Original Article

Breast cancer lymphoscintigraphy: Factors associated with sentinel lymph node non visualization

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A B S T R A C T

Objective: To evaluate factors associated with non identification of the sentinel lymph node (SLN) in lymphoscintigraphy of breast cancer patients and analyze the relationship with SLN metastases.

Material and methods: A single-center, cross-sectional and retrospective study was performed. Forty patients with lymphoscintigraphy without sentinel lymph node identification (negative lymphoscintigraphy – NL) were enrolled. The control group included 184 patients with SLN identification (positive lymphoscintigraphy – PL).

Evaluated factors were age, body mass index (BMI), tumor size, histology, localization, preoperative breast lesion hookwire (harpoon) marking and SLN metastases.

The statistical analysis was performed with uni- and multivariate logistic regression models and matched-pairs analysis.

Results: Age (p = 0.036) or having BMI (p = 0.047) were the only factors significantly associated with NL. Being ≥ 60 years with a BMI ≥ 30 increased the odds of having a NL 2.3 and 3.8 times, respectively. Marking with hookwire seems to increase the likelihood of NL, but demonstrated statistical significance is lacking (p = 0.087). The other tested variables did not affect the examination result. When controlling for age, BMI and marking with the harpoon, a significant association between lymph node metastasis and NL was not found (p = 0.565).

Conclusions: The most important factors related with non identification of SLN in the patients were age, BMI and marking with hook wire. However, only the first two had statistical importance. When these variables were controlled, no association was found between NL and axillary metastases.

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Linfogammagrafía en el cáncer de mama: factores asociados con la no visualización del ganglio centinela

R E S U M E N

Objetivo: Evaluar los factores relacionados con la no identificación del ganglio centinela (GC) en la linfogammagrafía de pacientes con cáncer de mama y su relación con metástasis en el GC.

Material y métodos: Se realizó un estudio unicéntrico, transversal y retrospectivo. Se seleccionaron 40 pacientes con linfogammografías sin identificación de GC (linfogammagrafía negativa - LN). El grupo control incluyó 184 pacientes con identificación del GC (linfogammagrafía positiva - LP).

Los factores evaluados fueron: edad, índice de masa corporal (IMC), tamaño, histología, localización, marcación preoperatoria del tumor con arpón e metástasis en el GC. El análisis estadístico se realizó mediante modelos de regresión logística univariante y multivariante y análisis pareado.

Resultados: La edad (p = 0.036) y el IMC (p = 0.047) fueron los únicos factores asociados significativamente con LN. Tener edad ≥ 60 años o IMC ≥ 30 elevaron la probabilidad de tener una LN 2 y 3.8 veces, respectivamente.

La marcación con arpón parece aumentar la probabilidad de LN, pero sin significación estadística (p = 0.087). Las otras variables no influyeron el examen. Al controlar edad, IMC y marcación con arpón, no se encontró una asociación significativa entre metástasis de ganglios linfáticos y LN (p = 0.565).

Conclusión: Los factores más importantes relacionados con la no identificación del GC en las pacientes fueron edad, IMC y marcación con arpón, pero, sólo los dos primeros tuvieron relevancia estadística. Cuando se controló estas variables, no se encontró asociación entre LN y metástasis axilares.

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Introduction

It is widely accepted that sentinel lymph node (SLN) biopsy is an important procedure for the correct staging of patients with breast cancer. Furthermore, SLN biopsy allows a minimally invasive surgery, reducing the co-morbidities associated with axillary lymph node dissection. Lymphoscintigraphy is a simple and accurate method that helps identifying the sentinel lymph node.1–3

In a minority of cases lymphoscintigraphic detection of SLN is not possible. Failure to visualize a SLN increases the difficulty of surgical intervention.2,4 In some patients the hidden SLN will be intra-operatively detected, either by gamma-probe alone or by gamma-probe combined with blue dye. When SLN is not found, axillary lymph node dissection is recommended.5

Some authors argue that the main reason for SLN non identification is SLN metastatization because lymphatics may be blocked by cancer cells, not allowing colloid progression through lymphatic channels.1,6 On the other hand, there are studies that did not find significant association between negative lymphoscintigraphy and axillary metastases.5 During the past decade several other factors have been reported as being related to the failure of SLN identification in lymphoscintigraphy, namely, patient’s age and body mass index (BMI), as well as tumor characteristics.

In our tertiary cancer center we perform around 570 lymphoscintographies in breast cancer patients per year. The scintigraphic SLN identification rate in our department is 98% and the concordance of SLN detection both in lymphoscintigraphy and surgery is 95%.

The primary goal of this retrospective study was to analyze which factors could influence the lymphoscintigraphic SLN non identification and to study each factor separately. As a secondary analysis, we wanted to establish the relationship between lymphoscintigraphic failure in detecting the SLN and the existence of SLN metastases, by controlling for the previously defined variables that could influence the examination result.

Material and methods

We reviewed all lymphoscintographies performed in breast cancer patients between January 2010 and December 2013, corresponding to a total of 2200 examinations.

Lymphoscintigraphy was classified as:

- Positive lymphoscintigraphy (PL) – lymphoscintigraphy with SLN identification.
- Negative lymphoscintigraphy (NL) – lymphoscintigraphy without SLN identification.

During this period we found 40 NL and from the remaining large amount of PL, we only included 184 PL in our study. The PL group was considered as a “control group” and corresponded to all patients with SLN identification in lymphoscintigraphy consecutively performed between January and June 2011. This period was randomly selected, because the technique was similar between 2010 and 2013.

Inclusion and exclusion criteria:

We included all female patients, with unilateral breast carcinoma and without previous chemotherapy that performed lymphoscintigraphy in our department.

Lymphoscintigraphy was performed in patients with histological confirmation of invasive carcinoma or in situ carcinoma with high or intermediate grade, necrosis or other aggressive characteristics and that had no histological evidence of axillary metastases.1,4,7

Our study excluded patients with bilateral breast cancer, male patients and patients treated with neoadjuvant chemotherapy prior to lymphoscintigraphy, due to the small number of patients in these groups. We wanted to have a homogenous study group, avoiding extra factors that could influence the results.

Patients with very early stage breast cancer (low-grade ductal carcinoma in situ) who were not proposed to mastectomy, inflammatory breast cancer, histologically confirmed positive axillary or extra-axillary lymph nodes or patients with widespread metastases beyond surgical resection did not perform lymphoscintigraphy.

Lymphoscintigraphy and surgical technique of the sentinel lymph node:

All examinations were performed in our department using a subareolar injection of 55.5 MBq (1.5 mCi) of 99mTc-albumin nanocolloid, 0.5 mL, in the same quadrant of the breast lesion.

Immediately after the injection sequential anterior, lateral and antero-oblique planar images, with the patient in supine position, were acquired using a dual-head Siemens E. Cam® or Philips Brightview® gamma-cameras with low-energy and high-resolution collimators. The first axillary hotspot identified in the lymphoscintigraphic image was considered the SLN and its projection was marked in the skin using a point source of 99mTc and further confirmed with a gamma-probe (Eurorad Europrobe®).

When SLN was identified in lymphoscintigraphy, it was considered PL.

When the SLN was not visible in the first set of images, patients were encouraged to do breast massage and image acquisition was sequentially repeated until up to 4h after injection.

In our department we did not inject a second dose of radio-pharmaceutical because of time and logistic aspects. In the period of time included in our study we did not have SPECT/CT available.

When SLN was not identified in lymphoscintigraphy, it was considered NL.

Usually, lymphoscintigraphy and surgery took place on the same day. In the operating room and after anesthesia, the surgeon injected blue dye in the upper-external breast quadrant. SLN biopsy was guided by our cutaneous mark and the removed SLN was the lymph node with highest counts shown by the gamma-probe (Eurorad Europrobe®), the majority marked with blue dye. Occasionally, more than one lymph node was resected, namely when there were other lymph nodes with >10% of the SLN greatest activity and when clinically suspicious lymph nodes were found. The lymph nodes removed were measured with gamma detector probe to confirm that they were responsible for the activity detected during lymphoscintigraphy and surgery. After resection, the axillary region was explored with the gamma-probe to confirm that there was only residual radioactivity.

The SLN was separated and carefully identified, putted in formal and sent to the Department of Pathology, where the SLN protocol was performed. First a macroscopic study of the lymph node was made, then it was sliced following the longitudinal/vertical axis into approximately 2 mm serial sections. Afterwards the lymph node was fixed in paraffin blocks. Three-micro sections of each block were obtained, stained with hematoxylin–eosin and finally examined under the microscope. Immunohistochemical study was used in dubious cases.

Parameters analyzed:

We retrospectively collected information from the patients' medical records concerning the factors referred in the literature as being associated with SLN identification failure in
lymphoscintigraphy. The seven factors included in our study and the parameters analyzed for each factor, were:

1. patient age: <60 vs. ≥60 years-old;
2. body mass index (BMI): <30 vs. ≥30;
3. breast carcinoma size according to AJCC Cancer Staging Manual, 7th edition classification: T1a/b/c/ml, T2 or T3;
4. histologic type according to AJCC Cancer Staging Manual, 7th edition classification: invasive ductal carcinoma (IDC), intermediate or high grade ductal carcinoma in situ (DCIS G2/G3) or invasive carcinoma;
5. breast tumor localization: external quadrants, internal quadrants or other;
6. pre-operative hookwire marking of the breast lesion before lymphoscintigraphy: yes or no;
7. SLN metastases (both macro and micrometastases were considered SLN metastatization): yes or no.

### Statistical analysis

Clinical and demographic characterization was performed using descriptive statistics.

We tested for any association between SLN non detection in lymphoscintigraphy and all the factors previously mentioned by using both univariate and multivariate logistic regression. Multivariate analysis considered all factors for which an association with SLN non detection was identified in univariate analysis (p-value below 0.10 in the likelihood ratio test). In the secondary analysis, in which the predictor of main interest was SLN metastases, the association with SLN non detection in lymphoscintigraphy was evaluated adjusting for all relevant factors identified in the previous analysis.

To compare the SLN metastatization in the group of cases and in the group of controls, we also conducted a matched pairs analysis by matching each case with two control subjects of similar age (<60 vs. ≥60 years old) and BMI (<30 vs. ≥30). Matching was performed using random selection methods and was done by a third person who was blinded to the SLN metastatic status of the patients in the control group. The matched samples were then compared using Mantel–Haenszel method for multiple matched controls per case.

Statistical significance was assessed by two-tailed tests with an alpha level of 0.05, unless otherwise specified. All of the analysis were done with R software.

### Results

The study included 40 patients with negative lymphoscintigraphy and 184 patients with positive lymphoscintigraphy (SLN non detection rate was 2% in lymphoscintigraphy).

In spite of the different number of patients included in each group, both groups were similar in terms of BMI, tumor size, localization, histology and lymph node metastases. This means that the majority of patients had a BMI <30, the most common breast cancer was an invasive ductal carcinoma, T1, located in the external breast quadrants and the majority of patients had no axillary metastatization, as determined by histology.

When compared to PL group, the NL group had a higher percentage of patients older than 60 years and the majority of these patients had tumor breast hookwire marking.

The clinical and demographic characteristics of each group of patients are presented in Table 1.

The univariate analysis showed that the only factors with statistical significance associated with NL were age and BMI (p-values of 0.036 and 0.047, respectively). The hookwire mark seemed to have influence in the NL group (p-value = 0.887).

These results were further explored with multivariate analysis. The multivariate analysis showed that, controlling for the remaining variables, the odds of having a NL was 2 times higher in women older than 60, compared to the ones younger than 60 years (OR = 2.02, 95%CI = 0.96–4.24, p-value = 0.065), and 3.8 times higher in obese women (BMI ≥30) when compared to non obese (OR = 3.8, 95%CI = 1.10–13.01, p-value = 0.034). Hookwire mark of the breast tumour seemed to raise the odds of having a NL, nevertheless statistical significance could not be demonstrated when considering a significance value of 0.05 (OR = 1.69, 95%CI = 0.82–3.50, p-value = 0.156). The remaining factors (size, histology, localization and SLN metastases) did not seem to influence SLN identification.

The analysis regarding the association between lymph node metastatization and NL did not show significance when controlling for BMI, age and hookwire mark (OR = 1.29, 95%CI: 0.55–3.03, p = 0.565).

### Table 1

<table>
<thead>
<tr>
<th>Clinical and demographic characteristics of the two groups: negative lymphoscintigraphy (NL) and positive lymphoscintigraphy (PL) patients.</th>
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</thead>
<tbody>
<tr>
<td>Factors</td>
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<tr>
<td>Hookwire</td>
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<tr>
<td>No</td>
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<tr>
<td>Age</td>
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<tr>
<td>≥60 years</td>
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<tr>
<td>Body mass index</td>
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<td>Tumor size (TNM)</td>
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<td>T3</td>
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<td>Tumor histology</td>
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<td>DCIS</td>
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<td>IC</td>
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<td>Tumor localization</td>
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<tr>
<td>Internal quadrant</td>
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<tr>
<td>Other</td>
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<tr>
<td>SLN metastases</td>
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<td>No</td>
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Legend: n – number of patients; IDC – invasive ductal carcinoma; DCIS – intermediate or high grade ductal carcinoma in situ; IC – invasive carcinoma; SLN – sentinel lymph node.
In the matched pairs analysis the percentage of lymph node metastases was 23% in the case group (NL) and 19% in the control group (PL) and the estimated odds ratio was 1.33 (p-value = 0.607). Similarly to the multivariate analysis, these results also suggest that patients with NL may have an increase in the odds of SLN metastases compared to patients with PL, but no statistical significance was demonstrated.

Discussion

Since 2001 many factors have been described as being associated with the failure of SLN detection in lymphoscintigraphy (Table 2).

In spite of each paper describing the main factor that influenced the lymphoscintigraphy result in their study, generally they did not compare two groups of patients with similar characteristics (a control group was not defined) and did not analyze the axillary involvement after controlling the other factors that might influence the sentinel lymph node identification during lymphoscintigraphy, thus the possibility of confounding bias cannot be excluded. There is lack of information about the specific influence of each factor.

Our study selected factors related to patients and to tumor characteristics, which can influence the lymphoscintigraphy result. Besides, we also considered the presence or absence of axillary lymph nodes metastases. We analyzed each factor separately and, finally, controlling the most influencing variables, we studied the probability of axillary metastases. The obtained results were confirmed through uni- and multivariate analysis and case-control study, which strengthens the results.

From the seven factors studied, only age and BMI were significantly associated with NL in univariate analysis. The hookwire marking of the breast lesion prior to lymphoscintigraphy may have some impact, because of a tendency to increase the odds of having a NL was verified, however without any statistical confirmation. The other factors did not seem to influence the lymphoscintigraphy result.

In terms of older age influencing the non identification of the SLN, this result is in accordance with other studies\(^9,10,14\) that reported that older woman had an odds ratio of 1.07 of failure to identify the SLN in lymphoscintigraphy, while in our study the odds ratio was about 2. In elder people lymph nodes fat degeneration increases, lymphatic flow slows and radioactive colloid recognition and phagocytosis by macrophages or histiocytes within the lymph node may be reduced. These factors decrease the node’s capacity to retain the radioactive colloid.

Concerning the influence of high BMI in the non visualization of the SLN, our results are similar to those\(^5,12,14,41\) that reported a higher risk of failure to identify the SLN when BMI was higher than 28 (in our study we found an increasing risk when BMI was superior to 30).

The association between breast hookwire marking and failure of lymphoscintigraphic mapping existed in our study. The same result, but with statistical significance, was shown by Pritsivelis et al.\(^5\) They consider that an interval between breast hookwire marking and lymphoscintigraphy should be respected, to reduce the inflammation, edema and hematoma related to the procedure. Other aspect that can be related is the radiopharmaceutical injection site. Radiochemical migration might be reduced if the injection is intra or peri-tumoral, which means that it is near the hookwire mark. Therefore, it is important to do lymphoscintigraphy before hookwire marking.

In spite of tumor size, histology and localization being described in literature as common factors associated with NL, in our study we did not found statistical evidence regarding these factors. This may be due to the fact that the majority of the breast lesions were...
invasive ductal carcinoma (about 80%), T1 (about 68%) and were located in the external quadrants (about 60%). Furthermore, the percentage distribution between the PL and NL groups was very similar.

Despite current theories stating that there is a connection between NL and axillary lymph nodes metastasization, in our study, there was no significant difference between PL and NL groups, when controlling for the factors age, BMI and hookwire marking. We believe that this statistical methodology of controlling the most important factors that influence the examination results can avoid biases, and consequently, allows a more accurate analysis.

Some studies suggest that when there is no evidence of SLN, a second radiopharmaceutical injection at a different site\cite{16,17} or a hybrid SPECT/CT study\cite{7,18} should be performed. These procedures increase the SLN identification rate. As previously said, there are aspects related to patients that can influence the SLN in lymphoscintigraphy and, in these specific patients, one should bear in mind complementary procedures (second injection and SPECT/CT) to overcome this limitation.

Even considering the fact of being a retrospective study, our analysis included a large cohort of patients, a rigorous statistical analysis was applied and it was performed in a reference cancer center where protocols are defined and fully adhered to. Some of the published studies had a low SLN detection rate in lymphoscintigraphy (<90%), but the detection rate in our study was high (98%). All these aspects increase the confidence in our results.

Although SLN non identification is a rare situation, we stress the clinical impact of our results, mainly because they alert to the existence of factors, other than lymph node metastases, associated with a negative lymphoscintigraphy and influencing this result. Obviously, these factors cannot be assumed as contraindications for the examination, but they should be taken into account when nuclear medicine physician interprets lymphoscintigraphy and alternative strategies should be used when necessary (second injection, SPECT/CT, etc.). These factors may not only be associated to pathological characteristics of the cancer but, even more important, be related to patient characteristics, which are easily detected. In addition, these results express why it is advisable to perform lymphoscintigraphy before breast hookwire marking.

**Conclusions**

Our results show that there may be factors directly related to SLN non visualization in lymphoscintigraphy, namely the patient’s age, body mass index and breast cancer hookwire marking prior to lymphoscintigraphy.

When these factors were controlled, we did not find statistical evidence in the association between negative lymphoscintigraphy and lymph node metastases. Therefore, the empirical association between lymph node identification failure in lymphoscintigraphy and axillary metastasization should be considered with caution.

**Conflict of interest**

The authors declare no conflict of interest.

**References**