A new optical probe for the detection of the sentinel lymph node using patent blue V dye in breast cancer: A preliminary study

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Abstract. The present study presents a novel near-infrared optical probe for the sentinel lymph node (SLN) detection in breast cancer patients, based on the recording of scattered photons. The aim of this study was to improve the detection of patent blue V (PBV), a dye routinely injected during clinical practice. A combined injection of the dye and radioactive colloid was used in the 24 patients enrolled in the study. The clinical results of the *ex vivo* detection of 70 dye-marked SLNs are reported, subsequent to the injection of various quantities of PBV (0.25-2 ml). The accuracy and success rate of an isotopic probe for the detection of radioactive colloid tracer, the eye visibility threshold of the surgeon and the use of a new optical probe were examined. The radio-labeled and dye-marked sentinel lymph nodes were all detected by the radio-isotopic probe, as opposed to the 75% detected by the eye visibility threshold of the surgeon. The optical probe detected all of the nodes, regardless of the volume of the dye injected. The relative PBV concentration computed by the probe facing SLNs with infravisible/visually undetectable dye-mark was relatively constant at 5.5±1.4 μmol/l. The optical detection of the sentinel lymph nodes using PBV and the probe presented in this study have the potential to reduce the false negative detection rate. This instrument is likely to provide surgeons with a simple diagnostic tool, without significantly changing their surgical procedures.

Introduction

In France, approximately 40% of the newly diagnosed cancer in female patients is breast cancer. The mapping and excision of sentinel lymph nodes is the main minimally invasive technique for cancer staging. The morbidity associated with this technique is less compared to the axillary dissection (1). The procedure used in routine clinical practice is clearly defined. Combined blue dye and isotope injection in the periareolar area allows for the identification of the sentinel lymph node (SLN) at a higher success rate (2-4). Thus, the detection subsequent to skin incision in the armpit involves a nuclear probe. Blue dye, such as patent blue V (PBV), is used to provide visual guidance during the surgical procedure (5,6). Potential allergy to PBV dye has recently been thoroughly studied and severe anaphylaxis is rare, approximately 0.9% (7). However, this detection method involves an element of subjectivity and requires a learning curve on behalf of the surgeon (8).

Currently, attempts have been made to develop accurate and minimally invasive diagnostic and interventional tools. Thus, several optical methods are currently being developed in parallel with conventional methods, such as magnetic resonance imaging (MRI) (9), positron emission tomography (PET) (10) or ultrasound (11), to detect SLN. The use of tracers with radiocolloid metastable technetium 99 (^{99m}Tc) and fluorescent labeling (ICG) is widely suggested (12). Moreover, the photoacoustic approach using nanoparticules has increasingly been considered in several studies (13). However, at present, applications using these molecules are only used in experimental research and require prior approval from the sanitary authorities for marketing and use in routine clinical practice.

In this study, several approaches for SLN detection were investigated. After the combined isotope and PBV injection, we aimed to compare the accuracy and success rate using an isotopic probe for detection of the radiocolloid tracer, and the eye visibility threshold of the surgeon, as well as a new optical probe for dye detection. This prototype is based on four laser diodes and a photodiode detector to automatically detect the presence of PBV. Each laser diode has a specific wavelength to measure the relative concentration of chromophores present in the biological tissue such as PBV, haemoglobin and water. In a previous study, we had demonstrated that the use of four wavelengths allows for an accurate measurement of dye absorption (14). The objective of the present study was to determine the *ex vivo* applicability of the probe with the view to develop a new diagnostic tool for the surgeon. The ultimate goal was to reduce the possibility of false negatives during the surgical procedure and propose an alternative to the isotopic detection.

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Materials and methods

General. This prospective study was performed in the Department of Pathology of the Paul Strauss Cancer Center in Strasbourg (France), given that the prototype used has not yet been approved by authorities for use in the operating room.

Patients. In total, 24 patients (females with clinical T0 and T1, N0M0 invasive breast cancer) were enrolled in this preliminary study. Diagnosis was achieved by pre-operative core biopsy.

Exclusion criteria were pregnancy, ductal carcinoma *in situ*, multicentric tumors, inflammatory tumors, neoadjuvant chemotherapy and metastatic breast cancer. The mean patient age was 57 years (range, 41-79). The mean body mass index was 24.7 (range, 15.9-42.5).

Techniques. The patients underwent the same protocol. Initially, 0.2 ml of 11- to 30-MBq ^{99m}Tc-labeled sulfur colloid (Nanocis, Cis Bio International, Saclay, France) was injected into the periareolar area, 24 h prior to surgery. Breasts were massaged at the site of injection for 2-3 min to improve the diffusion of the radiocolloid. Patients had pre-operative lymphoscintigraphy using a γ-camera. Static images were acquired for ~10 min, between 30 min and 2 h post-injection, to locate the site of the identified drained lymph nodes. After anesthesia, PBV (Guerbet, Aulnay-Sous-Bois, France), was injected periareolarly 5-10 min before skin incision. The breast was massaged for 2-3 min to facilitate the diffusion and uptake of the dye. To obtain PBV-marked surgical sections with visible and invisible to the eye dye-uptake, decreasing dye volumes ranging from 2 (1 patient) to 1 (3 patients), to 0.5 (2 patients) and, then, 0.25 ml (16 patients) were injected. In addition, 2 patients, without dye injection, were included in the protocol as controls. Axillary skin was incised and a careful dissection was performed to search for blue-stained lymph nodes.

An intraoperative isotopic probe (Europrobe, Eurorad, Strasbourg, France) was used to help and guide the dissection. SLNs were identified *in vivo* when they were blue, had radioactive counts or both characteristics. Radioactive nodes were removed until the background radioactivity of the axilla was <10% of the *ex vivo* count of the hottest node removed.

When the excised nodes arrived at the Department of Pathology (Paul Strauss Cancer Center), the optical probe prototype was used on each surgical section to detect dye accumulation in the SLNs and assess its performance. This measurement was performed prior to examination (frozen sections). The pathologists were consulted as to whether they were able to detect blue-stained lymph nodes visually, and visual assessments were compared with the information obtained from the probe.

Subsequent to the SLN biopsy, the patients underwent a lumpectomy, while patients with involved SLN underwent a hyperectomy combined with axillary lymph node dissection of level I and II.

Optical probe prototype description. The present study commenced with the development of a first prototype, demonstrating the feasibility of optical dye detection using scattered photons (15,16). However, that optical probe did not completely discriminate between dye accumulation and other sources of tissue absorption changes, possibly encountered by surgeons *in vivo*.

Table I. Pre-operative patient data.

Patient data	No. of patients	%
Tumor location		
Supero extern	8	33.3
Supero intern	6	25.0
Supero median	2	8.3
Infero extern	1	4.2
Infero median	1	4.2
Equato extern	4	16.7
Equato intern	1	4.2
Retro mammary	1	4.2
Scintigraphy		
Axillary	18	75.0
Axillary double	4	16.7
Internal mammary	1	4.2
Axillary + internal mammary	1	4.2

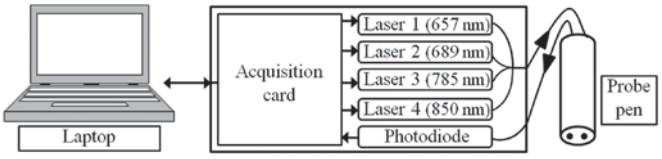

Figure 1. Schematic diagram of the four wavelength optical probe.

The new probe used in this study was described in detail by Tellier *et al* (14). It operates with four laser diode modules (I.L.E.E. AG, Urdorf, Switzerland), with an average power of 400 μW, emitting at four different wavelengths (657, 689, 785 and 850 nm). The laser beams are injected in a four-in-one furcated optical fiber with a common output, and are monitored via a National Instrument (NI USB-6229; National Instrument Crp., Austin, TX, USA) multifunction data acquisition (DAQ) module connected to a laptop via a USB port. Back-scattered photons are collected with a second fiber, detected by a photodiode (S5106; Hamamatsu, Hamamatsu City, Japan) and digitalized by the same NI DAQ module. The sample examined was illuminated with a sterilized stainless steel probe, including the 2 optical fibers, and placed 4 mm apart (Fig. 1).

The wavelength of 657 nm lies in the absorption band of PBV. Fig. 2 shows the absorption spectra of the PBV measured, the oxy-haemoglobin and the deoxy-haemoglobin, based on the website of the Oregon Medical Laser Center (17). The extinction coefficient of deoxy-haemoglobin was clearly higher compared to the oxy-haemoglobin, at 657 nm. Consequently, a decrease in blood oxygenation was likely to result in an increased absorption at this wavelength and be erroneously interpreted as increased optical absorption by the dye. To avoid such a misinterpretation, three other laser diodes were incorporated into the probe at 689,

Table II. Results of the SLN analysis.

Scintigraphy information	Biopsied SLNs, n	Histological status	No. of SLNs
Axillary SLN	48	Uninvolved	40
		Micrometastases	7
		Macrometastases	1
Axillary double	25	Uninvolved	23
		Micrometastases	0
		Macrometastases	2
Internal mammary	3	Uninvolved	2
		Micrometastases	1
		Macrometastases	0
Left axillary + intern mammary	2	Uninvolved	0
		Micrometastases	1
		Macrometastases	1

SLN, sentinel lymph node.

785 and 850 nm in order to measure PBV concentrations, taking blood oxygen saturation into account.

The laser diodes were modulated at different frequencies to separate the four components of the measured signal, by means of a spectral analysis. The absorption coefficients of the two forms of haemoglobin, as well as of the PBV dye were then computed to detect a relative PBV concentration in the tissues (14). This relative concentration was continuously displayed on the laptop screen, and was equal to the true concentration multiplied by a coefficient termed as the differential pathlength factor that varies as a function of the optical properties of the medium (18), its value ranging from ~5 to 10 for the tissues.

Results

Clinical data. The mean number of excised SLNs per patient was 3.3 (range, 1-8). The mean clinical and histopathological tumor size was 12.6 and 13.1 mm in diameter, respectively.

Pre-operative data are shown in Table I. In 75% of the patients, the marked node in the axillary area was detected with the γ camera. Moreover, in four patients lymphoscintigraphy detected two marked axillary lymph nodes.

When only one node was visualized, the mean of the excised sentinel lymph node was 2.7. That number increased to 6.3 when lymphoscintigraphy showed two axillary SLNs. No pathological metastases were found in SLN in 65 surgical sections (83.3%). Metastatic disease was found in 13 histopathological specimens (9 with micro- and 4 with macrometastases), originating from 10 patients (Table II). The histopathological characteristics of the tumor, Scarff-Bloom-Richardson (SBR) grade, oestrogen receptor, progesterone receptor, Ki-67 and HER2 are shown in Table III.

Detection results. Measurements were carried out ex vivo in the Department of Pathology (Paul Strauss Cancer Center) pending clearance for per-operatory use.

Table III. Histopathological patient characteristics.

Characteristics	No. of patients	%
SBR grade		
I	10	41.7
II	10	41.7
III	4	16.7
Oestrogen receptor		
Negative	2	8.3
Positive	22	91.7
Progesterone receptor		
Negative	3	12.5
Positive	21	87.5
Ki-67 (%)		
0-10	7	29.2
11-20	9	37.5
21-30	3	12.5
>30	4	16.7
Unknown	1	4.2
HER2		
Negative	21	87.5
Positive	3	12.5

SBR, Scarff-Bloom-Richardson.

All of the 78 nodes were detected by the isotopic probe. The 8 SLNs without dye marking were used to validate the probe, and as such did not yield any relative PBV concentration. Subsequently, only the 70 excised SLNs marked with PBV were considered. The dye-marked SLNs were detected by the optical probe, regardless of the visibility of the dye uptake. Subsequent to injection of 2, 1, 0.5 and 0.25 ml dye,

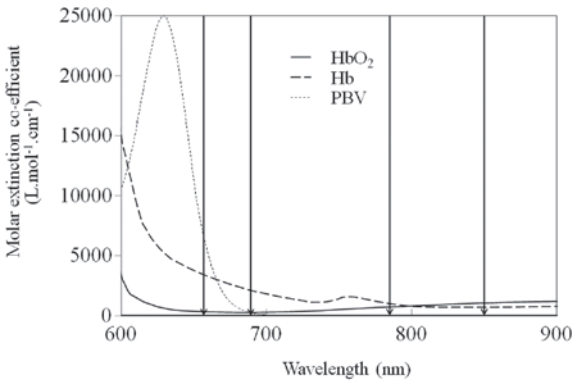

Figure 2. Molar extinction coefficient spectra of oxy-haemoglobin (solid line), deoxy-haemoglobin (dashed line) and PBV (dotted line) are shown. The four solid arrows show the wavelengths used in the optical probe.

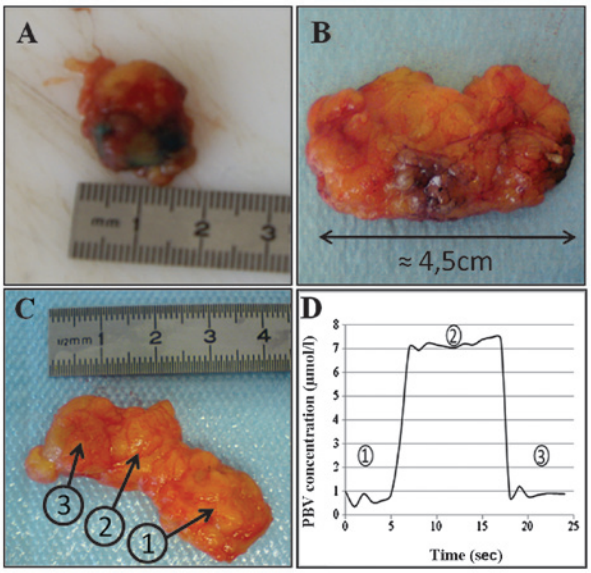

Figure 3. Visual differences between three biopsy samples after different injection volumes: (A) 2, (B) 0.5 and (C) 0.25 ml and (D) profile of the relative PBV concentrations during the displacement of the probe on the sample C containing a SLN in position 2.

3, 7, 6 and 54 SLNs were detected, respectively. The visual aspects of the surgical sections coloured by various PBV quantities are shown in Fig. 3.

For each surgical section and each injection volume the optical probe was able to detect the presence of exogenous dye in the tissue sample. However, only 53 nodes (i.e., 67.9%) were visually detected by the surgeon. Of the total number of excised nodes, 13 were micro- and macrometastatically involved. All of the nodes were detected by the isotopic and optical probe, whereas only 9 (69.2%) metastatic nodes were detected by visual inspection (Table IV).

Fig. 3C is an example of SLN localization using our optical prototype on a biopsy sample after a 0.25-ml dye injection. During the measurement, the probe was initially directed to the part of the excised tissue not containing the node. When the pen probe was placed in front of the SLN, dye was observed in the node. Detection occurred from 7 to 17 sec, as shown in Fig. 3D, with the PBV concentration profile increasing from 1 to 7 μmol/l. During the final seconds, the probe was not directed to the node any longer.

Table IV. Comparison of the sensibility detection of dye-marked SLNs.

Detection method	Total no. of SLNs (%)	No. of metastatic SLNs (%)
Europrobe	70 (100)	13 (100)
Optical probe	70 (100)	13 (100)
Surgeon eye	53 (67.9)	9 (69.2)

SLN, sentinel lymph node.

The 17 visually undetected nodes varied with respect to their histological status: 13 were uninvolved, 3 were micrometastatic and 1 was macrometastatic. One of the nodes was excised after the injection of 0.5 ml PBV, while the remaining nodes were excised after the injection of 0.25 ml PBV. To compare these nodes, only the 16 SLNs marked with 0.25 ml dye were considered.

In the 16 nodes measured with the probe, the mean relative PBV concentration was 5.5±1.4 μmol/l. The individual values were also analyzed as a function of the stage of metastatic invasion. The mean relative PBV concentration was 5.8±1.8 μmol/l in the 12 uninvolved nodes, 4.9±3.3 μmol/l in the 3 micrometastatic nodes and 4.3±0.5 μmol/l in the single macrometastatic node. Thus, the standard deviation corresponded to the signal variations detected during measurement. Although these results showed a low spreading of values in the sample categories, these data are not sufficient to establish a possible correlation between the histological status and relative dye concentration in the SLNs. The number of surgical sections available for the histological statuses (micro- and macrometastatic) was not adequate for an appropriate statistical analysis.

Discussion

The main advantage of the developed optical probe is its sensitivity in detecting PBV as used in routine clinical practice for SLN detection.

These experiments demonstrated that the instrument allowed for the measuring of the relative dye concentration in surgical sections. Currently, approximately 1% of the nodes are undetectable when using a radiotracer and a dye during sentinel lymph node biopsy (19). An increase in the optical sensitivity using a probe is most likely to reduce this false negative detection. In this case, optical detection was effective for each excised node, regardless of the dye concentration injected, even for the potentially lowest concentration used in the present study with a view to achieve a visually undetectable uptake. Upon visual inspection by clinicians only 53 nodes were detected. The mean relative PBV concentration was 5.5±1.4 μmol/l measured in the SLNs with visually undetectable/infravisible dye-mark had a relatively low dispersion. Detection with the probe was effective for patients with different clinical status and a good reproducibility.

The dye distribution in different nodes subsequent to injection of small concentrations of PBV was determined in previous pre-clinical studies with the present experimental setup (15). Those results, combined with the findings of the present study, open promising future perspectives in the operating room.

These experiments demonstrated the limited extent of SLN detection. This problem might be overcome by increasing one of the two following parameters: the source/detector separation and/or the laser intensities. Time-resolved optical methods also have the potential of being interesting alternatives to this approach. Being cost-effective, such techniques increase the extent of the detection, while improving the spatial resolution. (20).

The present results are promising, suggesting that the development of an opto-nuclear probe is an interesting approach to reduce false negative rates. This new technique might be used during node excision and provides surgeons with a simple diagnostic tool, without significantly changing their surgical practice. Additionally, the instrumentation and dye are cost-effective.

In conclusion, there is skin tattoo risk associated with the injection of the PBV, while decreasing the injected dose (by a factor 4) might certainly reduce this undesirable side-effect in patients.

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