Review Article

Breast biopsy techniques in a global setting—clinical practice review

Edibaldo Silva[1], Steven Meschter[2], Mona P. Tan[3]

[1]Division of Surgical Oncology, Department of Surgery, University of Nebraska Medical Centre, 986345 Nebraska Medical Centre, Omaha, NE, USA; [2]Department of Cytopathology, Geisinger Medical Laboratories, Danville, PA, USA; [3]Breast Surgical Oncology, JHPL, Mount Elizabeth Novena Hospital, Singapore, Singapore

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Correspondence to: Mona P. Tan, MBBS, FRCS (Ed). Breast Surgical Oncology, JHPL, Mount Elizabeth Novena Hospital, 38 Irrawaddy Road #10-60/62, Singapore 329563, Singapore. Email: jabezhopems@gmail.com.

Abstract: Breast cancer is a disease of global concern, regardless of economic status. A significant disparity in breast cancer care between low- and high-income countries is not unexpected, but consideration can be given to particular aspects of therapy to allow as much equitability as possible. One of these aspects involves biopsy of breast lesions. With available resources, management in developed countries focuses on dealing with screening and image-detected lesions. In such circumstances, advanced percutaneous biopsy techniques are utilized liberally. However, where resources are less forthcoming for mammographic screening, women frequently present with symptomatic, palpable and larger tumours. This scenario behooves the clinician to modify treatment approaches and yet use cost-effective management strategies. It is essential that thought is applied to breast biopsy technique used where there is cost-consciousness as it significantly influences subsequent therapy. Less expensive strategies like fine needle aspiration cytology (FNAC) and core needle biopsy (CNB), when performed with particular attention to technique, handling, transportation and preparation of biopsy specimens allows a high level of accuracy and provides adequate information for the next steps in treatment. This mini-review discusses the variation in biopsy approaches among lower and higher income areas and offers suggestions for appropriate breast biopsy strategies in resource-limited countries.

Keywords: Breast cancer; breast biopsy; fine needle aspiration biopsy; percutaneous breast biopsy; global concern

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Introduction

Breast cancer is a disease of global concern, regardless of economic status (1). There is therefore a need to address the diagnosis and treatment of breast cancer in countries with varying healthcare resources and capabilities. By virtue of asset limitations in low- to middle-income countries, clinicians are restricted in their diagnostic and therapeutic approaches, and may not be able to offer that which is commonplace and conventional in developed countries. Nonetheless, it is essential that women with breast cancer in these states receive appropriate care. The challenge is finding the balance between cost and efficacy of care in this setting.

Unlike the situation in developed countries where screening mammography is widely adopted, it is not routinely performed in less affluent countries. While continued improvement in technology is seen in high-income countries in the past two decades with the adoption of 3D tomo-mammography overriding digital mammography in many centres (2), screening programs

are rudimentary in low- to middle-income countries. This has naturally led to different clinical presentations of breast cancer. In countries without organized screening programs, the majority of women present with palpable breast cancers (3,4), while women in affluent countries present with image-detected lesions. Clinical strategies for diagnosis and treatment therefore necessarily differ.

Where image-detected breast lesions are commonplace, as in communities with mammographic screening, there is an opportunity for a non-interventional approach using short interval monitoring. This is a reasonable management strategy for mammographically low risk non-palpable lesions. In contrast, where screening is not readily available, women present with symptomatic lesions, the most common of which is a palpable breast lump. Population screening is associated with a survival benefit of 40% (2). It follows, therefore, that a lesion large enough to be palpable, if malignant, would bear a 40% poorer survival for the patient. The diagnostic process, hence, should be escalated and additional information may be reasonably attained through the use of the "triple test" assessment: abnormal exam, abnormal imaging and abnormal pathology. In the diagnostic management of breast lesions, when all three are concordant, the accuracy of diagnosis for breast lesions is reliable and optimal.

Thus, the contemporary universal principle of minimally invasive breast biopsy (MIBB) for preoperative percutaneous diagnosis can still be achieved in a reliable, expeditious and cost-effective manner in limited-resource communities (5). This may require the regular use of techniques which have fallen out of favour in developed countries for various reasons. Prior to a surgical biopsy, fine needle aspiration cytologic biopsy (FNAC) and core needle biopsy (CNB) (6), relatively inexpensive diagnostic procedures, may be employed routinely. More sophisticated instruments which may offer greater accuracy, like vacuum-assisted breast biopsy techniques, may or may not feature when there are cost constraints. Prudent use of resources entails a 'pyramid' approach, where the great majority of diagnostic procedures applied are the least expensive, with careful triage and selection of cases which require procedures of incremental cost. This necessarily takes into account the potential for harm at each level of the diagnostic process as well.

Despite the availability of a complete range of percutaneous biopsy devices, there might still be a need for surgical biopsies, particularly when the position of the lesion is not amenable to image-guided biopsy, when patient factors preclude its use, or where there is radiologic-pathologic discordance. In such cases, one may elect to perform an excision biopsy or empirical lumpectomy with clear margins. The latter can be performed if ductal-carcinoma-in-situ (DCIS) or cancer is suspected, with the objective of avoiding the cost of a second operation. A diagnostic excision biopsy should be performed only if percutaneous means have been exhausted or contraindicated, as the majority of biopsies will yield benign histology. Between 85–95% of breast lesions biopsied in developed countries have been reported to be benign (7). The cost to sustain such a volume of diagnostic procedures can be considerable. In the setting of low to middle income countries a reappraisal of the use of the triple test can optimize conservative care, control costs and rely on surgical biopsy only when rarely warranted.

Percutaneous breast biopsy

The use of percutaneous breast biopsies for diagnostic purposes in favour of open surgical procedures is well established (8). It allows better surgical planning and a reduction in the need for multiple surgical episodes for cost-effective treatment. It follows that this principle should be upheld as much as possible even in the context of cost constraints. The frequent use of FNAC as a first diagnostic procedure, followed by core biopsy where necessary, would be consistent with this philosophy. Where possible, image-guidance for these biopsies would be ideal. However, trained clinicians may perform these percutaneous biopsies with the help of clinical and tactile cues to achieve a reliably accurate diagnosis. Employing imaging-directed vacuum-assisted breast biopsy (VAB) instruments which may be routine in referral centres, may be a luxury and impractical where cost is an issue. The ensuing discussion keeps these tenets in mind.

Fine needle aspiration cytology (FNAC)

When applied appropriately, FNAC is an extremely useful diagnostic procedure, with its efficacy optimized in a multidisciplinary team setting (6,9,10). Clinicians working with trained cytopathologists can produce sensitivity levels of 99.7%, with low false negative rates (3,11). These high accuracy rates allow the establishment of same day 'on-the-spot' tissue diagnosis within multidisciplinary breast clinics. Such a workflow, as organized by the authors (ESL & SM) in a high volume academic centre, may be replicated in low-middle income countries where women

have to travel significant distances for medical care (3). Care can be appropriately triaged, where benign palpable lesions diagnosed through a concordant triple test based on clinical examination, imaging findings and adequate cytology, may be managed conservatively with follow-up. Where inconclusive or discordant, further testing with CNB is indicated. When all the elements of the triple test indicate malignancy, treatment decisions may be expedited depending on the circumstances of tumour presentation and resource availability.

Despite the increasing use of core biopsy, FNAC still remains as a useful and cost-effective means of diagnosis for breast lesions with similar sensitivity (97% *vs.* 97%), specificity (94% *vs.* 96%), diagnostic accuracy (95% *vs.* 96%) and negative predictive value (98% *vs.* 96%) as CNB, with lower complications (11). Frequent use of CNB for palpable lesions may have led to a reduced need for the clinician to be well trained in the use FNAC and for cytopathologists to develop confidence in interpreting smears (12). This diminution of skill may have led to poorer quality smears and hence lower accuracy rates, leading to a vicious cycle of decreased use, and perhaps finally abandonment (10,13). There may be a case for re-visiting the diminished use of FNAC and perhaps revive its routine use, especially for palpable breast lesions, in the light of increasing concerns about cost containment (8), a relevant consideration for low-middle income countries and developed countries.

While FNAC has the advantages of lower cost and rapid turnover, cytology is limited in its ability to confirm invasive disease. Therefore, when malignancy is diagnosed, a follow up CNB may be indicated. This is especially true for women who elect to undergo mastectomy directly or for whom neoadjuvant chemotherapy is planned. Not only does the additional test differentiate invasive disease from DCIS, it affords immunohistochemistry (IHC) assessment, as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) status are predicated on the presence or absence of documented invasion and the ability to identify them in the invasive component.

Axillary status is an important prognostic factor and its preoperative assessment should necessarily form part of the initial diagnostic work-up, for which FNAC performed, preferably under image-guidance, may be ideal. Malignant cells detected on cytology presumes invasion. As an alternative to CNB, adequate cellular material obtained through FNAC (see Section Handling of tissue samples) may offer the opportunity to interpret ER/PR and HER-2 status of the invasive component. This approach allows high accuracy rates regardless of economic status (14,15).

Core needle biopsy

Although FNAC may be used for image-detected lesions, CNB is preferred in this setting. Larger tissue samples with parenchymal elements, rather than cellular preparations can be submitted for standard histological processing. However, paraffin processing, unlike FNAC, cannot be performed within the same day, and turnover is longer but it has the advantage of being able to distinguish invasive from non-invasive disease and provide ER, PR, HER-2 status using the same tissue cores sent for diagnosis.

Due to the larger dimension of the core biopsy needles, a small incision and local anaesthesia is needed. Multiple passes are necessary, coupled with a rapid-fire mechanism may be uncomfortable for the patient. There is unsurprisingly a higher incidence of complications in comparison with FNAC, like haematoma formation post procedure and in rare instances, breach of the thoracic wall. There may also be sampling error when performing CNB.

Despite the acquisition of larger tissue samples, false negative results of 3–7% have been reported with CNB. In addition, lesions such as atypical ductal hyperplasia (ADH) and DCIS may be upstaged on excision biopsy (16). In the setting of low-middle income countries, CNB may be used as a confirmatory test when cytology results are inconclusive or inadequate. Furthermore, it may be performed on cytologically proven malignancies when either mastectomy or neoadjuvant chemotherapy is planned. If cytology were reported as carcinoma for a palpable lesion which is amenable to breast conserving surgery based on tumour to breast volume ratio, it would be reasonable to proceed with lumpectomy and sentinel lymph node biopsy rather than perform an additional CNB. Such a selective approach is cost-effective, at the same time minimizes axillary overtreatment since palpable DCIS is a risk factor for invasion and possible sentinel node metastasis.

The components of the triple test apply to the use of CNB as well as FNAC. Concordant findings with CNB harbour a low risk of false negatives and patients with such findings can undergo interval follow up with imaging and clinical review. This approach does allow considerable cost savings for the patients who are true negatives in low-middle income countries.

Vacuum-assisted breast biopsy devices

VAB devices were developed which allowed multiple tissue cores to be removed under direct visualization with a single pass, rather than several passes required during CNB (6). For smaller lesions, a minimally invasive, percutaneous excision can be performed. Conceptually attractive, it is able to reduce false negative results to a minimum but it is still unable to eliminate the underestimation of high risk benign lesions (16,17). Notwithstanding, there may be cost advantages in using this device to excise small lesions for a definitive diagnosis. Since it has excisional capabilities which address the issue of discordance for small lesions (<5 mm) with an eventual benign diagnosis (14), it reduces the need for excisional biopsy and follow-up. The challenge is to achieve financial equipoise in terms of the cost of investment for the equipment and optimum care for the patient with indeterminate image-detected lesions for which percutaneous excision is possible.

Surgical biopsy

Although from a pathology standpoint, a surgical biopsy provides the best material for complete evaluation, it is neither the most cost effective nor clinically expedient approach to adopt on a wide scale. Among the diagnostic biopsy modalities so far mentioned, FNAC, CNB and surgical biopsy, it is the general consensus that surgical biopsy should be seldom utilized. The financial burden of a surgical biopsy is significant and would expose most women to unwarranted harm since the majority of palpable lesions are benign and do not require surgical excision. Many are self-limiting and often resolve with time. Moreover, current management of locally advanced breast cancers (LABC) offers the option of pre-operative chemotherapy or estrogen ablation undertaken with the tumour in situ. Favorable clinical response with tumour downstaging in this setting may reduce the need for a deforming mastectomy. Complete clinical and pathologic resolution of the palpable cancer is accepted as a reliable prognostic sign which predicts long disease-free survival. Understanding the therapeutic strategies which necessarily follow a diagnosis of breast cancer, surgical excision as the primary diagnostic procedure should only be undertaken in exceptional circumstances.

In the case where the mammary lesion is screen-detected, there are very few situations where localization and surgical biopsy is warranted. These may include posteriorly sited lesions detected on mammography, or those close to the skin, where the use of stereotactic needle biopsies would be technically challenging. In the past, women with breast compression thickness of less than 30 mm were deemed to be unsuitable for stereotactic VAB. However, there are devices available now which enable a reduced aperture size of the instrument to surmount this issue. Despite these modifications, a small number of these women would still need to undergo image localization with excision biopsy for diagnosis.

In the event that a patient presents with a palpable lesion which has an incomplete or indeterminate triple test after initial FNAC and CNB, a few considerations need to be taken into account. Since contemporary data suggests that breast conservation treatment (BCT) results in superior survival outcome when compared to mastectomy (18,19), if resources allow, BCT would be the eventual goal. With this in mind, where a mass is small enough to be resected as part of an attempt at BCT, excision biopsy should be performed to achieve clear margins if possible. This is the preferred option to avoid a return to the operating room for margin re-excision if the lesion is diagnosed to be malignant. Careful incision planning and placement and repair of the ensuing defect will minimize impact on cosmetic outcome whether the lesion is diagnosed to be benign or malignant. The excision specimen as well as the tumor bed should be carefully oriented with either sutures, clips or ink according to availability and the preference of the treating team. While this offers a single surgical procedure, cost-effective approach for high-risk lesions like ADH and DCIS, it does not preclude the need for axillary staging for a possible invasive tumour.

In developing countries, some women may present with LABC which are too large to undergo BCT at the time of diagnosis. In such instances, especially in the presence of large, ulcerative or fungating tumours with or without distant metastasis, there may be a role for incisional biopsy under local anaesthesia for tissue diagnosis. Often, in non-T4 lesions, the next therapeutic step would be the initiation of neoadjuvant chemotherapy (NAC) Further local treatment would depend on response to NAC and the controversies regarding definitive surgery are not within the scope of this discussion.

Handling of tissue samples

Tissue samples from each biopsy technique have their own unique handling requirements to optimize histologic

interpretation. If they can be reasonably achieved within the scope of available funding, due attention should be given to these specific nuances.

Cytology

FNAC in principle is a very simple procedure but requires significant practice to reliably obtain diagnostic specimens. The technique is best executed with a 23- or 25-gauge needle using quick repeated passes through the lesion with minor directional changes with each pass. The tiny disengaged cored fragments are compacted into the needle under negative pressure using a 10 mL syringe which serves to increase yield while keeping the aspirated material in the needle and hub. The material obtained is dislodged onto a glass slide, smeared and spread out with a second slide, then either air-dried or placed in alcohol as the cytopathologist would prefer. The air-dried slides then are immediately stained using a Diff Quick stain and reviewed on site. The slide smears fixed immediately in 95% ethanol are taken to the laboratory and stained with a traditional Pap Stain. This process requires at least 60 minutes and is usually done in addition to the preparation of the additional cell block (24-hour turnaround). Every attempt is made to avoid introducing the contents, particularly blood or fluid, into the syringe as it would significantly dilute the cellular specimen risking an inadequately cellular smear. If the specimen gets into the syringe it is best removed by rinsing into saline or RPMI for cell block preparation. For cystic lesions or suspected abscesses, a larger gauge needle may be used to further aspirate the fluid contents within the breast. For bloody aspirates or infectious concerns, this can be then sent for cytology and/or culture and sensitivity.

Cell block preparation

Needle passes from the tumor rinsed directly into normal buffered saline or RPMI solution (cell culture media) are taken to the laboratory where they are centrifuged and the fragments concentrated into a cellular pellet. The supernatant fluid is removed and the pellet is resuspended in a small amount of pooled patient plasma. Thrombin is added and the subsequent clot containing the tissue fragments is placed into formalin. This is fixed for at least 6 hours, but not longer than 72 hours before being paraffin embedded, sectioned and Hematoxylin and Eosin (H&E) stained. These sections can be utilized for immunohistochemistry including ER/PR and HER-2 receptor assessment (20,21).

In the ideal circumstance of the pathologist performing the FNA and reviewing the smears on site, adequacy is determined by the interpretation of the smear. Malignant cells are diagnostic, regardless of number. A defined pattern of benign findings (bipolar naked nuclei) which indicate a diagnosis such as fibroadenoma or similar benign entities (apocrine metaplasia, fibrocystic changes, mastitis) would be considered adequate particularly if it is concordant with the triple test. Any acellular smears or normal structures (fat or histiocytes) in the presence of a palpable mass would not offer a definitive diagnosis and are considered unsatisfactory regardless of volume. These cases would require either CNB or excisional biopsy as an alternative means of assessment.

Cases identified as "malignant C5" by FNAC would be significantly enhanced by the acquisition of tissue for ER/PR and HER-2 receptor evaluation. FNAC can reliably predict the presence of invasion in palpable lesions when the characteristic lesion desmoplasia perceived by the needle resistance ("gritty feel") is best appreciated by the operator. However, this is not uniformly reliable as some DCIS can present with a palpable lesion. Since the subsequent surgical management of the patient will be predicated on this distinction and because determination of HER-2 status is necessarily performed on the invasive component, the patient should undergo a CNB for the purpose of assessing frank invasion and more accurately determining HER-2 status (*Figure 1*). In patients with triple negative breast cancers and HER-2 positive cancers, the treatment decisions may favour NAC. Given the expense and potential morbidity of these treatments the CNB is preferred.

Core biopsy specimens

Tissue handling in the instance of a CNB is very critical to the outcome of determination of ER/PR and HER-2 evaluation (22). The specimen should be directly collected into formalin to achieve a cold ischemic time of less than 1 hour. Once in formalin, it should be fixed for at least 6 hours and no more than 72 hours before being embedded in paraffin, sectioned and stained with H&E. These sections can be utilized for immunohistochemistry including ER/PR and HER-2 receptor assessment.

Excision biopsy specimens

Finally, there exists another problem with a diagnostic excision biopsy (23). For reliable pathologic assessment,

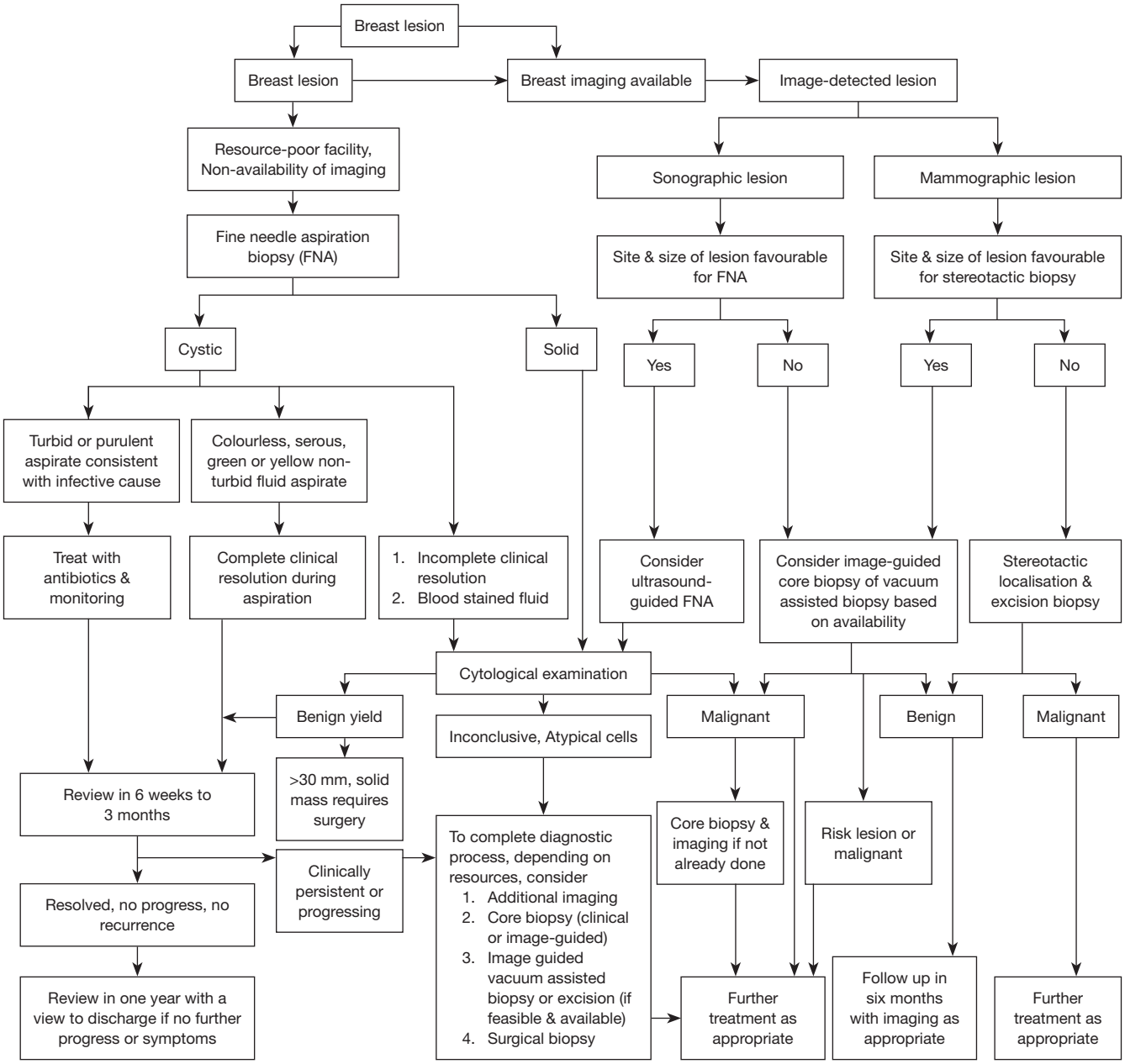

Figure 1 Algorithm for approach to biopsy of breast lesions according to resource availability.

strict tissue handling requirements demand appropriate tissue fixation (time to fixation or "cold ischemic time"). It should be borne in mind that duration of fixation in a large specimen can result in errors in assessment of ER/PR and HER-2 receptor status. These larger tissue specimens contain considerable amounts of fat and connective tissue. The size of the specimen also poses problems for adequate formalin penetration. Also, the time between obtaining the specimen and its addition to formalin is more difficult to control depending on the size of the specimen. These should be added to formalin no more than one hour after excision from the patient. The tissue should be sliced once directly through the tumor prior to submersion to ensure better formalin penetration. Similar to CNB specimens, tumour tissue should be fixed for a time period of between 6 and 72 hours before sections are paraffin embedded and

stained with H&E. Thin sections of this paraffin embedded tissue are suitable for immunohistochemistry and ER/PR and HER-2 receptor analysis.

Excision biopsy specimens also comprise another challenge for the pathologist which is accurate tissue orientation. Ideally the excised tissue sample should be oriented by the surgeon, which should allow reporting of all 6 margin surfaces of the tissue with distance of the tumor to each of these margins. Most pathologists prefer the surgeon inking each of the six faces of the specimen with colored inks. This reduces the potential for the pathologist to mis-orient the specimen in the laboratory. Many surgeons utilize a sequence of suture labels for orientation. This while less desirable is sufficient.

Discussion

Treatment selection and customization

Treatment should be individually tailored and optimized cost-effectively. FNAC has been shown to be a cost-effective approach and warrants consideration as part of the diagnostic algorithm (8). Subsequent use of CNB and VAB may be required but it needs to be borne in mind that these techniques, although allowing greater tissue acquisition, are still inherently associated with a real, albeit low, false negative result and a modest risk of upgrade. Indeterminate results in any form of percutaneous biopsy may still require a surgical biopsy as the final arbiter of diagnosis.

To minimize the need for surgical biopsy, the potential sequelae and cost associated with it, a tiered assessment of the FNAC may be used (C1-insufficient material, C2-benign, C3-atypical, C4-suspicious of malignancy C5-malignant) (24). Appropriate clinical application of FNAC and cytopathologist experience minimizes false negative results (24). This approach is particularly apposite for patients in countries with limited resources, a lack of screening facilities and often present with palpable disease. The rapid availability of diagnostic results adds a further benefit where travel for medical attention poses a significant challenge for the majority of the population. Treatment can be quickly planned according to the triple test results based on FNAC, rather than having to wait an extended 48–72 hours turn-over period for tissue diagnosis, as is the case with CNB or VAB. The small number of patients with non-diagnostic aspirates can be triaged to undergo an expedited CNB to reduce non-attendance at a second review. In this manner, false positives with FNAC can be reduced to an acceptable 2% or less (24).

Even in countries with limited resources, by following the suggested strategy discussed above, centralized comprehensive breast diagnostic centers can be organized with good diagnostic accuracy and outcomes (14). In turn, clinical skills for FNAC and cytopathology can be enhanced which forms a positive cycle of education. These capabilities may be supplemented with relatively inexpensive ultra-sonographic and mammographic equipment, useful both for image-guided biopsy techniques as well as screening.

Proposed algorithm for diagnostic approach

A recommended algorithm for diagnosis of breast disease is shown in *Figure 1*. Although this approach attempts to be as inclusive as possible in terms of socio-economic status, several considerations need to be made for a patient presenting with a palpable breast mass in a resource-limited country. Ideally, multidisciplinary care combines radiology, surgery, pathology, medical oncology and radiation oncology expertise. Plastic and reconstructive surgery, while good to have, may not be practicable where there are cost concerns. Therapeutic strategies may have to be modified.

Where the medical resources are extremely limited without the benefit of radiology and radiotherapy facilities, FNAC is the primary diagnostic modality for women with palpable lesions. If cytology is inconclusive or malignant, CNB should be performed as a confirmatory test. Even though BCT offers good survival outcomes, mastectomy may be the only practical surgical therapy if radiotherapy is not available. It is worth noting that in older patients with ER-positive tumours lumpectomy without radiotherapy is very effective.

If the entire suite of services for multidisciplinary care is available to a patient, then a staged diagnostic approach is undertaken, with mammogram and sonography being the first step. FNAC should not precede imaging as it may alter radiologic characteristics and confound interpretation. In this case, FNAC should follow imaging. Rarely, for lesions with benign but highly cellular cytopathology aspirates, a CNB may be needed for further evaluation. Often these highly cellular benign lesions may require a wide excision particularly if they present with a history of a rapidly growing mass. If cytology yields malignant cells, CNB of the breast tumour with FNAC of any identified axillary lymph nodes with clip placements would be the next step for purposes of NAC for tumour downstaging. This allows an attempt at BCT once NAC is completed. Adjuvant

radiotherapy is a standard part of BCT. As radiotherapy facilities have high initial costs, these may not be readily acquired in low-middle income countries and has to be borne in mind when planning surgery.

Conclusions

Healthcare cost is a perennial issue in the management of complicated medical problems such as breast cancer. At one end of the spectrum, high-income countries have few issues with availability of technology to offer optimum diagnostic strategies, but over-utilization and overconsumption of resources can spiral into uncontrolled spending, especially in the face of patient autonomy and litigation. Paradoxically, these can pose a significant barrier to proper healthcare delivery. In contrast, low-middle income countries frequently encounter the challenge of adequate funding to provide appropriate care. Perhaps the best way forward would be to tailor a rational approach for an equitable, sustainable and efficacious diagnostic strategy for breast cancer specific to a country's circumstance, which is the objective of the algorithm presented.

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Footnote

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