um grupo de pacientes no sul do brasil. **Anais Brasileiros de Dermatologia**, [S.L.], v. 79, n. 1, p. 53-60, 2004.

13- ROSSI, M. *et al*. Familial melanoma: diagnostic and management implications. **Dermatology Practical & Conceptual**, [S.L.], p. 10-16, 2019.

14- RIVITTI, Evandro A. **Manual de dermatologia clínica de Sampaio e Rivitti**. São Paulo: Artes Médicas, 2014.

15- PERERA, E. *et al.* Malignant Melanoma. **Healthcare**, [S.L.], v. 2, n. 1, p. 1-19, 2013.

16- BILLIS, A.; ALTEMANI, A. M.; ANDRADE, L. A.. **Patologia Diagnóstica de Tumores**. 3. ed. Campinas: Idb, 2017.

17- MASSI, Guido; LEBOIT, Philip E. **Histological Diagnosis of Nevi and Melanoma**. 2. ed.: Springer, 2014

18- SMOLLER, B. R. Histologic criteria for diagnosing primary cutaneous malignant melanoma. **Modern Pathology**, [S.L.], v. 19, n. 2, p. 34-40, 2006.

19- RONCATI, L.; VERGARI, B.; GAUDIO, A. The 'All-or-None Law' Applied to the Vertical Growth Phase of Cutaneous Malignant Melanoma. **Chonnam Medical Journal**, [S.L.], v. 53, n. 3, p. 234, 2017.

20- CLARK, Jr.; WALLACE, H. *et al.* The Histogenesis and Biologic Behavior of Primary Human Malignant Melanomas of the Skin. **Cancer Research**, [S.L.], v. 29, n. 3, p. 705-727, 1969.

21- BRESLOW, A. Thickness, Cross-Sectional Areas and Depth of Invasion in the Prognosis of Cutaneous Melanoma. **Annals of Surgery**, [S.L.], v. 172, n. 5, p. 902-908, 1970.

22- AMIN, Mahul B. **AJCC Cancer Staging Manual**. 8 ed. Chicago: Springer, 2017.

23- SWETTER, S. M. *et al*. Guidelines of care for the management of primary cutaneous melanoma. **Journal of the American Academy of Dermatology**. V. 80, n. 1, p. 208–50, 2019.

24- TEJERA-VAQUERIZO, A. *et al.* Protocolo de diagnóstico histológico para muestras de pacientes con melanoma cutáneo. Documento de consenso de la SEAP y la AEDV para el Registro Nacional de Melanoma. **Actas Dermo-Sifiliográficas**, [S.L.], v. 112, n. 1, p. 32-43, jan. 2021.

25- GERSHENWALD, J. E. *et al*. Melanoma staging: evidence-based changes in the american joint committee on cancer eighth edition cancer staging manual. CA**: A Cancer Journal for Clinicians**, [S.L.], v. 67, n. 6, p. 472-492, 2017.

26- SCOLYER, R. A. *et al.* Data Set for Pathology Reporting of Cutaneous Invasive Melanoma. **American Journal of Surgical Pathology**, [S.L.], v. 37, n. 12, p. 1797-1814, 2013.

27- SCOLYER, R. A. *et al*. Melanoma pathology reporting and staging. **Modern Pathology**, [S.L.], v. 33, n. 1, p. 15-24, 2019.

28- TAN, K. B. *et al*. Subungual melanoma: a study of 124 cases highlighting features of early lesions, potential pitfalls in diagnosis, and guidelines for histologic reporting. **The American Journal of Surgical Pathology**. [S.L.], v. 31, n. 12, p. 1902-12, 2007.

29- CHOPRA, K. *et al*. A Comprehensive Examination of Topographic Thickness of Skin in the Human Face. **Aesthetic Surgery Journal**. v.35, n.8, p. 1007-1013, 2015.

30- OLTULU, P. *et al*. Measurement of epidermis, dermis, and total skin thicknesses from six different body regions with a new ethical histometric technique. **Turkish Journal of Plastic Surgery**. v. 26, n. 2, p. 56-61, 2018.

31- WEI, X. *et al*. Prognostic impact of Breslow thickness in acral melanoma: A retrospective analysis. **Journal of the American Academy of Dermatology**. v. 87, n. 6, p. 1287–94, 2022.

32- ROBERTS, D.L.L. *et al*. U.K. guidelines for the management of cutaneous melanoma. **British Journal of Dermatology**, [S.L.], v. 146, n. 1, p. 7-17, 2002.

33- SWETTER, S. M. *et al.* Guidelines of care for the management of primary cutaneous melanoma. **Journal of The American Academy of Dermatology**, [S.L.], v. 80, n. 1, p. 208-250, 2019.

34- GUITART, J. *et al.* Histological Characteristics of Metastasizing Thin Melanomas. **Archives of Dermatology**, [S.L.], v. 138, n. 5, p. 603-608, 2002.

35 ANDERSSON, A. P.; DAHLSTRØM, K. K.; DRZEWIECKI, K. T. Prognosis of thin cutaneous head and neck melanoma. **European Journal of Surgical Oncology (EJSO)**, [S.L.], v. 22, n. 1, p. 55-57, 1996.

36- VILMER, C. *et al.* Thin melanomas with unusual aggressive behavior: a report of nine cases. **Journal of The American Academy Of Dermatology**, [S.L.], v. 34, n. 3, p. 439-444, 1996.

37- PLOEG, A. P. T. *et al.* Prognosis in patients with sentinel node-positive melanoma without immediate completion lymph node dissection. **British Journal of Surgery**, [S.L.], v. 99, n. 10, p. 1396-1405, 2012.

38- SARPA, H. G. *et al.* Prognostic Significance of Extent of Ulceration in Primary Cutaneous Melanoma. **American Journal of Surgical Pathology**, [S.L.], v. 30, n. 11, p. 1396-1400, 2006.

39- EGGER, M. E. *et al.* Lymphovascular Invasion as a Prognostic Factor in Melanoma. **The American Surgeon**, [S.L.], v. 77, n. 8, p. 992-997, 2011.

40- AZIMI, F. *et al.* Tumor-Infiltrating Lymphocyte Grade Is an Independent Predictor of Sentinel Lymph Node Status and Survival in Patients With Cutaneous Melanoma. **Journal of Clinical Oncology**, [S.L.], v. 30, n. 21, p. 2678-2683, 2012.

41- BACHMANN, I. M. *et al*. Tumor necrosis is associated with increased alphavbeta3integrin expression and poor prognosis in nodular cutaneous melanomas. **BMC Cancer**, [S.L.], v. 8, n. 1, p. 1-10, 2008.

42- OCANHA-XAVIER, J. P. XAVIER-JUNIOR, J. C. C.; MARQUES, M. E. A. Melanoma: clinical, evolutive and histopathological characteristics of a series of 136 cases. **Anais Brasileiros de Dermatologia**, [S.L.], v. 93, n. 3, p. 373-376, 2018.

43- CLARK, W. H. *et al*. A study of tumor progression: The precursor lesions of superficial spreading and nodular melanoma. **Human Pathology**. v. 15, n. 12, p. 1147-1165, 1984.

44- McDERMOTT, N. C. *et al*. Identification of vertical growth phase in malignant melanoma. A study of interobserver agreement. **American Journal of Clinical Pathology**. v. 110, n. 6, p. 753-7, 1998

45- ELDER, D. E. Melanoma progression. **Pathology**. v. 48, n. 2, p. 147-54, 2016

46- LAGA, A.C.; MURPHY, G.F; Cellular heterogeneity in vertical growth phase melanoma. **Archives of Pathology & Laboratory Medicine.** v. 134, n. 12, p. 1750-7, 2010.

47- SHIELDS, C. L. *et al*. American Joint Committee on Cancer Classification of Uveal Melanoma (Anatomic Stage) Predicts Prognosis in 7,731 Patients: The 2013 Zimmerman Lecture. **Ophthalmology.** v. 122, n. 6, p. 1180-6, 2015

48- CHENG, J.; Yan S. Prognostic variables in high-risk cutaneous squamous cell carcinoma: a review. **Journal of Cutaneous Pathology**. v. 43, n. 11, p. 994-1004, 2016.

49- MA, Q. *et al*. Prognostic significance of tumor size for primary invasive cutaneous melanoma: A population-based study, 2004-2016. **Cancer Medicine**. v. 9, n. 13, p. 4561-4571, 2020.

50- BAMFORD, M. *et al*. Comparison of the prognostic value of microscopically measured invasive width versus macroscopic width in cutaneous melanoma shows the superiority of microscopic invasive width measurement. **Journal of Cutaneous Pathology.** v. 49, n. 6, p. 536–42, 2022

51- LEE, Y. J. *et al*. Evaluation of Depth of Invasion and Tumor Thickness as a Prognostic Factor for Early-Stage Oral Squamous Cell Carcinoma: A Retrospective Study. **Diagnostics.** v. 12, n. 1, 2021.

52- SALAMA, A. M. *et al*. Depth of invasion versus tumour thickness in early oral tongue squamous cell carcinoma: which measurement is the most practical and predictive of outcome? **Histopathology.** v. 79, n. 3, p. 325–37, 2020

53- GHAZI, N. *et al*. Importance of depth of invasion in patients with oral squamous cell carcinoma: A review article. **Journal of Orofacial Sciences.** v. 10, n. 1, p. 3-6, 2018.

54- FERRINGER, T. Immunohistochemistry in Dermatopathology. **Archives Of Pathology & Laboratory Medicine**, [S.L.], v. 139, n. 1, p. 83-105, 2015.

55- SUN, X.; KAUFMAN, P. D. Ki-67: more than a proliferation marker. **Chromosoma**, [S.L.], v. 127, n. 2, p. 175-186, 2018.

56- CHEN, G*. et al.* The Prognostic Role of Ki-67/MIB-1 in Cervical Cancer: a systematic review with meta-analysis. **Medical Science Monitor**, [S.L.], v. 21, p. 882-889, 2015.

57- NIELSEN, P. S. *et al.* Proliferation indices of phosphohistone H3 and Ki67: strong prognostic markers in a consecutive cohort with stage i/ii melanoma. **Modern Pathology**, [S.L.], v. 26, n. 3, p. 404-413, 2012.

58- GIMOTTY, P. A. *et al.* Biologic and Prognostic Significance of Dermal Ki67 Expression, Mitoses, and Tumorigenicity in Thin Invasive Cutaneous Melanoma. **Journal Of Clinical Oncology**, [S.L.], v. 23, n. 31, p. 8048-8056, 2005.

59- UDOVIČIĆ-GAGULA, D. *et al*. Expression of Ki-67 and Estrogen Receptor Beta in Primary Cutaneous Melanoma as a Potential Indicator of Regional Lymph Node Positivity. **Applied Immunohistochemestry & Molecular Morphology**. v. 27, n. 1, p. 27-32, 2019.

60- HAZAN, C. *et al.* Evaluation of the proliferation marker MIB-1 in the prognosis of cutaneous malignant melanoma. **Cancer**, [S.L.], v. 95, n. 3, p. 634-640, 2002.

61- PARRA, O. *et al*. PRAME expression in cutaneous melanoma does not correlate with disease-specific survival. **Journal of Cutaneous Pathology** 2023;903–912.

62- VERHEIJEN, R. *et al*. Ki-67 detects a nuclear matrix-associated proliferation-related antigen. II. Localization in mitotic cells and association with chromosomes. **Journal of Cell Science**. Pt. 4, p: 531-40, 1989.

63- MATHESON, T. D.; KAUFMAN, P. D. The p150N domain of chromatin assembly factor-1 regulates Ki-67 accumulation on the mitotic perichromosomal layer. **Molecular Biology of the Cell.** v. 28, n. 1, p. 21-29, 2017.

64- DIAS, E. P. *et al*. A novel evaluation method for Ki-67 immunostaining in paraffin-embedded tissues. **Virchows Archiv**. v. 479, n. 1, p. 121-131, 2021.

65- IKEDA, H*. et al.* Characterization of an Antigen That Is Recognized on a Melanoma Showing Partial HLA Loss by CTL Expressing an NK Inhibitory Receptor. **Immunity**, [S.L.], v. 6, n. 2, p. 199-208, 1997.

66- GRADECKI, S. E.; SLINGLUFF, C. L.; GRU, A. A. PRAME expression in 155 cases of metastatic melanoma. **Journal Of Cutaneous Pathology**, [S.L.], v. 48, n. 4, p. 479-485, 2020.

67- EPPING, M. T. *et al.* The Human Tumor Antigen PRAME Is a Dominant Repressor of Retinoic Acid Receptor Signaling. **Cell**, [S.L.], v. 122, n. 6, p. 835-847, 2005.

68- AL-KHADAIRI, G.; DECOCK, J. Cancer Testis Antigens and Immunotherapy: where do we stand in the targeting of prame?. **Cancers**, [S.L.], v. 11, n. 7, p. 984, 2019.

69- FIELD, M. G. *et al.* PRAME as an Independent Biomarker for Metastasis in Uveal Melanoma. **Clinical Cancer Research**, [S.L.], v. 22, n. 5, p. 1234-1242, 2016.

70- DOOLAN, P. *et al.* Prevalence and prognostic and predictive relevance of PRAME in breast cancer. **Breast Cancer Research And Treatment**, [S.L.], v. 109, n. 2, p. 359-365, 2007.

71- NEUMANN, E. *et al.* Heterogeneous Expression of the Tumor-associated Antigens RAGE-1, PRAME, and Glycoprotein 75 in Human Renal Cell Carcinoma: Candidates for T-Cell-based Immunotherapies? **Cancer Research**, v. 58, n.18, p. 4090-4095, 1998.

72- ZHANG, W. *et al.* PRAME expression and promoter hypomethylation in epithelial ovarian cancer. **Oncotarget**, [S.L.], v. 7, n. 29, p. 45352-45369, 2016.

73 IURA, K. *et al.* Cancer-testis antigen expression in synovial sarcoma: ny-eso-1, prame, magea4, and magea1. **Human Pathology**, [S.L.], v. 61, p. 130-139, 2017.

74- LEZCANO, C. *et al.* PRAME Expression in Melanocytic Tumors. **American Journal Of Surgical Pathology**, [S.L.], v. 42, n. 11, p. 1456-1465, 2018.

75- LEZCANO, C. *et al.* Immunohistochemistry for PRAME in the Distinction of Nodal Nevi From Metastatic Melanoma. **American Journal Of Surgical Pathology**, [S.L.], v. 44, n. 4, p. 503-508, 2019.

76- TAN, P. *et al.* Expression and prognostic relevance of PRAME in primary osteosarcoma. **Biochemical And Biophysical Research Communications**, [S.L.], v. 419, n. 4, p. 801-808, 2012.

77- SZCZEPANSKI, M. J. *et al.* PRAME expression in head and neck cancer correlates with markers of poor prognosis and might help in selecting candidates for retinoid chemoprevention in pre-malignant lesions. **Oral Oncology**, [S.L.], v. 49, n. 2, p. 144-151, 2013.

78- GASSENMAIER M. *et al*. Diffuse PRAME Expression Is Highly Specific for Thin Melanomas in the Distinction from Severely Dysplastic Nevi but Does Not Distinguish Metastasizing from Non-Metastasizing Thin Melanomas. **Cancers.** v. 13, n. 15, 2021.

79- BELLO, G. *et al*. PRAME expression in 137 primary cutaneous melanomas and comparison with 38 related metastases. **Pathology - Research and Practice** , v. 251, 2023

80- HU, J. *et al*. Preferentially expressed antigen in melanoma immunohistochemistry as an adjunct for differential diagnosis in acral lentiginous melanoma and acral nevi. **Human Pathology,** v.120, p. 9–17, 2022.

81- RAWSON, R. V. *et al*. Diagnostic utility of PRAME, p53 and 5-hmC immunostaining for distinguishing melanomas from naevi, neurofibromas, scars and other histological mimics. **Pathology,** v. 54, n. 7, p. 863–873, 2022.

82- HORNICK, J. L. *et al*. PRAME Expression Is a Useful Tool in the Diagnosis of Primary and Metastatic Dedifferentiated and Undifferentiated Melanoma. **American Journal of Surgical Pathology,** v. 47, n. 12, p. 1390–1397, 2023.

ANEXOS

Prêmio SBP Categoria Oral – 6º Lugar, pelo trabalho apresentado durante o 33º Congresso Brasileiro de Patologia e 26º Congresso Brasileiro de Citopatologia, realizado de 03 a 06 de agosto de 2022, em Foz do Iguaçu – PR.



CERTIFICADO

33º CONGRESSO BRASILEIRO DE PATOLOGIA

FOZ DO IGUAÇU | 03 A 06 DE AGOSTO DE 2022

26º CONGRESSO BRASILEIRO DE CITOPATOLOGIA

Conferimos ao trabalho

VALOR DA DIMENSÃO MACROSCÓPICA DO MELANOMA CUTÂNEO NA PREDIÇÃO DO RESULTADO DO LINFONODO SENTINELA

Dos autores

MARCEL ARAKAKI ASATO; FRANCISCO ALVES MORAES NETO; MARCELO PADOVANI DE TOLEDO MORAES; JULIANA POLIZEL OCANHA-XAVIER; MARIANGELA ESTHER ALENCAR MARQUES; JOSÉ C NDIDO CALDEIRA XAVIER JÚNIOR

O Prêmio SBP Categoria Oral - 6º Lugar, pelo trabalho apresentado durante o **33º Congresso Brasileiro de Patologia e 26º Congresso Brasileiro de Citopatologia,** realizado de **03 a 06 de agosto de 2022,** em Foz do Iguaçu - PR.

Foz do Iguaçu, 06 de agosto de 2022.

Katia Ramos Moreira Leite
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Presidente da Sociedade Brasileira de Patologia

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Presidente do 33º Congresso Brasileiro de Patologia

Luiz Martins Collaço
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Presidente do 26º Congresso Brasileiro de Citopatologia

Sociedade Brasileira de PATOLOGIA

Terceiro Lugar na categoria Investigação, durante a 40ª Reunião Anual dos Dermatologistas Latinoamericanos, realizada de 27 a 30 de maio de 2023, no Viasoft Experience na Cidade de Curitiba – Paraná - Brasil



CERTIFICADO

RADLA BRASIL / 2023
27 A 30 DE MAIO DE 2023
CURITIBA / PARANÁ

Certificamos que o Trabalho Científico/ Pôster TI 9 - A aplicabilidade do DOI (Depth Of Invasion) para avaliação prognóstica no melanoma acral., autores: **Marcel Arakaki Asato, José Cândido Caldeira Xavier Júnior, Juliana Polizel Ocanha-Xavier e Francisco Alves Moares Neto** foi classificado em **TERCEIRO LUGAR** na categoria **Investigação,** durante a **40º Reunião Anual dos Dermatologistas Latinoamericanos,** realizada de 27 a 30 de maio de 2023, no Viasoft Experience na Cidade de Curitiba – Paraná – Brasil.

Curitiba, 30 de maio de 2023.

Realização: Apoio: RENNOVA O PODER DA ESCOLHA

Prof. Jesus Santamaria
Presidente do Comitê Científico

Prof. Hirofumi Uyeda
Presidente da 40º RADLA Brasil

Prêmio SBP Categoria Pôster, pelo trabalho apresentado durante o 34º Congresso Brasileiro de Patologia e 27º Congresso Brasileiro de Citopatologia, realizado de 29 de maio a 01 de junho de 2024, em Belém-PA.



34º Congresso Brasileiro de PATOLOGIA
27º Congresso Brasileiro de CITOPATOLOGIA
29/05 a 01/06/2024 Belém/PA

CERTIFICADO

Conferimos ao trabalho

A UTILIDADE DO PRAME E DO KI-67 COMO MARCADORES PROGNÓSTICOS NO MELANOMA CUTÂNEO

Dos autores

Marcel Arakaki Asato; Francisco Alves Moraes Neto; Marcelo Padovani de Toledo Moraes; Juliana Polizel Ocanha-xavier; Luiz Carlos Takita; Mariangela Esther Alencar Marques; José Cândido Caldeira Xavier-júnior;

O **Prêmio SBP Categoria Pôster**, pelo trabalho apresentado durante o **34° Congresso Brasileiro de Patologia e 27° Congresso Brasileiro de Citopatologia**, realizado de **29 de maio a 01 de junho de 2024**, em Belém - PA.

Belém, 01 de junho de 2024.

Dr. Clóvis Klock
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Sociedade Brasileira de PATOLOGIA

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Outros artigos publicados durante a realização do doutorado

1- XAVIER-JÚNIOR J. C. C., OCANHA-XAVIER J. P., ASATO M. A., BERNABÉ D. G. The 'AEIOU' system to identify primary oral melanoma. **Oral Oncology,** v. 124, p. 105670, 2022.

2- RUIVO A. G. B. R., ASATO M. A., FERRARI L. F. L., POLETTO D. R. P., OKIDA R. C., XAVIER-JÚNIOR J. C. C. Melanocytic Acral Nevus With Intraepidermal Ascent of Cells: Five Cases. **The American Journal of Dermatopathology**, v. 46, n. 5, p: 329-331, 2024.