Original Article

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Immunocytochemistry as An Adjunctive Diagnostic Tool in Cytology

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ABSTRACT

Background: Cytology is rapidly progressing from a primary screening investigation to a definitive diagnostic modality in increasing number of cases. This has been made possible by the use of ancillary tests such as immunochemistry, and molecular techniques. Over the years, the use of immunochemistry has evolved from being just an adjunct to diagnosis, to determining primary site of malignancy and exact typing of malignancies.

This study was conducted to find out the impact of immunochemistry performed on cell blocks and smears of varied cytology material including FNAC material and fluids.

Methods: It was a retrospective study. All cytology cases including FNAC material and fluids on which immunochemistry had been performed were retrieved from records. Immunochemistry was performed manually on cold acetone fixed smears, and cell-block preparations.

Result: A total of 50 cases were evaluated from past two years, including 10 cases of fluids, and 40 cases of FNAC material. Staining was done on cell blocks in 44 cases, and on smears in 6 cases. In 15 cases, immunochemistry was performed to find out the site of primary malignancy, of which in 11 cases, it was positively contributory in giving a list of possible primaries and in 4 cases, single definitive diagnosis was provided. In 27 cases, it helped in typing of malignancies, and providing additional information. It was non-contributory in 8 cases.

Conclusion: Immunochemistry, on smears as well as cell blocks is a useful adjunctive tool in cytology, therefore, it is advised to perform immunochemistry wherever possible.

Keywords: *Immunocytochemistry, Cell Block, Carcinoma of Unknown Primary*

Introduction

Cytological evaluation is a rapid, minimally invasive method of providing diagnosis. Although previously the efficacy of cytology was limited due to the reliance on cytomorphology alone, the utility has now expanded owing to the presence of multiple ancillary diagnostic methods such as immunocytochemistry, molecular diagnostic methods like PCR, etc. With the addition of immunocytochemistry, FNAC can enable us to provide definitive diagnosis in numerous cases posing a diagnostic dilemma, such as tumours of unknown primary, finding out the source of tumour in highly undifferentiated tumours, etc. Accurate typing of small round cell tumours, and distinction between adenocarcinoma and mesothelioma on fluid cytology are also important applications of immunocytochemistry. In this study, we have explored the various applications of immunocytochemistry and the advantages it provides as an adjunct to routine cytology.

Materials and Methods:

AIMS: To find out the impact of immunocytochemistry as an adjunctive tool in cytology.

The study was carried out in the department of pathology over a period of two years, from 2015 to 2017.

A total of 50 cases were evaluated from past two years, on which immunochemistry had been performed, including 10 cases of fluids, and 40 cases of FNAC material.

Immunocytochemistry had been performed by two different methods depending on the quantity and quality of available material. Cell blocks had been prepared from FNAC material and centrifuged fluid deposits in 44 cases. Immunocytochemistry was performed directly on smears in 6 cases.

Method of preparation of cell blocks

In case of FNAC, the aspirated material was put onto a slide (not smeared), and allowed to dry off completely into a clot. This button, thus prepared was wrapped in a filter paper and processed like small biopsy specimens. The cell yield was mostly adequate. Immunochemistry was then performed on the cell blocks as done routinely for histopathological blocks.

For preparing cell blocks from fluid specimens, they were centrifuged at a speed of 2500 rpm for 15 minutes. After removing the supernatant, the sediment was processed as described above to from a cell block, and required immunostaining was performed.

Methods of performing immunocytochemistry on smears:

In most cases, after performing FNAC, the slides on which immunocytochemistry had to be performed were subjected to overnight fixation in cold acetone. This was followed by H2O2 block, wherein the slides were immersed in 6-7% H2O2 in methanol for 30 minutes. (The concentration of H2O2 used was higher than used in conventional immunohistochemistry, 3%). This was followed by protein block for 15 minutes. The slides were then washed in Tris buffer for 15 minutes. Primary antibody was then applied left overnight in the fridge in a moist chamber. The next morning, the slides were washed in tris buffer. Primary antibody amplifier was then applied for 15 minutes. Next, the secondary antibody was applied for 30 minutes. After thorough Tris wash, DAB chromogen was applied, and the reaction was stopped when the colour developed, or upto 30 minutes.

In one case, due to scarcity of material, a pap stained slide was decolourised in methanol, and then subjected to immunocytochemistry as elaborated above. The immunochemistry staining was found to be satisfactory on this slide.

Result

Out of the 50 cases studied, immunochemistry was used to determine the site of primary in metastatic tumor of unknown primary in 15 cases. A panel of CK7, CK 20 was applied to determine the site of primary tumor. Out of these, in 6 cases, lung was found out to be the primary site of tumor, 2 cases were of metastatic papillary thyroid carcinoma, one of metastatic medullary carcinoma thyroid, and one of carcinoma breast. In these cases, site localization was made possible with the use of a basic panel of CK7, CK20, supplemented by various organ specific markers, and correlated with the clinical presentation and radiology. However, in 4 cases, no exact site could be determined and a list of few possible primaries were given.

There were 23 cases of poorly differentiated tumors, in which immunochemistry aided in the identification of the type of malignancy. 15 cases were found out to be adenocarcinoma (5 in fluid specimens), 5 cases of squamous cell carcinoma, one melanoma, and two lymphomas.

In one case of a small round cell tumor, immunocytochemistry was used to reach a definitive diagnosis of Ewing's sarcoma.

In two cases of thyroid malignancies there was a diagnostic dilemma between anaplastic carcinoma and anaplastic lymphoma. Using immunocytochemical panel of LCA, CK, Bcl-2, Bcl-6, CD20 and Vimentin, a final diagnosis was reached, which in both the cases was that of DLBCL thyroid.

In fluid cytology, there were three cases, in which it was difficult to distinguish adenocarcinoma from mesothelioma. Using an immunocytochemistry panel of Muc-1, Calretinin, WT-1, mesothelin, and MOC-31, a final diagnosis of adenocarcinoma was established (positive for Muc-1, MOC-31, and negative for calretinin and mesothelin).

There were a total of 6 cases (2 of fluids), in which immunochemistry did not yield any valuable information. This was because of scant/acellular preparation in 4 cases. In these cases, the cell blocks showed scant to no cellularity of tumor cells. In two cases (FNAC material), the immunocytochemistry was found to be non-contributory due to negative staining on smears.

Overall, in 88% (44 out of 50) cases, immunochemistry significantly helped in giving an exact definitive diagnosis (Table 1) (Figure 1) (Figure 2) (Figure 3).

Table 1: Comparison of immunochemistry aided diagnosis with cytological diagnosis alone

Cytological diagnosis	Diagnosis after immunochemistry
Metastatic poorly differentiated carcinoma (15) with unknown primary	-Metastatic SCC, lung (3) -Metastatic adenocarcinoma, lung (3) -Metastatic papillary carcinoma, thyroid (2) -Metastatic medullary carcinoma thyroid (1) -Metastatic adenocarcinoma, with possible list of primaries (4) -Metastatic carcinoma breast (2)
Poorly differentiated carcinoma, (23)	Adenocarcinoma (15) SCC (5) Melanoma (1) Lymphoma (2)
Small round cell tumor (1)	Ewing sarcoma (1)
Anaplastic carcinoma Vs anaplastic lymphoma, thyroid (2)	DLBCL thyroid, vimentin positive (2)
Adenocarcinoma Vs Mesothelioma (3)	Adenocarcinoma (3)

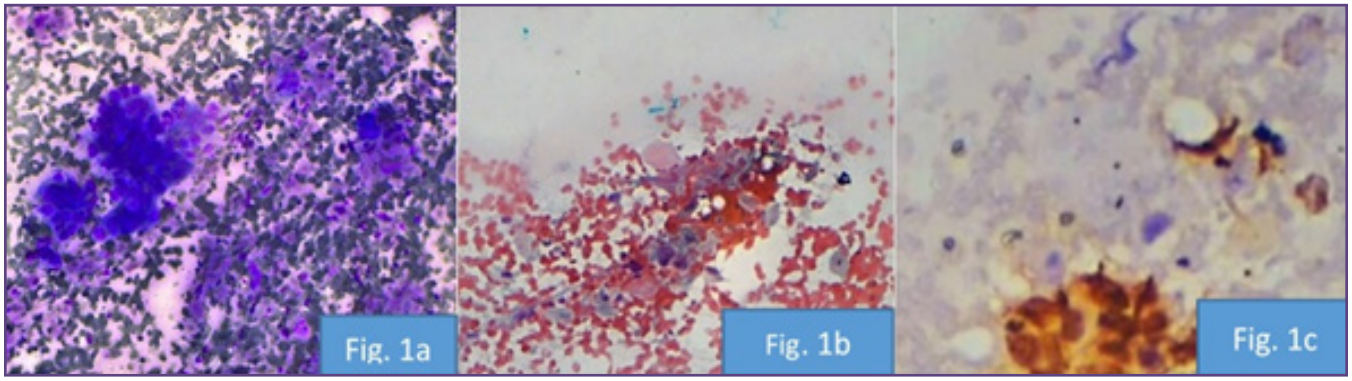

Fig. 1: FNAC done from scalp nodule. Fig. 1a. Giemsa stained FNA smear showing clusters and singly scattered cells with moderate cytoplasm, 20 X. Fig 1b. Pap stained FNA smear, 20 X. Fig. 1c. Immunocytochemistry showing cells positive for p63, 40X.

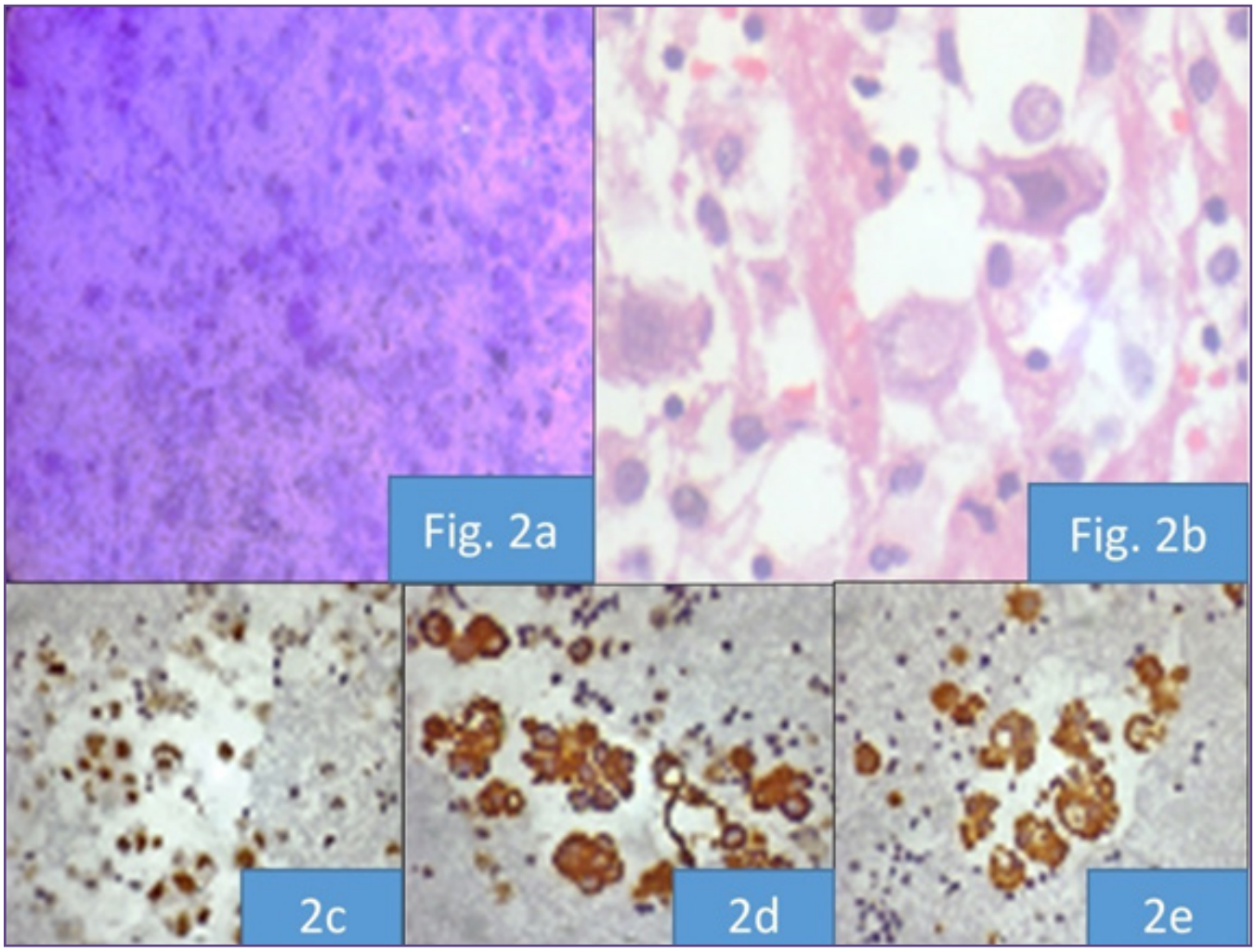

Fig. 2: Ascitic fluid preparations. Fig. 2a. Giemsa stained smear from ascitic fluid showing tumour cells, 20X. Fig. 2b. H&E stained cell block showing tumour cells with mucin, 40 X. Fig. 2c, 2d, 2e. IHC staining done on cell block showing positive staining for TTF-1, CK-7 (CK-20 was negative), and MOC-31 respectively.

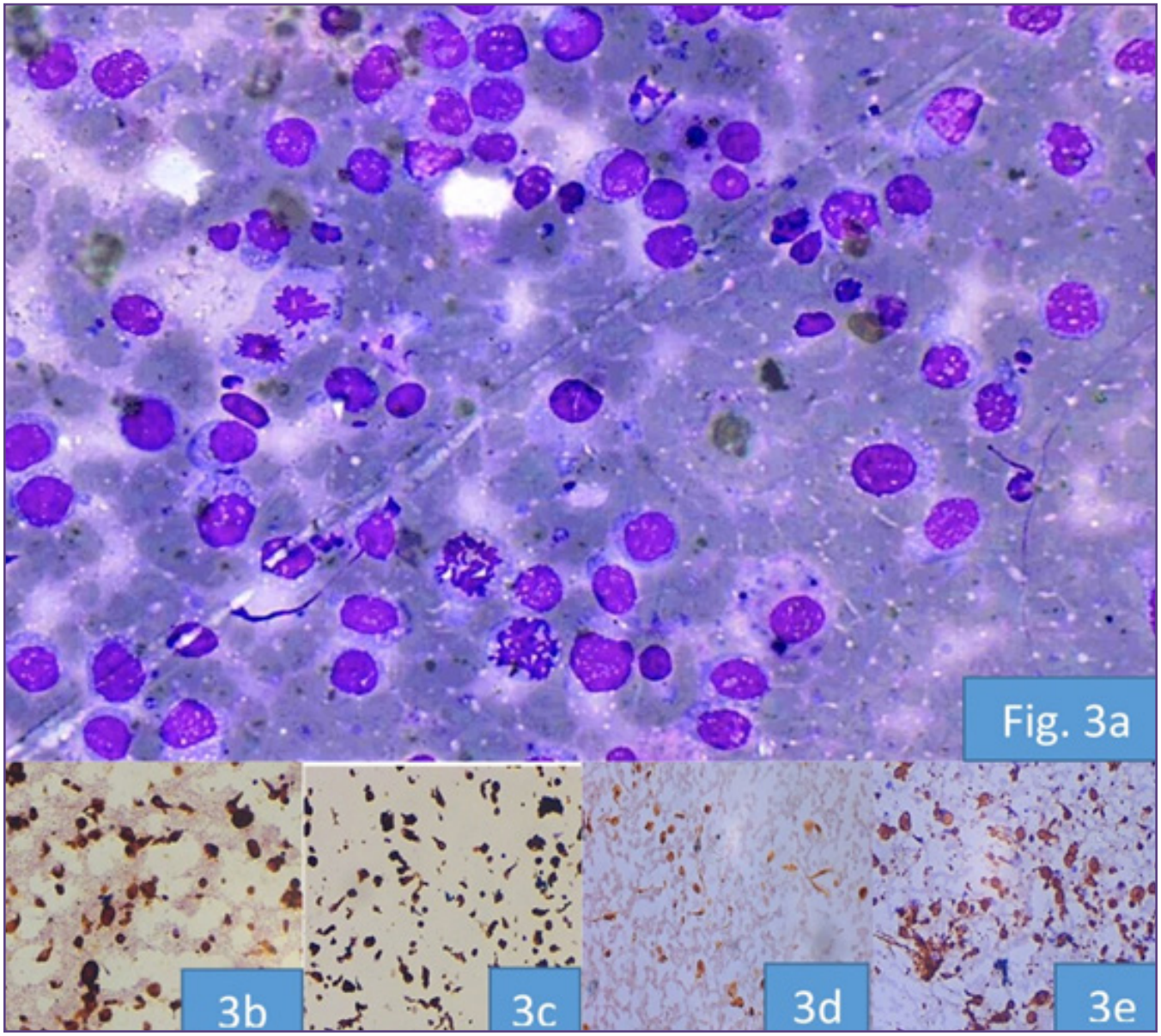

Fig. 3: FNA smears from diffuse thyroid swelling. 3a. Giemsa stained smear showing scattered cells with brisk mitoses, prominent nucleoli, D/D: Anaplastic carcinoma vs Anaplastic lymphoma. 3b, 3c, 3d, 3e: Immunocytochemistry smears showing positive staining for LCA, Bcl-6, CD-20 and Vimentin. Final diagnosis: Vimentin positive DLBCL thyroid.

Discussion

Cytology has come a long way from being a rapid screening tool to the preferred method for providing definitive diagnoses. The clinicians' expectations from cytologists are also rapidly rising to demand a definitive diagnosis, rather than tentative differentials in most cases. In this scenario, it becomes imperative to explore the potential of various ancillary techniques that can enable confident and exact diagnosis. In the era of molecular advancements, immunocytochemistry is just the most basic adjunctive tool for a cytology, yet it is also the one with the widest potential. In this study, we aimed to find out the impact of immunocytochemistry as an adjunctive tool.

Most of the studies on ICC have been done on lesions of specific sites. [1, 2] However, in our study we have included lesion from all sites, and samples of all types (FNAC material and fluids) making it more comprehensive.

In majority of cases studied (88%), immunochemistry helped in providing a definitive diagnosis, better than

by using cytology alone. Previous studies have found this figure to be around 69%. [3] The better results can be attributed to difference in techniques of performing immunocytochemistry and case to case variations to some extent.

The advantages of immunocytochemistry that we found out in our study was significant improvement in diagnosis without the need for unnecessary biopsy. In 15 cases of carcinomas of unknown primary, immunocytochemistry helped in determining the primary site of tumor. In 2 cases, of thyroid tumors, a distinction between carcinoma and lymphoma was made on FNAC alone, circumventing the need for biopsy in a precariously vascular organ, chemotherapy could be started for the patient. However, in one case, biopsy was performed only to confirm the diagnosis provided on FNAC.

In the remaining 12% cases (6 out of 50, with 4 fluid specimens) where immunochemistry failed to add valuable information, the reason was lack of cellularity of cell blocks in 4 cases (2 fluids). This is attributable to faults in preparation of cell blocks, especially in case of fluids. In the remaining two cases, there was negative staining which could not be repeated.

We evaluated both FNAC materials and fluid specimens, using two techniques of immunochemistry, cell blocks and direct on smear. Immunochemistry was found out to be more effective in FNAC specimens yielding accurate diagnosis in 95% cases, as compared to fluids where an accurate diagnosis was possible in 60% cases. This was due to lesser cellularity of cell blocks prepared from fluids. Although we performed immunochemistry directly on smear in only 6 cases, yet, in all these cases it was successful in yielding a diagnosis. In fact, in one case, de-staining a pap stained slide and using it for immunocytochemistry also yielded results. In cell blocks, however, there were problems in cellularity in a few cases. It may be due to variations in cellularity with multiple needle passes. In few cases, the FNAC smear had adequate cellularity, but the cell blocks were not cellular for tumor cells. This is in concordance with previous studies that have also determined direct smears to be better owing to confirmation of adequacy on unstained smears at the time of procedure itself. [4]

In two cases, the staining was negative and non-contributory. Both these cases were of FNAC material from which cell blocks had been prepared. This can be attributed to the inherent limitations of ICC, such as destruction of few epitopes in formalin fixed tissues that has also been previously reported. [5]

The major reasons for the non-contributory immunochemistry can be improved upon with either better cell block preparation or by performing immunochemistry on cold acetone fixed smears.

Conclusion

In the era of rapid advancements in cytology, immunochemistry is the simplest yet one of the most effective adjuncts to cytology. We strongly recommend the routine use of immunochemistry to aid cytological diagnosis.

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