Change of attitude in the surgical treatment of early breast cancer

Ph.D. Dissertation

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I. **Takács Tibor**, Szentpáli Károly, Paszt Attila, Ormándi Katalin, Lázár Máté, Pálka István, Kahán Zsuzsa, Lázár György: Az őrszem (sentinel) nyirokcsomó jelentősége in situ emlőcarcinoma sebészi kezelésében. *Magyar Onkológia* 2006; 50:247–51.

II. **Tibor Takács**, Attila Paszt, Károly Szentpáli, Katalin Ormándi, Máté Lázár, István Pálka, Zsuzsanna Kahán, György Lázár: Importance of Sentinel Lymph Node Biopsy in Surgical Therapy of in situ Breast Cancer. *Pathology & Oncology Research* 2009; 15:329-33. **IF: 1,152**

III. **Tibor Takács,** Attila Paszt, Zsolt Simonka, Szabolcs Ábrahám, Bernadett Borda, Aurél Ottlakán, Katalin Ormándi, Máté Lázár, András Vörös, Zsuzsanna Kahán, György Lázár: Radioguided occult lesion localization versus wire-guided lumpectomy in the treatment of non-palpable breast lesions. *Pathology & Oncology Research* 2013 19: 267-73. **IF:1.366**

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I. G. Cserni, G. Boross, R. Maráz, M.H.K. Leidenius, T.J. Meretoja, P.S. Heikkila, P. Regitnig, G. Luschin-Ebengreuth, J. Zgajnar, A. Perhavec, B. Gazic, G. Lázár, **T. Takács**, A. Vörös, R.A. Audisio: Multicentre validation of different predictive tools of non-sentinel lymph node involvement in breast cancer. *Surgical Oncology-Oxford* 2012; 21: 59-65. **IF: 2.444**

II. Gábor Cserni, Rita Bori, Róbert Maráz, Marjut H.K. Leidenius, Tuomo J. Meretoja, Paivi S. Heikkila, Peter Regitnig, Gero Luschin-Ebengreuth, Janez Zgajnar, Andraz Perhavec, Barbara Gazic, György Lázár, **Tibor Takács**, András Vörös, Riccardo A. Audisio: Multi-Institutional Comparison of Non-sentinel Lymph Node Predictive Tools in Breast Cancer Patients with High Predicted Risk of Further Axillary Metastasis *Patholologgy & Oncology Research* 2013; 19: 95-101. **IF:1.366**

III. Tuomo J. Meretoja, Marjut H.K. Leidenius, Päivi S. Heikkilä, Gábor Boross, István Sejben, Peter Regitnig, Gero Luschin-Ebengreuth, Janez Zgajnar, Andraz Perhavec, Barbara Gazic, György Lázár, **Tibor Takács**, András Vörös, Zuhair A. Saidan, Rana M. Nadeem, Isabella Castellano, Anna Sapino, Simonetta Bianchi, Vania Vezzosi, Emmanuel Barranger, Ruben Lousquy, Riccardo Arisio, Maria Pia Foschini, Shigeru Imoto, Hiroshi Kamma, Tove F. Tvedskov, Niels Kroman, May-Brit Jensen, Riccardo A. Audisio, Gábor Cserni: International Multicenter Tool to Predict the Risk of Nonsentinel Node Metastases in Breast Cancer. *Journal of the National Cancer Institute* 2012; 104: 1888-96. **IF:13.757**

IV. Tuomo J. Meretoja, R.A. Audisio, P.S. Heikkilä, R. Bori, I. Sejben, P. Regitnig, G. Luschin-Ebengreuth, J. Zgajnar, A. Perhavec, B. Gazic, G. Lázár, **T. Takács,** B. Kővári, Z.A. Saidan, R.M. Nadeem, I. Castellano, A. Sapino, S. Bianchi, V. Vezzosi, E. Barranger, R. Lousquy, R. Arisio, M.P. Foschini, S. Imoto, H. Kamma, T.F. Tvedskov, M.B. Jensen, G. Cserni, M.H.K. Leidenius: International multicenter tool to predict the risk of four or more tumor-positive axillary lymph nodes in breast cancer patients with sentinel node macrometastases. *Breast Cancer Research and Treatment* 2013; 138: 817-27. **IF:5.87**

List of abstracts related to the subject of the dissertation

1. MST kongresszus Budapest - 2006: Az őrszem (sentinel) nyirokcsomó jelentősége ductalis in situ emlőcarcinoma sebészi kezelésében. **Takács T.**, Szentpáli K., Paszt A., Lázár Gy.

2. II. Emlőrák szimpózium Szeged – 2007 Az őrszem (Sentinel) nyirokcsomó jelentősége in situ emlőcarcinoma sebészi kezelésében. **Takács T.**, Paszt A., Szentpáli K., Ormándi K., Lázár M., Lázár Gy.

3. MST Dél-Magyarországi Csoportjának tudományos ülése – 2008 Kecskemét Prognosztikai faktorok szerepe invasiv emlőcarcinomák hónalji nyirokcsomó metastasis képződésében. **Takács T.,** Paszt A., Simonka Zs., Török K., Lázár Gy.

4. III. Emlőrák szimpózium Szeged – 2009: Prognosztikai faktorok szerepe invasiv emlőrákok hónalji nyirokcsomó metastasis képződésében. **Takács T.,** Paszt A., Simonka Zs., Lázár Gy.

5. MST kongresszus – 2012: A radioizotópos és a dróthorog jelöléses lokalizálási módszer összehasonlítása nem tapintható emlőtumorok sebészi kezelésében **Takács T.,** Paszt A., Simonka Zs., Ábrahám Sz., Borda B., Ormándi K., Lázár M., Vörös A., Kahán Zs., Lázár Gy.

Abbreviations

ALND: Axillary Lymph Node Dissection

AUC: Area Under the Curve

BCS: Breast Conservative Surgery

CNB: Core-Needle Biopsy

DCIS: Ductal Cancer In Situ (of the breast)

DCISM: Ductal Cancer In Situ (of the breast) with Microinvasion

ER: Estrogen Receptor

FNAC Fine-Needle Aspiration Cytology

GWL: Guidewire Localization

HE: Hematoxylin-Eosin

HER-2: Human Epidermal Growth Factor Receptor-2

IDC: Invasive Ductal Carcinoma

IHC: Immunohistochemistry

ILC: Invasive Lobular Carcinoma

ITC: Isolated Tumor Cells

LCIS: Lobular Cancer In Situ (of the breast)

LVI: Lymphovascular Invasion

NSLN: Non-Sentinel Lymph Node

PR: Progesterone Receptor

ROLL: Radioguided Occult Lesion Localization

ROC: Receiver Operating Characteristic

RSL: Radioguided Seed Localization

SLN: Sentinel Lymph Node

SLNB: Sentinel Lymph Node Biopsy

Introduction

The extensive use of mammography for screening has resulted in the recognition of increasing numbers of malignant or malignancy-suspicious breast tumors in an early stage. Consequently, the ratio of non-palpable lesions has increased among early stage breast tumors; these lesions are detected during routine mammographic screening first[1]. In parallel with the extensive use of mammographic screening, the surgical treatment has changed as well: conservative breast surgery (quadrantectomy, lumpectomy, excision of the tumor) has replaced the earlier radical breast surgery, and axillary lymph node dissection (ALND) has likewise been replaced by sentinel lymph node biopsy (SLNB), which is currently the established method with which to assign the axillary lymph node status in early breast cancers.[2,3]

Basically, there are two methods used to localize non-palpable breast tumors. The method of guidewire localization (GWL) described by Kopans in 1980 has been used for decades in the preoperative localization of non-palpable breast lesions[4]. The radioguided occult lesion localization (ROLL) method was developed by Luini and colleagues in the Institute of Oncology in Milan and is used since 1996[5]. This method has become a standard marking procedure; however, GWL is still used by several Institutions.

For a century, ALND has been an essential component of the surgical treatment of invasive breast cancer. Axillary node status is one of the most important prognostic indicators in breast cancer, and of particular value in the choice of adjuvant therapy.[6,7] SLNB has been developed as a minimally invasive diagnostic procedure for the accurate preoperative staging of the axilla and ALND could be avoided if the patients do not have metastases in the Sentinel Lymph Nodes (SLNs). The technique was first used by Morton and colleagues with blue dye [8,9] and later by van der Veen and colleagues [10] with lymphoscintigraphy in the treatment of patients with melanoma. Similarly, this method can be applied in the treatment of breast cancers in early stage. If the SLN can be accurately identified, and the histological examination reveals no metastasis in the SLNs, the other nodes (non-SLNs - NSLN) in the axilla are unlikely to contain metastases and the unnecessary ALND can be avoided.[11]

Current practice guidelines recommended the ALND for breast cancer patients whose SLN contains metastatic cancer.[12,13,14] It is important to know that 40% to 70% of patients with positive SLNs are found to have no other NSLN metastases.[15,16] Therefore, these patients undergo unnecessary ALND without therapeutic benefit or additional information for the staging. Furthermore, completion ALND is associated with substantial morbidity affecting up to 39% of patients, with a nearly three-fold increased risk of lymphedema or regional sensory loss.[17,18] Based on these, it would be important to create a predictive model detecting patients who might benefit from ALND in case of SLN metastasis of the invasive breast tumor and this would provide additional information regarding tumor staging and would have additional therapeutic benefit as well.

In addition to early stage invasive breast tumors, the importance of in situ ductal cancers has increased. Before the widespread introduction of mammographic screening, only 1-2% of the recognized breast cancers comprised DCIS (Ductal Cancer in situ of the breast), but at present, the rate of detection by mammographic screening of non-palpable breast cancers is approximately 20%.[19,20] DCIS is defined as a non-invasive breast cancer, and is widely considered not to give metastases to the lymph nodes, so that ALND would comprise overtreatment.[20,21] Nonetheless, a number of studies have reported the detection of metastases in SLNs in patients with DCIS, though with a very low incidence. [22,23,24,25,26,27,28,29,30] In situ breast cancers require a novel approach in planning the surgical treatment as well, which suggests that SLNB may be omitted in such cases.

The purpose of our study

I. Comparing the methods of localization of non-palpable breast tumors (ROLL, GWL) (**Study 1)**.

II. Finding factors influencing NSLN metastasis, creating predictive nomograms, and comparing them with international nomograms **(Study 2)**.

III. Simplifying option of the surgical treatment of in situ breast tumors **(Study 3)**.

Patients and methods

Our studies were conducted in the Department of Surgery, Faculty of Medicine, University of Szeged.

Patients having a surgery using the GWL method (N = 69 patients) between January 1, 1997 and December 31, 2001 and the ROLL method (N = 321 patients) between January 1, 2002 and December 31, 2008 for having non-palpable unilateral malignant breast tumor and who had primary breast conservative surgery (BCS) were enrolled in **Study 1**.

Patients having a surgery using the ROLL method (N = 824) for having early stage unilateral malignant tumor between January 1, 2004 and December 31, 2011 who had primary breast tumor removal (BCS or mastectomy) and SLNB simultaneously were enrolled in **Study 2**.

Patients having a surgery using the ROLL method (N = 112) between January 1, 2002 and December 31, 2011 in case of whom the final histological examination confirmed unilateral in situ breast tumor were enrolled in **Study 3**.

1. WGL method

69 patients with a non-palpable malignant breast tumor were operated on in our institute following GWL between January 1, 1997 and December 31, 2001. During the intervention, a hook wire was introduced by the radiologist under radiographic or ultrasound guidance immediately before surgery. Direction of hook-wire insertion: for lesions in the upper quadrant/central region, preference is given to the cranio-caudal direction; for lesions in the lower quadrant, we prefer the lateral approach (these directions ensure the shortest way and at the same time are the most appropriate for the surgical incision). Exact positioning of the hook-wire (<5 mm) is important. The position of the wire was controlled by mammography. During the operation, the excision involved the tumor that was marked by a wire. The level of excision was the pectoral fascia. The excised specimen was marked with orientation stitches, and then specimen mammographic tests were performed.[4] Depending on the preoperative or final histological results, the procedure was supplemented with ALND because at that time, we did not use of SLNB. In the event of positive surgical margins, a supplementary operation (re-resection or mastectomy) was performed.

2. ROLL method

321 patients with non-palpable malignant breast tumor were operated on in our institute with the application of the ROLL method between January 1, 2002 and December 31, 2008. The ROLL technique and double marking SLNB were used simultaneously.[31] One day before the operation, 0.4 mL ^{99m}Tc-labeled human colloid albumin was injected into the tumor under radiographic or ultrasound guidance. 4 hours later, it was followed by a lymphoscintigraphic examination, and the projections of the SLNs on the skin were marked from two sides (SLN mapping). On the following day, 10 minutes before the operation, a second marker substance for the SLN, Patent Blue dye (2 mL) was injected into the subareolar region of the breast. During the operation, radiocolloid activity peak was identified with a gamma probe and the tumor was removed and the preoperative findings were also taken into account. The level of excision was the pectoral fascia. The excised specimen was marked with orientation stitches and controlled by specimen mammography. In patients with positive surgical margins, a second operation (re-resection or mastectomy) was performed. If metastases were detected in the SLNs, ALND was performed.

Radiologists recorded the time (minutes) required to localize the lesion with wire or isotope substance. Surgeons recorded the time (minutes) required for excisions (without axillary surgery).

3. Histological methods

Pathological examination of the removed breast tissue

During the pathological examination, the resection surface of the removed breast tissue was stained with various substances: anterior black (Indian ink), posterior blue (Alcian Blue) and superior red (Cadmium Red). The mass and the mediolateral, superoinferior and anteroposterior sizes of the removed tissue were measured. During the operation, a cylinder-form breast specimen was removed, and specimen volume could be calculated. After that, sections were cut, and the size of the tumor and its distance from the resection surface of the tissue were measured. During the procedure, at least 11 blocks were performed with the first superoinferior section being the macroblock. In this way, we were able to measure the size of the tumor and its distance from the anterior, posterior, superior and inferior resection surfaces.

The second block (medial) included the medial part of the tumor from the macroblock to the medial resection margin. With the help of this block, we were able to measure the distance between the tumor and the medial resection margin. The third block (lateral) included the lateral part of the tumor from the macroblock to the lateral resection margin providing the measurement of the distance between the tumor and the lateral resection margin. The shaves involved blocks 4–11. Besides traditional sections, we made 8 extra sections thus dividing the external surface of the removed tissue into 8 parts (superior, superomedial, superolateral, medial, lateral, inferomedial, inferolateral and inferior). By preparing these sections, our investigation of the resection margins became more precise. [32] During the examination were investigated the volume of removed specimen, the histological type of the tumor, the size of the tumor (T stage), the presence of in situ breast cancer around the invasive component, multifocality, the presence of lymphovascular invasion (LVI), histological grade, estrogen (ER) and progesterone receptor (PR) status and Human Epidermal Growth Factor Receptor-2 (HER-2) gene expression. The three diameters of the removed specimen (anteroposterior, mediolateral, superoinferior) were measured by pathologists, and the volume of the specimen was calculated with using an equation applied in case of an elliptical tissue cylinder. Pathologists considered multifocality where two or more invasive cancer foci could be found in the same quadrant of the breast and where there was no contact between the invasive focuses. Extensive in situ breast cancer around the invasive focus was defined in cases where the proportion of intraductal component was at least 25%, and intraductal focuses were present in the adjacent breast tissue as well. Microinvasive breast carcinoma (DCIS with microinvasion – DCISM) was defined if the extension of cancer cells beyond the basement membrane into the adjacent tissues is with no single focus larger than 1 mm in greatest dimension. For hormonal receptor status, 10% staining of cells by Immunohistochemical staining (IHC) was considered positive.

Pathological examination of the removed SLNs

SLNs were first examined using routine hematoxylin-eosin (HE) staining. If the metastasis can be confirmed by HE staining, additional processing was not performed. If this examination did not confirm metastasis, SLNs were evaluated in serial sections at intervals of 250 μm by means of HE. IHC staining was performed if the SLN was suspicious for

metastasis but HE was not able to identify the tumor cells accurately. Negative SLNs did not undergo IHC testing. The maximum dimension of the metastasis in each SLNs was measured since 2008, previously were described just the types of metastases (isolated tumor cells (ITC), micrometastasis or macrometastasis). Macrometastasis was defined if the SLN contains tumor metastasis in higher diameter than 2 mm. Micrometastasis was defined if the measure of SLN metastasis was between 0.2 and 2 mm. ITC metastasis in the SLN was defined if the measure of the metastasis was smaller than 0.2 mm. All SLNs were examined for extranodal extension. NSLNs from the ALND specimen were analyzed by routine HE staining only.

When comparing the GWL and the ROLL methods, we have taken into consideration the preoperative localization time, the operating time, the age of the patients, the pathological size of the tumor, the volume of the removed specimen, the ratio of the tumor size and the removed specimen volume, the number of positive surgical margins, the subsequent reoperations (reexcision or mastectomy) and the postoperative complications (wound infections). Furthermore, we investigated other factors, such as the presence of an extensive in situ breast carcinoma around the invasive cancer and the presence of multifocal tumors, as they could have an impact on the frequency of positive resection margins.

During the examination of the predictive factors of NSLN metastasis, the following variables were evaluated: tumor size, palpability, histological type of the tumor, grade of differentiation of the tumor, the presence of LVI, ER status, PR status, HER-2 status, number of removed SLNs, number of metastatic SLNs, the size of the metastasis in the SLNs, and presence of extranodal invasion.

During the evaluation of in situ tumors, the histological type, size, and grade of differentiation of the tumor, and the histological finding of the removed SLNs were examined.

4. Statistical analysis

For the comparison of continuous variables, t-test and one-way analysis of variance were used, as well as the Mann-Whitney in cases of non-normality. The normal distribution of samples was tested by using the Kolmogorov-Smirnov test. Categorical data were analyzed by using chi-square and Fisher's exact test. Multivariate analysis was performed by using logistic regression. SPSS version 20.0 (© 2012 SPSS Inc.) was used for statistical analysis. Significance was considered at $p<0.05$

Results

Study 1: Comparing the ROLL and the GWL methods

The final histological examination revealed 69 malignant lesions in the GWL group, and 321 malignant lesions in the ROLL group. Table 1 presents the histological results of the removed malignant breast lesions (Table 1).

Type of specimen	GWL (N=69)	ROLL (N=321)
LCIS	**–**	**3 (0.9%)**
DCIS	**5 (7.3%)**	**53 (16.5%)**
Papillary in situ breast cancer	**–**	**3 (0.9%)**
DCISM	**4 (5.8%)**	**4 (1.3%)**
IDC	**54 (78.3%)**	**218 (67.9%)**
ILC	**3 (4.3%)**	**20 (6.3%)**
Mucinous carcinoma	**2 (2.9%)**	**1 (0.3%)**
Tubular carcinoma	**–**	**7 (2.2%)**
Papillary carcinoma	**–**	**2 (0.6%)**
Phylloid carcinoma	**–**	**1 (0.3%)**
Mixed carcinoma	**–**	**9 (2.8%)**
Medullary carcinoma	**1 (1.4%)**	**–**

Table 1. Pathological features of the GWL and ROLL groups

Ultrasonographic guidance localization was performed in 58 cases using GWL method and in 277 cases using ROLL method. Radiographic guidance localization was performed in 11 cases using the GWL method and in 44 cases using the ROLL method. The localization time was significantly shorter in the ROLL group both with ultrasonographic guidance (5.7±1.4 min vs. 21.6±2.4 min, p=0.05) and with radiographic guidance (21.8±3.1 min vs. 41.6±3.8 min, p=0.021). It must be taken into consideration, however, that every time the GWL method was used, patients underwent mammography to verify the correct localization of the guidewire, which of course increased the localization time in all GWL cases. There was no significant difference in the operating time requirements (30.2±4.6 min vs. 30.7±4.7 min). The mean age of the patients was similar in both groups (59 yrs vs. 57.7 yrs). The removed breast specimen volume did not differ significantly between the GWL (89.5±116.3 cm^3) and the ROLL group (104.1±78.6 cm^3). The pathological tumor size and ratio of tumor size and

removed specimen did not show any significant difference between the GWL and the ROLL groups. The final pathological examination revealed 16 patients (23.2%) with a positive resection margin in the GWL group (n=69). Reoperations were performed on 14 of these patients (20.3%); 5 patients (7.2%) underwent breast reexcision and 9 patients (13.1%) mastectomy, and 2 patients refused consent to mastectomy. Residual tumor tissue was found by the histological examination in 6 patients (8.7%). In the ROLL group (n=321), positive resection margins were detected by the final pathological examination in 47 of the cases (14.6%). Reoperations were performed on 46 of these patients (14.3%); 24 patients (7.5%) underwent breast reexcision, and 22 patients (6.8%) underwent mastectomy. One patient refused mastectomy. Residual tumor tissue was found by the histological examination in 25 patients (7.8%). No significant difference was detected between the GWL and the ROLL groups in the frequency of positive resection margins. The incidence of postoperative complications (wound infections) did not differ significantly in the two groups (Table 2).

	GWL (N=69)	**ROLL (N=321)**	***p* value**
Duration of localization (mean ±SD), min **Radiographic guidance** **Ultrasound guidance**	 **41.6±3.8** **21.6±2.4**	 **21.8±3.1** **5.7±1.4**	 **0.021** **0.05**
Duration of surgical excision (mean ±SD), min	**30.2±4.6**	**30.7±4.7**	**NS**
Mean age, yr	**59**	**57.7**	**NS**
Removed breast specimen volume (mean ± SD), cm^3	**89.5±116.3**	**104.1±78.6**	**NS**
Pathological size of tumor (mean ± SD), mm	**12.4±8.6**	**15.2±11.2**	**NS**
Size of the tumor (cm)/specimen volume (cm^3)	**0.0237±0.0258**	**0.0181±0.0179**	**NS**
Involved surgical margin(s), n (%)	**16 (23.2%)**	**47 (14.6%)**	**NS**
Residual tumor, n (%)	**6 (8.7%)**	**25 (7.8%)**	**NS**
Wound infections, n (%)	**2 (2.9%)**	**3 (0.9%)**	**NS**

Table 2. Comparison of various factors between GWL and ROLL in malignant breast tumors (NS-not significant)

We have also taken further factors into consideration that influenced the frequency of positive resection margins. In the GWL group, 2 of the 69 patients (2.9%) had multifocal breast tumor, and another 4 patients (5.8%) had extensive in situ tumor components around

the invasive cancer. In the ROLL group 22 of the 321 patients (6.8%) had multifocal breast tumors, and 37 of the 321 patients (11.5%) had extensive in situ tumor components around the invasive cancer. The results of the histological analysis of the patients in the ROLL group indicated, that the size of the malignant lesion (p=0.021), the presence of a multifocal tumor (p=0.035), and the presence of an extensive in situ breast carcinoma around the invasive cancer (p=0.01) significantly increased the frequency of positive resection margins (Table 3).

	ROLL N=321 (100%)		***p* value**
	Involved surgical margins	**Clear margins**	
Patients, n (%)	**47 (14.6%)**	**274 (85.4%)**	
Mean age, yr	**55.6**	**58.1**	**NS**
Specimen volume (mean ± SD), cm^3	**119.9±104.7**	**111.7±73.4**	**NS**
Tumor size (mean ± SD), mm	**22.8±19.5**	**13.9±8.5**	**0.021**
Extensive DCIS present around the invasive cancer, n (%)	**12 (25.5%)**	**25 (9.1%)**	**0.01**
Multifocal lesion, n (%)	**8 (17%)**	**14 (5.1%)**	**0.035**

Table 3. Investigated features and surgical margin status in the ROLL group (NS-not significant)

In the GWL group the size of the tumors (p=0.05), the presence of an extensive in situ breast carcinoma around the invasive cancer (p=0.05) and the volume of the removed breast specimen (p=0.002) influenced the occurrence of unclear margins considerably. The frequency of reexcision was also higher for smaller specimens (Table 4).

	GWL n=69 (100%)		*p* value
	Involved surgical margins	**Clear margins**	
Patients, n (%)	**16 (23.2%)**	**53 (76.8%)**	
Mean age, yr	**58.4**	**60.6**	**NS**
Specimen volume (mean ± SD), cm^3	**66.4±40.3**	**96±127.1**	**0.002**
Tumor size (mean ± SD), mm	**17±11.6**	**11.2±7.4**	**0.05**
Extensive DCIS present around the invasive cancer, n (%)	**2 (12.5%)**	**2 (3.8%)**	**0.05**
Multifocal lesion, n (%)	**1 (6.3%)**	**1 (1.9%)**	**NS**

Table 4. Investigated features and surgical margin status in the GWL group (NS-not significant)

Study 2: Lymph node prognostic factors

Between January 1, 2004 and December 31, 2011, the final histological examination confirmed invasive carcinoma in case of 855 patients having a surgery using the ROLL method; in case of these patients, SLNB was also planned simultaneously. In 824 cases, marking of the SLN was successful (824/855 – 96.3%) using dual marking procedure, and successful SLNB was performed in all 824 cases. Based on the histological findings, the majority of the tumors was invasive ductal carcinoma (IDC) (79.1%), followed by invasive lobular carcinoma (ILC) (9.9%) and mixed type carcinoma (4.4%) (Table 5, Figure 1).

Histology	Number of cases (N)	%
IDC	**652**	**79.1**
ILC	**80**	**9.7**
Mixed carcinoma	**36**	**4.4**
Other less common carcinomas	**56**	**6.8**
Tubular carcinoma	**16**	**2**
Papillary carconoma	**9**	**1.1**
Medullar carcinoma	**8**	**1**
Mucinous carcinoma	**14**	**1.7**
Aplastic carcinoma	**1**	**0.1**
Undifferentiated carcinoma	**1**	**0.1**
Carcinosarcoma	**1**	**0.1**
Cribriform carcinoma	**1**	**0.2**
Neuroendocrine carcinoma	**1**	**0.1**
Metaplastic carcinoma	**2**	**0.2**
Micropapillar carcinoma	**1**	**0.1**
Epithelial carcinoma	**1**	**0.1**

Table 5. Histological findings of invasive breasts tumors

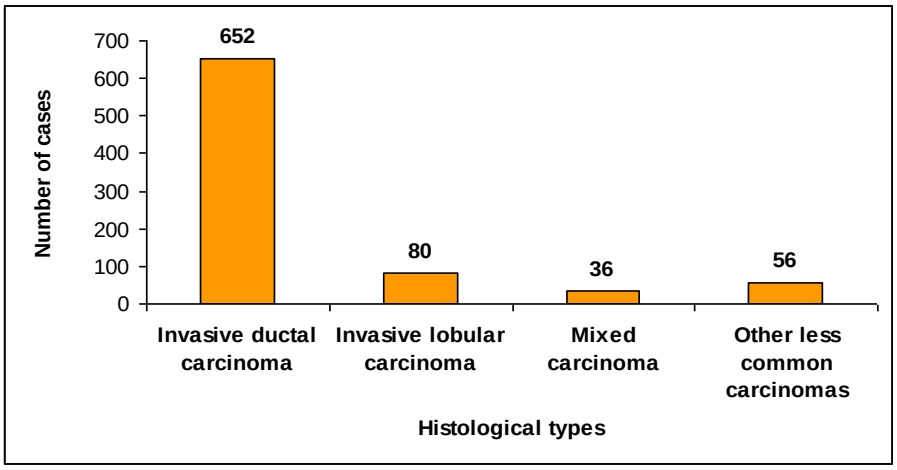


Figure 1. Histological types of breast tumors

A total of 1328 SLNs were removed from 824 patients, which means an average of 1.6 lymph nodes per patient. SLN metastasis was not confirmed by histology in 553 cases (553/824 – 67.1%), but in 271 cases (271/824 – 32.9%), SLN metastases were found. In 205 patients, complementary ALND was performed. In the other 66 cases, ALND was not performed based on the decision of the oncoteam regarding the patient's age and compliance, the size of the metastasis (ITC or micrometastasis), and in two cases, patients did not agree to perform the complementary surgery.

Then we studied the connection between primary tumor characteristics and NSLN metastasis in case of SLN positivity first with one-way analysis of variance in patients having complementary ALND (205 patients). In 70 patients (70/205 34.1%), additional metastasis was confirmed in the axillary lymph nodes by histological examination (Figure 2).

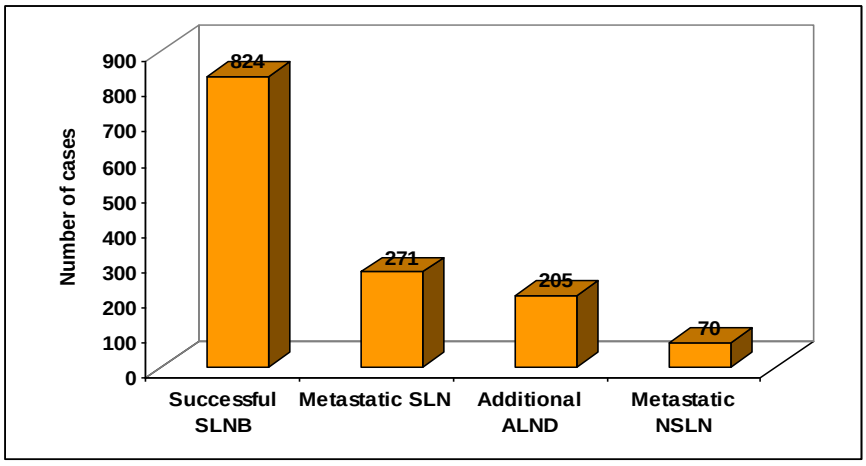


Figure 2. Histological finding of lymph node biopsies

Average age of the patients was significantly different between the NSLN negative and NSLN metastatic groups (55±11.1 years vs. 58.8±10.1 years, p=0.034). 75.6% (102/135) of NSLN negative patients had palpable tumor, while this ratio was 84.3% (59/70) in patients with NSLN metastasis, the difference was not significant (p=0.209). The presence of multifocal tumor did not influence the incidence of the metastasis in the NSLNs (NSLN negative: 11/135 – 8.1%, and NSLN positive 10/70 – 14.3%; p=0.224). The tumor size correlated with the incidence of the NSLN metastasis (NSLN negative: 18.8±8.1 mm vs. NSLN positive: 22.6±10.6 mm; p=0.019). Comparison was also made based on histological type. IDC was the most common (83.9%), followed by ILC (8.3%), and mixed type (lobular +

ductal) carcinoma (5.9%). The incidence of less common tumors (other histological tumors) was 2%. The histological types of the tumors did not influence the incidence of a NSLN metastasis (p=0.375). Studying the grade of histological differentiation revealed that the ratio of grade 1 tumors was 14.1% (19/135) in NSLN negative cases, and 8.6% (6/70) in NSLN positive patients. The incidence of NSLN metastasis was not different in grade 2 (NSLN negative: 64/135 - 47.4% vs. NSLN positive: 39/70 – 55.7%) or grade 3 tumors (NSLN negative: 52/135 – 38.5% vs. NSLN positive: 25/70 – 35.7%) either (p=0.397). The presence of LVI did not influence the incidence of additional NSLN metastasis (NSLN negative: 36/135 26.7% vs. NSLN positive: 17/70 24.3%; p=0.74). Neither ER positivity (NSLN negative: 107/135 – 79.2% vs. NSLN positive: 51/70 – 72.9%; p=0.274), nor PR positivity (NSLN negative: 101/135 – 74.8% vs. NSLN positive: 46/70 – 65.7%; p=0.24), nor HER-2 gene expression influenced the incidence of metastasis in the NSLN (NSLN negative: 24/135 – 17.8% vs. NSLN positive: 13/70 – 18.6%; p=0.848) (Table 6). The sizes of the metastasis in the removed SLNs were significantly different between the NSLN negative and positive groups (NSLN negative: 5.1±4.1 mm vs. NSLN positive: 8.1±4.6 mm, $p<0.0001$), however, there were only 148 patients in the latter group regarding the fact that before 2008, the proper size of the SLN metastasis was not routinely measured, only the type of the metastasis was determined (ITC-, micro- or macrometastasis). These groups were examined as well, but this comparison could be made with all 205 patients again. We found that in case of macrometastasis, the incidence of NSLN metastasis was significantly increased (p=0.013). Extracapsular spreading of the metastasis in the SLN did not influence the incidence of NSLN metastases (NSLN negative: 18/135 – 13.3% vs. NSLN positive: 16/70 – 22.9% p=0.112). The number of removed SLNs (p=0.37) and that of the SLNs containing a tumor (p=0.395) did not increase the risk of additional NSLN metastasis (Table 6-7).

Tumor characteristics	Patients with negative NSLN (N=135)	%	Patients with positive NSLN (N=70)	%	*p* value (univariate)	*p* value (multivariate)
Age (yr)	**55.1±11.1**		**58.8±10.1**		**0.034**	**0.073** *(1)* **0.057** *(3)*
Histology					**0.375**	
IDC	**117**	**86.7**	**55**	**78.6**		
ILC	**9**	**6.7**	**8**	**11.4**		
Mixed	**6**	**4.4**	**6**	**8.6**		
Other	**3**	**2.2**	**1**	**1.4**		
Palpability					**0.209**	
0 (no)	**33**	**24.4**	**11**	**15.7**		
1 (yes)	**102**	**75.6**	**59**	**84.3**		
Multifocality					**0.224**	
0 (no)	**124**	**91.9**	**60**	**85.7**		
1 (yes)	**11**	**8.1**	**10**	**14.3**		
Tumor size (mean ± SD), mm	**18.8±8.1**		**22.6±10.6**		**0.019**	**0.065** *(1)* **0.041** *(2)* **0.014** *(3)* **0.009** *(4)*
Grade					**0.397**	
1	**19**	**14.1**	**6**	**8.6**		
2	**64**	**47.4**	**39**	**55.7**		
3	**52**	**38.5**	**25**	**35.7**		
LVI					**0.74**	
0 (no)	**99**	**73.3**	**53**	**75.7**		
1 (yes)	**36**	**26.7**	**17**	**24.3**		
ER					**0.274**	
0 (no)	**24**	**17.8**	**17**	**24.3**		
1 (yes)	**107**	**79.2**	**51**	**72.9**		
Missing	**4**	**3**	**2**	**2.8**		
PR					**0.24**	
0 (no)	**32**	**23.7**	**22**	**31.4**		
1 (yes)	**101**	**74.8**	**46**	**65.7**		
Missing	**2**	**1.5**	**2**	**2.9**		
HER2					**0.848**	
0 (no)	**109**	**80.7**	**54**	**77.1**		
1 (yes)	**24**	**17.8**	**13**	**18.6**		
Missing	**2**	**1.5**	**3**	**4.3**		

Table 6. Importance of tumor prognostic factors in case of NSLN metastasis

Tumor characteristics	Patients with negative NSLN (N=135)	%	Patients with positive NSLN (N=70)	%	*p* value (univariate)	*p* value (multivariate)
Type of SLN metastasis					**0.013**	**0.047** *(3)* **0.03** *(4)*
ITC	**1**	**0.7**	**0**	**0**		
MIC	**21**	**15.6**	**3**	**4.3**		
MAC	**113**	**83.7**	**67**	**95.7**		
Size of SLN metastasis, (mean ± SD) mm, (N=148)	**5.1±4.1**		**8.1±4.6**		**<0.0001**	**0.002** *(1)* **0.001** *(2)*
Extracapsular invasion of SLN metastasis					**0.112**	
0 (no)	**117**	**86.7**	**54**	**77.1**		
1 (yes)	**18**	**13.3**	**16**	**22.9**		
No. of removed SLNs					**0.37**	
1	**63**	**46.7**	**42**	**60**		
2	**47**	**34.8**	**16**	**22.9**		
3	**20**	**14.8**	**10**	**14.3**		
4	**4**	**3**	**2**	**2.8**		
6	**1**	**0.7**	**0**	**0**		
No. of positive SLNs					**0.395**	
1	**109**	**80.8**	**53**	**75.7**		
2	**23**	**17**	**13**	**18.6**		
3	**2**	**1.5**	**3**	**4.3**		
4	**1**	**0.7**	**1**	**1.4**		

Table 7. Importance of SLN prognostic factors in case of NSLN metastasis

Factors confirmed to be significant with one-way analysis of variance are described in Figure 3.

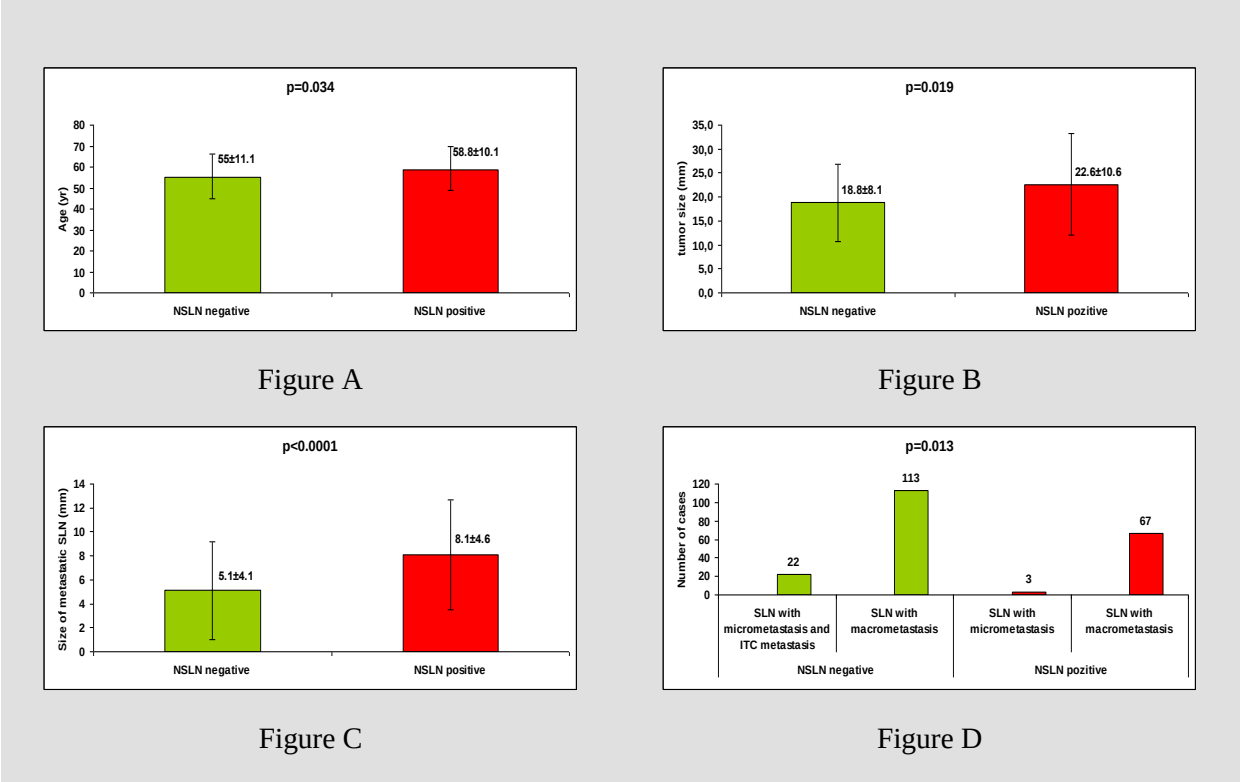


Figure 3. Age (Figure A), tumor size (Figure B), and size of SLN metastasis (Figure C; N=148 cases), and type of the SLN metastasis (Figure D; N=205 cases) in the NSLN negative and positive groups, figures include mean ± SD, significance values

Then factors found to be significant with one-way analysis of variance were tested with logistic regression (age, tumor size, size, and type of the SLN metastasis). More studies were performed. In our first study (1), if the size of the SLN metastasis was known and all three variables were included, age (p=0.073) and tumor size (p=0.065) did not influence the incidence of NSLN metastasis significantly, but the size of the SLN metastasis was found to be significant (p=0.002). The second study (2) was performed omitting the age from logistic regression. In this case, tumor size (p=0.041) and the size of the SLN metastasis (p=0.001) significantly influenced the incidence of metastasis in NSLN. In the third study (3), if the exact size of the SLN metastasis was not known only the type of it (macro-, or micrometastasis) and all three variables were included, age was strictly not significant (p=0.057), but the tumor size (p=0.014) and the type of the SLN metastasis were significantly (p=0.047) influencing the incidence of NSLN metastasis. The fourth study (4) was performed

similarly to the second one, age was not included in the analysis, in this case, tumor size (p=0.009) and the size of the SLN metastasis (p=0.03) significantly influenced the incidence of metastasis in NSLN (Table 6-7).

Based on our results, we tried to construct a model to predict the incidence of additional NSLN metastasis. The probability of the metastasis can be calculated by the following equitation if the size of the SLN metastasis is known in the first case:

$$P_{NSLNmet} = \frac{1}{1 + e^{-(0.034*a + 0.038*t + 0.144*s - 4.416)}}$$

in the second case:

$$P_{NSLNmet} = \frac{1}{1 + e^{-(0.042*t + 0.151*s - 2.562)}}$$

where: $P_{NSLNmet}$ *– the probability of NSLN metastasis*

a – age (year)

t – tumor size (mm)

s – size of the metastasis in the SLN (mm)

If the size of the SLN metastasis is not known only the type of it, the following equitation can be used to calculate the probability of NSLN metastasis in the third case:

$$P_{NSLNmet} = \frac{1}{1 + e^{-(0.028*a + 0.041*t - 1.283*m - 1.701)}}$$

in the fourth case:

$$P_{NSLNmet} = \frac{1}{1 + e^{-(0.043*t - 1.405*m - 0.016)}}$$

where: $P_{NSLNmet}$ *– the probability of NSLN metastasis*

a – age (year)

t – tumor size (mm)

m – type of the metastasis in the SLN (mac=1, mic=2)

Evaluation of the specificity and sensitivity of the first model using the Receiver Operating Characteristic (ROC) curve showed that its predictive value – based on the AUC=0.735 (Area Under the Curve) value is considered to be moderate, while the predictive value of the second model using the ROC curve similarly was somewhat lower (AUC=0.717) than the first one (Figure 4). Analysis of the third and fourth models with the ROC curve showed low predictive values (AUC=0.66 and 0.638), it practically cannot be used to predict NSLN metastasis (Figure 5).

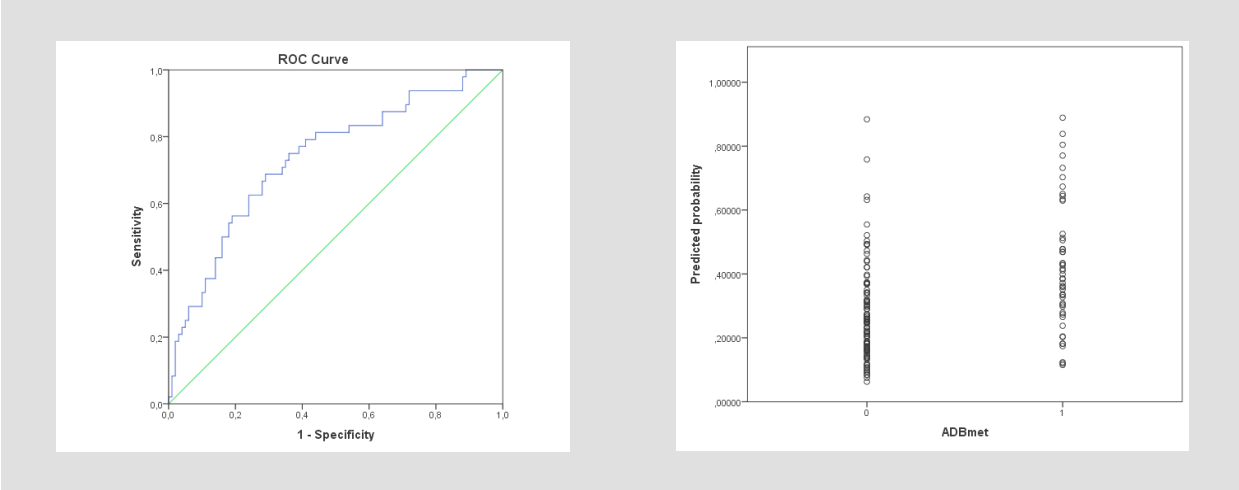


Figure A

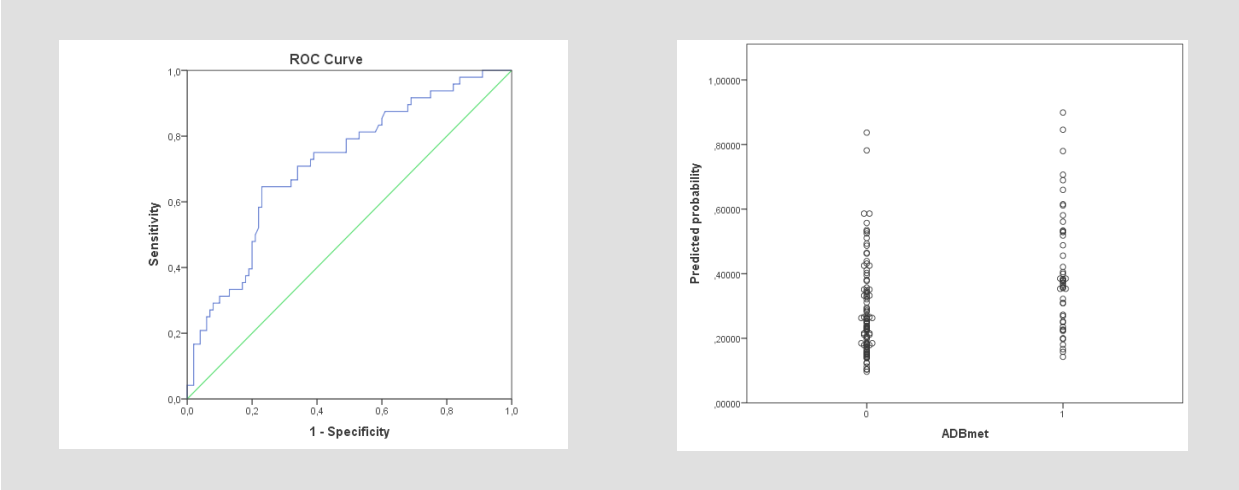


Figure B

Figure 4. Diagram presenting the reliability of the predictive models regarding specificity and sensitivity and predicted and actual incidence of NSLN metastases (ALND or ABD met); Figure A in case of the three-variable model (AUC=0.735), Figure B in case of the two-variable model (AUC=0.717) if the exact size of the SLN metastasis is known

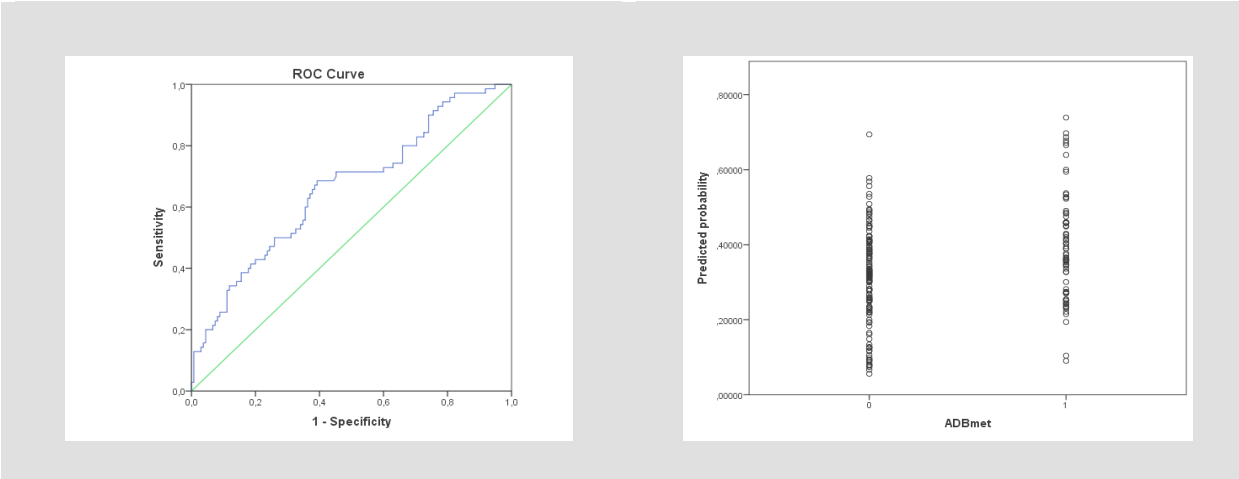


Figure A

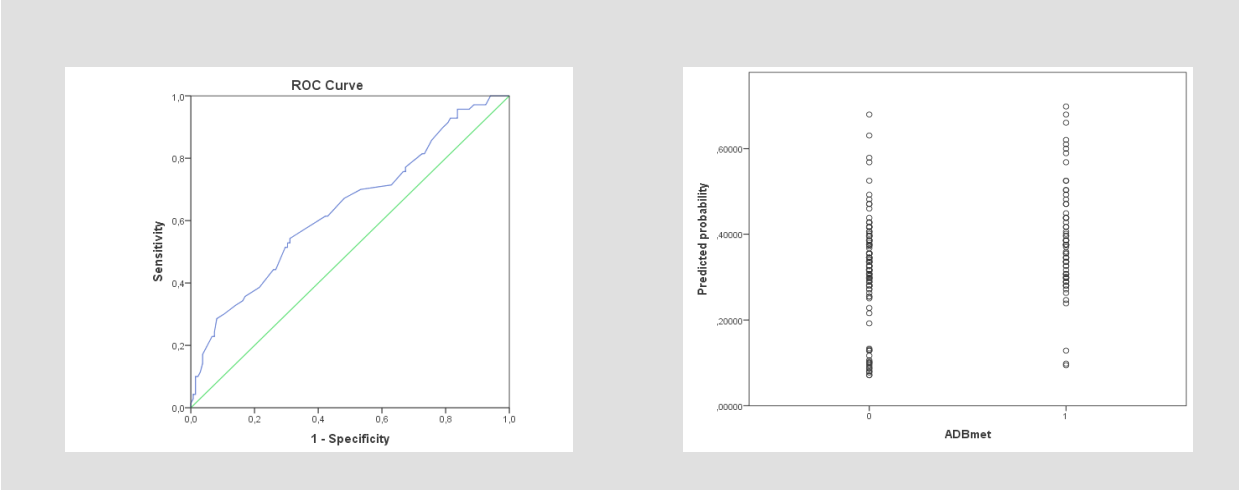


Figure B

Figure 5. Diagram presenting the reliability of predictive values of the models regarding specificity and sensitivity and predicted and actual incidence of NSLN metastases (ALND or ABD met); Figure A in case of the three-variable model (AUC=0.66), Figure B in case of the two-variable model (AUC=0.638) if only the type of the SLN metastasis (macro-, or micrometastasis) is known

Further examination of the first predictive nomogram revealed that in patients with lower risk for NSLN metastasis, the nomogram is relatively more accurate in predicting the NSLN metastasis. Using a cut-off value of 0.27 in case of the predictive curve, the predictive value of the model could be increased, a sensitivity of 62.5 % and a specificity of 75 % could be achieved, this is the most accurate range of the nomogram. We tried to improve sensitivity

and specificity similarly in case of the other nomograms as well; the results are seen in Table 8.

Nomogram	AUC	Cut off value	Sensitivity (%)	Specificity (%)
1. Diagram	**0.735**	**0.27**	**62.5**	**75**
2. Diagram	**0.717**	**0.35**	**55.4**	**81.3**
3. Diagram	**0.66**	**0.34**	**69.6**	**67**
4. Diagram	**0.638**	**0.36**	**57.1**	**64.4**

Table 8. Most accurate predictive ranges of the nomograms

In summary, in case of SLN positivity, the patient's age, the tumor size, and the size (type) of the SLN metastasis influenced the NSLN metastasis according to the one-way analysis of variance. Multivariate analyses of cases where the size of the SLN metastasis is known showed that only the size of the SLN metastasis was strictly significant, age and tumor size showed only borderline significance. If the age was not included in the multivariate analysis, the tumor size and the size of the SLN metastasis were significant factors. If only the type of the SLN metastasis is known, multivariate analysis showed borderline significance in case of the age, but the tumor size and the type of the SLN metastasis were still significant, even if the age was not included in the analysis. The predictive value of the models is moderate if the size of the SLN metastasis is known, while it is poor if only the type of the SLN metastasis is known. In case of patients with lower risk for NSLN metastasis, the predictive value of the nomogram is relatively more accurate.

Study 3: Importance of SLNB in case of in situ breast carcinomas

Between January 1, 2002 and December 31, 2011, patients having surgery using the ROLL method with a final histological examination confirming in situ breast tumor (N = 112 patients) were enrolled in the study. The median age of the patients was 55.2 years (range 30–78 years). 75 patients (75/112, 67%) underwent preoperative fine-needle aspiration cytology (FNAC) and 23 patients (23/112, 20.5%) underwent core-needle biopsy (CNB). Preoperative histological results were not available in 13 patients. FNAC was not informative (C1) in 28 of 75 patients (28/75, 37.3%), 3 patients (3/75, 4%) had a benign breast disease (C2), 5 (5/75, 6.7%) had atypical breast disease (C3), 19 (19/75, 25.3%) gave the suspicion of malignant disease (C4) and only in 20 (20/75, 26.7%) were malignant cells identified in the sample (C5). Of the 23 patients investigated by CNB, malignant breast cancer (pure DCIS) was identified in 17 (17/23 73.9%), invasive cancer in 2 (2/23, 8.7%), atypical breast disease in 2 (2/23 8.7%) and benign breast disease in 2 (2/23, 8.7%) cases (Table 9).

Results of FNAC	**N**	**%**
No cells detected (C1)	28	25.0
Benign disease (C2)	3	2.7
Atypical disease (C3)	5	4.4
Suspected malignancy (C4)	19	17.0
Malignant cells (C5)	20	17.8
Results of CNB		
Benign disease (B2)	2	1.8
Atypical disease (B3)	2	1.8
Malignant disease pure in situ cancer (B5a)	17	15.2
Malignant disease in situ and invasive cancer (B5a+b)	2	1.8
Excision	1	0.9
Data not available	13	11.6

Table 9. Results of preoperative histological diagnosis

The final histological examination verified lobular in situ breast cancer (LCIS) in 4 of the 112 patients (4/112, 3.6%), pure DCIS in 96 (96/112, 85.7%), papillary in situ cancer in 3 (3/112, 2.7%) and DCISM in 9 (9/112, 8%) patients (Table 10). 36 patients (36/112, 32.1%) had palpable tumor. DCIS breast cancers have a number of histological subtypes (solid, cribriform, papillary, micropapillary and comedo). The most important factors are the presence of comedo necrosis and the grade of the tumor cells. In 76 of the 108 pure DCIS patients (76/108, 70.4%), the tumor was of high grade (Grade III), in 11 (11/108, 10.2%)

cases, it was of intermediate grade (Grade II), and in 21 (21/108, 19.4%) cases, it was of low grade (Grade I).

Histological type	**N**	**%**
LCIS	4	3.6
DCISM	9	8.0
Pure DCIS	96	85.7
Papillary in situ cancer	3	2.7

Table 10. Final pathologic diagnosis

Simultaneous SLNB was planned in 108 of the 112 patients (108/112, 96.4%), while 4 (4/112 3.6%) patients underwent only wide excision. In 8 of 108 patients (8/108, 7.4%), SLNs were not identified; axillary sampling or ALND was performed to remove the axillary lymph nodes at level I-II. 100 patients underwent successful SLNB (100/108, 92.6%). In 95 cases, SLNs were evaluated in serial sections at intervals of 250 μm with using HE, while in 5 cases SLNs were processed only as routine axillary lymph nodes. First, during the evaluation of the 95 cases, a total of 147 sentinel lymph nodes were examined (an average of 1.5 lymph nodes per patient, range 1–5). Metastasis was not confirmed in SLNs processed with serial sectioning. In 11 cases, additional axillary lymph nodes were removed besides successful SLNB; metastasis was not confirmed in the removed axillary lymph nodes. These lymph nodes were processed with routine HE staining. In the other 13 cases (8 cases of ALND – processed with routine HE method + 5 cases of successful SLNB, but processed with routine HE method), metastasis was not confirmed either.

26 of the 112 (26/112, 23.2%) patients required a second complementary operation: mastectomy in 12 cases, and reexcision in 14. Residual tumor was verified in 10 patients treated with mastectomy and 7 patients treated with reexcision. One patient treated with reexcision underwent mastectomy because of positive resection margin during the reexcision (Table 11).

Complementary surgery	**Number of cases**	**%**	**Residual tumor (N)**	**%**
Reexcision	14	12.5	7	6.3
Mastectomy	12	10.7	10	8.9

Table 11. Reoperations and results in case of patients with in situ breast cancer

Discussion

The extensive use of mammography has resulted in the increased detection rate of early-stage non-palpable malignant breast tumors. Both the GWL and the ROLL methods are widely applied in surgical therapy to reveal and to remove non-palpable breast tumors. The GWL method is the more widespread technique in use today despite some well-known disadvantages: [1] radiologically, the guidewire placement is a difficult procedure to carry out; spontaneous wire displacement, and inability to reposition can occur as well. [2] The procedure is traumatic, causing discomfort and pain to the patient; furthermore, the wire must remain in place until the operation. [3] The surgical excision of a wire-located lesion with clear margins is a technically difficult procedure. There is obvious interference with the incision line and the surgical approach, and the wire can be accidentally transected as well. The ROLL method was developed to overcome some of the disadvantages of the GWL technique. Its reported advantages include precise localization, accurate surgical removal, higher rate of clear margins, reduced size of the excised specimen, better concentricity of the lesion, less patient discomfort, shorter operating time, and reduced numbers of reoperations, with an accompanying reduction in costs. Despite the fact that the ROLL technique has been available for more than 10 years now, only a few studies have been published about it. The GWL and the ROLL techniques have been compared only in 11 clinical studies and subjects were randomized only in 4 studies (Table 12).[33,34,35,36,37,38,39,40,41,42,43]

Authors	Year	Patients, N		Involved surgical margins %		*p* value
		ROLL	GWL	ROLL	GWL	
Luini et al. [33]	1999	30	30	0	0	NS
Rampaul et al.®[34]	2004	48	47	NA	NA	NA
Ronka et al. [35]	2004	64	14	8	28.6	0.03
Gallegos-Hernandez et al.[36]	2004	65	67	16.9	35.8	0.014
Zgajnar et al.[37]	2005	51	92	29.4	55.2	0.005
Nadeem et al. [38]	2005	65	65	17	43	0.001
Thind et al. [39]	2005	70	70	16.2	40	0.002
Strnad et al. [40]	2006	21	12	NA	NA	NA
Moreno et al.® [41]	2008	61	59	10	12.5	NS
Medina-Franco et al.® [42]	2008	50	50	11.1	37.5	0.04
Martinez et al. ® [43]	2009	66	68	10.6	17.6	NS
Present study	2012	321	69	14.6	23.2	NS

Table 12. Comparison of international results on the complete excision rates with the ROLL and the GWL techniques (®-randomized, NA-not available, NS-not significant)

Altogether 6 trials have described a significant difference regarding the clear resection margins in favor of the ROLL technique.[35,36,37,38,39,42] 3 studies have found that the volume/weight ratio of the excised specimen was lower in the ROLL group than in the GWL group.[35,37,41] Several studies have confirmed the well-known advantages of the ROLL method, such as better cosmetic results [38,39,41], less perioperative pain [34,41] and shorter localization time.[38,39,42] The newest systematic review demonstrated that radioguided localization techniques (including ROLL and radioguided seed localization (RSL) methods) produce lower positive resection margin rates and consequently fewer reoperations. However, this review was limited by its small size and the quality of the randomized controlled trials.[44] A recently published multicenter, randomized, controlled trial compared the RSL method to a standard GWL technique in the detection of non-palpable invasive and in situ breast

carcinomas.[45] In contrast to other trials, the positive resection margins and the reoperation rates were similar in both techniques, but the operating time was shorter when using RSL. It is important to know that in the RSL method, a radio-opaque titanium seed containing an ^{125}I-isotope was used, therefore it is not exactly equivalent to the classic ROLL method.

In the present study, we did not find any significant differences between the two compared methods in respect of the proportion of the average volume of the removed specimen, the proportion of positive surgical margins, the incidence of residual tumors (removed during a second operation), or the frequency of postoperative wound infections. Preoperative localization time was significantly lower in the ROLL group, but there was no significant difference in the duration of surgical excision. Nevertheless, our surgeons found the ROLL method technically easier. International study results suggest a higher rate of successful primary tumor excision (clear resection margins) with the use of the ROLL method (Table 12). Higher clear resection margin rates were found in the ROLL group in our study as well, but the difference was not significant statistically (85.4% vs. 76.8%). Although the average removed specimen volume and the pathological tumor size were higher in the ROLL group than in the GWL group, they were not significantly different. We did not find any significant difference in the ratio of the tumor size and the removed specimen volume. This indicates that a relatively smaller specimen can be removed safely using the ROLL method for the same tumor size. Another important advantage of the ROLL technique is that it allows concomitant removal of the invasive breast lesion and the SLN(s). Furthermore, our investigation revealed that by applying the ROLL method, the involved surgical margin was influenced by the tumor size, by the existence of a multifocal tumor, and by the presence of an extensive in situ breast cancer around the invasive tumor. In case of the GWL technique, the frequency of positive resection margins was influenced by the tumor size, by the presence of an extensive in situ breast cancer around the invasive tumor and by a lower specimen volume. It is important to emphasize that the size of the tumor was bigger and the specimen volume was lower in the GWL group with positive resection margins. Therefore, the ratio of the tumor size and the removed specimen volume are more indicative factors of the occurrence of a positive resection margin than just the size of the tumor or the removed specimen volume itself alone. Several studies have proved that the frequency of a positive resection margin is

significantly increased by the size of the tumor[46.47], by the presence of an extensive in situ tumor around the invasive tumor[46,48], by the presence of multifocal tumors[46,49] and by the volume of the removed specimen.[46,49] Considering these facts, it is noteworthy that the most important predictive factor of a local tumor recurrence in breast-conserving surgery is a positive resection margin.[50,51,52] If the final surgical margins are negative, the 5-year risk of local failure is 2–7%, whereas with positive margins, this risk can rise up to 16% or even higher[53,54,55,56]. In addition to the surgical margin status, factors such as young age, large tumor size, adjuvant chemotherapy and hormonal therapy, and positive ALNs are all significant independent predictors of locoregional recurrence.[57]

International results show that both the GWL and the ROLL methods are suitable for the localization and subsequent removal of non-palpable breast tumors. We have come to the same conclusion in our study. However, the ROLL method has more advantages, such as shorter localization time, more accurate surgical excisions and less discomfort to the patient. We recommend that the ROLL method should be used for the localization of non-palpable breast tumors if preoperative examinations prove the presence of an invasive breast cancer and SLNB is also to be considered. We would recommend the use of the GWL technique in cases with extensive microcalcifications and when SLNB is not going to be performed (pure DCIS, radial scar, etc.).

Besides BCSs, SLNB, which is an accepted indicator of the axillary lymph node status has come to the front. In accordance with previous clinical practice, complementary ALND was routinely performed in case of metastatic SLN.[12,13,14] However, several studies have highlighted that it was unnecessary in approximately 2/3 of the cases as additional metastasis was not detected in the removed lymph nodes.[15,16] Increased risk for the occurrence of ALND related complications, as well as the additional cost of the surgery and the treatment of potential complications indicate the development of methods that may predict the probability of additional axillary metastasis. This method may help in preventing or at least reducing the number of ALNDs performed unnecessarily in case of SLN positivity. Eight NSLN metastasis predictive models are used in the clinical practice currently.[58,59,60,61,62,63,64,65] The prospective study of Van Zee and colleagues (2003) studied the patients of the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York. 1075 patients with primary invasive

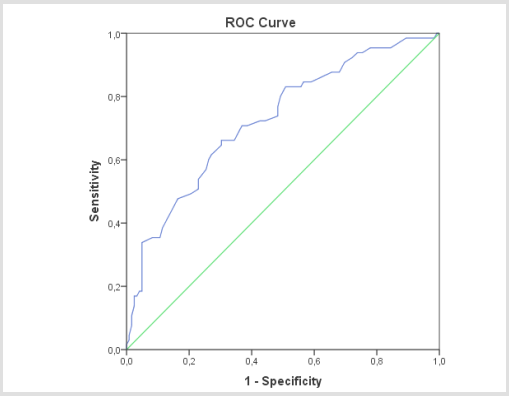
breast tumor and SLN metastasis were studied in 6 years. All patients had complementary ALND surgery. Multiparametric logistic regression was used to perform a predictive nomogram to predict the NSLN metastasis. The nomogram is available online at www.mskcc.org/nomograms. From the studied factors, tumor size, presence of LVI, presence of multifocal tumor, method of detection of SLN metastasis (frozen section, routine HE, SS, IHC), and number of positive and negative SLNs correlated with the incidence of the NSLN metastasis. A drawback of the study is that the examination of the axillary lymph nodes was performed with routine HE method; in case of SS, this number would have been higher. Exact size of SLN metastasis was missing, however, the method of detection may correlate with this. The most important disadvantage of the study is that the model does not determine when ALND should be performed, it only predicts the probability of the metastasis.[58] In a prospective study of Hwang and colleagues (2003), 131 patients of the MD Anderson Cancer Center, Texas were evaluated similarly. Their results showed that the tumor size, presence of LVI, the size of metastasis in the SLN increased the occurrence of NSLN metastasis, and the number of removed SLNs was a significant negative predictor for NSLN metastasis. The predictive nomogram is available online as well (http://www3.mdanderson.org/app/medcalc/bc_nomogram2/index.cfm?pagename=nsln). The developed score system includes a positive and negative predictive value besides sensitivity and specificity. The disadvantage of the model is that sensitivity was decreased in case of higher values, and specificity was reduced in case of lower scores.[59] Degnim and colleagues (2005) studied 574 patients having invasive breast tumor with clinically negative axillary status in the Mayo Clinic and University of Michigan were assessed under similar circumstances, and age, tumor size, size of the SLN metastasis, ER positivity, extracaspular spreading, number of positive and negative SLNs correlated statistically significantly with the NSLN metastasis. The method is simpler and uses more easily available clinicopathological factors compared with the MSKCC nomogram.[60] Barranger and workgroup (2005) enrolled 71 patients with SLN metastasis in their analysis similarly, they evaluated the tumor size, type of the SLN metastasis (presence of macrometastasis), and the ratio of removed positive and negative SLNs in the final NSLN metastasis predictive model.[61] Chapgar and colleagues (2006) included 1253 patients in their multicenter, prospective database, and they found that the tumor size (T), and the number and ratio of positive SLNs influenced the presence of

additional NSLN metastasis. A novel factor was that they studied the experience of the surgeon and differences among various regions as well. The drawback of the study was that ER, PR, HER2 status, grade, LVI, and size of SLN metastasis were not examined.[62] Khort and colleagues published a prospective multicenter (16 institutions) study in 2008 examining 285 patients under similar circumstances and found that the tumor size, presence of LVI, and the size of the SLN metastasis influenced metastasis formation in NSLNs. This model was the first emphasizing synergistic interactions between factors (LVI and size of SLN metastasis, as well as between tumor size and size of SLN metastasis). The predictive model (Stanford nomogram) is available online as well (http://www3-hrpdcc.stanford,edu/nsln-calculator).[63] In 2009, Houvenaeghel studied 909 cases in a retrospective multicenter (16 institutions) study with similar criteria, however only cases of SLN micrometastasis were evaluated. The study showed that the tumor size, detection method of the SLN micrometastasis, presence of LVI, and the histological type of the tumor influenced the development of NSLN metastasis. Omission of ALND could be recommended only in case of minimal risk for a low probability of NSLN metastasis (<10%).[64] Coufal and colleagues (2009) enrolled 330 patients in a similar way with similar criteria to develop a predictive model which was validated in a population of 383 patients operated on for having breast tumor in the Department of Surgery in the Kecskemét Hospital and who met the criteria. The final predictive model included tumor size, histological type, multifocality, presence of LVI, size of SLN metastasis, extranodal spreading, and the ratio of positive SLNs.[65] The most commonly used nomograms and evaluated and significant factors are summarized in Table 13.

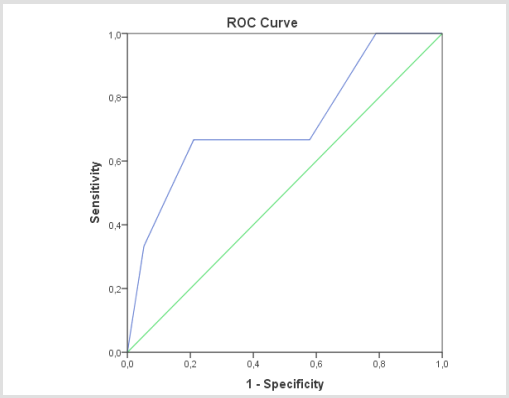
Nomograms / Variables	MSKCC[58]	MDA[59]	Mayo[60]	Tennon score[61]	Luisville[62]	Stanford[63]	French micrometastasis[64]	Masaryk[65]
Age	No	No	Yes	No	No	No	No	No
Tumor size	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Categorical	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Continuous	Yes	No	No	No	No	Yes	No	No
Tumor type	Yes	No	No	No	No	No	Yes	Yes
Nuclear grade	Yes	No	No	No	No	No	No	No
LVI	Yes	Yes	No	No	No	Yes	Yes	Yes
ER status	Yes	No	Yes	No	No	No	No	No
Multifocality	Yes	No	No	No	No	No	No	Yes
No of pos.SLNs	Yes	No	Yes	No	Yes	No	No	No
No of neg.SLNs	Yes	No	Yes	No	No	No	No	No
No of SLNs	No	Yes	No	No	No	No	No	No
Rate of pos. SLNs	No	No	No	Yes	Yes	No	No	Yes
Detection of SLN met.	Yes	No	No	No	No	No	Yes	No
Size of SLN met.	No	Yes	Yes	Yes	No	Yes	No	Yes
Categorical	No	Yes	Yes	Yes	No	Yes	No	Yes
Continuous	No	No	No	No	No	No	No	No
Extracapsular spreading	No	No	Yes	No	No	No	No	Yes

Table 13. Variables included in the different predictive models tested

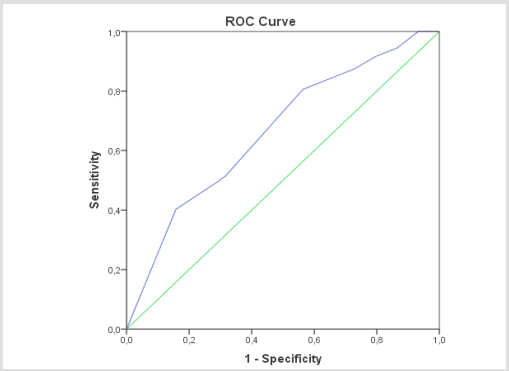
. Evaluation of the nomograms revealed that these models are not better predictive systems in predicting NSLN metastasis in our patient population either. Based on our studies, the most reliable methods are the Chapgar (AUC: 0.766) and MSKCC nomograms (AUC: 0.726) (Figure 6-7).



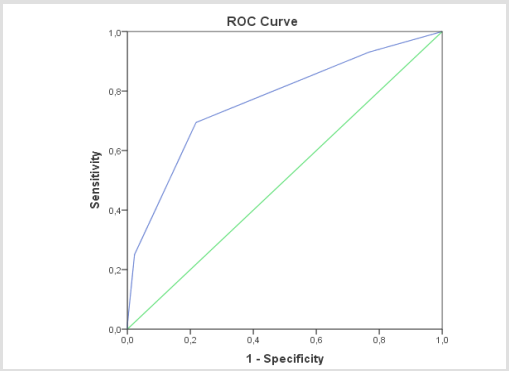
MSKCC nomogram



French micrometastasis nomogram

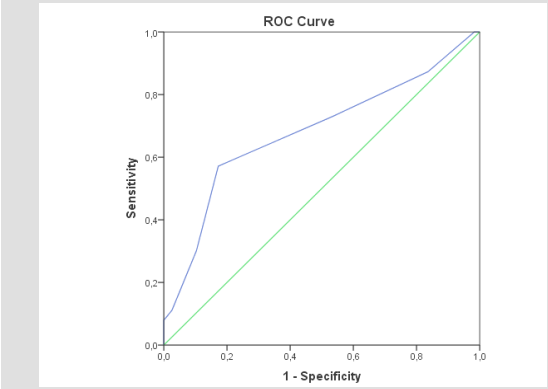


Tenon score nomogram

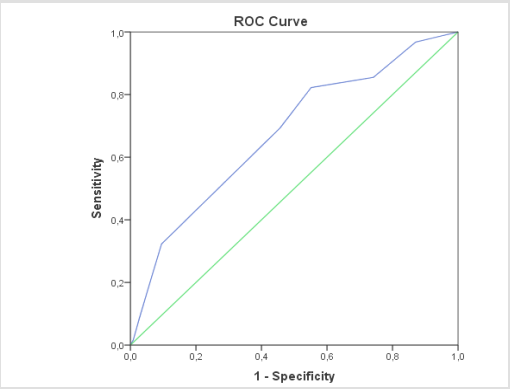


Luisville prediction nomogram

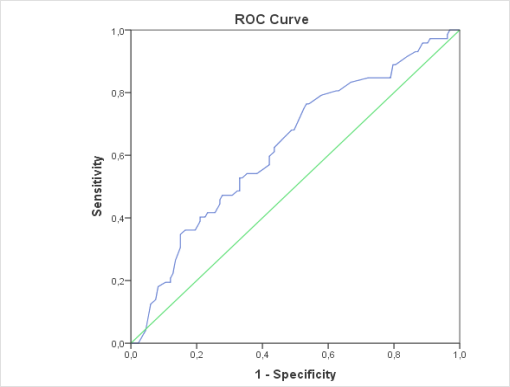
Figure 6. Evaluation of the sensitivity and specificity of predictive nomograms in our patients 1.



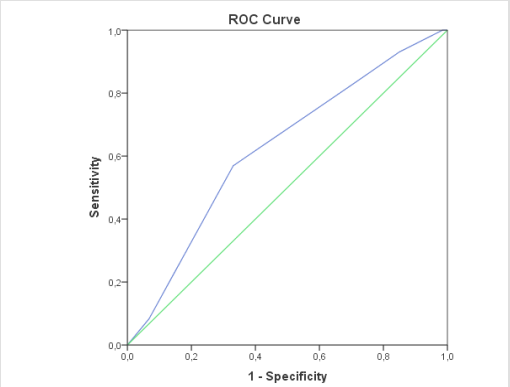
Mayo nomogram



Masaryck nomogram



Stanford nomogram



MDA nomogram

Figure 7. Evaluation of the sensitivity and specificity of predictive nomograms in our patients 2.

Cserni and colleagues (2012) studied and compared the above described 8 nomograms and their predictive values in their multicenter study using the clinical data of the University of Szeged as well. 200 patients having invasive breast tumor and positive SLN and in case of whom ALND was performed were enrolled in the study from all centers (a total of 1000 patients). A low risk value for NSLN metastasis was assigned to the nomograms, and its predictive value was studied and compared. Note that clinicopathological examination of the SLN, or even the processing of the primary tumor may be different, therefore inter-institutional difference was detected, which is important in identifying the low risk group as

well. Therefore, validation of the selected method is recommended in an institutional level and the most appropriate one should be used.[66] In another study of Cserni and colleagues (2012), predictive nomograms were used to identify patients with high risk for NSLN metastasis (>50%) who would benefit from ALND.[67] 1000 patients having invasive breast tumor and SLN metastasis as well as ALND were enrolled (200 from the University of Szeged) in the study. Patients with micrometastatic SLNs were tested separately. They results showed that identification of high risk patients is much worse than that of patients with low risk for NSLN metastasis. There were inter-institutional differences of nomograms as well regarding positive predictive values. Therefore, the nomograms should be validated and selected that is most suitable for the institution. In case of SLN micrometastasis, the risk of additional NSLN metastasis is low irrespective of the fact that the patient is in the high or low risk group, so in such cases, ALND is not recommended. The recommendation of the 2011 St Gallen Consensus conference is similar as well.[68] Meretoja and colleagues performed a new retrospective study with 200 cases from each 5 centers (including the University of Szeged) examining the same factors. Their aim was to prepare a predictive model for NSLN metastasis. After this, an internal (500 cases) and an external (1068 cases) validation was performed. Logistic regression was used with the data of the original 1000 patients and the probability of NSLN metastasis was determined with a mathematical model including LVI, mutifocality, HER2 status, number of negative and positive SLNs, tumor size, size of the SLN metastasis, and extracapsular spreading as significant variables. (The model is available online at http://www.hus.fi/breastsurgery/prediktivemodell.)[69] The gold standard in case of SLN positivity is performing ALND, the role of ALND has recently become controversial in selected cases. One of the most important studies examining this was the Z0011 trial performed by an American surgeon-oncologist team and was described by Guliano and colleagues in 2010 including patients with invasive breast tumor with T1-2, N0, M0 clinical stage in case of whom SLNB was performed in addition to the removal of the tumor, and macro-, or micrometastasis was found in the SLN. Pregnant women and patients receiving neoadjuvant therapy were excluded from the study. Patients were randomized into two groups, complementary ALND was performed in one group, and there were no additional surgical interventions or special complementary treatments in the other group. Finally, 388 patients having ALND were compared with 425 patients who did not have ALND. The

average duration of the follow-up was 6.3 years. The ratio of locoregional recurrence was 3.4% of the total patient population. There were no significant differences in local recurrence or regional recurrence (ipsilateral axilla), or the average time until the recurrence between the two groups. Members of both groups received systemic adjuvant oncological therapy (hormone therapy and chemotherapy) in similar ratio. Type of chemotherapy was similar as well. The ratio of locoregional recurrence was not significantly different in case of patients receiving systemic adjuvant oncology treatment and patients not receiving such treatment. Consequently, completing ALND was not beneficial in locoregional control even if tumor-containing lymph nodes were removed.[70] The effect of ALND on survival was also studied in this patient population. 5-year survival was similar between the two groups. 5-year disease-free survival was not significantly different either. Incidence of surgical complications (paresthesia, wound infection, seroma, lymphedema) increased in the ALND group. [71]

In our patient population, 271 patients were confirmed to have SLN metastasis, 205 ALND procedures were performed, and 70 patients in 205 had additional confirmed metastasis. Based on our study results it can be concluded that in our patient population, additional NSLN metastasis was influenced by the age of patients, the tumor size and the type (size) of the SLN metastasis among the studied prognostic factors in case of SLN metastasis. A precise predictive nomogram to predict NSLN metastasis could not be created. The predictive value of the nomogram is better when the size of the SLN metastasis is known compared to when only the type of the SLN metastasis is known. The predictive nomogram is relatively more precise in identifying NSLN metastasis in patients with lower risk for NSLN metastasis however, it is still not suitable for precise prognosis.

An increasing number of cases of malignant or malignant-suspicious non-palpable breast disease have been recognized since the introduction of mammographic screening. The same holds for the incidence of DCIS among early detected breast cancers.[19,20] DCIS is a non-invasive breast cancer, and is therefore not expected to give metastases. The conference organized in the USA in 1999 accepted the suggestion that it was unnecessary to perform ALND if the diagnosis was pure DCIS.[20] However, some authors consider that SLNB in pure DCIS is controversial, even though this might appear unnecessary. A number of studies have been published on this issue. It was reported by the H. Lee Moffitt Cancer Center in 2000 that

5 of 87 patients (5/87, 5.7%) had metastases in the SLNs.[29] These results led to their proposal to perform SLN biopsy in patients with pure DCIS. In 2003, the European Institute of Oncology Team reported metastases in the SLNs in 7 of 223 patients with pure DCIS (7/223, 3.1%). 6 of the 7 patients underwent ALND, but other metastases were not detected.[27] They published new results in 2005, with an SLN positivity rate lower than 2 years previously (9/508, 1.8%)[72]. Results from Padova indicated that only 1 of 102 patients (1/102, 1%) had metastasis in the SLNs and this was micrometastasis.[24] Similar findings were published by the Cleveland Clinic Breast Center (3/134, 2%), but it is important that only 41 of those patients underwent SLNB, and the other 93 axillary sampling. 1 of the 41 patients (1/41, 2%) exhibited SLN positivity.[26] The New Orleans Ochsner Clinic Foundation investigated 44 patients with pure DCIS and found no metastasis in the SLNs.[73] Other studies evaluated the incidence of SLN metastasis in DCIS and DCISM cases as well. These results can be seen in Table 14 as well.[21,28,30,74] Thus, the rate of SLN positivity in these literature reports ranged from 0% to 12% in patients with pure DCIS and from 10 to 16% in those with DCISM (Table 14).

Reference	N	SLN positivity	%
Intra, M[27] (DCIS)	**223**	**7**	**3.1**
Pendas, S[29] (DCIS)	**87**	**5**	**5.7**
Klauber-De More,N[22] (DCIS)	**76**	**9**	**11.8**
Veronesi, P.[72] (DCIS)	**508**	**9**	**1.8**
Zavagno, G[24] (DCIS)	**102**	**1**	**1**
Kelly, TA[26] (DCIS)	**134**	**3**	**2.2**
Farkas, EA[73] (DCIS)	**44**	**0**	**0**
Wilkie C[30] (DCIS)	**552**	**27**	**5**
Intra[28] (DCISM)	**41**	**4**	**9.7**
Klauber-De More, N21 (DCISM)	**31**	**3**	**10**
Wilkie C[30] (DCISM)	**51**	**7**	**13.7**
Camp R[74] (DCIS + DCISM)	**43**	**7**	**16.3**

Table 14. Literature results on SLN positivity rate in DCIS and DCISM patients

How can a tumor be defined as non-invasive, which gives metastasis to the lymph nodes? One explanation may be an inappropriate histological diagnosis. A microinvasive or invasive focus that can give metastasis cannot be detected in the specimen besides the DCIS. An accurate preoperative histological diagnosis is important if the patient is suspected of having DCIS breast cancer. The main preoperative histological method in our institute is FNAC, but this is not appropriate for the identification of DCIS preoperatively. FNAC was not informative (C1) in 37.3% of our patients in whom *in situ* breast cancer was detected and malignant cells (C5) were observed in only 26.7%, but the presence of DCIS could not be diagnosed. CNB is a more effective method than FNAC, but FNAC is the primary preoperative histological method in Hungary because of its cheapness.[75] The literature indicates that CNB is not a reliable method either. A group from Tampa investigated 613 DCIS patients: 290 (290/613, 47%) underwent preoperative CNB, 301 (301/613, 49%) had excisional biopsy and 9 (9/613, 2%) had FNAC. DCISM was detected in 62 patients. 20 of the 62 patients (20/62, 32%) underwent CNB, 40 (40/62, 65%) had excisional biopsy and 2 (2/62, 3%) had FNAC. The final histological examination indicated that 15 of the 301 patients (15/301, 5%) with excisional biopsy had a proven invasive component besides the DCIS. The rate in CNB was higher (38/290, 13%). The rate in preoperative DCISM patients was higher: 4 of the 40 (4/40, 10%) patients with a preoperative excisional biopsy and 6 of the 20 (6/20, 30%) patients with a preoperative CNB had a proven invasive component in the sample.[30] The reliability of CNB has likewise been investigated (Table 15). [76,77,78] These results demonstrated that CNB is not a perfect method with which to detect pure DCIS, because there can be an invasive component in the specimen (range 13-38%) besides the DCIS.

Reference	**N**	**N (wrong)**	**%**
Wilkie C [30]	**290**	**38**	**13**
Kurniawan E [76]	**375**	**65**	**17.3**
Mittendorf MEA[77]	**30**	**6**	**20**
Goyal A [78]	**587**	**220**	**38**

Table 15. Literature results on CNB reliability in DCIS patients

Another important circumstance is the pathological examination of the SLNs. In our institute SLNs have been examined by HE serial sectioning at 250 μm intervals and by IHC,

which is an effective method to verify micrometastasis (<2 mm) and ITC metastasis (<0.2 mm) in the SLNs. The more detailed the preparation of the SLN, the greater the chance that metastasis will be found in it, and this too can cause different results concerning SLN positivity. A study from the Bethesda National Cancer Institute reported that the rate of SLN positivity detected by IHC in the range of 2-13% when the diagnosis was high-grade DCIS, and in the range of 8-20% when it was DCISM; [79] this was in contrast with the earlier ALND method, which revealed an average 2% positivity in the lymph nodes. The studies from the New York Columbia University and the Netherlands Cancer Institute furnished similar results. These studies investigated patients with a long–term follow-up (127 months) and found that, as compared with SLN negative patients, the survival time was not influenced by the presence of micrometastasis or ITC metastasis in the SLNs if this was detected only by IHC. Accordingly, these patients did not require other surgical treatment.[80] It is important that patients with SLN positivity underwent ALND, and other metastases were not detected in the removed lymph nodes. In the majority of the SLN positive patients, only micrometastasis was detected. These results suggest that axillary block dissection is unnecessary.

The literature and our own experience lead us not to recommend SLNB in all patients with DCIS. SLNB can be necessary in certain circumstances: If the preoperative histological diagnosis indicates a microinvasive focus in the sample, then SLNB is necessary simultaneously. We suggest that, if the final histological examination indicates an invasive or microinvasive focus, SLNB should be recommended as a second step. It is further suggested that, if the indication is an extended DCIS tumor, and the patients must be treated with mastectomy, then simultaneous SLNB is recommended, because an invasive or microinvasive focus cannot be detected in the tumor and SLNB is impossible after mastectomy. If the histological examination detects micrometastasis or ITC metastasis in the SLNs, no other operation is necessary. A correct preoperative diagnosis is very important. If the preoperative histological diagnosis is based only on FNAC (together with mammography and breast ultrasound), we have two choices. We can perform an SLNB, which does not cause significant morbidity, but is expensive, or we can base the necessity of SLNB on the final histological diagnosis, and perform it as a second step.

Summary and new results

- Our results proved that both the GWL and the ROLL methods are suitable for the localization and subsequent removal of non-palpable breast tumors. The most important advantage of the ROLL is the shorter localization time.

- We recommend that the ROLL method should be used for the localization of non-palpable breast tumors if preoperative examinations prove the presence of an invasive breast cancer and SLNB is also to be considered. We would recommend the use of the GWL technique in cases with extensive microcalcifications and when SLNB is not going to be performed (pure DCIS, radial scar, etc.).

- The incidence of NSLN metastasis of invasive breast tumors was influenced significantly only by the age of the patients, the size of the tumor, and the size (type) of the metastasis in the SLN in case of SLN positivity.

- The studied clinical and the histological characteristics cannot be used to create a predictive nomogram that would predict the incidence of NSLN metastasis with good specificity and sensitivity. However, the developed predictive nomograms predict the incidence of NSLN metastasis more precisely in case of lower predictive risk; it is not suitable to exactly predict the incidence in this range either, so the probability of NSLN metastasis cannot be precisely predicated with the currently studied factors.

- Evaluation of the eight international NSLN predictive nomograms in our patients showed that these nomograms are not superior either and therefore cannot be used to predict NSLN metastasis reliably.

- Our studies confirm that in case of pure DCIS, there were no metastases in the SLNs, SLNB may be avoided in such cases. In case of extensive in situ breast cancers, if the primary surgery is mastectomy, SLNB should be performed simultaneously.

- If the final histological examination indicates an invasive or microinvasive focus, SLNB should be recommended as a second step.

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