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Patent Blue V dye: Time to say goodbye?

K. Gomez[1], J. Ansell[2], S. Goyal[3], R.E. Mansel[3]. [1]Nevill Hall Hospital, Oncoplastic Surgical Breast Unit, Abergavenny, United Kingdom; [2]Bristol Royal Infirmary, Surgery, Bristol, United Kingdom; [3]University Hospital of Wales, Academic Breast Unit, Cardiff, United Kingdom

Background: Patent Blue V is used in conjunction with Technetium-99 in the detection of sentinel lymph nodes. It is a food-dye that has provoked a variety of hypersensitivity reactions, including anaphylaxis, when used intravenously. The aim of our study was to assess if the improvements in the techniques of sentinel lymph node imaging have made the continued routine use of Patent Blue V unnecessary in the 21st century.
 Materials and Methods: A cohort of 349 consecutive patients for this retrospective audit was derived from a combination of hospital admission data and operative theatre lists over a 12-month period from a University Hospital and a District General Hospital. All patients underwent intradermal injection of Technetium-99 either the day before or on the morning of surgery, while Patent Blue V was injected in the peri-areolar region of the index quadrant at the time of surgery. All patients had a gamma-camera image taken prior to surgery to visualise the sentinel node.
 Results: 98.2% of our harvested sentinel lymph nodes were detected using a gamma-probe, while only 79% were stained blue. Our lymph node positivity rate is 21.5%. Less than 1% of sentinel lymph nodes were stained blue but undetectable using a gamma-probe and in only 1 patient, from 349 consecutive patients, was there a positive node that was stained blue but 'cold' radioactively.
 Conclusions: Our results show that as refinements in techniques of both nuclear imaging and radiological localisation of sentinel lymph nodes occurs, the use of Patent Blue V should not be as prescriptive as previously reported. We suggest the judicious use of Patent Blue V be limited to those rare situations where there are no detectable sentinel nodes pre-operatively using a gamma-camera.

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A new scoring system for predicting non-sentinel lymph node status using only clinicopathological variables proved at pre- or intra-operation

N. Wada[1], T. Shimada[1], S. Fujii[2]. [1]National Cancer Center Hospital East, Breast Surgery, Kashiwa, Japan; [2]Research Center for Innovative Oncology National Cancer Center, Pathology, Kashiwa, Japan

Background: Axillary lymph node dissection (ALND) is the standard treatment for patients with positive sentinel lymph node (SLN), but only about a half of these patients have positive non-SLN. Several models have been developed to predict non-SLN status. However, almost all models include some permanent pathological variables revealed after the operation. The purpose of our investigation was to develop a new scoring system for predicting non-SLN status using only clinicopathological variables proved at pre- or intra-operation.
 Patients and Methods: This study was based on a prospective database of 1320 patients who underwent SLN biopsy for cT1−2N0M0 invasive breast cancer, of whom 247 (19%) patients had positive SLN(s) proved by intra-operative frozen section analysis and subsequently underwent ALND. All clinical and pathological variables were collected and analyzed according to non-SLN status (negative non-SLN; n = 141 vs. positive non-SLN; n = 106). Univariate analysis showed that clinical T stage (p = 0.033), size of metastasis in the SLN (>2 mm) (p < 0.001), the proportion of positive SLN among harvested SLNs (p = 0.002), presence of lymphatic invasion (p = 0.003) and presence of vascular invasion (p = 0.029) were significant predictive variables of positive non-SLN. The first three variables of those could be proved at pre- or intra-operation. We developed a scoring system (range 0−7) to predict the likelihood of positive non-SLN. Each patients were assigned a score based on the sum of three variables: clinical T stage (T1a, b = 0, T1c = 1, and T2 = 2), size of metastasis (⩽2 mm = 0, >2 mm = 3), the proportion of positive SLN (<50% = 0, 51−99% = 1, and 100% = 2).
 Results: Twenty-nine (12%) of the 247 patients had a score of 3 or less. Among these 29 patients, 3 had at least one positive non-SLN. With a score cut-off of 3, the negative predictive value was 90% (26/29) and the false-negative rate was 3% (3/106). By comparison, the number of patients with a score of 4 or less was fifty-nine (24%). Among these patients, 13 had at least one positive non-SLN. In this instance, the negative predictive value and the false-negative rate were 78% (46/59) and 12% (13/106), respectively.
 Conclusions: This scoring system incorporating these valuables may help determine which patients would benefit from additional axillary surgery, intraoperatively. If we accept the false negative rate of 12% in a score cut-off of 4, one quarter the patients with positive SLN could omit routine ALND.

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Real-time polymerase chain reaction to detect micrometastases in sentinel nodes

A. Moreno[1], J.M. Roman[1], M. De la Puente[1], M. Vidaurreta[2], M. Maestro[2], M.J. Merchan[1], V. Furio[3], A. Prieto[4], J.A. Vidart[5], J.A. Asenjo[3]. [1]Hospital Clinico San Carlos, Gynecology Breast Service, Madrid, Spain; [2]Hospital Clinico San Carlos, Molecular Biology, Madrid, Spain; [3]Hospital Clinico San Carlos, Pathology, Madrid, Spain; [4]Hospital Clinico San Carlos, Nuclear medicine, Madrid, Spain; [5]Hospital Clinico San Carlos, Gynecology Service, Madrid, Spain

Background: Sentinel node biopsy permits detection of micrometastases, but they are often diagnosed postoperatively, so a second operation must be performed.
 Materials and Methods: 390 consecutive cases of negative sentinel node biopsy (no malignancy shown in routine intraoperative frozen section) were studied by: 1. paraffin embeded tissue:hematoxilin-eosin staining (HE), 2. real-time polymerase chain reaction (intraoperative) (RTPCR), 3. inmunohistochemical study (IHQ) and 4. postoperative polymerase chain reaction (PCR). These four groups were compared in terms of diagnostic cabability to detect malignancy using mammaglobin and other gene expression markers to obtain quantitative information on gene expression.
 Results: This trial has been designed to detect metastases of a clinically relevant size (0.2 mm or greater), nodes may be found that are histologically positive (isolated tumor cells or clusters <0.2 mm) and assay negative.

	Percentage of diagnosis of malignancy	p
HE	2%	0.001
RTPCR	12%	
IHQ	8.5%	
PCR	12.6%	

A second comparison was made between RTPCR and PCR groups, and no statistic difference was encountered.
 Conclusions: RTPCR has shown to be a rapid, reliable, method that accurately reflects the presence or absence of clinically actionable metastases in SLNs, and can be used intra-operatively during a SLND.

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Radioisotope count in preoperative lymphoscintigraphy predicts sentinel lymph node metastasis in patients with early breast cancer

T. Nagao[1], T. Kinoshita[1], M. Kikuyama[1], T. Hojo[1], S. Akashi-Tanaka[1], H. Kuriara[2], H. Tsuda[3]. [1]National cancer center hospital, Breast surgery, Tokyo, Japan; [2]National cancer center hospital, Radiology, Tokyo, Japan; [3]National cancer center hospital, Pathology, Tokyo, Japan

Background: Preoperative lymphoscintigraphy (LPG) is used in sentinel lymph node biopsy (SNB) for patients with early breast cancer. But in LPG, the clinical significance of radioisotope (RI) count is unknown.
 Material and Methods: 129 patients with clinically node-negative breast cancer were performed LPG before SNB. ^{99m}Tc-colloid (0.3 ml, 74MBq) was injected into the periareolar site and into subcutaneous tissue around the primary tumor. LPG was underwent immediately after injection (early phase) and after 3 hours (delay phase). Before SNB, hot spots were counted and measured RI count in each phase of scintigraphic image (in vivo). After SNB, the removed SNs were measured RI count (ex vivo) and evaluated histological state. The relation between RI counts (in vivo and ex vivo) and histological state was analyzed.
 Results: In all the patients, SNs were identified by combining methods of radio-colloid and dye. There were 29 cases with SN metastasis (pN+) and 100 cases with no metastasis (pN−). Between pN+ and pN− group, there was no significant difference about age, body mass index (BMI), tumor size and mean number of SNs. The frequency of lymphovascular invasion was significantly higher in pN+ group. In 129 cases, the number of SNs that was visualized in both phases of LPG was 168 (45 in pN+ and 123 in pN−). From evaluation of location and histological state about all the 168 SNs, there were 37 SNs with positive for metastasis (n+) and 131 SNs with negative (n−). In 37 SNs, there were 17 SNs with macrometastasis (n+macro) and 20 with micrometastasis (n+micro). RI count (ex vivo) was not significant about histological state. From RI count of LPG (in vivo), retention index [(delay-early)/early] was calculated about each SN and analyzed relationships to histological state, age and BMI. Mean retention index was lower in n+ (p < 0.01), age ⩾65 (p = 0.08) and BMI ⩾25 (p < 0.01) group. There was no significant difference between n+macro and n+micro groups.

Conclusions: Low retention index in LPG means radioisotope accumulation to SN is impaired. Low retention index in n+ suggests that metastatic tumor cells may obstruct lymphatic pathway to SN. The results suggest that retention index in preoperative LPG can be useful factor for predicting sentinel lymph node metastasis.

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One-step nucleic-acid amplification (OSNA) for sentinel node intraoperative diagnosis: advantages from the classical procedures

L. Bernet[1], M. Martinez-Benaclocha[1], R. Cano Muñoz[2], F. Sevilla Chica[1], B. Ballester Sapiña[3], J. Torró[4]. [1]Hospital Lluís Alcanyís, Pathology, Valencia, Spain; [2]Hospital de la Ribera, Pathology, Valencia, Spain; [3]Hospital de la Ribera, Surgery, Valencia, Spain; [4]Hospital Lluís Alcanyís, Surgery, Valencia, Spain

Background: Classical protocols for intraoperative diagnosis of sentinel node can be either histopathologic study of one or more sections of frozen tissue with or without inmunohistochemical technics or touch imprint cytology. In case of post-operative study the same histopathologic procedure is followed.

The one-step-nucleic-acid-amplification (OSNA) is a new procedure that detects mRNA of the Cytokeratin 19 and can be used for the intraoperative diagnosis of the sentinel node in breast cancer.

Material and Methods: We compare the results of histopathologic procedure in 478 sentinel nodes of breast cancer from the Ribera hospital with the OSNA results in 177 lymph sentinel nodes from the Lluís Alcanyís hospital.

The histopathlogic procedure consisted in the intraoperative study of pairs of frozen tissue sections, one of them stained with H/E and the other with Cytokeratin AE1/AE3 if the H/E result was negative.

The OSNA procedure consisted in the intraoperative homogenization of whole lymph node in a stabilizing solution of mRNA and next, it's amplification in a RD-100/® equipment.

Results: In the histopathological study, we found 116 metastatic cases (24.2%) where 15.6% were macro-metastasis, 5.8% micro-metastases and 2.7% ITC.

If we consider only the metastastic group, we found 64.6% macrometastases, 24.1% micro-metastases and 11.2% ITC.

The mean time for the intraoperative procedure was 49min.

In the OSNA one, we found 18.1% metastatic cases, with 5.6% macrometastases, 9.1% micro-metastases and 3.4% ITC. In the metastatic group, the macro-metastasis percentage was 31.2%, micro-metastatic percentage 50% and ITC 18.7%.

The mean of intraoperative time was 31min.

Conclusions:

1. The OSNA procedure diagnoses more micro-metastases and ITC than the classical histological procedure.
2. OSNA assay saves a mean of 18 minutes in the whole process.

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Predictors of non-sentinel node metastasis in patients with breast cancer after sentinel node micrometastasis

L. Galli[1], V. Mariotti[1], J. Lesaffer[1], L. Orzalesi[1], V. Vezzosi[2], R. Simoncini[1], D. Casella[1], E. Palma[1], S. Aldrovandi[1], L. Cataliotti[1]. [1]Azienda Ospedaliero-Universitaria Careggi, Department of Breast Surgery, Firenze, Italy; [2]Azienda Ospedaliero-Universitaria Careggi, Department of Human Pathology and Oncology, Firenze, Italy

Background: Sentinel lymph node biopsy (SLNB) is nowadays an overall accepted method for axillary lymph node mapping in patients with invasive breast cancer. The decision whether to proceed in case of negative regional lymph nodes or macrometastasis is clear. However in the case of sentinel lymph node micrometastasis the decision is still a subject of debate.

The aim of this retrospective study is to describe the significant factors correlated with involvement of non-sentinel lymph nodes (NSLN) in patients with sentinel lymph node micrometastasis.

Materials and Methods: We reviewed 226 patients in our institution who underwent a SLNB for invasive breast cancer from January 1999 to October 2009 with micrometastatic involvement in the sentinel lymph node. All patients underwent a completion axillary lymph node dissection (ALND).

Age of the patient, histopathological features of the primary tumor as well as the presence of involvement of non-sentinel lymph nodes were recorded.

Results: 31 cases (13.7%) showed involvement of the NSLN. In an univariate analysis, young age of the patient and grading of the primary tumor were associated with having positive NSLN findings. When all factors are included only grading of the primary tumor was a significant predictor of NSLN metastasis (p = 0.027).

Size of the tumor and vascular invasion were not significant associated with positive NSLN findings.

Table. Multivariate analysis°: association between lymph-nodes positivity and selected clinicopathological characteristics; ORs and 95% confidence intervals

Variable	Reference cat.	p-value	OR	95% CI
Age (groups)	<35 years	0.207	0.75	0.47–1.18
Er_Pr receptor status	negative	0.126	1.88	0.84–4.24
pT	1a,b,mic	0.291	0.71	0.38–1.34
Grading	1	**0.027**	**2.33**	**1.10–4.97**
Vascular invasion	positive	0.139	1.91	0.81–4.51
ErbB2	0,1,2	0.671	0.76	0.22–2.68
Ki67	≤30	0.622	0.72	0.19–2.70
Focality	1	0.344	1.32	0.74–2.35
Histotype	1	0.515	1.15	0.76–1.73

Conclusion: Grading of the primary tumor is the most important independent predictor of NSLN metastasis in case of sentinel node micrometastasis. Although there is a trend towards omitting full ALND for sentinel node micrometastasis, in our opinion a full ALND is still recommended in case of high-grade primary breast cancer and certainly in young patients.

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One Step Nucleic Acid Amplification (OSNA) assay for molecular detection of sentinel lymph node metastases in early breast cancer classified according to molecular subtypes: an observational prospective study

S. Buglioni[1], B. Casini[1], M. Marino[1], P. Perri[2], I. Terrenato[3], F. Di Filippo[4], F. Marandino[1]. [1]Regina Elena Cancer Institute, Pathology Department, Roma, Italy; [2]Regina Elena Cancer Institute, First Department of Surgery, Roma, Italy; [3]Regina Elena Cancer Institute, Department of Biostatistics, Roma, Italy; [4]Regina Elena Cancer Institute, First Department of Surgery, Roma, Italy

Background: The new molecular diagnostic tool OSNA has recently been validated and adopted in our Institute as routinary intraoperative test for metastases detection in sentinel lymph nodes (SLNs) of breast cancer (BC) patients. The aims of this study in a prospective series of early BC patients, were: (1) to evaluate the feasibility of intra-operative assessment of SLN using the OSNA system in our department; (2) to find out whether the performance of the OSNA method was comparable to post-operative histologic procedures; (3) to investigate the relationship between SLN status determined using OSNA method, and conventional bio-pathological factors taking into account the novel molecular BC classification: luminal A (LA), luminal B (LB), HER2 (HS), and triple-negative (TN); (4) to identify a subgroup of patients with positive SLN with higher risk of non-sentinel lymph nodes (NSLNs) metastatic involvement.

Materials and Methods: A prospective series of 416 consecutive SLNs from 327 BC patients was evaluated. The OSNA assay follows a short sample preparation step and subsequent rapid amplification of cytokeratin 19 (CK19) mRNA based on reverse transcription loop-mediated isothermal amplification. Each SLN was immediately divided into four slices. Two alternate slides were used for the OSNA assay. The remaining two slides were investigated by six-level histology. The results of these two methods were then compared. This series of BC patients were divided into four main subtypes taking in account the novel BC classification based on the immunohistochemistry phenotypic patterns identified by a few protein biomarkers. The relationship between SLN/NSLNs status and the molecular subtypes were analyzed by multiple correspondence analysis (MCA).

Results: The overall concordance of OSNA with histopathology was 95% with a specificity of 95% and sensitivity of 94%. The complex relationships among the bio-pathological variables analyzed by MCA showed that the metastatic involvement of NSLNs is associated with SLNs with a high copy numbers of CK19 mRNA (>5000) and HS subtype tumors.

Conclusions: This molecular assay can raise the standard of care for patient management as its accuracy is similar to that of standard postoperative histology with the advantage of being standardized, objective, and fast enough for intraoperative use. In our series of early BC patients with positive SLN the risk of NSLNs metastases was higher in the group of patients with HS subtype tumor.