Analysis of Inconclusive Breast FNA by Triple Test

I. Mansoor,I. Zahrani (Department of Histopathology, King Abdul Aziz University Hospital, Jeddah, Saudia Arabia.)

Abstract

Objective: To evaluate the efficiency of triple test for non-diagnostic (inconclusive and inadequate) FNAs of palpable breast lesions and to compare our results with the literature.
Methods:A retrospective analysis was done on cases that were having cyto-histological correlation with clinical and radiological follow up. Out of 446 FNAs performed at our institute during the period of 15 years, only 16 cases were discovered fulfilling the above criteria and these were reviewed blindly and scored. The triple test was applied to each case. Each component of the triple test was assigned 1, 2, or 3 points for a benign, suspicious, or malignant result, respectively, yielding a total triple test score (TTS) of these 16 cases.
Results: Three out of 16 specimens were unsatisfactory and rest 13 cases inconclusive (i.e., suspicious) cytologically, diagnosed as having scant atypical cells. All malignant caSes scored above 6 except one case that scored 5. The two benign cases scored I and 3. On histological follow up 2 of the unsatisfactory cases turned out as fibroadenoma (scored I and 3) and one as malignant (scored 5). Among inconclusive cases all turned out to be malignant on histological follow up (all scored 6 and above). High clinical suspicion was present in all 14 cases that were proved in histological follow up. Radiological diagnosis of malignancy was present in 11 of 14 malignant cases, suspicious in two malignant cases and in I of 2 fibroadenoma cases. All cases underwent surgical removal of the mass as a result of clinical or radiological suspicion.
Conclusion: Triple test can reliably guide the evaluation and treatment of palpable breast masses. Masses that score 6 points or higher are malignant and should undergo definitive therapy; masses that score 4 points or lower are benign and may be clinically followed up. Only those masses that score 5 points require open biopsy. Our results were in concordance with other lager studies published in the literature. We recommend utilizing the three diagnostic parameters of cytology, clinical findings and radiology, the triple test, to achieve the best diagnostic accuracy in breast FNAs and patient management (JPMA 52:25, 2002).

Introduction

Fine needle aspiration (FNA) of palpable breast lesion is a fast and cost effective method that can be done as an office procedure, requires little special equipment, causes minimal morbidity and has excellent patient acceptance[1-8]. Many investigators have reported FNA to diagnose breast masses[7,8] as an accepted diagnostic method5. However, the fine needle aspiration has some unavoidable limitations. The false negative rate, a limitation of FNA cytology, has become the center of focus in litigation cases, accounting for 10% of all pathology-related malpractice claims[9]. Many of these cases have been the direct result of inadequate specimen collection, often related to misdirected sampling of the lesions and

occasionally to mis-interpretation of the cells present. Some acellular breast FNA specimens from a fibrous nodule or an adipose mass are unavoidable due to the nature of these lesions. Definition of specimen adequacy is currently one of the most controversial issues regarding of breast lesions due to the complexity of these breast nodules. Several studies have addressed the issue of spec-imen adequacy; however, opinions vary between different authors and there are no clearly defined criteria. In order to consider a breast FNA specimen adequate, Layfield et al proposed a "cutpoint" of at least six clusters of epithelial cells per case composed of at least five cells each, or the presence of a prominent number of bipolar cells[10]. Others require similar criteria i.e., the presence of at least four to six epithelial cell groups composed of 5-10 cells per slide or case[11-15]. For some authors, diagnostic adequacy is not based on quantitating the number of epithelial cells or cell clusters present, but is a combination of factors. These factors include history, clinical assessment and radiological findings in addition to microscopic evaluatio[16-18]. Nevertheless, cytologic criteria, such as cellularity and other malignant criteria are used to minimize the rate of false negative cases. Some studies have discussed many factors contributing to non-diagnostic specimens and the false negative rate in non-palpable and palpable breast lesions[12]. These shortcomings of FNA can be reduced if we take into account the clinical and radiological findings as well, making it a triple test. The "triple testt' for palpable breast lesions consists of physical examination, mammography and fine-needle aspiration. There are many studies in literature that have shown that the triple test was 100% accurate in the diagnosis of palpable breast lesions when all three elements were concordant[19-25]. However, Fine-needle aspiration is the most reliable element of the triple test in cases where the elements of the test were nonconcordant. Many of these studies originated in effective diagnostic expert laboratories with experienced cytopathologists in Europe and no such study has been reported from this region. Although number of cases in our study is much lower but intention is to open a way for other larger studies from our region.

Material and Methods

Our study group consisted of 16 cases of female breast lesions. These were derived from four hundred and forty-six FNAs of the breast performed as a preoperative screening and diagnostic test at King Abdulaziz University Hospital, during the time frame of 16 years (January 1984 to December 1999). Selection criterion that led to these 16 cases, specified that the patients having inconclusive or inadequate FNA, had a subsequent excisional biopsy with clinical and radiological follow up. All the smears for these cases, stained with Geimsa stain, H&E and Papanicolaous stain were reviewed blindly and cytologically scored. Medical records of these 16 patients were reviewed and the clinical history and examination findings, results of mammography and tissue biopsy were obtained and all these three parameters i.e., cytology, clinical and radiological findings were assigned 1, 2, or 3 points for a benign, suspicious, or malignant result, respectively, yielding a total triple test score. The three unsatisfactory (inadequate) cases were reviewed and quantified for number and size of epithelial cell groups. In assessing the specimen adequacy, we used criteria similar to those established by Layfield et al10, requiring the presence of a minimum of six clusters of epithelial cells, each cluster composed of at least five cells. The original non-diagnostic cytological results, reviewed

cytological results, histological results, clinical suspicion of malignancy and radiological findings are all compared in Table for these 16 cases.

Results

A retrospective study was performed evaluating the efficiency of triple test for non-diagnostic (inconclusive and inadequate) FNAs of palpable breast lesions that were having cyto-histological correlation, clinical and radiological follow up. Sixteen cases were found fulfilling the selection criterion. The triple test was applied to each case. Three out 16 specimens were unsatisfactory (Inadequate) and rest 13 cases were inconclusive (i.e. suspicious) cytologically, diagnosed as having scant atypical cells. All malignant cases scored above 6 except one case that scored 5. The two benign cases scored 1 and 3. On histological follow up 2 of the unsatisfactory cases came out as fibroadenoma (scored I and 3) and one as malignant (scored 5). Among inconclusive cases all came out to be malignant on histological follow up (all scored 6 and above). High clinical suspicion was present in all 14 cases that were proved malignant in histological follow up. Radiological diagnosis of malignancy was present in 11 of 14 malignant cases and was suspicious in two malignant cases and in 1 of 2 fibroadenoma cases. All cases underwent surgical removal of the mass as a result of clinical or radiological suspicion (Table).

Table . Summarized result of analysis of inconclusive breast FNA by triple test scores and cytological score.

Serial No.	Hospital NO.	loss of cell adhesion	High cellularity	Striped bipolar	Increase cell size	Pleomorphism	Variable nuclear chromatin and	Lymphocyte response	Single cells with intact cytoplasm	Irregular angulated atypical cells	Necrosis	Signet ring cells	Single cells without cytoplasm	Overcrowded 3D clusters	Apocrine cells	Stromal element	Cytological Dx.	Clinical Diagnosis	Radiological Diagnosis	Triple Test Score	Histopathological Diagnosis
1	1662/86	+	-	-	-	+	-	-	-	-	-	-	-	++	-	-	Atypical cells	+++	+++	8	Comedo Ca.
2	1460/87	++	+	-	++	+++	+	-	-	+	-	-	++	++	-	-	Suspecious	+++	+++	8	Inf. Ductal Ca.
3	2427/87	+	+	-	+	+	-	-	+	+	-	-	+	++	-	-	Suspecious	+++	+++	8	Inf. Ductal Ca.
4	2075/90	++	+++	-	+	++	+++	-	++	+	-	-	++	+	-	-	Atypical Cell	+++	+++	8	Undifferentiated Ca.
5	25/91	++	+++	-	++	++	++	-	++	++	-	-	++	++	-	-	Suspecious	+++	+++	8	Inf. Ductal Ca.
6	83/91	++	+++	-	+	++	+	-	++	++	-	-	+	++	-	++	Suspecious	+++	+++	8	Inf. Ductal Ca.
7	19/94	++	++	-	++	+	-	+	+	-	-	-	+	+	-	++	Atypical cells	+++	+++	8	Inf. Ductal Ca.
8	126/94	++	++	-	++	++	+++	++	++	++	-	-	++	+	-	-	Suspecious	+++	+++	8	Inf. Ductal Ca.
9	41/97	+	++	-	++	+	-	-	++	++		-	++	++	-	-	Suspecious	+++	+++	8	Inf. Ductal Ca.
10	103/97	++	+	-	++	++	+	-	++	++		-	+	-	+	++	Suspecious	+++	+++	8	Inf. Ductal Ca.
11	112/97	+++	++	-	++	+++	++	-	+++	++	+	++	++	+	-	-	Suspecious	+++	+++	8	Inf. Lobular Ca.
12	0003/99	++	+	-	++	+	+	-	+	+	+	-	+	+	-	-	Suspecious	+++	++	6	Inf. Ductal Ca.
13	155/99	+++	+++	-	++	+	+	-	+++	+	-	-	+	+++	-	-	Suspecious	+++	++	6	Inf. Ductal Ca.
14	137/91	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	Unsatisfactor	+++	-	5	Inf. Ductal Ca.
15	1746/90	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Unsatisfactor	-	++	3	Fibroadenoma
16	1489/90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Unsatisfactor	-	-	1	Fibroadenoma

+=mild, ++=moderate, +++=abundant. Clinical and radiological diagnosis:

Discussion

The “triple test” for palpable breast lesions consists of physical examination, mammography and fine-needle aspiration has cumulative high accuracy rate over the three individual tests[19-25]. But the Fine-needle aspiration is the most reliable element of the triple test with negative predictive value and sensitivity of range 95-99% and 96-98%, respectively as reported in literature[7,8]. However, the fine needle aspiration does have some unavoidable limitations. The false negative rate, a limitation of FNA cytology, has become the center of focus in litigation cases, accounting for 10% of all pathology-

related malpractice claims[9]. Major factors contributing to a false negative cytologic diagnosis include inadequate cellularity, that may be due to mis-directed sampling and misinterpretation of cells. Of primary importance is obtaining an adequate specimen having sufficient cells to represent the true picture. However, specific cytologic criteria for FNA specimens that qualify' as adequate have not been clearly defined for nonpalpable or palpable breast lesions[19]. Should adequacy be based on the number of epithelial cell groups, the number of aspirates during FNA procedures or a specific diagnosis that may match the clinical fmdings? Should all specimens that lack epithelial component be considered nondiagnostic? Failure to obtain a representative sample is the major cause of false negative diagnoses, while misinterpretation of the smear accounts for the majority of false positives[26].

Various causes of inadequate aspirates and unsatisfactory smears are, aspirators insufficient needle maneuvers, needle withdrawal under negative pressure, lack of forcible specimen ejection onto the slide and less than optimal fixation methods, leading to unsatisfactory specimens". However, some inadequate aspirates are unavoidable even for the most skilled operators. Reports indi-cate a median nondiagnostic rate of 34% (range 3-80%) for breast FNA samples, with more than 50% of these aspirates representing a fibrous nodule or lipoma of the breast[27]. No epithelial clusters will be aspirated from fibrotic or fatty breast tissue regardless of the number of FNAs performed. In our previous study the unsatisfactory rate was 4%, inconclusive rate was 18% and overall nondiagnostic rate 21.6%. The three of our unsatisfactory cases were histologically 2-fibroadenoma and 1-ductal carcinoma respectively.

On the other 13 inconclusive (cytologically suspicious) cases, blind cytological analysis was done and each case was scored by a single pathologist for different malignant cytological diagnostic criteria into + ,++, +++, to define the major cause of inconclusive diagnosis. Later all these cases were compared with their clinical and radiological findings to determine the overall diagnostic accuracy of these three parameters.

On cytological review it was seen that in all the inconclusive cases the criteria favoring malignancy were present but in variable degrees. However it seems that low cellularity (- in 1, + in 4, ++ in 4 and +++ in 4), very mild pleomorphism (+ in 5, ++ in 5 and +++ in 2), lack of prominent nucleoli and little cell adhesions (+ in 3, ++ in 8 and +++ in 2), are the important criteria responsible for limiting the frank diagnosis of malignancy. (Table 1). The pathologist should consider each single criterion individually and grade them. If over all picture is having more malignant criteria positive then the diagnosis of malignancy should be considered.

Of the 16 cases we analyzed there were 14 malignant and two benign cases proved on histological diagnosis. Among the malignant cases there were 10 cases of invasive ductal carcinoma, one of comedo carcinoma, one of lobular carcinoma and one of undifferentiated carcinoma. Among the benign cases both were fibroadenoma. On analyzing the clinical records it was found that high clinical suspicion was present in all 14 malignant cases. So clinical suspicion was truly positive in 100% of cases. On mammography malignancy was diagnosed in 11 out of 14 malignant cases. Suspicion for malignancy was present in 3 cases. All these 11 radiologically diagnostic and two out of three radiologically suspicious cases were true positive, as they finally proved malignant on histological examination. Of the 3 malignant cases not picked up on radiological examination, one was lobular carcinoma, 1 comedocarcinoma and one invasive ductal

carcinoma. So radiological suspicion was true positive in 78.5% and falsely negative in 21.4% cases. All benign cases were negative on radiological examination. Now if we analyze the three parameters together, we will observe that atleast two parameters were truly positive in all proved malignant lesions and similarly atleast two parameters were truly negative in the benign lesions.

On the basis of above evidence a cytopathologist viewing a breast fine needle aspirate may make a diagnosis based on only the microscopic findings or may take assistance from clinical and radiologic findings into consideration, especially if the same physician has taken the FNA or when clinical impression has been mentioned by the aspirator on the FNA request. Implementing the triple test thus will increase the sensitivity and specificity of the final diagnosis. The triple test can be coordinated by any of the patient's physicians either, the cytopathologist, the clinician coordinating the patient's case or the collaboration of both physicians. If there is a discrepancy between any of these results, we recommend a surgical biopsy and clinical follow-up. FNA performed by well-trained, highly experienced physicians and in combination with the triple test will achieve the most accurate results in the diagnosis of palpable breast lesions.

A lot of studies have discussed the high accuracy and cost effectiveness of the triple test in management of breast lesions[19-25]. Veto et al showed that the triple test was 100% accurate in the diagnosis of palpable breast lesions when all three, elements were concordant[24]. Cost analysis revealed that elimination of confirmatory open biopsy in such cases and also in cases where the fine-needle aspiration and an other element of the test had a suspicious or malignant result, could yield an average per-case cost savings of up to $1,412 compared to triple test followed by routine confirmatory open biopsy[24]. Triple test scoring can also help in evaluating suspicious cases, according to Morris et al., masses that score 6 points or higher are malignant and should undergo defmitive therapy; masses that score 4 points or lower are benign and may be clinically followed up. Only those masses that score 5 points require open biopsy[20].

Another large study by Schuhmann et a!. analyzed 608 malignant and 224 benign cases by triple test to fmd out whether the triple diagnostic test, can replace surgical biopsy and thereby reduce the number of unnecessary biopsies[23]. In his study all lesions triple-diagnosed as malignant were histologically proved to be malignant, i.e., there were no false positive results. The rate of false negative results was found to be within the range reported for false negative results in fresh frozen sections. Based on these results he stated that the dogmatic statement "every palpable mass in the breast must be excised" should be replaced by the recommendation "every palpable mass must be assessed and clarified". A great number of retrospectively unnecessary biopsies can be avoided by a systematic use of the triple diagnosis. The diagnostic safety of this method is close to that of open biopsy. In all cases where positive or negative concordant triplets are found, histological confirmation by biopsy can be avoided. Patients with benign lesions can be thoroughly followed up by repeated physical and radiological examinations. Patients with triple diagnostic malignant results can be adequately treated. Lesions for which triple diagnosis yields neither benign nor malignant, must be biopsied. This is also necessary in all cases with suspicious findings in mammography without a palpable mass, if the equipment for stereotactic or ultrasound-guided biopsies is not available[23].

Now a new modification in this test has been introduced called Modified Triple Test (MTT: physical examination, ultrasonography instead of mammography and fine-needle

aspiration) that has appeared to be more affective and accurate in young breast lesions[21]. In conclusion we recommend utilizing the three diagnostic parameters of cytology, clinical findings and radiology, the triple test, to achieve the best diagnostic accuracy in breast FNAs and patient management.

References

1.Al-Kaisi N. The spectrum of the "gray zone" in breast cytology: a review of 186 cases of atypical and suspicious cytology. Acta Cytol., I 994;38:898-908.
2.Alvarez PLF, Velasco JRR, Heros CA, Zapatero H. La puncion-aspiracion con aguja finade Ia mama coma tecnica diagnostica preoperatoria: evaluacion del metodo y revison de Ia literatura. Rev. Clin. Esp., 1987;181 :480-5.
3.Atamdede Fl, Isaacs SF1. The role of fine needle aspiration in the diagnosis of breast lesions, Gynecol. Oncol., 1993;50:159-63.
4.Barrows OH, Anderson TJ, Lamb JL, Dixon JM: Fine-needle aspiration of breast cancer: relationship of clinical factors to cytology results in 689 primary malignancies. Cancer, 1986;58:1493-8.
5.Linsk JA, Franzen S (eds.). Breast aspiration in clinical aspiration cytology. Philadelphia: Lippincott, 1983,
6.Maygarden SJ, Novotny DB, Johnson DE, Frable WJ. Subclassiflcation of benign breast disease by fine needle aspiration cytology: comparison of cytologic and histologic findings in 265 palpable breast masses. Acta CytoL, I 994;38: 115-29.
7.Norton LW, Davis JR, Wiews JL, et al. Accuracy of aspiration cytology in detecting breast cancer. Surgery, l984;96':806-14.
8.Robbins OF. Brothers JH, Eberhart WF. Is aspiration biopsy of breast cancer dangerous to the patient? Cancer, 1974;7:774-8.
9.Troxel DB, Sabella JD. Problem areas in pathology practice uncovered by a review of malpractice claims. Am, J. Surg. Pathol., 1994:18:821-31.
10.Layfield U, Mooney EE, Glasgcow B, Hirschowitz SH, Coogan A. What constitutes an adequate smear in fine needle aspiration cytology of the breast? Cancer Cytol., 1997:81:16-21.
11.Kline IS. Adequacy and aspirates from the breast: a philo-sophical approach. Diagn. Cytopathol., 1995; 13:470-2.
12.Moriarty A. Fine-needle biopsy of breast when is enough, enough? Diagn Cytopathol., 1995:13:373-4.
13.Scopa CD, Koukouras D, Androulakis J, Bonikos D: Sources of diagnosti discrepancies in fine needle aspiration of the breast. Diagn. Cytopathol. 1991:7:546-8.
14.Sneige N, Staerkel GA, Caraway NP, et al A plea for uniform terminology and reporting of breast fine needle aspirates: the M. D. Anderson Cancer Center proposal. Acta Cytol., 1994:38:971-2.
15.Sneige N. Should specimen adequacy be determined by the opinion of the aspirator or by the cells on the slides? Cancer Cytopathol.,1997:81 :3-5.
16.Layfield LJ. Glascow BJ, Cramer H: Fine needle aspiration in the management of breast masses. Pathol. Ann., 1989:24:43-62.
17.National Cancer Institute. The uniform approach to breast fine needle aspiration biopsy: a synopsis; developed and approved at a National Cancer Institute-sponsored

conference, Bethesda, Maryland, September 9 and 10. Acta. Cytol., 1996:40:1120-26.
18.Stanley MW, Abele 3, Kline T, et al. What constitutes adequate sampling of palpable breast lesions that appear benign by clinical and mammographic criteria? Diagn. Cytopathol., 1995:13:473-87.
19.Salami N, Hirschowitz SU, Nieberg RK, Apple SK . Triple test approach to inadequate fine needle aspiration biopsies of palpable breast lesions. Acta. Cytol., 1999;43:339-43.
20.Morris A, Pommier RF, Schmidt WA, et al . Accurate evaluation of palpable breast masses by the triple test score. Arch Surg., 1998;133:930-4.
21.Vetto iT, Pommier RF, Schmidt WA, et at. Diagnosis of palpable breast lesions in younger women by the modified triple test is accurate and cost-effective. Arch Surg., 1996 ; 131:967-72;
22.Steinberg JL, Trudeau ME, Ryder DE, et at. Combined fine-needle aspiration, physical examination and mammography in the diagnosis of palpable breast masses: their relation to outcome for women with primary breast cancer. Can. J. Surg., 1996;39:302-l I.
23.Schuhmann R, Hubner F, Brose C, et al. The value of aspiration cytology within the scope of triple diagnosis of palpable breast changes. Geburtshilfe Frauenheilkd., 1 995;55:553-8.
24.Vetto 3, Pommier R, Schmidt W, et at. Use of the “triple test” for palpable breast lesions yields high diagnostic accuracy and cost savings. Am. J. Surg., 1995;1 69:519-22.
25.Johanson A, Sager EM. Local recurrence of breast cancer after breast preserving surgery: experiences with a triple test as a routine control. Tidsskr Nor Laegeforen., I 992;1 12:760-2.
26.Silverman JF: Breast. In: Bibbo M. (ed). Comprehensive Cytopathology. Philadelphia, WB Saunders, 1991, pp.703-7.
27.Pisano ED, Tsimikas J, Fajardo LL, et at. RDOG-5: Stereotactic fine needle aspiration (FNA) and core needle biopsy (CNB) in the work-up of lesions detected by mammography: Insufficient sample rate across multiple centers in a nationwide trial. American Society of Cytopathology. American College of Radiology National Breast Cancer Conference, Dallas, April 29,1996.