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The Fast-Track Biopsy (FTB): Description of a Rapid Histology and Immunohistochemistry Method for Evaluation of Preoperative Breast Core Biopsies

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Thirty-six core breast biopsies from 32 patients were paraffin embedded by use of an automated microwave processor. In addition, a quick immunohistochemical method was used in selected cases. The quality of the hematoxylin and eosin (H&E) slides was very satisfactory, as were also the immunohistochemical stains for ER, PR, and Ki67 when compared to those obtained with the use of a conventional automated immunostainer. The time required to process the tissue to the final H&E stage averaged 2 hours 52 minutes, and the immunohistochemical method required 90 to 100 minutes. This procedure, which we named "fast-track biopsy" (FTB), is quick enough to be competitive with FNAC (fine-needle aspiration biopsy) in terms of turnaround-times. The superiority of core biopsy over FNA in terms of the morphologic information provided is widely acknowledged, the only major argument currently mentioned in favor of FNAC being the shorter duration of the procedure. With the advent of FTB, it would appear that even this last remaining advantage has been erased.
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In recent years the diagnostic approach to breast lesions has changed dramatically. The use of intraoperative frozen sections has undergone a marked decline following the adoption of preoperative diagnostic strategies that include fine-needle aspiration cytology (FNAC) and needle core biopsy (NBC). These offer several advantages, not only because they are simple procedures applicable to outpatients, but also because they allow management decisions by the patient and the surgeon before hospital admission, with consequent reduction of the patient's anxiety. FNAC is performed in 2 situations: palpable masses, where the procedure is usually performed free hand; and nonpalpable masses, in which the procedure is image guided. A 2% false-negative rate is regarded as acceptable in ultrasound-guided procedures of nonpalpable breast lesions [1], but it has been stated that if in doubt it is preferable to defer the diagnosis and rely on NBC, as the combination of the 2 methods provides a correct preoperative diagnosis in most cases [2].

The advantages of NBC over FNAC are self-evident, as NBC allows simultaneous evaluation of cytology and architecture. This is more obvious with

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the nonpalpable lesions in which a stereotactic approach is favored, especially in areas containing microcalcifications. With NBC a definite diagnosis of an *in situ* or invasive lesion can be rendered, and the extent, quality, and size of microcalcifications are better assessed. Furthermore, grading of the tumor is possible, reaching a level of agreement between core and excisional biopsy of 84% in grade III invasive carcinomas. The agreement rate in tumor typing is 74% [3]. Finally, ER and PR can be evaluated immunohistochemically, even if the agreement rate between core and excision specimen is at most 73% and 42%, respectively [4].

Many of the above-cited parameters cannot be assessed by FNAC.

The disadvantages of NBC over FNAC are the rare phenomenon of dislodgement of epithelium by the needle [5] and the length of the NBC procedure, which requires an average of at least 24 hours (inclusive of fixation), compared to about 1 hour for FNAC (under ideal conditions).

Here we describe a technique that employs a controlled microwave processing procedure lasting about 2 hours and 30 minutes, inclusive of fixation time through staining. This technique has been named fast-track biopsy (FTB) to emphasize the fact that the diagnosis is obtained within a short period and that fast immunohistochemistry can be added to the diagnostic procedure.

It seems to us that this technique further weakens the practical value of FNAC in the evaluation of breast lesions.

Materials and Methods

Thirty-six consecutive core biopsies from 32 patients were obtained from the outpatient clinic at Bellaria Hospital, University of Bologna, Italy, from November 2004 to January 2005. From 2 to 5 cores for each case were processed. Patients were 30 women and 2 men, ranging in age from 23 to 91 years (mean 57.2 years). Sixteen specimens were from the left and 20 from the right side. Cases were all "freehand" core biopsies obtained from palpable breast masses.

For processing, a microwave, the instrument used for the study here described is the vacuum-assisted histoprocessor model RHS-1 manifactured and distributed by (Milestone Srl , Sorisole, Bergamo, Italy; e-mail *medical@milestonesrl.com*; *www.milestonesrl.com*) was used, in which automatic microwave power selection, processing time, temperature, and vacuum are computer-controlled and independent of operator.

Ten trial specimens were additionally studied with the same method. These trial specimens, obtained from core biopsies after mastectomies (3 cases) or quadrantectomies (7 cases) for invasive carcinoma, were processed before the 36 consecutive specimens in order to optimize, whenever required, the processing schedule recommended by the manufacturer.

The complete protocol we employed for microwave processing of breast cores is the following: specimens are immersed for 20 minutes in 10% buffered formalin (50°C), followed by an external, nonmicrowave rinse in reagent alcohol to remove excess formalin and water from the cassettes. This is followed by 15 minutes of microwaving in 100% ethanol (65°C) and 16 minutes in 100% isopropanol (68°C). Samples are then exposed to a brief vacuum microwave drying step of 1.5 minutes with 100 Watts of energy under 600 mBar of vacuum. Microwave paraffin infiltration is then performed for 25 minutes, with increasing increments of vacuum down to 100 mBar, to assist the infiltration process at 65°C.

Sections from paraffin blocks are stained with hematoxylin and eosin (H&E). The total time for processing includes 1 hour 30 minutes for processing/embedding, 30 minutes for sectioning, and an additional 30 minutes for H&E staining, totaling 2 hours and 30 minutes.

Invasive carcinomas can be immunostained, when required (i.e., for estrogen [ER] and progesterone [PR] receptors as well as Ki 67) with an UltraVision detection system antipolyvalent method (Lab vision, Fremont, CA), using monoclonal rabbit antibodies (NeoMarkers, Fremont, CA) directed against estrogen receptors (ER SP1) diluted 1:200; progesterone receptors (PR SP2) diluted 1:200; and Ki-67 (SP6) antibodies diluted 1:200. Dewaxing and antigen retrieval are performed at the same time with W-CAP TEC buffer pH 8.0 system (Bio Optica, Milan, Italy). The slides are placed into a warm bath at 95°C for 15 minutes and in the same buffer at room temperature (RT) for another 15 minutes. The slides are rehydrated with distilled water. To reduce nonspecific background staining due to endogenous peroxidase, slides are incubated in hydrogen peroxide for 10 minutes. After 2 washes, primary antibodies are applied for 10 minutes and then after 4 washes in buffer PBS to apply biotinylated goat antipolyvalent for 10 minutes at room temperature. Sections are washed 4 times in buffer, streptavidin peroxidase is applied, and slides are incubated for 10 minutes at room temperature, and rinsed 4 times in buffer. One to 2 drops (40–100 uL) DAB chromogen

applied to the tissue. After a 5–10 minutes incubation, sections are counterstained and coverslipped by using a permanent resin mounting medium.

The reagents in this kit constitute a labeled streptavidin-biotin immunoenzymatic antigen detection system. This technique involves the sequential incubation of the specimen with an unconjugated primary antibody specific to the target antigen, a biotinylated secondary antibody that reacts with the primary antibody, enzyme-labeled streptavidin, and substrate chromogen.

Sections from the same cases were stained in parallel with an automated immunostainer (Ventana Benchmark). In conclusion, the immunohistochemical procedure here described lasts 90–100 minutes, as opposed to 220 minutes with the automated immunostainer.

The reporting system used for the present cases was based on the 5 histologic categories recommended by the UK National Health Service Screening Program and adopted by the Breast Screening European Commission [6]. ER and PR were considered positive when the nuclear staining exceeded 10% of the total neoplastic cell proliferation. The percentage of positive cells was reported to assess the Ki 67 score.

Results

The H&E quality of the biopsies did not differ from that of the traditionally processed specimens (Figs. 1–3), and no difference was perceived in terms of nuclear and cytoplasmic staining. Three cases were regarded as B1, and 13 cases were diagnosed as B2; these included fibroadenomas and epitheliosis (epithelial hyperplasia of usual type). Two cases were regarded as B3; these included a ductal adenoma and a cellular fibroadenoma that was not possible to distinguish from a phyllodes tumor. No case was considered B4, while B5 cases were the most numerous (18 cases). These included 12 invasive duct carcinoma NOS , 1 invasive mucinous carcinoma, 3 invasive lobular carcinomas, and 2 cases of *in situ* duct carcinoma. The time of the FTB ranged from 2 hours and 30 minutes to 4 hours (mean 2 hours and 54 minutes). During the last month of the study (January 2005) the processing time was stabilized at 2 hours and 30 minutes.

For immunohistochemistry, 8 B5 cases were processed. The processing time of the fast immunohistochemical method ranged from 90 to 100 minutes; i.e., it was consistently shorter than that of the automated immunostainer, which ranged from 220

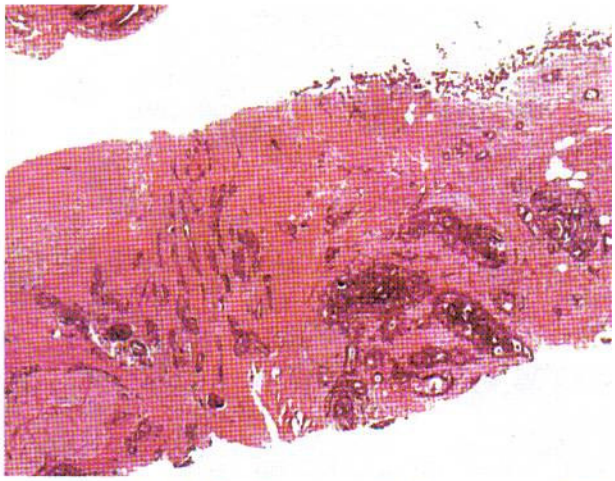

Fig. 1. Case 21: Well-differentiated (G1) type *in situ* DCIS with invasive carcinoma (G2). This case was assigned to category B5 (FTB technique).

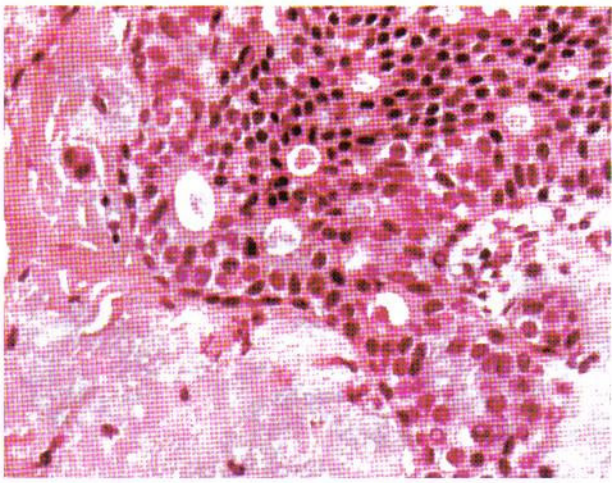

Fig. 2. Case 21: Higher magnification of the DCIS area. Notice the optimal preservation of the nuclei and cytoplasm (FTB technique).

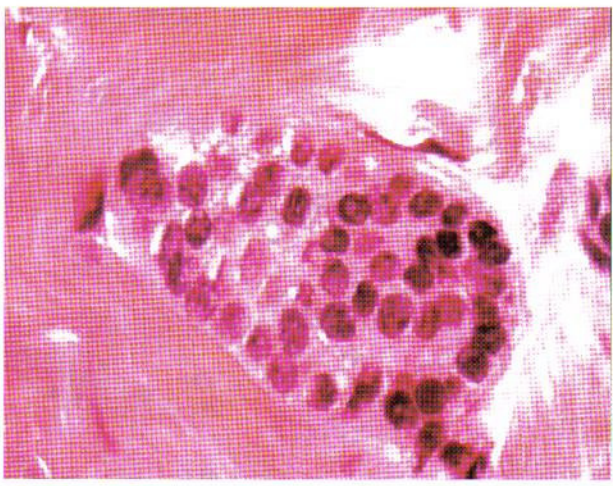

Fig. 3. Case 21: Invasive DCI component with round-to-

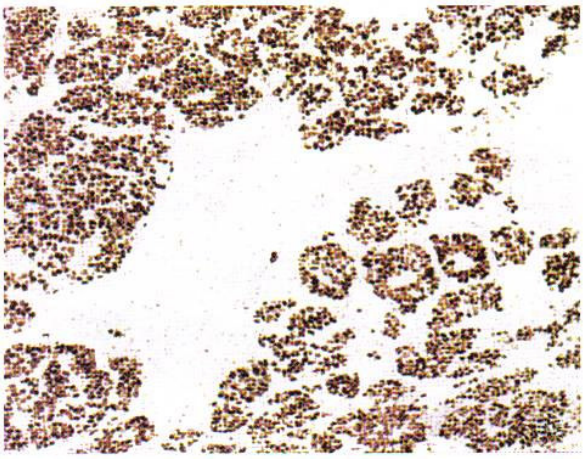

Fig. 4. Case 23: Numerous tumor cells are positive for ER as evidenced with the Ultra Vision system.

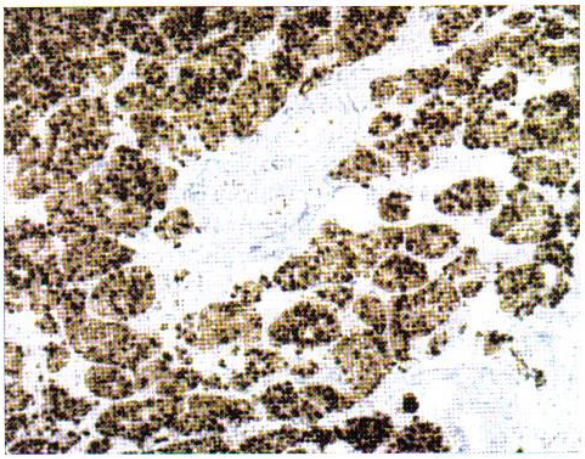

Fig. 5. Case 23: Same results for ER are obtained with the automated immunostainer Ventana.

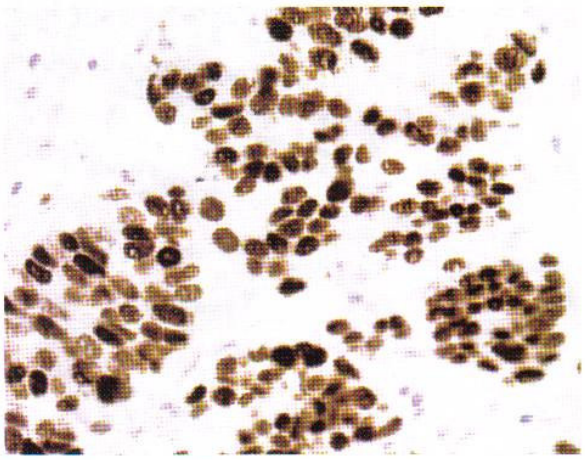

Fig. 6. Case 23: ER at higher power as stained with the Ultra Vision system.

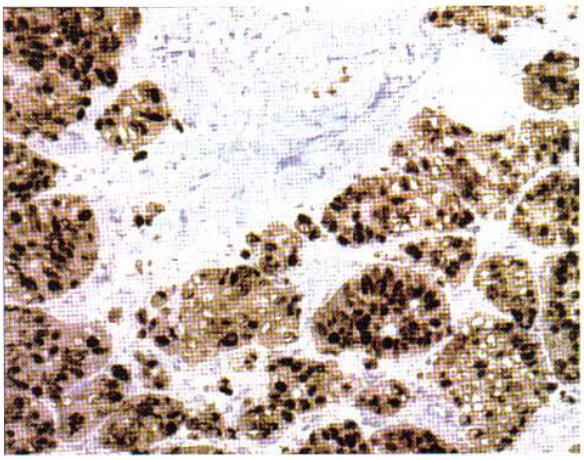

Fig. 7. Case 23: Same results are obtained for ER with the automated immunostainer Ventana.

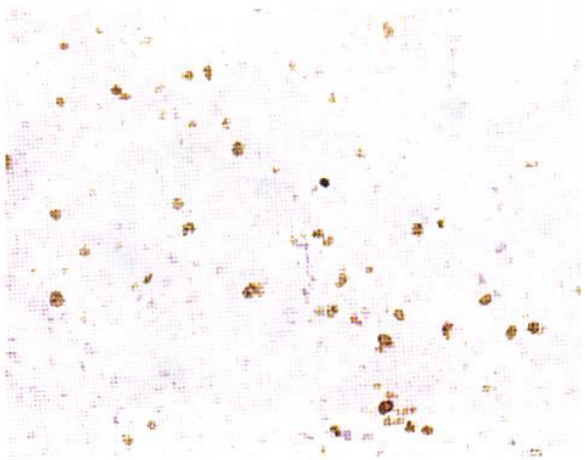

Fig. 8. Case 23: Three to 5% of cells are stained for Ki67 (Ultra Vision).

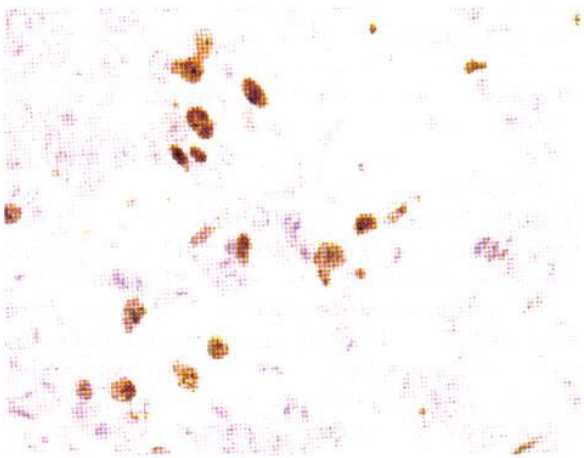

Fig. 9. Case 23: Same results are obtained with immunostainer Ventana.

When the quality and intensity of the staining obtained with the fast procedure were compared to those of the automated immunostainer, no differences between the 2 procedures were noted (Figs. 4–9). ER was positive in 7 cases (range 70% to 90% of the total neoplastic proliferation), whereas PR was positive in 8 cases (range 20% to 90%). Ki 67 positivity ranged from 5% to 60% of the total neoplastic proliferation (mean 16.2%).

Discussion

The process of fixation constitutes the foundation of any histologic permanent routine procedures, in the sense that it prevents autolysis and putrefaction.

Heat was the first form of tissue fixation, well known to Marcello Malpighi, who used a preheated iron to fix the tongues of animals [7]. Malpighi later realized that heat conduction is poor in biological material and changed his approach introducing as fixative grappa [7], a distilled form of wine 70° proof.

Mayers [8] demonstrated that microwave heating overcomes the limitation of poor heat conduction and that microwave energy at a frequency of 2.4 gigaherz per second penetrates several centimeters into the tissues [8]. It was later shown that microwave irradiation applied to tissues produced excellent preservation of cytomorphology and that this method had no deleterious effects on special techniques including most of the antigens evaluated by immunohistochemistry and most organelles examinated by electron microscopy [9].

The history of microwave histoprocessing has been documented by numerous papers [10] and includes the use of cooking microwave ovens applied to biopsy material to speed up tissue processing for transplant biopsies [10]. The limit of microwave processing is due to the fact that it is difficult to reach and keep constant temperatures in a specific time frame, a fact that led in the past to the loss of tissue. This has been overcome by the computerized system we have used that keeps constant preprogrammed temperatures within a specified time.

Microwave accelerates the procedures of histoprocessing as it optimizes fixation; it requires fewer steps for dehydration and clearing and paraffin impregnation can be performed at lower temperatures (i.e., 68°C).

These feature became well evident in our core biopsy material, which was consistently processed in a very short time (2.5 hours, including sectioning and staining), the total processing procedure lasting 77.2 minutes till embedding. ER, PR, and Ki 67 antigens appeared well preserved in the cases stained for immunohistochemistry. The same was true for the keratins and smooth muscle actin stains that are routinely performed in our lab but that were not included in the present study. The quality of staining with the quick method we used appeared as satisfactory as the results obtained by the automatic autostainer.

Therefore, it seems that the present procedure of processing core biopsies is reliable and gives satisfactory histologic and immunohistochemistry results. Furthermore, it is fast enough (4 hours inclusive of immunohistochemistry) as to be competitive with FNAC.

The actual time required for the pathologist to reach a final diagnosis on an H&E slide is faster in core biopsies than FNAC in most of the cases, as in the latter method the diagnosis requires careful scanning of several slides in search of neoplastic cells.

Therefore, it appears that the processing times for the 2 procedures are practically superimposable, and consequently the advantages of microwave-processed core biopsies are far superior to those offered by FNAC.

Now that the processing times for the 2 procedures have become practically superimposable with the advent of FTB, we would conclude that the overall superiority of FNAC over NCB cannot longer be denied.

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