Pathology - Research and Practice 215 (2019) 152582

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Potential predictive value of cofilin-1 for metastasis occurrence in a small cohort of Argentinian patients with mid-low Breslow thickness melanoma

Irene L. Ibañez[a,b,*], Francisco M. Grings[b], Candelaria Bracalente[b], Adriana R. Rinflerch[c], Victoria Volonteri[d], Mauro A.A. Castro[e], Fabio Klamt[f,g], Hebe Durán[a,b,h]

[a] National Atomic Energy Commission, (CNEA), Constituyentes Atomic Center, Research, Applications Management, Av. General, B1650KNA, San Martín, Buenos Aires, Argentina
[b] National Scientific and Technical Research Council (CONICET), C1425FQB, CABA, Argentina
[c] Italian Hospital of Buenos Aires (HIBA), Department of Dermatology, Experimental Dermatology, C1199ABB, CABA, Argentina
[d] Italian Hospital of Buenos Aires (HIBA), Department of Anatomic Pathology, C1199ABB, CABA, Argentina
[e] Laboratory of Bioinformatics, Curitiba (PR), Federal University of Paraná (UFPR), Professional and Technological Education Sector, Polytechnic Center Laboratory of Bioinformatics and Systems Biology, PPG-Bioinformática Jardim das Américas, 81531-970, Curitiba, PR, Brazil
[f] Federal University of Rio Grande do Sul (UFRGS), Institute of Basic Health Sciences (ICBS), Department of Biochemistry, Laboratory of Cellular Biochemistry, 90035-003, Porto Alegre, RS, Brazil
[g] National Institutes of Science & Technology, Translational Medicine (INCT-TM), 90035-903, Porto Alegre, RS, Brazil
[h] National University of San Martin, Miguelete Campus, School of Science and Technology, B1650KNA, Villa Lynch, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Cofilin-1
Melanoma
Metastasis
Breslow thickness
Bayesian analysis

ABSTRACT

Nowadays, histopathological criteria for melanocytic lesions are the mainstay prognostic factors for melanoma. However, there are cases in which these parameters fall short to predict melanoma spread.

We recently demonstrated a correlation of cofilin-1 levels, a key protein for tumor invasion, with different histopathological parameters associated with melanoma malignancy as well as a negative correlation with survival. In order to broaden our previous findings, we aim to estimate the probability of a melanoma to metastasize as a function of both a conventional histopathological parameter (Breslow thickness, BT) and cofilin-1's immunohistochemical expression levels, which we propose as a potential marker for metastasis.

We used a Bayesian approach to analyze clinical and cofilin-1 datasets formerly obtained from a patients' small cohort diagnosed with malignant melanocytic lesions since 2000 until 2008; classified at different tumor stages with or without detected metastasis and with at least 5 years of clinical follow-up.

Low BT values exhibited wide variance to predict metastasis occurrence, while the differential diagnostic value of cofilin-1 confirmed BT diagnosis or resulted more precise to predict outcome. Particularly, the probability of metastasis estimation improved when cofilin-1 was combined with BT for specific cases, where BT displayed large uncertainties.

Our analysis and the cofilin-1 determination provided statistically significant prognostic value in mid-low BT melanomas, which could complement further evaluation criteria to assist diagnosis and treatment decision-making. Moreover, the combined use of cofilin-1 with BT, if validated in follow-up studies, would be feasible to help patients' selection for treatment and optimize health resources.

Abreviations: A.U., arbitrary units; BT, Breslow thickness; HIBA, Italian Hospital of Buenos Aires; IHC, immunohistochemical; MCMC, Markov chain Monte Carlo; met, metastasis; O.D., optical density; *p*, probability of metastasis; P(met| BT), probability of metastasis given BT; P(met |cofilin-1), probability of metastasis given cofilin-1; P(met| BT,cofilin-1), probability of metastasis given both BT and cofilin-1; PPPM, predictive, preventive and personalized medicine; TCGA, The Cancer Genome Atlas

* Corresponding autor at: Comisión Nacional de Energía Atómica, Gerencia de Investigación y Aplicaciones, Av. General Paz 1499, B1650KNA, San Martín, Provincia de Buenos Aires, Argentina.

E-mail addresses: irenuliz@yahoo.com (I.L. Ibañez), francisco.m.grings@gmail.com (F.M. Grings), candybraca@gmail.com (C. Bracalente), adriana.rinflerch@hospitalitaliano.org.ar (A.R. Rinflerch), victoria.volonteri@hospitalitaliano.org.ar (V. Volonteri), mauro.a.castro@gmail.com (M.A.A. Castro), 00025267@ufrgs.br (F. Klamt), hduran@cnea.gov.ar (H. Durán).

https://doi.org/10.1016/j.prp.2019.152582
Received 28 June 2019; Received in revised form 26 July 2019; Accepted 3 August 2019

1. Introduction

Melanoma has showed a three-fold increase in incidence rates over the last forty years. Almost 75,000 new cases of melanoma in situ [40] and 91,270 new cases of invasive melanoma are expected to be diagnosed in 2018 [1]. Meanwhile, the melanoma occurrence rate in Argentina increases at 3% ratio per year and it is estimated that in 2017 in this country about 10 persons died from this disease per week [2]. Nowadays, the mainstay prognostic factors for melanoma are mainly based on histopathological parameters, such as Breslow's thickness (Breslow thickness, BT), ulceration, mitotic rate and lymph node positivity [16], which allow estimating melanoma staging [4]. However, they cannot univocally identify which individuals will progress in each staging group [23,35]. Besides, a quarter of melanoma deaths corresponds to 70% of early stage melanomas (BT ≤ 1 mm) [21,47]. Thus, additional biomarkers able to estimate the individual evolution of melanoma are required to monitor disease progression, recurrence, and treatment response [12,22,26,39].

In the context of predictive, preventive and personalized medicine (PPPM) [17], in-depth research of novel biomarkers to evaluate their predictive value for melanoma spreading is relevant. Nevertheless, typical clinical datasets for a given endpoint are commonly of modest size (tens to hundreds of patients), becoming disadvantageous in biomarker research since traditional statistical methods often show no obvious trend for small size samples. This statistical mismatch becomes worse as more of the intracellular molecular machinery complexity is revealed [32]. However a Bayesian approach can improve the analysis of small size samples and facilitate biomarker research [42]. Bayesian methods can provide less biased estimates, increased efficiency, and an enhanced ability to determine nonnull effects when they are present. This statistical methodology do not rely on asymptotics, a property that can be an obstacle when using frequentist methods in small sample contexts. With Bayesian methods such as Markov chain Monte Carlo (MCMC), the inference quality is controlled by the number of samples taken approaching infinity. Thus, conversely to frequentist methods, Bayesian approaches try to respond what are the values of the parameters, considering that all parameters are random and that the data are fixed [28].

Proteins involved in transforming melanoma cells into a migratory phenotype have potential as prognostic markers of metastasis. Among the lot of molecules that could be related to metastasis, we selected cofilin-1, a key mediator protein involved in cytoskeleton remodeling and migration by inducing cycles of actin polymerization/depolymerization [7,8]. In addition, as an effector of transforming growth factor-β, cofilin-1 largely contributes to epithelial mesenchymal transition programming which confers invasive and metastatic properties during cancer progression [27]. Cofilin-1 has been proposed as prognosis marker related to metastasis in lung [9,30] and breast cancer [34,45]. Regarding melanoma, migrating cells with metastatic ability exhibited high levels of cofilin-1 as compared with non-migratory neither metastatic controls [6]. Besides, increased levels were found in metastatic lymph node vs. matched human primary cutaneous melanoma of the same patients [19]. Recently, we demonstrated a correlation between cofilin-1 levels and melanoma malignant features, plus an inverse correlation with survival [5].

Therefore, the goal of this study is to model the probability of metastasis occurrence in melanoma as a function of both BT and cofilin-1′s immunohistochemical (IHC) expression levels using a Bayesian statistical approach. We also studied the predictive value of both parameters for metastasis occurrence in order to evaluate to which degree cofilin-1 levels could be used to upgrade BT prognosis and to benefit higher-risk patients for melanoma spread with a closer clinical follow-up.

2. Materials and methods

2.1. Clinical and Cofilin-1 immunohistochemical expression datasets

Clinical and IHC data were collected from our previous study as described in [5]. The pathological diagnoses were reviewed and classified by two independent pathologists, according to World Health Organization criteria. The Strengthening the Reporting of Observational Studies in Epidemiology guidelines were used (Supplementary material) [5,43,44]. REMARK guidelines [29] and the requirements set out by Simon et al. [41] were followed. Since we focused herein on the cofilin-1 value as a metastasis predictor, inclusion criteria were melanoma in situ (as non-metastatic control) and primary cutaneous melanoma from patients classified at different tumor stages (I-IV). It was taking into account an available clinical follow-up of at least 5 years and the detection or not of metastasis, reducing to 28 cases the original cohort. Data of undetermined tumor stages and nevi were excluded.

This work implemented The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human samples. The research program was approved by the Research Protocols Ethics Committee of the HIBA (#1922).

Cofilin-1′s IHC expression levels were quantified as described in [30] and expressed as optical density (O.D.) mean values in arbitrary units (A.U). Methodology is detailed in [5]. Briefly, tissue sections were processed following a conventional immunohistochemistry protocol. Rabbit polyclonal anti-cofilin-1 antibody (Abcam, ab42824) was used. In all cases, negative controls were obtained omitting the primary antibody, representing the background staining value in optical density (O.D.) measurements. Tissue sections obtained from normal skin and from breast cancer were used as negative and positive control tissues [30,45], respectively. The intensity of cofilin-1 IHC reaction was quantitatively measured in images obtained with an Olympus BX51 microscope coupled to a CCD camera (Olympus DP70). Five images were captured for each case on the same day by a single observer. Images were quantified as previously described [30], using Image *J* software (National Institutes of Health, Bethesda, USA) [36].

2.2. Statistical analysis

The association between BT or cofilin-1 and metastasis occurrence was assessed with the non-parametric Spearman's Rho test, p-values less than 0.05 were considered as statistically significant.

Modeling: We followed a Bayesian approach to study the predictive power of BT, cofilin-1 and BT + cofilin-1 to estimate the metastasis (met) occurrence. Considering that a priori, the probability of metastasis (p) occurrence increases as both BT and cofilin-1 levels increase, the idea was to model the p given BT (P(met | BT)), cofilin-1 (P(met |cofilin-1)) and both BT and cofilin-1 (P(met | BT,cofilin-1)), because there wasn't a strict cutoff point for which P(met) = 1. In contrast, the zone 1 mm < BT < 4 mm and 0.06 O.D. < cofilin-1 < 0.13 O.D. seems to be characterized by a non-deterministic relation between BT and cofilin-1. Thus, it was reasonable to ask for the p for a given value of BT and cofilin-1.

Since the observed effect can be understood as occurrence or non-occurrence, a Bernoulli random variable was chosen (where 1 means metastasis occurrence and 0 means metastasis non-occurrence). The only parameter of this distribution p should be a function of both BT and cofilin-1 values. Evidently, this function requires to be bounded in the [0–1] interval. However, we need an additional assumption: the p should be a monotonically increasing function of both BT and cofilin-1. This hypothesis is supported by observational data.

Given these constraints, a logistic function was selected to model the dependence of p as a function of BT (Eq. 1) and cofilin-1 (Eq. 2)

$$p(BT) = \frac{1}{1 + e^{\beta 1 + BT * \alpha 1}} \quad (1)$$

$$p(cofilin-1) = \frac{1}{1 + e^{\beta 2 + cofilin - 1 * \alpha 2}} \quad (2)$$

where β corresponds to the value of met(cofilin-1) for which $p = 0.5$ and α controls the steepness of the dependency. We considered β the cutoff point for the given biomarker, and α, the sensitivity, since very low values of α will indicate no dependence with the studied biomarker. A priori, we know that α should be negative in order to obtain an increase of p as a function of the biomarker, and that β should be a positive value, since it is a cutoff of both positively defined values. Therefore, we assumed both sets of β and α uniformly distributed as,

$$P(\alpha) = -U[0, \alpha_{max}]$$

$$P(\beta) = U[0, \beta_{max}]$$

where $\beta_{max} =$ max(BT or cofilin-1) and $\alpha_{max} = 25$ (a very large value, impossible for this dataset). Thereby, we included non-informative priors that will not influence our results. In summary, we modeled the observations as Bernoulli distributed with p, in which p itself is a function of BT(or cofilin-1) as given in Eq. 1 and Eq. 2, with hyperparameters β and α uniformly distributed to biologically possible scenarios.

Finally, we modeled P(met | BT,cofilin-1). The metastasis occurrence was also modeled as a Bernoulli random variable, but p should be a function of both BT and cofilin-1. In this scenario, we assumed that the effects of both BT and cofilin-1 are independent and,

$$p(BT,\ cofilin-1) = \frac{1}{1 + e^{\beta 1 + BT * \alpha 1}} \frac{1}{1 + e^{\beta 2 + cofilin - 1 * \alpha 2}} \quad (3)$$

Certainly, we are not stating that there is no statistical dependence between BT and cofilin-1, we just considered they are logically independent (e.g. if there is relation between them, we don't know the causal mechanisms explaining that, therefore we cannot model them).

Implementation: Modeling was developed in Python using the module pymc3 [33] that implements MCMC algorithms to perform all the estimations given explicit distributions to the parameters and explicit models that links them. Using the Metropolis-Hastings algorithm, the script generates 10^6 samples of the posterior distribution, then removes the burn in samples (10^5 samples) and decimates the result in order to obtain independent posterior samples. Convergence of the chains were evaluated using Geweke scores (-2/2 interval) and checking the decay of the autocorrelation function for every variable.

3. Results

3.1. Datasets description

Table 1 summarized available data used. The average age of patients with or without detected metastasis was 71.8 ± 15.2 and 65.5 ± 18.5 years, respectively. Regarding sex, 54.5% women vs. 45.5% men were patients with detected metastasis, and 50% women vs. 50% men were patients without detected metastasis. No significant differences about age and sex were found between groups.

Visibly, large BT values seem to be univocally related to large p. In this sense, the association between the two variables was statistically significant (Spearman's Rho: $R_s = 0.74$, $p_{(2\text{-tailed})} < 0.001$). However, low BT values do not necessarily correspond to low p (Fig. 1), since there are several metastasis cases near the typical threshold BT ~ 2 mm informed in the literature [46]. About cofilin-1, a significant association between its levels and p was observed (Spearman's Rho: $R_s = 0.48$ and $p_{(2\text{-tailed})} = 0.01$).

3.2. Predictive value of BT, cofilin-1 and BT + cofilin-1 for metastasis occurrence

Regarding the posterior P(met | BT) for the available data, the mean p increases as BT increases (Fig. 2a). A value of $p = 0.5$ corresponds to BT = 2 mm and the probability quickly increases as BT increase. Large values of BT are characterized by an almost certain p. The 95% confidence interval (95% CI) represents the interval for every value of BT that contains 95% of the posterior distribution, e.g. at BT = 2 mm, we can be 95% sure that the p lies between 0.18 and 0.85. The analysis shows that there is almost certainty of metastasis for BT > 8 mm, but for lower values of BT the variance of P(met | BT) is wide. This is related to patients that presented low BT values (~1-2 mm) but developed metastasis nevertheless (e.g. HIBA-14 and HIBA-18). Therefore, supported by the literature [13], large BT values display no ambiguous diagnostics, but low values cannot be safely used to predict metastasis occurrence (CI~65%).

Table 1
Clinical data available for this study of the patients' cohort with malignant melanocytic lesions and the correspondent cofilin-1 IHC expression levels.

Patient ID	Tumor Stage (TNM)	Detected Metastasis	Breslow Thickness (mm)	Cofilin-1 IHC Levels (mean O.D. A.U. ± SD)
HIBA-1	IV	Yes	14	0.092 ± 0.007
HIBA-2	IV	Yes	10	0.104 ± 0.015
HIBA-3	III	Yes	11	0.102 ± 0.016
HIBA-4	IV	Yes	2.5	0.105 ± 0.018
HIBA-5	IV	Yes	3.8	0.128 ± 0.011
HIBA-6	IV	Yes	11	0.092 ± 0.010
HIBA-7	IIB	No	3.2	0.076 ± 0.004
HIBA-8	IV	Yes	11.5	0.052 ± 0.010
HIBA-9	IV	Yes	12	0.075 ± 0.017
HIBA-10	IV	Yes	2	0.098 ± 0.006
HIBA-11	IA	No	1	0.082 ± 0.018
HIBA-12	IB	No	1	0.040 ± 0.024
HIBA-13	IA	No	1	0.072 ± 0.008
HIBA-14	IV	Yes	0.9	0.080 ± 0.006
HIBA-15	IB	No	1	0.126 ± 0.014
HIBA-16	IB	No	1	0.096 ± 0.013
HIBA-17	IA	No	1	0.084 ± 0.023
HIBA-18	III	Yes	1.2	0.065 ± 0.019
HIBA-19	IB	No	1.55	0.062 ± 0.016
HIBA-20	IA	No	1	0.065 ± 0.019
HIBA-21	0	No	0	0.055 ± 0.001
HIBA-22	0	No	0	0.009 ± 0.008
HIBA-23	0	No	0	0.011 ± 0.010
HIBA-24	0	No	0	0.027 ± 0.036
HIBA-25	0	No	0	0.041 ± 0.010
HIBA-26	0	No	0	0.023 ± 0.025
HIBA-27	0	No	0	0.092 ± 0.022
HIBA-28	0	No	0	0.075 ± 0.020

The analysis of the posterior P(met | cofilin-1) shows qualitatively similar results to the ones for BT, but with key quantitative differences (Fig. 2b). The mean p increases less sharply than in the BT case. A value of $p = 0.5$ corresponds to cofilin-1 = 0.09 O.D. More importantly, there is no rapid saturation for high or low values of cofilin-1. This is associated with relatively large but constant variance along the range (CI~40%), particularly near the cutoff at 0.09 O.D. The variance is larger than the one obtained using BT for large values of BT, but lower for the case of low values of BT.

Remarkably, for low BT values, the variance of P(met | cofilin-1) is lower than the one for P(met | BT), revealing the potential value of cofilin-1 as metastasis predictor. Therefore, we expect a differential sensitivity of P(met | BT,cofilin-1) to cofilin-1 for low and moderate values of BT.

Then, we used the formalism depicted in Eq. 3 to estimate the combined power of both BT and cofilin-1 to evaluate the metastasis occurrence, P(met | BT, cofilin-1). This probability corresponds to a two dimensional surface, so we performed three cuts of the surface as a function of BT for low, medium and large values of cofilin-1 (Fig. 3).

The trend of the p as a function of BT remains similar. However, the mean value of p as a function of BT exhibits an offset that is a function of cofilin-1 values. For instance, for a low value of cofilin-1 (0.009

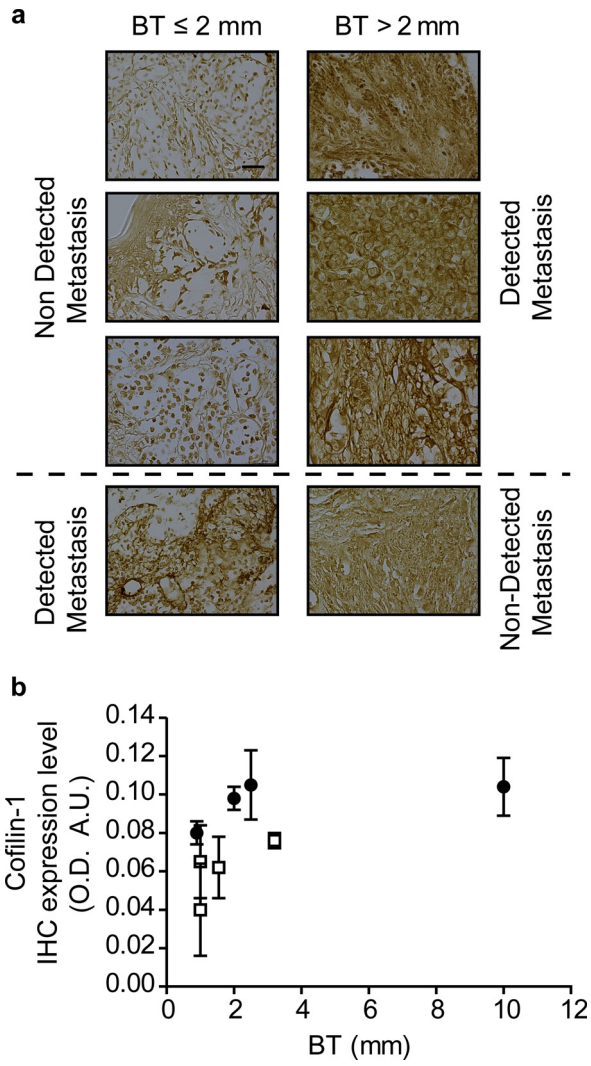

Fig. 1. Illustrative examples of the association between cofilin-1's IHC expression levels or BT values with patient metastasis occurrence. a) Representative images of cofilin-1 immunostaining in formalin-fixed paraffin-embedded tissue sections of malignant melanocytic lesions of patients with BT ≤ 2 vs. BT > 2 and detected vs. non-detected metastasis. Bar represents 20 μm. b) Patient metastasis occurrence (circles: yes, squares: no) as a function of BT values (mm) and cofilin-1's IHC expression levels (mean O.D. A.U.) in the cases showed in a).

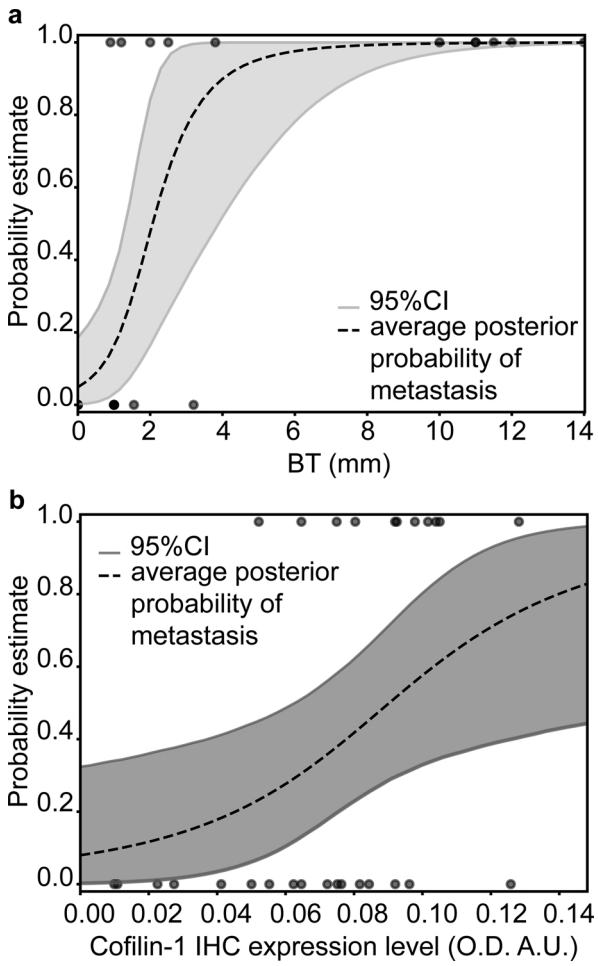

Fig. 2. Posterior probability of metastasis as a function of BT or cofilin-1 levels. a) P(met | BT) as a function of BT. 95% confidence interval is also informed. Patient occurrence ('1') or non-occurrence ('0') of metastasis as a function of BT is also informed. b) P(met | cofilin-1) as a function of cofilin-1. 95% confidence interval (CI) is also informed. Patient occurrence ('1') or non-occurrence ('0') of metastasis as a function of cofilin-1 is also informed.

O.D.), the value of $p = 0.5$ corresponds to BT = 4 mm, which decreases to BT = 2.5 mm for a medium value (0.078 O.D.) and reaches the minimum BT = 1.2 mm for a large value of cofilin-1 (0.148 O.D.). Therefore, for different values of cofilin-1, the differential diagnostic value of cofilin-1 can be illustrated as an improvement of the diagnosis of BT alone. Particularly, the probability of a metastatic incident, considering a mid-low range BT, decreases or increases if the levels of cofilin-1 exhibit a low or a high value, respectively.

Interestingly, the CI of the posterior distributions is generally larger than in the cases of estimations based on a single parameter (BT or cofilin-1). This is typical of Bayesian approaches, in which this increase in uncertainty is related to the lack of values of (BT, cofilin-1) in some regions of the (BT, cofilin-1) plane. Thus, Bayesian analysis automatically propagates the uncertainties exhibited in the data to the posterior distribution.

3.3. Study of specific cases

The previous analysis (Fig. 3), although interesting to study the predictive power of cofilin-1, is related to minimums and maximums of cofilin-1. However, for our dataset, in these extreme cases, BT alone would have been able to correctly estimate the p. This is related to some underlying correlation between cofilin-1 and BT for extreme cases. Nonetheless it is expected to find differential diagnosis for more complex cases, related to intermediate values of BT.

In order to illustrate this potential improvement of a combined BT + cofilin-1 scheme in the estimation of the p, we analyzed two cases in our dataset (Fig. 4), specifically selected in zones in which BT estimation showed large uncertainties.

Case #1: HIBA-7 is a patient with no detected metastasis, BT = 3.2 mm and cofilin-1 = 0.076 O.D. This is interesting because for the results of our dataset, using only BT to estimate P(met | BT), the mean p is 0.8 (0.36-0.98, 95% CI). However, when compute P(met | BT, cofilin-1), the below average value of cofilin-1 reduces the mean p to 0.75 (0.20-0.97, 95% CI). More importantly, the CI now stretches down to 0.2, softening the BT diagnosis by indicating more uncertainty than previously expected.

Case #2: HIBA-10 is a patient with detected metastasis, BT = 2 mm and cofilin-1 = 0.098 O.D. For the results of our dataset using only BT, the mean p is 0.46 (0.17-0.82, 95% CI), but when we estimate P(met | BT, cofilin-1) p increases to 0.58 (0.22-0.91, 95% CI). This is again related to the value of cofilin-1, in this case a little above the average value.

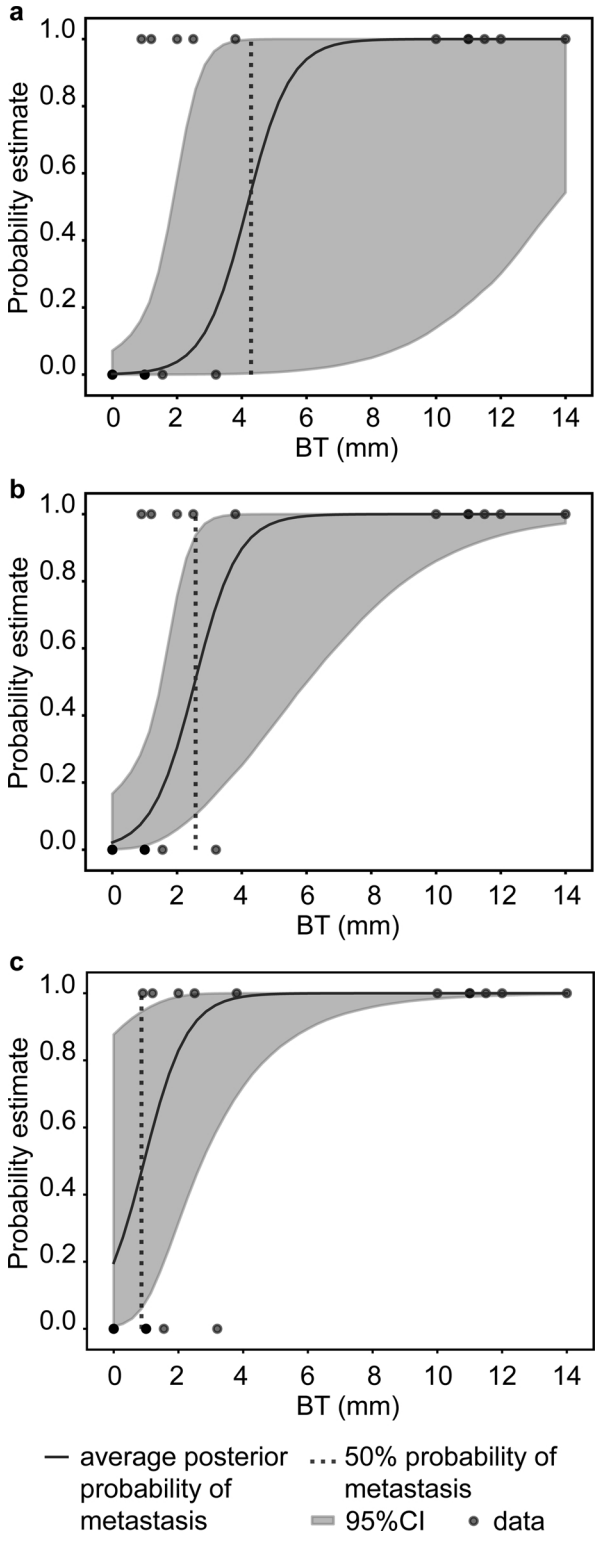

Fig. 3. Posterior probability P(met | BT, cofilin-1) as a function of BT for a) low (up, cofilin-1 = 0.009 O.D.), b) medium (middle, cofilin-1 = 0.078 O.D.) and c) large (down, cofilin-1 = 0.148 O.D.) values of cofilin-1. 95% confidence interval (CI) and patient occurrence ('1') or non-occurrence ('0') of metastasis is also informed.

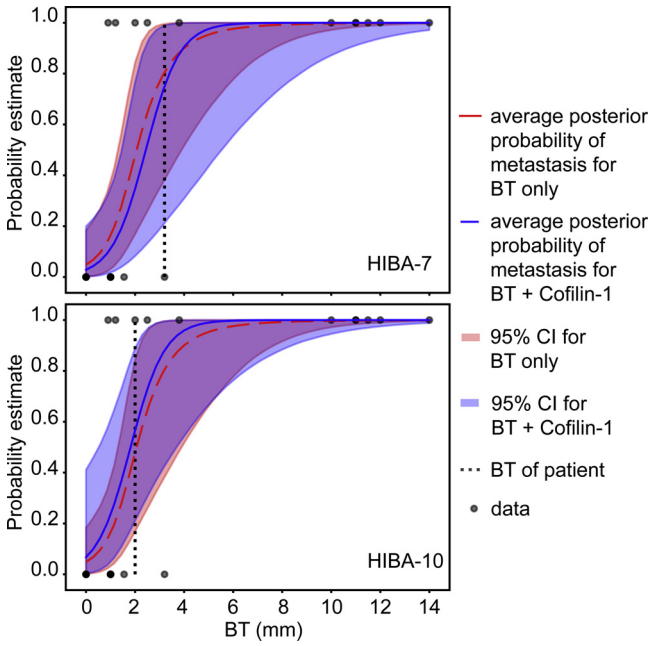

Fig. 4. Posterior probability of metastasis as a function of BT or combined BT and cofilin-1 in specific cases. Patients HIBA-7 and HIBA-10: (red) P(met | BT) as a function of BT for the inference scheme based on BT only; (blue) P(met | BT, cofilin-1) as a function of BT for cofilin-1 = 0.076 O.D. and 0.097 O.D. for HIBA-7 and HIBA-10 respectively. 95% confidence interval (CI) for both inference schemes and patient occurrence ('1') or non-occurrence ('0') of metastasis is also informed.

4. Discussion

Patients with melanomas with low values of BT generally have favorable outcomes, but there is a subgroup of them who will display recurrence or metastatic disease [13,47]. Our statistical analysis showed that we can set a cutoff for BT and the probability of metastasis occurrence considering the cofilin-1 level, adding evidence to the role of this protein as a prognostic biomarker in melanoma. In agreement with our results, high levels of cofilin-1 were associated with cancer progression and poor prognosis in different types of cancer [9,24,30,31,34,38,48]. Moreover, the LIMK/cofilin pathway and SSH1 were suggested as promising tumor biomarkers and therapeutic targets in human colorectal cancer, considering their role in tumor progression and chemoresistance [3]. Among the few studies that evaluate cofilin-1 in melanoma patients [5,19] and in keeping with our results, cofilin-1 was also described differentially expressed in metastatic lymph nodes vs matched human primary cutaneous melanomas of the same patients. Although that study made focus in other two proteins, it is worth to in deep analyze cofilin-1 and the other seven differentially expressed proteins [19]. This could support the use of cofilin-1 for metastasis screening studies. In this context, the cofilin-1's IHC expression levels could be determined in patients with mid-low BT in order to perform a tight clinical follow-up and improve treatment decision-making.

One of the main limitations of this study was the availability of patient samples with the required clinical data. However, statistically significant results were obtained. Certainly, a large dataset would reduce the CI. In this sense, the TCGA Research Network (http://cancergenome.nih.gov/) exhibits data from 479 melanoma samples of a cohort of 471 patients [10,15]. However, cofilin-1 is displayed at mRNA level (*CFL1*) and the results could be slightly different from the ones showed here, obtained at protein level by immunohistochemistry. Nevertheless, it is noteworthy that melanomas with BT > 2 mm exhibited higher *CFL1* mRNA expression z-scores (RNA Seq V2 RSEM) than those with BT < 2 mm in the TCGA cohort [5]. Moreover, Kaplan-Meier survival curves analysis showed that those patients with high *CFL1* mRNA expression levels had lower survival rate [5]. This supports that cofilin-1 can be associated with a worse prognosis in melanoma. Herein, we observed that although *CFL1* mRNA was only up-regulated

in 6% of the TCGA melanoma cohort, 53.8% of those patients exhibited disease spread into the lymph nodes (some patients were excluded of the analysis, two because lymph nodes couldn't be assessed and one for missing data).

Concerning the clinical use of biomarkers for melanoma, array comparative genomic hybridization (CGH) and fluorescence in-situ hybridization (FISH) studies proposed the combination of probes for 6p2518, 6q23, Cep6, and 11q13 as a good discriminator between melanoma and nevi. The probes panel used, identified inter-chromosomal rearrangements in chromosome 6 with gains in 6p25 (*RREB1*) and losses in 6q23 (*MYB*), as well as gains in 11q13 (*CCND1*) [14]. However, the diagnostic accuracy of CGH and FISH techniques is far from being absolute. Thus, these techniques have been sought to provide adjunctive diagnostic information for pathologists confronting histopathologically ambiguous melanocytic lesions [14]. Similarly, we propose the determination of cofilin-1 IHC levels not as the absolute biomarker to identify melanomas with high probability of metastasize, but to offer additional data which could help in the decision-making process about melanoma patients. Remarkably, *CCND1* and *CFL1* share the genomic location at 11q13.

In order to achieve targeted prevention and personalized treatments for a tumor like melanoma which is highly heterogeneous and one of the most aggressive and complex cancers [18], it is crucial to integrate the information obtained of our analysis with others biomarkers to evaluate the probability of a melanoma to metastasize. Currently, the multi-omics technologies made significant achievements in cancer research and clinically relevant results. These technologies will surely accelerate the cancer research with the improvement of technical limitations and ultimately benefit more cancer patients, worldwide [25]. Meanwhile, omics research should be understandable to other disciplines in order to maximize applicability [20]. In addition, other aspects are also needed to be considered, such as the patients' daily habits together with other factors, like age, sex, family history, lifestyle, etc. to create a model, in order to achieve a more accurate prediction of tumor and individualized treatment [11]. Unfortunately, this type of information is not always available to be included in a study, particularly when archival formalin-fixed paraffin-embedded tissue sections are used, like in our study. We perceive this as another limitation. However, we tried herein to simplify the analysis in order to evaluate the value of cofilin-1 as a predictive biomarker of metastasis in melanoma and how would help this value to a main parameter as it is BT, taking into account the complexity of the disease.

The term biomarker when used in translational research discussions, often refers to a marker used to speed up or aid in diagnosis or monitoring and advance into personalized medicine. Considering that a biomarker discovery path is long and challenging, the pace is often slow and arduous. In order to have clinical translation, a biomarker need to provide clinically relevant data beyond what is available or supply the same information at a lower cost, either financially or in measurable patient risk [37]. In the roadmap of biomarker discovery, first there is a question to address: "Is there an unmet clinical need?". When the answer is: "Yes", a reproducible assay must be available and used to define the markers distribution in the target population of the emerging potential candidates. Afterwards, this must be tested against the "gold standard" for diagnosis. If added diagnostic accuracy exists, then biomarker performance should be validated [37]. In keeping with this, we showed a potential candidate, cofilin-1, which could be easily measured in a reproducible manner following a conventional immunohistochemistry protocol in formalin-fixed paraffin-embedded tissue sections of melanoma patients. In this sense, there were proposed scenarios in which using archived specimens to validate biomarkers may be of considerable value [41]. Next, we tested cofilin-1 vs BT as "gold standard" for diagnosis and we demonstrated an improvement in the estimation of the probability of metastasis by combining cofilin-1 with BT, particularly when the estimation of this parameter exhibits large uncertainties. Thus, considering the potential of our findings, we are aware that a next step is to validate these results in an independent study before the assay and the prediction model are used clinically.

5. Conclusion

Although it is clear that validation of these promising results in a larger cohort of patients is required, we are taking a step-forward in determining the potential predictive value of cofilin-1 for metastatic spread of melanomas with mid-low BT, which could assist the diagnosis, follow-up and treatment of patients. Moreover, we explained this dependency in terms of the observed role of cofilin-1 in the metastatic process. Furthermore, Bayesian approach for modeling the predictive power of a conventional parameter and a proposed biomarker in a low-medium size clinical dataset, could allow a significant probability estimation to develop a bad outcome incident in other cancer studies. In a PPPM scenario, and if the combined use of cofilin-1 with BT is validated in follow-up studies, the type of methodology required is feasible to implement in a Pathology laboratory. A test like this could provide adjunctive information to conventional diagnosis studies and to novel emerging ones in order to benefit the selection of patients for treatments and on the other hand, could help to control the health budget of oncology.

Funding

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica, Argentina (Cooperación Internacional PICT-CABBIO 2014-0818, PICT 2014-1557 and PICT 2017-2478); MCTI/CNPQ/CBAB Cooperação Internacional em Biotecnologia, Brasil (465113/2014-1); Comisión Nacional de Energía Atómica, Argentina; Fundación Florencio Fiorini, Argentina.

Ethical approval

All the patient investigations conformed to the principles outlined in the Declaration of Helsinki and have been performed with the permission #1922 released by the responsible Ethic's Committee of the Hospital Italiano de Buenos Aires (HIBA), which concluded that informed consent for participation in this study was not required due the use of archival formalin-fixed paraffin-embedded tissue sections. Thus, patient's consent for publication is not applicable. However, additional privacy protection measures were taken. This study was performed with archival formalin-fixed paraffin-embedded tissue sections of benign and malignant melanocytic lesions from patients diagnosed between 2000 and 2008 with clinical follow-up of at least 5 years available. These samples were obtained from the Pathological Anatomy Service, Hospital Italiano de Buenos Aires, Argentina (HIBA). This article does not contain any studies with animals performed by any of the authors.

Data availability statement

All data generated or analyzed during this study are included in this manuscript (and its supplementary information files).

Consent for publication

The corresponding author has the consent from all authors to publish the manuscript in Pathology - Research and Practice and all authors have read and approved the manuscript.

CRediT authorship contribution statement

Irene L. Ibañez: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Francisco M. Grings:** Conceptualization,

Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Candelaria Bracalente:** Investigation, Methodology, Writing - review & editing. **Adriana R. Rinflerch:** Investigation, Methodology, Resources, Writing - review & editing. **Victoria Volonteri:** Investigation, Methodology, Resources, Writing - review & editing. **Mauro A.A. Castro:** Methodology, Supervision, Writing - review & editing. **Fabio Klamt:** Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - review & editing. **Hebe Durán:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors wish to thank to Instituto de Ciencias Básicas y Medicina Experimental, Instituto Universitario Hospital Italiano; and to the members of Departamento de Radiobiología and Laboratorio de Radiodosimetría Biológica, CNEA for their assistance. The authors also thank to the Head and staff from Dermatología Experimental, Servicio de Dermatología, Hospital Italiano de Buenos Aires (HIBA), particularly, Dr. Gastón N. Galimberti, and to the Head and staff from Servicio de Anatomía Patológica, HIBA.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152582.

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