Clinical Significance of Fine Needle Aspiration in Managing Patients with Breast Lesions

Konstantinos Dinas¹, Georgios C. Pratilas¹, Maria Nasioutziki¹, Eleftherios Vavoulidis¹, Vasileios Makris¹, Panagiotis D. Loufopoulos², Matthias Kalder³

¹ Second Department of Obstetrics and Gynecology, Aristotle University, Thessaloniki, Ippokrateio Hospital of Thessaloniki, Greece
² First Department of General Surgery, School of Medicine, Aristotle University, Thessaloniki, Papageorgiou Hospital of Thessaloniki, Greece
³ Department of Gynecology and Obstetrics, Philipps-University Marburg, Marburg, Germany.

Correspondence:
Georgios C. Pratilas, 49 Konstantinoupolioe Str., 54642 Thessaloniki, Greece
E-mail: georgepratilas978@gmail.com
Tel: +306945542878

Received: 21 Sept 2017
Accepted: 12 Nov 2017
Published Online: 02 Jan 2018
Published: 28 Sept 2018

Key words: FNA cytology, NCB, breast disease, benign, malignant, LBC, methylation

Citation: Dinas K, Pratilas GC, Nasioutziki M, Vavoulidis E, Makris V, Loufopoulos PD, Kalder M. Clinical significance of fine needle aspiration in managing patients with breast lesions. Folia Med (Plovdiv) 2018;60(3):364-72.
doi: 10.2478/folmed-2018-0002

BACKGROUND

It is an international consensus today that breast lesions should be managed with triple assessment.¹,² This involves clinical, radiologic, and pathologic (cytopathological or histopathological) assessments.

Regarding the pathologic assessment either fine needle aspiration cytology (FNAC) or needle core biopsy (NCB) is performed as a first modality, depending on the center and the lesion’s characteristics.¹ Excisional biopsy is not considered an acceptable first line diagnostic method today.³

As each axis of the prementioned triple assessment system involves specialists from different fields, reporting systems have been developed to facilitate communication. Breast imaging reporting and data system (BIRADS) has been issued by the American College of Radiology. The European Guidelines for quality assurance in breast cancer screening and diagnosis define reporting systems for cytopathology: C1 unsatisfactory, C2 benign, C3 atypical/probably benign, C4 suspicious for malignancy, C5 malignant and histopathology: B1 normal tissue or inadequate material, B2 benign, B3 indeterminate, B4 suspicious for malignancy, B5 malignant.¹,²

The review attempts to identify the role that FNAC has today in investigating breast lesions but also to present potential uses of this method that are currently under research and investigation.

MATERIALS AND METHODS

PubMed was searched for articles concerning prospective, retrospective and review studies about clinical applications of FNAC.
identify literature involving: clinical aspects of FNAC, axillary techniques with FNAC application, diagnostic buildup of breast lesions. Afterwards they were combined into an organized search limited in English from 01/01/1990 and to up to date. The reference lists of included studies were investigated to identify additional references.

RESULTS - DISCUSSION

INDICATIONS

There is no general consensus regarding the role of FNAC in the management of breast lesions. Over the past years the main indication for performing it is verification of benign disease in breast masses. Thus the majority (around 70%) of patients who will have FNAC will be discharged at home. Besides that, it also plays an important role in malignancy management, even though it must be taken in account that FNAC can present a weakness in assessing C1, C3, C4 lesions where benignity or malignancy are not always evident. In these cases the supplementary use of NCB is indicated.

EFFECTIVENESS

When a cytopathologist is in close cooperation with the health professional performing the FNAC, results can be given in a very short time. In cases of a benign disease it offers a one-stop assessment with either the discharge of the patient, or a programmed follow up. This immediate reassurance is one of the main benefits of this method towards the patients. Combined with the minimal pain and discomfort and the decreased risk of complications, FNAC could be characterized as the most patient-friendly option.

FNAC accuracy for benign disease (C2) is very high when it is performed by experienced cytopathologists. Previous studies have demonstrated that the sensitivity, specificity, and accuracy of breast FNAC all ranged from 77% to 100%.4

Lately immunocytochemistry (ICC) has a role in the diagnosis and treatment of breast pathology. It is especially promising in identifying lesions belonging to the ‘gray zone’. Antibodies that are mainly used to discriminate between ductal and lobular cancer involve E-cadherin, p120 catenin, CK34BE12 (keratin 903) and CK8 (CAM5.2). The expression of high molecular weight cytokeratins is used in discriminating between ductal hyperplasia and ductal in situ carcinoma.5-7 Furthermore, myoepithelial markers like CK5/6, CK14, SMA, CK17, calponin, SMMHC, S100 and mainly p63 are possible to contribute to diagnostic practice, since histologically the hallmark of invasion is the lack of myoepithelial cells (MECs). It should be noted, though, that p63 is expressed in certain types of invasive breast carcinoma. In cases with difficulty in evaluation, a combination of MEC markers such as p63, SMMHC and calponin is suggested.8,9

Regarding cases with malignancy FNAC has a different role. In a European discussion forum the majority of participants agreed that when triple assessment is concordant, final treatment of breast cancer can be planned based only on FNAC, without the need for NCB or excisional biopsy.2 However, it was stated that due to false negative results, other components of the triple assessment system need to enhance its efficacy and diagnostic yield. Despite this, a lot of health professionals would not agree, and would request a NCB to plan the final treatment.2

It has been suggested in the past that one of the main drawbacks of FNAC, was that it did not provide suitable and adequate material for reliable examination of prognostic and predictive biomarkers. Research on this issue gave encouraging results with good correlation between cytologic and histologic assessment of these markers, and in particular estrogen(ER) and progesterone(PR) receptors.10 Some authors advocate that methanol-fixed cytospins prepared from FNAC samples ensure highly reliable ICC assessment of ERs – the concordance between these samples and formalin-fixed paraffin-embedded (FFPE) was 100% –, whereas Papanikolaou-stained cytospins or smears are conditionally suitable because of the small risk of false negative results – the concordance of FNA to FFPE was 94%.11 These results have been improved even further with the use of liquid based cytology (LBC). LBC can also be used for molecular examinations and tests such as flow cytometry and immunocytochemistry (ICC) as long as strict conditions and guidelines in scoring and methodology are met.12-16 Comparisons of ER and PR statuses from FNAC and CNB showed that both methods give similar results. A concordance was found between the two tests of 98% for ER (kappa = 0.93) and 96% for PR (kappa = 0.91).17 HER-2 expression on FNAC samples seems to demonstrate various results in regards of overall accuracy in comparison to those of histological evaluation, in use for preoperative planning of treatment. HER-2 expression on cytological preparations was insufficiently reliable for clinical use, whereas HER-2 gene amplification determined by FISH demonstrated strong and consistent correlation with HER-2 status on tissue samples. Other studies have demonstrated
the high level of agreement for HER-2 expression on both LBC (TP) cytology specimens and the corresponding tissue samples concluding that LBC (TP) technique can be routinely used for the biological characterization of invasive breast cancer.18 Nevertheless in cases where HER-2 status is uncertain, the use of cell block techniques is a reliable practice in preoperative practice for treatment.19,20 The status of HER-2 on FNAC must be evaluated for breast cancer patients to receive anti HER-2 therapy, by either ICC or ISH or a combination of both, based on the updated ASCOP/CAP recommendations for HER-2 testing.21

As for the case of the Ki-67 biomarker, its main indication for clinical use, apart from its predictive/prognostic value when applied on the final surgical specimen, are cases of premenopausal women with tumors that would benefit from neoadjuvant chemotherapy.22 Ki 67 expression analysis is clinically performed today in histopathological samples. It must be mentioned though that it is has been applied on LBC cell block material, as part of trials, with strong positive correlation between immunohistochemistry and ICC results.18,23 An important point about Ki-67 is that no established quality assurance schemes are in place to assure that the procedures for its analysis could achieve good reproducibility and reliably interpretable results.24 Thus, Ki 67 measurement is not currently standardized among laboratories.22 Another study suggests that determining the immunocytochemical Ki-67 expression may play a role in further investigation of suspicious or indeterminate cytological reportings and in helping to differentiate malignancy from benignity in these cases.25

LBC can be used successfully for extraction of mRNA or DNA from the acquired sample. Thus this makes possible gene expression analyses to be performed.26-28 Moreover, LBC could play a role for tumor cell bank. This seems to be feasible, as with long-time storage of samples at -20°C and -80°C, there was no significant loss of immunoreactivity of PR and ER.29

FNAC can expand its laboratory application range with cell block cytology. FNA material cell blocks are useful in the assessment of hormone receptor status and HER-2, especially in cases of locally advanced breast cancer for planning neoadjuvant therapy. The technique involves centrifugation to concentrate cells from the liquid medium of LBC. The material acquired is then embedded in a polymer gel and then processed in a paraffin block, which can be used to produce classic biopsy slides. An evolution of this method is the Cellient system that automates and standardizes the whole procedure using ThinPrep technology. This method is attractive for ancillary techniques or long-time cell conservation. With this application, FNAC acquired material is suitable for use with techniques as fluorescence, chromogenic and silver in situ hybridization (FISH, CISH, SISH) and ICC as well as used in tissue samples. It has been shown that there is very good concordance compared to classic tissue samples and that these techniques can also help when HER2 status is uncertain in ICC.30,31 In all the above settings it is highly recommended to have good quality cell blocks and quality control of their interpretation.19

In neoadjuvant chemotherapy the commonly identified predictive markers are ER, PR, HER2 and Ki-67. FNAC has proved its efficacy in the first three of these and can be used for neoadjuvant planning when Ki-67 is not required. Based on the prementioned potentials for Ki-67, mainly through the application of LBC and Cellient techniques, FNAC could be proved to have a more generalized part in neoadjuvant chemotherapy in the future.32

Another field where research is done is the clinical usefulness of determining the methylation status of gene promoters, associated with different types of breast cancer, on FNAC samples. The main concept is to assess whether screening for a certain panel of methylated gene loci would increase the diagnostic accuracy of FNAC in distinguishing between benign and malignant lesions.33 A relevant study investigated a panel of three genes: RARbeta2, RASSF1A, and cyclin D2. The results were promising as 96% of invasive carcinoma cases had at least one of the genes methylated, whereas methylation occurred less frequently in benign lesions and carcinoma in situ: 42% and 76%, respectively.34 Another study comparing a total of twenty-three gene promoters managed to distinguish a panel of four of them: CCND2, RASSF1A, APC, and HIN1. The results showed that when three or four of these genes were positive for methylation, breast cancer was identified in 100% of cases.35 One further step was attempted by trying to evaluate the prognostic value of quantitative methylation of three gene promoters: APC, CCND2, and RASSF1A. The conclusion was that RASSF1A methylation was significantly and independently associated with worse disease-free survival in the final multivariate analysis.36 Thus, further research on the role of gene promoter methylation, might not only improve the accuracy of
FNAC, but more importantly will provide new tools for the management and treatment of breast cancer.

Another clinical role of FNAC is the investigation of axillary lymph nodes in patients diagnosed with breast cancer. Preoperative lymph node FNAC assists with staging the disease, selecting patients for sentinel node biopsy or axillary lymph node dissection, as well as planning for neoadjuvant therapy in cases of advanced disease. In studies for axillary status assessment, FNAC showed high specificity and sensitivity (88.0% and 97.8%).

False positive results are practically non-existent, while false negative may be encountered in cases where the lymph node is partially involved with micrometastases, or isolated tumor cells. The use of cytokeratin stains in this case is controversial, and the clinical significance of such findings is unclear. However, cytokeratin stains may be helpful especially in lobular carcinoma.

FNAC has also a role in the diagnosis and investigation of distant metastatic disease or recurrence. This becomes very important as commonly only primary tumor tissue is analyzed. Proof exists that the expression of estrogen, progesterone receptors and HER2 may be significantly different between the primary tumor and the metastatic lesions. The ConvertHer, BRITS, DESTINY trials as well as other studies show variable discordances in receptor status between primary tumors and metastatic lesions that may be as high as 40% for PR, 36% for ER and 20% for HER2. This can have an important impact in therapeutic decisions. Based on these discordances antitumor treatment is changed: in DESTINY trial 14% had modified treatment, in BRITS 17.5% and in ConvertHer 8%. All routine markers in breast cancer can be investigated on adequate FNAC material from metastatic lesions, provided that it is performed under proper standardization and quality assurance.

In the identification of breast primary, markers that aid include ER, GCDFP-15, MGB and more recently GATA3 as a sensitive and superior to others breast-specific available markers. This panel of immunomarkers is used in the work-up of tumors of an unknown primary.

Being minimally invasive, FNAC can be performed repetitively, permitting in this way evaluation of the chronological evolution in expression of tumor biomarkers. This is why it is a serious candidate for the chronological follow-up of neoadjuvant chemotherapy response.

LIMITATIONS
FNAC would not be the first option for investigation of very small lesions < 10 mm and NCB is preferred instead. There is also discussion about its role in non-palpable lesions where the use of US guidance is considered a necessary supplement, as it improves significantly the method’s efficiency. But apart from very small lesions, the use of US guidance as a routine has shown to increase the method’s overall accuracy: in a relevant study FNAC guided by US resulted in the reduction of patients requiring NCB by 87%, thus US guidance is recommended.

FNAC is not indicated when microcalcifications are present in the radiological assessment. Microcalcifications are technically difficult to sample and due to its nature, inadequate sample is usually aspirated. The major issue when using FNAC in the management of microcalcifications is the unreliability to make definitive diagnosis of benignity. In such cases NCB is indicated. Improved results are achieved with stereotactic guidance and specimen radiography to confirm the presence of the representative calcification.

FNAC as a cytological method is limited by the lack of full histological features and thus does not offer hard evidence in regards of the basement membrane invasion in cases of microinvasive cancer. Differentiating ductal carcinoma in situ (DCIS) from invasive disease is of great importance. FNAC cannot reliably distinguish between them.

Several studies have reported cytological criteria to predict invasion. These involve infiltration in fragments of fat and fibrous tissue, proliferation of fibroblasts and elastoid stromal fragments. The overall sensitivity and specificity of these criteria are low. 38% of invasive carcinomas are negative for them and 29% of DCIS showed at least one.

On the other hand, even though NCB as a histopathological examination offers information about the tissue architecture it also has its limitations. Its sensitivity in DCIS ranged from 55% to 95%, but more importantly 8 to 44% of DCIS when excised were reported as infiltrating carcinoma as a conclusion NCB cannot reliably rule out invasion in biopsies showing DCIS.

Another area with FNAC limitations are papillary lesions. They range from benign duct papilloma to papillary carcinoma. There are no specific radiologic findings that can differentiate benign and malignant lesions. As a result diagnosis relies mainly on histologic evaluation which can be challenging. Criteria suggested to distinguish benign and malignant papillary lesions are not established, as
the overall accuracy remains poor. Similar issues apply to NCB. According to these findings the excision of all papillary lesions is recommended.

Atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) have a 5- and 11-fold risk, respectively to develop malignancy. Both FNAC and NCB have diagnostic limitations because they may not be able to distinguish between ALH and LCIS on the one hand and invasive lobular carcinoma on the other. Even though there is consensus to close observe LCIS, excision is recommended when it is associated with a mass, calcifications, histologic overlap with DCIS and radiologic-pathologic discordance.

In mucinous lesions, FNAC can diagnose the majority of colloid carcinomas, but aspirates poor in cells, even without atypia, cannot rule out malignancy. Excision of these lesions is preferable.

Hemorrhagic cyst aspirations, particularly in the older patient, should be further investigated as carcinomas. In these cases an image-guided FNA of the cyst wall would be helpful. Cellular fibroadenomas, intramammary lymph nodes, lactational changes and rare tumors such as the adenomyoepithelioma may also be misinterpreted as malignant.

**Comparison to Needle Core Biopsy**

It is not well established if FNAC or NCB is better, more accurate and more efficient in the management of breast lesions. The methods should be considered supplementary, with NCB considered as the second step in investigation of breast lesions when indicated.

The usefulness of FNAC in the current management of breast lesions is questioned. The main argument against FNAC is that NCB offers better results and thus should be used directly as the first step in assessing the pathology of the lesion. Additionally, even though NCB as an individual examination is more expensive than FNAC, as part of an organized health system it is more cost-effective. This is justified by adding the cost of FNAC investigations that will need to be complemented by NCB.

The sensitivity and specificity of FNAC and NCB have been extensively evaluated, partly comparing directly the two methods. A recent review showed a significant amount of heterogeneous results, with differences between centers. NCB had generally consistent accuracy between centers but this was not the case with FNAC. Sensitivity ranged from 35% to 95% for FNAC and 85% to 100% for NCB. Specificity was 48-100% and 86-100%, respectively.

Some studies included in the abovementioned review argue against FNAC because of the high rate of inadequate/inconclusive samples when compared to NCB, but many factors differed significantly between these centers. Indeed when looking at the data from centers with great and continuous experience with FNAC the results are excellent. A study on 1238 cases over 11 years showed sensitivity, specificity, overall accuracy, false negative and false positive results of FNAC for diagnosing breast carcinoma: 97.72%, 99.4%, 97.94%, 2.28%, and 0.6%, respectively. Another study on 229 cases showed overall diagnostic accuracy 98.9%, specificity and sensitivity 99.3 and 96.7%, respectively. Overall positive predictive values and negative predictive values were 99.3 and 96.7%.

FNAC is more operator dependent than NCB and significantly subjected to the skills of the operator, making proper experience and skill training important. This reduces the incidence of inadequate and improper samples. Inexperienced physicians performing FNAC missed 25% of cancers in contrary to experienced and trained ones that missed only 2%. Sampling error was identified as the cause of this difference. Further the cytopathologist must have adequate training and significant experience in interpreting the sample properly, as this results in reduction of inconclusive samples.

It should be noted that NCB is more painful and has more frequent complications as hematoma, bruising, and, very rare, pneumothorax. It takes longer as a procedure, is slower in obtaining results, requires more equipment and is more expensive. Also it has its own limitations, as it is technically difficult to perform for lesions close to the chest wall, or near the areola area and cannot easily sample lymph nodes.

Despite this, it remains the examination of choice when FNAC cannot give a diagnosis. After a C1, C3, C4 result with FNAC, a repeat one should not be attempted, but instead NCB should follow, as also when there is an obvious differentiation between clinical/radiological and FNAC findings.

**Conclusions**

FNAC has been and still is challenged about its role in modern management of breast lesions. Guidelines today mandate that breast lesions should be managed with a triple assessment system consisting of clinical, radiological and pathological evaluation. A significant amount of specialists agree that FNAC should be the examination of first choice in mat-
ters of pathological evaluation.² It is an easily performed, patient-friendly and cheap examination. It can successfully and quite quickly filter out benign breast conditions without any further cost or stress to the patient. In cases with inconclusive results it should be followed by NCB that will provide further information. In the cases where malignancy is proven and when the other pillars of the triple system are in concordance final treatment can be planned. Modern developments in LBC upgrade its role in neoadjuvant chemotherapy too. But it should not be forgotten that in order to have satisfactory results, high levels of experience and training are required on both sides: the clinician acquiring the sample and the cytopathologist analyzing it. More essentially the two of them should work closely as a multidisciplinary team and FNAC should not be considered as an examination performed by an individual physician.

ACKNOWLEDGEMENTS

The authors declare no conflict of interest.

REFERENCES


21. Markman M. Breast cancer and HER2: Over-


Клиническое значение тонкоигольной аспирационной биопсии при исследовании больных с поражением молочной железы

Константинос Динас1, Георгиос К. Пратилас1, Мария Насиуцики1, Елефтериос Вавулидис1, Василиес Маркс1, Панайотис Д. Луфопулос2, Матиас Калдер3

1 Вторая кафедра акушерства и гинекологии, Университет Аристотеля, Больница „Гипократ”, Салоники, Греция
2 Первая кафедра общей хирургии, Медицинский факультет, Университет Аристотеля, Салоники, Больница „Папагеоргиу”, Салоники, Греция
3 Кафедра акушерства и гинекологии, Марбургский университет имени Филиппа, Марбург, Германия

Адрес для корреспонденции: Георгиос К. Пратилас, ул., Константинополес* № 49, 54642 Салоники, Греция
E-mail: georgepratilas978@gmail.com
Тел: +306945542878

Дата получения: 21 сентября 2017
Дата приемки: 12 ноября 2017
Дата интернет публикации: 02 января 2018

Введение: Цитология тонкоигольной аспирационной биопсии (ЦТИАБ) была и остаётся спорным методом в лечении поражений молочной желез. Стандарт в настоящее время требует, чтобы контроль над поражениями молочной железы выполнялся с помощью тройной системы оценки, состоящей из клинической, рентгенологической и патологической оценки.

Цель: В этой статье делается попытка уточнить, может ли ЦТИАБ играть роль инструмента в этой оценке.

Материалы и методы: Мы осуществили поиск в системе PubMed статей о перспективных, ретроспективных и обзорных клинических применениях ЦТИАБ.
Дата публикации: 28 сентября 2018

Ключевые слова: цитология, ТИА, толстоигольная режущая биопсия (ТИРБ), заболевания молочной железы, доброкачественный, злокачественный, цитологическое исследование (Liquid based cytology- LBC), метилирование


Результаты: Специалисты подтверждают, и есть данные о том, что ЦТИАБ может быть первым предпочтительным исследованием при патологической оценке.

Выводы: Данное исследование осуществляется легко, является щадящим для больного и недорогим. Оно может успешно и достаточно быстро фильтровать доброкачественные состояния молочной железы без дополнительного стресса или расходов для больных. В случае неубедительных результатов или неадекватных образцов, за ним следует толстоигольная режущая биопсия (ТИРБ). Она имеет ограничения как метод. Она не может предоставить непосредственную информацию об устройстве ткани и идентифицировать определённые состояния молочной железы. Современное развитие цитологического исследования также способствует его роли в неоадъювантной химиотерапии. Для установления удовлетворительных результатов необходим большой опыт.