Utility of immunochemistry in cytology

ABSTRACT

Background: The role played by cytology in primary diagnosis is undeniable. With improved management protocols and targeted therapy, the need for accurate diagnosis has become mandatory. Immunochemistry and molecular techniques are increasingly being used on limited tissue samples.

Aims: This study was conducted to find out the impact of immunocytochemistry (ICC) on cytology material in cytology practice.

Materials and Methods: Immunocytochemistry was done on alcohol-fixed smears and cell-block preparations. It was done with i6000 BioGenex autostainer using BioGenex reagents.

Results: A total of 148 cases occurring over a period of 3 years (September 2010-June 2013) were analyzed. Staining was done on cytology smears in 77 cases and on cell-block sections in 71 cases. ICC helped in diagnosis in 8 cases, confirmed the diagnosis in 26 cases, helped in subtyping in 60 cases, and helped in prognostication in 6 cases. ICC has altered the diagnosis in two cases. It was noncontributory in 43 cases, and the material was inadequate in three cases.

Conclusion: In 102 cases (69%), ICC proved to be a useful adjunct in the diagnosis and prognostication; hence, its use is recommended in practice to aid in cytology services.

Key words: Cell block, cytology, immunochemistry, utility

Introduction

Fine-needle aspiration cytology (FNAC) is a rapid and convenient method of diagnosing any accessible lesion. With improved management protocols and targeted therapy, the need for accurate diagnosis has become mandatory. Immunochemistry and molecular techniques are increasingly being used on limited tissue samples.[1]

Immunocytochemistry (ICC) is helpful to exclude pertinent differential diagnosis and permit a reliable preoperative diagnosis of tumor in doubtful cases. The study was conducted to find out the impact of ICC on cytology material to make a specific diagnosis and to study the limitations of ICC.

Materials and Methods

Cytology material used included alcohol-fixed smears and cell-block preparations. Immunocytochemistry was done with i6000 BioGenex autostainer (California, USA) using BioGenex reagents. The control samples taken and the conditions in which ICC was carried out were similar to that of immunohistochemistry. Results were categorized as follows: helped in diagnosis, confirmed the diagnosis, altered the
diagnosis, helped in subtyping, helped in prognostication, and noncontributory [Figure 1].

**Results**

The study period was September 2010 to June 2013. A total of 148 cases were analyzed; staining was done on cytology smears in 77 (52.1%) cases and on cell blocks in 71 (47.9%) cases. Twenty-eight cases (18.9%) had subsequent or previous histologic evaluation. Out of 148 cases, ICC was contributory in 69% of the cases. It has helped in the diagnosis in 8 cases (5.4%), helped in subtyping in 60 cases (40.5%) [Figure 2], helped in prognostication in 6 cases (4.0%) [Figure 3], and confirmed the diagnosis in 26 cases (17.5%). In two cases (1.6%), the cytomorphologic diagnosis was altered after ICC. However, ICC was noncontributory in 46 cases (31%), of which smears were 29, cell blocks were 14. Material was inadequate in three cases (2%).

Among these 102 cases, 56 cases had cell-block preparations including 5 fluids. The remaining 49 cases were without cell blocks and these included 19 fluids. In 21 cases, single marker was done. Immunohistochemistry (IHC) on histopathology specimens was available in 28 cases [Table 1].

**Discussion**

In this era of targeted therapy and personalized treatment protocols, the role of cytologist and pathologists is very vital. The role of cytology improved from screening in cervical smears to diagnosis, prognostication, and currently predictive aspects of pathology.[2] In the diagnosis of malignant tumors, specific subtyping is necessary to find out the tumors that have better prognosis or the ones that respond to specific therapies.[3] Efforts are on to get more from the limited tissue samples. ICC and molecular techniques are being tried on limited tissue samples.[1]

Majority of studies on ICC are on lesions at specific sites.[4–7] Maximum number of studies is on effusion cytology trying to validate the role of several ICC markers.[8–13] Subtyping of non small cell carcinoma into squamous cell

**Table 1: Correlation between immunohistochemistry (IHC) and immunocytochemistry (ICC) (N = 28)**

| Diagnostic category         | Number of cases (%) |
|----------------------------|---------------------|
| Confirmed                  | 16 (57.1)           |
| Subcategorized             | 7 (25)              |
| Altered the diagnosis      | 2 (7.1)             |
| No representative tissue on cytology | 2 (7.1)             |
| No tissue on histology     | 1 (3.6)             |

![Figure 1: Analysis of immunocytochemistry in all the samples based on their relevance (N = 148)](image1)

![Figure 2: Fine needle aspiration cytology from a case of nonsmall cell lung carcinoma. (a) Cytology smear shows cluster of atypical cells with pleomorphic nuclei (MGG, ×400). (b) Cell-block preparation showing nests of atypical cells (H and E, ×100). (c) ICC with CK7 showing strong cytoplasmic positivity (HRP POLYMER, ×100). (d) ICC with p63-strong nuclear positivity (HRP POLYMER × 100)](image2)

![Figure 3: A case of metastatic breast carcinoma. (a) Lesional cells seen in clusters and vague acini in a hemorrhagic background (MGG, ×400). (b) ICC with estrogen receptor (ER) showing grade 2 positivity (HRP POLYMER, ×400). (c) ICC with progesterone receptor (PR) showing grade 3 positivity (HRP POLYMER, ×100)](image3)
carcinoma, adenocarcinoma, adenosquamous or large cell neuroendocrine could be done by ICC.[14-18]

ICC has some inherent technical problems.[19,20] In one case, ICC with calcitonin was noncontributory that later came positive in histology. Hence, a negative result may not be contributory when the morphology strongly points a diagnosis.

ICC was done on smears in 52.1% of the cases in the present study. Background artifacts, limited panel, and ethanol or prior staining may adversely influence the results in smears.[20,21] ICC on cell block has the advantage of being able to perform IHC with proper controls and repeatability on sections for multiple markers. Cell block also helps in retaining a banked archive for future studies.[17]

However, effective sampling during dedicated fine-needle aspiration passes for cell-block preparation and the variability in cellularity might be a problematic issue as reported by Roh et al. We had a similar experience; however, we noted inadequacy of material for cell-block preparation in only 3 cases (2%) as compared with 37% of cases described by Roh et al. Another concern is that formalin may destroy some epitopes in paraffin-embedded tissues.[18]

Mandal et al. studied the role of ICC in undifferentiated neoplasms; ICC on cell block may not have 100% accuracy. Direct smears have the advantage of confirmation of adequacy on unstained smears at the time of procedure itself.[17] ICC is a rapid and sensitive method for the diagnosis and classification of lesions. As the sample is limited, conventional light microscopy should not be ignored for the judicious selection of antibodies.[1] A combination of morphologic examination, immunochemistry, and clinicopathological findings can further improve the rate of positive diagnosis.

Wallace and Rassl et al. studied endoscopic ultrasound guided bronchial cytology and correlated it with histopathology.[6] They observed that the cytology alone could subclassify nonsmall cell lung cancer in 44.4% of the cases which rose to 64.1% with cytology and cell block and further increased to 84.6% when ICC is added.

ICC can also help in prognostication. Sahebali et al. studied the role of Ki-67 in liquid based cytology samples and found it to be helpful in identifying high grade squamous intraepithelial lesion (HSIL) and human papillomavirus (HPV) 16 positive samples.[22,23]

In one of our earlier studies on direct smears for hormone receptor status of breast cancer by ICC, there was low sensitivity and negative predictive value.[14] The present study has better results probably because we have used rabbit monoclonal antibodies for estrogen and progesterone receptors (earlier mouse monoclonal was used). It is also possible that the number of breast cancer cases for hormone receptor included in this study is less. Two cases of thyroid aspirate, one for calcitonin and the other for thyroglobulin, were negative on cell block. However, they were positive on histopathology sections. Though the cell-block preparation for other antibodies did not pose problems in the present study, it appears that some antibodies require more care in antigen retrieval.

Fowler discouraged the use of single marker for ICC.[20] However, in the present study, due to the paucity of material, single marker was used in 21 cases. Most commonly used single marker was chromogranin (4) followed by CD99 (3). Single marker ICC was noncontributory in 9 (42.9%) cases; however, it helped in subtyping in 7 (33.3%) cases, in confirming the diagnosis in 4 (19%) cases, and in prognostication in 1 (4.8%) case.

The most useful marker overall was pancytokeratin that was used in 61 cases. It was contributory in 44 cases. Cytokeratin 7 (CK7) was contributory in 13 out of 21 cases in which it was used. Thyroid transcription factor1 (TTF1) helped in giving a conclusive opinion in 33 out of 148 cases. Other important conclusive markers were chromogranin (17 cases), p63 (13 cases), cluster of differentiation 99 (CD99) (12 cases), and neuron-specific enolase (NSE) (9 cases). Hematological markers were useful in 20 cases. CD20 was used in 17 cases and was conclusive in 12 cases; leucocyte common antigen (LCA) was used in 15 cases and was contributory in 13 cases.

Vimentin was contributory in 10 out of 11 cases in which it was used. Carcinoembryonic antigen (CEA) was contributory in 9 out of 14 cases.

ICC was most useful in fluids (24 cases, 5 of which had cell-block preparation). It helped in differentiating reactive mesothelial cells from adenocarcinoma. Similar utility in fluids was demonstrated by Kitazume et al.[24] Broad based studies like the present study on the utility of ICC in cytology are fewer. Mao et al. conducted a similar study and the results were comparable to the present study.[25]

The most common solid tissue on which ICC was performed is lymph node (20 cases) followed by soft-tissue swellings (17 cases) and lung masses (12). Of the 17 soft-tissue swellings, 12 were abdominal masses. The utility of ICC in giving a
conclusive result to solid organ cases also had a similar profile.

In the present study, technical difficulties formed 12.8% of the total cases. Scant material was responsible for limiting the panel and noncontributory results. In spite of the above limitations, ICC was proved to be a useful adjunct in the diagnosis, subtyping, and prognostication in majority of the cases.

Among the cases which had IHC correlation (n=28), ICC diagnosis was confirmed in 57.1% cases and further subtyping was done by using IHC in 25% cases. In seven cases (25%), further subtyping could be done. The major utility of ICC in our study was in subtyping malignancies (40.5%). Nevertheless, complete subtyping was not possible in many cases due to insufficient material. One follow-up case of Ewing’s sarcoma was treated and showed no residual tumor at the time of histopathology.

It may be concluded from the study that the primary diagnosis could be done on morphology alone in a significant number of cases. However, in the era of targeted therapy and due to the need to deliver accurate treatment with minimal invasive procedures, ICC seems to be a very useful option. It must also be emphasized that there is a need to obtain high-quality cytology material for a completely satisfactory result.

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Conflicts of interest
There are no conflicts of interest.

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