

In vivo localization of occult lesions and margins in breast carcinoma using radio-fluorescence: a new hybrid technique

Antônio César Pereira^{1*} , Sonia Marta Moriguchi² , Mara Costa Dutra³ ,
Rogério Bizinoto Ferreira⁴ , Alexandre Marchiori Xavier de Jesus⁵ ,
Délio Marques Conde⁶ , Sebastião Alves Pinto⁷ , Jorge Rodolfo Beingolea⁸ 

ABSTRACT

Objective: The aim of this study was to present a new technique for hybrid marking of non-palpable breast lesions and *in vivo* evaluation of surgical margins, called Fluorescence and Seed for Hybrid Intraoperative Evaluation. **Methods:** Seven women with non-palpable breast lesions and suspected or confirmed malignancy underwent prior iodine-125 seed implantation and peripheral intravenous administration of indocyanine green 30 min before surgery. A hybrid gamma probe with an optonuclear probe was used to detect gamma radiation in the lesions and, sequentially, the fluorescence mode, in the same lesion and its margins, after its removal. **Results:** This method distinguished, in real time, one benign and six malignant lesions, guiding the removal, identifying the remaining neoplastic area in the surgical bed, and allowing its intraoperative enlargement. **Conclusion:** This pilot study evaluates the feasibility of this new technique in identifying the primary lesion and controlling surgical margins using hybrid technology.

KEYWORDS: breast cancer; surgical margins; indocyanine green, nuclear medicine.

INTRODUCTION

Breast cancer is the second-highest incidence in the world and the first among women, representing a major public health problem worldwide¹.

Extreme changes in the surgical approach to breast cancer have occurred significantly in recent years. Minimally invasive surgeries emerged thanks to advances in technology, which have helped oncological surgeons to operate on increasingly smaller lesions detected only in imaging tests².

In recent decades, nuclear medicine has become a great ally in the surgical field as a result of the development of the portable gamma radiation detector (gamma probe), which introduced studies based on the sentinel lymph node (SLN)³, extending to the radioguided localization of non-palpable breast lesions (Radioguided Occult Lesion Localization), initially using

radiopharmaceutical and, later, sealed sources of iodine-125, known as iodine-125 seeds^{4,5}. More recently, hybrid tracers, which contain integrated radioactive and fluorescent markers, have been introduced to allow the detection of SLN^{6,7}. Currently, the most promising tracers for this technique are considered to be colloids labeled with technetium-99m and indocyanine green (ICG).

ICG, which has been used since 1950⁸, is a blood pooling agent that has a different delivery behavior between normal and cancer vasculature. In normal tissue, ICG acts as an indicator of blood flow in the narrow capillaries of normal vessels. However, in tumors, it can act with a diffusible (extravascular) flow caused by greater extravasation resulting from the increase in capillarity, thus intensifying the accumulation of the substance at the site⁹.

This pilot study aims to describe the development of a new hybrid technique for marking and locating non-palpable lesions

¹Centro de Diagnóstico por Imagem – Goiânia (GO), Brazil.

²Universidade Estadual Paulista, Faculty of Medicine of Botucatu – Botucatu (SP), Brazil.

³Santa Casa de Misericórdia de Goiânia – Goiânia (GO), Brazil.

⁴Hospital Estadual Alberto Rassi – Goiânia (GO), Brazil.

⁵Instituto de Mastologia e Oncologia – Goiânia (GO), Brazil.

⁶Universidade Federal de Goiás – Goiânia (GO), Brazil.

⁷Instituto Goiano de Oncologia e Hematologia – Goiânia (GO), Brazil.

⁸Universidade de São Paulo – São Paulo (SP) Brazil.

*Corresponding author: dr.antoniocesar@yahoo.com.br

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and evaluating surgical margins in real time, called Fluorescence and Seed for Hybrid Intraoperative Evaluation (FLASHIE), using hybrid gamma probes to detect seed iodine-125 and ICG.

METHODS

This is a cross-sectional, interventional pilot study with prospective data collection from patients with suspected or confirmed non-palpable breast cancer lesions between January and February 2018, after approval by the local Research Ethics Committee.

Seven patients over 18 years of age were included, one with a pre-operative diagnosis of benignity and the other six with a malignant biopsy, after signing the informed consent form.

The inclusion criteria were patients investigated by fine needle aspiration, core biopsy, or mammotomy, with an indication for radioguided lumpectomy. Patients with associated excisional biopsy, liver disease, uremia, asthma, a history of allergy to iodine or seafood, and previous anaphylactic reaction to dye injection were excluded from the study.

This is a convenience sample due to the restricted research development period of 1 month, when the researchers had the Europrobe Optonuclear equipment available for the work on loan from the manufacturer Eurorad in partnership with the commercial representative in Brazil, Eckert and Ziegler. Initially, the study was scheduled to last 3 months, but due to customs delays, it was restricted to 1 month.

Occult lesions were marked by implanting an iodine-125 seed in the center of each patient's breast lesion in a procedure guided by ultrasound (nodules) or stereotactic (microcalcifications) between 1 and 5 days before surgery. The correct apposition of the seeds was confirmed by mammographic images in two projections and planar scintigraphic images of the thoracic region in the anterior and lateral projections ipsilateral to the affected breast, acquired on the day of implantation or 1 day before surgery.

The seeds consist of a sealed titanium source of 4×1 mm in diameter with an iodine-125 filament included with 0.2 mCi activity and energy of 27–35 KeV (IPEN, São Paulo, Brazil). Scintigraphic images were captured in Symbia E (Siemens, Germany) or Millennium MG (General Electric, USA) gamma cameras with 500 Kctg, 256×256 matrix, photopeak centered at 30 keV, and ±10 keV window.

On the day of surgery, 30 min before the surgical intervention, 5 mg of ICG (ICV[®] Ophthalmos, São Paulo, Brazil) was injected intravenously. During surgical exploration, an optonuclear probe for open surgery (Europrobe 3.2 Optonuclear, Euromedical Instruments, Eckbolsheim, France) was used with the combination of a traditional gamma probe and a 769 nm narrowband laser excitation source to remove the tumor lesion (Figures 1 and 2). The probe was prepared with a sterile plastic cover to be handled by the mastologist.

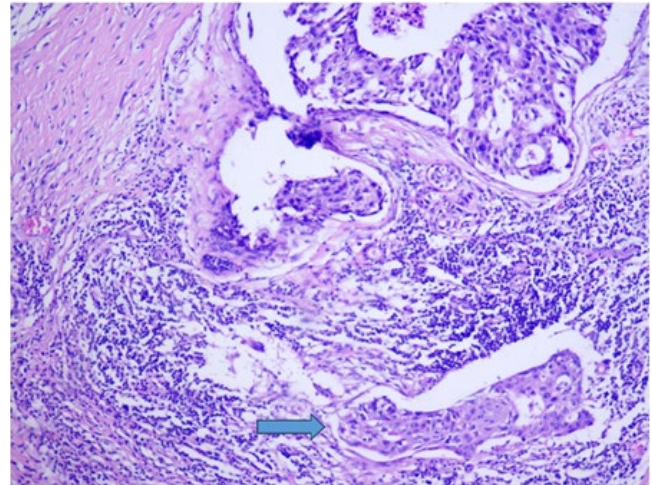


Figure 1. Anatomopathological study of the neoplastic area in the posterior region of the surgical bed (FI=15) after the removal of the primary lesion in patient 3. Color: H&E. Objective: 40×. The blue arrow indicates the presence of microinvasive carcinoma.

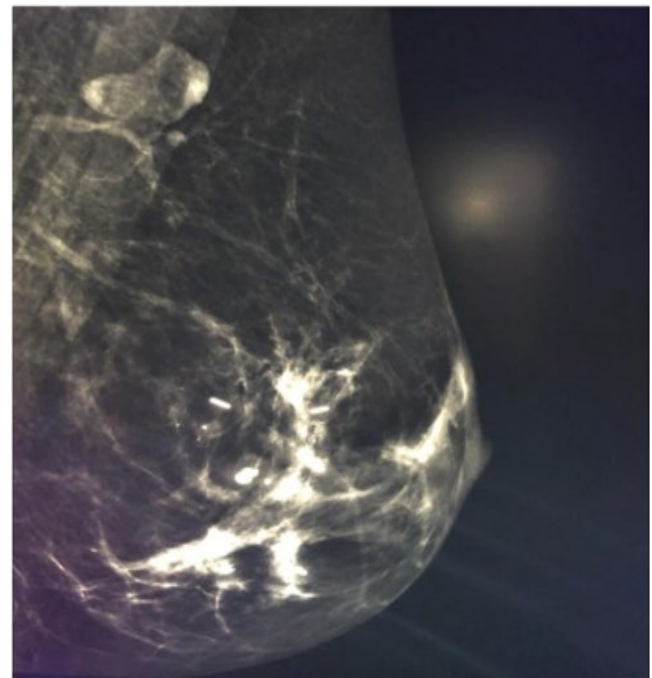


Figure 2. Mammography of patient 3 shows areas of microcalcifications marked by iodine-125 seed. No radiographically suspicious area was identified in the region posterior to the marked area.

During dissection, the lesion was localized initially, guided by the iodine-125 seed by the probe in conventional gamma-ray reading mode. Once the lesion was located, the probe's readout mode was switched to fluorescence. The areas defined in the preoperative images were analyzed by ICG fluorescence, and the reading was performed in ambient light.

Tumor fluorescence readings were performed intraoperatively (*in vivo*) and perioperatively (*ex vivo*). Once the lesion was removed, a new reading was performed at the margins of the lesion and in the tumor bed. A quantitative parameter was used to classify positivity for malignancy both in the primary lesion and in the surgical bed.

The fluorescence index (FI) was adopted, which consists of dividing the fluorescence count measured in the tumor by the fluorescence count measured in the healthy tissue surrounding the tumor and is considered positive when $FI \geq 3$, similar to that adopted in the study carried out by Duarte et al.¹⁰ at the Universidade de Campinas – Brazil, in a technique called Radioguided Intraoperative Margin Evaluation, in which a radiopharmaceutical (^{99m}Tc-sestamibi) was also used during surgical procedures to check if the resection margins were negative.

The excised material was sent to the Pathology Service for cytology and freezing analysis by imprint. Part of the sample was submitted to a histopathological study, fixed by formalin and paraffin, and a late anatomopathological study (AP) by staining with hematoxylin and eosin. The specimen was also subjected to automated immunohistochemistry (IMHQ) with HIER PTLINK antigen retrieval incubation and development in AUTOSTAINERLINK48/DAKO.

The surgical specimens were generally classified based on the absence or presence of malignancy, whereas in this study,

the cases were classified based on whether or not the margins and surgical bed were affected. For ethical reasons, the patients were named numerically.

RESULTS

Seven women, aged between 53 and 72 years (an average of 63.7 years), were included in this study. Seven lesions were removed, all guided by the radioactive seeds and located with the gamma probe. Most of them had a primary malignant lesion. Only one patient (n# 2) was confirmed as having a benign lesion (complex sclerosing papilloma), with FI=1. There was a predominance of nodular lesions, some with associated microcalcifications and in situ components. All primary malignant lesions presented $FI \geq 3$. Margins with a larger FI were malignant or small. ICG was more assertive than freezing in identifying compromised margins, based on AP. Table 1 presents this information.

Patient 3 presented FI=4 in the primary lesion, marked by iodine-125 seed, and in a deeper area, relatively distant from the marked area, FI=15 was measured. Due to the high FI, we opted for additional surgical expansion in this region. The AP confirmed malignancy. During the surgical procedure, in the frozen section, the margin of the enlarged region was considered compromised by the pathologist, so it was enlarged again, although there was no increase in the fluorescence reading in this surgical bed (FI=1).

Table 1. Correlation between the findings of fluorescence index, anatomopathological study/immunohistochemical study, and surgical margins of breast lesions.

Patient	Characteristics of the injury	Pathology	In situ	Local	FI	Freezing	AP/IMHQ	Assertiveness FI	Assertiveness freezing
1	Nodule	IDC GI* Luminal A	Not observed	Lesion	3	Malignant	Malignant	Yes	Yes
				Margin	1	Free	Free	Yes	Yes
2	Nodule	Complex sclerosing papilloma	Unrealized	Lesion	1	Benign	Benign	Yes	Yes
				Margin	NA	NA	NA	NA	NA
3	Nodule	Multifocal CDmI (3 foci) Hybrid luminal	Extensive (99.5% of the lesion)	Lesion	4	Malignant	Malignant	Yes	Yes
				Deep posterior region	15	Compromised	Compromised	Yes	Yes
				Margin	1	Compromised	Negative	Yes	No
4	Nodule	IDC GI* Luminal B	Scarce	Lesion	4	Malignant	Malignant	Yes	Yes
				Margin	1	Compromised	Free	Yes	No
5	Nodule	IDC Luminal A	Not observed	Lesion	7	Malignant	Malignant	Yes	Yes
				Posterior margin	6	Free	Exiguous	NA	No
6	Nodule + microcalcifications	IDC Luminal A	Extensive (>25% of the area)	Lesion	4	Malignant	Malignant	Yes	Yes
				Margin	1	Free	Free	Yes	Yes
7	Microcalcifications	IDC GI* Luminal A	Extensive (99.5% of the lesion)	Lesion	4	NR	Malignant	Yes	NA
				Posterior margin	5	NA	Exiguous	NA	NA
				Margin	4	NR	NR	NR	NR

*Scarff-Richardson-Bloom. In situ: associated in situ component; FI: fluorescence index; AP: anatomopathological study; IMHQ: immunohistochemical study; IDC: invasive ductal carcinoma; GI: grade I; NA: not evaluated; CDmI: microinvasive ductal carcinoma; GI: grade II; NR: not carried out.

After the AP with paraffin, the frozen section study was re-read, and the posterior margin was retrospectively considered free, a result reinforced by the histopathological reading of the new margin enlargement, which was also negative for malignancy. Figures 1 and 2 show, respectively, AP confirming micro-invasive carcinoma and the absence of suspicion of malignancy on mammography at the site with FI=15.

Patient 7 presented a heterogeneous area with cysts, indistinct margins, with intervening calcifications and a posterior acoustic shadow in the left breast on imaging examination, and a core biopsy was performed. The AP identified fibrocystic mastopathy containing areas of usual ductal hyperplasia and an atypical 1.0 mm area. This dubious finding indicated the removal of the entire lesion.

During the location of the suspicious area, with the iodine-125 seed, the ICG reading showed FI=4 at the seed location and FI=5 at the posterior margin in the ex vivo measurement of the piece. The reading corresponding to this region on the surgical bed also measured FI=4. As there was no diagnosis of a malignant lesion yet, freezing was not performed. Although the margin presented $FI \geq 3$, the mastologist preferred not to enlarge this area, opting not to remove it and wait for the AP result.

In this case, invasive ductal mammary carcinoma, grade III (Scarff-Richardson-Bloom), with an extensive carcinoma in situ component, corresponding to 90% of the lesion, which measured 2.0×1.8 cm in its largest dimensions, with an invasive area of 0.7×0.5 cm and multifocal lobular cancerization, was present.

This result was confirmed by immunohistochemistry (IMHQ) as invasive mammary carcinoma, luminal A subtype. The margins were free but narrow in the posterior, medial, and inferior regions, so it was decided to investigate the sentinel lymph node without enlarging the margins.

Patient 4 had infiltrating breast carcinoma, grade II (Scarff-Richardson-Bloom), which during the surgical procedure indicated FI=4 in the lesion and FI=1 in the margins, that is, without identifying malignancy. Freezing showed compromised margins on the anterior and inferior surfaces, which indicated enlargement of the margins. The AP showed free margins, indicating a false-positive result for the frozen section and a true-negative result for the ICG evaluation.

Patient 5 had infiltrating breast carcinoma, grade II (Scarff-Richardson-Bloom) on core biopsy, and during the surgical procedure, she presented FI=7 in the lesion and a small area in the posterior region of the surgical bed with FI=6, which showed a small posterior margin (1.0 mm).

Patients 1 and 6 obtained, respectively, FI=3 and FI=4 in primary lesions and frozen-free margins, with FI=1, concordant findings.

DISCUSSION

Despite the low sample size of this pilot study, good results were obtained in the evaluation of this technique using hybrid marking.

The assertiveness of ICG in primary tumor lesions was precise, distinguishing malignant from benign lesions.

It is clear that the series must be increased, since in the literature there is a description of false-positive results due to mastitis where there is increased microvascular permeability, which allows the extravasation of macromolecules, and also in epidermal cysts, the latter being an uncommon entity¹¹. The diagnostic association with other investigation methods helps in differentiating these lesions.

Breast-conserving surgery via partial mastectomy is an increasingly used option in the treatment of patients with invasive or in situ carcinoma³, but the assessment of margins can be difficult. Frozen freezing and AP are used to prevent residual disease after surgery and have an impact on the risk of breast tumor recurrence^{2,12}.

In this study, three of six patients presented an extensive in situ component in a large part of the lesion, which was marked by ICG. Hagen et al.¹³ also reported the case of a patient in whom there were two nearby lesions, identified as well-differentiated invasive ductal carcinomas, accompanied by a low-grade ductal carcinoma in situ, which was evidenced on fluorescence mammography with ICG. The presence of multifocality was observed in patient 3. Perhaps, the extremely high FI present in the remaining tissue is associated with the secondary multifocal focus.

St. John et al.¹⁴ concluded in their meta-analysis that the diagnostic accuracy of frozen section and cytology studies is currently unparalleled. According to these authors, to become a disruptive technology, emerging techniques will need to compete with this level of precision and provide significant improvements, such as speed of results, cost-benefit, and accessibility of information to the surgeon, to allow rapid operational decision-making, which must be accurate and appropriate. In this context, the ICG fluorescence technique could play an important role, as it evaluates the margin in real time.

The exact determination of the extent of breast carcinoma is increasingly important for the breast surgeon. If a technique underestimates the actual size of the lesion, it may lead the surgeon to a more conservative procedure, leaving residual disease, and, if overestimated, it may induce the removal of normal tissue, compromising the cosmetic aspect of the surgery.

Holland et al.¹⁵ showed foci of carcinomas more than 2 cm away from the main tumor in 43% of cases and more than 4 cm in 10% of them. In the sample studied, although not identified by other imaging methods or frozen section studies, a distant neoplastic area was identified by the fluorescence technique when reading the surgical bed, making it possible to enlarge the margin in real time.

There were also two patients (5 and 7) who presented small margins, and the FI was high in their respective regions, favoring the good correlation of the technique with the changes seen in the AP, indicating that the clinical integration of these two powerful technologies, interventionalists with molecular imaging potential, can act in synergy and add important characteristics

of both techniques, including the ability to microscopically evaluate lesions and their margins, both in vivo and ex vivo^{16,17}.

Furthermore, the analysis of surgical margins can potentially be expanded to evaluate other organs and even use other fluorescent dyes, as reported by Xiao et al.¹⁸, who used sodium fluorescein for fluorescent-guided surgeries for the excision of brain metastases from breast cancer.

Among the limitations of the study, the main one was the short period of availability of equipment for the project, which was scheduled for 3 months, but was carried out in 1 month due to bureaucratic problems with customs clearance, which directly impacted the sample size and, consequently, the development of research.

The small sample size, included for convenience, collected a heterogeneous sample of breast lesions, not allowing similar lesions to be grouped to investigate the existence of a pattern of accumulation of ICG and FI in different types of lesions. It seems that in multifocal and non-nodular lesions, the importance of using this technique is greater due to the limitations of other imaging techniques. New studies must be conducted to provide answers and evaluate the real impact of this technology on various breast injuries.

CONCLUSIONS

Within the limitations of the study, it was concluded that the proposed technique, FLASHIE, is feasible and quite promising.

Preliminary results indicated the potential to advance the state of the art in cancer surgical techniques, with the possibility of accurate detection of occult primary lesions and an indication of residual disease after tumor removal, in real time, especially in those areas not detected by diagnostic techniques and management of margins already established.

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AUTHORS' CONTRIBUTION

ACP: conceptualization, data curation, investigation, methodology, resources, project administration, visualization, writing – original draft. SMM: data curation, investigation, writing – review & editing. MCD: data curation, formal analysis, investigation, validation, writing – original draft. RBF: data curation, methodology, research, validation, visualization. AMXJ: data curation, methodology, research, validation, visualization. DMC: investigation, validation, visualization. SAP: data curation, validation, visualization. JRB: data curation, investigation, validation, writing – review & editing.

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