Prediction of Non-sentinel Node Status in Patients with Melanoma and Positive Sentinel Node Biopsy: An Italian Melanoma Intergroup (IMI) Study

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ABSTRACT

Background and Purpose. Approximately 20% of melanoma patients harbor metastases in non-sentinel nodes (NSNs) after a positive sentinel node biopsy (SNB), and recent evidence questions the therapeutic benefit of completion lymph node dissection (CLND). We built a nomogram for prediction of NSN status in melanoma patients with positive SNB.

Methods. Data on anthropometric and clinicopathological features of patients with cutaneous melanoma who underwent CLND after a positive SNB were collected from nine Italian centers. Multivariate logistic regression was utilized to identify predictors of NSN status in a training set, while model efficiency was validated in a validation set.

Results. Data were available for 1220 patients treated from 2000 through 2016. In the training set ($n = 810$), the risk of NSN involvement was higher when (1) the primary melanoma is thicker or (2) sited in the trunk/head and neck; (3) fewer nodes are excised and (4) more nodes are involved; and (5) the lymph node metastasis is larger or (6) is deeply located. The model showed high discrimination (area under the receiver operating characteristic curve 0.74, 95% confidence interval [CI] 0.70–0.79) and calibration (Brier score 0.16, 95% CI 0.15–0.17) performance in the validation set ($n = 410$). The nomogram including these six clinicopathological variables performed significantly better than five other previously published models in terms of both discrimination and calibration.

Conclusions. Our nomogram could be useful for follow-up personalization in clinical practice, and for patient risk stratification while conducting clinical trials or analyzing their results.

Sentinel node biopsy (SNB) has become a standard procedure in the prognostic staging of patients with cutaneous melanoma.[1,2] Currently, patients with a positive SNB are submitted to completion lymph node dissection (CLND) because of the risk of harboring metastatic non-

sentinel nodes (NSNs) in the same lymphatic basin.[1,2] On the other hand, the findings of the German DeCOG trial[3] and the international MSLT-II trial[4] suggest that CLND following positive SNB provides no significant survival advantage, although it might still be useful for local disease control purposes. Overall, the clinical usefulness of CLND is called into question and CLND might be abandoned.

Nevertheless, the status of NSN plays a significant prognostic role as it contributes to the determination of the number of positive lymph nodes (a key feature in the current TNM staging system) and maintains a prognostic value independently of the sentinel node status.[5]

Using current inclusion criteria for SNB (usually primary tumor thickness $\geq$ 1 mm), the proportion of patients with metastatic NSNs is relatively low (10–30%).[1,5] Therefore, the therapeutic role of CLND might be difficult to be uncovered because most patients with metastatic sentinel nodes (75–85%) are made locally disease-free by the SNB procedure itself, and are thus unlikely to benefit from CLND. In other words, data from these patients might act as a confounding factor in the survival analyses of trials performed to date.

Moreover, some available evidence supports the benefit of SNB-guided surgery in terms of disease-free survival,[6] which calls for a therapeutic role of surgery, at least in improving locoregional disease control.

If patient risk of harboring metastatic disease in NSNs could be reliably predicted on an individual basis, physicians would be able to identify patients at high risk of disease progression (prognostic value), which might represent an important piece of information for both therapeutic (e.g. adjuvant treatment), follow-up (i.e. personalization of type and frequency of controls), and research purposes (e.g. risk stratification and selection of patients to be enrolled in clinical trials).

In order to address this issue, we carried out a multicenter national study to build up a multivariable model predicting the status of NSN in patients with melanoma and positive SNB. The results were utilized to set up a nomogram for practical use.

PATIENTS AND METHODS

Study design

This was a retrospective study based on prospectively maintained databases curated by nine Italian centers belonging to the Italian Melanoma Intergroup (IMI). Our aim was to create a model for predicting NSN status in patients with sentinel node-positive melanoma.

Besides anthropometric data (age, sex, and primary tumor site, i.e. limbs, head and neck, trunk), we collected the following pathological data on patients who underwent CLND after positive SNB: primary tumor thickness (millimeters), primary tumor mitotic rate (number per squared millimeter), primary tumor regression (present vs. absent), primary tumor ulceration (present vs. absent), primary tumor angiolymphatic invasion (present vs. absent), number of sentinel nodes excised, number of metastatic sentinel nodes, site of sentinel node metastasis (subcapsular, parenchymal, combined [subcapsular + parenchymal], multifocal, extensive), size of sentinel node metastasis (millimeters), and NSN status (positive vs. negative).

The main inclusion criterion for SNB was pT1b or higher primary tumor stage (thickness $\geq$ 1 or $<$ 1 mm) associated with either primary tumor ulceration or mitotic rate $\geq$ 1 per squared millimeter. The main inclusion criteria for CLND were positive SNB and lack of evidence (clinical and radiological) for metastatic disease (all patients were M0).

The pathology protocols to assess primary melanoma features, sentinel node, and NSN status were shared by participating centers as members of the IMI.[7]

The patients' series was randomly split into a training set and a validation set (with a 2:1 ratio), and the model coefficients were used to set up a nomogram for clinical use.

The diagnostic efficiency of our nomogram was compared with that of other prediction models already proposed in the international literature.

Statistical Analysis

Differences between the features of patients with and without NSN metastasis were investigated using the Mann–Whitney test and the Pearson Chi-square test, as appropriate.

In order to build a predictive model, the association between covariates of interest and NSN status was assessed using multivariate binary logistic regression in the training set. The functional form of continuous covariates was investigated using fractional polynomials, as suggested by Sauerbrei et al.[8]

We used the Akaike information criterion ($AIC = 2n - 2\log L$, where n is the number of model coefficients and L is the model likelihood) for backward variable selection.[9] Statistically significant coefficients generated by the best model (lowest AIC value) were employed to build an NSN status prediction nomogram. The performance of the prediction model was evaluated in the validation set. To this aim, we assessed two parameters[10]: (1) discrimination (i.e. the ability of correctly separating positive subjects from negative subjects) was graphically presented (by plotting sensitivity vs. 1-specificity by means of a non-parametric receiver operating

characteristic [ROC] curve) and quantified (by calculating the area under the curve [AUC], which corresponds to the overall model accuracy, ranging from 0 to 1, where 1 indicates perfect discrimination, 0.5 the same discrimination of a coin flip, and 0 a perfect inverse discrimination); and (2) calibration (the agreement between observed and predicted risk) was graphically presented by using the locally weighted scatterplot smoothing (LOWESS) method,[11] and quantified by means of the Brier score (a measure of disagreement between the observed binary outcome and the predicted probability, as defined by the average squared error, ranging from 0 in the case of perfect calibration, to 0.25 in the case of maximum disagreement). As an alternative method of assessing model performance, we also used the bootstrap method (1000 replications). Briefly, random samples drawn with replacement from the original data set are created with the same size as the original series; the performance index of the model built on the entire cohort is always better than the average of the indices calculated in each replication. The difference between the two is an estimate of the model overfit (optimism), and the average value of the indices is considered the unbiased estimate of how well the model would perform in future data sets.

All tests were two-sided, and the alpha level of significance was set at 5%. All analyses were performed using Stata 11.2 SE software (StataCorp LLC, College Station, TX, USA).

RESULTS

We collected data from 1220 patients treated between January 2000 and December 2016 at nine participating centers (mean number of patients per center 135; range 48–328). Patient and tumor characteristics are reported in Table 1, while univariate and multivariate analysis in the training set ($n = 810$) are reported in Tables 2 and 3, respectively.

The multivariate analysis showed that the risk of harboring metastatic NSN was higher when (1) the primary melanoma is thicker or (2) sited in the trunk/head and neck (compared with the limbs); (3) fewer sentinel nodes are excised; (4) more sentinel nodes are metastatic; and (5) the sentinel node metastasis is larger or (6) located deeper (see Table 2 for details).

Mitotic rate, primary tumor ulceration, and regression, which resulted in being significantly associated with NSN status on univariate analysis (Table 2), were not retained by the multivariate model (Table 3).

Age, sex, and angiolymphatic invasion did not result in a significant association with NSN status, either in univariate or multivariate analysis. Unknown data related to five

TABLE 1 Main features of 1220 patients with cutaneous melanoma and metastatic sentinel lymph node(s) who underwent completion lymph node dissection

Age (years)	
Median (IQR)	58 (46–69)
Sex	
Males	686 (56.2)
Females	534 (43.8)
Primary tumor site	
Limbs	612 (50.1)
Trunk	548 (44.9)
Head and neck	60 (5.0)
Primary tumor thickness (mm)	
Median (IQR)	2.8 (1.7–4.4)
Mitotic rate[a]	
Median (IQR)	3^{2-8}
Unknown	275 (22.5)
Histological regression	
Yes	208 (17.0)
No	979 (80.2)
Unknown	33 (2.8)
Angiolymphatic invasion	
Yes	110 (9.0)
No	760 (62.3)
Unknown	350 (28.7)
Ulceration	
Yes	555 (45.5)
No	622 (51.0)
Unknown	43 (3.5)
Sentinel nodes excised	
One	554 (45.4)
Two	367 (30.1)
Three or more	299 (24.5)
Metastatic sentinel nodes	
One	1007 (82.5)
Two	184 (15.1)
Three or more	29 (2.4)
Site of sentinel node metastasis	
Subcapsular	394 (32.3)
Parenchymal	302 (24.7)
Combined	109 (8.9)
Multifocal	71 (5.8)
Extensive	96 (7.9)
Unknown	248 (20.4)
Size of sentinel node metastasis (mm)	
Median (IQR)	1.5 (0.5–3.6)
Non-sentinel node status[b]	
Positive	311 (25.5)
Negative	909 (74.5)

Data are expressed as *n* (%) unless otherwise specified

IQR interquartile range

[a]Number/squared millimeter η

[b]Based on completion lymph node dissection

TABLE 2 Univariate analysis comparing patients with metastatic NSNs versus those with non-metastatic NSNs, in the training set ($n = 810$)

	Positive NSNs [$n = 219$]	Negative NSNs [$n = 591$]	p value[a]
Age (years)			
Median (IQR)	60 (47–71)	57 (45–69)	0.132
Sex			
Males	124 (56.6)	327 (55.3)	0.741
Females	95 (43.4)	264 (44.7)	
Primary tumor site			
Limbs	124 (56.6)	279 (47.2)	0.012
Trunk	82 (37.4)	289 (48.9)	
Head and neck	13 (6.0)	23 (3.9)	
Primary tumor thickness (mm)			
Median	3.6	2.5	< 0.0001
Mitotic rate[b]			
Median	4.5	3	0.003
Unknown	49 (22.4)	134 (22.7)	0.928
Histological regression			
Yes	30	118	0.032
No	186	456	0.219
Unknown	3	17	
Angiolymphatic invasion			
Yes	21 (9.6)	51 (8.7)	0.761
No	137 (62.5)	362 (61.2)	0.53
Unknown	61 (27.9)	178 (30.1)	
Ulceration			
Yes	118 (53.9)	258 (43.6)	0.016
No	96 (43.8)	309 (52.3)	0.226
Unknown	5 (2.3)	24 (4.1)	
Sentinel nodes excised			
One	118 (53.9)	245 (41.4)	0.004
Two	60 (27.4)	186 (31.5)	
Three or more	41 (18.7)	160 (27.1)	
Metastatic sentinel nodes			
One	171 (78.1)	498 (84.3)	0.002
Two	36 (16.4)	85 (14.4)	
Three or more	12 (5.5)	8 (1.3)	
Site of sentinel node metastasis			
Subcapsular	41 (18.7)	229 (38.7)	< 0.001
Parenchymal	56 (25.6)	145 (24.5)	0.189
Combined	19 (8.7)	48 (8.1)	
Multifocal	16 (7.3)	29 (4.9)	
Extensive	36 (16.4)	27 (4.6)	
Unknown	51 (23.3)	113 (19.1)	
Size of sentinel node metastasis (mm)			
Median (IQR)	3 (1–8)	1.1 (0.4–3)	< 0.0001

Data are expressed as n (%) unless otherwise specified

IQR interquartile range, *NSNs* non-sentinel nodes

[a]Mann–Whitney or Fisher's test, as appropriate

[b]Number/squared millimeter

TABLE 3 Multivariate regression analysis for the identification of non-sentinel node status predictors in the training set ($n = 810$)

	OR (95% CI)	p value
Body site		
Trunk/head and neck[a]	1 (reference)	–
Limbs	0.61 (0.42–0.87)	0.007
Primary tumor thickness (mm)		
First FP transformation[b]	7.92 (2.61–24.03)	0
Second FP transformation[c]	0.68 (0.49–0.94)	0.019
Sentinel nodes excised		
1	1 (reference)	–
2	0.61 (0.39–0.95)	0.027
3 or more	0.40 (0.24–0.66)	0
Metastatic sentinel nodes		
1	1 (reference)	–
2	1.42 (1.01–2.01)	0.049
3 or more	5.26 (1.76–15.73)	0.003
Site of sentinel node metastasis		
Subcapsular	1 (reference)	–
Parenchymal/combined[a]	1.67 (1.05–2.66)	0.03
Multifocal	2.20 (1.03–4.68)	0.041
Extensive	3.31 (1.67–6.56)	0.001
Unknown	1.74 (1.00–3.02)	0.051
Size of sentinel node metastasis (mm)		
First FP transformation[d]	27.14 (6.18–119.18)	0
Second FP transformation[e]	0.29 (0.16–0.51)	0

OR odds ratio, *CI* confidence interval, *FP* fractional polynomial

[a]The two categories showed the same risk and were thus considered jointly in the final model

[b]$X^{0.5} - 0.598$, where X is tumor thickness/10

[c]$X^{3} - 0.046$, where X is tumor thickness/10

[d]$X^{0.5} - 1.076$, where X is equal to (metastasis diameter + 0.099)/100

[e]$X^{0.5}*\ln(X) - 0.1578$, where X is equal to (metastasis diameter + 0.099)/100

pathological features, with their frequency ranging from 2.8 to 28.7% (Table 1); these data were evenly distributed between patients with metastatic and non-metastatic NSNs (Table 2).

Of note, primary tumor thickness and maximum diameter of sentinel node metastasis did not have a linear relationship with the outcome, thus their fractional polynomial transformation was used in the predictive model to relax the linearity assumption of logistic regression.

Model performance, which was assessed in the validation set ($n = 410$), was quite satisfactory both in terms of discrimination (AUC 0.74, 95% CI 0.70–0.77; see also the ROC curve in Electronic Supplementary Fig. 1) and calibration (Brier score 0.16, 95% CI 0.15–0.17; see also the calibration plot in Electronic Supplementary Fig. 2). Very similar results were yielded by using the bootstrap method (data not shown). A nomogram for clinical and research purposes was built using the coefficients estimated by the multivariate model (Fig. 1).

The literature search identified five studies presenting alternative predictive models, built using two to five clinicopathological variables observed in 171–343 patients.[12–16] We tested the performance of the five models in our validation set, and our model compared favorably with the others in terms of both discrimination and calibration (see Table 4).

DISCUSSION

We presented the results of a multicenter study aimed at building a model to predict the status of NSNs in melanoma patients with metastatic sentinel node. By combining the information of six clinicopathological variables (primary tumor site and thickness, number of excised and involved sentinel nodes, as well as site and size of tumor

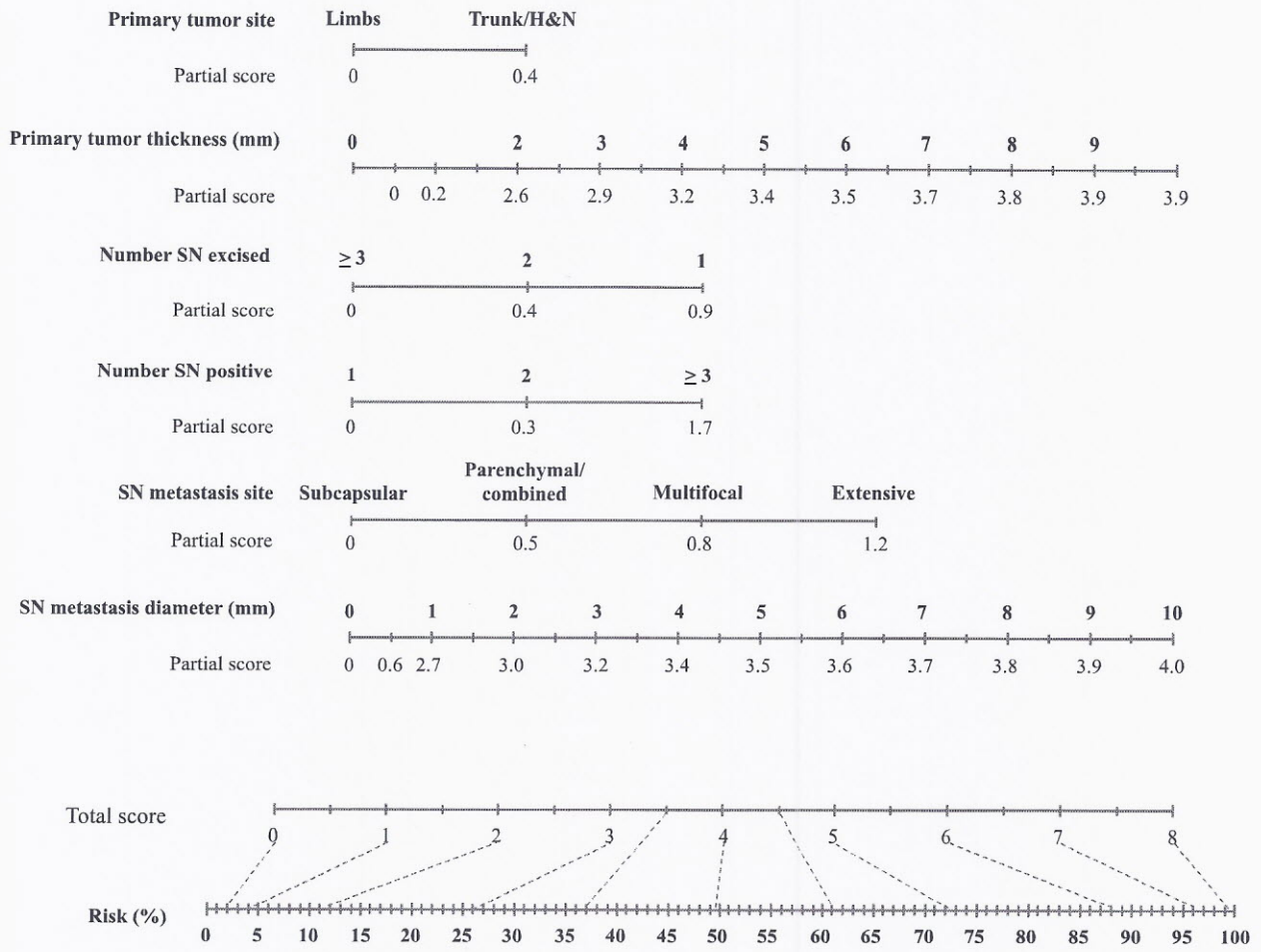

FIG. 1 Nomogram for the prediction of NSN status. For each patient, the risk of harboring metastatic melanoma in his/her NSN (bottom line) is estimated by calculating a total score, which in turn is calculated by adding up the partial score for each of the six clinicopathological features identified by the multivariate model (see text for more details). *H&N* head and neck, *SN* sentinel node(s), *NSN* non-sentinel node

deposits in the lymph node), the model can predict the status of NSNs with clinically valuable accuracy (overall accuracy in the validation set, 74%). To the best of our knowledge, this is the largest series ($n = 1220$) to be published with this aim. Previous reports have addressed the same issue using a smaller series of patients treated at single centers without a validation cohort.[12–16] The authors of the five identified studies utilized two to five clinicopathological features to build their models, and, interestingly, all included at least one primary tumor feature (except the study by Murali and colleagues)[16] and one sentinel node metastasis characteristic, whereas an anthropometric variable (patient sex) contributed to the predictive model in only two cases.[12,16]

Direct comparison showed that our model performed better than the other five models in terms of both discrimination and calibration (Table 4). For practical purposes, we also generated a nomogram to enable physicians to easily implement the results of this model in the clinical setting (Fig. 1).

Overall, our findings indicate that it is possible to reliably identify patients at high risk of harboring residual disease in the NSNs of patients with sentinel node-positive melanoma using easy-to-obtain clinicopathological information.

The survival results of the DeCOG and MSLT-II trials might lead to abandoning the use of CLND in melanoma patients with positive sentinel node(s), leaving room for radical lymphadenectomy only if patients develop clinically evident nodal metastasis. However, patients submitted to CLND to date are characterized by a relatively low risk of harboring metastatic NSNs (averaging 20%), whereas the availability of predictive tools capable of identifying high-risk patients could confer new importance to the surgical removal of NSNs.

TABLE 4 The performance of our prediction model, as well as five other previously published models tested in our validation set

Model	Parameters	Patients	Discrimination AUC (95% CI)[a]	p value[b]	Calibration Brier score (95% CI)[a]	p value[b]
Present	Primary tumor thickness Primary tumor site SN metastasis diameter SN metastasis site Number SN positive Number SN excised	Training set, $n = 810$ Validation set, $n = 410$	0.74 (0.70–0.79)	–	0.16 (0.15–0.17)	–
Lee et al.[15]	Primary tumor thickness SN metastasis diameter	$n = 191$	0.65 (0.60–0.70)	0.0001	0.19 (0.18–0.20)	0.0002
Gershenwald et al.[14]	Primary tumor thickness SN metastasis area Number SN excised	$n = 343$	0.65 (0.60–0.70)	0.0001	0.18 (0.17–0.20)	0.014
Kibrité et al.[12]	Primary tumor thickness SN metastasis diameter	$n = 171$	0.65 (0.60–0.70)	0.0001	0.19 (0.18–0.20)	0.0002
Sabel et al.[13]	Sex Primary tumor thickness Number SN positive SN metastasis site	$n = 221$	0.67 (0.63–0.74)	0.001	0.18 (0.16–0.20)	0.034
Murali et al.[16]	Sex Primary tumor regression Number SN positive SN metastasis site SN metastasis diameter	$n = 309$	0.65 (0.60–0.70)	0.0001	0.18 (0.17–0.19)	0.012

P values refer to the comparison of each of the five models with our present model in terms of both discrimination and calibration

SN sentinel node, *AUC* area under the receiver operating characteristic curve, *CI* confidence interval

[a]See text for more details

[b]The p value refers to the test comparing each previously published model with the present model

[c]Thickness was included in the model as a log-transformed value

In fact, there might be more than one reason to candidate high-risk patients to CLND.

First, knowing the actual number of positive lymph nodes in the regional lymphatic basin (which can only be assessed with a CLND) remains a key piece of information while staging melanoma using the American Joint Committee on Cancer (AJCC) TNM system; therefore, for high-risk patients, the prognostic judgment based on SNB alone is likely to significantly change after CLND.

More precise risk stratification is important for adequate patient information on the severity of the disease and for correct adjustment of the results of multivariate analysis within the frame of clinical trials, and is especially useful for selecting patients who can benefit most from adjuvant therapy. In this latter regard, the efficacy of drugs such as interferon-α[17] and ipilimumab[18] is likely to be more easily observed in patients with a higher risk of harboring minimal residual disease (such as patients with a higher risk of metastatic NSNs).

As mentioned above, patients submitted to CLND to date are characterized by a relatively low risk of harboring metastatic NSNs (ranging from 10 to 30%),[1] which might make it difficult to demonstrate a survival advantage from CLND since most patients are rendered locoregional disease-free at the time of the SNB. In this regard, it should be remembered that the German DeCOG trial was prematurely stopped, leading to an underpowered survival analysis acknowledged by the authors themselves.[4] Tools dedicated to the prediction of the NSN status would allow investigators to conduct surgical trials testing the hypothesis that CLND provides a survival advantage in a more homogeneous subgroup of patients with a higher risk of harboring tumor deposits in their NSNs (compared with patients at lower risk, such as those selected using the current criteria).

As a corollary of the above issue, an additional value of such a predictive tool is that it would enable the physician to discuss with the patient the likelihood of metastasis in

the NSN, and thus the convenience of performing CLND after a positive SNB. As a matter of fact, a patient with an estimated risk equal to or greater than, for instance, 30%, might be suggested to undergo CLND with the aim of improving locoregional disease control. Moreover, the definition of the body site (e.g. a specific lymph node basin) at high risk of disease relapse could also enable physicians to personalize patient follow-up in terms of targeted radiological assessments and frequency of periodical controls.

Finally, we acknowledge at least two main limitations of our tool. First, we could not validate our results in an external series of patients, as would be desirable.[19] Second, although the predictive model is fairly accurate, there is still much to work to do prior to obtaining a fully reliable tool. For instance, if the cutoff for patients to be considered at high risk of harboring melanoma cells in their NSNs (which entails an average 45% risk in our series) was set at 30%, the sensitivity, specificity, and positive and negative predictive values would be 65, 76, 50 and 85%, respectively, implying that, should the nomogram be applied to our series, approximately one-third of patients would be classified as high risk and would thus be operated on (CLND). Half of these patients would carry metastatic NSNS, which means that approximately two-thirds of all patients actually harboring metastatic disease in their NSNs would be submitted to CLND.

In this regard, new information (such as biomarkers assessed in the primary tumor, sentinel node, and peripheral blood)[20–22] is eagerly needed in order to improve the efficiency of predictive models, as we have recently reviewed for melanoma.[23]

CONCLUSION

We propose a nomogram for the prediction of NSN status of patients with sentinel node-positive cutaneous melanoma, which could be useful for patient counseling in routine clinical practice, as well as for risk stratification within the frame of clinical trials.

DISCLOSURE Carlo Riccardo Rossi, Simone Mocellin, Luca Giovanni Campana, Lorenzo Borgognoni, Serena Sestini, Giuseppe Giudice, Corrado Caracò, Adriana Cordova, Nicola Solari, Dario Piazzalunga, Paolo Carcoforo, Pietro Quaglino, Virginia Caliendo, and Simone Ribero have declared no conflicts of interest.

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