Technical method

Histological localisation of mammographic abnormalities in breast biopsy specimens

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In the last decade there have been increasing numbers of breast screening programmes, resulting in the detection of non-palpable mammographic abnormalities, which are accepted as valid indications for surgical biopsy.¹⁻³

Although specimen radiography is regarded as an essential procedure to prove that any lesion identified by mammography has been excised,^{4,5} in none of the techniques described for dissecting the biopsies does the biopsy tissue remain in a constant position for both radiography and subsequent dissection after fixation.^{3,6-9} Complex, or potentially complex, labelling procedures and multiple specimen radiographs following dissection may be required to locate any abnormal area, and the practice of slicing a specimen into thin blocks prior to fixation ("bread-loading") leads to tissue distortion and diminishes the correlation between the radiographic and histological appearances. Isolation of an area containing microcalcification by dissection from the surrounding tissue may be inadvisable, as it is now well recognised that a benign lesion containing microcalcification may be adjacent to an area of in-situ or invasive carcinoma^{1,5,10} (Fig. 1).

This paper describes a simple method of investigating breast biopsies by specimen radiography and accurately localising the lesion for histological examination of the mammographically abnormal areas.

Material and methods

When the biopsy is being taken, the area in question is localised by the surgeon by compression of the breast, simulating the compression used in producing the standard cephalocaudal and lateral mammograms, referring to these mammograms on a viewing box in theatre.

Preoperative localisation techniques such as needle

Fig. 1 Microcalcification (arrowed) in a focus of sclerosing adenosis on the left, with an invasive carcinoma on the right $\times 60$.

localisation or dye injection have not been used. Besides the hazard of exposing the breasts to further x-rays, dye injection also causes a risk of obscuring the macroscopic appearances of some lesions, and of disrupting and distorting the tissues for subsequent histological examination, which often involves interpreting subtle tissue and cell changes.

After excision, the biopsy specimen is affixed to a 6 mm thick balsa wood board by four small hypodermic needles. A metal "L" at the top left hand corner serves as a radiopaque marker. (Fig. 2). The

Fig. 2 Biopsy tissue mounted on a balsa wood board prior to fixation.

specimen is x-rayed on a Senographe fx clinical mammography unit, using a 14×9 cm cone positioned 2 cm above the biopsy. For specimens less than 1 cm and for those between 1–3 cm in maximum thickness, exposures of 10mA 22kV and 12A 24kV respectively have been found to give the greatest clarity, using Kodak Min R, a vacuum-packed film backed by an intensifying screen.

After comparison of the specimen radiographs with the original mammograms, confirmation may be given to the surgeon that the abnormal area is included in the biopsy within 10–15 min of excision. At this stage, prior to routine fixation, small amounts of fresh tissue may be taken for electron microscopy or immunohistological examination. The specimen is disturbed as little as possible, so that no areas critical to diagnosis are removed. Frozen sections are not performed in these specimens for the same reason.¹¹

For routine fixation, the board and attached breast tissue are inverted and floated in a bath of formol saline for 24–48 h (depending on the volume of tissue) allowing fixation with minimal tissue distortion.

A tracing of the specimen radiograph is made on clear polyacetate film using a permanent pen marker. The tracing includes the main features of the radiograph, the margins of the biopsy, an outline of the abnormal area plus the locating needles and the radio-opaque "L" marker to assist in orientation.

After fixation the specimen is carefully removed from the board by loosening the needles, and the tracing inserted underneath. When the tissue has been accurately superimposed on the tracing, the needles are reinserted (Fig. 3). Using the bevels of the needles as reference points, 4 mm thick blocks are obtained by cutting down, through the specimen, onto the abnormal area marked on the polyacetate film. The areas from which the blocks are taken are

Fig. 3 A tracing inserted underneath the biopsy tissue following fixation, indicating the area of interest.

identified from, then marked on, the tracing. This allows the histology to be accurately correlated with the radiographic appearances. In addition, the tracing aids in reorientation should further blocks be required after the initial dissection. Very small lesions can be accurately localised by this technique (Figs. 4, 5, 6).

Balsa wood has been found to be the best material for supporting the biopsy tissue, as it contains no metallic impurities, does not interfere with the identification of microcalcific particles or other densities within the specimen, and is relatively uniformly radiolucent. A Kodak Indicator for Technique Operation (ITO) was used to compare

Fig. 4 A small focus of microcalcification (arrowed) is noted on a mammogram.

Fig. 5 The calcification is clearly seen in the specimen radiograph.

Fig. 6 Histological sections show microcalcification associated with benign epithelial hyperplasia in this case $\times 150$.

films taken with and without the board underneath over the range of exposures used, and no significant difference in the definition of the various materials in the ITO was present proving that balsa wood is an ideal material on which to mount the specimen.

Results

One hundred and twenty-seven specimens from a 'well-woman' breast cancer screening trial have been processed, and in 104 of these the area indicated on the mammograms has been identified in both the specimen radiograph and histological sections (Table). In three cases no abnormality was identified

A breakdown of the histological findings in the 127 mammographic abnormalities examined by this method

Mammographic abnormalities	Total numbers	Percentage of total
Invasive carcinoma	14	11.0
In-situ carcinoma	6	4.7
Severe atypia/carcinoma in-situ	3	2.4
Epitheliosis and epithelial hyperplasia	34	26.8
Fibrocystic disease without epitheliosis	39	30.7
Fibroadenomas	15	11.8
Papillomas	4	3.1
Fibrosis and involution	5	3.9
Fat necrosis	3	2.4
Fibroelastic scar	1	0.8
No lesion identified	3	2.4

in either the specimen radiograph or on histological examination. The mammograms in these cases showed poorly defined areas of increased radio-density, not associated with microcalcification.

Conclusion

The method described here offers advantages over any yet described. One of the most important is that the biopsy tissue still corresponds to the specimen radiograph, and distortion during fixation is minimised as the tissue is restrained by the needles. When breast tissue is allowed to float freely in fixative, orientation becomes difficult or impossible.

The high degree of correlation between the radiographic and histological appearances at any particular point is important if there is more than one abnormal area within a specimen, or if diffuse parenchymal changes are to be studied. The method also allows the location and study of mammographically normal tissue surrounding any particular lesion.

A tracing of the specimen radiograph means dissection of the tissue is accurately controlled by clearly indicating abnormal areas, eliminating guesswork and providing a permanent record of the blocks taken.

The accuracy of localisation means that a lesion may be identified using the minimum number of blocks, saving time and expense in processing all of the biopsy tissue. Using this technique it is unlikely that a small malignancy lying adjacent to a benign lesion containing microcalcification would be missed, as transverse blocks are taken through the width of the specimen, at radiologically abnormal areas.

The materials used are easily available and inexpensive. The method may be easily incorporated into the routine histopathology laboratory service, provided facilities for the rapid transport of the specimen from the operating theatre to the radiology department and pathology laboratory are available.

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Letters to the Editor

Histological criteria for childhood coeliac disease

I am writing to confirm the findings by Dr MN Marsh on high mitotic indices¹ in small intestinal mucosal biopsy of patients with gluten-sensitive enteropathy.

I would like to emphasise that in our series a high mitotic index has been one of the most useful pointers in differentiating coeliac disease from other causes of "flat mucosa," including that of cow's milk intolerance. The three important morphological criteria used by us in childhood coeliac disease are as follows: (a) a flat mucosa with elongated crypts (the latter is absent in most cases of cow's milk intolerance), giving the mucosa an appearance of a normal or occasionally increased thickness (> 550 μ m); (b) infiltration of the surface enterocytes by an increased number of lymphocytes (emperipolesis) and a mitotic index of the lymphocytes of greater than 0.3%; (c) abnormal fat absorption pattern, characterised by accumulation of fat blobs in the supranuclear spaces of the surface enterocytes, which is easily detected by any special fat stain.

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- ¹ Marsh MN. Studies of intestinal lymphoid tissue. IV—The predictive value of raised mitotic indices among jejunal epithelial lymphocytes in the diagnosis of gluten-sensitive enteropathy. *J Clin Pathol* 1982;**35**:517–25.

The in vitro responses of *Bacteroides fragilis* to Moxalactam, Cefotaxime, Cefmetazole, Josamycin and erythromycin

Although the sensitivity of *Bacteroides fragilis* to Moxalactam, Cefotaxime, Cef-

metazole, Josamycin and erythromycin has been described little has been reported about the bactericidal effect of these antibiotics against this organism. Such an effect may be of considerable importance when the treatment of highly susceptible patients—such as the immunosuppressed—is being considered. Furthermore, little is known about the in vitro and in vivo acquisition of resistance of *B fragilis* to these drugs. The results of our observations may, therefore, be of interest.

We have studied the susceptibility of two clinically isolated strains of *B fragilis* ss *fragilis* (strains A and B) and one of *B fragilis* ss *vulgatus* (strain C) to Moxalactam, Cefotaxime, Cefmetazole, Josamycin and erythromycin. Tubes of brucella-enriched broth medium containing serial dilutions of the various antimicrobial drugs were inoculated with approximately 10^7 CFU/ml of the strains under test. Anaerobic incubation at 35°C was carried out over 24 h. Subcultures were made using a standard loop, to blood agar. The minimum inhibitory concentration (MIC) was defined as the minimum antibiotic concentration preventing bacterial growth detectable by the naked eye after 24 h incubation. The minimum bactericidal concentration (MBC) was arbitrarily defined as the minimum concentration of the drug producing a 99.9% reduction of the inoculum.

The MIC and MBC values for Moxalactam, Cefotaxime, Cefmetazole, Josamycin and erythromycin against the three strains

of *Bacteroides* tested are shown in the Table. Cefotaxime showed poor activity against the three strains when compared with the other four drugs. Moxalactam, Cefotaxime and Cefmetazole were bactericidal at concentrations equal to or only slightly higher than their inhibitory values. Although sterilisation of the cultures was not achieved—even with concentrations 30–500 times higher than the particular MIC—the average reduction was from 10^7 CFU/ml to 10^3 CFU/ml. The effect of Josamycin and erythromycin was entirely bacteriostatic failing to reduce the initial inoculum at concentrations 30–100 times higher than their MICs.

Resistance to the antibiotics studied was not induced in the *Bacteroides* strains following a single exposure at concentrations below, equal to, or above the MICs. Resistance following a single exposure, although uncommon, has been shown to occur with clindamycin¹ and in *Bacteroides* isolated from faeces of patients receiving this drug.²

In summary, considerable bactericidal activity against *B fragilis* has been demonstrated by Moxalactam, Cefotaxime and Cefmetazole although each drug produced "persisters"; these are defined as (bacterial) cells which survive exposure to ostensibly lethal concentrations of bactericidal antibiotics but whose progeny remain fully sensitive to the drug.³ *B fragilis* organisms demonstrating such phenotype resistance may play an important part in

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of five antimicrobials against *Bacteroides fragilis* (mg/l)

	Moxalactam		Cefotaxime		Cefmetazole		Josamycin		erythromycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B fragilis A	2	4	64	256	4	4	0.25	>32	0.25	>32
B fragilis B	2	2	32	256	4	16	0.5	>32	1	>32
B fragilis C	2	4	32	512	4	4	0.12	>32	0.12	>32