Sentinel node total tumour load as a predictive factor for non-sentinel node status in early breast cancer patients – The porttle study

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ABSTRACT

OSNA is a molecular assay for the detection of sentinel node metastasis. TTL emerged as a concept that seems to accurately predict the status of the NSN. Authors tried to confirm this motion.

This is a retrospective and multicentric study that analyzed 2164 patients, 579 of whom had positive SN and completion AD. Logistic regression models were performed in order to identify a suitable cutoff to identify patients who benefit from AD.

Univariate and multivariate regression analysis showed a relationship between TTL>30000 and the presence of NSN metastasis (OR 2.84, CI 1.99–4.08, $p < 0.001$). Logistic regression indicated that the cutoff of 30000 copies/μL better discriminates patients with NSN positivity and allows wide use of these criteria.

This cutoff value may safely assist clinicians and patients to decide to proceed or not with an AD.

1. Introduction

Lymph node staging (pN) is still considered an important prognostic factor in breast cancer patients. In recent years, this goal is achieved through sentinel lymph node (SN) biopsy and pathologic analysis. The finding of a negative sentinel node means a reliable pN0 patient [1]. In the case of a positive sentinel node, the next clinical step is being challenged [2–4].

In the last few years, a positive SN implies an axillary lymph node dissection (AD). But, in the majority of the patients, the non-sentinel axillary nodes (NSN) removed in this additional surgery are negative; in those cases, AD will be an useless procedure and the cause of important morbidity.

When the SN metastasis is of small volume (micrometastasis) there is no need to proceed to an AD; this option is supported by the observations of the IBCSG 23–01 trial [5]. But if the SN metastasis is a macrometastasis, the clinical practices diverge.

ACOSOG Z0011 results are being adopted by many leading cancer institutions and guidelines. Nevertheless, the trial does not apply to total mastectomy patients (that represents 40–50% of BC surgical treatment in many institutions) and the management of the trial, and its outcomes, are being questioned by many authors [6,7].

In the meantime, several institutions have developed, and applied, different nomograms or clinical decision rules to predict the risk of

Table 1
Demographic information.

	All cases n = 2164				SN positive plus Axillary Dissection cases n = 579			
	Mean	Median	Range	%	Mean	Median	Range	%
Cases per institution	271	246	18–606		72.4	84.5	8–111	
Age (y) (at diagnosis)	59.4	60	21–92		58.3	58	21–87	
Tumour size (mm)	23	18	1–110		22.4	20	1–80	
Number of SN excised	1.9	2	1–8		2.2	2	1–7	
Number of positive SN	0.6	0	0–5		1.5	1	1–5	
NSN excised	4.3	0	0–47		15	14	1–47	
Axillary dissection performed								
Yes				30.7%				100%
No				69.3%				0%
Micrometastasis (SN)				21.6%				27.1%
Macrometastasis (SN)				22.0%				72.9%
Type of Surgery								
Partial mastectomy				62.1%				49.1%
Total mastectomy				37.9%				50.9%
Number of positive SN								
0				56.4%				0%
1				31.9%				65.8%
2				9.0%				24.9%
3				2.0%				6.9%
4				0.6%				2.2%
5				0.1%				0.2%
Histologic type								
NST				78.5%				79.8%
Lobular				9.8%				11.6%
Medular				1.0%				0.9%
Micropapilar				0.8%				1.0%
Tubular				1.3%				0.5%
Metaplastic				0.1%				0%
Papilar				0.1%				0%
Other				8.1%				5.7%
Unknown				0.4%				0.5%
pT (TNM)								
Tis				0.6%				0%
T1a				5.5%				3.5%
T1b				16.8%				7.6%
T1c				45.3%				41.1%
T2				28.7%				42.5%
T3				2.0%				3.5%
Unknown				1.2%				1.9%
pN (TNM)								
0				54.7%				0%
1				39.2%				78.9%
2				5.0%				17.6%
3				0.9%				3.5%
Unknown				0.2%				0%
Grade								
1				24.3%				18.8%
2				51.8%				57.0%
3				19.6%				20.0%
Unknown				4.2%				4.1%
LVI observed								
Yes				19.3%				36.6%
No				75.2%				57.7%
Unknown				5.5%				5.7%
Multifocality/multicentricity								
Yes				17.6%				23.3%
No				77.9%				71.2%
Unknown				4.5%				5.5%
Estrogen receptors								
Positive				84.5%				86.9%
Negative				10.6%				8.6%
Unknown				4.9%				4.5%
Progesterone receptors								
Positive				72.3%				76.2%
Negative				23.2%				19.9%
Unknown				4.5%				4.0%
HER 2 status								
Positive				11.1%				13.3%
Negative				83.5%				81.3%
Inconclusive				2.4%				2.6%
Unknown				3.0%				2.8%
Received chemotherapy								

Table 1 (*continued*)

	All cases n = 2164				SN positive plus Axillary Dissection cases n = 579			
	Mean	Median	Range	%	Mean	Median	Range	%
Yes				48.8%				75.1%
No				50.7%				24.0%
Unknown				0.5%				0.9%
Received breast/thoracic wall radiotherapy								
Yes				58.9%				63.2%
No				23.5%				20.4%
Unknown				17.6%				16.4%
Received lymph drainage areas radiotherapy								
Yes				17.2%				46.9%
No				58.7%				28.2%
Unknown				24.1%				24.9%
Received Endocrine therapy								
Yes				73.2%				76.9%
No				9.5%				6.7%
Unknown				17.3%				16.4%
Tamoxifen				44.7%				42.6%
Anastrozole				19.8%				17.0%
Letrozole				30.4%				33.3%
Exemestane				1.7%				2.1%

SN-sentinel node; NSN-non sentinel node; NST-no special type; TNM-tumor, node, metastasis; LVI-lymph vascular invasion; HER 2-human epidermal receptor 2.

having positive NSN in the presence of a SN macrometastasis. Those tools incorporated many patient, cancer and SN variables, such as the size of the tumor, the presence of lymph-vascular invasion, the presence of multifocality, and so on [8,9].

The One Step Nucleic Acid Amplification (OSNA) is a molecular assessment for the presence of metastasis in the SN, based on the RT-PCR quantification of CK 19 mRNA copies. This emerging technique is being adopted by an increasing number of centers and has the advantage of being semi-quantitative, reproducible, standardized and of analyzing the whole SN [10]. The concept of Total Tumour Load (TTL) is being studied and it seems to accurately predict the status of the NSN, thus being another important tool for clinical decisions on pN1a breast cancer patients [11].

The aim of this study is to determine the predictive power of TTL to identify positive NSN patients.

Table 2

Non-Sentinel Node status according to SN metastasis size/volume.

OSNA assay	NSN positive n (%)	NSN negative n (%)	Total n (%)
Macrometastasis	202 (47.9)	220 (52.1)	422 (72.9)
Micrometastasis	37 (23.6)	120 (76.4)	157 (27.1)
Total	239 (41.3)	340 (58.7)	579

NSN-non sentinel node.

2. Methods

2.1. Type of study

This is an observational, retrospective and multicentric study, carried out in Portugal. We analyzed cases treated from September 2010 to December 2016 (76 months). Eight participating hospitals gathered information on 2164 consecutive c/uT1-3 c/uN0 invasive breast cancer patients, whose SN was analyzed by OSNA assay. Patients receiving neoadjuvant treatment were excluded. Variables collected were age at diagnosis, tumor size, grade and histologic type, multifocality, lymph-vascular invasion (LVI), type of surgical treatment, number of biopsied SN, number of positive SN, number of mRNA CK19 copies per SN, number of excised NSN in the axillary dissection, number of positive NSN, ER, PR and Her2 status, Total Tumor Load (TTL), adjuvant treatments, local, regional or distant recurrence and vital status.

2.2. Surgery and pathology

Sentinel nodes were identified under combined techniques, using Radioisotope plus Patent blue V dye or Indocyanine Green fluorescence plus Patent blue V dye or Superparamagnetic Iron Oxide. According to institutional guidelines, SN were analyzed immediately after biopsy, during the breast surgical procedure, or in a deferred way. Again, according to each institutional guidelines, micro or macrometastasis in the

Table 3

testing different tools to assist the clinical decision.

	HSJ-CDR 2012 [9]	Z0011 [2]	TTL 15000 [14]	CDR + TTL 15000	LVI + TTL 15000	TTL 20000	TTL 30000	TTL 50000	TTL 100000	TTL 200000
n	548	568	579	533	546	579	579	579	579	579
Number (%) of patients potentially benefiting from the decision criterion for AD vs no-AD	146 (27)	156 (27)	246 (43)	84 (16)	167 (31)	262 (45)	296 (51)	329 (57)	373 (64)	411 (71)
Sensitivity (%)	77,3	25,1	72,8	89,1	80,1	70,3	64,8	57,7	50,2	41,8
Specificity (%)	29,4	70,9	53,2	19,2	38,5	56,2	62,4	67,1	74,7	80
PPV (%)	43,3	37,8	52,3	43,7	47,8	53,0	54,8	55,2	58,3	59,5
NPV (%)	65,1	57,3	73,6	71,4	74,3	72,9	71,6	69,3	68,1	66,2
Accuracy (%)	49,1	51,9	61,3	48,0	55,9	62,0	63,4	63,2	64,6	64,3
AUC	0,534	0479	0,630	0536	0,591	0.632	0.636	0.624	0.625	0.609

HSJ-CDR-Hospital São João-Clinical Decision Rule; TTL-Total Tumor Load; LVI-lymph vascular invasion; AD-axillary dissection; PPV-positive predictive value; NPV-negative predictive value; AUC-area under the curve.

Table 4

Univariate and Multivariate analysis.

	Positive Non-Sentinel nodes		Univariate analysis		Multivariate analysis	
	No	Yes				
	n (%)	n (%)	OR (IC95%)	p	OR (IC95%)	p
Age (mean, years)	58,2	58,4	1,00 (0,99–1,01)	0,856		
Tumour size (path)						
< 2 cm	199 (58,5)	103 (43,1)	REF			
≥ 2 cm	134 (39,4)	132 (55,2)	1,90 (1,36–2,67)	<0,001	1,66 (1,15–2,38)	0,006
Unknown	7 (2,1)	4 (1,7)				
Lymphvascular Invasion						
No	213 (62,6)	121 (50,6)	REF			
Yes	109 (32,1)	103 (43,1)	1,66 (1,17–2,36)	0,004	1,36 (0,93–2,00)	0,112
Unknown	18 (5,3)	15 (6,3)				
Multicentricity/Multifocality						
No	247 (72,6)	165 (69,0)	REF			
Yes	74 (21,8)	61 (25,5)	1,23 (0,83–1,83)	0,293		
Unknown	19 (5,6)	13 (5,5)				
Tumour grade (path)						
I	77 (22,7)	32 (13,4)	REF			
II/III	252 (74,1)	194 (81,2)	1,85 (1,18–2,91)	0,008	1,69 (1,04–2,77)	0,036
Unknown	11 (3,2)	13 (5,4)				
Oestrogen receptors						
Negative	25 (7,3)	25 (10,5)	REF			
Positive	295 (86,8)	208 (87,0)	0,70 (0,39–1,26)	0,239		
Unknown	20 (5,9)	6 (2,5)				
Progesterone receptors						
Negative	65 (19,1)	50 (20,9)	REF			
Positive	258 (75,9)	183 (76,6)	0,92 (0,61–1,40)	0,701		
Unknown	17 (5,0)	6 (2,5)				
HER2						
Negative	259 (76,2)	212 (88,7)	REF			
Positive	54 (15,9)	23 (9,6)	0,52 (0,31–0,88)	0,014	0,45 (0,26–0,78)	0,004
Unknown	27 (7,9)	4 (1,7)				
Number of positive Sentinel Nodes						
≤ 2	315 (92,6)	210 (87,9)	REF			
> 2	25 (7,4)	29 (12,1)	1,74 (0,99–3,05)	0,054		
CK19 copy number classification						
ITC	7 (2,1)	2 (0,8)	REF			
Micrometastasis	113 (33,2)	35 (14,6)	1,08 (0,22–5,46)	0,922		
Macrometastasis	220 (64,7)	202 (84,6)	3,21 (0,66–15,65)	0,148		
Z0011						
No	236 (69,4)	176 (73,6)	REF			
Yes	97 (28,5)	59 (24,7)	0,82 (0,56–1,19)	0,290		
Desconhecido	7 (2,1)	4 (1,7)				
HSJ-CDR						
No	95 (27,9)	51 (21,3)	REF			
Yes	228 (67,1)	174 (72,8)	1,42 (0,96–2,11)	0,080		
Unknown	17 (5,0)	14 (5,9)				
TTL (CK 19 copy number)						
≤ 30000	212 (62,4)	84 (35,2)	REF			
> 30000	128 (37,6)	155 (64,8)	3,06 (2,16–4,31)	<0,001	2,84 (1,99–4,08)	<0,001

OR-odds ratio; HER 2-Human Epidermal Receptor 2; REF-reference; CK-cytokeratin; HSJ-CDR-Hospital São João-Clinical Decision Rule; TTL-total tumor load.

SN may imply a level I-II axillary dissection or the absence of axillary dissection. Non-Sentinel nodes were assessed by current histological and immunohistochemical methods. Molecular subtype was defined according to St Gallen's Consensus Conference [12].

Sentinel nodes were assessed by OSNA assay, as described elsewhere [11]. According to the number of calculated CK19 mRNA copies/μL the results are defined as "macrometastasis" (more than 5000 copies/μL, "micrometastasis" (250–5000 copies/μL) and "non-metastasis" (less than 250 copies/μL). The TTL was defined as the amount of CK19 mRNA (copies/μL) in all positive SN (including micrometastasis but not SN with less than 250 copies/μL) [10].

2.3. Statistics

Data were analyzed descriptively, with frequencies used for categorical variables and medians for continuous variables. Chi-square tests were used to compare patients with at least one positive NSN with patients having all NSN negative. An estimation of the area under the curve (AUC) and the ROC diagram were performed in order to identify the most accurate test and the TTL cut-off that maximized sensitivity and specificity to predict NSN positivity. Logistic regression was used to identify variables associated with positive NSN. Multivariate analysis included only significant variables ($p < 0.05$) on univariate analysis. Database and statistical analysis were done on IBM SPSS Statistics 24

Table 5

Testing the TTL concept.

Published paper	Patients studied (SN+ followed by AD)	AUC results
Espinosa Bravo 2013 [11]	108	0.714
Peg 2013 [14]	697	0.709
Deambrogio 2014 [33]	194	0.69
Rubio 2014 [34]	697 (definition series)	0.755
	436 (validation series)	0.678
Piñero Madrona 2014 [35]	797 (nomogram)	0.78
	797 (TTL only)	0.69
Kubota 2016 [36]	134	0.708
Di Fillipo 2016 [37]	1495 (unique SN highest number of copies)	0.65
Nabais 2017 [17]	58	0.805
Terrenato 2017 [26]	318 (unique SN highest number of copies)	0.765
Shimazu 2018 [38]	623 (to identify at least one NSN+)	0.70
	623 (to identify 4 + NSN+)	0.69
Sa-Nguanraksa 2019 [39]	89 (tumour size + TTL nomogram)	0.801
	89 (tumour size + LVI + TTL nomogram)	0.849
Present study	579 (TTL 15k)	0.630
	579 (TTL 30k)	0.636
	579 (TTL 200k)	0.609

SN-sentinel node; AD-axillary dissection; AUC-area under the curve; TTL-total tumor load; NSN-non sentinel node.

(Chicago, Illinois, USA) and Stata 15 (College Station, Texas, USA).

2.4. Ethical considerations

This study was approved by the Ethics Committee of each participating institution.

3. Results

The median follow-up time was 37 months (range: 1–76). The median number of patients included by each participating center was 246 (range: 18–606). Additional demographic information may be found in Table 1. From the total sample we selected every patient that had at least one positive SN and had been submitted to axillary dissection; all analysis were conducted on this sample of 579 patients.

Pathologic median tumor size was 18 mm (range: 1–110 mm). The median number of SN biopsied per patient was 2 (range: 1–8). Sentinel node metastasis was present in 44.8% of all the cases, of whom 30.7% were submitted to AD.

From our study sample of 579 SN positive patients, 157 (27.1%) had micrometastasis (CK19 mRNA copies/μL between 250 and 5000) and 422 (72.9%) had macrometastasis (CK19 mRNA copies/μL more than 5000). Among the micrometastasis group (n = 157) there were 37 (23.6%) with positive NSN whereas in the macrometastasis group (n = 422) there were 202 (47.9%) with positive NSN (p < 0.000) (Table 2).

We tried to assess different tests that best correlates and predicts the presence of additional positive NSN. In this way, we tested the results of the application of a Clinical Decision Rule that is being used from 2012 in one of the participating centers [13], the ACOSOG Z0011 criteria [2], the results of the SOLO study [14], proposing a cutoff value of 15000 copies, against different cutoff levels, such as 20000, 30000, 100000 and 200000 copies (Table 3). Considering the application of these criteria to a breast cancer population, in order to spare the most to an AD, we verified that the most restrictive ones were HSJ-CDR + TTL 15000 (patients having less than 15000 CK 19 copies, and a unifocal pT1 tumour without LVI), the Z0011criteria (pT1 tumor treated conservatively, with breast radiotherapy and systemic treatment and 1 or 2 positive SN) and the HSJ-CDR (patients having a unifocal pT1 tumour without LVI), that could be applied 16%, 27% and 27%, respectively (Table 3).

Using only the TTL as a predictor, the cut-off of 30000 copies was identified as the one that maximizes sensitivity and specificity to predict NSN positivity. (Graph 1).

Univariate logistic regression showed that tumor size (T1 vs T2-3), lymph vascular invasion, grade (1 vs 2–3), HER2 status and the TTL (=<30000 vs >30000 copies) were associated with the presence of additional metastasis in the non-sentinel lymph nodes (Table 4).

The multivariate logistic regression analysis showed that the TTL cutoff level of 30000 is an independent predictor of metastatic NSN, with an OR of 2.84 (p < 0.001), with balanced results in the binary classification tests and having a broad clinical application (Table 3, Table 4).
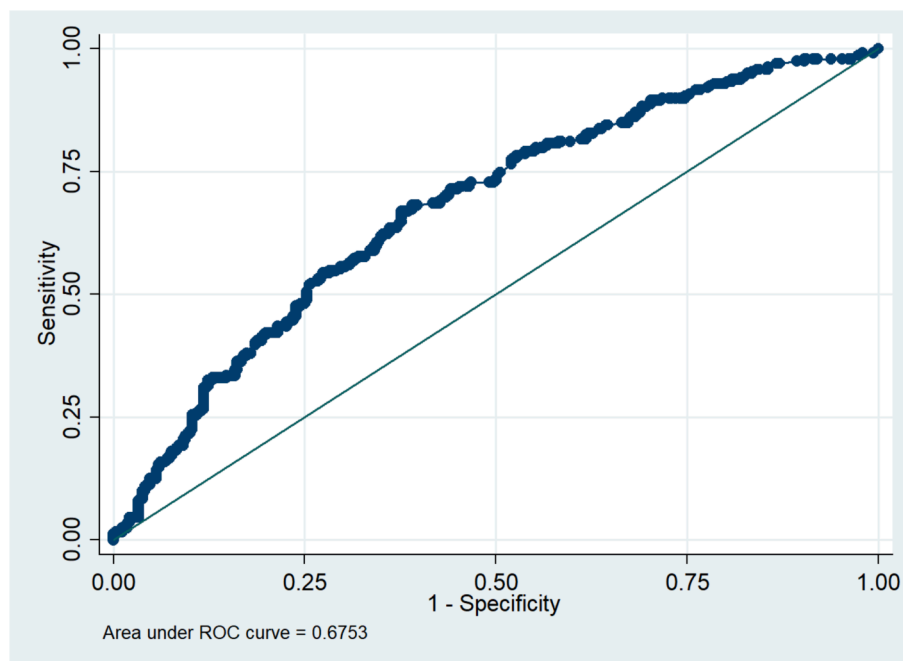

Fig. 1. Area under the curve (30000 copies/μL).

4. Discussion

OSNA is an accurate method for the detection of SN metastasis, either intra- or post-operative, that is being clinically applied from 2007 [10,15,16]. It determines quantitatively the amount of cytokeratin 19 mRNA copies present in the SN; these values correlates to the absence of metastasis (less than 250 copies/μL), to the presence of micrometastases (250–5000 copies/μL) or the presence of macrometastasis (more than 5000 copies/μL).

Total tumor load in OSNA-analyzed SN is a concept that was defined in 2013, and several studies that relate TTL to NSN metastasis can be traced from then [11,14,17].

Since the beginning of the development of the SN concept, authors realize that only 40–60% of the SN positive patients will have additional NSN metastasis [18,19]. Due to that finding, countless research projects and papers addressed that theme, trying to identify variables strongly related to NSN positivity or creating mathematical tools to help surgeons and patients to decide whether to do an AD [8,9,20–22].

The size of the SN metastasis has been ubiquitous among those studies, either in its area or in its volume meaning [23]. Other variables showed a deep relation to NSN metastasis, such as the size of the primary tumor and the presence of multifocality or LVI [9,20].

Nevertheless, the use of SN-dependent variables has been questioned [9], mainly because of the high inter-observer variability in the traditional H-E SN assessment, as well as in the interpretation of the SN concept.

The use of the OSNA assay has emerged as an automated, uniform, standardized and reproducible method, which analyses the whole SN and quantifies the tumor load in every SN. So, it turns to be the ideal method to deliver SN-dependent results that could be applied in the prediction of NSN positivity.

Several authors studied and confirmed the strong relationship between the tumor load (CK19 number of copies/μL) in the SN and the presence of metastatic NSN (Table 5), but the concept of TTL brings a more comprehensive approach to the issue.

Our results agree with those. The concept of TTL is predictive by itself and in the context of the 30000 copies cut-off.

Typically, in these types of predictive studies, to define the risk of metastasis in NSN, the authors value NPV and FN rate more, so as not to risk leaving metastatic lymph nodes in otherwise surgically untreated (no AD) axilla.

However, nowadays, every pN + breast cancer patient should receive adjuvant systemic chemo and/or endocrine treatment and, in some cases, the regional nodes will, advisedly, receive external radiation treatment. Besides that, currently, breast centers add up an axillary ultrasound to the initial clinical staging procedure (and, in some cases, a breast MRI), that excludes gross axillary metastatic involvement. This means that, if left untreated, axillary nodal metastases will be mostly of small volume and will be targeted by the systemic or radiotherapy treatment. Even more, we know from previous studies that the clinical impact of regional recurrence is lower than expected [4,24,25].

Interestingly, Terrenato et al. [26] proposed a cut-off of only 2150 mRNA CK19 copies/μL to decide to complete an AD. This cut-off yields a FN rate of only 1.6%, but at the expense of a higher FP rate, which means that many patients will have AD without any clinical benefit and encompassing a smaller group of patients.

Nowadays, the focus should be "in what cases should we do an AD" instead of "in what cases can we omit an AD", valuing more the PPV and the FP rate. It is well known the impact of the long-term morbidity of the Axillary Dissection and the breast surgical oncologist should be aware of it [27,28].

Our results suggest that a TTL cut-off of 30000 mRNA CK19 copies/μL to decide to complete an AD is safe and balanced, with an accuracy rate of 63.4% and a PPV of 54.8%, covering almost half of the cases in any given breast cancer center.

Using TTL as the sole criterion to decide to perform or not an AD makes the decision process easier. It does not depend on other variables, some of them may not be available at the time of the operation, nor on complex nomograms. The more variables needed to decide, the more restrictive the process will be, as fewer patients will be covered, so diminishing its clinical interest.

One advantage of the OSNA assay is that its definitive results may be known intra-operatively and this allow the surgical team to decide whether to complete the AD [29,30]. Some studies addressed the time needed for the result issuance, defined as 30–40 min from the excision of the SN [31] and others the costs of having a second, deferred, operation for AD [32]. So, TTL may be known, and used, during the primary breast tumor operation.

A limitation of the present study is its retrospective and multicentric design. Nevertheless, these features give the study a real-world evidence. Moreover, our AUC results (0.636) fall into the "poor discrimination" category, which compares poorly with the results depicted in Table 5.

In this cohort of 2164 patients, 579 (26.8%) were submitted to AD; of these, only 239 (11.0%) may have benefited from the procedure, from the curative or the staging point-of-view. Together with other patient and tumour features, the authors propose the use of a TTL cutoff of 30000 mRNA CK19 copies/μL to decide to complete an AD, in order to try to achieve a maximum of 10% complete axillary dissections within an early breast cancer population(See. Fig. 1).

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