Laboratory handling of impalpable breast lesions: A review

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Introduction

With the accidental discovery of mammography by Stafford L Warren in 1926 (published six years later),¹ a new tool became available for clinically examining breasts for small carcinomas. The use of mammography in screening for breast cancer was first suggested in 1956,² and the original trial, the Hospital Insurance Plan (HIP) for women in New York, was inaugurated in 1963.³ Since the early mammograms, an enormous improvement in quality has occurred⁴ making it possible to detect small and impalpable breast carcinomas.

The implementation of the United Kingdom Breast Screening Programme up to the end of 1990 will result in an estimated 15 000 biopsies a year⁵; of these, at least 40% will be impalpable lesions,⁶ localised by the radiologist before surgical excision.

Several radiological methods are available to guide the surgeon in his or her search for mammographic abnormalities. These range from simple painted skin markers such as methylene blue⁷ or silver nitrate⁸ to sophisticated hooks,^{9,10} and include the injection of coloured dyes.¹¹ A common mistake is for the pathologist to assume that the marking is precise. In fact the marker provides only a fixed point for reference. The surgeon is directed to the abnormality by the radiologist, who issues a written report stating the relation of the marker to the abnormality (fig 1).¹² The surgical excision must be confirmed by specimen radiology⁵ and further tissue may need to be excised.⁵

In handling these specimens the pathologist faces three problems. First, there is often no palpable or obvious visible abnormality. This means that blocks for histological analysis cannot be selected by usual methods, and therefore either all the tissue must be blocked or the abnormality needs to be located by another means. Secondly, any histological abnormality detected must correspond to the radiological lesion detected on the original mammogram which may be rather subtle. Thirdly, histological analysis may indicate a borderline lesion.¹³

Any method should thus involve block selection by specimen radiology and prime fixation is mandatory. The economic use of the laboratory technician and the pathologist's time also needs to be considered.

General guidelines about specimen handling have been included in the Sloane reports.^{14,15} Other recent reviews have placed more emphasis on logistical and organisational

Figure 1 Photoradiograph showing a mammo-lok hook in its final position, lying near to but not within an area of suspicious microcalcification (circled).

difficulties.^{16,17} Anderson's article deals particularly with the problems of specimen handling.¹⁸ This review details simple practical methods of dealing with impalpable screened specimens.

Specimen radiology

A whole specimen radiograph needs to be performed immediately after the biopsy specimen is resected to confirm removal of the radiographic abnormality. A copy of the specimen mammogram can be made at this point, at minimal extra cost. One copy should remain in the patient's notes as a record of excision, the second is for the pathologist's use. A second set of mammograms is performed by the pathologist after fixation and slicing of the specimens.

Specimen radiograms can be produced in several ways. The most convenient method is to use a self-contained table-top unit designed

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for the purpose. Such units include the Faxitron 43855A with option A02 (Hewlett-Packard, USA) and the JKJ Micro specimen unit (Xerox Medical Systems, USA). Careful calibration of both machines by qualified radiographers is essential to obtain optimal performance, but the machines can be operated by other staff. These units may be sited in the departments of pathology, radiology, or in the operating theatres. If one machine is situated at a site distant from the pathology department it may be desirable to have a second machine for the pathologist's use. For convenience, a rapid process film that can be processed automatically by the department of radiology should be used as this avoids the problem of maintenance of individual processing units.

Individual settings for the machines vary according to the thickness and density of the tissue. As a rough guide the voltage should be between 25 and 35 kilovolts, and the exposure time two or three seconds on the Faxitron, and 20 to 40 seconds using the Xerox machine. The machine should be calibrated (and maintained) by experienced radiographers. A table of settings can be left with the machine so that laboratory technicians can take high quality x-ray pictures.

Alternatively a straightforward clinical mammogram machine can be used by placing the biopsy specimen directly on to the stage and reducing the exposure time by one half to one quarter of that needed for clinical mammography.^{19 20} This machine does, however, need to be operated by a qualified radiographer. If no clinical mammogram machine is available adequate visualisation of microcalcification can be obtained on a standard x-ray machine set at a low voltage (Low R, Frenchay Hospital, personal communication). As the technical quality is inferior to mammography, however, this technique is not suitable for use as a check of surgical excision. It might be useful to the pathologist for taking x ray pictures of the specimen to aid block selection.

Radiological interpretation will be a new experience for many pathologists. In the initial stages guidance may be required from the radiologists involved in the screening programme. Some pathologists may find it easier to compare their specimen mammograms with the patients' preoperative mammograms. This will ensure that the abnormality they see in the specimen mammogram is the same as that in the original film.

Most mamographic lesions will be areas of microcalcifications but some will concern soft tissue abnormalities, such as distortion of the architecture, increased vascularity, or an area of increased density (fig 2). Microcalcifications vary in their histological appearance. Most are concentrically arranged deposits of calcium hydroxyapatite— $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ —which are readily detected. A few calcific mamographic lesions (composed of Weddellite, calcium oxalate dihydrate) are not stained by haematoxylin and eosin and can

Figure 2 Whole specimen mammogram showing two carcinomas. The hook lies close to an area of microcalcification with the characteristic differences in particle size and density seen in malignant calcification. The second lesion is the circular soft tissue density showing an irregular outline and distortion of the surrounding tissue. The linear shadow is the skin ellipse.

only be recognised by using cross polarised light. These forms are easily overlooked unless the pathologist is alerted to their presence.^{21 22}

Many women requiring biopsies will have more than one radiographic abnormality but only one suspicious area. It is vital to take blocks for histological analysis from this mammographically suspicious area, even if there is also an obvious visible or other radiological abnormality.

Marking of resection margins

During the processing of breast biopsy specimens it is important to mark accurately the margins of surgical excision. With the trend towards conservative treatment for malignant breast lesions some surgeons require differential marking of the resection margins of excisional biopsy specimens. The biopsy specimen is orientated for the pathologist by the surgeon using sutures of different colours, or with differing numbers of sutures or knots.^{23 24} If the skin is included the number of marking sutures may be reduced by the use of an orientating arrow painted on the skin.²⁵

When marking these specimens it is important to use a radiolucent material. Unfortunately, inorganic artists' pigments,²⁶ and to a

lesser extent, silver nitrate,²⁷ both show up on sliced specimen mammograms, thus confusing radiological detail. India ink is possible but not ideal: it is slow to dry and more seriously, spreads into the planes of the underlying connective tissue.²⁷ Although drying problems may be obviated by mounting India ink in acetone,^{28,29} by itself India ink does not permit identification of the individual planes of excision. The same disadvantage applies to alcian blue³⁰ and TippEx,³¹ the latter also being radio-opaque.

Differential marking of breast biopsy specimens has previously been achieved using inorganic artists' pigments,²⁶ but as mentioned above these are not suitable for subsequent soft-tissue x ray pictures. We have recently developed a method of differential marking using coloured gelatins.³²

Methods of localising the mammographic lesion

Several methods can be used to find an invisible abnormality in a biopsy specimen. The simplest but most expensive would be to process all the tissue. Few laboratories would relish the amount of work generated by this method. Some pathologists remain resistant to the use of x ray pictures to locate the abnormality, feeling that this is time consuming. The tissue around the tip of the hook is therefore blocked, and if no abnormality is found in the initial blocks more tissue is taken from the specimen. This method is only effective if the hook is accurately placed in the abnormal area.

We feel that the use of specimen mammography to localise the abnormality in the biopsy specimen before the block is selected is essential. There are two basic methods using mammography. They are outlined below.

BREAD SLICING METHODS

Our modified "bread slicing" technique is based on the original suggestions by Helen Ingleby, dating from the 1930s, for handling breast biopsy specimens.³³

The fresh or partially fixed specimen, along with a copy of the specimen mammogram is received in the laboratory. The specimen mammogram acts as a check of surgical excision. The specimen is then examined macroscopically and the excision margins identified and marked. After overnight fixation the lesion is bisected along the line of the hook to show the cut surface and the tip of the hook (fig 3). If incision shows a discrete lesion corresponding to the mammographic abnormality this can be sampled directly. Further slicing is performed thereafter. This time slicing is perpendicular to the line of the hook and parallel in the two halves of the biopsy specimen (fig 4). This produces many semicircular slices. The aim of this is to produce slices thin and small enough to fit into a cassette. To aid fine slicing a jig can be used.³⁴

The numbered slices are then placed in a compartmentalised container with a lid, which is filled with formol saline (fig 5). Each slice is then x rayed.³⁵ After studying the x ray pictures

Figure 3 After overnight fixation in formol saline the specimen is bisected along the track of the localisation wire as indicated by the arrow (left). This produces two pieces, one (A) containing the hook, the other (B) without the hook (right).

blocks are taken to include the areas of mammographic abnormality, the adjacent normal breast, and the resection margins. Any visible abnormality can also be sampled. The site of all blocks in the specimen should be carefully noted. The specimen can be stored in a plastic bag sealed into individual compartments (fig 6).

It has been suggested by some authors that this or similar methods are time consuming and inconvenient due to the need for repeat specimen radiology.³⁶ We find that in routine use this is not a real problem.

The slicing techniques need no special equipment other than access to a mammo-

Figure 4 After further overnight fixation the halves are sectioned at 2-4 mm intervals at 90° to the original cut. The sections are parallel in each half of the biopsy specimen.

Figure 5 Metal container with removable dividing strips. This can be used for transporting the sliced biopsy specimen to the x-ray facilities and for short term storage.

Figure 6 Sliced specimen after blocks have been selected for histological analysis. Each slice has a separate compartment in the plastic bag, the number is indicated on its outside. The specimen can be stored indefinitely in this way.

graphic machine. Bisecting the lesion and an extra day of formalin fixation provide high quality histological results. Methodological slicing and taking x ray pictures mean that a good three dimensional picture of the lesion is obtained, with accurate correlation between histology and radiology. Taking further blocks is extremely straightforward.

GRID METHODS

This approach is based on the principle that the intact specimen is attached to a wooden, card, or perspex board in the operating theatre. The specimen mammography is performed on this board and sections can then be taken from the specimen directly. The advantages are that only one specimen radiograph needs to be performed and that the radiologist can indicate the area to be blocked on the specimen mammogram.

Some methods have used tracings of the abnormality from the x ray picture to localise the lesion,³⁷ while others use a card or grid with radiodense markings on it to provide a fixed reference point for histological sampling.^{36 38}

The most sophisticated method consists of attaching the fresh specimen in the operating theatre to a commercially available perspex grid, which has 1.0 centimetre spaced metal wires running vertically and horizontally through it. These are visible as a grid on the x ray picture, and blocks can be taken from the radiological abnormality (fig 7).³⁶ The surgeon can use sutures to indicate which margin is which, and appropriate blocks can be taken from each.

The main advantage of the "grid" method is the obviation of specimen mammography in the laboratory. Tissue distortion is also claimed by some to be less than in more traditional methods of fixation.³⁶

Major disadvantages of the grid method are, however, the danger of movement of the biopsy specimen on the grid between x ray and dissection, and the depth of substantial biopsy specimens. In addition, there is the difficulty of taking further blocks. Other logistical problems include the need for the surgeons to use the grid and securing an adequate supply in the operating theatre.

Variations in technique

It is probably inappropriate to perform frozen section examination for diagnostic purposes. Techniques are described in the American scientific journals for removing representative tissue using radiographically guided biopsy.³⁵ This approach most certainly makes the biopsy specimen difficult to handle subsequently.

If fresh tissue is required for advanced bioassay, immunohistochemical or genetic methods of examination, it is possible to fine slice the biopsy specimen and to freeze down a proportion of the slices. Unfortunately, this makes subsequent handling of the biopsy specimen extremely difficult and is not to be recommended for general use. The relatively small size of some biopsy specimens and the strictly focal nature of borderline lesions and microinvasion make the loss of vital diagnostic

tissue a distinct possibility.

Gibbs has favoured an entirely different approach to the examination of breast biopsy specimens. The biopsy specimen is transversely sliced at 5 mm intervals. Specimen mammography is performed and the appropriate slices are processed, sectioned, mounted and stained in their entirety to produce whole-slice mounts.^{39 40} None the less it must be pointed out that this procedure requires the use of a specialised heavy microtome and the production of high quality sections for the assessment of possible borderline lesions. Neither requirement is readily achieved.

Nevertheless, this method clearly has the advantage of producing a complete section of the whole biopsy specimen with a clear record of the resection margins. We feel, however, that the disadvantages preclude its routine use. Special cassettes and containers for automatic processing are needed, and sections are cut on a heavy duty sledge microtome which is technically demanding. The quality of the sections produced, unless highly skilled technicians are available, may be inferior, thus making diagnosis more difficult. The method has the dual disadvantages of increased cost and potentially poorer quality of the sections.

Conclusions

The handling of mammographically detected lesions is a complex and time consuming procedure whatever method is used. If accurate and meaningful results are to be obtained it is important to have a consistent method for handling these specimens.

It is impossible to process these specimens properly without specimen radiology. It is essential that the diagnostic blocks are taken from the abnormal area identified on the original mammogram by the radiologist.

Marking of the surgical resection margins is necessary, before the lesion is dissected in any way.

As these lesions may be "borderline" good fixation is vital.

We recommend a two-stage bread-slicing method for handling these specimens, although it is a little more time consuming and requires a second set of specimen radiographs. The advantages are the lack of any specialist equipment, ease of block selection, excellent correlation between the radiology and histology and the simplicity of taking extra blocks.

The use of frozen section diagnosis is not advised for general use.

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