

Original article

Intra-operative touch preparation cytology following lumpectomy for breast cancer: A series of 400 procedures

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ABSTRACT

Aims: Achieving negative margins is essential in conservative treatment for breast cancer. The conventional method for intra-operative assessment of resection margins is gross or histological examination of frozen sections. We describe and evaluate the contribution of an original intra-operative touch preparation cytology (IOTPC) technique (400 procedures) performed on 396 patients.

Materials and methods: IOTPC consists of touching glass slides to the surfaces of interest after gently pressing the spatially localized specimen taken according to predetermined conditions. The result is conveyed to the surgeon immediately and compared with the conventional histological findings after embedding in paraffin.

Results: The average response time is 10 min, which renders the technique compatible with standard operating room procedures and its cost is reasonable. The method has a sensitivity of 88.6%, specificity of 92.2%, positive predictive value of 73.6%, negative predictive value of 97%, and correlation with paraffin section histology of 91.5%. Only 5 true false negatives were found in this series and the technique prevented 11.75% of secondary re-excision procedures for positive margins.

Conclusion: IOTPC is a reliable extemporaneous method for assessing surgical margins in conservative treatment for breast cancer and a useful tool for surgeons.

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Introduction

In the last 30 years, local treatment combining wide breast-conserving resection and radiotherapy (Breast-Conserving Therapy) has become the standard treatment for early stage breast cancer.¹ This therapy has enabled breast conservation and long-term safety in cancer.^{2,3} Local recurrence is the main risk of BCT, varying from 6 to 24% depending on the length of the series, with an annual rate of 1%, even when radiotherapy is administered.^{2,4} With an extensive in situ component, invasion of the margins around the lumpectomy cavity is the main factor of local recurrence after conservative treatment.^{2,5} The difficulty with breast-

conserving treatment is therefore reconciling an objectively satisfactory cosmetic result⁶ with excision to healthy tissue.^{4,7} This explains why a large number of patients undergo surgical re-excision for inadequate margins (up to 20 or 30%), in particular young patients with a clinically palpable lesion, and presenting with an extensive in situ component or a lobular subtype.^{8,9} An intra-operative assessment of resection margin status must enable directed resections to be made, thus reducing the deferred surgical re-excision rate and cosmetic, psychological and economic prejudice.⁸ The most widely used technique in clinical practice is gross examination, which is sometimes verified by an X-ray examination, with a margin of clearance of healthy tissue at least 1 cm thickness. Frozen section margin assessment is gradually falling into disuse as it is time-consuming, costly and difficult to perform in an adipose region and is also a source of material loss.^{10,11} We present an imprint cytology technique (intra-operative touch preparation cytology, IOTPC) and correlate findings for a series of 400 procedures with conventional analysis of paraffin-embedded operative specimens which remains the gold standard.

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Materials and methods

Materials

This controlled prospective study was conducted at the Eugène Marquis Comprehensive Cancer Center between January 1, 2004 and December 31, 2005. Seven hundred and thirty-six patients underwent breast-conserving treatment for unifocal breast cancer. Patients with multifocal lesions presenting with ipsilateral recurrence and having received neo-adjuvant treatment were excluded from the study. Four hundred procedures were thus performed on 396 patients (4 cases of bilateral BCT), which led to the collection of 1665 slides for cytological investigation, all of which were examined during the procedures.

The characteristics of the operated patients were comparable with other series of women having received breast-conserving treatment. The average age was 58.6 years (range: 29–88), 260 of the 384 patients whose hormonal status was known were menopausal (65%), 117 were premenopausal (29.25%), 7 had disorders associated with the perimenopausal period (1.75%), and 28 patients had a personal history of contralateral breast cancer (4 of which during the study period). The procedures were divided into 258 breast-conserving procedures for clinically palpable lumps (64.5%) and 142 lumpectomies for nonpalpable lesions (35.5%). The mean volume of operative specimens was 104.1 cm³ (range: 1.5–701.25 cm³). The mean size of invasive lesions (pT, histological measurement of the operative specimen by the pathologist) was 16 mm (range: 2–50 mm), and the size of in situ lesions was 18.9 mm (range: 4–70 mm). Histologically, the distribution was 89% ductal lesions (pure IDC 19%, pure DCIS 11%, IDC with in situ component 59%) and 11% lobular lesions (pure ILC 3%, associated with in situ lesions 5%, and pure LCIS 3%). The size of the tumors according to the histological characteristics is summarized in Table 1. Deferred surgical re-excision was performed when, on final examination, the minimum margin was less than or equal to 2 mm, based on a local recurrence rate of 7% at 10 years after conservative treatment followed by radiation therapy.¹²

The statistical analysis was conducted using the SAS[®] software package (Statistical Analysis System, SAS Institute Inc. Cary, USA). The χ^2 test was used for qualitative variables and the Student test was applied for comparison of mean values. The tests were considered positive when $p \leq 0.05$.

Methods

Surgical resection of operative specimens included en bloc skin excision of the lesion (palpable or localized by ultrasound or radiological examination the day before the procedure by hook

wire left in contact with the radio signal) to the *pectoralis major*. The operative specimen was localized by the surgeon in the 3 spatial planes and referred to the pathologist who dried it on absorbent paper. A numbered glass slide was applied to each surface of interest (superior, inferior, anterior–posterior, lateral) after a gentle press on the specimen. If the side measured more than 5 cm or thickness exceeded 2 cm, 2 glass slides were used to cover the entire surface. The slides were room-dried and stained with Toluidine blue prior to interpretation. The results reported to the surgeon were expressed as: (a) acellular slides corresponding to healthy tissue (due to absence of normal epithelial cell desquamation¹³); (b) presence of benign cells (macrophages, columnar or apocrine metaplasia cells, Fig. 1); or (c) suspect positive slide in the presence of malignant cells (Fig. 2). The precise site of the lesions was deduced from their localization on the slide with anatomical mapping to the specimen for performing guided resection. In the case of resection, also oriented by the surgeon, the cytological procedure was reproduced until standardization of the imprint cytology technique was achieved.

Histological examination was performed after fixing and embedding in paraffin. The specimen was inked with different colors (1 color per specimen surface), then sectioned into 3-mm slices in a frontal plane to enable us to best analyze the 4 surfaces of interest. The inked resection margins were analyzed in 3-mm slices perpendicular to the lumpectomy bed.

Results

The pathologists analyzed 1665 slides corresponding to a total of 400 breast-conserving procedures and lumpectomies performed, and compared their cytological findings with those of the histological examinations on paraffin sections.

The total time of the procedure was on average 10 min (range: 7–17 min). Correlation between IOTPC and histological examination was 91.5%, with sensitivity and specificity of 88.6% and 92.2%, respectively. The positive predictive values (PPV) and negative predictive values (NPV) were respectively 73.6% and 97.0% (Table 2). In 9 in 400 cases, the cytological finding was falsely negative, representing 2.31% of total findings. For 4 patients, the final histological examination showed tumor cells in contact with at least one margin of the specimen (true false negatives). In the other 5 cases, the margins of the analyzed paraffin-embedded specimen were less than or equal to 2 mm, resulting in deferred surgical re-excision. Ductal carcinoma in situ was significantly more represented in the false negatives than in the other cases ($p < 0.005$), particularly when its size was greater than or equal to 30 mm ($p < 0.005$). In addition, the histological grade of ductal carcinoma in situ influenced the results. The IOTPC technique recognized all the cases of pure grade 3 ductal carcinoma in situ with involved margins, whereas sensitivity was only 72% in grade 1 and 2 ductal carcinoma in situ cases (Table 2). IOTPC was falsely positive (positive imprint cytology with in sano margins on paraffin specimen examination) in 6.25% of cases (25/400). We observed twice as many false positives in the lobular carcinoma population (10.2%) as in the ductal carcinoma ones and particularly in the case of histological grade 3 (5.6%) although the results were not statistically significant given the small size of the population (Table 2). On the other hand, the presence of fibrocystic mastopathy lesions in the tumor significantly increased ($p < 0.001$) the percentage of false positives (48% versus 17%). We also observed IOTPC requiring more than 4 slides to be linked to a statistically higher false positive rate ($p = 0.0019$). Finally, the pathologists observed that the imprint cytology cases considered “difficult” (no objective classification could be put forward) were a source of false positives. This was particularly the case when the

Table 1
Characteristics of the tumors (histological type, grade, histological size of the tumor and the margins).

	SBR1 ^a	SBR2 ^a	SBR3 ^a	Size of the tumor (mm – min–max)	Distance between tumor and margins (mm)
DCI & DCIS	97 ^a	89 ^a	49 ^a	28.5 (1–55) 35.4 (2–70)	7.9 7.1
DCI	40	18	17	21.5 (3–55)	14
DCIS	11	16	14	23.5 (0.9–70)	4.5
LCI & LCIS	9 ^a	6 ^a	4 ^a	14.5 (1–28) 27.5 (8–35)	5.9
LCIS	6	1	1		

DCI/IS: ductal carcinoma invasive and in situ. LCI/LCIS: lobular carcinoma invasive and in situ.

^a Scarff-Bloom-Richardson Grade of the invasive component.

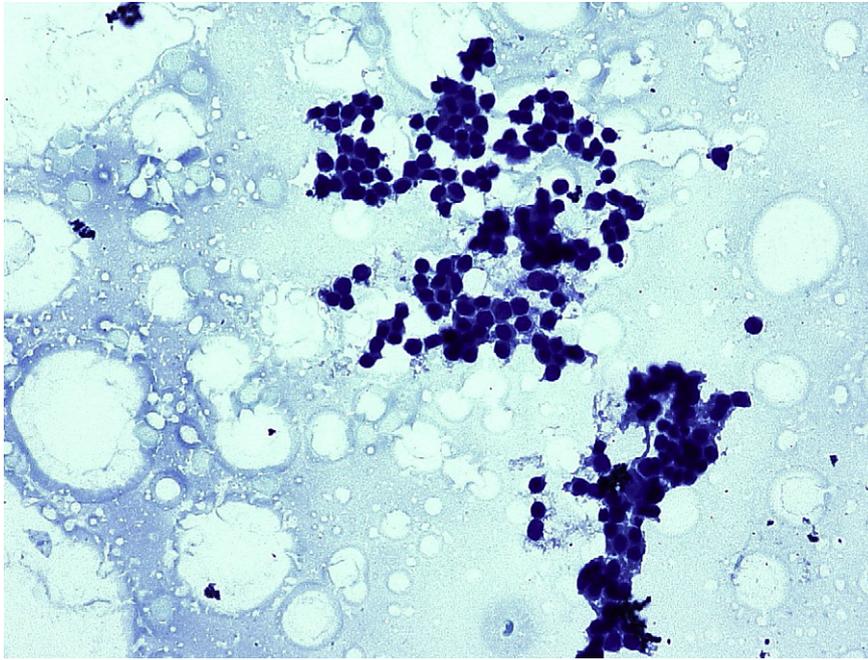


Fig. 1. Cluster of cells from typical epithelial hyperplasia (HES $\times 40$).

slides revealed cells presenting minimal cytoplasmic and nuclear anomalies, making these clusters difficult to classify, and above all when the slides collected a limited number of cells.

During the procedures, 153 intra-operative re-excisions were performed, i.e. 38.25% for the entire study. In 56.8% of cases, they were performed at the pathologist's request (87/153), in which case tumor residue was found in the final histological examination in 71.2% (62/87). Re-excisions that were not performed by the surgeon on cytological grounds were undertaken either systematically before IOTPC, or after cytology despite a negative finding (66/153). In 93.9% of cases, resections not requested by the pathologist were free of tumor residue (62/66).

In this series of 400 operated patients, the overall surgical re-excision rate was 13.25% for all cases, i.e. 53 secondary surgical re-excisions in 50 patients, corresponding to 23 mastectomies and 30 re-excision procedures. In 47 patients (of the total 400, i.e. 11.75%), imprint cytology prevented secondary surgical re-excision thanks to oriented intra-operative re-excision with a lesser cosmetic impact.

Discussion

In our study of 400 imprint cytology procedures performed on a series of 396 patients, we observed a positive predictive value of

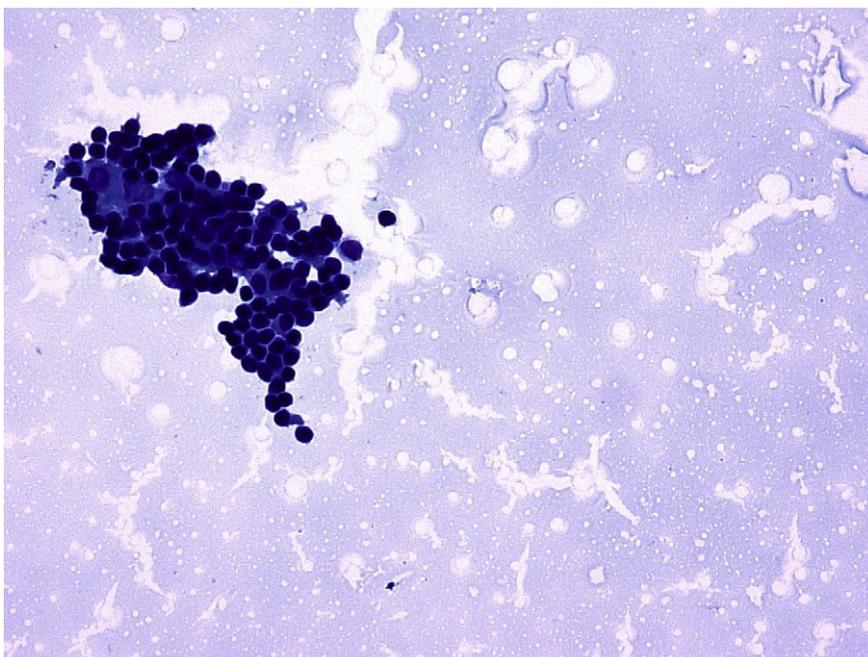


Fig. 2. Cluster of malignant cells (HES $\times 40$).

Table 2
Results of IOTPC procedures according to the histologic type.

Histology	True negative	True positive	False negative	False positive	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Total (n=)
DCIS & DCI	170	44	8	13	86.2	92.3	77.1	96	235
SBR1 ^a	71	16	4	6	86.8	92.6	75	96.5	97
SBR2 ^a	66	17	1	5					89
SBR3 ^a	33	11	3	2	84.6	92.9	77.2	95.5	49
DCIS	22	15	3	1	88.2	95.8	93.7	92	41
SBR1	8	1	2	0	72.7	100	100	84.2	11
SBR2	8	7	1	0					16
SBR3	6	7	0	1	100	85.7	87.5	100	14
DCI	65	4	0	6	100	91.5	40	100	75
SBR1	32	3	0	5	100	89	33.3	100	40
SBR2	17	0	0	1					18
SBR3	16	1	0	0	100	100	100	100	17
LCI & LCIS	12	4	0	3	100	80	57.1	100	19
SBR1 ^a	5	3	0	1					9
SBR2 ^a	6	0	0	0					6
SBR3 ^a	1	1	0	2					4
LCI	17	2	0	1	100	94.4	66.6	100	20
SBR1	7	1	0	0					8
SBR2	10	1	0	1					12
SBR3	0	0	0	0					0
LCIS	8	1	0	1	100	88.8	50	100	10
SBR1	6	1	0	0					7
SBR2	1	0	0	1					2
SBR3	1	0	0	0					1
Total	304	71	9	26	88.6	92.2	73.6	97	400

DCI/IS: ductal carcinoma invasive and in situ. LCI/LCIS: lobular carcinoma invasive and in situ. PPV and NPV: positive and negative predictive value.

^a Scarff-Bloom-Richardson Grade of the invasive component.

73.6% for the procedure, which enabled us to perform oriented resection intra-operatively and thus avoid, in 11.75% of cases, a second deferred surgical procedure to extend margins, and a negative predictive value of 97.0%, which prevented unnecessary surgical excisions and potential cosmetic damage.

Surgical margin status is a key to the success of conservative treatment in terms of local control and overall survival.¹⁴ This is due to the high rate of residual disease found after BCT with positive margins.^{15,16} Specimen radiograms and gross examinations are cheap and fast techniques for assessing resection adequacy with high sensitivity and specificity, but are of limited accuracy in the presence of an extensive in situ component or lobular histological subtype. Several studies analyzing the excision margins in intra-operative cytology revealed a sensitivity of 78–91% and a specificity of 86–100%,^{11,17–19} but the histological control methods are not specified. Histological section analysis alone can determine resection margin status. However, in the particular case of breast cancer, this concept has several limits: (a) it is technically impossible to analyze the entire surface of an operative specimen using conventional histology (given that a histological section is 5 μ thickness, 4000 tissue sections would need to be examined to represent the surface of a specimen measuring 2 cm in length²⁰); the pathologist must therefore select regions of interest on macroscopic data, which is a source of sampling error; (b) frozen sections result in specimen damage and therefore a loss of material which can no longer be used for the final histological examination and give rise to artifacts of interpretation related to the technique. Adipose tissue is difficult to freeze and fat specimens with irregular surfaces facilitate the running of ink into the adipose lobules making the histological interpretation far more tricky, and leading to false positives.^{18,21} An analysis of the cavity walls following breast-conserving surgery has been proposed, thus reducing the postoperative re-excision rate,¹⁰ but this procedure is long and costly. In 1991, Cox²² introduced Imprint Cytology for the intra-operative assessment of resection margins. The results obtained with this technique correlate with those from several teams^{23–27} and compare with our results (Table 3). Saarella²⁸ alone observes

poor sensitivity and specificity, which are probably related to the absence of patient selection since multifocal tumors are included. Furthermore, the accuracy of IOTPC was assessed by Cox for whom the rates of local recurrence after BCT evaluated by IOTPC are 2.7% as against 14.6% after BCT evaluated by extemporaneous frozen section analysis²⁹ and after a follow-up period of 3.5 years. In our study, the sensitivity of IOTPC is higher in invasive lesions than in in situ carcinomas, regardless of the cellular grade, whereas this purely cytological examination does not provide any indication of invasive disease or otherwise. This may be explained by the fact that (a) surgeons perform wider resections in palpable lesions as opposed to nonpalpable lesions, which are mostly related to in situ carcinoma; (b) the margins of in situ lesions are highly irregular: cell cohesion is usually referred to benign and in situ lesions in cytology, but in case of invasive tumors the cells on the smear are grouped together in a large cluster of malignant cells. IOTPC may therefore fail to recognize an invaded margin of a single mammary duct containing malignant cells in the presence of a few isolated neoplastic cells, whereas invasive lesions tend to have more regular margins. The false negatives are most often explained by failure to adhere to the glass slide in the case of adipose tissue (particularly in the case of colloid carcinoma or comedo-type intraductal

Table 3
Comparative results of IOTPC between the principal series.

Author, patients (n=)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Correlation with histology on paraffin section (%)
Klimberg, ¹ n = 83	96.4	100	100	99.3	99.3
Creager, ² n = 137	80	85	40	97	85
Bakhshander, ³ n = 100	97	99	84	99	NA
Our series, n = 396	88.6	92.2	73.6	97.0	91.5

carcinoma) or by the presence of tissue folds which conceal tumor lesions due to a flap-like phenomenon. False negatives are rare, even in invasive low-grade or in situ lesions, as pathologists recommend re-excision on principle if in doubt about cells with minor abnormalities. The sources of false positives are to be found in slide interpretation errors. Experience and regular mammary cytology procedures are therefore necessary for mastering this technique.²⁴ Immunostaining techniques (MIC-1 and E-Cadherin) can be applied to IOTPC in order to improve performance but pose a problem owing to their cost and practical implementation.³⁰ The principal criticism made about IOTPC is that it is limited to the margins of the BCT procedure and is thus incapable of assessing the safety distance needed between the limits of the tumor and those of the operative specimen.¹⁴ In our numerically large series, we only observed this problem in 5 patients in whom the safety distance between the BCT margins and the tumor was less than 3 mm resulting in re-excision (1.25% of cases). This is probably due to the fact that the operative specimen is pressed by the pathologist when touching the slide to it. Thus, particularly in the case of carcinoma in situ or invasive carcinoma surrounded by carcinoma in situ, in carcinoma with vascular and lymphatic emboli where pressing causes the malignant cells to seep out of the infiltrated ducts or vessels, and in lobular carcinoma where the cells have lost their adhesive power, malignant cells are easily found on IOTPC slides. This point is particularly interesting, notably as the limits of in situ and lobular lesions, and the extension of vascular and lymphatic emboli are poorly assessed by conventional extemporaneous histology and that these particular cases are often found in the case of positive margins.^{31,32} The second limitation of the IOTPC procedure is the lack of information provided by the technique about the size and the invasive or non-invasive nature of the extent of the lesion.²⁷ This information, which could be useful to the surgeon as a guide to determining the extent of the intra-operative re-excision, can be deduced for the size alone from the number of positive glass slides. IOTPC offers a certain number of advantages: (a) it can be used to analyze the resection limits over the entire surface of the specimen; (b) it does not alter the structure of the operative specimen and therefore has no effect on the quality of the final histological examination; and (c) the time required to perform the procedure is acceptable for the operating room environment.²⁴

Few studies have compared IOTPC and conventional extemporaneous margin assessment. The 2 series that present a large number of cases^{33,34} show no statistically significant difference between the two methods. The sensitivity and specificity of the frozen section technique vary from 77 to 100% and from 91 to 100%, according to whether the specimen scrape or cavity margin protocol was used.^{18,19,35,36} In a recent series of 264 conservative procedures, Cabioglu¹⁴ reported a sensitivity of 77.8%, a specificity of 91.7%, and a concordance rate of 87.4% with paraffin section histology for conventional extemporaneous margin analysis on frozen sections. A different study of 290 patients reported similar results (sensitivity = 73.08%, specificity = 99.59%, PPV = 91.94%, NPV = 98.2% and concordance = 98.02%) with a resulting low local recurrence rate (2.74% for a mean follow-up of 53.4 years).³⁷ The teams that routinely use an extemporaneous margin technique resort to a significant number of intra-operative resections. In our study, this resection rate is 38.25%, i.e. similar to that required by conventional extemporaneous examinations (above 50% for the MD Anderson Cancer Center¹⁴ or 40.8% for Pinotti³⁸). Thus, in our study, the overall surgical re-intervention rate is 13.25% and we estimate having avoided a second procedure in 11.8% of patients.

Our work has several limitations: (a) the evaluation of sensitivity and specificity of our technique is not referred to the first resection specimen but to the final results after the operation: but it can be noted that in 93.9% of cases, resections not requested by the

pathologist (performed before or after the IOTPC procedure) were free of tumor residue; (b) the impact of our technique is not measured on the clinically relevant criterion of local recurrence. However, our results are close to those of Olson³⁷ who notes a positive impact of IOTPC; (c) a true comparison with the extemporaneous histological examination after freezing can only be considered as part of a randomized study; (d) the results presented reflect our team's long-standing interest in cytology for which interpretation requires a learning curve³⁹; and (e) a medico-economic assessment of the technique has yet to be conducted.

In this series of 400 procedures in selected patients to whom conservative treatment could be proposed (early stage, unifocal tumors), IOTPC is a simple technique for pathologists with experience in mammary cytology, requires little time and resources, and is effective. In addition, the technique that we are proposing (gentle press on the operative specimen and application of glass slides to the 4 margins) is particularly suited to tumors for which conventional extemporaneous histology is often deficient and which therefore runs the risk of secondary re-excision. It would be worthwhile to conduct a comparative study with histological examination after prospective randomized freezing in order to carry out a medical and medico-economic comparison of the 2 techniques.

Conflict of interest statement

None declared.

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