Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2022**; 49(4): 86
https://doi.org/10.31083/j.ceog4904086

Original Research

Intraoperative predictive model for the detection of metastasis in non-sentinel axillary lymph nodes

José Antonio García-Mejido[1,2,*], Miguel Sanchez-Sevilla[1], Rocio García-Jimenez[1], Ana Fernández-Palacín[3,*], José Antonio-Sainz[1,2]

[1]Department of Obstetrics and Gynecology, Valme University Hospital, 41014 Sevilla, Spain
[2]Department of Obstetrics and Gynecology, University of Seville, 41004 Sevilla, Spain
[3]Biostatistics Unit, Department of Preventive Medicine and Public Health, University of Seville, 41004 Sevilla, Spain
*Correspondence: jgmejido@us.es (José Antonio García-Mejido); afp@us.es (Ana Fernández-Palacín)
Academic Editor: Michael H. Dahan
Submitted: 26 July 2021 Revised: 7 September 2021 Accepted: 22 September 2021 Published: 8 April 2022

Abstract

Background: To design a software-applied predictive model relating patients clinical and pathological traits associated with sentinel lymph-node total tumor load to individually establish the need to perform an axillary lymph-node dissection. **Methods**: Retrospective observational study including 127 patients with breast cancer in which a sentinel lymph-node biopsy was performed with the one step nucleic acid amplification method and a subsequent axillary lymph-node dissection. We created various binary multivariate logistic regression models using non-automated methods to predict the presence of metastasis in non-sentinel lymph-nodes, including Log total tumor load, immunohistochemistry, multicentricity and progesterone receptors. These parameters were progressively added according to the simplicity of their evaluation and their predictive value to detect metastasis in non-sentinel lymph-nodes. **Results**: The final model was selected for having maximum discriminatory capability, good calibration, along with parsimony and interpretability. The binary logistic regression model chosen was the one which identified the variables Log total tumor load, immunohistochemistry, multicentricity and progesterone receptors as predictors of metastasis in non-sentinel lymph-nodes. Harrell's C-index obtained from the area under the curve of the predicted probabilities by Model 4 was 0.77 (95% CI, 0.689–0.85; $p < 0.0005$). **Conclusions**: the combination of total tumor load, immunohistochemistry, multicentricity and progesterone receptors can predict 77% of patients with metastasis in non-sentinel lymph-nodes and said prediction may be made intraoperatively in a feasible manner.

Keywords: Breast cancer; One-step nucleic acid amplification; Sentinel lymph-node; Non-sentinel lymph-node metastasis; Axillary lymph-node dissection; Total tumor load

1. Introduction

Breast cancer is a frequent entity whose management has evolved in recent years. Nowadays there is a tendency towards more conservative techniques. In the last decade of the 20th century, sentinel lymph node (SLN) biopsy replaced systematic axillary lymph node dissection (ALND), becoming the standard procedure for staging the axilla in breast cancer patients with clinically node-negative axilla [1–4]. This caused a decrease of ALND rates [5] along with its associated comorbidity [6]. In aims to assess semi-quantitatively the state of the SLN, the "One Step Nucleic Acid Amplification" (OSNA) method was proposed [7–11]. It is based on the quantification of Cytokeratin 19 (CK19) mRNA, which is expressed in more than 95% of breast cancer cases [12], and it is associated with total tumor load (TTL). The OSNA method shows a rentability comparable to conventional histologic techniques, and greatly benefit patients with clinically node-negative axilla [10,11].

Determination of the different values of SLN TTL allows a different surgical approach to the axilla. In cases with an undetectable or low TTL, the ALND may be safely avoided [13–15]. Patients with detected micrometastasis in SLN have a disease-free and overall survival comparable to those who received an ALND [16]. Moreover, patients with a T1-2 tumor who had macrometastasis in two or less SLN and received conservative surgery, radiotherapy and adjuvant systemic therapy, showed similar results in terms of survival [13].

On the other hand, TTL of CK19 mARN correlates with the presence of metastasis in non-sentinel lymph nodes (NSLN), thus it is considered the most important predictive factor for the presence of metastasis in NSLN [17]. This is the reason why several cut-off points have been published for SLN TTL to determine when to perform an ALND [18–20]. Said cut-off points vary between 2150 copies, established by Terretano *et al.* [20], and 15,000 copies of CK19 mARN, defined by Peg *et al.* [19]. However, SNL TTL is not the only predictive factor for metastasis in NSLN. Previous studies tried to identify predictive factors for metastasis in NSLN in aims avoid ALND [21–24]. Clinical and pathological factors have been described in aims to improve the predictive capability of SNL TTL. In this regard, we consider that a unique cut-off point of CK19 mARN copies would not be enough to predict the probabilities of

metastasis in NSLN [25], given that there are other factors to be considered. Thus, our objective is to design a software-applied predictive model relating patients clinical and pathological traits associated with SNL TTL to individually establish the need to perform an ALND.

2. Materials and methods

An observational retrospective study was carried out, including 127 patients with breast cancer in which a SLN biopsy was performed with the OSNA method and a subsequent ALND. Patients were consecutively recruited between October 1st 2010 and April 31st 2018.

Inclusion criteria were as follow: patients who had surgery for an invasive T1-3 breast carcinoma, which expressed CK19, with clinically node-negative axilla, and a normal preoperative axillar ultrasound or a lymph node biopsy with no evidence of metastasis. Patients with neoadjuvant chemotherapy treatment, previous ipsilateral axillary surgery, cancer recurrence or extensive in situ ductal carcinoma, were excluded from the study.

Variables studied were: age, menopausal status, menopause age, parity, number of births, ALND, tumor size, tumor histology type (ductal, lobular or others), multicentricity (presence of 2 or more tumor foci in different quadrants of the same breast or foci more than 5 centimeters from the primary focus), multifocality, lymphovascular invasion, tumor histological grade according to Modified Bloom-Richardson (tubules, nuclei and mitosis), estrogen receptors (ER), progesterone receptors (PR), y cHer-2 protein (HER2), Ki-67, SLN (macro or micrometastasis), NSLN (presence or absence of metastasis) and SLN TTL. Immunohistochemistry (IHC) classification was based in previously stablished criteria [26]: "Luminal A-like" (all of: ER and PR positive, HER2 negative and Ki-67 low); "Luminal B-like HER2 negative" (ER positive, HER2 negative and at least one of: Ki-67 high, PR negative or low); "Luminal B-like HER2 positive" (ER positive, HER2 overexpressed or amplified, any Ki-67, any PR); "HER2 positive non-luminal" (HER2 over- expressed or amplified, ER and PR absent); "Triple negative ductal" (ER and PR absent, HER2 negative).

Prior to surgery, and after the breast cancer diagnosis, all patients were assessed with an axillar ultrasound. Axillary lymph nodes suspicious for metastasis were those who shown cortical thickening of 2–3 mm, focal bulge, round shape, partial or complete absence of the fatty hilium, non-hiliar cortical blood flow, or a complete or partial replacement by tumoral tissue. A core needle biopsy was performed in patients with suspicious lymph-nodes. If the presence of metastasis was confirmed patients were excluded from the study.

During surgery, SLN biopsy was performed according to the established protocol in our unit, marking the node with a radiopharmaceutical and blue dye. The radiopharmaceutical used was 99mTc albumin nanocolloid, which was injected in the intradermic periareolar area a day prior to surgery. The dye used was methylene blue. Once the patient was under anesthesia, 2 mL of methylene blue was injected in the four quadrants of the periareolar area (0.5 mL per quadrant). Once the SLN was located and extirped, it was sent to the Anatomy Pathology unit for application of the OSNA method according to the existing literature [9]. The amplification rate was assessed by specthophotometry, and the number of CK19 mARN copies was calculated in relation to a standard curve. Macrometastasis were defined as the existence of more than 5000 copies/μL, micrometastasis as 250–5000 copies/μL, and absence of metastasis as less than 250 copies/μL [9].

Afterwards a Level II ALND was performed and NSLN were histologically assessed after being processed with a hematoxylin and eosin stain. Tissue blocks of NSLN were selected with a width of 3 microns, in a 200 microns interval, to determine the presence or absence of metastasis.

2.1 Statistical analysis

Statistical analysis was performed with the statistics software IBM SPSS version 22 (IBM, Armonk, NY, USA). Mean and standard deviations were determined for numeric variables, while percentages were used for qualitative variables. Comparisons of numeric variables was evaluated using Student's *t*-test, while the Chi-square test was used for comparisons of qualitative variables. Individual predictive values were evaluated using a receiver operating characteristic curve and the area under the curve (AUC). All statistic comparisons were performed with a two-tailed test, and statistical significance was set at 0.05.

2.2 Evaluation of logistic regression models

We created various binary multivariate logistic regression models using non-automated methods to predict the presence of metastasis in NSLD, including Log TTL, immunohistochemical (IHC), multicentricity and progesterone receptors (PR). These parameters were progressively added according to the simplicity of their evaluation and their predictive value to detect metastasis in NSLN.

We implemented and compared four binary logistic regression models (Table 1). A goodness-of-fit test was applied (logarithmic probability of –2) as well as the Hosmer and Lemeshow test for each model. Then we determined the Harrell's C-index (a statistics index to measure the goodness of fit for regression models, which analyzes its capability to discriminate the presence or absence or the event) for those models with an adequate fit to evaluate their discriminatory capability (obtained as the AUC of the predicted probabilities predicted by the model). The slope and calibration graph were also obtained.

The final model was selected for its maximum discriminative capability and calibration graph, according to the principles of parsimony and interpretability. Models were calibrated by the slope and calibration graph. Once the

Table 1. Predictive parameters evaluated in the binary logistic regression.

Model	Parameters included in the predictive model
Model 1	Log TTL
Model 2	Log TTL and IHC
Model 3	Log TTL, IHC and multicentricity
Model 4	Log TTL, IHQ, multicentricity and PR

TTL, total tumor load; IHC, immunohistochemical; PR, progesterone receptors.

definitive binary multivariate regression model was identified, we developed a software to predict the presence of metastasis in NSLN with the objective to make this model applicable for clinical practice.

3. Results

3.1 Study population

A total of 127 patients who required an ALND were recruited. Of the population studied, 37% (47/127) had metastasis in NSLN, while 63% (80/127) did not. Characteristics of both groups are shown in Tables 2,3,4. Multicentricity is more frequent in ALND with metastatic NSLN (23.4% vs 10.0%; *p*: 0.069). IHC classification showed that Luminal A-like tumors were more frequent in patients with no metastatic NSLN (57.4% vs 48.1%; *p*: 0.359). When comparing axillary surgery characteristics between both groups, we observed that patients with metastatic NSLN has higher rates of macrometastasis (93.6% vs 63.7%; *p*: 0.005) and TTL (917772.13 ± 249668.03 vs 335574.25 ± 892050.91; *p*: 0.005).

3.2 Predictive models for metastasis in NSLN

We used several binary logistic regression models to predict the presence of metastasis in NSLN. Harrell's C-index values of models oscillated between 0.68 and 0.77, determined as the AUC of the probability predicted (Table 5). Bivariate logistic regression models were performed linking metastasis in NSLN (positive/negative) and every single one of the identified variables as prognostics of positivity or negativity of metastasis in NSLN. These models led us to selecting the variable Log TTL in Model 1. The addition of variables in the subsequent models was made attending to the increase of the predictive capability of the models, their calibration (Hosmer-Lemeshow) and their discrimination capability (Harrell's C-index). The final model was selected for having maximum discriminatory capability, good calibration, along with parsimony and interpretability. The binary logistic regression model chosen was the one which identified the variables Log TTL, IHC, multicentricity and PR as predictors of metastasis in NSLN. Thus, these were the variables included in the final multivariate analysis, as can be seen in Table 5.

Harrell's C-index obtained from the AUC of the predicted probabilities by Model 4 was 0.77 (95% CI, 0.689–0.85; $p < 0.0005$) (Fig. 1). Calibration of the chosen model was evaluated calculating the slope and calibration graph which was 0,97 (95% CI, 0.59–1.35; $p < 0.0005$) (Fig. 2).

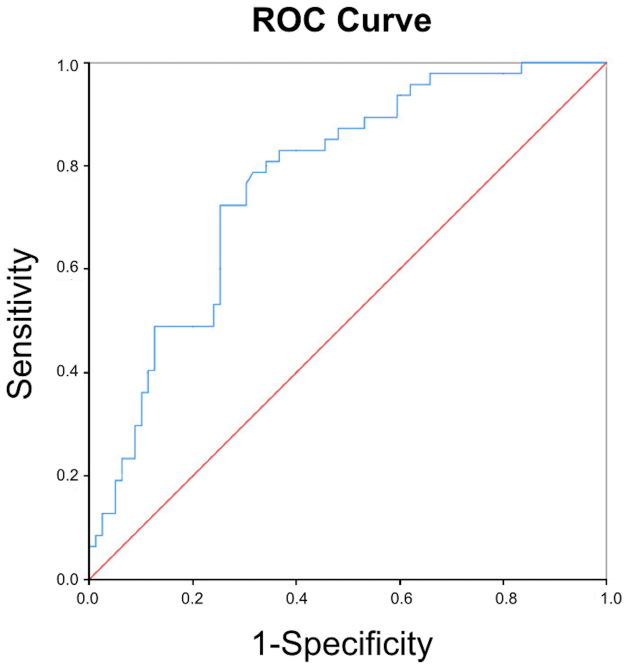

Fig. 1. ROC curve for logistic regression model obtained from the association between Log TTL, IHQ, multicentricity and PR. Area under ROC curve 0.770 (95% CI, 0.688–0.852; $p < 0.0005$).

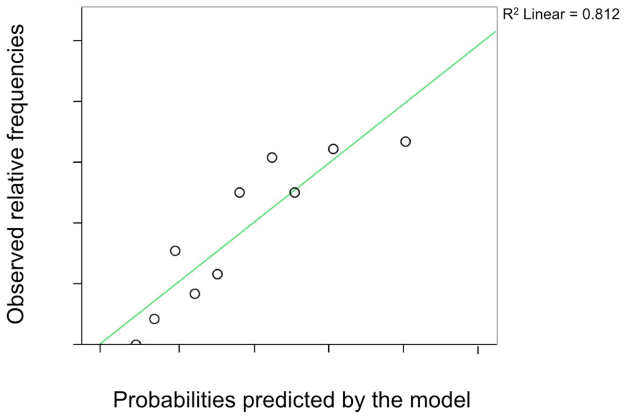

Fig. 2. Calibration graph of original logistic regression model obtained from the association between Log TTL, IHQ, multicentricity and PR.

4. Discussion

The main finding of our study is that a model based in the TTL, IHC, multicentricity and PR can predict 77% of patients with metastasis in NSLN. Given the simplicity of

Table 2. General characteristics of the patients studied.

	Mean ± SD o %				
	ALND with metastatic NSLN (n: 47)	ALND without metastatic NSLN (n: 80)	*p*	OR (95% CI)	*p*
Age	54.21 ± 10.49	57.18 ± 13.90	0.325	0.98 (0.95; 1.00)	0.208
Menopausal status	27/47 (57.4%)	43/80 (53.8%)	0.715	1.16 (0.56; 2.40)	0.686
Age of menopause	49.33 ± 3.35	47.84 ± 5.21	0.158	1.07 (0.96; 1.18)	0.217
Parity	43/47 (91.5%)	71/80 (88.8%)	0.766	1.36 (0.39; 4.70)	0.624
Number of births	2.56 ± 1.18	2.45 ± 1.40	0.381	1.06 (0.80; 1.41)	0.673

ALND, axillary lymph node dissection; NSLN, nonsentinel lymph nodes.

Table 3. Pathological characteristics of the tumor according to the presence of metastases in NSLN.

	Mean ± SD o %				
	ALND with metastatic NSLN (n: 47)	ALND without metastatic NSLN (n: 80)	*p*	OR (95% CI)	*p*
Tumor size	20.29 ± 9.13	20.67 ± 10.69	0.641	0.99 (0.96; 1.03)	0.838
Histological type					
Ductal	32/47 (68.1%)	64/80 (80.0%)	0.074	2.50 (0.28; 22.31)	0.412
Lobular	14/47 (29.8%)	11/80 (13.8%)		6.36 (0.65; 62.69)	0.113
Others	1/47 (2.1%)	5/80 (6.2%)			
Multicentricity	11/47 (23.4%)	8/80 (10.0%)	0.069	2.75 (1.02; 7.44)	0.046
Multifocality	5/47 (10.6%)	8/80 (10.0%)	1	1.07 (0.33; 3.49)	0.909
Lymphovascular invasion	15/47 (31.9%)	27/80 (33.8%)	1	0.92 (0.43; 1.98)	0.832
Tumor histological grade					
1	8/47 (17.0%)	9/80 (11.4%)	0.664		
2	24/47 (51.1%)	44/80 (55.7%)			
3	15/47 (31.9%)	26/80 (32.9%)			
Tubules					
1	3/47 (6.4%)	2/80 (2.5%)	0.279		
2	10/47 (21.3%)	26/80 (32.9%)			
3	34/47 (72.3%)	51/80 (64.6%)			
Nuclei					
1	2/47 (4.3%)	1/80 (1.3%)	0.419		
2	21/47 (44.7%)	30/80 (38.0%)			
3	24/47 (51.1%)	48/80 (60.7%)			
Mitosis					
1	27/47 (57.4%)	40/80 (50.6%)	0.383		
2	14/47 (29.8%)	21/80 (26.6%)			
3	6/47 (12.8%)	18/80 (22.8%)			

ALND, axillary lymph node dissection; NSLN, nonsentinel lymph nodes.

this model, which includes only 4 parameters (TTL, IHC, multicentricity an PR), it is easy to apply intraoperatively in any breast cancer unit. When applying this proposed predictive model, any breast surgeon can easily predict the probability of metastasis in NSLN and then decide whether to perform an ALND during the surgery act (Fig. 3).

Up until recently, nomograms have been based in SLN TTL, establishing different AUC ranging between 0.66 and 0.86 [20,27–33]. In our work, we described an AUC of 0.77 (95% CI, 0.688–0.852; $p < 0.0005$), similar to those previously published, although we included clinical and pathological factors that may affect the probability of metastasis in NSLN. In a previous study, our group described an AUC of 0.651 using only the TTL, rising to 0.752 [25] when adding clinical and pathological factors (multicentricity, IHC and RP). Thus, we believe that clinical and pathological factors are of the utmost importance for the design of the presented model, for they allow us to individually assess each patient for the need of performing an ALND.

There have been publications of several cut-offs points for the copies of CK19 mARN in SLN TTL to decide on the ALND. Heilmann *et al.* [34] defined 7900 copies/μL as the cut-off point, with a sensibility of 91% and specificity of 61%. In their work, they combined histologic techniques and the OSNA method for the processing of SLN, which might alter the number of copies. In several other publications, we can observe that different cut-off points for the SLN TTL in the prediction of metastasis in NSLN have progressively decrease in recent years [18–20]. Peg *et al.* [19] defined in 2013 a high cut-off points of 15,000 copies (Sensibility: 76.7%, Specificity: 55%; Positive predictive value: 41.1%; Negative predictive value: 85.5%). Later, Ambrogio *et al.* [18] established a cut-off point of 7700 copies (Sensibility: 78%, Specificity: 57%; Positive

Table 4. Immunohistochemical profile of the tumor according to the presence of metastases in NSLN.

	Mean ± SD o %				
	ALND with metastatic NSLN (n: 47)	ALND without metastatic NSLN (n: 47)	*p*	OR (95% CI)	*p*
IHC					
Luminal A-like	27/47 (57.4%)	38/80 (48.1%)	0.811		
Luminal B-like HER2 negative	17/47 (36.2%)	31/80 (39.2%)			
Luminal B-like HER2 positive	2/47 (4.3%)	6/80 (7.6%)			
HER2 positive nonluminal	0/47 (0%)	1/80 (1.3%)			
Triple-negative	1/47 (2.1%)	3/80 (3.8%)			
IHC (Grouped)					
Luminal A-like	27/47 (57.4%)	38/80 (48.1%)	0.359	0.69 (0.33; 1.42)	0.311
No Luminal A-like	20/47 (42.6%)	41/80 (51.9%)			
ER	46/47 (97.9%)	76/80 (95.0%)	0.651	2.42 (0.26; 22.33)	0.435
PR	37/47 (78.7%)	69/80 (86.3%)	0.325	1.69 (0.66; 4.36)	0.274
HER2 positive	2/47 (4.3%)	7/80 (8.9%)	0.482	0.46 (0.10; 2.30)	0.342
Ki 67 (%) (Grouped)					
≤20%	33/47 (70.2%)	47/80 (59.5%)	0.255	0.62 (0.29; 1.34)	0.228
>20%	14/47 (29.8 %)	32/80 (40.5%)			
SLN					
Micrometastasis	3/47 (6.4%)	29/80 (63.7%)	<0.0005	8.34 (2.38; 29.26)	0.001
Macrometastasis	44/47 (93.6%)	51/80 (63.7%)			
TTL	917772.13 ± 249668.03	335574.25 ± 892050.91	0.005	1 (1.00; 1.00)	0.120
Log TTL	5.11 ± 0.99	4.28 ± 1.26	0.005	1.31 (1.31; 2.61)	<0.0005

ALND, axillary lymph node dissection; NSLN, nonsentinel lymph nodes; SLN, sentinel lymph node; IHC, immunohistochemical; ER, estrogen receptors; PR, progesterone receptors; TTL, total tumor load.

Table 5. Evaluation of the various selected models.

Models	Variables	OR 95% CI	Calibration (Homer-Lemeshow) *p*	Discrimination (Harrel's C-index 95% CI)
1	Log TTL	1.85 (1.31; 2.61)	0.555	0.68 (0.59; 0.78)
2	Log TTL	1.89 (1.34; 2.68)	0.708	0.70 (0.61; 0.79)
	IHC	0.56 (0.26; 1.23)		
3	Log TTL	1.87 (1.31; 2.66)	0.347	0.73 (0.64; 0.82)
	IHC	0.58 (0.26; 1.28)		
	MC	0.43 (0.15; 1.23)		
4	Log TTL	2.14 (1.45; 3.17)	0.151	0.77 (0.69; 0.85)
	IHC	0.30 (0.11; 0.78)		
	MC	0.34 (0.11; 1.07)		
	PR	6.68 (1.79; 24.87)		

predictive value: 50%; Negative predictive value: 83%), similar to the cut-off point of 7294 copies published by our group [25]. Terretano *et al*. [20] presented in 2017 the lowest cut-off point of 2150 copies sensibility: 94.9%, Specificity: 51.4%; Positive predictive value: 46.5%; negative predictive value: 95.8%). These variations among values of the SLN TTL for the prediction of metastasis in NSLN indicate that none of them are superior to the rest, and we should use other associated parameters. Thus, our model included the previously mentioned variables to individually assess the need of an ALND.

The association between clinical and pathological factors and the risk of metastasis in NSLN have been established in several studies, like the HER-2 status or the histological and nuclear grade described by Meretoja *et al*. [35]. Tumor size is also considered an important factor for metastasis in NSLN [36–38]. Furthermore, some factors have even been included in nomogram like the association of the TTL with tumor size, lymphovascular invasion, HER-2 status and number of metastatic SLN [28].

In contrast, Shimazu *et al*. [29] stated that nomograms using several pathological parameters are not practical for intraoperative decision-making. However, we consider that a simplify model like the one presented may be quite useful given the feasibility of use during surgery with a high predictive capacity.

We consider our study to have many strengths, one of them being the considerable sample size, with the inclusion of a representative sample of breast cancer patients undergoing ALND. Another notorious strength of our study is the

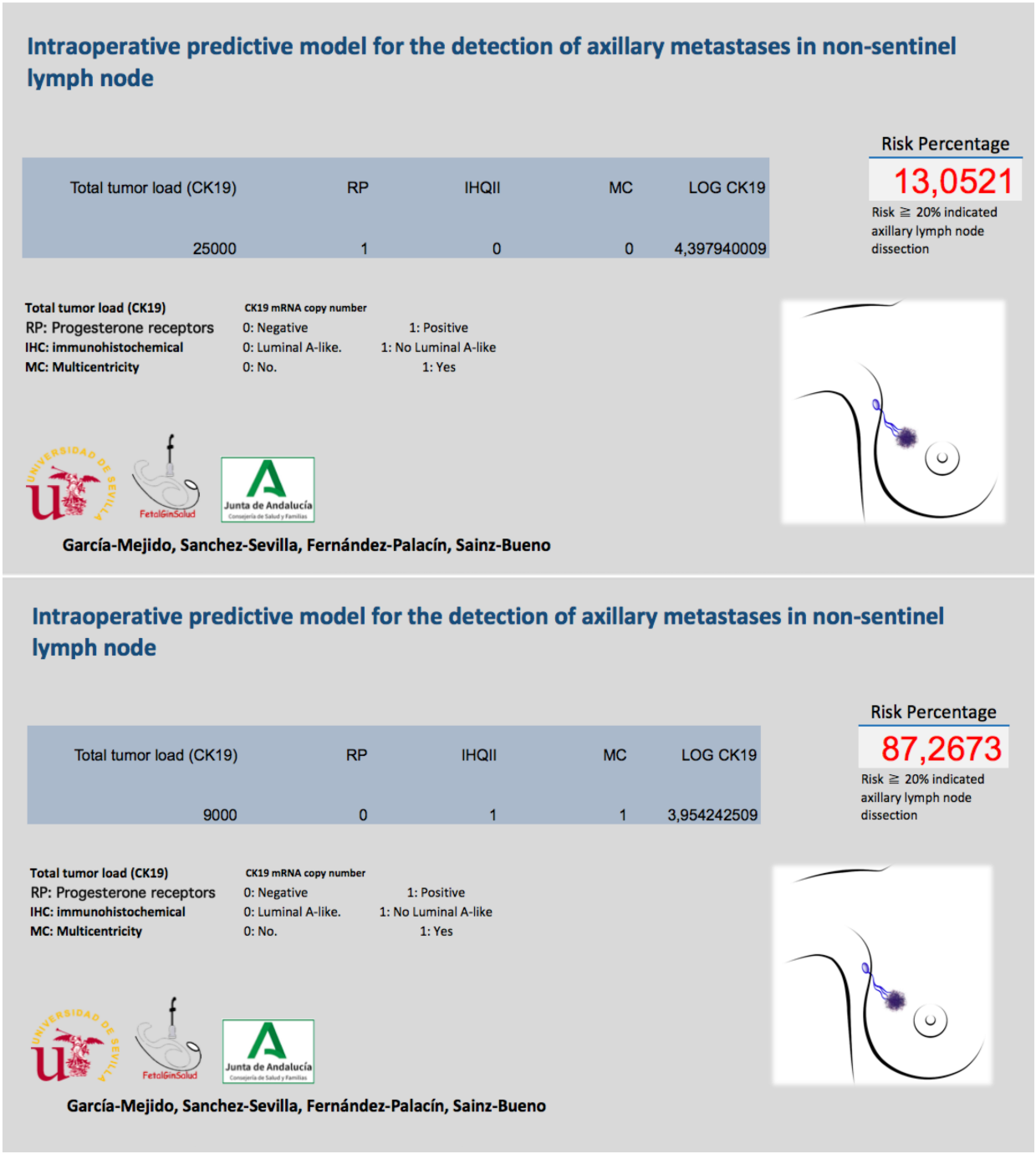

Fig. 3. Example of using the binary model based on total tumor load (TTL), IHC, MC and PR as a predictor for metastasis in non-sentinel lymph-nodes. PR (progesterone receptors): 0 = Presence of progesterone receptors; 1 = No presence of progesterone receptors. IHC (Immunohistochemistry): 0 = Luminal A-like; 1 = No Luminal A-like. MC (multicentricity): 0 = multicentric tumor; 1 = Non-multicentric tumor.

fact that this proposed model allows for individual assessment of the need to perform ALND in the operating room, that can be made in a quick, effective and simple way without extending surgery time. Nonetheless, our study also has some limitations. We consider that a prospective design, along with the need for external validation might be considered for future studies. In addition, it would require an external validation to be able to apply it in routine practice.

5. Conclusions

In conclusion, the combination of TTL, IHC, multicentricity and PR can predict 77% of patients with metastasis in NSLN and said prediction may be made intraoperatively in a feasible manner.

Abbreviations

SLN, Sentinel lymph node; ALND, Axillary lymph node dissection; OSNA, One step nucleic acid amplification; CK19, Cytokeratin 19; TTL, Total tumor load; NSLN, Non-sentinel lymph nodes; IHC, Immunohistochemical; ER, Estrogen receptors; PR, Progesterone receptors; MC, Multicentricity.

Author contributions

JAGM and JAS designed the research study. RGJ performed the research. AFP analyzed the data. JAGM, MSS, RGJ, and JAS wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki , and the protocol was approved (on January 29th 2019) by Andalucia's Board of Biomedicine Ethics Committee (registration number: 1004-N-18).

Acknowledgment

Thanks to all the peer reviewers for their opinions and suggestions.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Veronesi U, Paganelli G, Viale G, Luini A, Zurrida S, Galimberti V, *et al*. Sentinel lymph node biopsy as a staging procedure in breast cancer: update of a randomized controlled study. The Lancet Oncology. 2006; 7: 983–990.

[2] Benson JR, della Rovere GQ. Management of the axilla in women with breast cancer. The Lancet Oncology. 2007; 8: 331–348.

[3] Golshan M, Martin WJ, Dowlatshahi K. Sentinel lymph node biopsy lowers the rate of lymphedema when compared with standard axillary lymph node dissection. The American Surgeon. 2003; 69: 209–11; discussion 212.

[4] Reitsamer R, Peintinger F, Prokop E, Menzel C, Cimpoca W, Rettenbacher L. Sentinel lymph node biopsy alone without axillary lymph node dissection–follow up of sentinel lymph node negative breast cancer patients. European Journal of Surgical Oncology. 2003; 29: 221–223.

[5] Garcia-Etienne CA, Mansel RE, Tomatis M, Heil J, Biganzoli L, Ferrari A, *et al*. Trends in axillary lymph node dissection for early-stage breast cáncer in Europe: Impact of evidence on practice. Breast. 2019; 45: 89–96.

[6] Veronesi U, Paganelli G, Galimberti V, Viale G, Zurrida S, Bedoni M, *et al*. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. Lancet. 1997; 349: 1864–1867.

[7] Schem C, Maass N, Bauerschlag DO, Carstensen MH, Löning T, Roder C, *et al*. One-step nucleic acid amplification-a molecular method for the detection of lymph node metastases in breast cancer patients; results of the German study group. Virchows Archiv. 2009; 454: 203–210.

[8] Tamaki Y, Akiyama F, Iwase T, Kaneko T, Tsuda H, Sato K, *et al*. Molecular detection of lymph node metastases in breast cancer patients: results of a multicenter trial using the one-step nucleic acid amplification assay. Clinical Cancer Research. 2009; 15: 2879–2884.

[9] Tsujimoto M, Nakabayashi K, Yoshidome K, Kaneko T, Iwase T, Akiyama F, *et al*. One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients. Clinical Cancer Research. 2007; 13: 4807–4816.

[10] Raia-Barjat T, Trombert B, Khaddage A, Douchet C, Seffert P, Peoc'h M, *et al*. OSNA (one-step nucleic acid amplification) sentinel lymph node intraoperative molecular analysis in breast cancer: a cost-benefit analysis. Medical Oncology. 2014; 31: 322.

[11] Brambilla T, Fiamengo B, Tinterri C, Testori A, Grassi MM, Sciarra A, *et al*. One-Step Nucleic Acid Amplification in Breast Cancer Sentinel Lymph Node: a Single Institutional Experience and a Short Review. Frontiers in Medicine. 2015; 2: 37.

[12] Chaudhry A, Williams S, Cook J, Jenkins M, Sohail M, Calder C, *et al*. The real-time intra-operative evaluation of sentinel lymph nodes in breast cancer patients using one Step Nucleic Acid Amplification (OSNA) and implications for clinical decision-making. European Journal of Surgical Oncology. 2014; 40: 150–157.

[13] Giuliano AE, Hunt KK, Ballman KV, Beitsch PD, Whitworth PW, Blumencranz PW, *et al*. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. The Journal of the American Medical Association. 2011; 305: 569–575.

[14] Koca B, Kuru B, Ozen N, Yoruker S, Bek Y. A Breast Cancer Nomogram for Prediction of Non-Sentinel Node Metastasis—Validation of Fourteen Existing Models. Asian Pacific Journal of Cancer Prevention. 2014; 15: 1481–1488.

[15] Glechner A, Wöckel A, Gartlehner G, Thaler K, Strobelberger M, Griebler U, *et al*. Sentinel lymph node dissection only versus complete axillary lymph node dissection in early invasive breast cancer: a systematic review and meta-analysis. European Journal of Cancer. 2013; 49: 812–825.

[16] Galimberti V, Cole BF, Zurrida S, Viale G, Luini A, Veronesi P, *et al*. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastases (IBCSG 23-01): a phase 3 randomised controlled trial. The Lancet Oncology. 2013; 14: 297–305.

[17] Ohi Y, Umekita Y, Sagara Y, Rai Y, Yotsumoto D, Matsukata A, *et al*. Whole sentinel lymph node analysis by a molecular assay predicts axillary node status in breast cancer. British Journal of Cancer. 2012; 107: 1239–1243.

[18] Deambrogio C, Castellano I, Paganotti A, Zorini EO, Corsi F, Bussone R, *et al*. A new clinical cut-off of cytokeratin 19 mRNA copy number in sentinel lymph node better identifies patients eligible for axillary lymph node dissection in breast cancer. Journal of Clinical Pathology. 2014; 67: 702–706.

[19] Peg V, Espinosa-Bravo M, Vieites B, Vilardell F, Antúnez JR, de Salas MS, *et al*. Intraoperative molecular analysis of total tumor load in sentinel lymph node: a new predictor of axillary status in early breast cancer patients. Breast Cancer Research and Treatment. 2013; 139: 87–93.

[20] Terrenato I, D'Alicandro V, Casini B, Perracchio L, Rollo F, De Salvo L, *et al*. A cut-off of 2150 cytokeratin 19 mRNA copy number in sentinel lymph node may be a powerful predictor of

non-sentinel lymph node status in breast cancer patients. PLoS ONE. 2017; 12: e0171517.
[21] van la Parra RFD, Peer PGM, Ernst MF, Bosscha K. Meta-analysis of predictive factors for non-sentinel lymph node metastases in breast cancer patients with a positive SLN. European Journal of Surgical Oncology. 2011; 37: 290–299.
[22] Van Zee KJ, Manasseh DE, Bevilacqua JLB, Boolbol SK, Fey JV, Tan LK, *et al*. A nomogram for predicting the likelihood of additional nodal metastases in breast cancer patients with a positive sentinel node biopsy. Annals of Surgical Oncology. 2003; 10: 1140–1151.
[23] Houvenaeghel G, Nos C, Giard S, Mignotte H, Esterni B, Jacquemier J, *et al*. A nomogram predictive of non-sentinel lymph node involvement in breast cancer patients with a sentinel lymph node micrometastasis. European Journal of Surgical Oncology. 2009; 35: 690–695.
[24] Mittendorf EA, Hunt KK, Boughey JC, Bassett R, Degnim AC, Harrell R, *et al*. Incorporation of sentinel lymph node metastasis size into a nomogram predicting nonsentinel lymph node involvement in breast cancer patients with a positive sentinel lymph node. Annals of Surgery. 2012; 255: 109–115.
[25] García-Mejido JA, Sánchez-Sevilla M, González-Martínez J., Fenández-Palacín A, Sainz-Bueno JA. ¿Pueden los factores clínicopatológicos mejorar la predicción de metástasis en ganglios linfáticos no centinelas en pacientes con cáncer de mama?. CIR. (in press)
[26] Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, *et al*. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2015; 26: v8–v30.
[27] Teramoto A, Shimazu K, Naoi Y, Shimomura A, Shimoda M, Kagara N, *et al*. One-step nucleic acid amplification assay for intraoperative prediction of non-sentinel lymph node metastasis in breast cancer patients with sentinel lymph node metastasis. Breast. 2014; 23: 579–585.
[28] Rubio IT, Espinosa-Bravo M, Rodrigo M, Amparo Viguri Diaz M, Hardisson D, Sagasta A, *et al*. Nomogram including the total tumoral load in the sentinel nodes assessed by one-step nucleic acid amplification as a new factor for predicting nonsentinel lymph node metastasis in breast cancer patients. Breast Cancer Research and Treatment. 2014; 147: 371–380.
[29] Shimazu K, Sato N, Ogiya A, Sota Y, Yotsumoto D, Ishikawa T, *et al*. Intraoperative Nomograms, Based on one-Step Nucleic Acid Amplification, for Prediction of Non-sentinel Node Metastasis and Four or more Axillary Node Metastases in Breast Cancer Patients with Sentinel Node Metastasis. Annals of Surgical Oncology. 2018; 25: 2603–2611.
[30] Di Filippo F, Giannarelli D, Bouteille C, Bernet L, Cano R, Cunnick G, *et al*. Elaboration of a nomogram to predict non sentinel node status in breast cancer patients with positive sentinel node, intra-operatively assessed with one step nucleic acid amplification method. Journal of Experimental & Clinical Cancer Research. 2015; 34: 136.
[31] Sa-nguanraksa D, O-charoenrat E, Kulprom A, Samarnthai N, Lohsiriwat V, Nimpoonsri K, *et al*. Nomogram to predict non-sentinel lymph node status using total tumor load determined by one-step nucleic acid amplification: first report from Thailand. Breast Cancer. 2019; 26: 471–477.
[32] Nabais C, Figueiredo J, Lopes P, Martins M, Araújo A. Total tumor load assessed by one-step nucleic acid amplification assay as an intraoperative predictor for non-sentinel lymph node metastasis in breast cancer. Breast. 2017; 32: 33–36.
[33] Fung V, Kohlhardt S, Vergani P, Zardin GJ, Williams NR. Intraoperative prediction of the two axillary lymph node macrometastases threshold in patients with breast cancer using a one-step nucleic acid cytokeratin-19 amplification assay. Molecular and Clinical Oncology. 2017; 7: 755–762.
[34] Heilmann T, Mathiak M, Hofmann J, Mundhenke C, van Mackelenbergh M, Alkatout I, *et al*. Intra-operative use of one-step nucleic acid amplification (OSNA) for detection of the tumor load of sentinel lymph nodes in breast cancer patients. Journal of Cancer Research and Clinical Oncology. 2013; 139: 1649–1655.
[35] Meretoja TJ, Leidenius MHK, Heikkilä PS, Boross G, Sejben I, Regitnig P, *et al*. International multicenter tool to predict the risk of nonsentinel node metastases in breast cancer. Journal of the National Cancer Institute. 2012; 104: 1888–1896.
[36] Degnim AC, Reynolds C, Pantvaidya G, Zakaria S, Hoskin T, Barnes S, *et al*. Nonsentinel node metastasis in breast cancer patients: assessment of an existing and a new predictive nomogram. American Journal of Surgery. 2005; 190: 543–550.
[37] Ozmen V, Karanlik H, Cabioglu N, Igci A, Kecer M, Asoglu O, *et al*. Factors predicting the sentinel and non-sentinel lymph node metastases in breast cancer. Breast Cancer Research and Treatment. 2006; 95: 1–6.
[38] Joseph K. Predictors of Nonsentinel Node Metastasis in Patients with Breast Cancer after Sentinel Node Metastasis. Archives of Surgery. 2004; 139: 648.