

Original article:**Role of cell block immunohistochemistry in early diagnosis of lymphoma.***Prabir Kumar Saha¹, Ferdousy Begum², Syed Muhammad Baqui Billah¹***Abstract**

Background: Cell block immunohistochemistry (IHC) is an important adjunct for diagnosis of tumour. However, its usefulness in the diagnosis of lymphoma is yet to be established.

Objective: We wanted to assess the reliability of cell block IHC in the diagnosis and classification of lymphoma.

Methods: This cross-sectional study was carried out among 17 patients with enlarged lymphnode in cytopathology department of Bangabandhu Sheikh Mujib Medical University. **Results:** IHC could give either positive or negative result in 12 cases, leaving 5 cases as inconclusive, which lead us to remove them from analysis. Cell block IHC diagnosed 9 cases (75%) cases as positive for lymphoma and 3 cases (25%) as negative for lymphoma. The sensitivity, specificity, accuracy, strength, positive predictive value and negative predictive value of cell block IHC were 100% when assessed with final diagnosis based on histopathology and clinical outcome. **Conclusion:** Cell block IHC could be a useful tool for early diagnosis of lymphoma.

Keywords: immunohistochemistry, histopathology, cell block, lymphoma, antibody.

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Introduction

Lymphoid neoplasm is a diverse, complex array of neoplasms of B-cell, T-cell and NK cell. Used for proliferations as discrete tissue mass, the disparity of global cancer and outcome between developed and developing countries are pretty wide¹. Diagnosing neoplastic and non-neoplastic lesions through histopathology combined with immunohistochemistry (IHC) has been used to detect lymphoid neoplasm². World Health Organization now emphasizes multiparametric approach which includes morphological, phenotypic and genomic features and clinical syndromes in classification of haematopoietic and lymphoid malignancies³. B-cell cancers are most common, with a 4% incidence of cancer cases each year globally⁴. Being prevalent in developed countries like USA, Australia, New

Zealand, and Europe, the diagnostic procedure is also being practiced in developing countries⁵. USA alone accounts for 70,000 new cases per year, which comprises about 6% of all cancers in the particular country⁶. The gold standard for diagnosing lymphoma is tissue excision and biopsy coupled with immunohistochemistry⁶. Sometimes tissue excision and biopsy are not possible eg. mediastinal, retroperitoneal or ocular lymphadenopathy⁷, where fine needle aspiration cytology is considered to be the method of choice. But accurate classification is not possible without immunocytochemistry⁸. Cell block IHC can be considered for early diagnosis of lymphoma for better treatment plan in these cases⁹.

We aimed to assess the diagnostic accuracy of cell block IHC against histopathology followed by IHC to refute the null hypothesis that cell block

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IHC is not effective as an early diagnostic tool for lymphoma.

Materials and methods

We included 30 individuals of clinically suspected lymphoproliferative disorder at the department of pathology of Bangabandhu Sheikh Mujib Medical University (BSMMU) from March 2014 to February 2016. Patients with enlarged lymph node of greater than 1.5 cm and more than 2 months' duration were included excluding those who were previously diagnosed as cases of malignancy other than lymphoma. Cell block samples were collected from selected patients with all aseptic precautions at the department of pathology of BSMMU with a 22 gauge needle on a syringe with application of vacuum. The aspirates were directly fixed in 10% buffered formalin and used for cell block preparation by bacterial agar technique. The prepared cell blocks were sent to the pathology laboratory of BSMMU and kept in 10% formalin in a properly labeled container assigned with a laboratory number and was fixed overnight. In the laboratory, tissue processing, paraffin embedding, sectioning of the paraffin blocks, H & E staining were done according to the standard protocol followed at BSMMU and were assessed for histopathological diagnosis. Subsequently each cell block with corresponding histopathology slides were sent to IHC laboratory. Primary IHC antibodies were selected. CD 20, CD 3, CD15, CD 30, Td, C 79 α , Pancytokeratin, CD5, CD 10, CD 34 and BCL 2 antibodies were applied according to selection based on cyto and histomorphology. Cellblock IHC was done in the IHC laboratory, BSMMU according to standard protocol followed by DAKO EnVision™ Flex, High p^H detection system, peroxidase/Dab⁺, Rabbit/Mouse technique.

All IHC cases were run with appropriate positive and negative controls. Sections of appendix with follicular hyperplasia were selected as control for CD 20, CD 3, CD 15, CD 30, CD 79 α , CD 5, CD 10, CD 34 and BCL 2. Thymus was used as control for TdT and skin for pancytokeratin (AE₁/AE₃).

Out of 30 cases, cell block IHC was performed in 17 cases. Cell block IHC was not done in 13 cases (43.3%) due to negative for lymphoma diagnosis.

Cell block IHC diagnosed 9 cases (52.9.2%) as positive for lymphoma and 3 cases (17.6%) as negative for lymphoma. Cell block IHC was inconclusive in 5 cases (29.5%). So we removed these 5 records keeping only 12 samples for final analysis. The sensitivity, specificity, positive predict value, negative predictive value and accuracy or efficacy were evaluated to compare cell block IHC and final diagnosis.

Results

The reasons for inconclusive diagnosis in cell block IHC were crush artifact, inadequate sample and extensive fibrosis, necessity of application of extensive antibody panel, improper fixation and sometimes IHC technique fault. The positive cases were non Hodgkin lymphoma, classical Hodgkin lymphoma, unspecified T cell lymphoma and nodal marginal zone B cell lymphoma. Although it was possible to diagnose Hodgkin or non Hodgkin lymphoma and lymphoma of B or T cell lineage, further subclassification was not possible with cell block IHC due to scanty material obtained with fine needle aspiration technique. The negative for lymphoma cases diagnosed by cell block IHC were reactive lymphadenitis and metastatic carcinoma (Table 1).

Table 1: Frequency distribution of the study patients by cell block IHC diagnosis (n=30)

Cell block IHC diagnosis	Number of patients	Percentage
Positive for Lymphoma	9	52.9
Negative for Lymphoma	3	17.6
Inconclusive	5	29.5
Inflammatory cases	13	

Comparison between cell block IHC diagnosis and final diagnosis

All of nine cases positive for lymphoma by cell block IHC diagnosis were finally diagnosed as positive for lymphoma (true positive). Out of three cases of negative for lymphoma by IHC on cell block all 3 cases were finally diagnosed as negative for lymphoma (true negative) (Table 2). There was no false positive or false negative test result with cell block IHC. The Yates' corrected χ^2 value was 7.26, $p = 0.007$.

Table 2: Comparison between cell block IHC diagnosis and final diagnosis based on histopathology, IHC and clinical follow-up (n=12)

Cell block IHC	Final Dx		Total
	Positive for lymphoma	Negative for lymphoma	
+ve	9	0	9
-ve	0	3	3
Total	9	3	12

Table 3: Sensitivity, specificity, accuracy, positive and negative predictive values of the cell block IHC diagnosis and Final diagnosis based on histopathology, IHC and clinical follow-up (n=12)

The sensitivity, specificity, accuracy, strength, positive predictive value and negative predictive value of cell block IHC were 100% compared to the standard diagnostic procedure (Table 3).

Test of Validity	Percentage
Sensitivity	100.0
Specificity	100.0
Accuracy	100.0
Strength	100.0
Positive predictive value	100.0
Negative predictive value	100.0

Discussion

The 100% sensitivity, specificity, accuracy, strength, positive predictive value and negative predictive value of cell block IHC diagnosis in our study bears promising indication in favor of IHC. Albeit, the investigation should be dealt with caution as our sample size was very small. Even we are optimistic about the findings of the study as we get support from other researchers¹⁰ indicating high sensitivity (99.0%), specificity (95.9%), PPV (97.1%), and NPV (98.6%) for the lymphoma and benign reactive hyperplasia. Cell block technique offers an important advantage of obtaining multiple section of the same material for routine¹¹.

FNA might not yield enough information for accurate diagnosis in some cases, while cell block

preparations can be a good alternative to better detecting the pathology (Mayall2000, Goyal2020). Even cell block technique appeared to be superior over conventional smear as found by Honnappa et al¹².

Adequate material is essential for diagnosis from cell block. To be adequate, we have to keep aspirating until the aspirates enter the barrel from the nozzle. In lymph node aspiration there is possibility of bloody aspirate if we want to take more material/ for adequate specimen aspiration. Some recommends 22.5 G needle to aspirate adequate material. In my observation bloody aspirate can be minimized by taking two or more aspirations from different site of a large lymph node (>2cm). But it is not yet possible to aspirate adequate material in lymphomas where fibrosis is the main feature (e.g. Nodular sclerosis classical Hodgkin lymphoma).

Crushing and other artifacts are major limitations of cell block histopathology where materials are very limited. To minimize crushing artifact, the needle should be taken out of the nozzle and the fixative should be taken into the barrel by suction without needle as soon as the aspiration was done. Buffered formalin should be used as fixative. The aspirates then should be dropped into the filter paper and the tissue fragments should then be carefully taken off and put into the 3% agar. Centrifugation may be used.

FNA followed by cell block IHC could be performed in cases of debilitating patients or when patient was in critically ill conditions and patient could not undergo biopsy or procedures which requires general anesthesia¹³. This safe technique would allow more sections which would improve the diagnostic ability. Moreover, IHC has been tried by different researchers as evidenced by good interrater agreement¹⁴ over other techniques.

Lymphoma diagnosis is currently aided by flow cytometry which is a common and effective adjunct to FNAB. Cell block IHC had several advantages over flow cytometry as it is relatively easy and requires inexpensive preparation. IHC studies can be done without extensive training where histological morphology could be obtained in immunohistological stained slides, offering higher diagnostic accuracy for Hodgkin lymphoma and some T-cell lymphomas. It also allows preservation as archival tissue for complementary analyses, reclassification, and research purposes¹⁰.

IHC appears to be a good tool to detect lymphoma from nonneoplastic cases. We recommend that cell block IHC should be practiced routinely to rule out the possibility of lymphoma to those cases which are clinically and cytologically equivocal. The clinical workup is a challenge to clinicians in those there is clinical suspicion for lymphoma but the cytology appears benign. The ability of IHC to detect lymphoma could establish its importance had we done further study with bigger sample size.

Conclusion

Our study reaffirm that cell block IHC is a highly sensitive and specific tool for diagnosis of lymphoma and could be a reliable and useful adjunct to fine needle aspiration cytology. We recommend to further the study with large sample size and

multicenter collaboration to establish its usefulness.

Ethical Approval: We got the ethical approval from institutional review board of BSMMU before starting the study.

Conflict of interest: The authors declare no conflict of interest.

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Individual Authors Contributions: PS designed the study, FB mentored the whole procedure of the study, SB did the statistical analysis and necessary modification of the script. All the authors contributed to script writing, review and finalization of the draft.

Reference

- Hewamana S, Kandabadage L, Skandarajah T, Peiris N, Abeyaratne S, Arseculeratne G, et al. Applicability of protocols from high-income countries in a resource limited setting; real world data of histopathology, clinical features and long-term outcome of Hodgkin Lymphoma in Sri Lanka. *EClinicalMedicine*. 2021; 38: 100998.
- Dave DV, Patel DB, Popat DVC. Comparison of immunohistochemistry with conventional histopathology for evaluation of lymphnodes. *International Journal of Clinical and Diagnostic Pathology*. 2022;5:80-4.
- Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leukemia*. 2022;36:1720-48.
- Masnikosa R, Piric D, Post JM, Cvetkovic Z, Petrovic S, Paunovic M, et al. Disturbed Plasma Lipidomic Profiles in Females with Diffuse Large B-Cell Lymphoma: A Pilot Study. *Cancers (Basel)*. 2023;15.
- Conte S, Aldien AS, Jette S, LeBeau J, Alli S, Netchiporouk E, et al. Skin Cancer Prevention across the G7, Australia and New Zealand: A Review of Legislation and Guidelines. *Curr Oncol*. 2023;30:6019-40.
- Khanom KH, Tarafder S, Sattar H. Flow Cytometry Immunophenotypic Diagnosis of B-Cell Non-Hodgkin Lymphomas on Fine-Needle Aspirate of Lymph Node. *Clin Cancer Investig J*. 2019;8:155-60.
- Mahajan VK, Sharma V, Sharma N, Rani R. Kikuchi-Fujimoto disease: A comprehensive review. *World J Clin Cases*. 2023;11:3664-79.
- Keane GC, Keane AM, Diederich R, Kennard K, Duncavage EJ, Myckatyn TM. The evaluation of the delayed swollen breast in patients with a history of breast implants. *Front Oncol*. 2023;13:1174173.
- Shidham VB, Layfield LJ. Cell-blocks and immunohistochemistry. *Cytojournal*. 2021;18:2.
- Zhang S, Yu X, Zheng Y, Yang Y, Xie J, Zhou X. Value of fine needle aspiration cell blocks in the diagnosis and classification of lymphoma. *Int j Clin Exp Pathol*. 2014;7:7717-25.
- Cozzolino I, Varone V, Picardi M, Baldi C, Memoli D, Ciancia G, et al. CD10, BCL6, and MUM1 expression in diffuse large B-cell lymphoma on FNA samples. *Cancer Cytopathol*. 2016;124:135-43.
- Honnappa S, S GB, B S. Cytological Analysis of Body Fluids and Comparison of Precision in Diagnosis Between Conventional Smear and Cell Block Along with Clinical Correlation. *Annals of Pathology and Laboratory Medicine*. 2019;6:A90-5.
- Karabachev AD, Brundage WJ, Sajisevi MB, Ciolino AL. Feasibility of fine needle aspiration for diagnosis of b-cell lymphoma of the thyroid: a case series and review of the literature. *Diagn Pathol*. 2023;18:69.
- Sampaio F, Marrinhas C, Fonte Oliveira L, Malhao F, Lopes C, Gregorio H, et al. Detection of Lymphoid Markers (CD3 and PAX5) for Immunophenotyping in Dogs and Cats: Comparison of Stained Cytology Slides and Matched Cell Blocks. *Vet Sci*. 2023;10.