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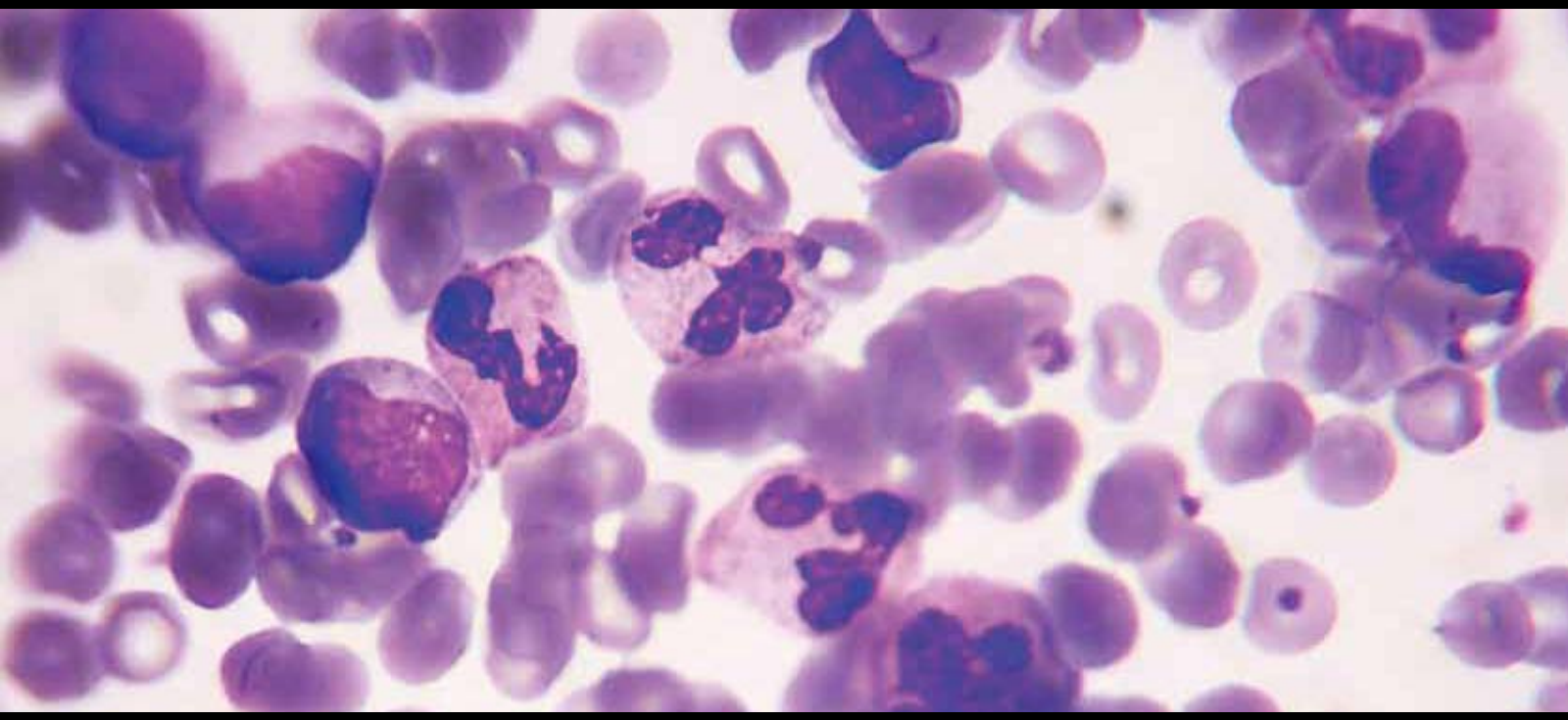
Fine-Needle Aspiration Cytology: An Advancing Horizon

Guest Editors: Darshana Jhala, Aileen Wee, Gary Tse, and Zubair Baloch



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Editorial Board

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Editorial

Fine-Needle Aspiration Cytology: An Advancing Horizon

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The current practice of "Fine Needle Aspiration Cytology (FNAC) has established itself as an important modality in the diagnosis and management of superficial and deep seated lesions throughout the body. With this wide employment of FNAC, the cytopathologists play a pivotal role in the management and therapeutic decisions that are based on the diagnosis of these limited FNAC samples. Furthermore, the use of FNAC samples for biomarker research is advancing rapidly and is being widely investigated and applied for the treatment and prognostic purposes.

In this special issue, we have made an attempt to provide a "flavor" of the current practice of FNAC with an emphasis on correlation of tissue biopsies with PET results, FNAC of breast, liver, pulmonary, and head and neck lesions. Similarly, the use of FNAC in the diagnosis of Langerhans cell histiocytosis, neuritic leprosy, and granulomatous inflammation is also discussed.

With the increasing use of image-assisted FNAC, pathologists are now an integral part in the diagnosis and management of the deep seated lesions. In lieu of this, it has become important for the pathologists to be aware of the limitations of different imaging techniques. The paper on the correlation of tissue biopsies with PET results discusses the limitations of an increased SUV value on PET scan.

The use of fine needle aspiration cytology has been proven to give fast, economical, and valuable diagnosis of palpable breast lumps. In this issue, the role of FNAC in the evaluation of breast lump in a high patient volume center is addressed with emphasis on the importance of skill and training for both pathologists and technicians to prevent suboptimal sampling, thus, increasing the reliability of the procedure. Another important but not commonly discussed aspect of breast FNAC—the nonmalignant categories—is also addressed, with a review of the cytomorphology of benign breast lumps, some of which could be mistaken for malignancy due to the diaphanous appearance and overlapping cytologic features. The false negative and false positive FNAC is further discussed in detail so as to avert misinterpretation. These provide practical information for readers when dealing with FNAC of breast lesions.

The paper on liver FNAC covers various aspects and discusses the role of FNAC in liver lesions. There is an active debate about the preoperative/pretransplantation diagnostic role of FNAC of hepatocellular carcinoma (HCC) and precursor lesions, especially in the face of advances in dynamic imaging techniques. New trends in personalized molecular targeted therapy require better characterization and prediction of HCC behavior. FNAC biopsy technique is still the most minimally invasive approach for the procurement of tumor and peritumoral tissue for molecular studies. Thus, in the near future, hepatic FNAC is likely to become a point of care in the management of HCC patients, especially inoperable cases.

In the current era of personalized medicine, the FNAC diagnosis of nonsmall cell carcinoma for a pulmonary nodule is no longer considered an adequate diagnosis. Pathologists are often required to further subclassify these in to adenocarcinoma and squamous cell carcinoma. With the increasing use of image-assisted FNAC including endobronchial

ultrasound guided FNAC (EBUS-FNA), cytologic or small biopsy material has become the only form of tissue available for diagnosis. The paper on FNAC of pulmonary lesions reviews the current concepts in the suitability and accuracy of FNAC in lung cancers including diagnosis, classification, use of ancillary techniques, and prognostic marker assessment.

FNAC is a valuable technique in the workup of nodules and masses arising within the head and neck region. It is primarily utilized to confirm or exclude the diagnosis of malignancy involving head and neck organs especially lymph nodes, thyroid, and salivary glands. It has been shown that FNAC of salivary gland lesions is a valuable way to preoperatively assess lesional tissue, determine the need for surgical intervention, and assist in planning the appropriate surgical approach prior to resection. In this issue, the manuscript on cytologic diagnosis of mucoepidermoid carcinoma discusses the role of FNAC in the diagnosis of mucoepidermoid carcinoma (MEC), the common malignant tumor affecting parotid gland. In addition, it also brings forth how the rare and recently described oncocytic variant can pose problems in the diagnosis of MEC.

In conclusion, this special issue includes a potpourri of topics which provides a thoughtful glimpse into various techniques, diagnostic ability, and limitations of the current practice of FNAC.

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Research Article

Correlation of Tissue Biopsy and Fine Needle Aspiration Cytology with Positron Emission Tomography Results

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F-18-fluorodeoxyglucose (FDG) Positron Emission Tomography (PET) scans are positive in any condition which increases metabolism in a mass or tissue and are therefore not specific for neoplastic conditions. The use of an SUV cutoff value of 2.5 may not always help discriminate between benign and malignant cases. For a practicing cytopathologist doing adequacy checks during an image-guided procedure, it may be of value to be aware that elevated SUV values are not always indicative of a malignant process, and vice versa.

1. Introduction

Positron Emission Tomography (PET) is a form of nuclear medicine technology that measures bodily functions, such as blood flow, oxygen use, and glucose metabolism. The procedure utilizes a radioactive "tracer" substance, which is typically injected into the bloodstream. This radioactive material accumulates in organs and decays by the emission of gamma rays. These are captured by a PET scanner, and, with the aid of a computer, an image is generated. Unlike other imaging modalities, PET studies are less directed toward depicting anatomy and structure and more concerned with depicting physiologic processes [1]. This includes the rates of glycolytic metabolism or levels of other various chemical activities that are often high in malignancy.

A commonly used tracer substance is the glucose analogue, F-18-fluorodeoxyglucose (FDG). Areas of greater intensity, called "hot spots," indicate where large amounts of the radiotracer have accumulated and where there is a high level of glucose hypermetabolism. Less intense areas, or "cold spots," indicate a smaller concentration of radiotracer. FDG utilization can be used to semiquantify metabolic activity via the generation of "standardized uptake values," or SUVs [2]. These "hot" and "cold" spots correlate to higher or lower SUVs, respectively.

PET has found wide-spread application in the field of oncology, where it is used in the differential diagnosis, staging and therapy monitoring of oncologic disease [3]. However, there are limitations to the procedure. Body positioning, as well as movement during the procedure, has been shown to affect the results [4]. Additionally, altered metabolic rates or chemical balances may yield false results. A PET scan may be positive in any condition that results in the elevated metabolism of a mass or tissue. This could include inflammatory states, as well as other benign processes [5]. Therefore, PET is not specific for neoplastic states. If a lesion is identified by a PET scan, it may need to undergo a biopsy to determine benign nature versus malignancy. The reported sensitivity and specificity varies greatly among studies, and, in many instances, there is a lack of histologic confirmation. The correlation of tissue diagnoses with PET scan-identified lesions in our institution is unknown. The aim of our study was to evaluate the overall accuracy of positive PET scans at detecting malignant lesions (i.e., the number of positive PET cases confirmed malignant by tissue diagnosis).

Table 1: Type of diagnostic procedure.

Procedure	Total
Biopsy	30
TBNA	30
CT FNA	22
Superficial FNA	6
US FNA	6
BRUSH/WASH	1
Total	95

FNA: fine needle aspiration, TBNA: transbronchial needle aspiration, CT FNA: computerized tomography guided FNA, US FNA: ultrasound guided FNA, BRUSH/WASH: bronchial brush/wash done during bronchoscopy.

2. Study Design

We searched the electronic records of Veteran Affairs Medical Center, Houston, Texas to identify patients that had a fine needle aspiration (FNA) or tissue biopsy performed as a consequence of a PET-positive result, over a twenty-four-month period. Cases where biopsy or FNA procedure preceded the PET scan were not included in the study. "PET impression" was defined as a qualitative evaluation of the visually recognized focal area of hypermetabolism. PET impression was divided into four categories: positive, negative, suspicious, and indeterminate. Positive PET impression was defined as a well-defined focus of abnormal FDG uptake, more active than the surrounding tissue, and with an SUV more than 2.5. Areas with no activity or activity less than that of the adjacent tissue were identified as negative by PET impression [6, 7]. Cases in which there was any FDG uptake qualifying as focal hypermetabolism with SUV less than 2.5 or SUV more than 2.5 but visually not focally hypermetabolic were classified as "suspicious" by PET impression. Indeterminate was any uptake or lesion which could not be classified as above. All PET studies were evaluated for PET impression by one Nuclear Medicine Physician for benignancy versus malignancy blinded to the tissue diagnosis. SUV was measured in the focus of hypermetabolism by drawing the region of interest (ROI) for all cases. SUV of 2.5 or greater is reported to be more indicative of malignant rather than benign lesions [8, 9]. Correlation of tissue diagnoses with the PET impression and the standard uptake value (SUV) using 2.5 as a cutoff was performed and the sensitivity and specificity calculated. Cytology or biopsy specimens obtained from PET-negative results were obtained from patients who had a PET-positive lesion with a concurrent positive biopsy of that site. In addition, some of these patients had biopsies of other locations (that were PET negative) for staging purposes. "PET grouped" refers to the analysis of the sum of both PET impression positive and PET impression suspicious groups. Cases with PET inconclusive interpretation were not included in the specificity or sensitivity analysis.

3. Statistical Methods

Basic statistical analysis to calculate percentage, specificity, and sensitivity were done using Microsoft Office Excel 2007.

Table 2: Organ sites where PET scan and tissue diagnosis were performed.

Site	Total
Lung	36
Lymph node	
Lung	23
Neck	5
Paratracheal	2
Axilla	2
Mediastinum	2
Supraclavicular	1
Groin	1
Bone	
Rib	3
Vertebral	1
Parotid gland	2
Skin	2
Chest wall	1
Colon	1
Epiglottis	1
Esophagus	1
Soft tissue	
Gluteal	1
Supraclavicular	1
Kidney	1
Liver	1
Mediastinum	1
Neck mass	1
Orbit	1
Oropharynx	1
Rectum	1
Minor salivary gland	1
Thyroid	1
Total	95

Differences in proportions among SUV value and biopsy results were calculated using Student's t-test. Receiver operating characteristic (ROC) curve was used to evaluate SUV and biopsy-FNA results. An ROC curve is a plot of the true positive fraction (sensitivity) versus the false positive fraction (one minus the specificity). ROC curves were constructed for the whole group of cases and controls. The area under the ROC curve (AUC) was also calculated (Statistica Version 8, Statsoft, Tulsa, OK).

4. Results

A total of 1383 biopsies and FNA cytologies were found in our electronic records, of which 95 had tissue and available corresponding preceding PET scan to be included for the final analysis. Most diagnostic procedures resulted in cytology specimens (from fine needle aspirations, $n = 65$); the type of diagnostic procedure is detailed in Table 1, and the organ location, where PET scan and tissue diagnosis were performed, is depicted in Table 2. These 95 procedures

Table 3: Distribution of cases according to PET impression and Bx/FNA result.

	Biopsy-FNA				
PET impression	Positive	Negative	Nondiagnostic	Inconclusive	Total
Positive	37 (80%)	8 (18%)	1 (2%)	0	46 (49%)
Negative	0 (0%)	24 (100%)	0	0	24 (25%)
Suspicious	9 (43%)	10 (47%)	1 (5%)	1 (5%)	21 (22%)
Inconclusive	2 (50%)	2 (50%)	0	0	4 (4%)
Total	51	41	2	1	95

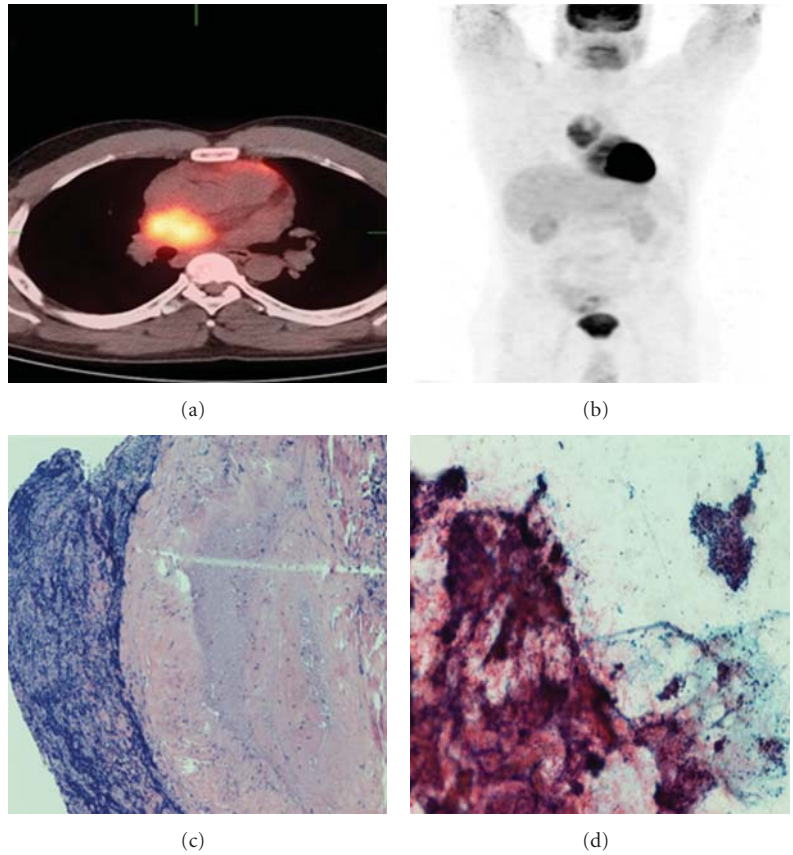

Figure 1: A case showing PET scan diagnosed as "positive for neoplastic process" and a corresponding negative biopsy. (a, b) Intense FDG radiotracer uptake in the mediastinal lymph node. (c) Surgical specimen showing caseating granuloma (H&E 100x). (d) FNA showing absence of malignant cells and clusters of epithelioid cells admixed with lymphocytes and debris (Papanicolaou 100x).

were performed in 54 patients, of which 53 were male and 1 female (this reflects usual demographics of a Veterans Affairs hospital, where most patients are male). The average age was 66.5 years at time of diagnosis (42–88 years old). The average time that elapsed between PET scan and diagnostic procedure was 36 days (0–288 days).

Forty-six (49%) lesions were interpreted as positive on PET scan, of which 37 (80%) were malignant, 8 (18%) benign, and 1 (2%) nondiagnostic on cytology or biopsy. Twenty-four cases (25%) had a negative PET scan, all of which were benign on cytology or biopsy. Twenty-one (22%) lesions were interpreted as suspicious on PET scan, of which 9 (43%) were malignant, 10 (47%) were benign, 1 (5%) rendered nondiagnostic material, and 1 (5%) inconclusive result on cytology or biopsy. A total of 4 cases (4%) were interpreted as inconclusive on PET scan, of which 2 (50%) were diagnosed as positive and 2 (50%) as negative on cytology (Table 3).

PET-positive/FNA-biopsy negative cases were found in 8 procedures performed on 6 cases. These corresponded to 5 lung lesions and 3 lymph nodes. On pathologic exam, these cases showed either no pathologic change (one case), necrotizing granuloma (one case; Figure 1), or chronic inflammatory changes (three cases). In addition, 10 PET suspicious-biopsy/FNA negative cases were identified showing reactive changes, inflammation, aspiration pneumonia, reactive lymphoid hyperplasia, and a villous adenoma.

Table 4: Distribution of the cases according to PET SUV and Biopsy-FNA result.

	Biopsy				
SUV	Positive	Negative	Suspicious	Inconclusive	Total
>2.5	39 (66%)	19 (32%)	0	1 (2%)	58
<2.5	7 (22%)	21 (68%)	3 (10%)	0	31
Not available	5 (83%)	1 (17%)	0	0	6
Total	51	41	2	1	95

Table 5: Clinical characteristics of the 8 biopsy-FNA positive procedures with SUV < 2.5.

Patient	Age	Organ	Procedure	PET impression	SUV	Diagnosis
1	68	Supraclavicular lymph node	US FNA	Inconclusive	1.5	Metastatic adenocarcinoma
2	77	Mediastinal lymph node	TBNA	Suspicious	2.1	Poorly differentiated squamous cell carcinoma
3	77	Lung	TBNA	Suspicious	2.4	Basaloid carcinoma
4	82	Axillary lymph node	CT FNA	Suspicious	2.3	B-cell lymphoma, follicular type
5	42	Neck lymph node	CT FNA	Suspicious	2.1	Small lymphocytic lymphoma
6	55	Paratracheal lymph node	Biopsy	Negative	0	Poorly differentiated squamous cell carcinoma
6		Paratracheal lymph node	Biopsy	Negative	0	Poorly differentiated squamous cell carcinoma
6		Lung	Biopsy	Negative	0	Poorly differentiated squamous cell carcinoma

US FNA: ultrasound guided FNA, TBNA: transbronchial needle aspirate, CT FNA: CT-guided FNA.

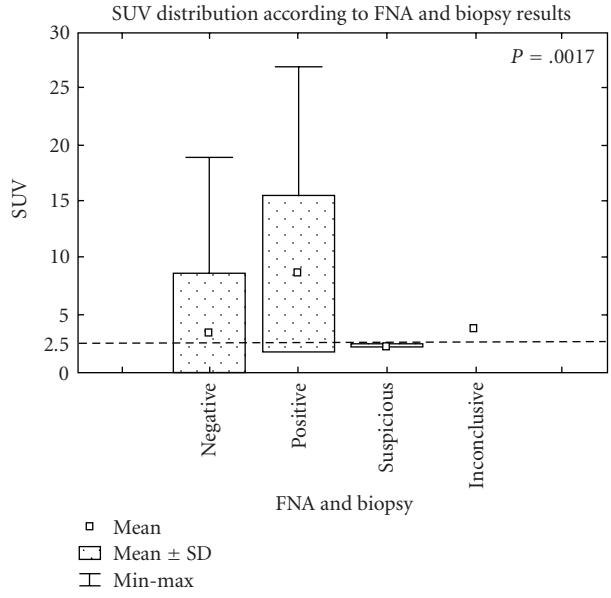

Figure 2: Distribution of SUV according to biopsy result.

The correlation between SUV and biopsy results is shown in Table 4. A total of 19 cases (32%) with SUV > 2.5 had a negative FNA or biopsy result. These cases were diagnosed as negative for malignancy, reactive lymph nodes, villous adenoma, and necrotizing granuloma. Seven procedures (22%) with an SUV < 2.5 with a positive FNA or biopsy corresponded to 6 patients (Table 5). The first case was a metastatic adenocarcinoma consistent with a pancreatic primary. By immunohistochemistry the tumor cells were cytokeratin 7 positive, cytokeratin 20 negative, and TTF-1 negative. The patient was found to have a 7.3 cm pancreatic head mass by imaging studies. The remaining cases included a poorly differentiated squamous cell carcinoma, basaloid carcinoma of the lung, B-cell lymphoma follicular type, small lymphocytic lymphoma, and poorly differentiated squamous cell carcinoma (Table 5).

A box plot showing the SUV distribution according to the biopsy/FNA result is shown in Figure 2. The average SUV for the negative biopsy group was 3.3 (0–18.9, SD: 5.2) and for the positive biopsy group 8.6 (0–26.9, SD: 6.7). There was a significant overlap among negative and positive cases as shown in Figure 2. However, the difference between negative and positive biopsy groups was statistically significant ($P < .0017$). In concordance, the ROC curve shows that an SUV cutoff of 2.5 has a significant discriminatory value (Figure 3). The calculated area under the curve was 82.3%.

The overall sensitivity and specificity for PET impression was 100% and 75%, respectively (Table 6). When biopsy and FNA results are correlated with SUV, the overall sensitivity and specificity were 84% and 52%, respectively (Table 6). Overall, the sensitivity and specificity were higher for PET impression compared to the SUV. When suspicious and inconclusive cases were grouped together with the positive results, the sensitivity dropped, while the specificity remained basically unchanged (Table 6).

5. Discussion

In this study we analyzed the correlation between pathology diagnosis (obtained either by FNA or biopsy) and the corresponding PET scan result. We found that the sensitivity and specificity were higher for PET impression (qualitative interpretation of a PET scan which takes into account visual

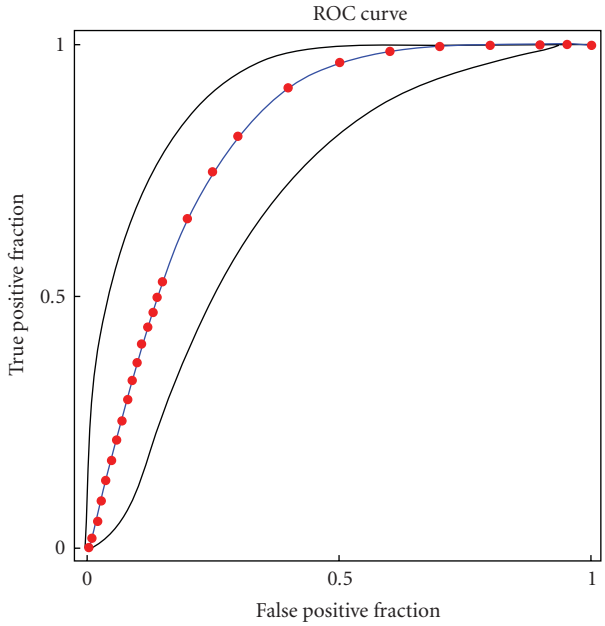

Figure 3: ROC curve.

interpretation of FDG uptake coupled with SUV value) compared to the SUV alone (quantitative measure of FDG tracer uptake with a cutoff value of 2.5) [8, 9].

Currently, the application of Positron Emission Tomography (PET) in the diagnosis, staging, and monitoring of therapeutic response has gained wide acceptance in the field of oncology. Metabolism of the most commonly used tracer, F-18-fluorodeoxyglucose (FDG), can be used to semiquantify metabolic activity in tissues of interest via generation of "standardized uptake values," or SUVs. Standardized uptake values of greater than 2.5 are reported to be more indicative of malignant conditions [8, 9]. However, studies comparing PET impression and SUVs with FNA or biopsy outcomes are sparse. In 2007, Pansare et al. performed a retrospective analysis of PET scan SUV with final FNA results [10]. Using an SUV cutoff of 2.5, their findings showed that, for lesions with an SUV > 2.5, 87% proved to be malignant and 13% benign on tissue diagnosis. Of the lesions with an SUV < 2.5, 54.5% showed benign cytology and 45.5% malignant cytology. The reported sensitivity, specificity, positive predictive values (PPV), and negative predictive value was 84%, 60%, 87%, and 56%, respectively. In comparison to Pansare's findings, the sensitivity and specificity from our study are higher. The different characteristics of the patient population, organ sites, and method of collecting the specimens as well as variation in PET analysis may account for these differences. In particular, the number of true negative and false negative cases may be difficult to obtain. Normally, tissue sites that are negative on PET scan are not biopsied or are biopsied rarely. Therefore, the total number of true negative and false negative cases is difficult to assess and can be variable among studies. In our study, these cases (PET positive/biopsy-FNA negative) were obtained from patients who had a PET-positive lesion

Table 6: Comparison of the sensitivity and specificity for PET and SUV.

	Pet impression	SUV	PET grouped*
Sensitivity	100%	84%	100%
Specificity	75%	52%	57%

* "PET grouped" refers to the analysis of the sum of both PET impression positive and PET impression suspicious groups.

with a concurrent positive biopsy of that site, and in addition these same patients had biopsies of other locations (that were PET negative), for staging purposes. The number of these cases was small and may not accurately reflect the true correlation of PET diagnosis and pathologic diagnosis. Other considerations that may contribute to differences among studies are organ site, type of tumor, size of the lesion, and metabolic state of the tumor cells. For example, the reported sensitivity and specificity PET CT of lung lesions is 96% (range: 83–100%) and 79% (range: 52–100%) [11, 12], for colorectal cancer 97% (95–99%) and 75.6% (64–88%) [13, 14], for Hodgkin lymphomas 84% (71–92%) and 90% (84–94%) [15], non-Hodgkin lymphomas 72% (61–82%) and 100% (97–100%) [15], esophageal tumors 51% (27–93%) and 84% (41.7–95.2%) [16, 17], and head and neck tumors 98% (88–100%) and 92% (75–100%), respectively [18–20]. One of the limitations of our study was the enrollment of patients with known history of cancer. Further studies, including more homogeneous and larger cohort of patients stratified by anatomic site and histologic diagnosis are needed to further characterize and define SUV cutoff values for particular organ system in our patient population.

In our study, we also assessed the correlation between PET impression with the final tissue diagnosis. For lesions diagnosed by PET impression as positive, 80% proved malignant and 18% benign on cytology or biopsy. One case (2%) with a PET-positive impression was signed out as nondiagnostic on cytology. All 24 cases that were diagnosed as negative by PET impression were diagnosed as benign on cytology or biopsy. The overall sensitivity and specificity was 100% and 75%, respectively. In comparison to the results found by Pansare, the sensitivity when utilizing PET impression was roughly the same; however, the specificity appears notably higher (75% versus 60%) [10].

The SUV threshold of 2.5 has been used in most studies to discriminate benign from malignant lesions [21, 22]. However, receiver operation characteristic analysis has shown that a highest diagnostic accuracy can be achieved using SUV thresholds of 4.4 or higher [23–25]. On the other hand, such high threshold would significantly increase the false negative rates and may have suboptimal clinical impact [26]. According to one study, one can omit surgical staging in patients with a PET-negative mediastinum [27]. Furthermore, in our study, an SUV of 2.5 does not seem to segregate positive and negative cases adequately. Even though the ROC curve analysis showed a significant discriminatory value and cases with a negative biopsy result tend to have a significantly lower SUV (mean 3.3) compared to positive

biopsy result (mean 8.2), there was a significant overlap in the overall distribution of the SUV among these two groups as shown on the box plot analysis. Overall, PET impression was more accurate in determining whether a lesion was benign or malignant than the SUV value alone. Some studies have reported the use of different SUV cutoff values to better discriminate benign versus malignant lesion [28]. The use of a single universal SUV cutoff may not always be appropriate.

Regarding false positive results, eight PET-positive/biopsy-FNA negative cases corresponded to 6 patients with 5 lung lesions and 3 lymph nodes. Two cases showed inflammatory changes, and the remaining 6 cases were diagnosed as negative for malignancy. Benign conditions that cause increased glucose uptake can result in elevated SUV [5]. Nonspecific inflammatory lesions in lymph nodes, as well as various infectious etiologies, have been shown to cause SUV elevation which can sometimes be misleading [5], implying a malignant process. Among others, some examples include sarcoidosis, lymph node with follicular hyperplasia, tuberculosis, histoplasmosis, and aspergillosis [5]. The proposed mechanism responsible for this phenomenon is increased glucose uptake by inflammatory cells (e.g., neutrophils and macrophages) within the lesional tissue [5]. This seems a plausible explanation for our false positive PET results.

In our study we did not encounter any false negative PET studies (PET-negative/biopsy-FNA positive cases). Studies have reported the sensitivity of the PET scan ranging from 50% to over 90% while in our study was 100% [29]. In the literature false negative PET scans have been reported in well-differentiated adenocarcinomas, purportedly due to low glucose metabolism and/or low tumor cell density [5]. Examples of low-grade lesions possibly yielding false negative results via PET could include bronchioalveolar lung carcinoma or small lymphocytic lymphoma. It has also been speculated that this false negative result may be due to the presence of necrosis in high-grade lesions [10]. In addition, organ location, histologic tumor type, metabolic status of the patient, and size of the lesions, among others, may also account for the wide range of reported sensitivity [29].

In conclusion, our study indicates that an SUV cutoff value of 2.5 does not always adequately discriminate between malignant and benign processes, as confirmed by follow-up tissue diagnosis. While a negative PET study most likely excludes a malignant process, a positive PET scan may be due to either a malignant process or reactive/inflammatory condition, and therefore it may be useful to undertake further diagnostic attempts (such as FNA) to better define the lesion. For a practicing cytopathologist doing adequacy checks during an image-guided procedure, it may be of value to be aware that elevated SUV values are not always indicative of a malignant process, and vice versa. This, among other factors (such as, but not limited to, cellularity and presence of lesional tissue), may help in determining the number of required passes to get adequate diagnostic material. The observed difference in our findings and other studies highlights the need for additional investigation in this area, especially investigating specific organ systems, specific site, and specific diagnostic categories on a larger number of patients and correlating with PET scan readings.

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Case Report

Diagnosis of Langerhans Cell Histiocytosis on Fine Needle Aspiration Cytology: A Case Report and Review of the Cytology Literature

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A case of multifocal Langerhans cell histiocytosis in a two-year-old child is presented where fine needle aspiration was helpful in achieving a rapid and accurate diagnosis in an appropriate clinical and radiological setting. This can avoid unnecessary biopsy and guide the management especially where access to histopathology is limited. The highly characteristic common and rare cytological features are highlighted with focus on differential diagnoses and causes of pitfalls.

1. Introduction

Langerhans cell histiocytosis (LCH) is a rare disease affecting predominantly children. It can present as a solitary lesion requiring no treatment or as a multisystem, life-threatening disorder necessitating aggressive therapy [1].

We present a case of LCH in a child where fine needle aspiration (FNA) was helpful in establishing a rapid and correct diagnosis in correlation with radiology. The purpose is to highlight common and rare cytological features. This will add to the pathologist's confidence in rendering a rapid and accurate cytologic diagnosis, avoid unnecessary biopsy and guide appropriate management. This is especially valuable in a setting where cytopathologist expertise may not be easily available and histopathology services are located only in big cities and are inaccessible to patients in rural areas due to the long distance and high cost involved.

2. Case Report

A two-year-old female child presented to the outreach centre of our university hospital with swellings on right frontal and occipital regions of skull for the last one year. On examination, these were fluctuant, ill-defined soft tissue masses which measured 2×2 and 3×3 cms, respectively. In addition, a cervical lymph node was palpable on the left side measuring 1×1 cms. It was firm, tender, and slightly mobile. The patient had no fever or loss of weight. The liver and spleen were not palpable.

Peripheral blood film showed microcytic hypochromic anemia. Hemoglobin was 10 gm/dL. Differential count showed 19% monocytosis with 38.3% neutrophils, 38.9% lymphocytes, 2.5% eosinophils, and 1.3% basophils. Platelet count was normal. The initial clinical impression favored a malignant lesion. The patient was referred for FNA.

FNA from lymph node yielded whitish aspirate. FNA from right frontal and occipital masses yielded 0.5 mL and 1 mL hemorrhagic fluid, respectively. The fluid was centrifuged to make smears from the sediment. Ethanol-fixed smears and air-dried smears were prepared and stained with Papanicolaou and Giemsa method, respectively. The remaining sediment was processed to make cell block for immunochemistry.

Smears were highly cellular and showed numerous atypical histiocytes as the predominant cell type scattered singly and in loosely cohesive clusters. These were admixed with a polymorphic population of eosinophils, neutrophils, lymphocytes, plasma cells, foamy histiocytes, and multinucleated reactive histiocytic giant cells (Figure 1). Smears from both the swellings in the skull and cervical lymph node

were morphologically similar except that atypical histiocytes were less in number and eosinophils were more abundant in smears from lymph node as compared to smears from skull lesions.

The atypical histiocytes were large cells with moderate to abundant, pale blue cytoplasm and an eccentric or central round to oval, vesicular nuclei. Prominent nuclear indentations and grooves (with a coffee bean appearance) were observed which were best seen in Papanicolaou stain (Figure 2). Some showed intranuclear pseudoinclusions. Nucleoli were absent. These cells displayed marked pleomorphism with variation in size and shape of cells and nuclei. Occasional mitoses were seen. Some of these cells showed cytoplasmic processes. Most were mononuclear, and some were binucleate or multinucleated. The multinucleated giant cells had complex folded nuclei similar to mononuclear atypical histiocytes and were easily differentiated from reactive multinucleated histiocytic giant cells.

The multinucleated reactive histiocytic giant cells contained numerous indented vesicular nuclei in abundant cytoplasm. They also contained hemosiderin in smears from skull masses (Figure 1). In addition, many rhomboid and needle-shaped Charcot-Leyden crystals were seen both extracellular (Figure 3(a)) and intracellular in the giant cells (Figure 3(b)). The atypical histiocytes stained positive for both cytoplasmic and nuclear S-100 protein. The cytologic findings were highly suggestive of LCH.

At this point, a plain X-ray was requested which showed two lytic lesions corresponding to occipital and frontal swellings. Subsequently, computerized tomogram (CT) with 3D reconstruction showed lytic lesions which were clearly demarcated "punched-out" lesions (the classic geographic skull) in frontal and occipital regions. There was associated homogenous soft tissue swelling of the scalp but no breech of the dura. No other systemic involvement was found.

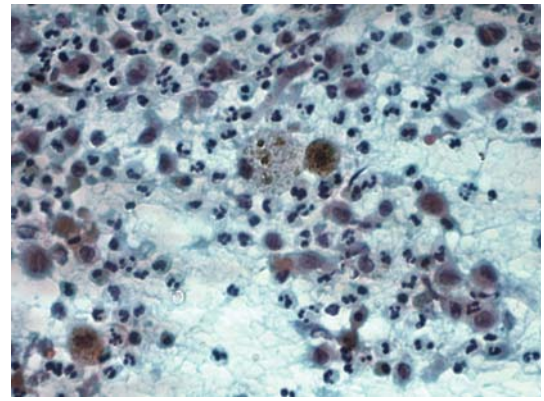


Figure 1: FNA smear from frontal mass showing single and loosely clustered Langerhans cells admixed with neutrophils, lymphocytes and, reactive histiocytes. Two foamy macrophages containing hemosiderin are seen in the centre (Papanicolaou stain, original magnification, ×400).

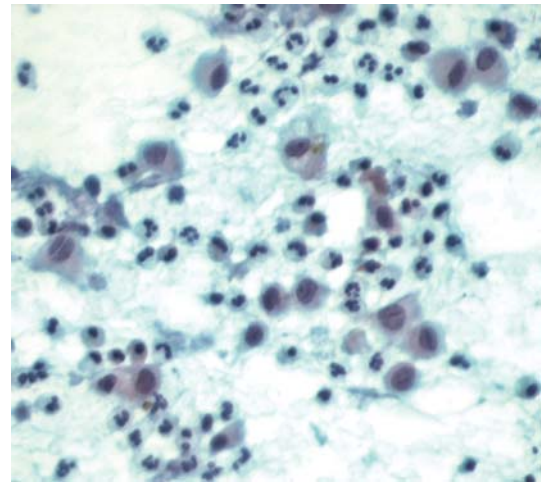


Figure 2: Langerhans cells with moderate to abundant cytoplasm and prominent nuclear grooves (Papanicolaou stain, original magnification, ×400).

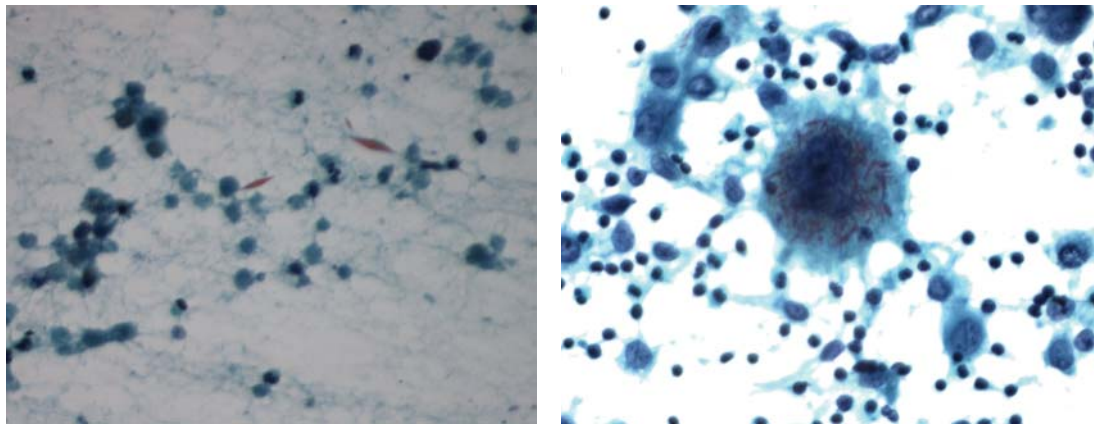
3. Discussion

LCH is a rare disease, and the estimated annual incidence ranges from 0.5 to 5.4 cases per million persons [2]. In the past, the disorder was referred to as histiocytosis X and had three variants: eosinophilic granuloma, Hand-Schuller-Christian disease and Letterer-Siwe syndrome. These three conditions are believed to represent different expressions of the same disorder, now known as LCH [3, 4].

An ongoing debate exists over whether this is a reactive or neoplastic process [2]. The disease is characterized by a clonal proliferation of the antigen-presenting dendritic cell called the Langerhans cell (LC) [5, 6]. The proliferation may be induced by a viral infection, a defect in T cell-macrophage interaction, and/or a cytokine-driven process mediated by tumor necrosis factor, interleukin 11, and leukemia inhibitory factor [2, 7–10].

LCH may occur at any age, although the majority of the cases are diagnosed in children from newborn to 15 years. There is no significant gender difference. The clinical spectrum varies from a solitary lesion, to multifocal unisystem to multisystem lesions with related symptoms. The unifocal form usually involves the bone, often seen in children between 5 and 15 years old. Systemic LCH is more common in children under 2 years of age. The multifocal unisystem form almost always occurs in the bone. Any bone can be involved, but more than 50% of lesions occur in the skull, spine, pelvis, ribs, and mandible. The multifocal multisystem form involves many organs, including the bone, skin, liver, spleen, hematopoietic system, and lymph node [2, 11]. The lymph node involvement in LCH can be seen as a part of a systemic disease or as a localized lesion, although isolated nodal involvement is rare. Lymph node may also enlarge as a reaction to bone or skin lesions [12].

Traditionally, the diagnosis of LCH is based on hematologic and histologic criteria [2, 4, 13–15]. Enough experience has accumulated in accurate cytological diagnosis of LCH in various body sites on the basis of characteristic cytological features in the presence of appropriate clinical and radiological setting as evident from several case reports



(a) (b)

Figure 3: (a) Extracellular rhomboid Charcot-Leyden crystals (Papanicolaou stain, original magnification, x400). (b) Macrophages with several ingested Charcot-Leyden crystals (Papanicolaou stain, original magnification, x400).

and case series [16–31]. Study of these shows that cytology closely reflects histomorphology. Ancillary studies may not be always necessary for diagnosis in appropriate setting [32].

The classical cytological features include high cellularity composed of sheets and many isolated LCs seen admixed with polymorphous population of numerous eosinophils, neutrophils, lymphocytes, plasma cells, multinucleated giant cells, and macrophages. The key to the diagnosis is to identify the LC through its characteristic features, namely, nuclear grooves and nuclear pseudoinclusions. They show variable degree of pleomorphism and mitotic activity [17, 18, 22, 26, 29]. Presence of dendrite-like cytoplasmic processes in LCs is a rare but characteristic feature [22, 33, 34]. Sometimes the LCs are few or nuclear grooves not very prominent or lack cytopalsmic processes. Degree of eosinophil infiltration varies in different areas of LCH lesion and different organs, thus their number can vary from scant to abundant in cytology smears [22]. Their presence can help attract attention to the diagnosis. In our case, eosinophils were more abundant in lymph node smears as compared to skull lesions which had more of LCs and reactive histiocytes.

Presence of Charcot-Leyden crystals singly and in bunches within the macropahges, giant cells, and extarcellularly was a unique feature in our case and has been reported very rarely [20, 27, 29, 31]. Charcot-Leyden crystals are crystalloids containing eosinophil membrane protein formed from rupture of eosinophil's granules. They indicate tissue eosinophilia and may help in drawing attention to the LCH diagnosis.

The diagnosis of LCH in our patient was made on the basis of FNA which showed characteristic (both common and rare) features of LCH. This was corroborated by characteristic radiology and clinical findings. In this case, CT showed lytic lesions in the skull bones having sharp borders with a punched-out appearance. Destruction of both the inner and outer tables results in a double-contour or beveled-edge appearance which is a typical feature in the diagnosis of LCH [35, 36].

The cytologic diagnosis may be missed due to lack of familiarity with its cytological features among pathologists or due to the lack of characteristic cytological findings resulting from a sampling error. Therefore it is prudent on the part of the pathologist to consider this diagnosis only in an appropriate clinical and radiological setting. It is also necessary to be familiar with cytological features of other differential diagnoses.

In the present case, the most common differential diagnoses of skull lesions clinically included Ewing's sarcoma, non-Hodgkin lymphoma, and osteomyelitis. Ewing's sarcoma and non-Hodgkin lymphoma are characterized by monotonous population of small round blue cells. In acute osteomyelitis, the neutrophils form a prominent component. The reactive histiocytes are seen and can be easily distinguished due to the absence of distinctive features of LCs. Chronic osteomyeltis shows predominantly plasma cells and lymphocytes. Plasma cells and neutrophils are infrequent in LCH.

Sinus histiocytosis with massive lymphadenopathy (SHML) involves primarily the cervical nodes, but its histiocytes are morphologically quite different from those of LCH. In SHML, the histiocytes have abundant cytoplasm, exhibiting hematopoietic phagocytosis and prominent nucleoli [28].

Secondary hyperplasia of the LCs is associated with lymphomas, especially with Hodgkin's disease and lung tumors. Care should be taken to differentiate these hyperplastic Langerhans cells from atypical LCs of LCH. Rarely, LCH can be associated with another malignancy such as malignant lymphoma, leukemia, or metastatic neoplasm [37, 38]. These need to be excluded after a diligent search for malignant cells with obvious cytologic atypia in the smear. Malignancies with tumor cells commonly having nuclear grooves or pseudoinclusions should also be considered, such as malignant melanoma and papillary thyroid carcinoma.

LCs show positivity for S-100, PNA (peanut agglutinin), MHC class II, CD1a, and langerin (CD207) [2]. Our case

showed positivity for S-100 protein. CD1a and langerin are not available in our lab. The Birbeck granule is their distinctive ultrastructural hallmark [2]. Electron microscopy was not performed in our patient and was not considered essential for diagnosis as also suggested by other authors [32].

Patients with apparently restricted LCH need careful staging of their disease to ensure that the lesions are not part of a more extensive process. FNA can be used to establish the extent of disease or recurrence of LCH [18]. In children with multiple swellings as in our case, FNA, being minimally invasive, is particularly suitable to sample all swellings in detecting the extent of involvement. For localized lesions in the skeletally immature patients, a simple, minimally invasive form of treatment with a low rate of complication is desirable. In view of this and the possibility of spontaneous resolution in localized disease, FNA alone could be used to confirm the diagnosis.

To conclude, the present case highlights the role of FNA in the diagnosis of the rare disease of LCH in a child with usual clinical presentation. The cytologic features of LCH are highly characteristic to suggest a diagnosis in an appropriate clinical setting with classical radiological findings. A high index of suspicion, awareness of common and rare cytological features of LCH, its differential diagnoses, and causes of diagnostic pitfalls is necessary. This can obviate the need of biopsy and electron microscopy. Immunochemistry if available can be performed on cell block.

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Clinical Study

A Cytohistologic Correlation of Mucoepidermoid Carcinoma: Emphasizing the Rare Oncocytic Variant

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It is well-known that the morphological variability of mucoepidermoid carcinoma (MEC) of the salivary glands may lead to interpretative difficulties on fine-needle aspiration (FNA) diagnosis. In this study we identify morphologic features that may be useful in the FNA diagnosis of MEC. The cohort included 23 cases of MEC; cytology and histology slides were reviewed and assessed for % cystic component, extracellular mucin, mucous and intermediate cells, oncocytes, cells with foamy/clear cytoplasm, keratinized cells and lymphocytes. On FNA 12/23 (52%) cases were diagnosed as consistent with or suggestive of MEC; 6/23 (26%) as salivary gland neoplasm and 5/23 (22%) as no tumor seen. The cystic component was ≥50% in 18/23 (78%) and <50% in 5 cases. The features prevalent in FNA and histology were: mucous cells (96% and 91%), extracellular mucin (91% both), intermediate cells (100 and 83%), lymphocytes (96 and 78%) and cells with foamy/clear cytoplasm (74% both). Oncocytes were seen in 43 and 22% and keratinized cells in 48 and 13% cases. Cases with oncocytes and lymphocytes were interpreted as favor Warthin's tumor on FNA. Presence of mucous cells, cells with foamy/clear cytoplasm, intermediate cells and lymphocytes in a mucinous background are diagnostic indicators of MEC; presence of oncocytes should not refrain from diagnosing MEC in FNA specimens.

1. Background

Fine needle aspiration (FNA) of salivary gland lesions is a valuable tool to pre-operatively diagnose/assess lesional tissue, determine the need for surgical intervention and assist in planning the appropriate surgical approach prior to resection. This technique is safe and effective with some studies demonstrating overall sensitivity, specificity and accuracy of 92%, 100%, and 98%, respectively [1–6]; however, the employment of FNA to diagnose mass lesions of the salivary glands remains controversial [1, 6]. The proponents believe that it can provide accurate diagnosis in many common tumors such as pleomorphic adenoma, distinguish benign from malignant lesions and prevent surgical intervention in cases with inflammatory lesions, lymphoma and certain metastatic tumors. The opponents of the use of FNA in salivary gland lesions believe that this procedure carries a high false negative rate and may fail to diagnose specific type of tumor. Many studies have shown that the latter is most likely due to the inherent morphologic variability that is, overlapping architectural patterns and nuclear cytology seen within these tumors, which can lead to interpretative difficulties on cytologic examination [1, 2, 7–10].

Mucoepidermoid carcinoma (MEC) is the most common malignant neoplasm of the salivary gland origin and accounts for 5% to 10% of all salivary gland neoplasms [11]. The majority of MEC occur in the parotid gland resulting in accessibility to biopsy by FNA; however, at times the diagnosis of MEC (mainly low-grade tumors by FNA) can be difficult due to overlapping cytomorphology with benign lesions [2, 3, 7, 8, 12, 13]. Therefore, given the common occurrence and heterogeneity of MEC, proper sampling and awareness of its morphologic complexity is critical to an accurate diagnosis.

The difficulty in the cytologic diagnosis of MEC is related, in part, to the histologic grade of this tumor [7, 12].

High grade neoplasms are more easily recognizable as malignant and, therefore, more likely to receive the appropriate preoperative management [11]. By contrast, low-grade neoplasms are less easily recognizable as malignant and, therefore, under-diagnosis could result in treatment delays or inappropriate pre-operative management [14]. Numerous of grading schemes have been devised to differentiate between low, intermediate, and high-grade MEC [11, 14]. A scheme, proposed by Brandwein et al., assigned a numerical score to specific histologic features and adding these scores to determine the histologic grade. The accumulation of malignant features such as (tumor-type necrosis, nuclear pleomorphism, and high mitotic activity) results in a higher score [11]. Given the consequences of under-diagnosing MEC (such as treatment delays or inappropriate surgical approach), and the challenges of diagnosing MEC by cytology, in this study we attempt to identify the morphologic features that may be most useful in the FNA diagnosis of MEC, particularly of low-grade neoplasms.

2. Materials and Methods

In this retrospective study, 23 cases of MEC with preoperative FNA, were evaluated. The patient's ranged in age from 18 to 79 years and received surgical care at the Hospital of the University of Pennsylvania between 1995 and 2008. Cytology and histology slides and clinicopathologic features were reviewed in each case. The cases were assessed for the following features: % cystic component, nuclear atypia, necrosis, extracellular mucin, mucus cells, intermediate cells, oncocytes, and cells with foamy/clear cytoplasm, keratinized cells and lymphocytes. A histologic grade of low, intermediate or high was assessed in each case. The morphologic features noted by cytologic examination were compared to the original cytologic diagnosis in an effort to assess which features were the most consistent/reproducible in providing an accurate cytologic diagnosis and which histomorphologic features were associated with under-diagnosis of MEC by cytologic examination.

3. Results

In this study, 22/23 (96%) of, MECs arose in the parotid gland (average size 1.9 cm) and one (4%) from a minor salivary gland in the tongue. On FNA, 7/23 (30%) cases were diagnosed as consistent with, 5/23 (22%) as suggestive of MEC; 6/23 (26%) as salivary gland neoplasm and 5/23 (22%) as no tumor seen. In the six cases diagnosed as salivary gland neoplasm on FNA, two were diagnosed as favor acinic cell carcinoma (2/6), two were diagnosed as favor Warthin tumor (2/6), one was diagnosed as neoplasm with squamous differentiation (1/6) and another was diagnosed as favor benign mixed tumor versus mucoepidermoid carcinoma or adenoid cystic carcinoma (1/6). On histologic examination, the tumor grade was low in 13/23 (56%), intermediate 9/23 (39%) and high in 1/23 (4%) cases; neural invasion was seen in 4/23 (17%) and lymph node metastasis in 1/23 (4%) cases. The cystic component was ≥50% in 18/23 (78%) cases (see Tables 1 and 3). The morphologic features prevalent in both histology and FNA specimens included: mucus cells (96 and 91%), presence of extracellular mucin (91% both), intermediate cells (100 and 83%), lymphocytes (96 and 78%), and cells with foamy/clear cytoplasm (73% both). Oncocytic cells were seen in 43 and 22% and keratinized cells in 48 and 13% cases (see Table 2).

An intraoperative frozen section was performed in 9/23 (39%) cases. The frozen section diagnoses were: MEC 4 cases, low-grade carcinoma 1, adenocarcinoma 1, consistent with Warthin tumor 1, cystic neoplasm 1, and no tumor seen 1 case. The average time interval between FNA and surgery was 5 weeks in 12 cases diagnosed as consistent with or suggestive of MEC. The average time interval was 4 weeks in 6 cases diagnosed as salivary gland neoplasm and 22 weeks in cases where FNA failed to identify tumor (see Tables 1 and 3).

4. Discussion

Mucoepidermoid carcinoma is the most common malignant salivary gland tumor. It is usually composed of varying amounts of epidermoid (squamoid) cells, intermediate cells, and mucocytes (often seen lining the microcysts). The combination of these cellular elements in varying proportions can lead to complex histologic patterns causing diagnostic challenges [11, 12]. The MEC are usually graded as low grade/welldifferentiated (tumor exhibiting greater than 50% of mucous elements), intermediate grade (10–50% of mucous elements and high grade (less than 10% of mucous elements). The histopathologic grading is usually used as the main prognostic indicator; however, some of the low-grade tumors can follow an aggressive clinical course [11, 14–18]. Furthermore, some experts believe that a tumor grading system of low and high grade is more reproducible as compared to the 3 category system [11].

Similar to histology, the diagnosis of low-grade MEC by FNA can be challenging due to spatial heterogeneity and multiple histologic components. Therefore, adequate sampling of various components within the tumor is essential to arrive at correct diagnosis [7, 12].

In our study, the morphologic features most prevalent in both the cytologic and histologic specimens of MEC were mucus cells (pseudo-goblet cells) and presence of extracellular mucin (both >90%). In addition, intermediate cells (100% in histology and 83% in cytology) and lymphocytes (96% in histology and 78% in cytology) were also commonly noted. The presence of oncocytic cells (43% in histology and 22% in cytology) and squamous/epidermoid cells (48% in histology and 13% in cytology) were less commonly seen.

Oncocytic cells were seen 10/23 (43%) cases in histology and 5/23 (22%) of cytology cases. One case with oncocytic cells was interpreted on FNA as "salivary gland neoplasm favor Warthin tumor", 2 as MEC, 1 as acinic cell carcinoma and 1 as suggestive of MEC. On re-review, all contained varying amounts of extracellular mucin, mucous cells and intermediate cells; pseudo-goblet cells/clear cells were seen in 3 cases. The case originally classified as Warthin tumors also contained an excess of lymphocytes. Oncocytic cells have

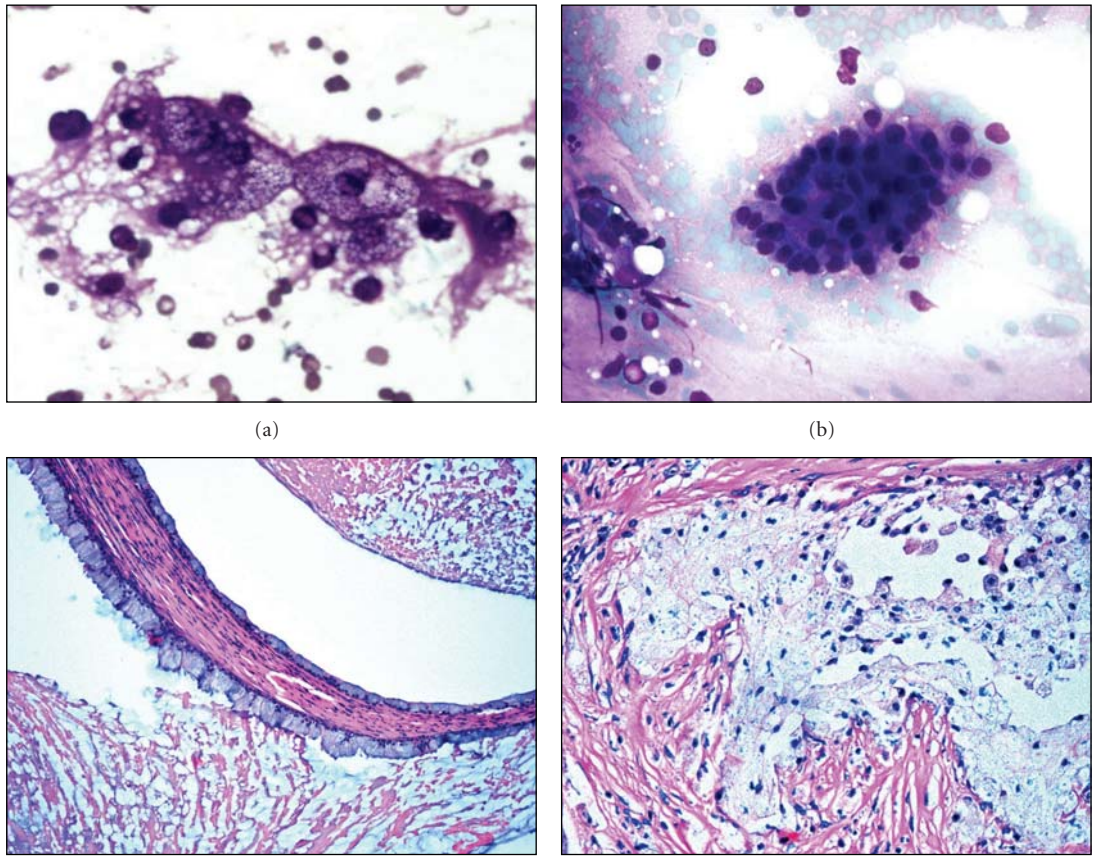

Figure 1: (a and b): High-power magnification of a Diff-Quik stained FNA specimen of low-grade mucoepidermoid carcinoma showing histiocyte-like (clear) cells with vacuolated cytoplasm (a) and fragments of glandular-type cells (b). (c and d) High-power magnification of H&E stained histologic section of a low-grade mucoepidermoid carcinoma showing glandular-type (mucous) cells lining a microcyst (c) and a cluster of histiocyte-like (clear) cells (d).

Table 1: Clinicopathologic data on 23 patients with mucoepidermoid carcinoma.

FNA-Dx % cases	Gender (M : F)	Avg-Age (yrs)	Avg-Size (cm)	Location	Avg-time between FNA & resection
CW/SO MEC (12/23) 52%	8 : 4	49	1.6	11/12 parotid, 1 tongue	5 weeks
SGN (6/23) 26%	4 : 2	52	2.5	6/6 parotid	4 weeks
Neg or inflamm (5/23) 22%	1 : 4	50	1.6	5/5 parotid	22 weeks

Dx:Diagnosis, Avg:Average, CW:Consistent with, SO:Suggestive of, SGN:salivary gland neoplasm, Neg:Negative, Inflamm:Inflammatory.

Table 2: Key morphologic features observed in histology and FNA specimens.

Cell type observed	% in Histology	% in FNA
Mucous (pseudogoblet) cells	(22/23) 96%	(21/23) 91%
Lymphocytes	(22/23) 96%	(18/23) 78%
Clear cells	(17/23) 74%	(17/23) 74%
Intermediate cells	(23/23) 100%	(19/23) 83%
Keratinized squamous cells	(11/23) 48%	(3/23) 13%
Oncocytes	(10/23) 43%	(5/23) 22%

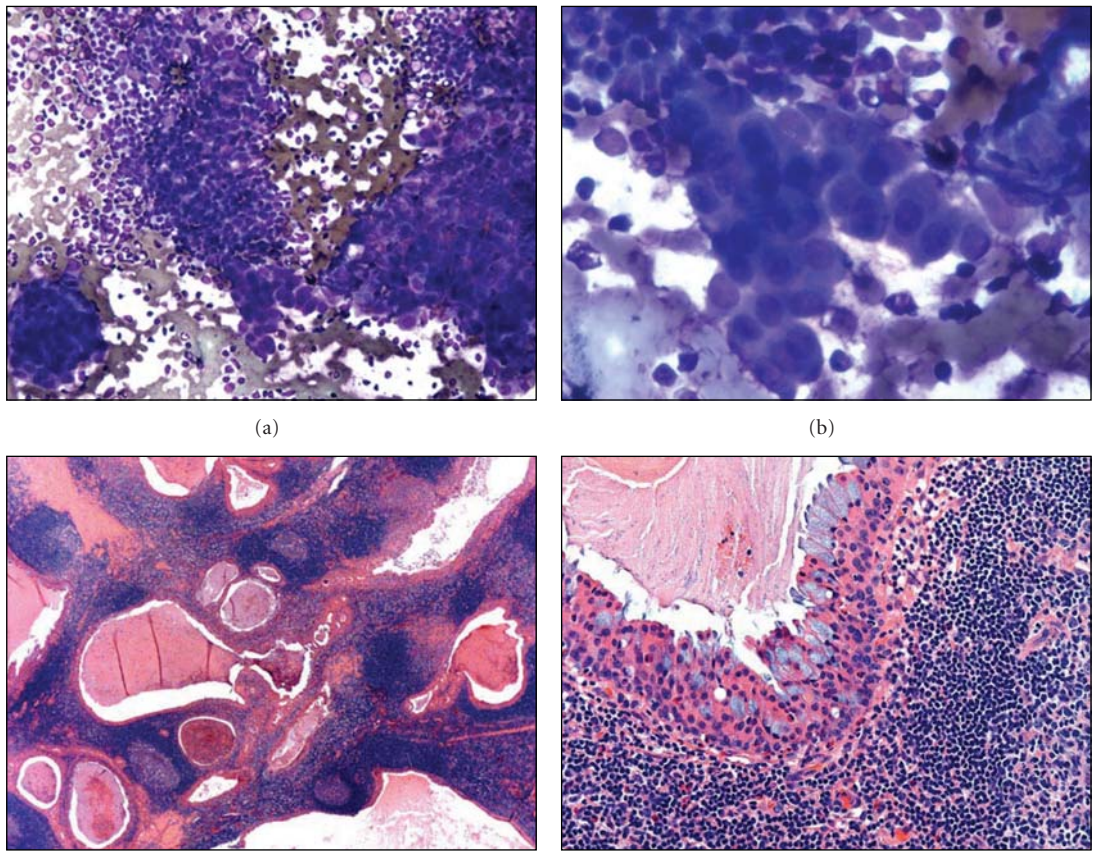

Figure 2: (a and b): Low-power magnification of a Diff-Quik stained FNA specimen showing oncocytic fragments present in a background lymphocytes (mistaken interpreted as Warthin's tumor) and high-power magnification of oncocytic fragments intermixed with lymphocytes. (c and d): Low-power magnification of H&E stained histologic section of a low-grade mucoepidermoid carcinoma showing a numerous cystic space lined by oncocytic epithelium in a background of reactive lymphoid follicles (c) and high-power magnification shows a microcyst lined by oncocytes and mucous cells (this case was misinterpreted as Warthins tumor on frozen section) (d).

Table 3: Cytologic/Histologic diagnosis comparison.

FNA DX (23 cases)	FS DX (9/23 cases)	Histologic grade
C/W MEC (7/23)	MEC (3/5)	LGMEC (2/7)
	Adenocarcinoma (1/5)	IGMEC (4/7)
	Cystic Neop with papillary-features (1/5)	HGMEC (1/7)
S/O MEC (5/23)	SO MEC (1/2)	LGMEC (3/5)
	NTS (1/2)	IGMEC (2/5)
SGN: (6/23)	None performed	
Favor WT (2/6)		LGMEC (1/2)
		IGMEC (1/2)
Favor ACC(2/6)		LGMEC (2/2)
Favor BMT versus MEC or ADCC (1/6)		LGMEC (1/1)
Neop with squ-feat (1/6)		IGMEC (1/1)
Neg or inflamm (5/23)	LG-CA (1/2)	LGMEC (4/5)
	WT (1/2)	IGMEC (1/5)

Dx:Diagnosis, Avg:Average, CW:Consistent with, SO:Suggestive of, SGN:salivary gland neoplasm, Neg:Negative, Inflamm:Inflammatory.

been reported to occur in MEC; in addition, a rare variant of MEC known as oncocytic MEC has been described [19–22]. These tumors are composed exclusively of oncocytic cells arranged in nests and sheets in sclerotic stroma with variable number of chronic inflammatory cells [22]. The majority of the oncocytic MEC described in the literature lack or contain minimal squamous/epidermoid cells. On re-review we believe, based on the criteria described by Weinreb et.al, 5 of 10 cases represent MEC containing oncocytic cells as one the cellular components while 5 of 10 cases represent true oncocytic variant of MEC [22].

We believe that the most helpful features in differentiating MEC containing oncocytic cells from other salivary gland lesions in FNA specimens is the presence of extracellular mucin, mucous cells and pseudo-goblet/clear cells.

Since a major difficulty in utilizing FNA to diagnose MEC is related to sampling, [7, 12] we believe it is most useful to identify various cellular and acellular components and formulate a differential diagnosis based on a few criteria including: nuclear atypia, metaplastic changes/cell type present (squamous, oncocytic, basal, or myoepithelial cells), presence or absence of lymphocytes, and presence of extracellular material (necrotic debris, chondromyxoid matrix or Mucin). Given the overlapping morphologic features of many salivary gland neoplasms, immunostains are rarely useful in differentiating the various salivary gland neoplasms [7, 23].

To add to these challenges, it has been shown that metaplastic/reparative changes can occur in benign salivary gland neoplasms due to physical trauma induced by FNA [24]. These changes include squamous metaplasia, infarction and necrosis, subepithelial stromal hyalinization, acute and chronic hemorrhage, inflammation with multinucleated giant cells, granulation tissue with subsequent fibrosis; cholesterol cleft formation, pseudoxanthomatous reaction, and microcystic degeneration. Thus, a repeat FNA of a salivary gland lesion containing above-mentioned reactive/reparative changes can pose an even greater challenge to the cytopathologist in the diagnosis of low-grade mucoepidermoid carcinoma; therefore, most clinicians will recommend surgery after an FNA diagnosis of salivary gland neoplasm [24].

Several studies have discussed the utility of intraoperative consultation in the surgical management of salivary gland lesions. Studies from our institution have shown that the diagnostic accuracy of FNA and frozen section are comparable for the interpretation of salivary gland neoplasms, and the accuracy of both is increased when used in conjunction [6]. In the current study, 12/23 (52%) cases were diagnosed as either consistent with or suggestive of MEC and 6/23 (26%) cases as salivary gland neoplasm. An intraoperative frozen section was performed in 9/23 (39%) cases, and of these, 8 (89%) cases were classified either as carcinoma or neoplasm (MEC 3, low-grade carcinoma 1, suggestive of MEC 1, adenocarcinoma 1, consistent with Warthin tumor 1, cystic neoplasm 1 and no tumor seen 1 case). Based on these data, the diagnostic accuracy of FNA is close to that of frozen section for the diagnosis of salivary gland neoplasms (78% (18/23) versus 89% (8/9)) and carcinomas (52% (12/23) versus 67% (6/9)). These findings support, as suggested by other authors, the combined use of intraoperative frozen section with FNA in the evaluation of salivary gland neoplasms [6].

5. Conclusions

We believe that the cytologic diagnosis of low-grade MEC remains challenging due to overlapping cytomorphologic features seen in other salivary gland lesions. Among the many cytologic features described, presence of extracellular mucin, mucous cells, and intermediate cells should raise the suspicion of MEC. In addition, oncocytic cells can occur in varying proportions in some cases of MEC and in the presence of above-mentioned features should not dissuade one from making or suggesting a diagnosis of this tumor in FNA specimens.

Competing Interests

None of the authors of this paper have any competing interests to report.

Authors Contributions

All authors (TW, VAL, KTM & ZWB) participated equally in the research design, slide review, drafting and approval of the final manuscript. All authors read and approved the final manuscript.

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Research Article

Use of Fine-Needle Aspiration in the Evaluation of Breast Lumps

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Background. A study was designed to see the role of fine needle aspiration cytology (FNAC) in palpable breast lumps. *Materials and Methods*. Four hundred and twenty five (425) patients came to the Department of Pathology King Edward Medical University, Lahore in four years for FNAC of their palpable breast masses from June 2006 to June 2010. FNAC diagnosis was compared with histological diagnosis to see the accuracy of fine needle aspiration cytology for neoplastic lesions. *Results*. There were 271/425 benign, 120/425 malignant, and 32/425 suspicious smears. Inadequate samples were repeated twice or thrice, and the degree of success was improved with consecutive repeating approaches. The frequency of inadequacy declined from 86 to 18, and 2 for first, second and third attempts, respectively. The number of repeats increased the diagnostic accuracy of aspirates which is statistically significant ($P = .000$). Invasive ductal carcinoma was the most commonly reported lesion with maximum incidence in the 4th, 5th, and 6th decades followed by invasive lobular carcinoma and other malignant lesions. The sensitivity, specificity, accuracy, negative predictive value, and the positive predictive value of FNAC was 98%, 100%, 98%, 100%, and 97%, respectively. *Conclusion*. FNAC serves as a rapid, economical, and reliable tool for the diagnosis of palpable breast lesions because the cytopathological examination of these lesions before operation or treatment serves as an important diagnostic modality.

1. Introduction

All breast lesions are not malignant, and all the benign lesions do not progress to cancer; however the accuracy of diagnosis can be increased by a combination of preoperative tests (like physical examination, mammography, fine-needle aspiration cytology, and core needle biopsy). These modalities are more accurate, reliable, and acceptable when compared with a single adopted diagnostic procedure despite of having their own technical limitations [1, 2].

"As fine-needle aspiration (FNA) has become a critical component in the investigation of palpable breast masses; false-negative diagnoses have become a major concern, prompting re-evaluation of the definition of specimen adequacy. Although cytopathologists agree that a number of parameters relate to the adequacy of an FNA specimen, there is no concsensus on the role of epithelial cell quantitation in the determination of an adequate FNA. To better understand the significance of epithelial cellularity, false-negative FNA samples from palpable breast lesions were reviewed" [3].

"Fine-needle aspiration (FNA) biopsy is an established and highly accurate method for diagnosing breast lesions." The use of core biopsy (CB) is being increasingly advertised but its procedure is more cumbersome, expensive and time consuming as compared to FNA procedure [4–6]. Core Biopsy or tru cut needle biopsy is not widely used because of its complications, interpretation, and time-consuming results; therefore palpable breast lesions can be accurately diagnosed by triple test only (FNAC, physical examination and Mammography) [7].

"Although fine-needle aspiration (FNA) biopsy of the breast has been shown to be a safe and accurate technique, many surgeons question whether it is reliable enough to replace excisional biopsy. If FNA biopsy is followed by an excisional biopsy for confirmation, it would seem that the cost of diagnostic workup would be increased, but it has been

seen that FNA biopsy is cost effective even when followed by an excisional or frozen section biopsy for confirmation. It is considered safe and reasonable to expand its use to smaller hospitals where the personnel may be initially less experienced with the technique" [8, 9].

It is considered a successful and less complicated procedure with excellent results; however the main factors influencing success should be considered before its procedure to increase its accuracy and these are "the aspirator, the small size of many cancers, and the occult nature of the lesions seen only on mammography" [10].

Fine-needle aspiration cytology (FNAC) is widely used in Pakistan as a reliable, rapid, cost-effective, complication free, and an accurate diagnostic modality for the evaluation or management of breast lumps. A study was conducted to see the usefulness of fine-needle aspiration (FNA) in screening of palpable breast masses at the Department of Pathology, King Edward medical University, Lahore, Pakistan.

2. Material and Methods

King Edward Medical University is the largest Medical Institute of Asia and Pakistan, comprising of four tertiary care hospitals with record numbers of yearly registered patients coming from all parts of the country. We have two programs at our institution: one is screening of all palpable lumps for early detection of malignancy and onsite diagnostic program for all referral cases waiting for surgery and treatment.

A total of 425 patients came to the Department of Pathology in four years for FNAC of their palpable breast masses from June 2006 to June 2010.

Out of these 425 patients, 338 biopsies were collected for comparison as inflammatory, and inadequate lesions were excluded.

2.1. Patients Protocol. A designed proforma was used to collect the consent and data of patients. History of lactation and pregnancy was included in the proforma. All the lesions were followed for biopsy except inflammatory and inadequate samples. The inadequate lesions were advised a repeat FNAC. Both benign and malignant tumors were followed up. Patients were divided into groups, and their mean age was calculated.

2.2. Procedure for Fine-Needle Aspiration Cytology (FNAC). A written consent was taken before performing the FNAC. FNA was done using a 23 gauge needle and 10 mL disposable syringe of Becton and Dickinson Pakistan (Pvt) for each prick and for each patient. No local anesthetic was used, and the needle was inserted into the palpable lesions, either once or twice depending upon the size of the nodule. FNA gave us cytological diagnoses, and we visualized at individual cells but cellular structures and not at macrotissue architecture like a histological specimen would. Cellular material was aspirated into a syringe and expelled onto slides. Four to five slides were prepared for each patient. A small or medium-sized drop of aspirate was put near the frosted end of a slide that was placed on a table. A second slide was used to spread the aspirated material in the same manner used to prepare a peripheral blood smear. All the smears were wet fixed in 95% methanol, and the air dried smears were stained with two stains Hematoxylin & Eosin (H&E), and Papanicolaou stains. The procedure was done within one hour, and the reports were signed out within 2-3 hours.

2.2.1. Four Groups Were Defined for the FNAC Diagnosis

(1) Inadequate
(2) Non neoplastic lesions
(3) Suspicious of malignancy
(4) Positive for malignancy.

NB: in the calculations, groups 3-4 were malignant.

2.3. Preparation of Cell Blocks. Remaining aspirates were used for cell blocks, by putting 10% formalin in the syringes used for FNA.

2.4. Procedure for Histopathology. Inflammatory (85) and Inadequate (2) smears were not followed for their biopsies. Remaining 338 (benign smears, suspicious, and malignant) smears were followed for biopsies (76 mastectomies and 262 lumpectomies). These biopsy specimens were fixed in 10% formalin for 24 hours and then grossed in the Department of Pathology by consultant histopathologists. The gross and cut section findings were noted. Several bits were taken from appropriate sites for processing and paraffin embedding. From each block, sections were cut at 4-5 microns thickness and stained by H&E.

2.5. Criteria for Selection of Patients. Inclusion criteria.

(1) All females with unknown primary diagnosis of breast mass.
(2) Patients consented for inclusion in study according to designed proforma.

Exclusion criteria.

(1) Patients with recurrent malignancy.
(2) Patients who underwent FNAC but did not undergo subsequent histopathological diagnosis.
(3) Patients in whom FNAC was either acellular or non-diagnostic or inflammatory.
(4) Past or current chemo-therapeutic or prevention treatment.
(5) Male patients with breast cancer and gynecomastia.

2.6. Statistical Analysis. Data was computerized with window SPSS version16. Specificity, sensitivity, accuracy, and predictive values were calculated, P values were also calculated, while correlation was seen by Pearson's correlation curve.

3. Results

A total 425 fine-needle aspirates (FNAs) were carried out over a period of four years in the Department of Pathology.

Table 1: Age of the patients presenting with lump breast.

Age in years ($n = 425$)	Inadequate	Inadequate on first repeat	Inadequate second repeat	Benign lesions		Suspicious for malignancy	Malignant
				Inflammatory	Benign proliferative lesions		
A 16–20 ($n = 23$)	4	1	0	4 (4.7%)	10 (5.8%)	6 (16.6%)	3 (2.3%)
B 21–30 ($n = 76$)	12	2	0	10 (11.8%)	50 (29.2%)	3 (8.3%)	13 (10%)
C 31–40 ($n = 166$)	27	5	1	52 (61%)	79 (46%)	7 (20%)	27 (20.6%)
D 41–50 (86)	23	4	0	14 (16.5%)	30 (17.5%)	11 (30.5%)	31 (23.6%)
E 51–60 ($n = 53$)	17	5	1	5 (5.8%)	2 (1.2%)	7 (20%)	38 (29%)
F 61–70 ($n = 16$)	2	1	0	0	0	1 (2.8%)	15 (11.5%)
G <70 years ($n = 5$)	1	0	0	0	0	1 (2.8%)	4 (3%)
Total	86	18	2	85 (20%)	171 (40%)	36 (8.4%)	131 (31%)

Note: the number of repeat increased the diagnosis of aspirates which is statistically significant ($P = .000$).

Table 2: Comparison of distribution of inflammatory lesions.

Inflammatory Lesions ($n = 85$)	Cytology	Histopathology
Acute suppurative Mastitis	20 (23.5%)	Excluded
Acute mastitis	26 (30.5%)	Excluded
Non-tuberculosis granulomatous mastitis	15 (17.6%)	Excluded
Chronic nonspecific mastitis	12 (14%)	Excluded
Duct ectasia	8 (9%)	Excluded
Tuberculosis	2 (2.3%)	Excluded
Fat necrosis	2 (2.3%)	Excluded

Table 3: Comparison of distribution of inflammatory lesions.

Benign Lesions on cytology ($n = 171$)	Cytology	Histopathology						
		FA	FSD	FAN	FEH	AEH	BPh	Malignant
Fibroadenomas	70 (41%)	60	6	3	0	0	1	0
Fibrocystic disease	90 (52.6%)	10	70	5	3	1	1	0
Benign proliferative diseases	6 (9%)	0	0	2	3	0	1	0
Benign phyllodes	5 (3%)	2	0	0	0	0	3	0
Total	171	72	76	10	6	1	6	0

FA: fibroadenoma, FCD: fibrocystic disease, FAN: fibroadenosis, AEH: atypical epithelial hyperplasia, FEH: florid epithelial Hyperplasia, and BPh: Benign Phyllodes.

Table 4: Histopathological diagnosis of suspicious and malignancy smears.

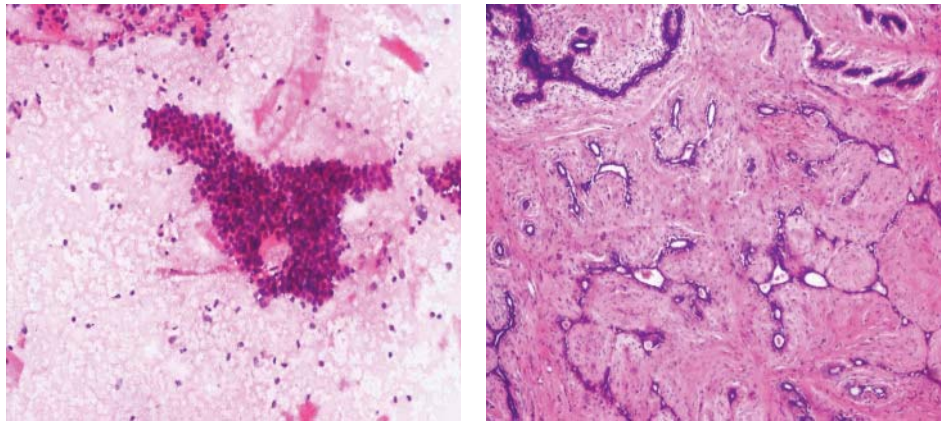
Cytology	Histopathology											
	Malignant results									Benign results		
	DCIS	IDC	LCIS	ILC	MC	MDC	MTC	Lymph	MPhy	FA	FAN	SCA
Suspicious for malignancy ($n = 36$; 8.4%)	15	10	4	4	0	0	0	0	0	1	2	1
Malignant lesions ($n = 131$; 30.8%)	4	100	2	16	2	1	1	1	4	0	0	0

FA: fibroadenoma, FAN: fibroadenosis, SCA: sclerosing adenosis, DCIS: ductal carcinoma Insitu. IDC: invasive ductal carcinoma, LCIS: lobular carcinoma In situ, ILC: invasive lobular carcinoma, MC: mucinous carcinoma, MDC: medullar carcinoma, MTC: metaplastic carcinoma, Lymph: lymphoma: MPh: malignant phyllodes.

Table 5: The Diagnostic accuracy of FNAC in histologically correlated cases.

FNAC result	Histopathological diagnosis		Total
Positive for malignancy	163 (TP)	4 (FP)	167
Negative for malignancy	0 (FN)	171 (TN)	171
Total	163	175	338

Sensitivity: 100%, Specificity = 98%, Accuracy = 98%, PPV = 97%, NPV = 100%.



(a) (b)

Figure 1: (a) Photomicrograph on FNAC of a benign smear (H&E stain 10x) of Fibroadenoma showing sheet of regularly arrange epithelial cells (Stag horn pattern) and numerous bare nuclei. (b) Comparison on Histopathology (10x H&E) of fibroadenoma breast.

There were 85 inflammatory lesions and 2 smears inconclusive. Remaining 338 (benign smears, suspicious, and malignant) smears were followed for biopsies (76 mastectomies and 262 lumpectomies). There were total of 256/425 (60%) benign lesions comprising of 85 (20%) inflammatory aspirates and 171(40%) benign proliferative lesions. Cytological diagnoses of other aspirations were reviewed, and lesions were classified into four diagnostic classes revealing 171 benign proliferative lesions, 131 malignant, 36 suspicious, and 2 inadequate smears (Table 4).

3.1. Followup of Inadequate Smears. Inadequate samples were repeated twice and degree of success was improved with two consecutive approaches with an interval of 24 hours in subsequent repeats. There were 86, 18, and 2 inadequate smears in first, second, and third approach. The number of repeats increased the diagnostic accuracy which is statistically significant ($P = .000$). There were maximum lesions during the reproductive age groups, and most of these were benign. Maximum malignant lesions were seen in older age groups. This relationship was statistically significant when compared by Pearson correlation curve. A positive correlation was observed between benign and malignant lesions ($r = 0.95$, $P < .0001$) (Table 1, Figure 9). The age of the patients ranged between 16 to >70 years. The youngest patient diagnosed as invasive ductal carcinoma (IDC) was seen at 16 years of age. In our study, most of the benign lesions were reported (29.2% & 46%) in 3rd and 4th decades of life, while maximum numbers of malignant lesions (23.6 and 29%) were reported in 5th and 6th decades (Table 1).

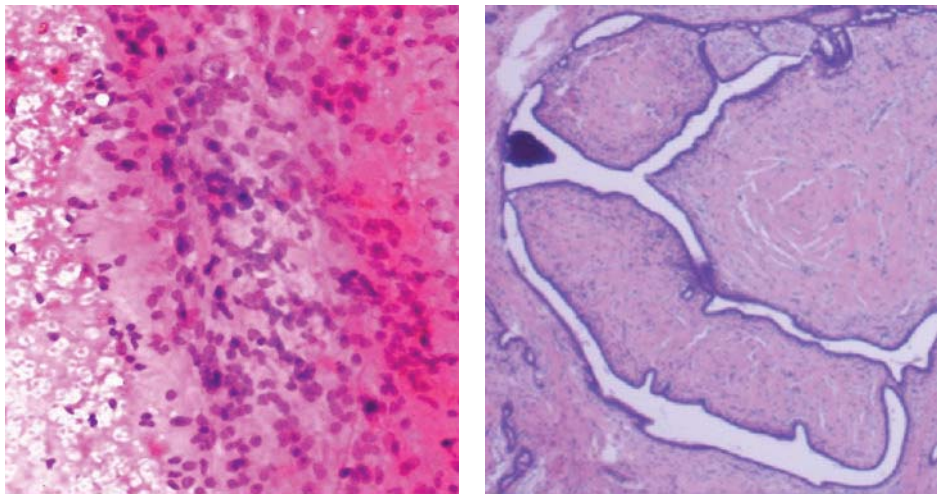
There were 23.6%, 30.6%, 17.6%, 14%, 9%, 2.3%, and 2.3% lesions of acute suppurative mastitis, acute mastitis, non-tuberculous mastitis, chronic nonspecific mastitis, duct ectasia, tuberculous mastitis, and fat necrosis, respectively. The diagnosis of these lesions was made by cytology and histology of cell blocks (Table 2).

In benign proliferative breast lesions, we were very conscious about over-diagnosis, therefore our reporting style was as "smear negative for malignant lesions", and for further analysis we advised an excision biopsy. For definite diagnosis our reports were limited to Fibroadenoma, fibrocystic change disease, benign proliferative disease, and Phyllodes tumors. For uncertain categories we only reported "benign proliferative lesions". This class of diseases constituted the largest group of lesions with maximum incidence in the 3rd, 4th and 5th decades of life (Tables 1 and 3).

Invasive ductal carcinoma was the most common malignant lesion reported in our study with a maximum incidence in the 4th, 5th, and 6th decades, followed by invasive lobular carcinoma and other malignant lesions (Table 5). Following histopathologic correlation with FNA, we calculated the sensitivity, specificity, and positive predictive values.

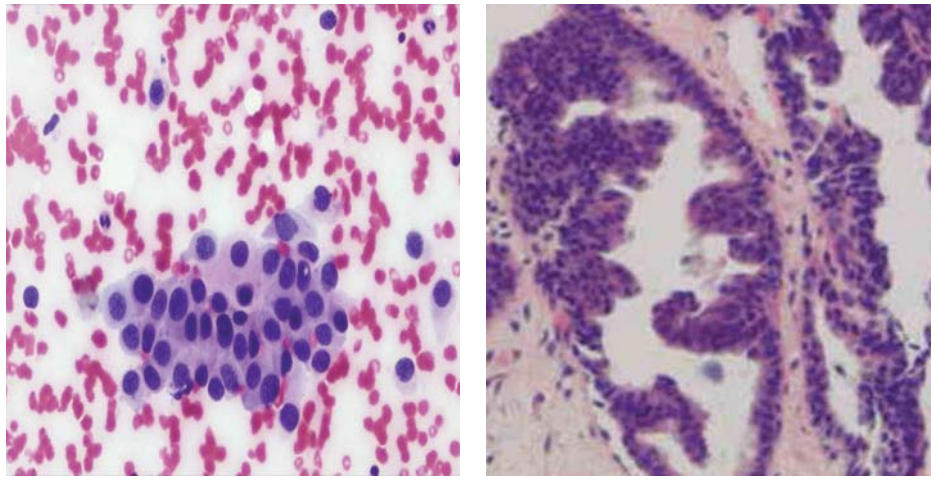
There were 4 false positive cases and no false negative cases in this study. False positives were observed in the suspicious lesions.

False positives noted mainly in the interpretation of suspicious smears or with atypical features, were due to uniformly enlarged nuclei with prominent nucleoli, occasional marked nuclear enlargement, and moderate pleomorphism seen in fibrocystic disease or fibroadenoma. Regarding



(a) (b)

Figure 2: (a) Photomicrograph on FNAC of a benign smear of Benign Phyllodes (H&E stain 20x) showing cellular smear of spindled stromal cells. (b) Comparison on Histopathology of Benign Phyllodes.



(a) (b)

Figure 3: (a) Photomicrograph on FNAC of a suspicious smear (H&E stain 40x) of breast showing increased cellularity with mild pleomorphism and discohesion but lacking other features of malignancy. (b) Comparison on histopathology of atypical ductal hyperplasia.

benign proliferative and malignant lesions no false positivity was seen. Therefore, in the this study, the sensitivity was 100%, specificity 98%, accuracy 98%, negative predictive value 100%, and the positive predictive value was 97% (Table 5).

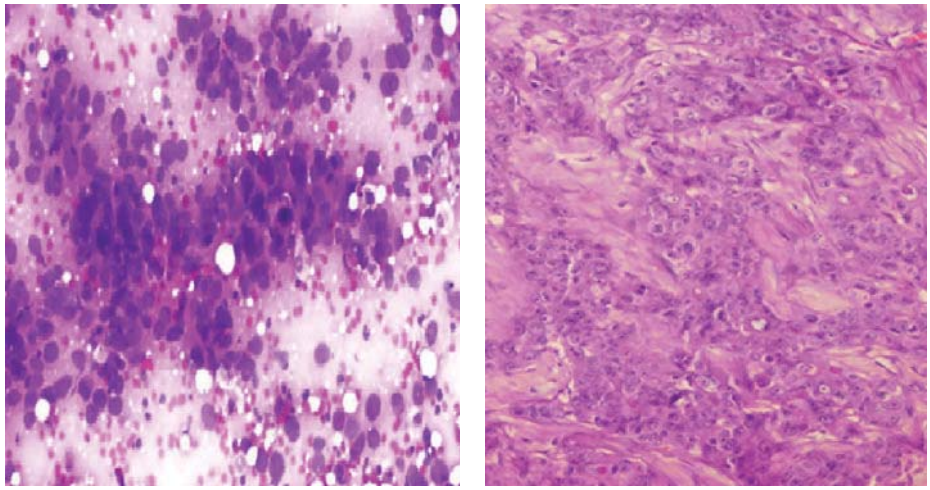
All the FNAC smears are compared with histopathology and the details given in Figures 1, 2, 3, 4, 5, 6, 7, and 8.

4. Discussion

Fine-needle aspiration cytology is widely used in the diagnosis of breast cancer because it is an excellent, safe, and cost-effective diagnostic procedure. One can get on site immediate report with minimal cost using inexpensive equipments and a simple technique. The most significant advantage of FNAC is the high degree of accuracy, rapid results, and a less invasive procedure than a tissue biopsy. FNAC of the breast can reduce the number of open breast biopsies [11–14].

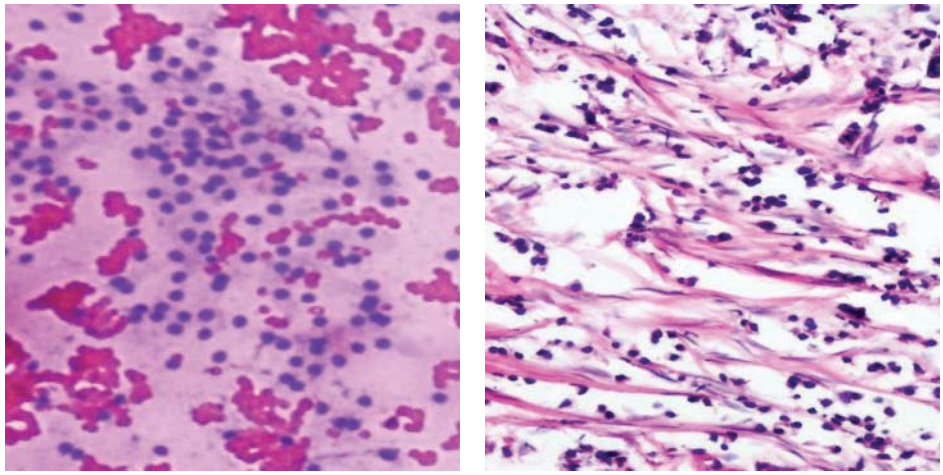
The frequency of inadequate cases are variable is different studies ranging from 0 to 57.2% depending on various factors. The main causes for inadequate smears may be due to either lack of technical experience in performing FNA, preparation, and fixation of smears. FNA of ill-defined masses like or lesions with hyalinization and deeply located lumps may also be contributed to the inconclusive diagnosis [15, 16].

Many inflammatory breast lesions create confusion as these are presented as a palpable mass. "Mammographic, sonographic, and magnetic resonance imaging findings may not always distinguish some of the benign lesions like duct ectasia, fat necrosis from a malignant lesion." Fine-needle aspiration (FNA) is a well-accepted diagnostic modality and



(a) (b)

Figure 4: (a) Photomicrograph on FNAC of a malignant smear of ductal carcinoma (40x Pap stain) showing variation in nuclear shape and size, with decreased intercellular cohesion and dirty background (b) Comparison on histopathology of invasive ductal carcinoma

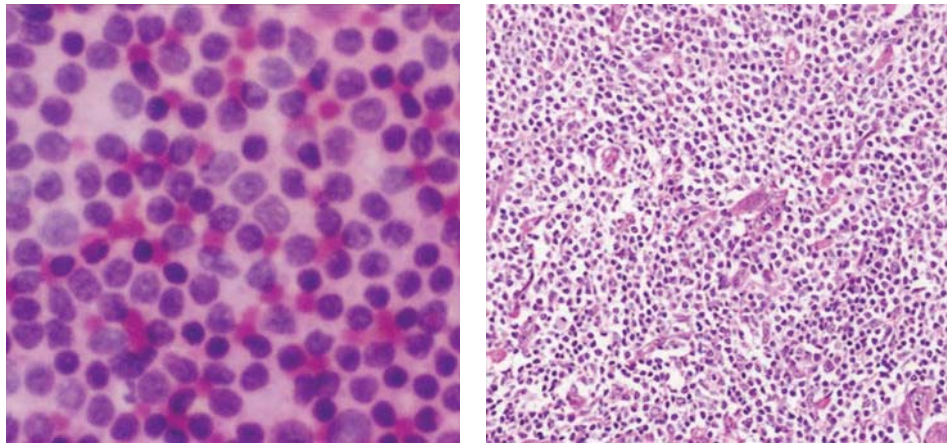


(a) (b)

Figure 5: (a) Photomicrograph on FNAC of a malignant smear's depicting cytological features of lobular carcinoma (20x H&E stain). Groups of small, round, uniform cells with distinct cell membrane and discohesion. (b) Comparison on histopathology showing uniform cells arranged in alveolar pattern of lobular carcinoma.

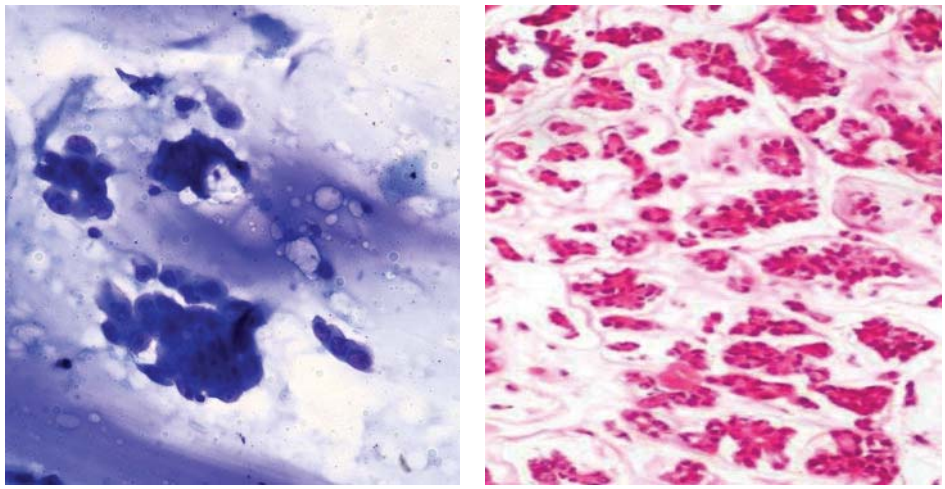
procedure for the diagnosis of inflammatory swellings of breasts. We are using this technique in such lesions, and results are variably accepted by our consultants and clinicians with varying degrees of acceptance rates, accuracy, and results. Fine-needle aspiration is the most accurate diagnostic modality for these lesions and cell blocks accentuate the reliability of the diagnosis in these benign inflammatory and curable lesions without requirement of excision biopsy or other second-line investigations. In this study, these were reported as benign inflammatory diseases and their histopathologies was followed and were further categorized into different lesions. Cell blocks were prepared after making the required smears and were processed for histopathology [17–19].

There were 85 (20%) cases of benign inflammatory lesions, and the majority of these were of acute and chronic mastitis. "Granulomatous mastitis is a rare chronic inflammatory breast lesion that mimics carcinoma clinically and radiologically" [19–21]. There were 2 (2.3%) cases of tuberculosis; definitive diagnosis of the tuberculous mastitis was based on identification of typical histological features under microscopy and detection of tubercle bacilli on Ziehl-Neelsen stain. There were 8 (9%) patients of duct ectasia whose histological diagnosis was based on observing dilatation of major ducts which contained eosinophilic granular secretions and foamy histiocytes both within the duct epithelium and in the lumen. Two cases of fat necrosis were also reported on histopathology, characterized by "anucleated fat cells surrounded by histiocytic giant cells and foamy macrophages". Our findings are consistent with Nemenqani et al. that primary tuberculous mastitis is very rare in Pakistan [19].



(a) (b)

Figure 6: (a) Photomicrograph on FNAC of a malignant smear of Non-Hodgkin Lymphoma (H&E stain 40x). Sheets of noncohesive cells with scanty cytoplasm. (b) Comparison on histopathology of non Hodgkin's lymphoma (40x H&E).



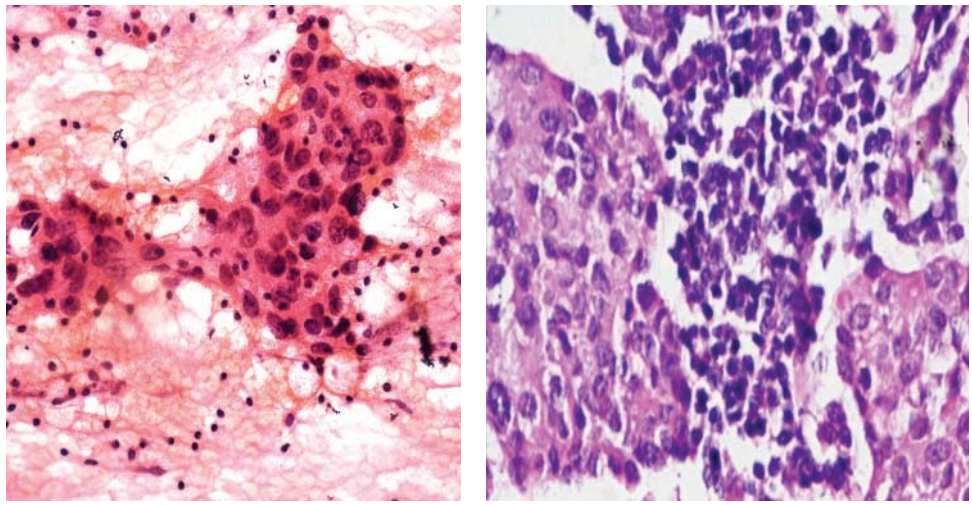
(a) (b)

Figure 7: (a) Photomicrograph on FNAC of a malignant smear's of colloid carcinoma (20x Giemsa stain) with loose clusters of epithelial cells against abundant mucin. (b) Comparison on histopathology of mucinous carcinoma breast (10x H&E).

FNAC has some pitfalls in the diagnosis of Fibrocystic disease (FCD), adenosis, epithelial hyperplasia with or without atypia, apocrine metaplasia, radial scar, and papilloma "[22]. Fibroadenoma and these benign lesions are more common in our setup. Various types of adenosis have also been described, of which sclerosing adenosis and microglandular adenosis merit detailed description and most of these lesions mimic malignant lesions" [23].

In this study, 338 FNA aspirations were correlated with histopathology to evaluate the diagnostic sensitivity, specificity, and accuracy of this diagnostic modality. Among these lesions 171 (40%) were benign proliferative lesions. In our center, we further categorized these lesions into three main groups; namely fibroadenoma, fibrocystic disease, and benign proliferative diseases. The spindle cell lesions were diagnosed as benign Phyllodes on cytology reports. These results were confirmed by histopathology from the cell blocks and tru-cut biopsies. In our study, no malignancy was seen while few discrepancies were seen in making final categories like out of 70 FNAC diagnosed FA, there were 60, 6,3, and 1 FA, FCD (When there were mixture of cysts, fibrosis, and proliferating ductal epithelium), FAN (overgrowth of both fibrous stroma, and of epithelial elements, i.e., ducts and lobules, in differing proportions), and benign Phyllodes on histopathology. From 90 FCD, there were 70 FCD on histopathology while other were 10, 5, 3, 1, and 1 cases of FA, FAN, florid epithelial hyperplasia (FEH), atypical epithelial hyperplasia (AEH), and benign Phyllodes, respectively.

In our experience, FNAC results are more reliable regarding malignant lesions; however the category of "Suspicious for Malignant Lesions" needs histopathological evaluation before performing surgical measures. Self-assessment, mammography, and tru-cut biopsy may help in the accuracy of these lesions [2].



(a) (b)

Figure 8: (a) Photomicrograph on FNAC of a malignant smear of medullary carcinoma (H&E stain at 40x) showing large to medium sized cells with large nucleoli with syncytial pattern against lymphoplasmacytic cells. (b) Comparison on Histopathology of Medullar carcinoma Breast (40x H&E).

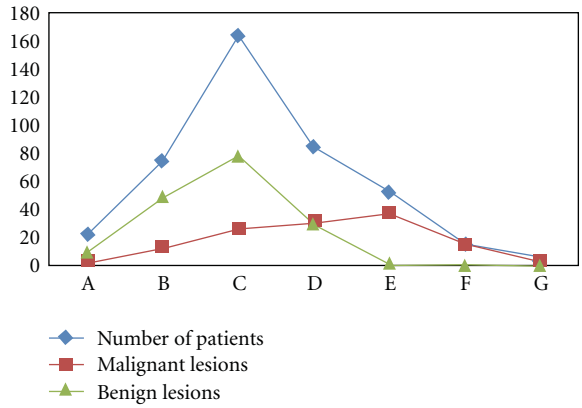


Figure 9: Correlation between Benign and malignant lesions in different Age groups of patients presented with lump breast. Note: A positive correlation was observed between Benign and malignant lesions ($r = 0.95$, $P < .0001$).

It is widely accepted that FNA is a less traumatic and easy technique than core needle biopsy because we repeated the FNAC in case of inadequate smears without any delay, difficulty, trauma, and getting highly accurate results. This statement is not applicable for open biopsy as it is a time consuming and cumbersome technique which requires fixation, processings, staining and so forth. It is also expensive procedure costing Rs 700 (9.5 USD) as compared to Rs 200 (2.5 USD) for each FNA while it is also expensive in advanced countries. In a study Rubin et al. has mentioned a saving of 1000$ with this cost effective procedures [4]. In our study the accuracy of FNA aspiration was increased by repeating the process within 24 hours and was found to be significant ($P = .000$). There were many reasons for inadequate smears like size, type of lesions, experience of the technical staff, and cooperation of patients in our study.

We have a proper and permanent FNAC modality room, onsite service, technical trained staff, expert consultants, and a large number of postgraduate students available for FNA service. We provide 90% free services; however, our charges are 1.2 USD for the affording cases. We have direct interaction with the clinicians, patients, and our laboratory technical staff. In our study, no false negative cases were reported when compared with histopathology.

Only four cases were observed as being false positives (1.7%). False positives were interpreted as "suspicious for malignancy" that were later on reported as benign proliferative lesions on histopathology. All benign and malignant reported aspirates showed 100% accuracy. In this study the sensitivity was 100%, and the specificity was only 98% because of false positives when compared with the histological reports.

There is a wide range of false results regarding the credibility of FNAC. The rate of false negatives, false positives varied from 0% to 10% in various studies [4, 24, 25]. Our results are consistent with our previous study and other studies in Pakistan and other areas. "The overall results in our previous study for sensitivity, specificity, accuracy, PPV, and NPV were 97%, 100%, 97%, 100%, and 87%, respectively" [2]. The reasons for such a large range of variable results are multifactorial and the main factors being the small number of cases of FNA published, lack of onsite service, and coordination between surgeons, radiologists and pathologists [24, 26].

5. Conclusion

The cytological examination of breast lesions prior to surgical treatment serves as a rapid, economical, and valuable diagnostic tool. Adhering to the principle of "Triple test," and

acquisition of technical, observational, and interpretative skills will further enhance the diagnostic accuracy of proliferative conditions with atypia or suspicious lesions of breast.

6. Recommendations

(1) Proper training and skilled courses should be arranged for the technical staff because it increases the reliability and accuracy of the test.

(2) No overemphasis should be made in the reporting of FNA, and very careful strict criteria should be adapted like advised repeat FNAC, cell block preparation, biopsy or correlation with clinical findings and so forth. Three tier system should be used instead of 5 classes of the breast cytology, for example, benign, suspicious, and malignant. FNAC should be repeated when inadequate and improper smears are prepared.

(3) When the results are obviously benign, patients should be reassured and can be prevented from undergoing unnecessary surgery while in the case of a clearly malignant smears, surgery and other treatment should be started without any delay.

(4) For the gray areas, for example, suspicious and inadequate smears, either repetition of the FNA or a surgical biopsy should be recommended.

Conflict of Interests

There is no conflict of interests.

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Research Article

Evaluation for Granulomatous Inflammation on Fine Needle Aspiration Cytology Using Special Stains

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Background. Tuberculosis is the commonest infectious disease in the developing world. Many diagnostic tests are devised for its detection including direct smear examination. This study was designed to determine the frequency of cases positive for AFB and positive for fungus in patients diagnosed to have granulomatous inflammation on Fine Needle Aspiration Cytology using special stains. *Materials and Methods*. A descriptive cross-sectional survey was done on 100 cases of granulomatous inflammation consistent with tuberculosis diagnosed on fine needle aspiration cytology at the Department of Pathology, King Edward Medical University, Lahore. After reporting granulomatous inflammation on Hematoxylin & Eosin staining of aspirates from FNAC, some unstained slides were subjected to special stains, like ZN, GMS, and PAS. Cases positive for AFB on ZN stain and fungus on GMS/PAS were noted down along with their frequency and percentages. *Results*. Forty-four cases (44%) of AFB positive smears were reported in granulomatous inflammation while only 5% cases of fungus were reported down. Cervical lymph nodes were the most commonly involved site (87%), and females were affected more (62%) than males. Most cases of AFB-positive smears were associated with caseation necrosis (93%). *Conclusion*. Special stains should be done on all granulomatous inflammation cases seen on FNAC for confirmation of TB and ruling out other infectious causes.

1. Introduction

Tuberculosis is playing havoc throughout the world, and this is especially true for the developing countries. Every year 8 million new cases are seen and 2 million deaths occur because of Tuberculosis [1]. In Pakistan, the estimated incidence of Tuberculosis is 181/100000 [2]. Tuberculosis (TB) carries a high risk of morbidity and mortality. TB has widespread involvement and rarely any tissue or organ is not involved by it. Most common is the pulmonary involvement [3] which has caused numerous deaths in the past. It can also involve the appendix [4], small and large intestine [5], skin [6], soft tissues, lymph nodes [7], genitourinary tract [8], and brain [9]. The dilemma does not end here and many other unusual organs are also involved [10].

The histology of TB is a characteristic showing granuloma formation by epithelioid histiocytes and Langhan's type Giant cells with or without caseation necrosis. This pattern is also preserved somehow in cytology specimens [11]. Infectious causes most notably presenting with granulomatous inflammation is Mycobacterium Tuberculosis with a reported frequency of 59.4% [11] and fungal causes [12, 13] with a reported frequency of 20.4% [14]. Other common causes include Sarcoidosis [15], Wegener's granulomatosis [16], Actinomycosis [17], Crohn's diseases [18], Histoplasmosis [19], foreign body, and Langerhans cell histiocytosis [20].

Pertaining to a broad differential diagnosis, the diagnosis of tuberculosis remains a challenge. History and clinical examination are always very helpful. Many diagnostic tests are in practice. Every test has its own sensitivity and specificity and limitations. The commonly performed tests include examination of sputum for Acid Fast Bacilli [21], Cultures for Mycobacterium tuberculosis [22], Fine Needle Aspiration Cytology (FNAC) [23], Biopsy, and PCR [24].

Fine Needle Aspiration Cytology is a minimally invasive and time-saving procedure, which helps in the diagnosis of number of diseases especially in palpable nodules of

breast, lymph node disorders [25], thyroid [26], and palpable skin and subcutaneous nodules. It has become very popular nowadays among physicians and surgeons because of its benefits. In clinical practice, it helps them to reach a diagnosis or at least plan beforehand the proper management of the patient. As we have already discussed that granulomatous inflammation is not diagnostic of TB, many others causes must be ruled out before giving ATT. However, in the clinical scenario if a patient is diagnosed as granulomatous inflammation, then antituberculous treatment (ATT) is started at the first point in our setup. Statistically this behavior may be right but this is not in accordance with the reality. We come to encounter cases which have taken ATT for at least 9 months but still these symptoms persist. Reassessment is done, and later the patient is diagnosed as suffering from fungus, sarcoidosis, or some other granulomatous disease. Some special stains are very helpful in this regard, like Gomori Methenamine silver stains (GMS), Giemsa stain, Periodic acid Schiff (PAS), and Zeihl Neelson's stain (ZN stain) [27].

In the present study, granulomatous inflammation consistent with Tuberculosis diagnosed on FNAC will be analyzed using special stains like ZN (Ziehl Neelson's) and GMS (Gomori Methenamine Silver) stains. This will help to confirm tuberculosis in cases which will be positive for Acid Fast Bacilli on ZN staining. Positive GMS/PAS staining will confirm in the fungal causes of granulomatous inflammation including Mucormycosis, Blastomycosis, Cryptococcosis, and Candidiasis. There is a limitation of this study that not all causes of granulomatous inflammation can be ruled out since the ancillary investigations needed to diagnose them are not available in our setup.

The rationale of this study is that cases diagnosed wrongly as TB can turn out to be fungus and can be picked by GMS/PAS. These patients can thus be saved from long painful and harmful side effects of expensive ATT (Antituberculous therapy). This benefit alone is worth mentioning for the usefulness of this study, and this would be further reaffirmed by those who have experienced taking ATT for 9 months in their life without having TB. On ZN staining, the positive AFB cases would help the physicians to start treatment of TB, very confidently. Moreover, the work done to assess the frequency of different infectious agents in granulomatous inflammation especially fungal causes is very old, and this study would bridge a gap between newer studies done on this topic.

2. Material and Methods

2.1. Setting. The study was conducted at Pathology Department of King Edward Medical University and Mayo Hospital Lahore. The department receives 10,000 surgical specimens and 3000 cytology specimens including FNAC annually.

2.2. Duration. Six months.

2.3. Sample Size. Sample size of 100 cases was calculated with 95% confidence level, 8% margin of error, and taking expected percentage of positive cases of fungus on GMS/PAS that is, 20.4% in diagnosed cases of granulomatous inflammation.

2.4. Sampling Technique. Nonprobability purposive sampling.

2.5. Inclusion Criteria

(1) Cases diagnosed on FNAC as granulomatous inflammation consistent with tuberculosis as per operational definitions.

(2) Cases in which FNAC was done on Lymph node, skin swellings, subcutaneous swellings, and Lung masses

2.6. Exclusion Criteria

(1) Pyogenic inflammation seen on microscopy as extensive neutrophilic infiltration.

(2) Acellular smears/smears with crushed morphology or poorly stained slides will be excluded.

(3) Previously diagnosed cases and cases already getting ATT.

2.7. Study Design. Descriptive cross-sectional survey.

2.8. Operational Definitions

2.8.1. Granulomatous Inflammation. It is defined on cytology as aggregates of epitheloid cells forming a granuloma with or without necrosis. Sometimes multinucleated giant cells are also seen.

2.8.2. Positive for AFB. On ZN staining the acid fast bacilli would be labeled when we find pink, beaded, and rod-shaped organisms after comparing with control samples.

2.8.3. Positive for Fungus. On GMS staining, presence of black colored septated or nonseptated hyphae (depending upon the species of Fungus) or spores against a greenish background would be labeled as positive for fungus. On PAS stain, presence of red- or purple-colored septated or nonseptated hyphae or spores would be labeled as positive for fungus.

2.9. Data Collection Procedure. Patients fulfilling inclusion and exclusion criterion were selected from Fine needle aspiration cytology specimens received during the study period. After informed consent of patients and noting down the demographic data, the *hematoxylin* and Eosin staining was done. Two extra unstained slides were smeared from aspiration material. One slide was stained by GMS stain. Some cases were stained with Periodic Acid Schiff stain (PAS). Steps of PAS staining are as follows: similarly 2nd unstained slide was stained with Ziehl Nelson's stain. Commercially available positive and negative controls of ZN

Table 1: Distribution of age of patients.

Age of patient (years)	Granulomatous inflammation	% of granulomatous inflammation
1–10	3	3
11–20	44	44
21–30	31	31
31–40	11	11
41–50	8	8
51–60	2	2
61–70	0	0
71–80	1	1
Total mean = 25.14 ± 12.75	100	100

Table 2: Distribution of gender of patients.

Gender	Frequency	Percent (%)
Female	62	62
Male	38	38
Total	100	100

and GMS were used to compare and measure the consistency of staining technique. These smears were examined under the light microscope by a histopathology's. The findings of Hematoxylin and eosin staining were categorized as epithelioid granuloma with necrosis and epitheloid granuloma without necrosis. The finding of ZN staining was labeled as positive for AFB or negative for AFB. The finding of GMS was recorded as positive for fungus or negative for fungus.

2.10. Data Analysis. Data was analyzed by SPSS version 10. Age of patient was presented as mean and standard deviation. Gender, positive cases of AFB, and positive cases of fungus were presented as frequency and percentages.

3. Results

One hundred patients of granulomatous inflammation diagnosed on FNAC were taken. Granulomas were described as comprising of pale staining epithelioid cells which were round to oval to spindle against an eosinophilic background (Figure 1). Few degenerated epithelioid histiocytes were also seen in long-standing mycobacterial infection with caseation necrosis in the background (Figure 2). On Ziehl Neelson's staining, mycobacterium tuberculosis appeared as red/pink beaded rod-shaped bacteria against a blue background (Figures 2(a) and 2(b)). On PAS staining, fungus appears as purple hyphae which were segmented or nonsegmented depending on the species. Few spore forms with budding were also seen (Figure 2). On GMS stain, fungal hyphen appeared as black-colored forms which showed segmentation and some were nonsegmented (Figure 2).

In this study, 78% patients were below 30 years of age (Table 1). Mean age was 25.14 with standard deviation of 12.745. Females were affected more (68%) than males

Table 3: Frequency of positive smears of acid fast bacilli.

Acid fast bacilli	Frequency	Percent
Positive for AFB	44	44
Negative for AFB	56	56
Total	100	100

Table 4: Frequency of fungus and granulomatous inflammation.

Fungus	Frequency	Percent
Negative for fungus	95	95
Positive for fungus	05	05
Total	100	100

(Table 2). 44 out of 100 patients of granulomatous inflammation are positive for AFB (Table 3). There was an association between AFB positivity and caseation necrosis. We have found 41 out of total 44 AFB positive cases (93%) with caseation necrosis (Table 6), while 60% cases of fungus were related to caseation (Table 7). No definite relationship was seen between AFB and giant cells since 19 out of total 44 AFB positive cases were seen with caseation while rest 57% were without giant cells. Another finding was the involvement of specific lymph nodes regions. In 87% of cases, the most commonly involved group of lymph nodes was cervical lymph node (combining cervical and supraclavicular lymph nodes). If per auricular lymph nodes were included, then in 93% of cases the head and neck was the primary site of TB involvement (Table 5).

4. Discussion

Accurate and timely diagnosis together with effective TB treatment is the mainstay of TB care and control. A confirmed diagnosis of TB can only be given on isolating the M. tuberculosis or finding specific DNA sequence of the bacteria in aspirates. In the resource-poor countries, however, these tests are not within the reach of every individual. In these countries, cost-effective techniques for example, sputum smear microscopy and morphological features are the corner stone of TB diagnosis. In cases of extra pulmonary tuberculosis, fine needle aspiration cytology (FNAC) is a very useful and reliable test. In areas where tuberculosis is prevalent, diagnosis of TB can be made by seeing the morphological features. Granulomatous inflammation is the common histological presentation of tuberculosis. However, there are many other infectious and noninfectious causes which can lead to granulomatous inflammation. Second important infectious cause of granulomatous inflammation is fungus. In the present study, we tried to differentiate between granulomatous inflammation caused by TB and fungus, by using special stains.

Blind FNAC can approach safely the superficial lesions, including lymph nodes, skin, and soft tissue nodules. In our study, 98 (98%) cases were from lymph nodes. Many studies have diagnosed TB by aspiration form lymph nodes [7, 11, 26, 28, 29]. Cervical lymph node was the most

Table 5: Distribution lesions according to site of FNAC.

Site of FNAC	Frequency	Percent	Valid percent	Cumulative percent
Cervical lymph node	72	72.0	72.0	72.0
Peri-auricular lymph nodes	6	6.0	6.0	78.0
Supraclavicular lymph node	15	15.0	15.0	93.0
Axillary lymph node	3	3.0	3.0	96.0
Inguinal lymph node	2	2.0	2.0	98.0
Skin or subcutaneous lesion	1	1.0	1.0	99.0
Other sites	1	1.0	1.0	100.0
Total	100	100.0	100.0	

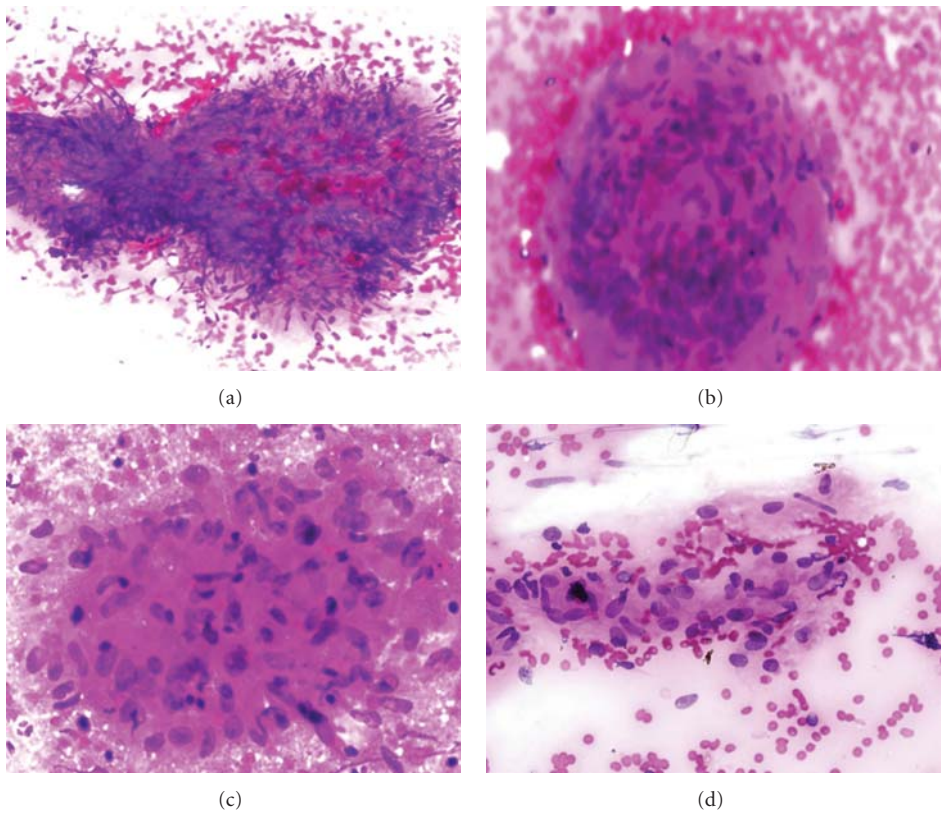

Figure 1: (a) Photomicrograph showing degenerated granulomas with caseation necrosis (H & E stain, 100x), (b) Granulomatous inflammation on FNAC (H & E stain, 200x) giant cells, (c) Granulomas comprising of epithelioid histiocytes with caseation necrosis (H & E stain, 200x), (d) Aggregates of pale staining epithelioid histiocytes (H & E, 400x).

Table 6: Relationship of acid fast bacilli with caseation necrosis.

	Acid fast bacilli		Total
	negative for AFB	positive for AFB	
Caseation necrosis			
Not present	28	3	31
Present	28	41	69
Total	56	44	100

Table 7: Frequency of caseation necrosis with fungus.

	Fungus		Total
	negative for fungus	positive for fungus	
Caseation necrosis			
Not present	29	2	31
Present	66	3	69
Total	95	5	100

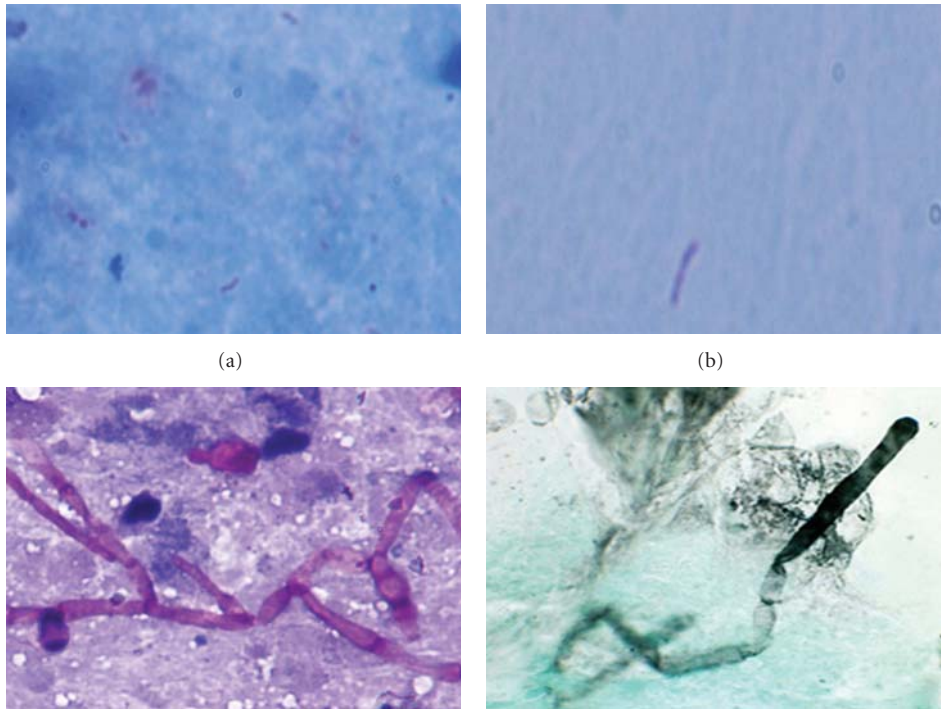

Figure 2: Photomicrographs in (a) Acid Fast Bacilli (Ziehl Nelson's stain, 400x), (b) Pink beaded rod against a blue background (ZN stain, 1000x), (c) Septated fungal hyphae and budding spore (at the top of photomicrograph), (PAS, 400x), (d) Black fungal hyphae against greenish background (GMS, 400x).

Table 8: Frequency of acid fast bacilli with giant cells.

	Acid fast bacilli		Total
	negative for AFB	positive for AFB	
Giant cells			
Not present	50	25	75
Present	6	19	25
Total	56	44	100

common site of involvement in studies followed by axillary lymph nodes [11, 21]. Our study was also consistent with above studies in terms of cervical lymph node involvement (87%) as the most common anatomic site of granulomatous inflammation. Periauricular lymph nodes were involved in 6% cases in our study and was the second most commonly involved. Female gender was a slightly more affected (62%) in current study and was in concordance with other studies [26]. However, there was slight male predominance in a study of Bezabih et al. [11]. Out of 100, 47% patients in this study were of 20 years or below and 62% were below 30. This finding was in accordance with Bezabih et al. in which 69% were below 30. Based on the facts, it can be inferred that tuberculosis was more commonly seen in young population [11].

One case of granulomatous inflammation was from skin (Table 5). Few studies from India have also discussed this aspect [30]. Numerous morphological variations in the granulomatous inflammation are seen. There were 69% cases with necrosis. The rest (31%) of cases were granulomatous inflammation without necrosis. The various morphological presentations of TB have been published locally [7]. International data also supports this variation and studies tried to correlate morphological findings with the AFB staining [11, 31].

The Acid Fast Bacilli positivity was labeled after finding red or pink rod-shaped bacteria with beaded appearance (Figures 2(a) and 2(b)). Regarding AFB positivity variable, results were seen and frequency ranges from 10% to 70% [26, 27, 30, 31].

In current study, out of 100 cases, 44 cases were positive for AFB (44%). This was in concordance with the international data of a large-scale study of 328 cases, out of which 152 cases (46.4%) were positive for AFB [21]. Similarly, our findings agree with Lau et al. who report 47% sensitivity for tuberculous abscess cases [26] and with Das et al. showing overall 45.8% rate of AFB positivity [32]. A study conducted in India shows an overall 27% AFB positivity [31], and the reason for this low AFB sensitivity was given: studies with higher AFB have adult subjects, in whom open tuberculosis and necrotic lesion were far more common. Examples of low yield of AFB were also due to treatment with antituberculosis drugs and presence of very few bacilli in the lymph node [31]. Some studies report very high frequency of AFB positivity. Bezabih et al. reported 59.4% of overall AFB positivity [30], and Vignesh et al. reported 53.3% sensitivity for single AFB smear [27].

In regions where TB is very common, the morphological findings of granulomatous inflammation is consistent with tuberculosis [30, 31]. Pakistan is also included among these countries along with India, Ethiopia, and other African countries. Since epithelioid granulomas, caseation necrosis, giant cells, and AFB positivity are specific for TB, so in these countries excision biopsy can be avoided and antituberculous treatment can be given straightaway [26]. Excision is not free of complication and is expensive and time consuming, thus it can delay the treatment. Above findings conclude that FNAC with special stains can solely help the physician to start the treatment.

There was an interesting finding in our study. AFB positivity was notablely and more commonly found in granulomatous inflammation with caseation necrosis. 41 out of 44 AFB positive cases associated with caseation necrosis (93%), in current study. This finding is consistently seen in previous studies [11, 21, 31]. Otherwise in some studies, it is claimed that instead of granulomatous inflammation, if only necrosis or abscess formation is seen, the AFB-positivity increases [26]. Dua et al. even documented 100% of AFB positive cases in this scenario [31]. Since in the inclusion criterion of our study we only selected cases with granulomatous inflammation with or without necrosis, but not cases only with necrosis, this aspect cannot be discussed in this study. Most of studies improved the technique of finding AFB by using fluorescence microscopy. They claimed at least 10% improvement in sensitivity and sensitivity if fluorescence microscopy is used as compared to direct smear examination [33, 34]. However, in resource-poor countries it would still take some time to gain wide acceptance.

Another interesting finding was that an acid fast bacillus was usually found extracellularly. Usually areas of microscopic degeneration, within or at the periphery of the granulomas, were most the common location to find AFB [21]. The morphology of these bacilli was short and stumpy rods with red beaded appearance. These findings correlated with those given by Rajasekaran et al. [35] and Ahmad et al. [21]. For early lesions of tuberculous lymphadenopathy, there is no evidence that chemotherapy (ATT) plus excision is superior than chemotherapy alone [26]. Moreover, the excision biopsy in tuberculous lymph nodes is hazardous since it may cause sinus formation. Therefore, FNAC finding of granulomatous inflammation and detection of AFB would be very specific and help the physicians to start ATT confidently, immediately as it is cost effective and economical.

The special stains GMS and PAS were used to detect the fungus, since it may present with same morphology as TB [14, 17, 36]. In this study, we found 5% cases of fungus presenting with granulomatous inflammation. After extensive search of the literature, only one study was found in which 20.4% cases of fungus occurred among 245 subjects [14]. Yet many other studies discussed fungus as a cause of granulomatous inflammation and published them as case reports [14, 36–39]. But these studies did not mention frequency or percentage of positive case of fungus. In this regard, the present study would bridge a gap and may become a source of future reference for further studies in this aspect. The main benefit we gained from this study was that these patients were diagnosed morphologically as "consistent with tuberculosis". However, the results via special stains established that it can be caused by fungus and not only by mycobacterium tuberculosis. Added benefit is that these patients would be safe from harmful side effects of prolonged ATT treatment. They can get antifungal treatment, and the disease can be cured. In this study, we did not classify species of fungus on these special stains for it may not be accurate. For this purpose, fungal cultures should be performed.

Recommendations

(1) Every case of granulomatous inflammation seen on aspiration cytology should be subjected to special stains like ZN, GMS/PAS. It would increase the diagnostic accuracy of this technique and help to differentiate between different infectious causes which can present with the same morphology.

(2) When physicians are confronted with enlarged lymph nodes, the node may be punctured with a sterile disposable needle, and if cheesy material is aspirated then the physician can strongly consider tuberculous adenitis in areas where tuberculosis and immunodeficiency states are rampant and pathology services are lacking.

(3) Patients who are not responding to empirical ATT should be considered for other causes of granulomatous inflammation other than TB, and proper workup should be done.

Limitation of Study

(1) This study does not include comparison with histology and other microbiological detection methods like culture and PCR, because of cost and unavailability issues.

(2) Our study did not comment on all the possible differential diagnosis of granulomatous inflammation, which requires sophisticated techniques and tertiary care laboratory services which are currently not available in our setup.

Future Studies. On the current issue, future studies should include comparison of direct smear microscopy of AFB with fluoroscopic evaluation. Moreover, the aspirate of FNAC should be subjected not only for special stains, but also for immunohistochemical stains, culture and PCR, and then compared for efficacy.

5. Conclusion

Fine needle aspiration cytology (FNAC) is very important investigation in the diagnosis of granulomatous inflammation. If it is supplemented with special stains like ZN, GMS, and PAS, it may help to differentiate between many infectious causes of granulomatous inflammation.

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Review Article

Fine-Needle Aspiration Biopsy of Hepatocellular Carcinoma and Related Hepatocellular Nodular Lesions in Cirrhosis: Controversies, Challenges, and Expectations

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The role of hepatic fine-needle aspiration (FNA) biopsy has evolved. Advances in imaging modalities have obviated the need for tissue confirmation in most hepatocellular carcinomas (HCCs). There is risk of needle-tract seeding. Increasingly, small nodules are being detected on ultrasound surveillance of high-risk patients. Diagnostic challenges associated with cirrhosis include distinction of benign hepatocellular nodules, namely, large regenerative nodules and dysplastic nodules, from reactive hepatocytes; and distinction of well-differentiated HCCs from benign hepatocellular nodules. This paper will discuss (i) controversies regarding preoperative/pretransplantation FNA diagnosis of HCC, (ii) update of biological evolution, nomenclature, and histopathologic criteria for diagnosis of precancerous nodules and small HCCs, and (iii) algorithmic approach to FNA diagnosis of hepatocellular nodules. Optimal results depend on dedicated radiologist-cytopathologist team, on-site cytology service; combined cytohistologic approach, immunohistochemistry, and clinicopathologic correlation. Hepatic FNA is likely to be incorporated as a point of care as we move towards personalized medicine.

1. Introduction

The incidence of hepatocellular carcinoma (HCC) has risen as a result of increased global burden of chronic liver disease due to hepatitis B and C virus infections, aflatoxin B1, alcoholism, and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis associated with the metabolic syndrome. It is now the sixth most common cancer worldwide and the third most common cause of cancer deaths [1]. The natural history in high-risk patients is the occurrence of dysplastic foci in a cirrhotic background from which precancerous dysplastic nodules may ensue with some transforming to become HCC [2, 3].

Much progress has been achieved over the years with regard to detection/screening, diagnosis, surveillance, and multimodal treatment approaches leading to improvement in prognosis of HCC [4, 5]. Surgery, and in particular liver transplantation, is considered the best option; but this is not widely available, and many patients still present with advanced disease. Increasing knowledge of molecular hepatocarcinogenesis has led to the advent of molecular targeted therapy [6]. Sorafenib, the antiproliferative and antiangiogenic multikinase inhibitor, has broadened the therapeutic horizon for advanced cases [5].

Early detection with appropriate therapy is still the optimal approach that offers the patient the best prognosis. High-risk patients undergo 6-monthly ultrasound screening and serum alpha-fetoprotein (AFP) assessment. Advances in dynamic imaging techniques have increased the accuracy of HCC diagnosis thus obviating the need for tissue confirmation [7–9]. The role of fine-needle aspiration biopsy (FNA) in the diagnosis of hepatocellular nodular lesions has evolved over the years. Smaller and smaller nodules are being detected on increasing surveillance of high-risk patients. Accurate tissue characterization of small well-differentiated hepatocellular nodular lesions (<2 cm) is very challenging and has significant therapeutic implications.

There are two schools of thought with regards to preoperative/pretransplant FNA diagnosis of HCC. An update of the biological evolution and histopathologic criteria for the

diagnosis of dysplastic nodules, small HCCs and "nodule-in-nodule" lesions is presented in tandem with clinically relevant nomenclature. An algorithmic approach to FNA diagnosis of HCC and hepatocellular nodular lesions is outlined. Current issues, controversies, challenges and future expectations are discussed. The focus is on hepatocellular nodular lesions associated with cirrhosis.

2. Fine-Needle Aspiration Biopsy from the Point of View of the Aspirator and Reader

"*Can indeterminate diagnoses of highly well-differentiated hepatocellular nodular lesions be reduced?*"

2.1. The Technique. There are various routes for performing FNA biopsy of the liver. Percutaneous (transabdominal) FNA biopsy performed under computed tomography (CT) or ultrasound (US) guidance has been adopted worldwide as a safe, efficient, and minimally invasive procedure for the diagnosis of focal liver lesions. It is useful for establishing inflammatory/infective conditions to ruling out or confirming malignancies, helping to distinguish primary from secondary lesions. This technique is especially advantageous in patients with advanced malignancies or who are poor surgical candidates. It can also be performed at laparoscopy or laparotomy under direct vision. The sensitivity and specificity of percutaneous FNA biopsy for detection of liver malignancy have been reported to be around 90% (range, 67%–100%) and 100%, respectively [9–11]. Sensitivity varies according to factors such as blind versus guided aspiration; number of passes; operator skill; size, location and consistency of the lesion; quality of smears; combined cytohistologic studies with ancillary testing; and reader expertise. The positive and negative predictive values and overall accuracy of FNA diagnosis for liver malignancy were reported in one large study to be 100%, 59.1%, and 92.4%, respectively [9]. False positives are rare.

Endoscopic ultrasound-guided FNA (EUS-FNA) is the latest diagnostic and staging tool. It is safe, accurate, and versatile but highly operator dependent. EUS-FNA can access left lobe of liver, hilum, proximal right lobe, gallbladder, extrahepatic biliary system, and perihilar lymph nodes. It is especially useful for small and deep-seated left lobe lesions below CT/MRI resolution or not easily accessible to percutaneous FNA. As such, it enhances staging of liver metastases [12], and facilitates early detection of multifocal HCC in cirrhosis, thereby allowing for accurate assessment of number of lesions (intrahepatic staging of HCC) for transplantation eligibility purposes [13, 14]. Another advantage is concurrent sampling of pancreas and liver lesions, confirming primary and metastatic malignancy in one single diagnostic encounter. EUS-FNA has high sensitivity (82%–94%) and specificity (90%–100%) for malignancy [15–17]. However, as with percutaneous FNA, it gives a better diagnostic yield with metastases than with well-differentiated hepatocellular nodular lesions.

The needle size is between 20 to 22 gauge. Aspiration needles with cutting mechanism enable microbiopsy cores to be procured. In some instances, FNA samples can be more representative than wide-bore core needle biopsies because although the cores are broader they are shorter, and the core biopsy procedure is limited in flexibility and accessibility. With fine needles, multiple aspirations (up to four passes) can be safely performed in different directions, provided there are no contraindications.

2.2. Tissue Samples. The types of tissue samples obtained with FNA technique include smears, needle rinse samples, cell blocks, and microbiopsies from which core imprints can be made. Smears are air-dried and stained with Diff-Quik and/or May-Grünwald-Giemsa (MGG) as well as fixed in 95% alcohol and stained by the Papanicolaou method. To overcome paucity of histologic material, particulate material is quickly retrieved from the glass slides with a scalpel prior to staining and formalin-fixed for paraffin-embedded cell block preparation. The resultant histologic sections allow for study of architecture and for special stains and immunohistochemistry. Immunocytochemical studies may be necessary if only smears are available.

Liquid-based cytology (LBC) of fine needle aspirates of HCC is not routinely accepted. Potential advantages include: (i) the aspirator can work alone putting the entire specimen into preservative solution, thus solving the problem of centralized pathology services receiving highly variable quality of sent-in smears; (ii) the smears are more representative as everything is collected on one slide with a portion stored in the permanent preservative solution for re-use when needed; (iii) multiple smears and cell block can be prepared for routine staining, immunostaining, and molecular tests; (iv) better preservation of cells; (v) shorter screening time as cells are concentrated in a 20-mm round area; and (vi) no interference from background elements [18–20]. Disadvantages include: (i) no on-site assessment of adequacy (unless split-sample method or double-aspiration protocol employed), (ii) no triage of specimens, (iii) no air-dried smears for Giemsa preparations, (iv) semiautomated processing requiring longer preparation time, (v) loss of information from removal of background elements, (vi) familiarity with cytologic artifacts and recognition of diagnostic pitfalls to avoid misinterpretations, and (vii) high cost. Under certain circumstances, smears of FNA material prepared by ThinPrep method may not be representative. It has been postulated that larger cell groups may settle out of the specimen so rapidly that they are not sucked onto the filter; or the groups may drop off the ThinPrep filter immediately after suction is removed and not be transferred to the prepared slide.

In the author's experience (unpublished data) with ThinPrep method for aspirates of HCC, the background tends to be devoid of red blood cells, debris, and bile; however, tumor diathesis, if excessive, is still discernible. The cellularity is much lower per slide than in conventional smears. The cell aggregates tend to be tighter, 3-dimensional, and decreased in size with partial/complete loss of cytoarchitecture, such as, arborizing trabecular structures, pseudoacinar rosettes, and peripheral and transgressing endothelium. Tumor cells appear generally smaller with nondescript/"angulated" shapes, denser cytoplasm, and frayed cell borders. Chromatin details, nucleoli, and intranuclear inclusions are more

defined but difficult to appreciate in cells that are not monolayered. Cell blocks can be prepared from liquid-based specimens with reasonable quality; however, integrity of the trabecular-sinusoidal arrangement may be compromised. Immunocytochemistry can be successfully applied to thin-layer cytology slides [20]. However, just as in conventional smears, immunocytochemical interpretation can be an issue, especially in smears with low yield, dispersed cellularity, and obscured nuclear details. The general impression is that it is more challenging to decipher the cytologic characteristics of HCC in LBC material than on conventional smears.

2.3. Diagnostic Challenges. The diagnostic challenges in FNA of focal liver lesions are (i) distinction of benign hepatocellular nodular lesions, namely, large regenerative nodule, dysplastic nodule, focal nodular hyperplasia, and hepatocellular adenoma from reactive hepatocytes; (ii) distinction of well-differentiated HCC from benign hepatocellular nodular lesions; (iii) distinction of poorly differentiated HCC from cholangiocarcinoma and metastatic carcinomas; (iv) determination of histogenesis of malignant tumor; and (v) determination of site of origin of malignant tumor [21]. At the well-differentiated end of the spectrum, cytohistologic features of malignancy are lacking whilst at the poorly differentiated end, clues to the cell lineage are found wanting (see Section 6).

2.4. Ways to Improve Accuracy Rates of FNA Biopsy Diagnoses. Optimal results are obtained with dedicated and experienced team comprising aspirator (radiologist- or endosonographer) and reader. An on-site cytology service with a cytopathologist or well-trained cytotechnologist is ideal [22, 23]. The service provides for rapid Diff-Quik-stained smears for assessment of sample adequacy, retrieval of particulate tissue for cell blocks, and triage of specimens for culture, flow cytometry and other ancillary tests, including molecular analysis. A combined cytohistologic approach is highly recommended with cell blocks and microbiopsies [24–26]. Immunohistochemistry has a great adjunctive role. The final diagnosis should be based on close clinicopathologic correlation. Resources should be provided to support training of cytotechnologists as well as for continuing professional development.

3. Fine Needle Aspiration Biopsy from the Point of View of the Hepato-Oncology Team

"To perform or not to perform FNA biopsy for confirmation of diagnosis?"

The last decade has seen much active debate over the role of FNA in the detection of HCC [7, 9, 27]. Advances in dynamic imaging techniques have increased the accuracy of HCC diagnosis. Recognition of the diagnostic value of contrast washout allowed for the refinement of the European Association for the Study of Liver 2000 Conference (EASL) guidelines and is reflected in the American Association for the Study of Liver Disease (AASLD) guidelines [7, 8]. Nodules larger than 2 cm occurring in cirrhotic livers are diagnosed as HCC if they show characteristic intense arterial profile with contrast washout in delayed venous phase on one dynamic imaging modality. Nodules measuring between 1 and 2 cm in cirrhotic livers require concurrence of two coincidental imaging modalities; otherwise, biopsy is recommended. The dilemma is whether to biopsy nodules < 1 cm. The EASL guidelines recommend the "wait and see" policy with 3-monthly US surveillance.

Those who oppose the performance of FNA biopsy cite the following reasons.

(1) *Advances in Dynamic Imaging Techniques are Sensitive and Accurate Enough for Establishing an HCC Diagnosis in most Nodules.* Advances in dynamic imaging modalities, such as contrast-enhanced US and dynamic magnetic resonance imaging (MRI), have yielded an accuracy, sensitivity, and specificity of 99.6%, 100%, and 98.9%, respectively, for the diagnosis of HCC [28]. The demonstration of an intensified contrast-enhanced arterial phase followed by delayed venous washout pattern is pathognomonic of HCC, thus, obviating the need for tissue confirmation. However, false positives do occur. According to Ghittoni et al, a diagnostic protocol based on imaging is like "a boat that leaks like a sieve." Intrahepatic cholangiocarcinoma may occasionally have a hypervascular imaging pattern mimicking HCC [29]. False negatives are more likely to happen, especially with insufficient neoarterialization of small hepatocellular nodules. Many of these nodules may prove to be malignant in the long run.

(2) *Adopt the "Wait and See" Policy for Hepatocellular Nodules Measuring <1 cm.* The current EASL and AASLD guidelines are to biopsy nodules between 1 to 2 cm if no definitive diagnosis of HCC is reached on two coincidental imaging modalities, and to adopt the "wait and see" policy with more frequent US surveillance for nodules <1 cm [7, 8]. Small well-differentiated hepatocellular nodular lesions associated with cirrhosis range from large regenerative nodule, to low- and high-grade dysplastic nodules and small HCCs (early and progressed types) [30]. Studies on the biological evolution of small HCCs reveal that they tend to start off as dysplastic foci of hepatocytes exhibiting large cell or small cell change. Large cell change is detected in up to 81% of cirrhotic liver explants [31]. The incidence of small cell change in cirrhotic livers varies considerably ranging up to 50% in explants [31]. These abnormal foci may develop into low-grade (with large cell change) and high-grade (with small cell change) dysplastic nodules. The precancerous nature of small cell change is supported by high proliferative activity in the hepatocytes and morphologic resemblance to early HCC [2].

Malignant transformation may occur in dysplastic or even regenerative nodules giving rise to nodule-in-nodule lesions. High-grade dysplastic nodules become malignant in a third of cases [32]. Cytodiagnosis of these small nodules and the distinction of high-grade dysplastic nodule from early HCC are extremely challenging even with the aid of reticulin stain and novel immunohistochemical markers. Although the specificity of FNA for HCC is close to 100%, its negative predictive value is low. Hence, patients with negative

biopsy findings should either undergo a second biopsy or enhanced surveillance [33].

It is the opinion of Caturelli et al that the prognostic implications of early diagnosis and treatment of HCC cannot justify this policy of "masterly inactivity" [27]. Firstly, it is incongruous to work on increasing detection of small nodules in high-risk patients and then recommend "masterly inactivity." Secondly, more than half of the nodules < 1 cm in cirrhotic livers prove to be HCCs (68%). Thirdly, US-guided FNA biopsies of hepatic nodules <1 cm in experienced hands with use of novel biomarkers and interpreted by expert reader yield correct diagnoses in about 90% of cases [34].

(3) *Risk of Needle-Tract Seeding*. The most contentious complication cited by detractors of the technique is the risk of needle-tract seeding turning a potentially operable case of HCC to a metastatic state [28]. Risk of implantation metastases after biopsy for malignancy in general is considered rare (0.003–0.009%); the incidence for HCC varies from 0.003% to 5% [34–36]. An overall incidence of 0.13% of HCC with soft tissue metastases was reported in one large study where a total of 18,227 person-times of FNA or percutaneous ethanol injection was performed on HCC patients [37]. The estimated rates of 18 and 22 G needle-induced seeding for HCC were 0.60% and 0.11%, respectively [37]. The interval between detected seeding and biopsy varies from several months to 3 years. Whilst some studies have shown that preoperative FNA has no statistically adverse effect on the operability, possibility of tumor spread, or long-term survival of HCC patients [38, 39], there are others which strongly maintain that pretransplant FNA diagnosis of HCC is not necessary [40, 41]. Seeding is usually noted with subcapsular tumors and those of high-grade malignancy; these tend to be tumors >2 cm. Hence, FNA of nodules 1 to 2 cm may be fairly free of seeding, and if these turn out to be HCC, they tend to be well differentiated.

(4) *Risk of Intraperitoneal Bleeding*. The major cause of death after percutaneous FNA is bleeding, mostly associated with severe cirrhosis with coagulopathy or large superficial tumors not covered by normal liver parenchyma [9]. A mortality rate of 0.018% was reported in a multi-institutional Italian series of 10,766 US-guided FNA biopsies [42]. Risk of bleeding is not a controversial contraindication.

(5) *Increased Risk of Tumor Recurrence and Posttransplantation Recurrence*. There is no clear evidence that, independent of tumor stage, patients who undergo FNA biopsy are at higher risk of tumor recurrence and posttransplantation recurrence due to biopsy-induced hematogenous dissemination of tumor cells [34, 36, 43]. It is possible that microinvasive tumor cell dissemination may have occurred prior to the procedure. Metachronous tumors may also arise from the residual oncogenic cirrhotic liver [4].

Those in favor of performing preoperative/pretransplant FNA cite the following reasons:

(1) *Serum AFP Has Low Sensitivity*. Serum AFP is the most commonly used serum biomarker in conjunction with US in screening programs. It has low sensitivity (45%) [44], and the level has to be significantly elevated (400 ug/L) to be of any value as a screening tool. It is also usually not elevated in patients with small HCCs. Hence. It is prudent to perform FNA so as not to miss an early case of HCC. Development of novel serum surrogate markers, such as, glypican-3 (a membrane proteoglycan) could prove useful [45].

(2) *To Allay Patient Anxiety Once a Liver Nodule Has Been Detected on Imaging*.

(3) *To Cut Down on Costs of Long-Term Imaging Surveillance in the Long Run*.

(4) *To Avoid a Futile Transplantation*. False-positive results from imaging techniques have occurred. The conundrum is to balance the risk of unnecessary surgery (2.5%) [28] against the risk of needle-tract seeding. The risk of seeding is overall lower than that of a futile transplantation with its attendant risks and life-long financial and medical issues.

(5) *Eligibility for Liver Transplantation*. Liver transplantation provides the best overall outcome in that it removes the possibility of metachronous lesions in a cirrhotic liver and restores liver function. Pretransplantation biopsy is strongly recommended for transplantation listing, if HCC is the only reason for transplant in a compensated cirrhotic case [33]. In fact, the confirmation of HCC favorably alters the patient's candidacy for liver transplantation. A previous biopsy should not be considered a contraindication for transplantation. Although the specificity of FNA for HCC is close to 100%, its negative predictive value is low. Hence, patients with negative biopsy findings should either undergo a second biopsy or enhanced surveillance [33].

(6) *For Immediate Institution of Anti-HCC Therapy When Lesion Is Still <2 cm*. The 5-year survival rate of early HCC is twice as high as that of progressed HCC [32]. It has been documented that 80% of small HCCs are already progressed and moderately differentiated with microinvasion of portal vein radicles (27%) and minute intrahepatic metastases or satellites (10%) at time of diagnosis [3]. Furthermore, 80% of patients with microinvasion and/or satellitosis suffer recurrence within the first 2 years of followup after surgery. In such cases, it would be prudent to perform FNA biopsy early, particularly in high-risk patients, so as not to miss that small window of opportunity for chance of cure.

(7) *For Appropriate Therapy in Non-HCC Cases*. If resection appears to be the best option, biopsy may or may not be performed. When palliative treatment is planned, biopsy is recommended to avoid unnecessary/inappropriate treatment [46].

(8) *Use of Coaxial Technique of Biopsy May Reduce Risk of Seeding*. A coaxial technique allows multiple samples to be obtained without repeated placement of the

needle; thus, potentially reducing the risk of needle-tract seeding. This approach helps to reduce the number of inadequate biopsies and is preferred for small and distant lesions. It is highly recommended but has yet to be evaluated [47].

(9) *FNA Biopsy as Point of Care*. Practices are likely to change. Prognostic factors to determine survival and recurrence rate include tumor size, localization, number of nodules, satellitosis, vascular invasion, and histologic grade. Tumor differentiation and vascular invasion show a strong correlation. Despite a potential bias due to sampling errors, FNA biopsy might help identify patients having well-differentiated HCCs with low risk of vascular invasion and good prognosis after transplantation.

Much research is being done in genomics and proteomics to determine the molecular signatures of HCCs. With the advent of molecular testing for better clinical tools for screening, diagnosis, surveillance, prediction of efficacy of treatment, monitoring of response, prognostication, and for rational targeted therapies, it is foreseen that FNA biopsy will be the most minimally invasive technique available to obtain samples of tumor and peritumoral tissues for molecular profiling [5, 6].

The issues that need to be addressed by hepatology teams were aptly put by Schölmerich and Schacherer [46], as: (i) how good is the technique and which is the preferred technical modality to perform a biopsy [efficacy of imaging-guided FNA], (ii) how dangerous is such a procedure and does it interfere with later treatment [complications and treatment options], and (iii) is a biopsy necessary and does it change the outcome [need for biopsy]? In the meantime, however, to biopsy or not to biopsy is a bedside decision to be made by the hepatology team, depending on the treatment options available. In developing/less developed countries where patients tend to present with more advanced disease, the practice of percutaneous US-guided FNA is still popular due to cost effectiveness, nonavailability of state-of-the-art imaging technologies, limited treatment options, and individual preference and expertise.

4. Nomenclature and Biological Evolution of Precancerous Lesions in Cirrhosis

Small hepatocellular nodules ($\leq$2 cm) can occur in cirrhotic or noncirrhotic livers. They comprise large regenerative nodules, dysplastic nodules, and small HCCs. Focal nodular hyperplasia and hepatocellular adenoma are well-differentiated hepatocellular nodular lesions occurring in noncirrhotic livers and will not be discussed in this review.

Recent studies on the biological evolution of hepatocellular nodules in cirrhosis have led to establishment of clinically relevant nomenclature for precancerous lesions and small HCCs [2, 6, 30]. Large cell change ("liver cell dysplasia") is defined as hepatocytes displaying corresponding nuclear and cellular enlargement with preserved nuclear-cytoplasmic ratio. Small cell change ("small cell dysplasia") is defined as hepatocytes exhibiting decreased cytoplasmic volume, cytoplasmic basophilia, mild nuclear pleomorphism, hyperchromasia, and increased nuclear-cytoplasmic ratio, giving an impression of nuclear crowding/increased cellular density. These groups of abnormal hepatocytes are referred to as dysplastic foci if they are <1 mm and dysplastic nodules if they are >1 mm in size. Dysplastic nodules can be low grade (large cell change) or high grade (small cell change). The precancerous nature of large cell change is still debatable. On the other hand, a high-grade dysplastic nodule indicates an increased risk for carcinoma development.

Small HCCs ($\leq$2 cm) are further categorized into early HCC (well-differentiated HCC with indistinct margins) and progressed HCC (well- to moderately differentiated HCC with distinct margins) [3, 30]. Distinctly nodular small HCCs usually contain well-developed unpaired arteries. Early HCCs may contain both portal tracts and unpaired arteries; similar features may be found in high-grade dysplastic nodules. Identification of high-grade dysplastic nodules and/or small HCCs should lead to treatment by local ablation, surgical resection or transplantation.

Awareness of the current morphologic criteria for the diagnosis of early HCC is helpful for the interpretation of small histologic samples and for choice of appropriate immunohistochemical panel. Early HCCs are characterized by the following histologic features [2, 30]: (i) increased cell density >2 times that of the surrounding tissue, with an increased nuclear-cytoplasmic ratio, (ii) irregular thin trabecular pattern (2 cells or more thick), (iii) pseudoglandular pattern, (iv) unpaired arteries, (v) diffuse fatty change, (vi) cytoplasmic basophilia or eosinophilia, (vii) sinusoidal capillarization, (viii) invasion of intratumoral portal tracts, and (ix) stromal invasion accompanied by lack of ductular reaction at the periphery of the nodules. Malignant transformation can occur within dysplastic or regenerative nodules, where any of the above features may be restricted to an expansile subnodule in the parent nodule ("nodule-in-nodule"). Under such circumstances, several passes in different directions during the FNA procedure may be required to overcome the focality of these proliferative foci.

Fatty change, which can be observed in any hepatocellular nodular lesion, is reported in 40% of early HCC [6]. Prevalence of fatty change decreases along with increasing tumor size as neoarterialization increases. Sinusoidal capillarization is present diffusely in HCC and diffusely/focally in high-grade dysplastic nodule. Stromal invasion is the most helpful feature in differentiating early HCC from high-grade dysplastic nodule. Microinvasion of portal vein radicles is not expected in early HCC but may be encountered in progressed HCC. Microbiopsies allow for assessment of architecture and stromal invasion.

5. Clinical Implications of Molecular Subclassification of Hepatocellular Carcinoma

Hepatocellular carcinoma has a complex molecular pathogenesis and morphologic heterogeneity. Genomic and proteomic studies have helped elucidate the molecular signatures of HCCs. Recent studies have also identified molecular

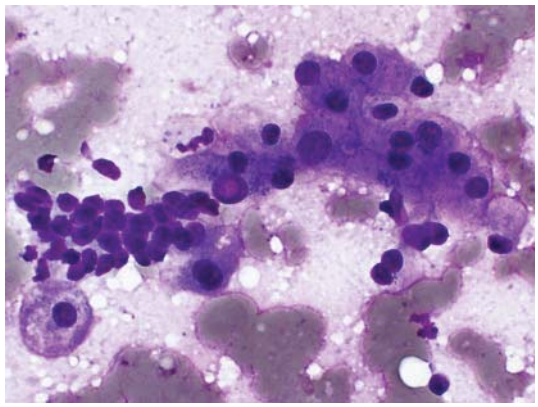


Figure 1: FNA of cirrhosis. Hyperplastic hepatocytes occur in 2-cell thick cords with closely adherent bile ductule. The polygonal hepatocytes exhibit cellular polymorphism with well-defined cell borders, ample granular cytoplasm, central round nucleus, and nuclear-cytoplasmic ratio of about 1/3. The ductule, indicative of hepatocellular-stromal interface restoration, consists of overlapping rows of small, ovoid, darkly-staining nuclei with nondiscernible cytoplasm. (Giemsa, ×400).

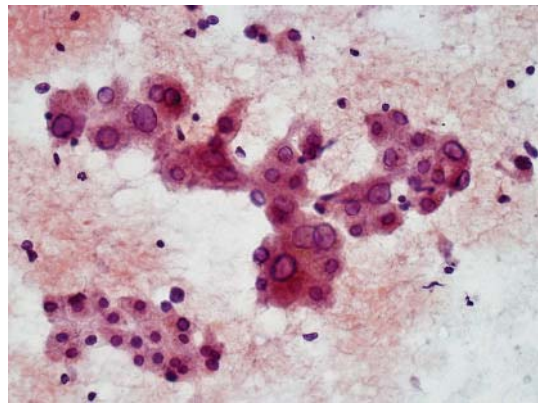


Figure 2: FNA of hepatocytes with large cell change. Two-cell-thick rows of hepatocytes show corresponding cellular and nuclear enlargement, maintaining normal nuclear-cytoplasmic ratio. Cellular polymorphism amongst sibling cells is clearly evident. Note intranuclear inclusions. Contrast with group of normal-sized hyperplastic hepatocytes (Papanicolaou, ×200).

changes in HCC which are potential markers of early HCC, such as glypican-3. Furthermore, a specific gene-expression signature of the peritumoral liver tissue was found to correlate with late recurrence and survival [48]. A molecular subclassification will soon be added to the conventional morphologic classification; the purpose of which is to identify predictors of aggressive/indolent behavior or features associated with sensitivity/resistance to novel therapies [6].

The future role of pathologists will be to interrogate the tumor and the peritumoral tissues and to be able to interpret this situation for clinical use. Apart from the main objective of developing personalized molecular targeted anticancer treatment protocols, such an approach will also help to identify patients at high risk for recurrence/further development of cancer. This will allow for selective intensified clinical followup with possible chemopreventive strategies (personalized preventive medicine) [5]. The combination of multiple targeted agents is the next logical step in the treatment of HCC due to the strong rationale to inhibit as many signalling pathways as possible in hepatocarcinogenesis [49]. This is also useful for further treatment of sorafenib-resistant cases. What all this translates into on the practical front is that tissue samples will have to be procured from various parts of the tumor, and in inoperable cases, the FNA biopsy technique provides the best approach to date for tissue procurement.

6. Algorithmic Approach to FNA Diagnosis of HCC and Associated Hepatocellular Nodular Lesions

Well known for its heterogeneity, HCC has variants and mixed lesions that may mimic other tumors. On the other hand, metastases to the liver are by far commoner than primary liver cancers. Apart from cystic and inflammatory conditions, the major diagnostic issues in FNA of focal liver lesions are highlighted in Section 2 under "Diagnostic challenges" [21].

6.1. FNA Biopsy of Benign Hepatocellular Nodular Lesions. Aspirates of *cirrhotic nodules, including large regenerative nodules,* show a polymorphous population of cells, comprising hyperplastic hepatocytes, bile ductal epithelium, endothelial cells, and Kupffer cells, accompanied by features of regeneration and repair (Figure 1) [26, 50]. Polygonal hepatocytes have well-defined cell borders, ample granular cytoplasm, central round nuclei with well-defined nuclear membrane, granular chromatin, and distinct nucleolus. Nonneoplastic hepatocytes exhibit polymorphism, that is, sibling cells display variation in cell size and shape with corresponding variation in nuclear size. The nuclear-cytoplasmic ratio is about 1/3 (if one were to eyeball the nuclear and cell diameters). Hyperplastic hepatocytes show binucleate forms and appear as short 2-cell thick cords rather than singly. Fatty change, when present, is best appreciated in Giemsa preparations as intracytoplasmic vacuoles or as dispersed bubbles leaked from ruptured cells. Bile ductal epithelium appears as small flat clusters of cohesive uniform cells with minimal pale cytoplasm and bland, equidistant round to ovoid nuclei, and lacking nucleoli. Bile ductular epithelium is indicative of parenchymal-stromal interface restoration, and its recognition in lesional material is tantamount to confirming the benign status of the hepatocellular nodular lesion. Bile ductules appear as curved double-stranded rows of epithelial cells exhibiting ovoid darkly-staining overlapping nuclei with nuclear disarray, high nuclear-cytoplasmic ratio and barely visible cytoplasm, mimicking adenocarcinoma. Elongated endothelial and comma-shaped Kupffer cell nuclei can be identified amongst the hepatocytes. Inflammatory cells, comprising predominantly of lymphocytes, and stromal fragments may be encountered in the background. Focal nodular hyperplasia gives similar restoration features.

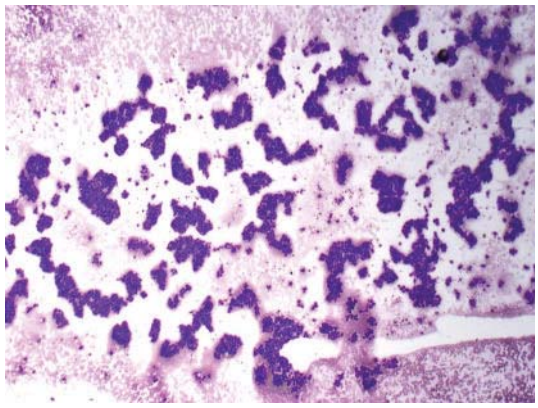


Figure 3: FNA of hepatocellular carcinoma. Low magnification view shows granular trails of irregularly shaped tumor aggregates (Giemsa, ×40).

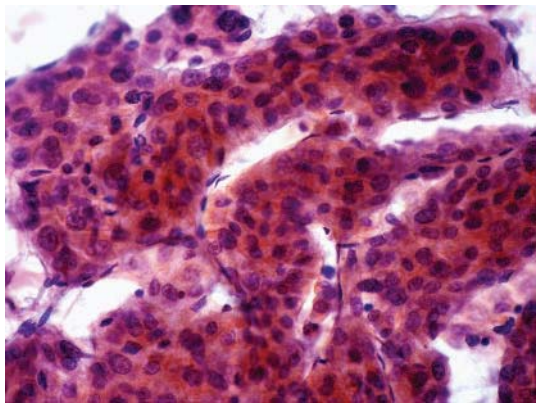


Figure 4: FNA of moderately differentiated hepatocellular carcinoma. Broad tongues of cohesive malignant hepatocytes wrapped by peripheral endothelium (Papanicolaou, ×400).

Aspirates of *low-grade dysplastic nodules* contain hepatocytes exhibiting large cell change with no/minimal nuclear atypia and normal nuclear-cytoplasmic ratio (Figure 2). Hepatocytes from *high-grade-dysplastic nodules* are small and monotonous with subtle increase in nuclear-cytoplasmic ratio; the nuclear size is fairly similar to that of normal hepatocytes but there is less cytoplasm, thus imparting an impression of nuclear crowding [51]. Dysplastic hepatocytes generally occur singly or in 1- to 2-cell thick cords. Fatty change may be present. Bile ductal and ductular epithelium and stromal fragments may be evident in the background. It is difficult to distinguish high-grade dysplastic nodule and early HCC purely on cytologic grounds.

6.2. FNA Biopsy of Classic Hepatocellular Carcinoma. Hepatocellular carcinomas are highly heterogeneous tumors with regard to differentiation, histologic patterns (trabecular-sinusoidal, pseudoacinar, and compact types), and cell morphology. As such, one should be fully cognizant of the challenges and limitations of FNA biopsy in the diagnosis of HCC. Several passes in different directions should be performed in large tumors to overcome diagnostic difficulties due to sampling bias. Accurate distinction of HCC and its variants from metastases is crucial for institution of appropriate therapy.

Cytologic features of HCC include [50]:

(i) *Hypercellular smears composed of trails of tumor cell clusters imparting a granular pattern of spread evident on gross inspection* (Figure 3).

(ii) *Irregular arborizing, broad, tongue-like cords* (*>2 cells thick*) *of cohesive malignant hepatocytes* (Figure 4).

(iii) *Peripheral endothelium wrapping broad cords* (Figure 4).

(iv) *Transgressing endothelium running across larger aggregates* (Figure 5(a)): basement membrane material looking like pink "tramlines" (indicative of sinusoidal capillarization) is best seen in Giemsa preparations.

(v) *Cohesion is the rule:* tendency to dissociation is observed in highly well-differentiated HCC due to narrow cords; and in poorly differentiated HCC where there is virtually absent reticulin.

(vi) *Pseudoacini containing bile or pale secretions* (Figure 5): polygonal neoplastic hepatocytes surround cystically dilated canaliculi.

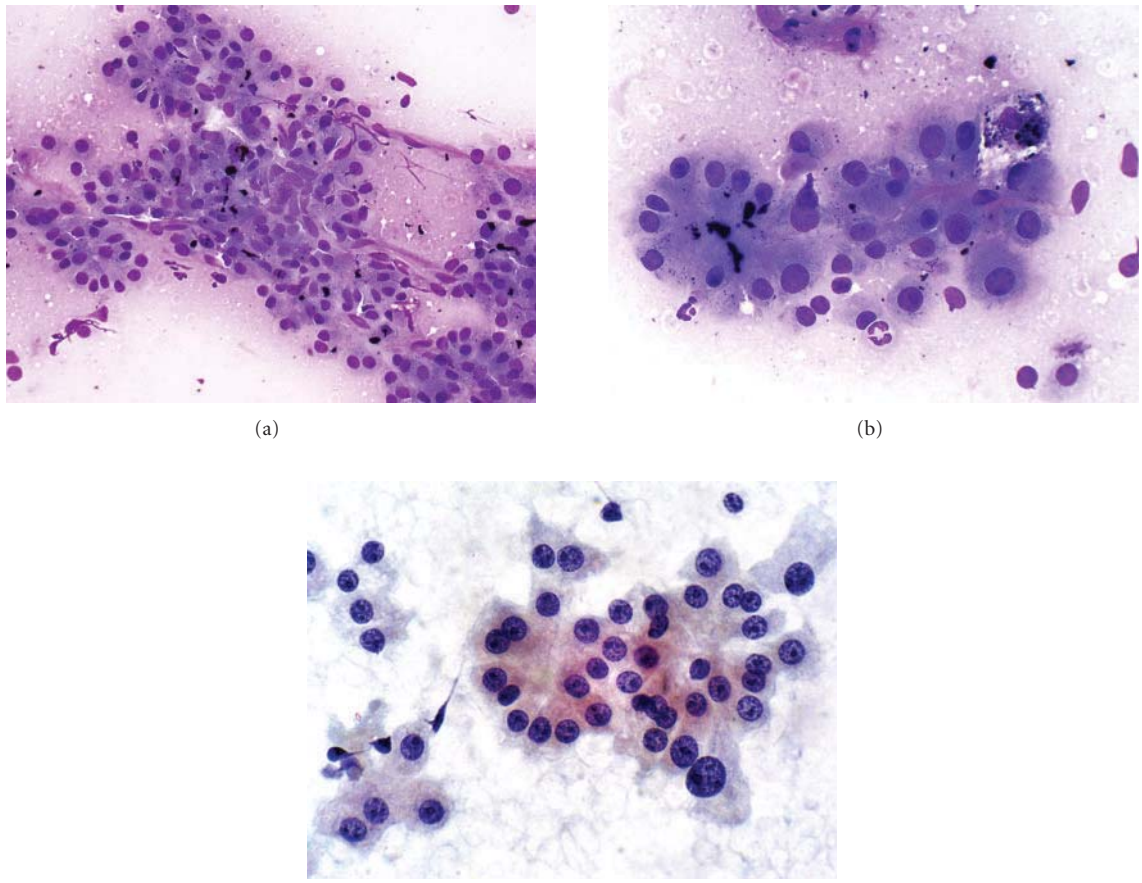


Figure 5: FNA of well-differentiated hepatocellular carcinoma. (a) Malignant hepatocytes exhibit pseudoacinar pattern with blackish bile plugs. Note transgressing endothelium with pink basement membrane material traversing tumor cells (Giemsa, ×200). (b) Rosette-like clusters of malignant hepatocytes form pseudoacini with blackish bile plugs within the dilated bile canaliculi. Some intracytoplasmic bile droplets are evident (Giemsa, ×400). (c) Similar pseudoacini are surrounded by malignant hepatocytes. The polygonal cells exhibit well-defined cell borders, ample granular cytoplasm, central round nucleus with well-defined nuclear membrane, distinct nucleolus and granular chromatin (Papanicolaou, ×400).

(vii) *Hepatocytic characteristics include polygonal cells with well-defined cell borders, ample dense granular cytoplasm, increased nuclear-cytoplasmic ratio* (>1/3), *central round nucleus, well-delineated nuclear membrane, prominent nucleolus, and fine, irregularly granular chromatin. Mitoses increase with nuclear grade* (Figures 5(b) and 5(c)): cytologic features of malignancy are wanting at the well-differentiated HCC end whereas clues to hepatocytic histogenesis are lacking at the poorly differentiated end.

(viii) *Tumor cells may be smaller, larger, or of the same size as nonneoplastic hepatocytes* (Figure 6): well-differentiated HCC cells tend to be conspicuous by their small size, monotony, subtle increase in nuclear-cytoplasmic ratio and nuclear crowding. Poorly differentiated HCC cells tend to be pleomorphic with thin nuclear membranes and irregular nuclear contours.

(ix) *Atypical bare hepatocytic nuclei may abound* (Figure 7).

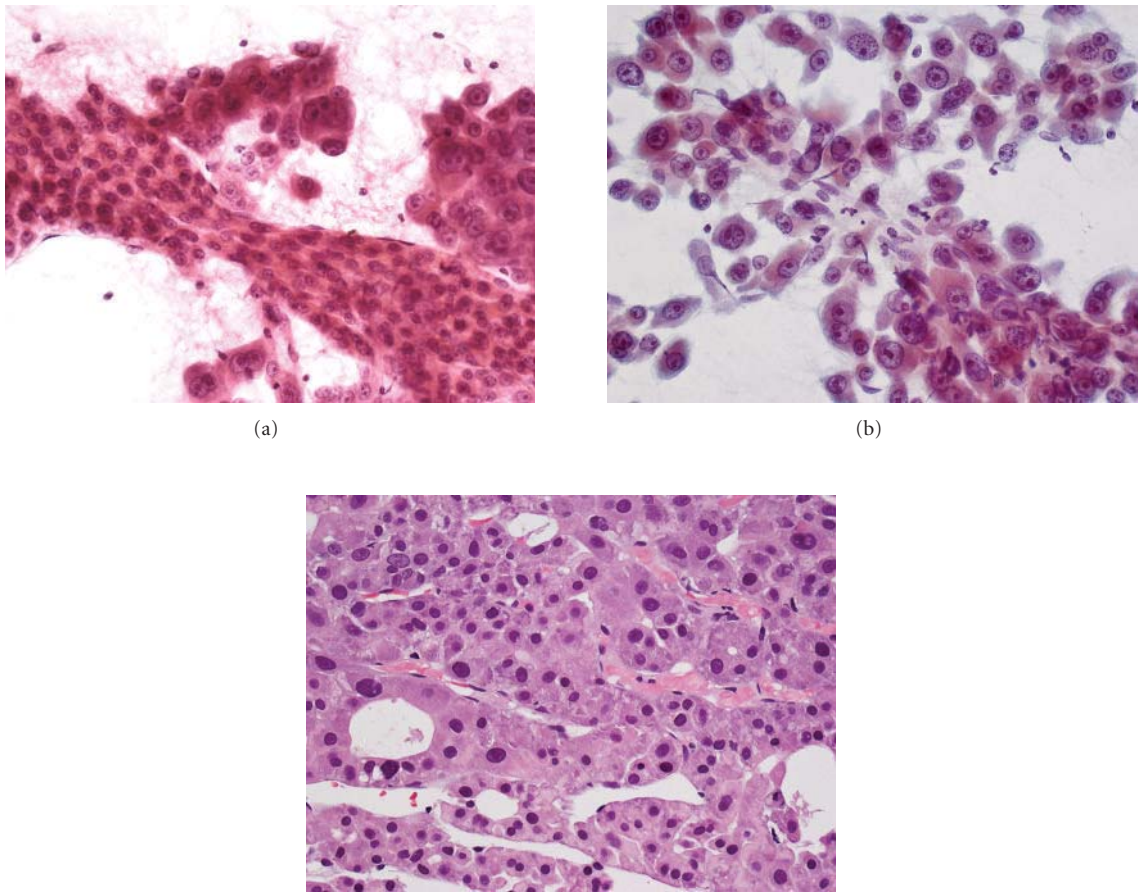
(x) *Multinucleated tumor giant cells may be of "osteoclastic" or pleomorphic type* (Figure 8): the former shows nuclear features akin to sibling tumor cells. Tumor giant cells may be found even in the lower grades of HCC. Their presence does not necessarily upgrade the tumor.

(xi) *Bile may be present within tumor cells or in canaliculi or pseudoacini* (Figure 5): bile appears as greenish-black intracytoplasmic droplets, ropey intracanalicular strands and blobs within pseudoacini; best detected in Giemsa-stained smears.

(xii) *Intracytoplasmic fat and glycogen vacuoles are common. Intracytoplasmic inclusions include hyaline, pale, and Mallory bodies* (Figure 7). *Intranuclear cytoplasmic inclusions are not specific.*

(xiii) *Bile duct epithelial cells, if present, are few and far apart. Background may be hemorrhagic and/or necrotic.*

Classic HCC is cytologically graded into well, moderately and poorly differentiated lesions based on nuclear grade.



(a) (b) (c)

Figure 6: FNA of hepatocellular carcinoma. (a) Two populations of malignant hepatocytes are seen. One population forms a distinct broad trabecula with peripheral endothelium. The other tumor cells are bigger, more pleomorphic, and less cohesive (Papanicolaou, ×200). (b) The more pleomorphic cells appear discohesive but still retain recognizable hepatocytic characteristics. Note transgressing endothelium (Papanicolaou, ×400). (c) Cell block shows trabecular-sinusoidal and pseudoacinar patterns. (H&E, ×200).

Well-differentiated HCC (Figure 9): Tumor cells closely resemble nonneoplastic hepatocytes in size, shape and nuc–lear and nucleolar appearances. The nuclear-cytoplasmic ratio is slightly higher. Mitoses are exceptional.

Moderately Differentiated HCC (Figure 4). Tumor cells bear a resemblance to nonneoplastic hepatocytes. The nuclear-cytoplasmic ratio is moderately high, the round to ovoid nuclei show moderate degrees of pleomorphism, nucleoli are prominent, and mitoses are identifiable.

Poorly Differentiated HCC (Figure 8). There is poor resemblance to hepatocytes. Tumor cells exhibit marked pleomorphism, less cytoplasm, very high nuclear-cytoplasmic ratios, thinner nuclear membranes with irregular nuclear contours, hyperchromasia, and numerous mitoses. Nucleoli may be prominent or absent. Multinucleated tumor giant cells are easily identified.

Cell Block/Microbiopsies. The histologic diagnosis of HCC is based on cyto-architectural features, such as cell atypia, cell crowding, trabecular thickness, and microacini. Establishment of trabeculae ≥3 cells thick is one of the most helpful features in diagnosis of highly well-differentiated HCC. Gomori's silver stain for reticulin fibers is useful in distinguishing HCC from benign hepatic processes [52]. The reticulin framework is abnormal or deficient in HCC, and this coupled with the presence of broad cords accounts for their friability during the retrieval process for cell block preparation. Immunohistochemistry plays a helpful adjunctive role.

6.3. FNA Biopsy of Variants of Hepatocellular Carcinoma. Adequate representative sampling to achieve accurate cyto-diagnosis of this heterogeneous malignancy will become more crucial when molecular subclassification of HCC is implemented for targeted therapy. The variations and variants of HCC include [53] the following.

(i) *HCC with Fatty Change* (Figure 10). Fatty change can occur in all sizes and grades of HCC. Highly well-differentiated HCC with fatty change can easily be overlooked for nonneoplastic hepatocytes from fatty liver or focal fatty change [50, 54].

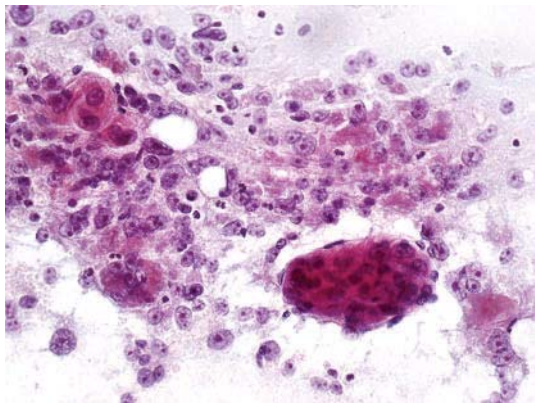
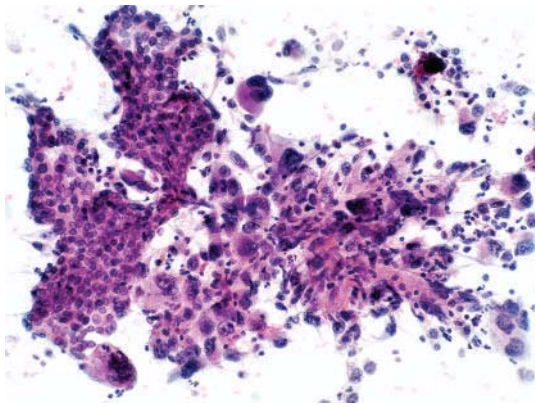


Figure 7: FNA of hepatocellular carcinoma. Atypical bare nuclei exhibit characteristic hepatocytic nuclear features. Mallory hyaline is seen as reddish clumpy intracytoplasmic material. Cross-section of a broad trabecula bordered by peripheral endothelium is evident (Papanicolaou, ×200).



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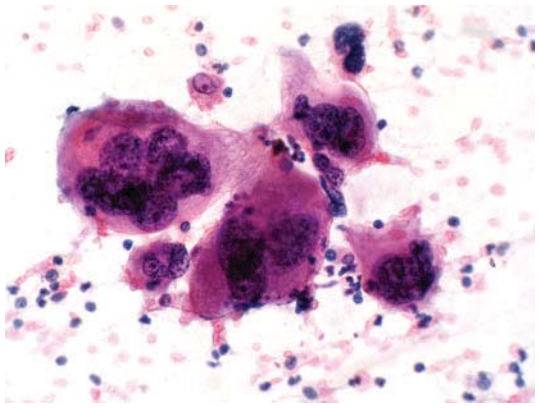


(b)

Figure 8: FNA of poorly differentiated hepatocellular carcinoma. (a) Two populations of tumor cells are seen. The better differentiated component is represented by a trabecula of malignant hepatocytes with peripheral endothelium. The other population consists of highly pleomorphic tumor cells with giant cells and bizarre nuclei; there is no apparent resemblance to hepatocytes (Papanicolaou, ×200). (b) Highly pleomorphic tumor giant cells exhibit multinucleation, bizarre nuclei, and hyperchromatism (Papanicolaou, ×400).

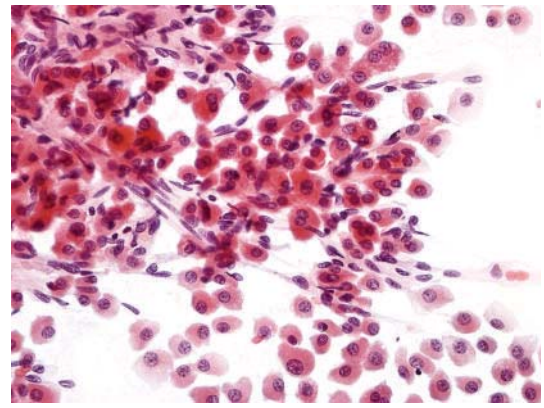
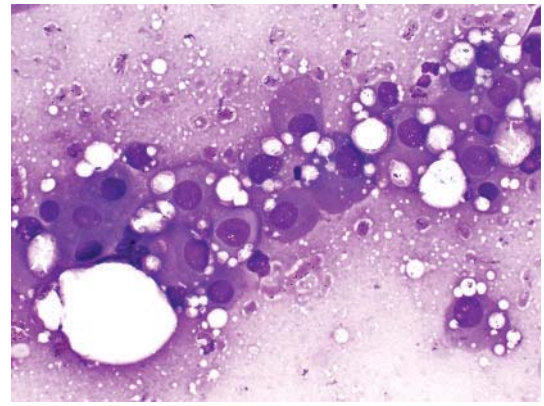


Figure 9: FNA of well-differentiated hepatocellular carcinoma. Small-sized malignant hepatocytes exhibit monotonous appearance with tendency to dissociation. The cells display well-defined cell borders, decreased dense cytoplasm, slightly eccentric nuclei, increased nuclear-cytoplasmic ratio, and impression of nuclear crowding. Transgressing endothelium abound (Papanicolaou, ×200).



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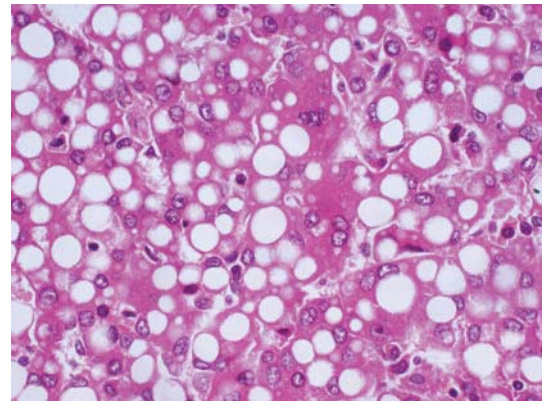
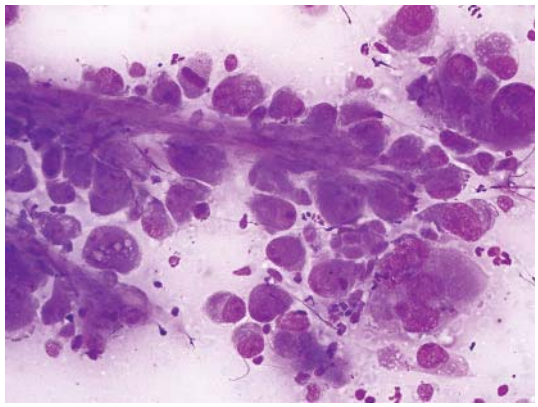


(b)

Figure 10: FNA of hepatocellular carcinoma with fatty change. (a) Malignant hepatocytes exhibit intracytoplasmic fat vacuoles of varying sizes. Lipid-containing bubbles are observed in the background (Giemsa, ×400). (b) Cell block shows fatty change in the tumor cells (H&E, ×200).



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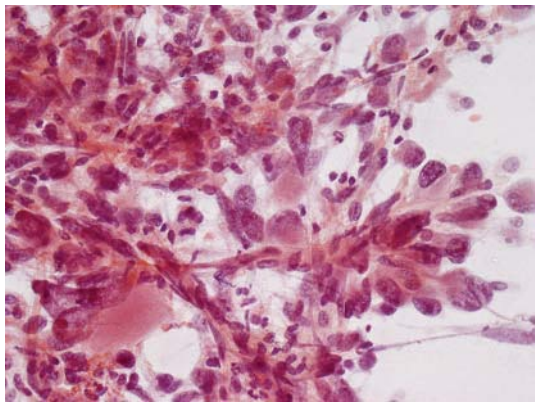


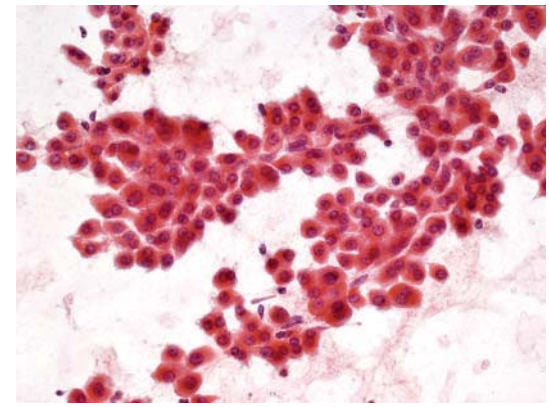
(b)

Figure 11: FNA of hepatocellular carcinoma with sarcomatoid change. (a) Loosely cohesive highly pleomorphic tumor cells exhibit sarcomatoid features. Note transgressing endothelium (Giemsa, ×400). (b) Spindle-shaped tumor cells and tumor giant cells bear no resemblance to hepatocytes. Transgressing endothelium abound (Papanicolaou, ×400).

(ii) *HCC with Clear Cell Change.* Malignant hepatocytes with glycogen-laden cytoplasm display a pale/clear bubbly appearance, best appreciated in Giemsa preparations [55].

(iii) *HCC with Small Cell Change.* The small tumor cells show scanty cytoplasm, round nuclei, high nuclear-cytoplasmic ratio, granular chromatin and small nucleolus. They mimic neuroendocrine tumors with a similar tendency to dissociation and microacinar formation [56, 57]; however, the "salt and pepper" chromatin of endocrine tumor cells is absent. Closer scrutiny of all available material may reveal more classic HCC features.

(iv) *HCC with Pleomorphic Features.* The pleomorphic tumor cells are poorly differentiated with a tendency towards dissociation. Scattered multinucleated tumor giant cells and necrosis may be present.



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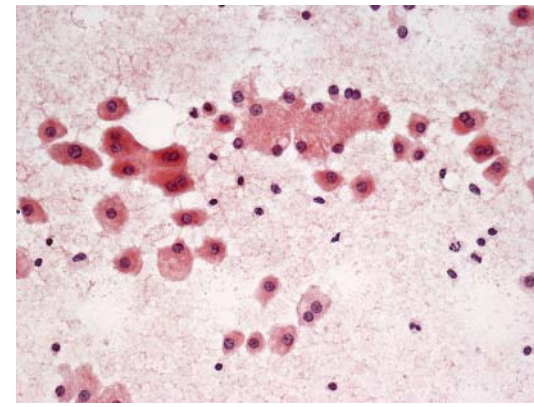


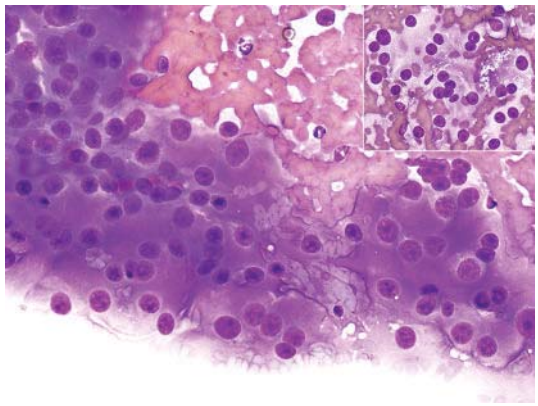
(b)

Figure 12: FNA of highly well-differentiated hepatocellular carcinoma from a "nodule-in-nodule" lesion. (a) Small and monotonous malignant hepatocytes exhibit decreased dense cytoplasm, central to slightly eccentric nuclei, increased nuclear-cytoplasmic ratio, and impression of nuclear crowding with closer inter-nuclear distances. Transgressing endothelium is present. Cytologic features are difficult to distinguish from those of a high-grade dysplastic nodule with small cell change. (Papanicolaou, ×200) (b) Two populations of dissociated hepatocytes are present. The malignant cells have dense eosinophilic cytoplasm with higher nuclear-cytoplasmic ratio. The nonneoplastic cells from the parent nodule have ample paler cytoplasm and normal nuclear-cytoplasmic ratio (Papanicolaou, ×200).

(v) *HCC with Spindle Cell Features* (Figure 11). The pleomorphic spindle-shaped cells are indistinguishable cytologically from sarcomatous cells [58]. (see Sarcomatoid variant of HCC).

(vi) *HCC with Giant Cell Features* (Figure 11). This pure variant is rare and has to be distinguished from giant cell sarcomas. Bizarre multinucleated giant cells with highly abnormal mitoses are present.

(vii) *HCC with Biliary Differentiation.* Some HCCs may contain tubular spaces surrounded by columnar cells with basal palisading nuclei, favoring true acinar differentiation. HCCs may exhibit focal CK19 positivity—this may imply poorer prognosis [59]. Such HCCs have to be separated from the rare mixed type of HCC-CC.



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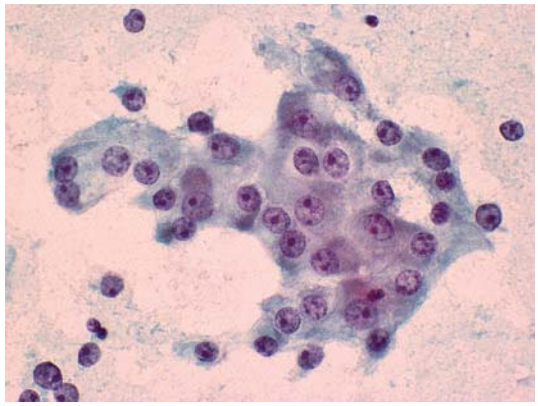

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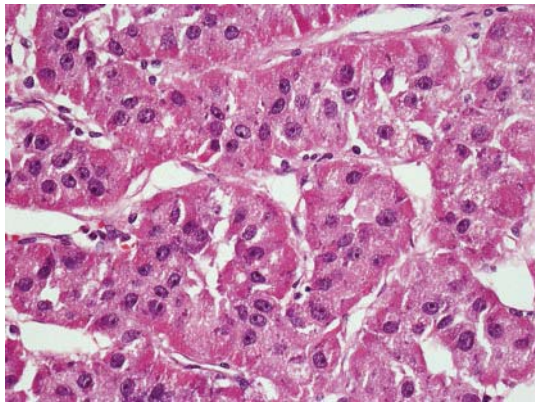


(c)

Figure 13: FNA of metastatic adrenocortical carcinoma to the liver. (a) Cohesive group of tumor cells with ample cytoplasm, central round nucleus, distinct nucleolus, and granular chromatin. (Giemsa, ×400). Inset: tumor cells contain fine lipid vacuoles in the cytoplasm (Giemsa, ×200). (b) Loosely cohesive tumor cells with nuclear features mimicking malignant hepatocytes (Papanicolaou, ×400). (c) Histology of adrenal tumor shows trabecular-sinusoidal pattern and polygonal cells with central round nucleus and nucleolus, mimicking HCC. The cytoplasm shows eosinophilic granularity (H&E, ×200).

6.3.1. Special Types

(i) *HCC, Fibrolamellar Variant.* The tumor is characterized by monotonous population of discohesive, large polygonal cells with abundant oncocytic granular cytoplasm [60, 61]. Individual cells are about three times larger than normal hepatocytes, as are nuclear and nucleolar sizes.The nuclear-cytoplasmic ratio is generally <1/3. Intracytoplasmic pale and hyaline bodies are common. Presence of collagenous bands is a distinctive clue.

(ii) *HCC, Scirrhous Type.* This type is uncommon and should not be confused with fibrolamellar carcinoma and cholangiocarcinoma.

(iii) *Undifferentiated Type.* The tumor cells can be loosely cohesive or show tendency to dissociation. They are pleomorphic and nondescript with no cytologic clues to their hepatocytic histogenesis.

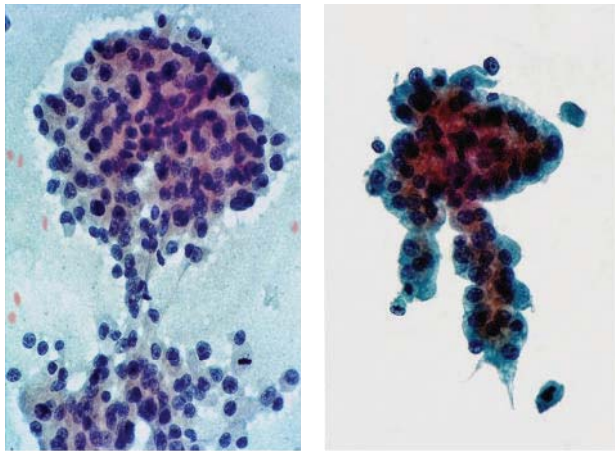
(iv) *Lymphoepithelioma-Like Carcinoma.* It is a rare type of HCC with small pleomorphic tumor cells admixed with abundant lymphocytes. In some cases, the tumor cells are positive for Epstein-Barr virus [53].

(v) *Sarcomatoid HCC.* The purely sarcomatoid variant is rare; it is more often seen in conjunction with tumor giant cells. Extensive sampling may reveal areas of conventional HCC [58].

6.3.2. Others

(i) *Combined Hepatocellular-Cholangiocarcinoma.* Although this combined (or mixed) tumor is characterized by an intimate admixture of HCC, cholangiocarcinoma, and a transitional component, not all components need be encountered in FNA material. HCC and cholangiocarcinoma are easily recognizable. However, transitional cells with features straddling HCC and adenocarcinoma may predominate [62, 63]. They may resemble malignant hepatocytes with trabecular arrangement but also exhibit acini with nuclear palisading. On the other hand, the transitional cells may display nuclear contour irregularities, indistinct nucleolus, and less granular cytoplasm. There may be difficulties distinguishing pseudoacini from true acini. Mucin may not be detected. The immunophenotype is often equivocal. A high index of suspicion is required for this cytodiagnosis.

FNA Biopsy of Highly Well-Differentiated Hepatocellular Carcinoma. Diagnostic accuracy is certainly a challenge at this end of the spectrum and often indeterminate reports are rendered. Cytologic features predictive of HCC include increased nuclear-cytoplasmic ratio, cellular monomorphism, nuclear crowding, trabeculae >2 cells thick, atypical naked hepatocytic nuclei, and lack of bile duct cells [26, 51, 64, 65]. Cytologic parameters distinguishing highly well-differentiated HCC from cirrhosis include well-defined cell borders, scant cytoplasm, monotonous cytoplasm, thick cytoplasm, eccentric nuclei, and increased nuclear-cytoplasmic ratio [66]. An intimate admixture of neoplastic



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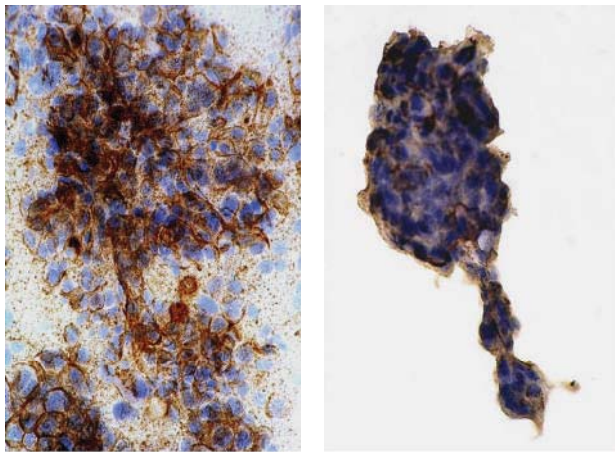

(b)

Figure 14: FNA of resected specimen of moderately differentiated hepatocellular carcinoma: conventional smears versus liquid-based cytology. (a) *Left panel*: Conventional smear shows larger and flatter aggregate of malignant hepatocytes. *Right panel*: ThinPrep smear shows a tighter, 3-dimensional cluster of malignant hepatocytes with trabeculae. The background is devoid of cells. Crisp nuclear details are better appreciated in monolayered cells. (Papanicolaou, ×400) (b) *Left panel*: Conventional smear shows malignant hepatocytes with bile canaliculi forming a delicate network of criss-crossing tubules highlighted by immunostaining with polyclonal CEA. *Right panel*: The canalicular network is more difficult to discern in the ThinPrep smear. (pCEA immunostain, ×400).

and nonneoplastic hepatocytes can be encountered in FNA of "nodule-in-nodule" lesions (Figure 12). Availability of cell blocks or microbiopsy material is very helpful for ancillary stains.

6.4. FNA Biopsy of Nonhepatocellular Mimics of Hepatocellular Carcinoma and Its Variants

6.4.1. Benign Entities

Hepatic Angiomyolipoma. May consist of epithelioid and/or spindle cells with increased vascularity but without obvious adipocytes. A high index of suspicion and familiarity with this entity go a long way to avoiding the common pitfall of labeling the epithelioid variant with clear or oncocytic cells as HCC [67, 68]. Immunoreactivity of the tumor cells with HMB45 and desmin will help clinch the diagnosis.

Inflammatory Pseudotumors. Pose a distinct diagnostic pitfall clinically, radiologically, and cytomorphologically. Cytologic findings are highly variable. The pitfall of interest is mistaking reactive hepatocytes for HCC [69]. One should exercise extreme caution in making a diagnosis of malignancy in the face of concomitant inflammation.

6.4.2. Malignant Entities. A practical approach to adopt when dealing with hepatic FNA of nonhepatocellular malignancies is to categorize them into the following cytomorphologic groups, namely, adenocarcinoma, squamous cell carcinoma, small round cell tumor, clear cell tumor, or malignancies characterized by pleomorphic, spindle, giant, or undifferentiated cells [21]. The initial cytologic assessment is crucial as it forms the impression upon which appropriate ancillary tests are ordered. Some of these cytomorphologic entities may occur de novo in the liver. At best, information gleaned from a precise cytodiagnosis can only favor a particular primary site. Close clinicopathologic correlation is mandatory.

Renal cell carcinoma, adrenocortical carcinoma, and melanoma are well-documented mimics of HCC (Figure 13). Metastatic and primary hepatic neuroendocrine tumors can occur [70]. The polygonal cell subtype of neuroendocrine tumor can mimic well-differentiated HCC whilst the small cell subtype can mimic small cell variant of HCC. Epithelioid and spindle variants of leiomyosarcoma/gastrointestinal stromal tumor may simulate HCC and its sarcomatoid variant, respectively [71, 72]. Rarely, extrahepatic AFP-producing hepatoid or nonhepatoid carcinomas, arising most commonly in the gastrointestinal tract, may occur. They have a proclivity for vascular permeation and liver metastases, giving rise to confusion with primary AFP-producing HCC [73].

7. Diagnostic Utility of Immunohistochemistry

An armamentarium of antibodies is available for the comparative immunohistochemical analysis of primary and metastatic tumors of the liver. A panel of immunostains has more discriminant value. Immunohistochemistry is preferred to immunocytochemistry. Careful light microscopic assessment of the histologic sections is important as judicious use of immunostains is imperative since material is limited. Double-staining protocols may help to optimize tissue usage.

Kakar et al. outlined best practice guidelines for use of immunohistochemistry in the differential diagnosis of hepatic lesions under specific clinical scenarios [74]. Stepwise logistic regression analysis has shown that the panel of glypican-3, HepPar1, MOC-31, and CK7 is most useful in diagnosing and distinguishing HCC from metastatic adenocarcinoma on FNA material, with accuracy rates of

90.5 and 91.7%, respectively [75–78]. In the HCC group, glypican-3 was the most sensitive (81%), whereas HepPar1 (71.4%) and polyclonal carcinoembryonic antigen (pCEA) (50%) were less sensitive. In the metastatic adenocarcinoma group, MOC-31 was most sensitive (79.2) followed by CK7 (41.7%).

In the context of hepatocellular nodular lesions, the objectives are twofold [79]: (i) to prove hepatocellular histogenesis and (ii) to demonstrate the malignant status of the hepatocytes. For the former, the panel should include Hep Par 1 [80–82], TTF-1 [83, 84], and pCEA or CD10 to demonstrate canalicular formation (Figure 14) [85]. For the latter, the panel should include glypican-3, glutamine synthetase (a target protein of β-catenin), and heat shock protein 70 (a chaperone stress protein); two out of three positivity of these novel biomarkers are taken as indicative of HCC [86–90]. The demonstration of AFP positivity points towards a malignant tumor of hepatocellular origin provided nonseminomatous germ cell tumors and extrahepatic AFP-producing carcinomas have been excluded. Unfortunately, this tumor marker has such low sensitivity that it is no longer recommended as part of the panel [74]. If histologic material is available, use of CD34 highlights diffuse sinusoidal capillarization in HCC [74, 79]. CK19 can be used to demonstrate the absence of ductular reaction at the periphery of small HCCs, thereby confirming stromal invasion at the hepatocellular-stromal interface. The clinicopathologic and prognostic relevance of CK7 or 19 expression in HCC as indicative of possible progenitor cell origin for the tumor is still being studied [59]. Immunohistochemical results should always be interpreted in the larger context of the case.

8. The Future

Our cytology role fits into the overall patient clinical pathway as FNA biopsy offers the potential immediacy of a diagnosis to the clinician who can then advise the patient and develop an appropriate next clinical step. The rapid turn-around time or at best reporting within 24 hours is ideal but for most practices this is not achievable with current resources. On-site cytology service provides immediate evaluation for adequacy and triage of specimens, which can be assessed by cytotechnologists rather than cytopathologists. The reduction in inadequate sample rates is important for overall cost effectiveness of the technique.

The future will see a paradigm shift in the perception of the role of FNA in HCC. New trends in personalized molecular targeted therapy require better characterization and prediction of HCC behavior [48]. The FNA biopsy technique is still the most minimally invasive approach for the procurement of tumor and peritumoral tissue for molecular studies. We foresee that in the near future hepatic FNA is likely to become a point of care in the management of HCC patients, especially inoperable cases.

Conflict of Interests

The author declares that there is no conflict of interests.

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Research Article

Fine Needle Aspiration Cytology in Diagnosis of Pure Neuritic Leprosy

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Leprosy is a chronic infection affecting mainly the skin and peripheral nerve. Pure neuritic form of this disease manifests by involvement of the nerve in the absence of skin lesions. Therefore, it can sometimes create a diagnostic problem. It often requires a nerve biopsy for diagnosis, which is an invasive procedure and may lead to neural deficit. Fine needle aspiration cytology (FNAC) of an affected nerve can be a valuable and less invasive procedure for the diagnosis of such cases. We report five suspected cases of pure neuritic Hansen's disease involving the common and superficial peroneal, ulnar, and median nerve, who underwent FNAC. Smears revealed nerve fibers infiltrated by chronic inflammatory cells in all cases, presence of epithelioid cells granulomas, and Langhans giant cells in three cases, and acid fast bacilli in two cases. In conclusion, FNAC is a safe, less invasive, and time saving procedure for the diagnosis of pure neuritic leprosy.

1. Introduction

About 4–8% of all leprosy is clinically limited to the peripheral nerve [1]. This form of leprosy is termed pure neuritic leprosy [1]. The other names given are: neural, pure neural, primary neural, primary neuritic, purely neural, purely neuritic, or polyneuritic leprosy [1]. The clinical features of leprotic nerve involvement include nerve enlargement, tenderness, pain, and sensory motor impairment [1]. These are not specific and may not always be present [1]. The most commonly affected nerves include the posterior tibial, peroneal, ulnar, and median nerves [1]. Diagnosing leprosy in the absence of typical dermatological features is challenging and requires histological confirmation [1]. This is often achieved using nerve biopsy [1]. Limitations of this technique are sampling error, low sensitivity, and permanent nerve deficit [1]. A technique that is simpler than nerve biopsy is needed to evaluate the nerve involvement, especially in pure neuritic leprosy (PNL) [1]. Only a few studies have evaluated the role of fine needle aspiration cytology (FNAC) of the nerve in the diagnosis of PNL [2–7]. Here we report five cases of PNL diagnosed by FNAC.

2. Material and Methods

2.1. Cases. Five cases with varied complaining features and thickened nerves without any cutaneous lesion were subjected for FNAC from the department of dermatology of our hospital between the periods of October 2004 to December 2008.

2.2. Methods. The cases were examined for most prominent site of thickened nerve. The area was cleaned with an alcohol swab. The prominent part of nerve was fixed by index finger and thumb of left hand, and the 22 G needle fitted in 10 mL disposable plastic syringe was inserted along the length of the nerve. The suction was applied and aspiration was performed using a single-puncture, multidirectional technique. The direction of the needle was always kept parallel to the length of the nerve so as to cause minimal damage to the nerve. The material aspirated was smeared on glass slides. Minimum three smears were made for each case. The wet smear was fixed in 95% ethanol and stained by Papanicolaou stain after 30 minutes of fixation. One of the dried smear was stained by May-Grünwald-Giemsa (MGG) stain, and the other dried

Table 1: Clinicocytological details of the cases of pure neuritic leprosy.

	C/N	Cl/f	Site of FNA	Cytological details								
				cellul	Ner frag	Sch cells	L	M	Epi cells	Gr	Casnecr	AFB
1	26/M	Num,	Rt com per	++	+	+	++	++	+	−	−	++
2	32/M	Sens def	Rt uln	+	+	+	++	++	−	−	−	++
3	45/F	Pain, pares	Lt med	++	++	++	+++	++	++	++	−	−
4	24/M	pain	Rt uln	++	++	++	+++	+	+++	+++	−	−
5	42/M	pain	Lt sup per	++	++	++	+++	+	+++	+++	−	−

C/N, Case Number; M, Male; F, Female; Cl/f, Clinical feature; Num, Numbness; Sens def, Sensory deficiency; pares, paresthesia; fna, fine needle aspiration; Rt, Right; Lt, Left; com per, common peroneal; uln, ulnar; med, median; sup per, superficial peroneal; cellul, cellularity; Ner frag, Nerve fragment; Sch, Schwann; L, Lymphocytes; M, Macrophages; Epi, Epithelioid; Gr, Granuloma; Cas necr, Caseous necrosis; AFB, Acid Fast Bacilli; (Cellularity: +, moderate, ++, good); (Nerve fragments and other cells: +, present; ++, moderate in number; +++; numerous); (AFB: ++, many).

smear was stained by Fite's stain to demonstrate acid fast bacilli (AFB). All these smears were studied for cytological details.

2.3. Cytological Examination. Both Papanicolaou and MGG stained smears were examined for cellularity, presence of nerve fiber, Schwann cells and nerve fiber infiltration by inflammatory cells, lymphocytes, macrophages, epithelioid cells, granuloma, giant cells, and caseous necrosis. The cellularity was quantified into moderate (+) and good (++). Nerve fragments, schwann cells, and inflammatory cells are quantified according to the presence of their number, and it was denoted as, present (+), moderate in number (++) and numerous (+++). Smear stained by Fite's stain was examined for the presence or absence of AFB. If the AFB was seen, it was quantified according to the presence of their number per high-power field. It was denoted as present (+), if occasional bacilli was seen after searching it in many high-power fields, and many (++) if many bacilli per high-power field were seen. Negative finding was denoted as absent (−).

3. Result

All the cases had mononeuropathy. All the clinicocytological details of these cases have been compiled in Table 1. Out of 5 cases, 4 were male and one was female. The age range was 24–42 years. All 5 cases showed nerve infiltration by chronic inflammatory cells (Figures 1 and 2). 3 cases showed epithelioid cell granuloma (Figure 3) and Langhans giant cell (Figure 4) without caseous necrosis, and Acid Fast Bacilli were kept under tuberculoid form and 2 cases with the absence of granuloma, giant cell, caseous necrosis but positive for AFB (Figure 5) were kept under borderline form of leprosy under Ridley-Jopling scale.

4. Discussion

Leprosy is common disease of India, Nepal, and Myanmar [1]. Although leprosy is a treatable disease, many patients will continue to experience significant nerve damage [1]; Patients with leprosy have high rates (56%) of established nerve damage at diagnosis, which frequently lead to disability [1]. In Nepal, about 7–16% of patients present with this form of leprosy [3].

Diagnosing leprosy relies on the identification of the typical clinical and histopathological involvement of the skin and nerves [1]. The absence of typical dermatological features greatly decreases clinical diagnostic accuracy and necessitates histological confirmation [1]. Skin biopsies from anesthetic areas may fail to show histological changes suggestive of leprosy in cases of pure neuritic leprosy [3]. The diagnosis of primary neuritic leprosy (PNL) and its differentiation from other causes of peripheral neuropathy is difficult, since acid-fast bacilli (AFB) smears and skin biopsy are negative from anesthetic areas [4]. A biopsy of the involved nerve is the only conclusive method of diagnosis [4]. Such a biopsy may not necessarily be free of complications when a large nerve is involved [4]. Nerve biopsy is limited by sampling errors, low sensitivity, and permanent nerve deficit, as still functioning nerves often need to be sacrificed [1]. However, nerve sparing techniques such as FNAC have been shown to maintain a high diagnostic yield when compared with standard biopsy and have less side effects [1]. Since this is a relatively "nerve sparing" procedure, this may allow the examination of motor nerves when sensory nerves are not involved or cannot be sampled [1]. No incidence of iatrogenic loss of motor, sensory function, or of local changes has been reported, following nerve FNAC [2–5]. Extensive medline search did not show any evidence of transmission of leprosy during FNA or nerve biopsy. However, we suggest that precaution should be always taken by using face mask and hand gloves, and one should remain very careful to prevent needle prick in each and every case during the procedure, because the transmission of leprosy by inoculation is well documented in the literature [11].

Fine needle aspiration has proved to be a simple technique to demonstrate inflammatory aspirate of lymphocytes, macrophages, epithelioid cells granulomas, Langhans giant cells, caseous necrosis, and AFB from the involved nerves in suspected cases of PNL [2–7]. Schwann cells arranged in a parallel fashion could be seen intimately mixed with granulomas [4]. The procedure is simple and minimally traumatic and has shown to provide valuable information, not only in the demonstration of leprotic inflammation,

Table 2: Cytomorphological classification of leprosy according to Ridley-Jopling spectrum.

Class	Singh et al. [8] (skin smear)	Prasad PV et al. [9] (skin smear)	Jaswal et al. [10] (skin smear)	Vijaikumar et al. [6] (nerve aspirate)
TT	Cellular smears, cohesive epithelioid cell granulomas, numerous lymphocytes not infiltrating the granuloma, no stainable AFB	Cellular material with predominantly lymphocyte population and histiocytes without epithelioid transformation, no stainable AFB	Cellular smears, cohesive epithelioid cell granulomas, numerous lymphocytes not infiltrating the granuloma. BI 0–3+	Good cellular aspirate· Cohesive epithelioid cell granuloma or lymphocytic cell collection· Predominantly epithelioid cells with predominant to moderate number of lymphocytes. Occasional giant cells and neutrophils· BI 0-1+.
BT	Same as TT	Cellular material with lymphocytes, histiocytes and epithelioid cells, foamy macrophages are not a feature, no stainable AFB.	Same as TT	Same as TT
BB			Fair cellular yields, poorly cohesive granuloma composed of an admixture of epithelioid cells and macrophages, few lymphocytes infiltrating the granulomas. BI 1-2+	Fair cellular aspirate· Mixed cellularity of predominantly nonfoamy macrophages, moderate number of epithelioid cells and lymphocytes. Macrophage granuloma· BI 2-3+.
BL	Moderate cellularity, singly dispersed macrophages with no epithelioid cells. Numerous lymphocytes diffusely scattered along with macrophages. BI 3-4+	Moderate cellularity, singly dispersed macrophages with no epithelioid cells. Numerous lymphocytes diffusely scattered along with macrophages. BI 3-4+	Moderate cellularity, singly dispersed macrophages with negative images, no epithelioid cells, numerous lymphocytes diffusely admixed with macrophages. BI 3-4+	Fair cellular aspirate· Predominantly lymphocytes and moderate number of foamy macrophages. BI. 4-5+.
LL	Heavy cellularity, numerous foamy macrophages in fatty background with a few lymphocytes. BI 5-6+	Heavy cellularity, numerous foamy macrophages in fatty background with a few lymphocytes. BI 5-6+	Heavy cellularity, numerous foamy macrophages in fatty background with intracellular and extracellular negative images, few lymphocytes. BI 4–6+	Fair to poor cellular aspirate· Predominantly foamy macrophages and few lymphocytes· BI 6+

TT, tuberculoid; BT, borderline tuberculoid; BB, borderline borderline; BL, borderline lepromatous; LL, lepromatous leprosy; BI, Bacillary index.

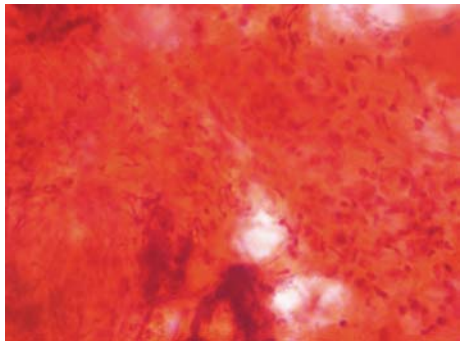

Figure 1: Nerve fragment showing infiltration by chronc inflammatory cells and granuloma (PAP, ×10).

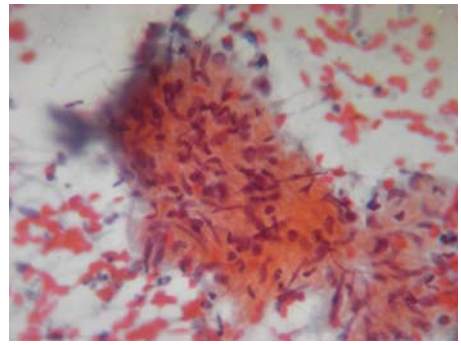

Figure 2: Spindle shaped Schwann cells infiltrated by chronc inflammatory cells comprising of macrophages, epithelioid cells and lymphocytes (PAP, ×40).

but also in the categorization of leprous neuritis along the Ridley-Jopling scale [2, 5, 6]. Cases with nerve involvement in leprosy classified leprous neuritis into paucibacillary (PB), borderline borderline (BB), borderline lepromatous (BL), and polar lepromatous leprosy (LL) types [6]. PNL in general will fall from typical tuberculoid to borderline lepromatous leprosy in the Ridley-Jopling classification [3]. A few cases of the Indeterminate [12] and lepromatous [13] form of pure neural leprosy have been also reported. Cytologically, tuberculoid PNL manifests with, either caseous necrotic

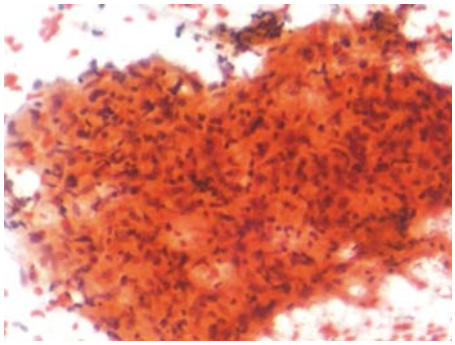

Figure 3: Smear showing epithelioid cells Granuloma (PAP, ×40).

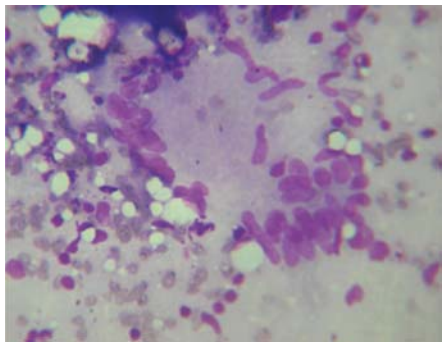

Figure 4: Smear showing Langhans giant cell (MGG, ×40).

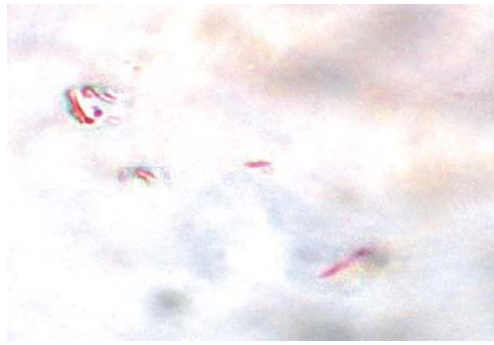

Figure 5: Smear showing AFB (Fite's Stain, Oil immersion).

material or epithelioid granulomas or a combination of both [2, 5]. Cutaneous involvement in leprosy is never associated with caseation, whereas the tuberculoid neuritic form of leprosy presents frequently with caseous necrosis [2, 5]. In the present study, 3 cases fall in tuberculoid and 2 cases in borderline lepromatous form of leprosy and managed accordingly, and caseous necrosis has not been found in any case. Like in its cutaneous counterpart, tuberculoid PNL is characterized by a high degree of cell mediated immunity (CMI) with intense granulomatous neuritis and no AFB [5, 12]. Borderline PNL is associated with a lower degree of CMI with several sites of neurologic impairment, with few, or many, AFB [5, 12]. A lepromatous PNL with low or absent CMI manifests with multiple lesions exhibiting numerous organisms within foamy histiocytes [12]. Indeterminate PNL is characterized by a few hypoesthetic or anesthetic patches, with little or no nerve involvement, few organisms, or no cutaneous changes [12].

Literature review of cytological findings of skin and nerve aspirates with Ridley-Jopling classification [6, 8–10] is tabulated in Table 2.

The accuracy of cytological classification along the Ridley-Jopling spectrum in nerve aspirate was found in 92% cases [6]. In the present study, we were able to classify all 5 cases according to criteria devised for interpreting the cytology of nerve aspirates [6]. However, a negative aspirate does not entirely rule out leprosy [6]. A strong concordance in tuberculoid (90%) and in lepromatous (93.7%) cases has been documented [14]. Mid-borderline cases of leprosy show a problem in proper diagnosis [14].

Nerve fragments comprising of Schwann cells cytologically simulate to epithelioid cell granuloma in low-power screening. It can be differentiated by morphological details made in high power. The Schwann cells are spindle-shaped cells of varying sizes with abundant, pale-staining cytoplasm with pulled out ends, and have oval, centrally or eccentrically, placed vesicular nuclei with ill-defined nucleoli [4]. Epithelioid cell granuloma is comprised by the collection of epithelioid cells. The epithelioid cells can be differentiated from Schwann cells by the presence of pale cytoplasm and vesicular elongated, drawn out, indented or folded nucleus, producing a shape reminiscent of a footprint. The nuclear chromatin is fine, and nucleoli are usually inconspicuous. The cytoplasmic margins are indistinct [4].

When the nerve involvement is solitary, the differential diagnosis includes tumors of the nerve sheath (neurofibromas and schwannomas), sarcoidosis, and sporotrichosis [15]. In sarcoidosis, the granulomas may be randomly dispersed from the roots to the distal nerve trunks and branches [15]. In these cases, involvement of neural tissue occurs after the expansion of a neighboring granuloma, while in leprosy the granulomas occur primarily in the nerve [15]. Moreover, sarcoidosis usually presents as a multifocal disease with multiple granulomas in several organs, mainly in the lung tissue [15]. The diagnosis of sporotrichosis can be suggested by the occurrence of several abscesses distributed along the lymphatic chains, but with no relation to the neural tissue [15]. In endemic area of leprosy, pure neuritic leprosy should always be considered in the investigation of a peripheral neuropathy [15]. We suggest that FNAC of the nerve will solve the problem to differentiate it from other lesions and can establish the conclusive diagnosis in these cases. FNAC of nerve sheath tumor shows benign spindle cells with palisaded long slender nuclei having pointed ends in fibrillary background. Sarcoidosis shows open granulomas with the absence of necrosis, acute, and chronic inflammatory cells and rarely the presence of asteroid bodies or Schaumann bodies in histiocytes and giant cells. Sporotrichosis shows suppurative granuloma with surrounding plasma cells and demonstration of fungal elements.

5. Conclusion

FNAC is safe, early, easy, less invasive, time saving, and cost effective procedure for the diagnosis of pure neuritic leprosy, and biopsy should be reserved only for inconclusive cases.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Review Article

Fine Needle Aspiration Cytology of the Breast: The Nonmalignant Categories

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Currently, accurate diagnosis of breast lesions depends on a triple assessment approach comprising clinical, imaging and pathologic examinations. Fine needle aspiration cytology (FNAC) is widely adopted for the pathologic assessment because of its accurracy and ease of use. While much has been written about the atypical and maliganant categories of FNAC diagnosis, little covers the non-malignanat category which represents a sheer number in all FNAC cases. Moreover, any false-negative diagnosis of the non-malignant cases may lead to missed diagnosis of cancer. This paper aims to discuss the issues of smear adequacy, the cytologic features of benign breast lesions and the dilemma of a false-negative aspirate. Much has been suggested about the smear adequacy criterion, including quantifying epithelial clusters, whereas others advocate basing adequacy on qualitative quantum of using noncellular features of FNAC. Various benign lesions could be easily diagnosed at FNAC; however, they have cytologic features overlapped with malignant lesions. False negativity of FNAC does occur; this could be caused by either "true" false-negative cases attributed to suboptimal sampling technique, poor localization of the mass or nonpalpable lesions or "false" false-negative cases due to interpretational errors. Though false-positive cases are less commonly found, they will also be discussed briefly.

1. Introduction

Fine needle aspiration cytology (FNAC) has become popular as a valuable tool in preoperative assessment of breast masses, and it shows high accuracy, sensitivity, and specificity. It has gained popularity due to its fast and easy approach, being inexpensive, and can be performed with little complications. To differentiate benign from malignant lesions is one of the major goals of FNAC. In the evaluation of breast masses, the time honored triple assessment combines clinical, radiological, and pathological information, and FNAC, together with core needle biopsy, is the initial pathological investigative methods of choice. Much confidence has been placed on this approach for it can obviate standard excisional biopsy when all three components of the triple test are conclusively negative or positive [1]. Nevertheless, in FNAC of breast lesions, there are instances where the differentiation of benign and malignant is not possible. This problem arises when paucity of specimen sampling is encountered or there is a morphological overlap between benign and malignant lesions (e.g., atypical hyperplasia and low-grade carcinoma in situ, or in papillary lesions). As a result and to accommodate these problematic areas, cytological reporting categories are used to objectively describe their features in cytological terms and to incorporate the groups with uncertainties. The most commonly used categorization is a five-tier system, with categories ranging from insufficient materials (C1), benign (C2), atypical (C3), suspicious of malignancy (C4), or frankly malignant (C5) (Table 1) [2]. This categorization helps the cytopathologists to define the uncertain areas, and the clinicians to offer further investigation like excisional biopsy judiciously. This categorization was initiated by the national coordinating committee for breast screening and the UK national breast screening program and serves as a common dialect among all breast health care professionals involved in breast management.

Table 1: Cytology reporting categories*.

C1 Inadequate
C2 Benign
C3 Atypia probably benign
C4 Suspicious of malignancy
C5 Malignant

* From Diagnostic Cytopathology of the Breast by Zakhour and Wells [2].

Under this categorization, C1 is inadequate aspirate smear due to hypocellularity, aspiration, smearing or staining errors. Most often, it is the degree of cellularity of the epithelial cells that is inadequate [2] (Figure 1). The exact definition of what constitutes an inadequate aspirate remains an enigma, and this subjective issue is best determined by the interpreter of the aspirate, whether or not a confident diagnosis could be made basing on the quantity of the materials aspirated. C2 category is for smears that are usually cellular, showing the characteristic patterns of different benign lesions. No atypical or malignant features are present. Usually duct configurations, myoepithelial cells, and bipolar nuclei are visible. Inflammatory background is commonly encountered. In contrast, C3 and C4 are the grey zones. C3 presents the characteristics of a benign smear and yet there are features that are not usually seen in clearly benign specimens such as cellular crowding, pleomorphism, and discohesion. C4 is reserved for aspirate where atypical features are obvious but factors such as poor preservation, hypocellularity, or components of a benign smear are present, thus precluding a firm malignant diagnosis to be made. This ambiguity shows the importance of correlation with other disciplines. It also emphasizes not to stretch the result of FNAC beyond the capabilities and experience of the interpreter to reduce both positive and negative errors [2]. C5 category consists of cellular aspirate with evidently malignant cytologic features. As much has been discussed on the atypical, suspicious, and malignant categories, this paper will be limited to the adequate (or inadequate) and benign categories together with the false negative and false positive cases.

2. Adequate FNAC

The adequacy of FNAC is dependent on multiple factors. The rate of inadequate aspiration ranges from 0.7% to 25.3% (Table 2), and this is influenced by the nature of the lesion, the available technology, and the experience and preference of the operator [2]. It was reported that the nature of the lesion was the most common cause of inadequacy of FNAC, accounting for 68% of the inadequate aspirates, followed by the experience of the aspirator that accounted for 32% of the inadequacy rate [3]. During the procedure, patient's cooperation is valuable, and a well-informed patient with good rapport with the operator for FNAC would greatly facilitate the procedure and improve the outcome in terms of adequacy. Thus, each procedure should be patterned and restricted to clinically and radiologically appropriate scenarios [2]. Some studies advocated that both aspirator and interpreter should ideally be the same, as the number of inadequate aspirates was far lower and the accuracy of diagnosis was higher when the same person aspirated and reported on the specimens [2, 4, 5]. The mean frequency of unsatisfactory aspirates by a nonpathologist was twice that when performed by a pathologist [6].

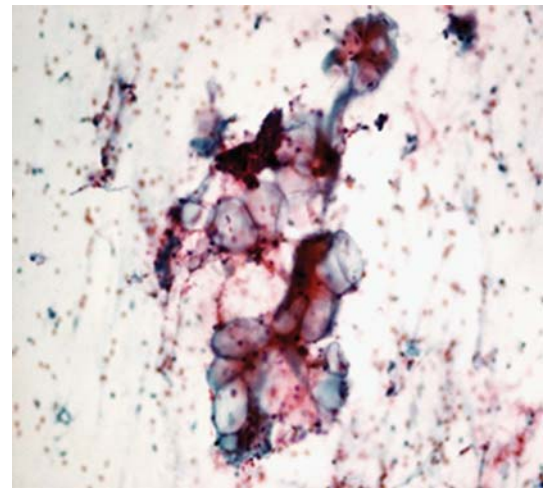

Figure 1: Photomicrograph of hypocellular smear, Pap, 10x. C1, Hypocellular smear.

Table 2: Inadequate FNA.

Authors	Inadequate cases (%)	Total number of cases
O'Neil et al. [24]	0.7%	697
Nguansangjam et al. [38]	4.2%	190
Rosa et al. [39]	8%	1583
Day et al. [40]	9%	831
Feichter et al. [41]	16.2%	1003
Zarbo et al. [6]	17%	13066
Park and Ham [37]	25.3%	699

Unanimous definition of specimen adequacy in breast FNAC has not been reached so far. The National Cancer Institute (NCI) definition of adequacy was one that led to resolution of a problem presented by a lesion in a particular patient's breast [7]. This definition was somewhat vague, being devoid of a quantifiable clause, but had the advantage of being very flexible and gave the aspirator the full mandate in deciding whether the cytologic features of the aspirate were consistent with the clinical findings and deemed adequate [8]. This would be particularly useful when both the aspirator, and interpreter of the sample were the same.

Most cytopathologists agree that a number of related parameters are significant determinants of the adequacy of breast FNAC, and these include clinical and imaging findings, size of the lesion, aspiration characteristics, experience of the aspirator, and the number of the needle passes [9]. Nevertheless, many authors considered epithelial cell clusters

Table 3: Benign FNA.

Authors	Benign cases (%)	Total number of cases
O'Neil et al. [24]	24%	697
Ishikawa et al. [25]	47.6%	382
Rosa et al. [39]	60%	1583
Feichter et al. [41]	68.1%	1003
Day et al. [40]	77.5%	831

as the most important adequacy criteria. Studies demonstrated that an appropriate number of epithelial cell clusters could be an important factor in lowering the false-negative diagnosis rate in palpable and nonpalpable breast masses [9–13]. It was further suggested that a cut-off of six epithelial cell clusters may provide a reasonable balance between reduction of false-negative FNAC smears and an increase in the rate of inadequate smears [13]. Since diagnosing malignancy involves evaluation of the cytologic features of the epithelial cells, quantification of epithelial cells in the smears is most likely helpful [9]. Other authors however, proposed not to require a minimum number of ductal epithelial cells as an adequacy criterion, and the assessment relied more on the noncellular features of FNA such as confidence and experience of the clinician or operator with regard to needle placement, resistance of the mass to the needle, and correlation with the clinical and physical findings [8], that is, using a triple assessment approach. Argument for this approach was that if one was to apply a specific number of ductal epithelial cell clusters [3–10], up to 35–40% of the true negative FNAC using a nonquantitative method would become unsatisfactory, forcing patients to undergo more expensive and possibly unnecessary work ups [8]. Typical examples would be in breast cysts, in which the aspirates usually yield histiocytes without epithelial cells. Rendering an inadequate diagnosis in an aspirate that collapsed a cyst yielding significant amount of serous fluid would make correlation with the clinical parameters difficult. Similar situations would be seen in postmastectomy scars with fibrosis, in which the hardened fibrotic area gives very low yield, and labeling such as inadequate may cause anxiety to the patients and prompt unnecessary subsequent investigations or excisions.

In reality, the issue is not in choosing to which school of thought should one affiliate in defining an adequate smear. A more practical approach is to consider the results of the triple test and the appearance of the epithelial clusters.

3. Benign FNAC

The bulk of breast FNAC diagnoses are benign, accounting for 24–77.5% of cases (Table 3). Fibrocystic changes present a spectrum of histological features that may sometimes be associated with calcification. Cystic changes represent a common finding. Characteristically, the size of the cyst varies in between consultation visits, giving the clinician further hint on its benign nature, especially when accompanied by imaging studies. In most situations, the aspirated cyst fluid may not be routinely submitted for cytologic evaluation,

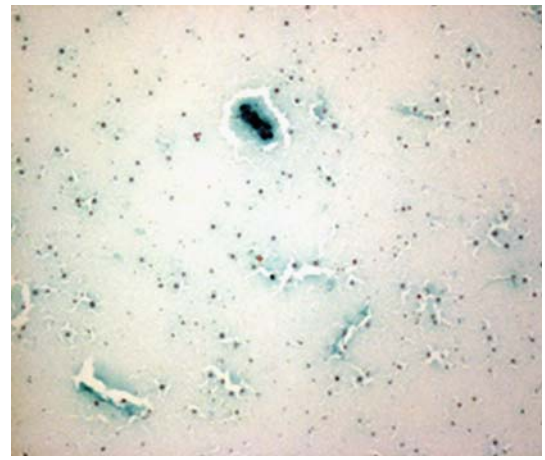

Figure 2: Photomicrograph of cyst contents composed of scattered macrophages and clusters of benign ductal cells, Pap, 4x. Cyst contents: scattered macrophages and few clusters of benign ductal cells.

except when the fluid is blood stained, cloudy, or turbid or when the masses remain uncollapsed and palpable after the aspiration. Most of the time, the smears would only show macrophages mixed with other inflammatory cells, confirming the cystic content nature of the lesion. Ductal epithelial and myoepithelial cells are also commonly seen in cyst aspirate, mostly as small balls and clusters mixed with the macrophages (Figure 2). Apocrine cells lining cyst cavity may exfoliate, showing the characteristic eosinophilic cytoplasm and round nuclei with distinct nucleoli. The above findings of apocrine cells, macrophages, and ductal cells are the characteristic features of a nonproliferative type of fibrocystic changes, which yields only scanty materials. When there is a significant epithelial proliferative component, sheets and tight clusters of cells are usually prominent. The presence of atypia in these cellular clusters may be further evaluated basing on cellular and nuclear spacing, multiple nucleoli, and character of chromatin materials. When these cytologic features are encountered, intraductal papilloma and fibroadenoma are some of the differentials that need to be ruled out. Though cytologically indistinguishable from proliferative fibrocystic changes, intraductal papilloma is often accompanied by clinical history of nipple discharge and a palpable subareolar mass.

Another potential source of confusion rises when there is the presence of proteinaceous fluid in the background, being associated with epithelial cells that are large, with enlarged nuclei, eosinophilic nucleoli, and vacuolated and wispy cytoplasm. The nuclear features may appear worrisome. Nevertheless, one should also be on the outlook for lactational changes, and the appropriate history has to be sought to avoid a false-positive diagnosis [2]. FNA plays a significant role when a discrete nodule appears during pregnancy or lactation. This spares the pregnant patient from the pain and complications of excision.

In more florid examples, thickening of the wall due to papillary apocrine change may cause papillary clusters with the same cytoplasmic and nuclear details to be present [2].

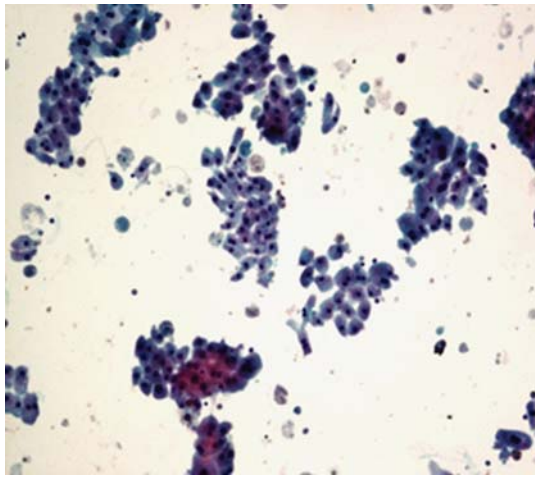

Figure 3: Photomicrograph of apocrine cells with granular cytoplasm and mild anisonucleosis, Pap, 10x. Apocrine cells: granular cytoplasm and mild anisonucleosis.

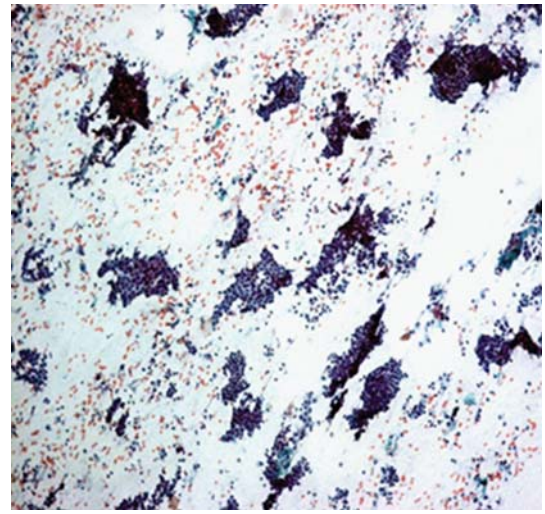

Figure 4: Photomicrograph of hypercellular smear with monolayered sheets of ductal cells in fibroadenoma, Pap, 10x. Fibroadenoma: hypercellular smear with monolayered sheets of ductal cells.

Not all smears from cyst aspirate are easy to evaluate. Apocrine cells, when degenerated will most often appear atypical especially if it has progressed over time to the phase of chromatin clumping with associated anisonucleosis [2], and these could potentially be labeled as suspicious (Figure 3). When there is infection or prior rupture of the cyst, the aspirated fluid may be turbid or milky. Such aspirates often contain degenerated cells and debris in an abundant background of inflammatory cells. In this situation, it needs to be differentiated from the rare squamous carcinoma, which may present with features akin to inflamed cyst.

Among the solid breast lesions, fibroadenoma is most common especially in women who are less than 40 years old. The clinical presentation is very characteristic, and correct clinical diagnosis can often be made. Radiologically, it is described as a low density mass with well-defined margins. Calcification may not often be present in fibroadenoma especially in the young age group, but among older population a popcorn calcification is characteristic [14]. Multiple fibroadenomas are seen in 15 to 20% of the cases [2].

FNAC diagnosis of fibroadenoma is highly accurate. Lopez-Ferrer reported a 79.3% predictive value out of 362 fibroadenoma aspirates with most diagnostic errors occurring in the older age group [15]. Cytologically, aspirates are hypercellular with characteristic monolayer sheets of benign-looking epithelial cells mixed with myoepithelial cells. These sheets are often described as "staghorn", having antler-like configuration on its edges (Figure 4). This pattern reflects the configuration of ducts as observed on histological sections [2, 16]. Cellular cohesiveness is often appreciated in the aspirate smear. Accompanying the epithelial cells are the fibrillar stromal materials which may vary in cellularity and sometimes show myxoid change (Figure 5). Commonly, the background of the aspirate is composed of numerous naked/bipolar nuclei (Figure 6). This is one of the characteristic cytologic features of fibroadenoma. The added presence of large number of bipolar nuclei in the background of smear

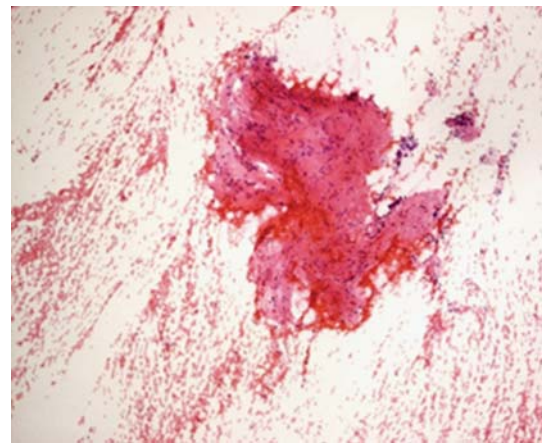

Figure 5: Photomicrograph of stromal fragments in fibroadenoma, H&E, 10x. Fibroadenoma: stromal fragments.

is a reliable feature in favor of fibroadenoma [2]. There are aspirates which may show less pronounced antler horns but this may represent sample from fibroadenoma with pericanalicular pattern. Branching of epithelial sheets is more common if the aspirated sample is from an intracanalicular form (Figure 7). The commonly encountered cytological features of fibroadenoma are fibromyxoid stroma, staghorn clusters, and numerous single bare nuclei, being seen in 92.7%, 73.6%, and 73.6% of cases, respectively [17]. These findings constitute the diagnostic triad for fibroadenoma. There are instances wherein the diagnosis of fibroadenoma on cytology is not straight forward. The absence of any components of the diagnostic triad and low cellularity are the common causes of pitfalls in missed cytodiagnosis of fibroadenoma [17] (Table 3). Giant cells are uncommonly seen in fibroadenomas (Figure 8). In the report of Kollur and El Hag, it showed an increased incidence, being present

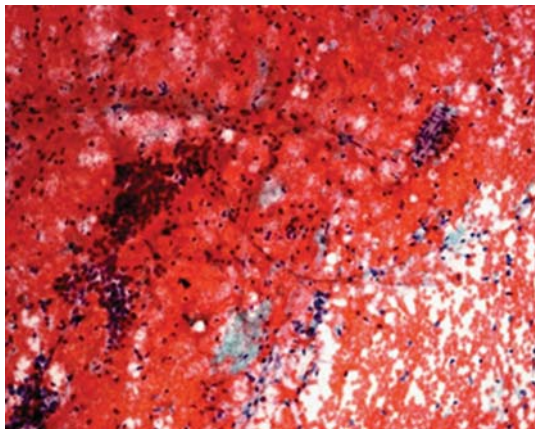

Figure 6: Photomicrograph of numerous bipolar cells in fibroadenoma, H&E, 10x. Fibroadenoma: bipolar cells in the background.

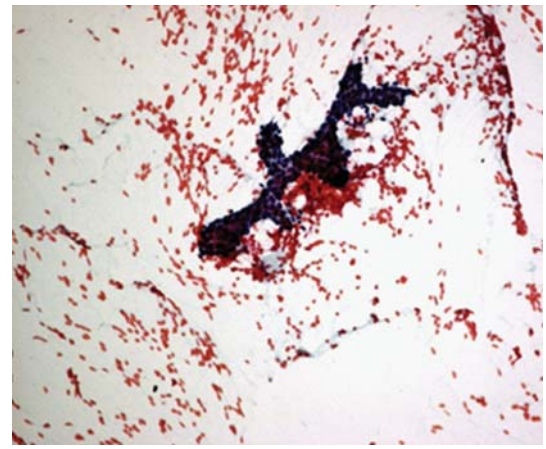

Figure 7: Photomicrograph of antler-horn configuration of ductal cells in fibroadenoma, Pap, 10x. Fibroadenoma: antler-horn configuration.

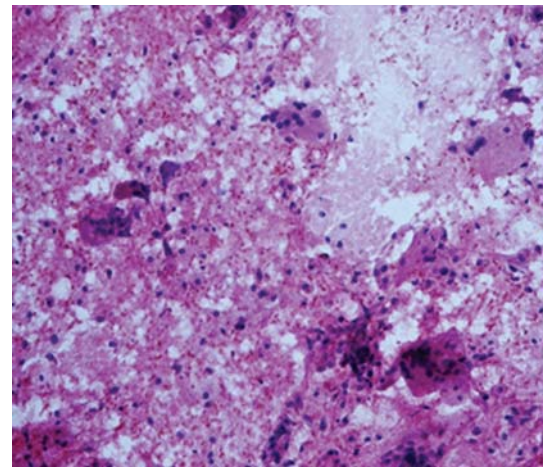

Figure 8: Photomicrograph of giant cells in fibroadenoma, H&E, 20x. Fibroadenoma: stromal giant cells.

in 31.8% of the aspirated cases [17]. These giant cells are variable in appearance, were thought to be stromal in origin [18, 19], and are of little prognostic significance. Most series reported the presence of these stromal giant cells being present in fibroepithelial lesions of the breast, but were more common in phyllodes tumor than fibroadenomas [18, 19]. Sometimes, giant cells may indicate an extra-tumoral reactive process in the surrounding breast tissue which may be due to palpation granuloma or fat necrosis [17]. It is a known fact that fibroadenoma is difficult to distinguish from phyllodes tumor using aspiration cytology but there are some features that are more characteristic to phyllodes tumors that will support its diagnosis on cytology. A cellular aspirate with numerous plump and spindly nuclei, pronounced of hypercellularity of stromal fragments, and presence of atypia are the key points that support a diagnosis of phyllodes tumor over fibroadenomas. However, these differentiating features may not be present in all cases. The presence of more stromal fragments over epithelial fragments (higher stromal epithelial ratio) and the presence of single columnar cells in the background are some of the "soft signs" reported for the identification of phyllodes tumor over fibroadenoma. In the extremely rare instance in which a malignant phyllodes tumor is encountered, the sarcomatous spindle cells within cellular stromal fragments may be definitive for the establishment of the diagnosis. Fibroadenomas also need to be differentiated from papillomas, by virtue of the fact that the latter show presence of small cell balls or clusters, with either staghorn or papillary configurations in the smears.

On the whole, FNAC showed a high sensitivity of up to 68–97% in fibroadenomas [15, 17], and it has been demonstrated that the overall cellularity, amount of bipolar nuclei, amount and architectural of epithelium, apocrine metaplasia, nuclear overlapping and pleomorphism, foam cells, and stroma are significant cytologic parameters in distinguishing fibroadenomas from papilloma, fat necrosis, fibrocystic changes, and duct ectasia [16].

Nipple discharge is one of the alarming complaints that would prompt patients to seek clinical consults. This represents, commonly, a papillary lesion involving one of the major lactiferous ducts [2]. Intraductal papillomas are usually solitary and most often found in the subareolar region. It is relatively common, accounting for 2.5% of all benign breast excisions [16]. It is seen as a well-defined mass which radiologically presents as low-density soft tissue mass with no surrounding architectural distortion or tissue response. Calcification, when present, is usually of the dystrophic and curvilinear type [2]. In addition, papillary fronds reminiscent of staghorn clusters can also be seen in papillomas. It was found that foam cells in association with these fronds is one of the more specific features of differentiating papilloma from fibroadenoma [2, 16]. Papillomas in FNAC may cause diagnostic problems. The accuracy of FNAC in diagnosing papillary lesions and differentiating benign and malignant papillary lesions is low [20]. Among the aspirates diagnosed as atypical, intraductal papilloma represents about 6% [21]. For papillomas, the typical FNAC picture of papillary fronds, cell balls, and columnar cells may not all be seen in the aspirate, which may also be complicated

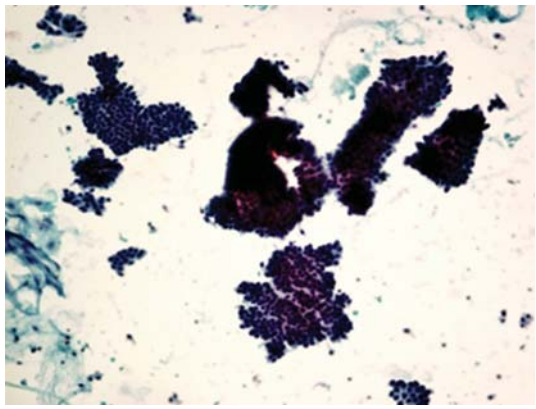

Figure 9: Photomicrograph of papillary fronds in papilloma, Pap, 10x. Papilloma: papillary fronds.

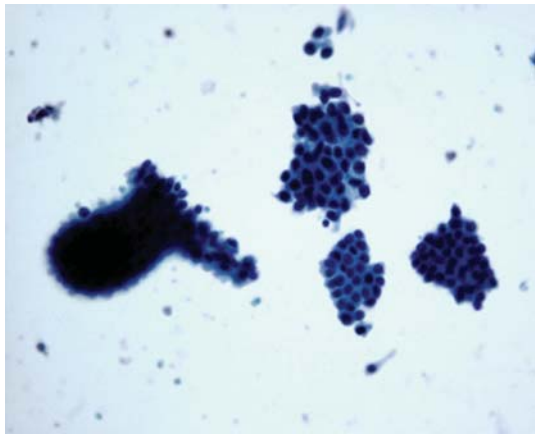

Figure 10: Photomicrograph of cell balls with cytologic atypia in papilloma, Pap, 20x. Papilloma: cell balls with cytologic atypia.

by a hemorrhagic background (Figures 9 and 10). At such the cytologic picture may raise the possibility of a malignancy. Problems also occur when the papillomas are complicated by epithelial hyperplasia, hyalinization, or apocrine changes as these may yield hypocellular to hypercellular smears with pleomorphic cells showing prominent and background necrotic debris [22, 23]. To date, there have been no well-defined cytological criteria to differentiate between benign and malignant papillary lesions. Their significant overlap in terms of architecture and cytological atypia is the primary reasons for not differentiating them cytologically [20, 21].

4. False-Negative FNAC

FNAC has irrefutably and significantly contributed to the reduction of excisional biopsies in the assessment of breast lesions, especially in the context of triple assessment [24]. Nevertheless, there still exists a significant false negative rate for FNAC, in the range of 1.2–10.6% (Table 4). These may lead to missed/delayed diagnosis and treatment [25], sometimes with adverse clinical outcome. This has become a major concern, prompting, on the side of the laboratory and pathologist, a re-evaluation of the adequacy limitation, referral system, and processing techniques. Previous studies have demonstrated that the sensitivity, specificity, and accuracy of breast FNAC all ranged from 77% to 100% [24, 26–29].

Table 4: False-negative FNA.

Authors	False negative cases (%)	Total number of cases
Rosa et al. [39]	1.2%	1583
O'Malley et al. [12]	1.6%	1005
O'Neil et al. [24]	1.9%	697
Ishikawa et al. [25]	2.2%	382
Arisio et al. [29]	3.9%	1601
Day et al. [40]	5.4%	831
Feichter et al. [41]	9%	1003
Park and Ham [37]	10%	699

The underlying causes for false negativity can be grouped into diagnostic errors and true false negative factors. Diagnostic errors can be attributed to lack of training, overload of cases, and miscorrelation with the patient's clinical and radiologic findings [6]. In the true false negative factors, the denominators are poor sampling technique, mislocalization of the tumor, or the presence of a well-defined tumor demonstrating minimal atypia [29, 30]. The widespread adoption of breast screening and advances in imaging techniques also resulted in the detection of small lesions, and understandably, FNAC of these small lesions has a significant risk of missing these lesions, leading to potentially false-negative results.

Nonpalpable lesions constitute a specific category of screen detected lesion. In one study, 21% of false negative breast FNAC was due to nonpalpable tumors [31]. The main problem associated with FNAC of nonpalpable lesions was the variable but sometimes unacceptably high rate [32] of inadequacy. An inadequacy rate as high as 34–58% had previously been reported [33, 34], and the lowest reported inadequacy rate was around 10% [35, 36]. Attaining adequacy in the aspirates of these nonpalpable lesions poses greater challenge because of their small sizes in many cases, as well as the presence of fibrotic component. Nowadays, management of these lesions always involves CT or ultrasound guidance to better define and localize the lesion upon aspiration. Apart from tumor size, tumor grade was also an important risk factor for false-negative FNAC (Table 5). In Bulgaresi's report of false-negative FNAC reporting, 24.3% were those from special types of tumor, 39% of which were low grade tumors [31]. The resemblance of lobular carcinoma to lymphocytes and its subtle cytologic atypia are well-known diagnostic problems. Ductal carcinoma, not otherwise specified (NOS) subtype, accounted for 2/3 of the cases false-negative cases in another series [37]. As a result, nonpalpable lesions constitute a specific "blind area" not amenable to FNAC, indeed most authors would recommend core needle biopsy for the work up of such lesions [32].

Table 5: Surgical followup in false-negative cases*.

Follow up tissue diagnosis	Number/percentage of cases
Atypical ductal hyperplasia	1 (5%)
Ductal carcinoma in situ	3 (16%)
Cribriform carcinoma	1 (5%)
Metaplastic carcinoma	1 (5%)
Infiltrating lobular carcinoma	6 (32%)
Infiltrating ductal carcinoma	7 (37%)
Total cases	19

* Rosa [39]. The value of fine needle aspiration biopsy in the diagnosis and prognostic assessment of palpable breast lesions.

Table 6: False-positive FNA.

Authors	False negative cases (%)	Total number of cases
Rosa et al. [39]	0%	1583
Day et al. [40]	0%	831
Arisio et al. [29]	0.3%	1601
Feichter et al. [41]	0.5%	1003
Park and Ham [37]	1%	699
Ishikawa et al. [25]	2%	382

5. False-Positive FNAC

False-positive diagnosis in aspiration cytology is significantly lower in incidence compared to false-negative cases. From the previous reports, false-positive cases range from 0% to 2% (Table 6), in most studies reporting a 100% positive predictive value. Among the reported cases, the common lesion giving a false-positive aspirate is ductal hyperplasia or lobular hyperplasia. This finding is also in consonance with previous reports that fibrocystic changes and pregnancy-related breast masses account for false-positive findings [25]. In most of the accounted cases, radiologic findings are mostly indeterminate for breast cancer, requiring confirmation by histology.

6. Summary

FNAC is an essential component in the preoperative management of breast lesions. Its accuracy, ease of use, and affordability are factors that cause its popularity. The advent of imaging technology together with the clinical expertise of the clinician contributed to its increased sensitivity. The adequacy of smears is influenced by the nature of the lesion, experience of the aspirator, and access to the available imaging modality. An adequate smear can be defined by either quantitative or qualitative means, with advocates for either approach. Nevertheless, the operators' experience and confidence in correlating with the clinical and radiologic findings, the cellularity of smears, and the aspiration technique are always helpful. Exceptions occur in cystic and fibrotic lesions that are inevitably hypocellular. Degenerative changes would render the smear to be difficult to interpret. Benign breast lesions are usually easy to diagnose when their characteristic cytologic patterns are obvious. Hypocellularity, degenerated apocrine cells, necrosis, and epithelial hyperplasia are some of the factors that may be encountered in evaluating a difficult smear, mimicking atypical or malignant lesions. The false-negative cases in breast FNAC, although few, are commonly due to poor sampling technique, poor tumor localization, and the presence of a well-differentiated histology of the tumor. Small tumor size and nonpalpable breast lesions are also commonly associated with false-negative and aspirate inadequacy. Thus in the interpretation of breast FNAC, all these factors should be considered before a benign diagnosis is being rendered.

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Research Article

Comparison of Fine Needle Aspiration Cytology and Thyroid Scan in Solitary Thyroid Nodule

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Objective. This was a comparative study between FNAC and thyroid scan used to diagnose the solitary thyroid nodule and histopathology was used as gold standard to compare the results of both modalities. We hypothesized that Fine needle aspiration cytology and thyroid scan diagnose solitary thyroid nodule (STN) as accurately as histopathology. *Materials and Methods*. This study comprised of 50 patients with solitary thyroid nodules (STN) presented to OPD. After clinical examination these patients were referred to Centre for Nuclear Medicine, Mayo Hospital Lahore for thyroid function tests and thyroid scan (TS). These patients underwent FNAC in the department of Pathology and surgery in Mayo Hospital. The cases were operated and evaluated for histopathological changes. *Results*. On thyroid scan, 40 patients (80%) having cold nodule were labeled as suspicious 10 patients (20%) had hot nodule. On FNAC 23 patients (46%) had benign lesion, 22 patients (44%) had indeterminate lesion and 5 patients (10%) had malignant lesions. On histopathology, 45 patients (90%) were confirmed to have benign lesions and 5 patients (10%), malignant lesions. After comparison of results of thyroid scan and FNAC with histopathology, the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of thyroid scan were 80%, 20%, 10%, 90% and 26%, respectively whereas those of FNAC were 80%, 97.7%, 80%, 97.7% and 96%, respectively. *Conclusion*. Fine needle aspiration was a significantly better predictor of malignancy than thyroid scan and resulted in a smaller proportion of excisions for benign nodules.

1. Introduction

Thyroid nodular (TN) lesions are a common clinical problem in the world. These are more common in women and in areas of iodine deficiency. Exposure to ionizing radiation in childhood and adolescence increases the risk of solitary thyroid nodule and thyroid carcinoma. In the United States, 4 to 7% of the adult population has a palpable thyroid nodule [1].

A solitary thyroid nodule is a palpable swelling in thyroid gland that has otherwise a normal appearance [2]. The majority of thyroid nodules are asymptomatic and only about 5 percent of all palpable nodules are found to be malignant. A variety of tests have been employed to separate benign from malignant thyroid nodules [2, 3].

These tests include isotope scanning and fine needle aspiration cytology. Combined use of isotope scanning, fine needle aspiration cytology, and histopathology of thyroid offers the best diagnostic strategy [4].

Isotope scanning was generally used to classify nodules into nonfunctioning (cold) or functioning (warm or hot) nodules. The scans used either Iodine123 or technetium Tc99m pertechnetate. Only 5 to 15% of the cold nodules are malignant [1, 5].

As the cost of 131 scintigraphy increases, the I-131 scintigraphy becomes less effective than the fine needle aspiration cytology strategy. In one study of solitary thyroid nodule, the specificity of scan turns out to be only 21.1%. So advice is there to avoid this technique as a routine test in patients with nontoxic thyroid nodules [6, 7] (6, 8).

Fine needle aspiration cytology of thyroid nodules is the single most sensitive, specific, and cost-effective method of investigation of thyroid nodules. Now it is safely and widely recommended for the preoperative selection of patients.

The major pitfall of this procedure is that fine needle aspiration cytology cannot differentiate between follicular adenoma and follicular carcinoma [5, 7].

Histopathology of the excised specimen showed multinodular goiter as the commonest lesion. In one study, fine needle aspiration cytology and thyroid scan offers the best preoperative assessment of solitary thyroid nodule. Histopathology later on confirms the preoperative diagnosis. As the incidence of solitary thyroid nodule is high in Pakistan, this study will help in early detection of thyroid lesions [8].

Many modalities are used for the diagnosis of solitary nodule in Pakistan but histopathology remained the gold standard for the comparison of all. In a recent study it has been seen that FNAC is a primary investigation of thyroid lesions and used in a patient with one or more thyroid nodule. FNAC is also advised for every patient for exclusion of cancer and in the initial management of patients. It is frequently used because it is inexpensive, sensitive, specific, and an accurate procedure; therefore it is adapted as an initial investigation of thyroid diseases in all tertiary hospitals in developing countries like Pakistan [9]. In Pakistan, before performing manual or guided FNAC, thyroid scan and thyroid function tests (TFTs) are performed for further management of solitary thyroid nodules. In this study we compared the individual efficacy of FNAC and thyroid scan in the management of solitary thyroid nodules. The nodules were marked after doing their scan by the nuclear center of Mayo Hospital taking histopathology as gold standard.

2. Material and Methods

This was a comparative cross-sectional study and carried out at the Departments of Surgery, Nuclear Medicine, and Pathology, King Edward Medical University, Lahore. It was conducted on 50 patients of solitary thyroid nodule in 06 months. A nonprobability purposive sampling technique was used for these patients.

Inclusion Criteria.

(1) Age 10 to 70 Years.

(2) Both genders.

(3) Patient presenting with solitary swelling arising from any lobe of thyroid selected by clinical palpation.

Exclusion Criteria.

(1) Patients with diffuse thyroid swelling.

(2) All toxic and multinodular goiters confirmed by clinical evaluation.

(3) Patients with history of any type of thyroid surgery (lobectomy or total thyroidectomy).

2.1. Data Collection. All patients presenting with solitary thyroid nodules in the OPD and fulfilling the inclusion criteria were included in this study. Informed consent from all the patients included in the study was taken. All the patients were recorded for their demographic features, that is, age, sex, and address (for followup). History of present illness with regard to symptoms and duration was recorded. They were examined for the signs related to the solitary thyroid swelling. All routine investigations and serum T3, T4, and TSH levels were performed by Radioimmunoassay (RIA), (normal range of T3, 2.5–5.8 pmol/L, T4, 11.5–23.0 pmol/L, and TSH, 0.2–4.0 mIU/L). Patients with solitary thyroid swelling underwent thyroid scan. Solitary thyroid nodules were selected and then FNAC was performed. The radionuclide scan and FNA were performed in conjunction as compared to FNAC alone.

Cytological diagnosis was categorized into four groups: negative for malignancy, indeterminate (suspicious) for malignancy, positive for malignancy, and inadequate. The cases were operated and evaluated for histopathological changes. The results of thyroid scan, fine needle aspiration cytology, and histopathology were compared. Histopathology was taken as gold standard. All these information were collected through proforma that is attached herewith.

2.2. Statistical Analysis. All the data was analyzed with SPSS version 11. The variables included were demographic information, routine investigations, thyroid scan, and thyroid function tests. For quantitative data, that is, thyroid function tests, duration and size of thyroid nodule, mean, and standard deviation were calculated. For qualitative data, that is, results of thyroid scan, fine needle aspiration cytology, and histopathology, percentages was calculated. A 2×2 table was used to calculate sensitivity, specificity, positive predictive value, negative predictive value, and accuracy.

Table 1: Distribution of subjects by age and sex.

Age Male: Female = 9:41 (18% and 82%)	Number	Percentage
10–20	04	8.0
21–30	22	44.0
31–40	14	28.0
41–50	04	8.0
51–60	05	10.0
61–70	01	2.0
Total	50	100.0
Mean ± SD	33.04 ± 12.29	
Male to female ration	1 : 4.6	

3. Results

The age of patients ranged from 10 to 70 years with mean age 33.04 ± 12.29 years. 41 patients (82%) were females, and 9 (18%) were males (male to female ratio 1 : 4.6) (Table 1).

Regarding thyroid function tests, 48 patients (96%) were euthyroid, 2 patients (4%) were hyperthyroid, and no patient was hypothyroid. The mean for serum T3, serum T4, and serum TSH were 4.00 ± 0.90, 16.47 ± 3.18, and 0.95 ± 0.85, respectively, (normal values given in Table 2). There were 40 patients (80%) who had cold nodule on thyroid scan of which 8 patients (16%) were male and 32 patients (64%)

Table 2: Distribution of subjects by thyroid function tests (RIA method).

	Serum T3 (2.5–5.8 pmol/L)		Serum T4 (11.5–23 pmol/L)		Serum TSH (0.2–4.0 mIU/L)	
	No.	%age	No.	%age	No.	%age
Normal	48	96.0	48	96.0	48	96.0
Increased	02	4.0	02	4.0	0	0
Decreased	0	0	0	0	02	4.0
Total	50	100.0	50	100.0	50	100.0

Table 3: Distribution of subjects by thyroid scan.

Thyroid scan	Male		Female		Total	
	No.	%age	No.	%age	No.	%age
Cold nodule	08	16.0	32	64.0	40	80.0
Hot nodule	01	2.0	09	18.0	10	20.0
Total	09	18.0	41	82.0	50	100.0

Table 4: Distribution of subjects by size of nodule ($n = 50$).

Size of nodule (cm)	Number	Benign 44	Malignant 6
1–5	35 (70.0%)	32	3
6–10	15 (30.0%)	12	3
>10	0	0	0
Total	50 (100.0%)	44	6
Mean ± SD	4.60 ± 1.63		

were female, while 10 patients (20%) had hot nodule on thyroid scan of which 1 patient (2%) was male and 9 patients (18%) were female (Tables 2 and 3).

The mean for size of thyroid nodule was 4.60 ± 1.63 cm. 35 patients (70%) had 1–5 cm sized thyroid nodules, and 15 patients (30%) had 6–10 cm thyroid nodules. No patient had thyroid nodule greater than 10 cm in size (Table 4).

Out of 50 patients, 23 patients (46%) were of benign FNAC (7 colloid cyst colloid, 2 multinodular goiter, 12 colloid goiter, and 2 chronic lymphocytic thyroiditis). 22 patients (44%) were of indeterminant including suspicious for malignancy FNAC of which 2 patients (4%) were males and 20 patients (40%) were females, and 5 patients (10%) were of malignant FNAC of which 1 patient (2%) was male and 4 patients (8%) were female. Out of the 23 patients with benign FNAC, 7 patients (30.4%) had colloid cyst, 2 (8.7%) males and 5 (21.73%) females; 2 patients (8.7%) had colloid goiter, 1 (4.35%) male and 1 (4.35%) female; 12 patients (52.17%) had colloid nodule, 3 (13.04%) males and 9 (39.13%) females, and 2 patients (8.7%) had lymphocytic thyroiditis, both females (Tables 4-5).

Out of the 5 patients with malignant FNAC, 3 patients (60%) had papillary carcinoma, 1 (20%) male and 2 (40%) females; 1 patient (20%) had medullary carcinoma who was female, and 1 patient (20%) had anaplastic carcinoma who was female (Table 4).

Out of the 22 patients with indeterminant FNAC, 3 patients (13.63%) had follicular lesion, all of them females, and 19 patients (86.37%) had follicular neoplasm, 2 (9.1%) males and 17 (77.27%) females (Table 4).

On histopathology, 45 patients (90%) were confirmed to have benign lesions and 5 patients (10%) malignant lesions. Out of 45 patients with benign lesions on histopathology, 7 patients (15.5%) had colloid cyst, 2 (4.4%) males and 5 (11.1%) females; 2 patients (4.4%) had colloid goiter, 1 (2.2%) male and 1 (2.2%) female; 12 patients (26.67%) had colloid nodule, 3 (6.67%) males and 9 (20%) females; 2 patients (4.4%) had chronic lymphocytic thyroiditis, both females; 18 patients (40%) had follicular adenoma, 2 (4.4%) males and 16 (35.5%) females; 1 patient (2.25%) had diffuse hyperplasia which was female, and 3 patients (6.67%) had hyperplastic nodule, all of whom were females. Out of 5 patients with malignant lesions on histopathology, 2 patients (40%) had pure papillary carcinoma, 1 (20%) male and 1 (20%) female; 1 patient (20%) had medullary carcinoma who was female, 1 patient (20%) had anaplastic carcinoma who was female, and 1 patient (20%) had angioinvasive follicular carcinoma (Table 4).

On comparison of results of thyroid scan with histopathology taken as gold standard, out of 50 patients, 4 patients were true positive, 9 patients true negative, 36 patients false positive, and one patient false negative (Table 6). The sensitivity of thyroid scan was found to be 80%, specificity 20%, diagnostic accuracy 26%, positive predictive value 10%, and negative predictive value 90% (Figure 1 and Table 8).

On comparison of results of FNAC with histopathology taken as gold standard, out of 50 patients, 4 patients were true positive, 44 patients true negative, one patient false positive, and one patient false negative (Tables 6-7). The sensitivity of FNAC was 80%, specificity 97.7%, diagnostic accuracy 96%, positive predictive value 80%, and negative predictive value 97.7% (Table 8).

Morphological comparison of different lesions on FNAC and Histopathology is shown in Figures 2–5.

4. Discussion

Fine needle aspiration cytology is a well-established technique for preoperative investigation of thyroid nodules. The technique is a noninvasive, cost-effective, and efficient

Table 5: Distribution of subjects by benign and malignant lesions FNAC and histopathology.

Classification (benign = 23 and malignant = 5)	FNAC ($n = 50$)	Histopathology ($n = 50$)
Colloid cyst	07 (30.43%)	7 (14%)
Multi nodular colloid goitre	02 (8.70%)	2 (4%)
Colloid goiter	12 (52.17%)	12 (24%)
Chroinic lymphocytic thyroiditis	02 (8.70%)	2 (4%)
Follicular lesions (neoplasm)	21	Nil
Follicular adenoma	—	18 (36%)
Diffuse hyperplasia	—	1 (2%)
Hyperplastic nodule	—	3 (6%)
Papillary carcinoma	03 (60.0%)	2 (4%)
Medullary carcinoma	01 (20.0%)	1 (2%)
Anaplastic carcinoma	01 (20.0%)	1 (2%)
Folicular carcinoma	—	1 (2%)

Table 6: Distribution of subjects by classification of FNAC $n = 50$.

Classification	FNAC			Histopathology
	Male No.	Female No.	Total	
Benign	06 (12.0%)	17 (34.0%)	23 (46.0%)	44 (88%)
Malignant	01 (2.0%)	04 (8.0%)	05 (10.0 %)	06 (12%)
Indeterminant	02 (4.0%)	20 (40.0%)	22 (44.0%)	—
Total	09 (18.0%)	41(82.0%)	50 (100.0%)	50 (100%)

Table 7: Comparison of distribution of subjects by classification of FNAC and histopathology.

Classification	FNAC	Histopathology
Benign	23 (46 %)	44 (88%)
Malignant	05 (10%)	06 (12%)
Indeterminant	22 (44%)	—
Total	50 (100%)	50 (1005)

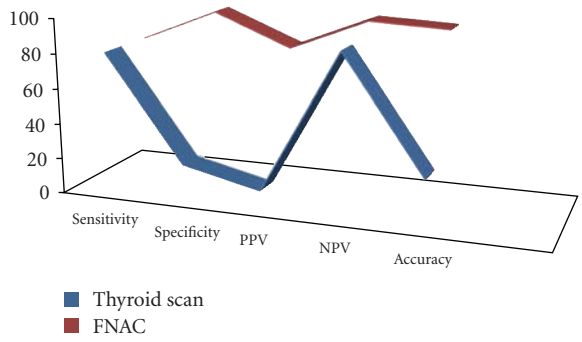


Figure 1: Comparison of sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of thyroid scan and fine needle aspiration cytology.

method of differentiating benign and malignant thyroid nodules [1, 10, 11]. Many investigators have shown that fine needle aspiration cytology is the single most sensitive, specific, and cost-effective method in the investigation of solitary thyroid nodules [12, 13]. Despite studies supporting the cost-effectiveness of fine needle aspiration cytology as the diagnostic test of choice, I-131 scintigraphy continues to be used by frontline providers as primary diagnostic tools in the management of patients with nodular thyroid diseases. Justifications for the continued use of this alternative diagnostic strategy usually range from historical practice patterns within institutions to faster turnaround time for results when compared with waiting for FNAC pathology reports. FNAC of thyroid is gaining popularity among pathologists and clinicians all over the world [14–17].

In our study, the age of patients ranged from 10 to 70 years with mean age 33.04 ± 12.29 years. The highest number of patients was aged between 21–30 years, that is, 22 (44%). In a study from Saudi Arabia, the mean age was 36.17 ± 12.3 years (range 15–67 years) which is very close to our study [18]. In this study, the female to male ratio was high. In our study, out of 50 patients 41 (82%) were females and only 9 (18%) were males. Female to male ratio was 5.2 : 1. These results are close to Hussain and Anwar, who found female to male ratio as 6.9 : 1 [3]. In our study, 40 patients (80%) had cold nodule on thyroid scan. 10 patients (20%) had hot nodule on thyroid scan. In a study from India, 77.77% patients had cold nodule on thyroid scan while 22.22% patients had hot nodule on thyroid scan, which is close to our study [19].

In the literature, it is clearly indicated that the nodule size is only a weak predictor of histological malignancy [12]. In our study, 35 patients (70%) had 1–5 cm sized thyroid

Table 8: Comparison of thyroid scan and FNAC with histopathology.

	Thyroid scan and histopathology		Total	FNAC and histopathology ($n = 50$)		Total
	Positive	Negative		Positive	Negative	
Positive	04 (TP)	36 (FP)	40	04	01	05
Negative	01 (FN)	09 (TN)	10	01	44	45
Total	05	45	50	05	45	50
Sensitivity = 80%, specificity = 20%, accuracy 26%, PPV = 10%, and NPV = 90%				Sensitivity = 80.0%, specificity = 97.7%, accuracy = 96.0%, PPV = 80.0%, and NPV = 97.7%		

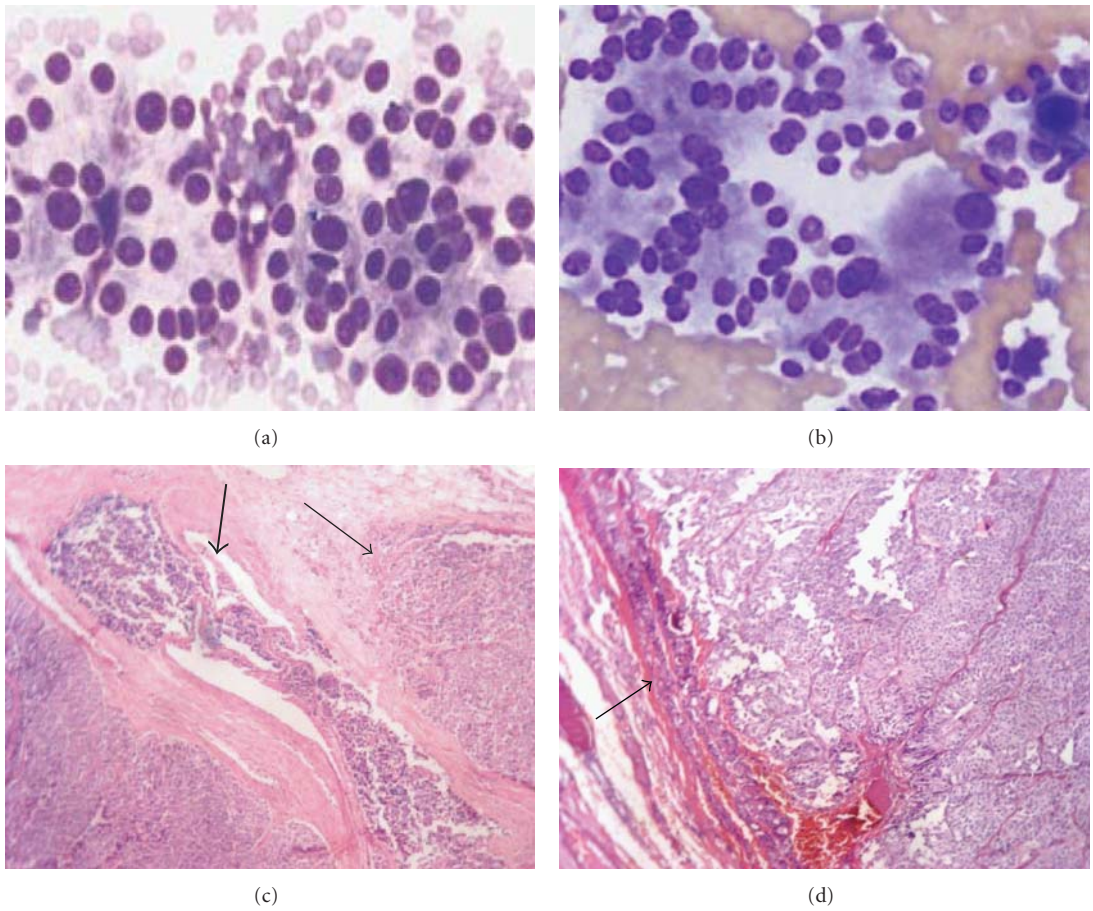
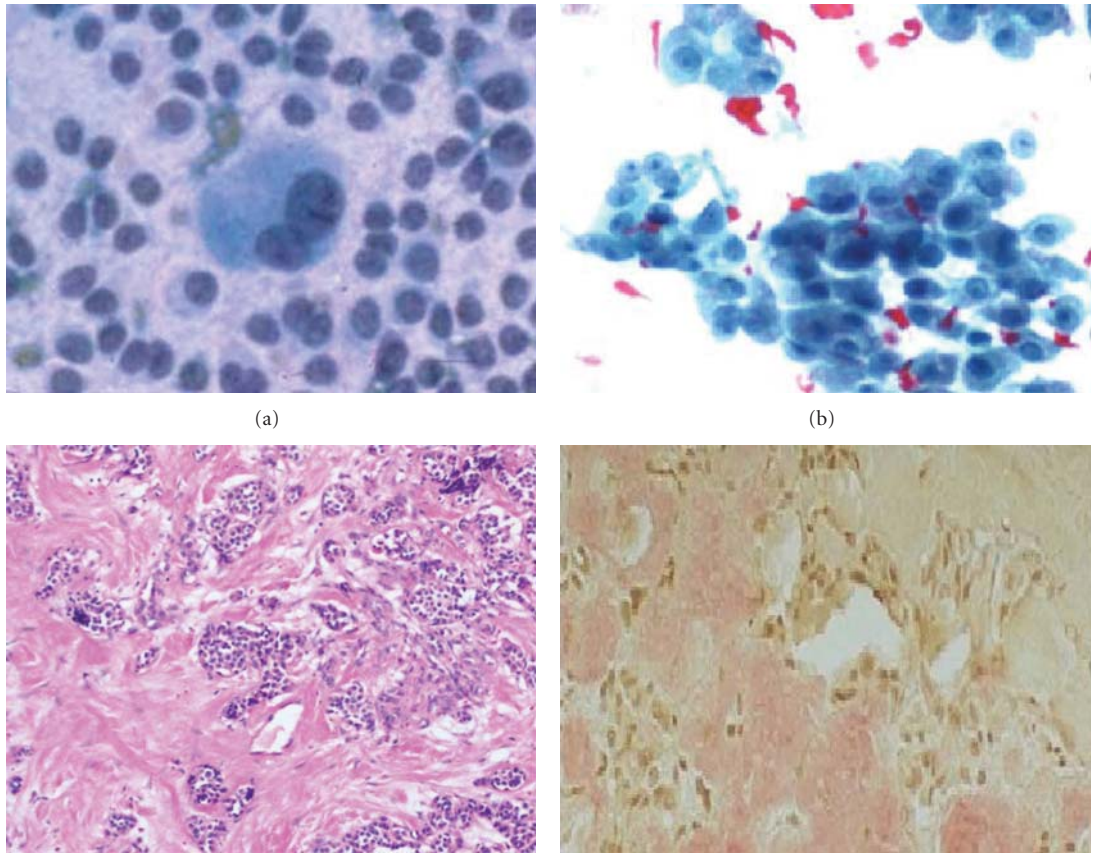


Figure 2: Photomicrograph of FNAC (a) and (b) (H&E) follicular neoplasms, showing marked cellularity, discohesion, single cells, Predominantly microfollicles and/or trabeculae, uniformly enlarged cells, crowding, scant colloid, marked nuclear atypia, mitosis and necrosis is uncommon. (c) Histopathology (H&E 10x) of follicular carcinoma showing capsular (thin arrow) and vascular invasion (thick arrow). (d) Follicular adenoma, (H&E 10x) where no capsular invasion is seen while histologic evidence of invasion is the gold standard of malignancy for the follicular lesions.

nodules and 15 patients (30%) had 6–10 cm thyroid nodules. The rate of malignancy was almost the same in our data, and the chances of malignancy were independent of its size. Our findings are consistent with other studies [20].

After comparison of our results of thyroid scan with histopathology, overall sensitivity of thyroid scan was found to be 80%, specificity 20%, positive predictive value 10%, and negative predictive value 90%. The overall accuracy was 26%. In one study of thyroid scan, it was reported to have sensitivity 100% and specificity 24% which is close to our study [19]. This shows that thyroid scan is more sensitive than specific in detecting thyroid malignancy. In our study, 23 patients (46%) were of benign FNAC. 22 patients (44%) were of indeterminant FNAC. 5 patients (10%) were of malignant FNAC. In a study from Pakistan, 39.47% of patients were of benign FNAC, 43.42% were of indeterminant FNAC, and 11.84% were of malignant FNAC, which is very close to our study [9].

Out of the 23 patients with benign FNAC, 7 patients (14%) had colloid cyst, 2 patients (4%) had colloid goiter,



(a) (b) (c) (d)

Figure 3: (a) and (b) Photomicrograph on FNAC (Pap) of medullary carcinoma with isolated, loosely cohesive, syncytial fragments, round, oval, cuboidal, plasmacytoid, spindle, round, multinucleated, nuclear inclusions and pale, fibrillar, calcitonin granules and extracellular amyloid. (c) Histopathology (10x H&E) of same case and (d) Congo red positivity.

12 patients (24%) had colloid nodule, and 2 patients (4%) had lymphocytic thyroiditis. All the patients with benign FNAC were confirmed to have the same results on histopathology. Out of the 22 patients with indeterminant FNAC, 3 patients (6%) had follicular lesion and 19 patients (38%) had follicular neoplasm. On histopathology, all 3 patients with follicular lesions were confirmed to have hyperplastic nodule, and out of the 19 patients with follicular neoplasm, 18 patients (36%) were confirmed to have follicular adenoma whereas 1 patient (2%) had follicular carcinoma. The intermediate findings were the main pitfalls of FNAC thyroid. This could be due to overdiagnosis on cytological reporting [9]. Our findings are consistent with Flanagan et al. [21].

Out of the 5 patients with malignant FNAC, 3 patients (6%) had papillary carcinoma (Figure 3), 1 patient (2%) had medullary carcinoma (Figure 2), and 1 patient (2%) had anaplastic carcinoma (Figure 4). On histopathology, out of 3 patients with papillary carcinoma, 2 patients (4%), were confirmed to have papillary carcinoma whereas 1 patient had diffuse hyperplasia. Patients with medullary carcinoma and anaplastic carcinoma on FNAC were found to have the same on histopathology. Therefore, in this study the concordance between the malignant FNAC diagnosis and histologic followup was 80%; this is comparable to other studies in the literature [9, 21–25].

FNAC is a sensitive and highly specific method of evaluating thyroid nodules for malignancy [22–25]. After comparison of our results of FNAC with histopathology, overall sensitivity of FNAC was 80%, specificity 97.7%, positive predictive value 80%, and negative predictive value 97.7%. The overall accuracy was 96%. Our results are consistent with results of other studies. In a review on FNAC of the thyroid nodule, it was reported to have sensitivity of 65–98% and a specificity of 72–100% [26–28]. In another study, the analysis of data revealed a sensitivity of 88.9% and specificity of 96.1% with diagnostic accuracy of 94.2%. This shows that FNAC is more specific than sensitive in detecting thyroid malignancy, and therefore, it is used as a reliable diagnostic test [1].

The false negative rate (FNR) is defined as the percentage of patients with benign cytology in whom malignant lesions are later confirmed on histopathology. Our results about false positive and false negative rates are consistent with the published guidelines of Papanicoloau society of cytopathology [29–31]. This guideline suggested that a false negative and false positive rate of $< 2\%$ and 3%, respectively, should be

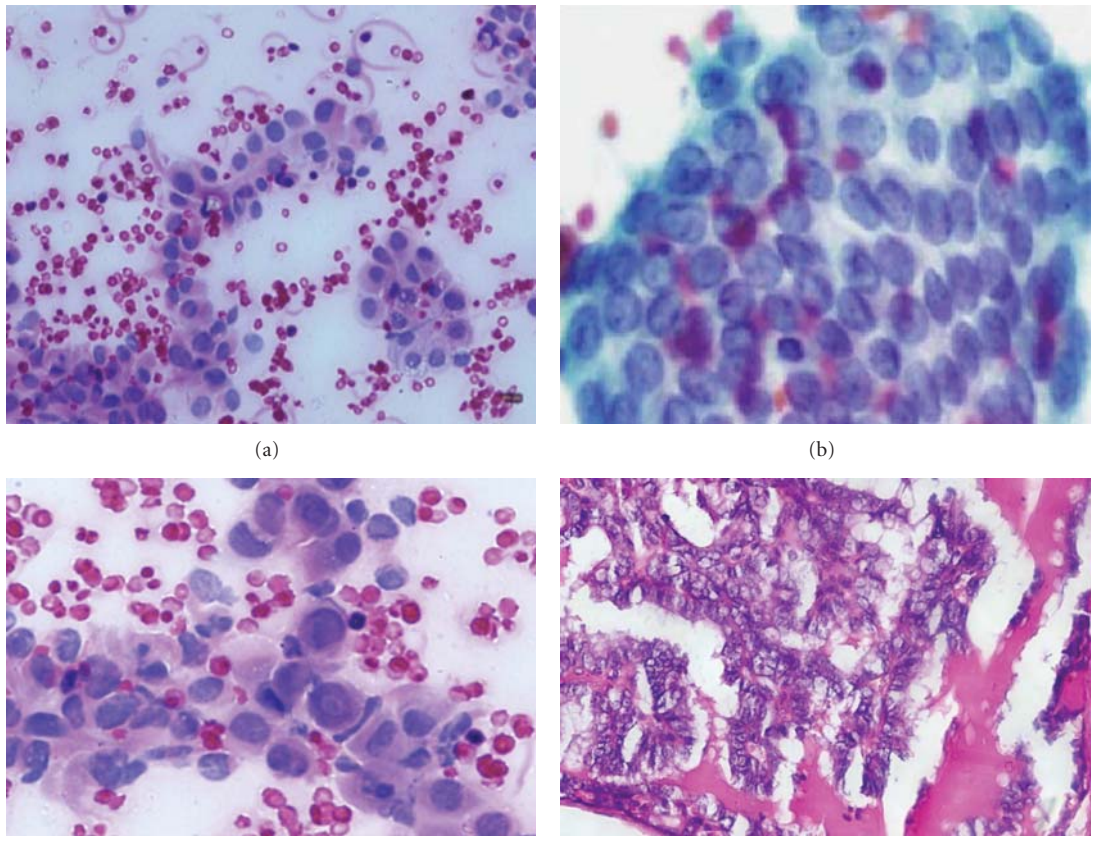


Figure 4: (a), (b), and (c) Photomicrographs of papillary carcinoma showing hypochromasia/pallor, nuclear grooves, intranuclear cytoplasmic inclusions, ovoid nucleus, and micronucleolus. (d) Histopathology of papillary cell carcinoma.

achieved [29]. In series of studies, FNR was reported ranging from 1.5% to 11.5% [32]. Ashcraft and van Herle noted that FNR results varied in reported series from 2% to 50% and that among 1330 patients, all of whom had a histological examination, the FNR was 1.7% [33]. In our series, we reported one case as false negative that translated to 2% FNR. This case was however confirmed on histopathology as follicular carcinoma. Our value is consistent with other studies of Boey and colleagues [34].

The false positive rate (FPR) indicates that a patient with malignant FNAC result was found on histological examination to have benign lesion. Caruso and Mezzaferri [32] reported less than 6% FPR while Campbell and Pillsbury [35] reported 1.2%. In our series, we reported 1 case as malignant on FNAC but it turned out to be diffuse hyperplasia on histopathology. The FPR is 2%, which agreed with other series that range 0–8%.

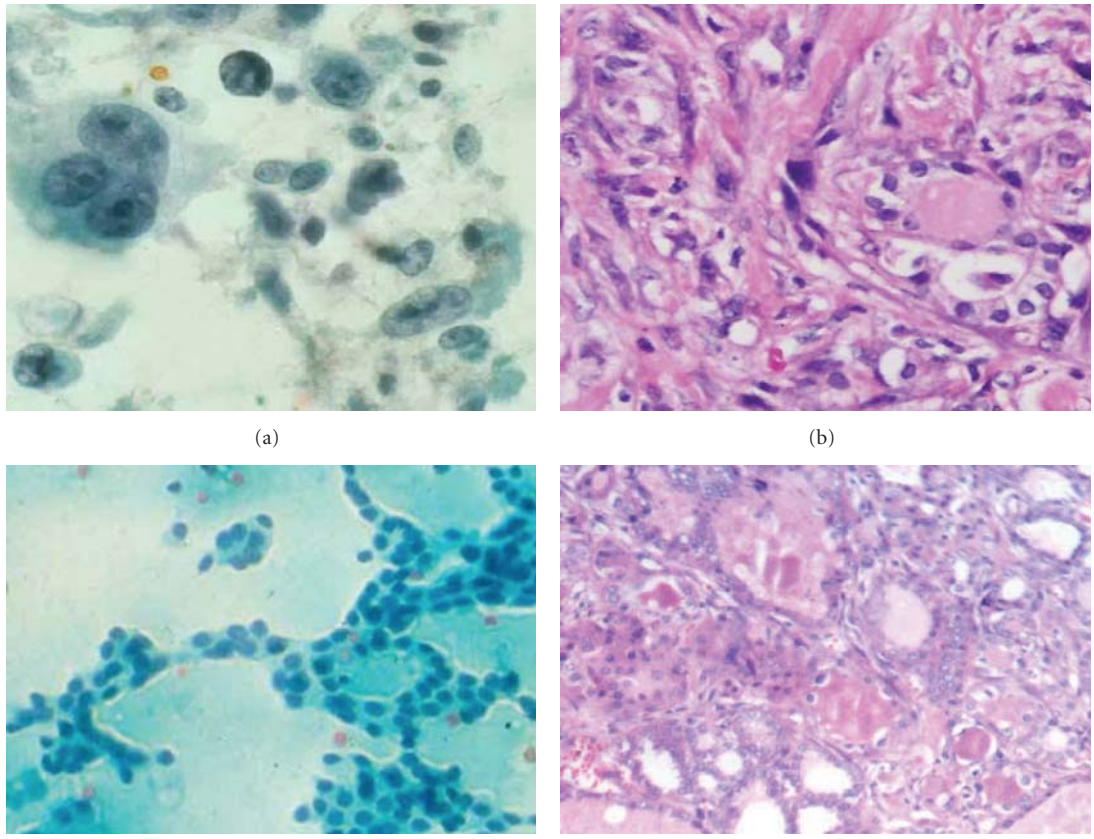
The overall accuracy for FNAC was 96%, which agrees with other studies of 95% [32]. In our study, sensitivity of FNAC was 80%, which was equivalent to the sensitivity of thyroid scan. However, specificity of FNAC was 97.7% compared to only 20% for thyroid scan. The very low specificity of thyroid scan might make it appear as a superfluous investigation compared to FNAC. The overall accuracy of FNAC was 96% while that of thyroid scan was only 26%.These findings are consistent with the data found worldwide [19].

In Pakistan as a routine matter, the evaluation of thyroid is carried out with FNA, the ultrasonography, and scanning with 131I and 99Tc. But before this procedure thyroid scans was the most common test in our setup, used to identify "hot" and "cold" lesions. Hot or warm nodules, about 5%, are seldom malignant, whereas cold or hypofunctional have 10% to 25% chances of being malignant. With the introduction of FNA, people are more relying on this procedure as an elected laboratory test: because it is easy, simple, nontraumatic, and very acceptable to the patients. We cannot undermine the usefulness.

FNAC should be advised for every patient for exclusion of cancer. As FNAC is an inexpensive, sensitive, specific, and accurate procedure, it should be adapted as an initial investigation of thyroid diseases in all tertiary hospitals in developing countries like Pakistan.

5. Conclusion

Fine needle aspiration cytology is more specific than sensitive whereas thyroid scan is more sensitive than specific in detecting thyroid malignancy. Fine needle aspiration cytology is highly accurate and better than thyroid scan in the evaluation



(a) (b)

(c) (d)

Figure 5: (a) Photomicrograph on FNAC (Pap stain 40x), anaplastic carcinoma showing pleomorphism in size and shape with giant or multinucleate forms, prominent nucleoli, nuclear inclusions, and vacuolated cytoplasm. (b) Histopathology (10x H&E) of anaplastic carcinoma. (c) FNAC (Pap stain 20x) of colloid goiter showing macrophages and uniform cuboidal cells of thyroid follicles. (d) Histopathology of colloid goiter (H&E 40x).

of solitary thyroid nodule. Therefore, FNAC should be adapted as an initial investigation of thyroid diseases in all tertiary hospitals. The information provided by thyroid scan has no significant bearing in the management of solitary thyroid nodule. FNAC provides useful information and may be used along with other clinical information to decide best form of treatment in a solitary thyroid nodule. The use of FNAC has reduced the number of patients with solitary thyroid nodules undergoing unnecessary surgery and has led to proper planning of surgery in malignant cases.

Conflict of Interests

The authors declared that there is no conflict of interests.

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Review Article

Advances in Fine Needle Aspiration Cytology for the Diagnosis of Pulmonary Carcinoma

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New developments in the field of thoracic oncology have challenged the way pathologists approach the diagnosis of pulmonary carcinoma. Nonsmall cell carcinoma is no longer an adequate diagnostic category. Pathologists are required to further classify tumors into adenocarcinoma and squamous cell carcinoma since specific therapies are now recommended depending on the histological tumor type. This change occurred following the discovery of specific molecular alterations that predict response to certain drugs and now molecular testing of tumor cells is often requested to direct therapy. The vast majority of lung cancer is diagnosed in advanced clinical stages, where cytologic or small biopsy material is the only form of tissue diagnosis, thus placing cytology, especially fine needle aspiration biopsy in the front line for management of patients with lung cancer. In this paper we will review the current concepts in the suitability and accuracy of fine needle aspiration biopsy, including diagnosis, classification, prognostic markers, and use of ancillary techniques.

1. Introduction

Pulmonary nodules discovered by an imaging technique present a relatively frequent clinical problem. A solitary pulmonary nodule is a common manifestation of a benign condition. However, in nodules larger than 2 cm, the incidence of a primary lung cancer ranges from 64 to 82% [1]. An early, accurate diagnosis is of paramount importance for initiating specific therapy for malignant lesions, and for avoiding unnecessary procedures for benign conditions. Thus, after clinical risk assessment tissue diagnosis is the next step in managing radiologically suspicious lung nodules. Direct tissue sampling for diagnosis is essential in most patients for decisions regarding treatment and can be accomplished by fine needle aspiration biopsy (FNAB), endoscopic or core needle biopsy, or surgical resection. Sampling of the lesion by FNAB can be performed via the airway (endobronchial transbronchial FNAB) or chest wall (CT-guided percutaneous FNAB). Transbronchial FNAB is useful for the diagnosis of primary pulmonary lesions that lie beneath the bronchial surface and for staging lung cancer patients by sampling mediastinal lymph nodes. FNAB has become recognized as a safe and effective diagnostic tool, as a result of improved aspiration biopsy tools and techniques, better control of complications, and increased experience of cytopathologists in interpreting aspirate specimens.

Most patients with lung cancer present with clinical advanced disease and therefore are not candidates for surgery with curative intent, but are rather treated with systemic therapies. In the age of personalized therapies, cytological material in the form of FNAB may be the only available diagnostic specimen, and the only material available for molecular studies, necessary for current therapeutic decision making. New recommendations for screening of high-risk populations [2] coupled with the ongoing development of minimally invasive techniques and procedures for sampling lung lesions will most likely further increase the need for accurate diagnosis and molecular characterization of malignant tumors on small biopsy specimens.

In this paper, we will cover current concepts and advances in FNAB of pulmonary carcinomas including diagnosis,

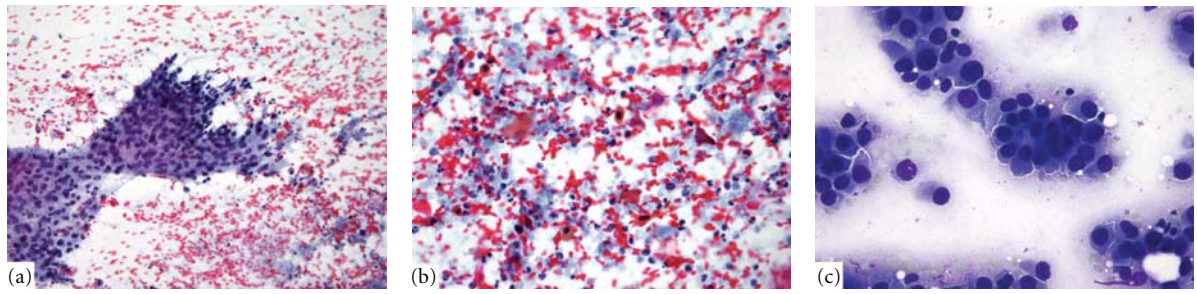

Figure 1: The characteristic morphologic patterns of tumor in smears. (a) SQC carcinoma showing flat sheet of polygonal, atypical cells and (b) orangeophilia on Pap stain demonstrating keratinization. (c) ADC with typical cytomorphology and formation of glandular structures.

classification, prognostic makers, and use of ancillary techniques.

2. Clinical Advances in the Management of Patients with Pulmonary Carcinoma

The current classification of lung cancer recognizes four major histological subtypes, namely, squamous cell carcinoma (SQC), adenocarcinoma (ADC), large-cell carcinoma (LCC), and small cell lung carcinoma (SCLC). Until recently, most of the cytological diagnosis of lung carcinoma was based on distinguishing SCLC from other tumors generally designated as nonsmall cell carcinoma (NSCLC), because these two categories were the most relevant for directing therapy. However, advances in thoracic medical oncology have led to a paradigm shift in NSCLC diagnosis, resulting in a new emphasis on accurate NSCLC subtyping. Specifically, two novel agents have challenged NSCLC as clinically relevant diagnostic category. It has been demonstrated that patients with the diagnosis of SQC are at increased risk for life-threatening complications if treated with bevacizumab, a humanized antibody against vascular endothelial growth factor (VEGF) [3]. In addition, in the case of pemetrexed, an antifolate that inhibits multiple enzymes in purine and pyrimidine synthesis, patients with SQC showed no response to the drug in comparison to a good response observed in patients with the diagnosis of non-squamous cell carcinoma [4]. For these reasons, these two new drugs are only recommended for use in patients with a diagnosis of non-squamous cell carcinoma. Other developments include the identification of genetic alterations which have been described almost exclusively in adenocarcinoma that confer susceptibility to therapeutic agents or resistance to chemotherapeutic drugs. For example, tumors with epidermal growth factor (*EGFR*) mutations have a better outcome and respond to the tyrosine kinase inhibitors erlotinib and gefitinib, as a first-line therapy, whereas patients without *EGFR* mutations seem to have a better outcome with standard chemotherapy [5]. Furthermore, translocation in the *EML4-ALK* gene has been described predominantly in adenocarcinomas. This translocation confers susceptibility to specific inhibitor, crizotinib that is currently undergoing clinical testing [6].

These advances in the understanding of molecular mechanisms underlying lung cancer and the development of new targeted therapies challenge the traditional diagnostic dichotomization between SCLC and NSCLC and prompt a more specific characterization of NSCLC into squamous or adenocarcinoma category. Traditionally, NSCLC subclassification has been based on morphologic assessment of routine H&E-stained histological specimens. Because cytology specimens, such as FNAB, differ in preparation and technique from traditional histology, the accuracy of subtyping these specimens has been challenged, yet there is considerable evidence supporting the utility of cytology in both subtyping NSCLC and providing material for predictive and prognostic studies.

3. Role of Immediate Assessment in the Accuracy of Lung FNA Cytological Diagnosis

Published reports reveal that the sensitivity of FNAB for the diagnosis of lung cancer ranges from 56 to over 90% whereas specificity is close to 100%. In nearly all these studies, the overall positive predictive value is nearly 99%. While the false positive rate is generally less than 1%, a negative result is less reliable with most studies reporting a false negative rate of around 10% [7, 8]. The major contribution to the relatively high false negative rate is failure to obtain diagnostic material, most commonly due to sampling error. Studies have shown that immediate on-site assessment is valuable in minimizing false negative diagnoses due to nondiagnostic material [9, 10]. Published series by Austin and Cohen show that immediate on-site assessment during FNAB was associated with a statistically significant increased diagnostic accuracy compared to cases without immediate assessment, 100% versus 80%, respectively [11]. During on site adequacy determination, smears from the aspirate are rapidly stained and are evaluated by a cytopathologist or cytotechnologist for cellularity and diagnostic yield. On-site adequacy evaluation also provides real-time communication of information including appropriate tissue triage recommendations for ancillary tests such as molecular testing, flow cytometry, cytogenetics, electron microscopy, and so forth. This interaction directly impacts clinical management during the critical

Table 1: Factors contributing to difficulty of cytologic subtyping of adenocarcinoma and squamous cell carcinoma.

$N = 165$	Cellularity		Differentiation[1]		Histologic type	
	Low	High	Well-moderately	Poorly	ADC	SQC
Correct definitive Subtyping ($n = 148$)	82%	94%	87%	69%	93%	70%
Difficulty in Subtyping ($n = 17$)[2]	18%	6%	13%	31%	7%	30%

[1] Grade of differentiation was based on resected specimens.
[2] Difficulty in subtyping encompasses cases which were incorrectly classified, unclassified, or underclassified/subtype favored by cytology.

diagnostic phase while the lesion can still be readily sampled.

4. Accurate Morphological Diagnosis of Subtypes of NSCLC

Morphology still remains the cornerstone in lung cancer classification. The World Health Organization classification of lung tumors through the 1999 edition did not address lung cancer diagnosis based on small biopsies and cytology [12]. In the 2004 World Health Organization classification, cytology was addressed for the first time, with descriptions of the morphological criteria for each type of pulmonary carcinoma [13]. In the new revised proposal [14], an entire section is dedicated to the classification of lung tumors based on small biopsy material including FNAB. This highlights the importance and recognition of the role that FNAB plays in the diagnosis and management of pulmonary carcinomas.

Lung cancer histological subtypes that are morphologically recognizable on cytology specimens are ADC, SQC, and SCLC, as well as carcinoid tumors. Other types of lung carcinoma such as large cell carcinoma and other rare variant as fetal type and colloid adenocarcinoma may be suspected on the basis of pure morphology but usually require evaluation of the surgically resected specimen for the final diagnosis.

Historically, it has been important to accurately identify SCLC as the treatment is different from NSCLC. Classical morphological features of SCLC such as nuclear molding, frequent mitoses, and absence of nucleoli are often distorted on a small biopsy specimen showing extensive crush artifact. In this setting, cytology has an edge over histology because of better preservation and fewer artifacts [15]. Cytology is highly accurate and a well-recognized method to distinguish SCLC from NSCLC. In a study of 259 consecutive lung FNAs by Delgado et al., SCLC was distinguished from NSCLC with accuracy of 96% [16].

Unlike the distinction of SCLC and NSCLC, feasibility of NSCLC subtyping in cytology has been controversial. However, based on a recent study from our institution, cytology provides several advantages over surgical specimens for the subtyping of NSCLC [17]. The key morphologic criteria for ADC versus SQC are glandular architecture versus keratinization, respectively. The Papanicolaou (Pap) stain has exquisite sensitivity for even minimal keratinization aiding in the distinction of SQC from ADC. The morphologic patterns which emerge in tumor smears provide a clue to a tumor subtype which may not be apparent in surgical specimens (Figure 1). In addition, due to immediate fixation, cytology provides greater nuclear and cytoplasmic resolution than histology. While in the majority of cases a line of differentiation can be clearly identified by morphology, difficulties arise in a subset of cases.

Table 2: Sensitivity and specificity of cytologic tumor subtyping.

$n = 183$	Sensitivity	Specificity	Accuracy
SQC versus non-SQC	87%	98%	97%
ADC versus non-ADC	98%	79%	93%

In a recent study, which included 165 cases with paired FNAB and resection diagnosis of ADC and SQC, we described some of the limiting factors for the interpretation and accurate classification in cytology specimens. The strongest predictors for difficulty in subtyping were poor differentiation of the tumor where distinguishing morphologic features are not apparent (Table 1), followed by scant cellularity. Nonkeratinizing poorly differentiated squamous cell carcinoma in particular is subject to misclassification by FNAB [18]. Another difficulty is presented by tumors with mixed histology, but true adenosquamous carcinomas are infrequent with reported incidence of 2 to 3% in published surgical series [19].

Despite these limitations, using morphology and occasional immunocytochemistry we observed that when faced with the need to subclassify NSCLC we performed with high concordance between cytology and resected specimens of 97% and 93% for identifying squamous and adenocarcinoma, respectively (Table 2). Despite the lower sensitivity for non-keratinizing SQC, the specificity of this diagnosis is very high, which means that the false-positive classification as SQC is extremely rare. Thus having proved overall high accuracy of cytology in distinguishing SQC versus non-SQC it was concluded that cytological specimens are suitable for guiding therapeutic decisions within these diagnostic categories [18].

5. Use of Immunohistochemistry Stains in the Characterization of NSCLC Subtypes

In tumors that do not show clear-cut signs of differentiation on light microscopy examination (Figure 2) further investigation by immunohistochemistry may highlight tumor cell lineage. In biopsies like FNAB, with limited material, the need to conserve material for possible mutational analysis and other prognostic markers obligates the use of the most

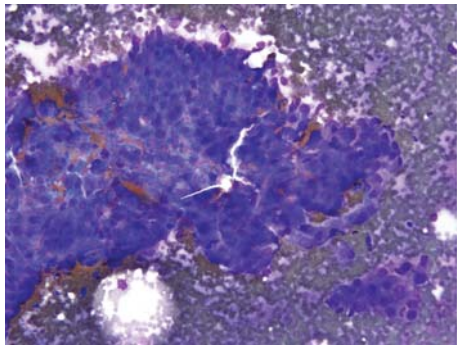

Figure 2: Poorly differentiated carcinoma lacking clear signs of differentiation on light microscopic examination. Further work up using limited panel of antibodies will differentiate tumor cell lineage in the majority of cases.

efficient and limited panel of immunohistochemical stains. Several recent publications have addressed the question of what is the best panel of markers to be used in the distinction between adenocarcinomas and squamous cell carcinoma. Results published by Wu et al. showed the advantages of immunocytochemistry in distinguishing poorly differentiated SQC from SCLC of the lung, and primary adenocarcinoma from metastatic tumors in cytology specimens. In their study, all poorly differentiated squamous cell carcinomas showed expression of p63 while negative for thyroid transcription factor-1 (TTF-1), whereas adenocarcinomas had the opposite staining pattern. Of note, SCLC had identical p63/TTF-1 expression profile as adenocarcinoma, but application of standard morphologic criteria and addition of neuroendocrine markers was sufficient for accurate classification [20]. Nicholson et al., in their recently published work that included 13 FNA cytology specimens, showed that a limited panel of TTF-1, CK5/6, and p63 together with a mucin stain, refined diagnosis of NSCLC to either ADC or SQC in 65% of cases [21].

In summary, most of the work has been concentrated on the expression of 3 markers TTF-1, p63, and a high-molecular-weight cytokeratin (HMWCK). The following algorithm, based on published data and our experience can guide the interpretation of the stains. A TTF-1 negative/p63-positive/HMWCK-positive profile is supportive of SQC, whereas any expression of TTF-1 is supportive of ADC. It is worth mentioning that coexpression of TTF-1 and p63 can be seen in adenocarcinomas. In most cases p63 expression in adenocarcinoma is patchy and weak. Negative staining for both p63 and TTF-1 usually rules out squamous cell carcinoma [22].

Napsin-A is an aspartic proteinase, involved in the maturation of the surfactant protein B and is expressed in the cytoplasm of cells of lung and kidney [23]. The staining is cytoplasmic and is strongly positive in up to 80% of primary lung adenocarcinomas. In the study of Stoll et al., 75 cytology cases were analyzed. It showed that the sensitivity and specificity of TTF-1 were each 81%. Napsin-A exceeded the specificity of TTF-1 at 96% with a lower sensitivity of 65%. In the study, the only carcinoma of nonlung origin in which Napsin-A was detected was renal cell carcinoma, suggesting that Napsin-A can be used as a surrogate marker in work up of poorly differentiated lung adenocarcinoma or an unknown primary tumor [24]. Desmocolin-3, constitutive protein of desmosomes, is found to be overexpressed in SQC of the lung [25, 26]. In the study by Monica et al., which included 31 cytological specimens originally classified as NSCLC, staining for desmocolin-3 and TTF-1 was mutually exclusive in tumors [26].

Several authors reported on the high sensitivity and specificity of miRNA expression in SQC of the lung, and its usefulness in differentiating ADC from SQC in small specimens [27, 28]. However, a recently published study does not support this observation [29]. The utility of these new approaches compared to standard markers needs to be evaluated further and validated, since a limited panel of immunohistochemical markers can reproduce the results obtained by the new molecular techniques [22].

6. Adequacy of Material Obtained by FNAB for Molecular Testing

In the recent years we have witnessed a revolution in our understanding of the molecular basis of NSCLC. These advances have led to development of multitude of commercially available prognostic and predictive biomarkers and targeted therapeutic agents. Despite these advances in treatment, the overall prognosis remains poor in patients with advanced disease. Personalizing therapy based on an individual tumor molecular profile can optimize efficacy with the available agents. Molecular determinants that guide treatment decision-making may have a prognostic or predictive function, and are commonly referred to as prognostic or predictive markers, respectively. Prognostic marker refers to a tumor characteristic that is useful for estimating a patient's outcome independent of therapeutic decisions. In contrast, predictive markers are useful in making therapeutic decisions. Mutations of *EGFR, KRAS*, and *EML4/ALK* translocations are mutually exclusive in lung ADC and identify tumor subsets with unique dependencies and drug sensitivities. *KRAS* mutation testing is utilized by some institutions to exclude *EGFR* mutations or an *EML4/ALK* translocation.

In particular *EGFR* mutation and *EML4/ALK* fusion gene testing have reached clinical validation and are incorporated into the current treatment paradigm [30]. *EML4/ALK* testing also appears to have clinical utility in identifying patients who could benefit from referral for a study targeting *ALK* inhibition, such as ongoing phase 3 studies of the small molecule tyrosine kinase inhibitor crizotinib.

There is increasing awareness that the quality of specimens, such as cytology, has a profound influence on molecular diagnostic test results. FNAB samples are exposed to a greater variety of cytopreparation methods than resected tissue and sample size and heterogeneity may have an effect on the downstream molecular test results [31]. Most molecular techniques including in situ hybridization, polymerase chain reaction (PCR), and transcriptional profiling can be done on FNAB specimens [32, 33]. Optimizing and standardizing

of FNAB sample preparation methods is needed to preserve biomolecular integrity to enable seamless integration into molecular testing.

At present, there are few studies that rigorously compare the cellular composition of FNA samples with the quantity and quality of the desired analyte (DNA, mRNA, or protein) or the robustness of the biomarker test utilizing the sample. Schuurbiers et al. in their study conclude that molecular testing of *EGFR* and *KRAS* on cytologic material obtained by endobronchial ultrasound-guided transbronchial FNAB is feasible and could be performed on 77% of their specimens [34]. Another study by Smouse et al. showed that 67% of cytology specimens were adequate for molecular testing with some of the samples having as little as 25% tumor cellularity [35].

We have found that in a large screen of various cytologic specimens (FNABs, effusions, and exfoliative specimens) submitted for *EGFR* or *KRAS* mutational *testing*, 98% of samples were suitable for analysis. We concluded that testing is feasible and that with rare exception all cell blocks subjectively interpreted as "adequate" for diagnosis by a pathologist yielded sufficient quantity and quality of DNA for mutational analysis. This finding is in agreement with several other studies [18]. In a study of consecutive specimens from our institution, it was found that 79% of cytology specimens and 89% of small biopsy specimens submitted for molecular testing were sufficiently cellular [36]. The rate of *EGFR* and *KRAS* mutations detected in cytologic specimens in the study was comparable to the rate detected in surgical specimens.

7. Use of FNAB for Prognostic Markers

The reported incidence of local or distant recurrence following surgical resection of early-stage NSCLC is around 36% [37]. The risk of recurrence is clearly linked to clinical stage but further biomarkers predictive of tumor recurrence are needed. A histological grading system based on the predominant histological patterns seen in pulmonary adenocarcinoma has been described recently [38]. In that study, patients with stage 1 pulmonary adenocarcinoma could be accurately stratified according to risk of recurrence of death of the disease into 3 tiers representing low, intermediate and high risk, thus indicating that an objective system of tumor grade had prognostic significance. In cytological material however, there was no reliable corresponding pattern of cellular aggregates to predict a histological pattern for the same tumor [39], therefore a grading system based on nuclear features has been developed and evaluated in FNAB of pulmonary adenocarcinoma. The cytology grading system is based on the nuclear size of neoplastic cells; pattern of chromatin distribution; and nuclear contour and it separates stage 1 pulmonary adenocarcinomas into two groups. Tumors with low nuclear scores show a lower risk of recurrence or death of the disease in contrast to tumors with high nuclear scores [40]. In conclusion, FNAB can provide significant prognostic information to clinicians managing patients with pulmonary adenocarcinoma.

EGFR mutations are the best predictor of response to *EGFR* kinase inhibitors in pulmonary adenocarcinoma. Recently, two antibodies that detect specifically mutated EGFR proteins have become commercially available. A recent study from our institution demonstrated that these antibodies show sensitivity of 95% for the detection of EGFR L858R mutation, and sensitivity of 85% for the detection of exon 19 deletions. They concluded that immunohistochemistry for mutated EGFR could be used as a screen method to identify candidates for therapy with EGFR tyrosine-kinase inhibitors [41].

An antibody that correlates with *ALK* gene rearrangement in NSCLC has also been reported recently with promising results [42, 43] however this antibody is not yet commercially available.

The use of these antibodies in cytological material has not yet been validated, but they may offer an invaluable tool for screening patients with lung cancer where cytological material is the only specimen available.

8. New Sampling Methods in Lung FNAB (EBUS and ENB)

A couple of new minimally invasive procedures have been recently developed as an alternative to standard approaches. One of them, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has recently emerged as a valid minimally invasive method for mediastinal staging of lung cancers and diagnostic workup of centrally located masses [44]. When used to sample mediastinal lymph nodes, at least a moderate number of lymphocytes must be present to ensure the adequacy of the specimen and avoid a false negative result. A large retrospective study performed at our institution found that EBUS-TBNA had 89% sensitivity and 100% specificity for malignant disease, revealing no major discrepancies between tumor subtypes rendered by EBUS-TBNA cytology and histology [45]. A study by Turnoy et al. showed that in patients with NSCLC without extrathoracic metastasis, EBUS-TBNA reduces the need for surgical staging by 68% with lower incidence of complications and no difference in diagnostic performance, thus establishing the procedure as a valid alternative to mediastinoscopy [46].

The two major limitations of standard flexible bronchoscopy are its inability to reach peripheral segments of the lung and the limited diagnostic yield from lesions less than 3 cm in diameter. The alternative to bronchoscopy is CT-guided percutaneous biopsy where the possible complications include hemorrhage and pneumothorax. Recently developed technology that is emerging in clinical practice essentially combines these two methods. Electromagnetic navigation is a localization device that assists in placing endobronchial accessories (e.g., forceps, brush, and needle) in the desired areas of the lung. The system uses low frequency electromagnetic waves and real-time 3D digital reconstruction of the previously obtained CT scan of the bronchial tree. Electromagnetic navigational bronchoscopy (ENB) systems were recently cleared by the US Food and Drug Administration to aid the physician in guiding endoscopic tools in the respiratory tract. This novel technical advance localizes and samples lesions in the lung

parenchyma and mediastinum that are beyond the reach of standard endoscopy [47]. Recent published studies showed the diagnostic yield of ENB for small peripheral lung lesions are in the range from 54% to 77%. Lamprecht et al. studied ENB sampling using rapid on site cytological evaluation during the procedure and it showed sensitivity and specificity of 84.6% and 100%, respectively. Citing potential drawbacks, they found that 33.3% of the cases with ENB sampling were falsely negative and definitive diagnosis had to be established by CT-guided biopsy or by surgery [48]. Of note, other authors have reported that presence of cytologist virtually eliminates the problem of inadequate samples [49, 50].

9. Conclusion

The field of thoracic oncology is going through a revolution with the advent of targeted therapy for the management of patients with lung cancer. FNAB is in many cases the only diagnostic specimen available for guiding therapeutic decisions. FNAB has proven to be an invaluable tool not only for diagnostic accuracy of pulmonary carcinomas classification, but also as a reliable and adequate source of material suitable for molecular analysis.

Conflict of Interests

The authors declare no conflict of interests.

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