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Original Article

Value of fine needle aspiration cell blocks in the diagnosis and classification of lymphoma

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Abstract: Fine needle aspiration biopsy (FNAB) is a simple yet accurate diagnostic procedure. However, the role of FNAB in lymphoma diagnosis and classification remains controversial. This study aimed to evaluate the value of FNAB cell blocks in the diagnosis and classification of lymphoma using our patented aspirator in a pencil-grip operation manner and a simplified cell block preparation method. We retrospectively reviewed 177 cases of lymph node and extranodal lymphoproliferative disorders that were diagnosed with cytomorphology, morphology, and immunohistochemistry of cell blocks. Of these, 83 were primary lymphoma; 14 were recurrent lymphoma; 8 were suspected as lymphoma, and 72 were benign reactive hyperplasia (BRH). Our analysis indicated 99.0% sensitivity, 95.9% specificity, 97.1% positive predictive value, and 98.6% negative predictive value in discriminating among primary/recurrent lymphoma and BRH. The diagnostic accuracy for sub-classification of lymphoma was 86.6% (84/97), with 77.8% (7/9) for classical Hodgkin's lymphoma and 87.5% (77/88) for non-Hodgkin's lymphoma. Our results implicated cell blocks as a reliable and useful adjunct to FNAB for the diagnosis and classification of lymphoma. Cytomorphology, morphology, and immunohistochemical studies of cell blocks offered very high accuracy in the diagnosis of lymphoma and allowed further sub-classification in many cases. Thus, patients with a definitive diagnosis and classification might avoid invasive and expensive surgical biopsy procedures.

Keywords: Fine needle aspiration biopsy, cell block, lymphoma, accuracy, immunohistochemistry

Introduction

Fine needle aspiration (FNA) biopsy (FNAB) is a simple, inexpensive, less traumatic, safe, and fast diagnostic procedure with high accuracy. It is usually the first choice to diagnose superficial lumps. However, the role of FNAB in lymphoma diagnosis and classification remains controversial. Additionally, cytological diagnosis of lymphoma via FNAB is followed by surgical biopsy in most cases. Over the last decade, several studies have reported the value of FNAB combined with flow cytometry immunophenotyping in the diagnosis and classification of lymphoma [1-10]. However, to our knowledge, there are no published studies on the diagnosis of lymphoproliferative disorders using cell blocks in a large patient cohort. Our extensive analysis on the efficiency of cytomorphology, morphology of cell blocks, and immunohistochemical studies using cell block sections in lymphoma diagnosis and sub-classification have highlighted the applicability of cell blocks.

The latest World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues [11] emphasizes a multiparametric approach to include morphological, phenotypic, and genetic features, as well as clinical syndromes. Morphology and immunophenotyping are sufficient for the diagnosis of most lymphoid neoplasms. We often perform FNAB as a preferred procedure for patients with superficial lumps at our hospital. In the past few years, we have routinely processed residual materials from cytological smear into cell blocks, which are composed of random cells and tissue fragments. They could provide morphology and partial histological structures, and can be sectioned for immunohistochemical staining, which offers reliable and stable results. From this perspective, cytological features, morphology, and immunophenotype of cell blocks represent fun-

Figure 1. Fine needle aspiration using our patented aspirator (Youyi type aspirator) was performed in a patient with lymph node enlargement in the right neck. The pencil-grip operation manner improved the sense of finger tips, whereas the automatically retained vacuum helped sample more tissue.

damental tools in the diagnosis and classification of lymphoma. In recent years, there have been an increasing number of lymphoma cases diagnosed by this approach at our hospital, and cell blocks have gradually been adapted as a routine ancillary technique to evaluate lymphoid lesions. The present study summarized our experience using cell blocks for the diagnosis of lymphoproliferative diseases and discussed the value and limitation of the method as well as its technical aspects.

Materials and methods

Case selection

We selected 177 cases of lymphoproliferative disorders diagnosed by cell block analysis from the database of Beijing Friendship Hospital, which is affiliated with the Capital Medical University Department of Pathology, between January 2010 and December 2013. Inclusion criteria included primary lymphoma, recurrent lymphoma, and suspected lymphoma with available surgical biopsy as controls. Either surgical biopsy or clinical follow-up data were used as controls for benign reactive hyperplasia (BRH) cases. All cases in our series were reevaluated, and the initial diagnosis was reconfirmed.

FNA and cell block preparation procedures

FNAB was performed without anesthesia using our patented 21 G (0.8 mm) aspirator (Youyi aspirator), which had a slot in the plunger and a latch on the bottom of the tube (**Figure 1**), in a pencil-grip operation manner. FNA specimens were collected by cytopathologists in more than 10 needle insertions, followed by direct smear and hematoxylin and eosin (H&E) staining. The remaining cells and tissue fragments were routinely processed into cell blocks using ethanol coagulation and formaldehyde fixation as previously described [12]. The procedure details are presented in **Figure 2**. All cell blocks were treated similarly to surgical biopsy specimens, including formalin fixation, paraffin embedding, and sectioning at 4-5 μm thickness, followed by H&E staining and immunohistochemistry.

Main diagnostic criteria for morphology and immunohistochemistry

The main diagnostic criteria for cytological smears and cell blocks were as follows: (1) BRH: smears mainly contained small lymphocytes and lymphocytes at different transformation stages; morphological analysis of cell blocks could include multiple round, semi-circular, or irregular lymphoid follicles with germinal centers and mantle zones; (2) Hodgkin's lymphoma: reactive background cells (including small lymphocytes, eosinophils, histiocytes, and plasma cells, etc.) were scattered among Reed-Sternberg cells and their mutant derivatives; morphology of cell blocks was the same as that of smears, but showed different levels of fibrosis; (3) non-Hodgkin's lymphoma (NHL): smears demonstrated monomorphic and immaturely differentiated lymphocytes; morphology of cell blocks showed relatively uniform cells and nuclear atypia. Additionally, follicular structures could be observed in follicular lymphoma (FL), whereas some marginal B-cell lymphoma/marginal zone lymphoma of mucosa-associated lymphoid tissue (MZL/MALT) cases might exhibit monocytoid cells.

A preliminary diagnosis was made on the basis of the diagnostic criteria of lymphocyte population cell size in the smear proposed by Koss et al. [13] and morphological characteristics of cell blocks. A panel of appropriate antibodies was then selected from the following for immunohistochemical staining: CD3, CD5, CD10, CD15, CD20, CD21, CD23, CD30, CD38, CD138, CD56, B-cell lymphoma (BCL)-2, BCL6, multiple myeloma oncogene (MUM)-1, Cyclin

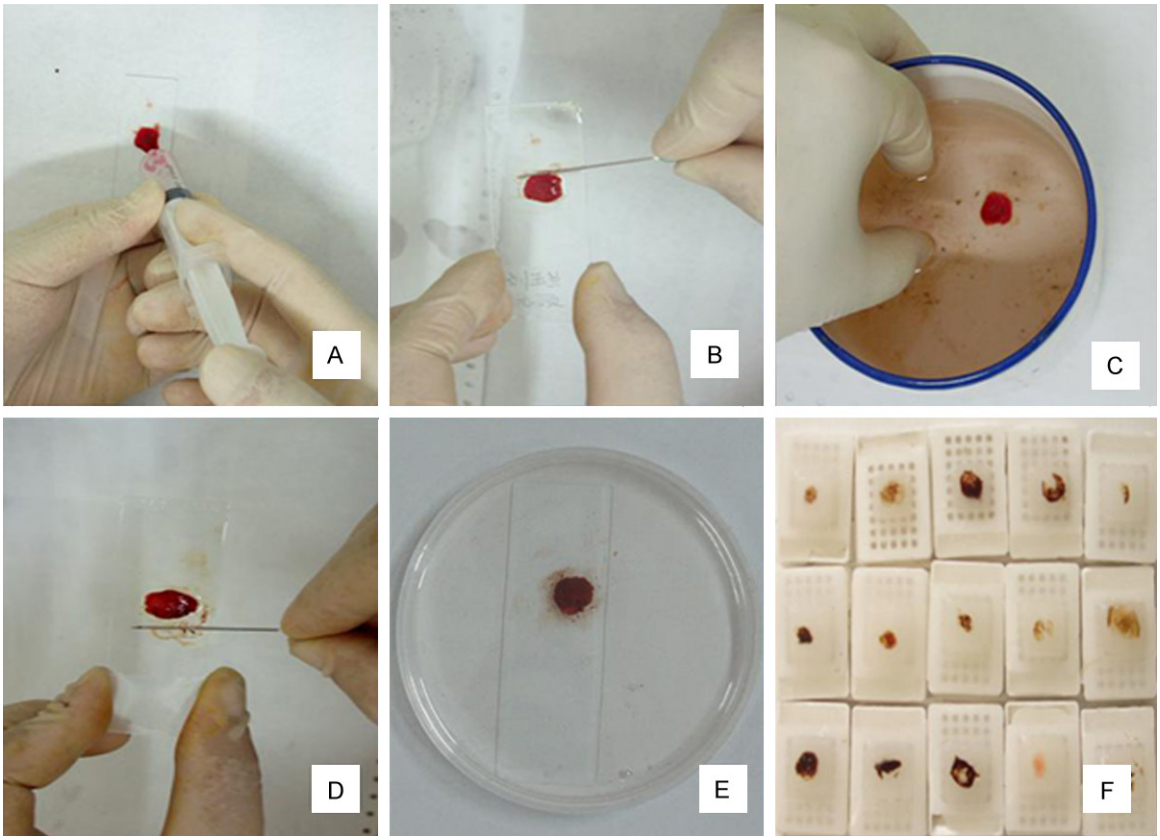

Figure 2. Flow chart of fine needle aspiration biopsy cell block preparation. A. Place the specimen from needle aspiration on a slide; B. Gather the sample using an aspirator; C. Immerse the slide in 95% ethanol for 30 to 60 seconds and set aside; D. Further trim the sample using an aspirator; E. Place the specimen in formalin fixative for 2 to 8 hours and remove the specimen from the slide using a razor blade; F. Wrap the specimen in lens cleaning paper, place it into a dehydration box, and it was ready for paraffin embedding.

Table 1. Correlation among diagnoses by fine needle aspiration biopsy, surgical biopsy, and follow-up

	No. of patients Surgical biopsy and follow-up		
FNAB diagnosis	(%)	Lymphoma	BRH
r-lymphoma	14 (7.9)	14	0
p-lymphoma	83 (46.9)	83	0
Suspicious lymphoma	8 (4.5)	5	3
BRH	72 (40.7)	1	71
Total	177 (100)	103	74

Abbreviations: FNAB, fine needle aspiration biopsy; r-lymphoma, recurrent lymphoma; p-lymphoma, primary lymphoma; BRH, benign reactive hyperplasia.

D1, anaplastic large-cell lymphoma (ALCL), T-cell-restricted intracellular antigen (TIA)-1, Granzyme-B, terminal deoxynucleotidyl transferase (TdT), and Ki-67.

Cytomorphology, morphology of cell blocks, and corresponding immunohistochemistry results were evaluated by both cytopathologists and hematopathologists to achieve a final diagnosis based on the latest WHO classification. The diagnostic results were divided into three categories, including a definitive diagnosis of lymphoma, suspected lymphoma, or BRH.

Statistical analysis

Using surgical biopsy or clinical follow-up data as controls, cases in which a definite diagnosis of BRH or lymphoma with or without further classification was made were considered truly negative and truly positive, whereas suspected lymphoma cases were considered positive. Statistical analysis was performed to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of this method in diagnosing lymphoma and benign reactive lymphoid hyperplasia, and to calculate the diagnostic accuracy of lymphoma sub-classification.

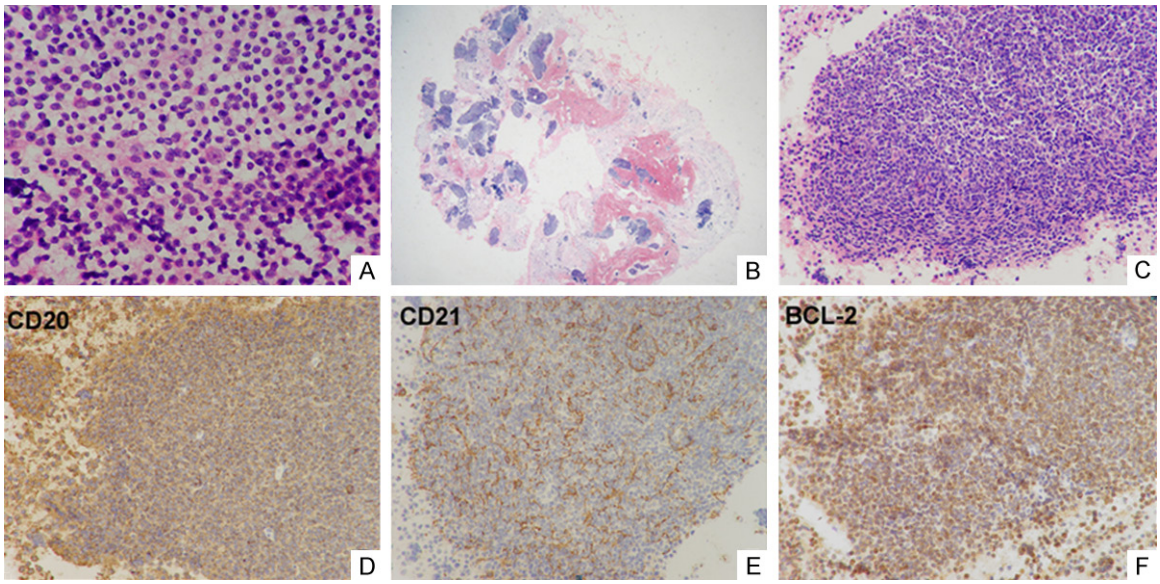

Figure 3. Follicular lymphoma. A. A cell smear contained medium-sized and a few scattered large-sized cells. Medium-sized cells were slightly larger than mature lymphocytes with an irregular nuclear membrane and unobvious nucleolus. Two to three nucleoli could be observed in the few scattered large-sized cells (hematoxylin and eosin [H&E] stain, 400× magnification). B. Sections from cell blocks demonstrated multiple small tissue fragments in low magnification (H&E stain, ×12.5). C. Sections from cell blocks demonstrated follicle-like structures constituted by centrocytes in high magnification (H&E stain, ×200). D. Immunohistochemistry of cell block sections demonstrated CD20 positivity (×200). E. Immunohistochemistry staining of CD21 in cell block sections demonstrated follicular dendritic cell network (×200). F. Immunohistochemistry of cell block sections revealed BCL-2 positivity (×200).

Results

The study cohort included 76 women and 101 men with an average age of 49 years (range, 2-83 years). The tissue samples consisted of 164 superficial lymph nodes and 13 superficial extra-lymph node lesions, which included the skin (5 cases), soft tissue (5 cases), tonsil (2 cases), and parotid gland (1 case). All patients were able to tolerate the procedure without anesthesia, allowing the cytopathologists to successfully perform FNA. Bleeding during aspiration was about 0.1-0.2 ml. During clinical follow-up of 96 patients after aspiration, only 1 had infection within 1 week after aspiration, and 2 experienced transient local swelling.

We performed aspiration using our patented aspirator in a pencil-grip operation manner, which enabled us to obtain more specimens for successful cell block preparation. The cell blocks had a diameter of 0.4-1.2 cm and a thickness of 0.2 cm. Tissue deformation was observed in only one cell block, whereas all others maintained adequate tissue structure and cellular morphology, allowing for morphological observation and immunohistological evaluation.

Of 177 FNAB cases, 83 (46.9%) were primary lymphoma, 14 (7.9%) were recurrent lymphoma, 8 (4.5%) were suspected as lymphoma, and 72 (40.7%) were BRH. All primary and recurrent lymphoma cases were confirmed by surgical biopsy with an accuracy of 100%. Of 8 suspected lymphoma cases, 5 were confirmed as NHL (3 FL, 1 MALT, and 1 peripheral T-cell lymphoma, not otherwise specified [PTL-NOS]), and 3 were BRH (2 florid follicular hyperplasia and 1 monocytoid B-cell hyperplasia). There were 11 BRH cases with available surgical biopsy results as controls, and 61 without surgical biopsy but available clinical follow-up data. Of 11 cases with surgical biopsy results, 10 were BRH and 1 was MZL (false negative). The results are summarized in **Table 1**.

In this study, 97 cases of lymphoma were definitively diagnosed by cell block analysis, including 9 classical Hodgkin's lymphoma (CHL) cases and 88 NHL cases. Using surgical biopsy results as controls, the accuracy of lymphoma sub-classification by cell block analysis was 86.6% (84/97), with 77.8% (7/9) accuracy for CHL subtype and 87.5% (77/88) for NHL, which included diffuse large B-cell lymphoma (DLBCL),

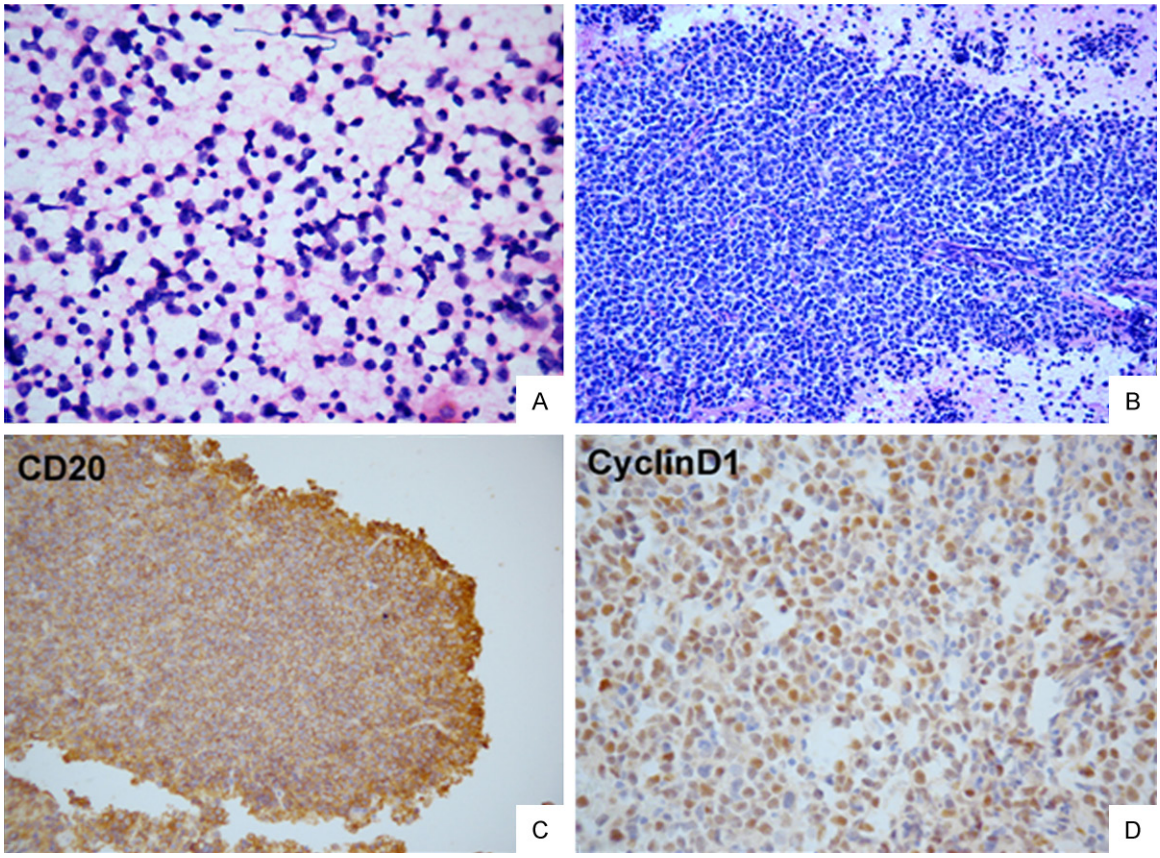

Figure 4. Mantle cell lymphoma. A. A cell smear consisted of monomorphic small- to medium-sized cells, similar to centrocytes (H&E stain, ×400). B. Cell block sections demonstrated medium-sized cell infiltration diffusely (H&E stain, ×200). C. Immunohistochemistry of cell block sections revealed CD20 positivity (×200). D. Immunohistochemistry of cell block sections demonstrated Cyclin D1 positivity (×400).

FL (**Figure 3**), mantle cell lymphoma (MCL, **Figure 4**), small lymphocytic lymphoma (CLL), ALCL, plasmacytoma, Burkitt lymphoma (BL), lymphoblastic lymphoma (LBL), and natural killer/T-cell lymphoma (NK/T). Of the 11 cases that could not be sub-classified, 6 were B-cell lymphoma (1 case, diagnosed as DLBCL by surgical biopsy, could not be sub-classified due to technical problems leaving the tissues deformed and unable to be evaluated by immunohistological markers; 5 cases were diagnosed as MZL/MALT by biopsy), and 5 were T-cell lymphoma (2 PTL-NOS cases and 3 angioimmunoblastic T-cell lymphoma [AITL] cases). The distribution of NHL sub-classification by FNAB and surgical biopsy is shown in **Table 2**.

Statistical analysis revealed 99.0% sensitivity, 95.9% specificity, 97.1% PPV, and 98.6% NPV for the discrimination among primary lymphoma, recurrent lymphoma, and BRH (**Table 3**).

Discussion

The major difference between surgical biopsy and FNAB is that FNAB yields smaller amounts of sample with partial or complete loss of histological structure, potentially causing its diagnostic limitations. We believe that improving the FNA technique and cell block preparation procedure has significant impacts on broadening the scope of applications for FNAB. Cell blocks are very useful not only for performing additional immunohistochemical studies to further sub-classify lymphomas but also for visualizing tissue architecture [14]. We herein summarize the clinical application of cell blocks in lymphoma diagnosis and classification, analyze the accuracy of diagnosis and possibility of classification, and discuss the application value of cell blocks in FNA diagnosis.

With regard to the efficiency of the method, we obtained very high sensitivity (99.0%), specific-

Table 2. Distribution of non-Hodgkin lymphoma sub-classification diagnosed by fine needle aspiration and surgical biopsy

FNAB	No. of patients	Surgical biopsy												
		DLBCL	FL	MCL	CLL	ALCL	PCT	T-LBL	B-LBL	BL	NK/T	PTL-NOS	AITL	MZL/ MALT
DLBCL	28	28												
FL	11		11											
CLL	9			9										
MCL	8				8									
ALCL	7					7								
PCT	3						3							
T-LBL	4							4						
B-LBL	3								3					
BL	2									2				
NK/T	2										2			
Unclassifiable	11	1										2	3	5
Total	88	29	11	9	8	7	3	4	3	2	2	2	3	5

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; CLL, small lymphocytic lymphoma; MCL, mantle cell lymphoma; ALCL, anaplastic large-cell lymphoma; PCT, plasmacytoma; T-LBL, T-lymphoblastic lymphoma; B-LBL, B-lymphoblastic lymphoma; BL, Burkitt lymphoma; NK/T, natural killer/T-cell lymphoma; PTL-NOS, peripheral T-cell lymphoma not otherwise specified; AITL, angioimmunoblastic T-cell lymphoma; MZL/MALT, marginal B-cell lymphoma/marginal zone lymphoma of mucosa-associated lymphoid tissue.

Table 3. Diagnostic reliability of fine needle aspiration biopsy as compared with surgical biopsy and follow-up

FNAB diagnosis	Surgical biopsy and follow-up		Total
	Positive	Negative	
Positive	102	3	105
Negative	1	71	72

Sensitivity 99.0%, specificity 95.9%, positive predictive value 97.1%, and negative predictive value 98.6%.

ity (95.9%), PPV (97.1%), and NPV (98.6%) for the discrimination between lymphoma and BRH, and we were able to accurately classify 7/9 CHL and 77/88 NHL cases. These findings were quite similar to the results of other studies confirming the accurate diagnosis and classification of lymphoma using FNAB combined with flow cytometry immunophenotyping [2, 4]. Therefore, cell blocks are a reliable and useful method to be utilized with FNAB for establishing a definitive lymphoma diagnosis.

Previous studies [15-17] suggested that diagnosis of CHL using cytomorphology alone was highly accurate (> 85%). However, the method yields relatively low accuracy for subtype diagnosis owing to the requirement of histological structure information. In our study, we examined CD30 and CD15 expression by immunohistochemical staining of cell block sections from CHL patients to establish a definitive diagnosis and distinguish CHL from morphologically similar conditions such as poorly differentiated carcinoma, melanoma, and ALCL. Although cell blocks could provide some structural information, subtype diagnosis might still be challenging as the overall structure cannot be observed in cell blocks. In this study, 2 of 9 CHL cases could not be accurately classified.

For B-cell NHL, diagnostic methods combining morphology and cell block immunohistochemistry offer high accuracy for lymphoma subtypes with characteristic morphology and specific immunophenotype, such as DLBCL, B-cell CLL, MCL, plasmacytoma, BL, and B-cell LBL. However, it is difficult to diagnose nonspecific lymphoma subtypes such as MZL/MALT. In our study, 5 cases of B-cell lymphoma, which could not be classified due to non-technical reasons, were proved to be MZL/MALT by surgical biopsy. In addition, we were able to confirm 10/11 BRH cases with surgical biopsy results. The remaining 1 case was misdiagnosed as BRH and subsequently confirmed by biopsy as MZL. Therefore, it remains challenging to diagnose MZL/MALT by FNAB even with the help of cell block immunohistochemistry.

In the classification of B-cell lymphoma by FNAB, the most challenging subtype is FL due to the need to observe follicular structure. One of the advantages of cell blocks is the ability to provide partial histological structure, which, in combination with immunohistochemistry findings (i.e. BCL-2 positivity in the germinal center), contributes to the diagnosis of FL. However, in clinical practice, as only part of the structure can be observed, it is sometimes difficult to distinguish between florid follicular hyperplasia and FL (especially when BCL-2 is negative). We encountered such a potential pitfall in our study when two cases of suspected lymphoma were confirmed as florid follicular hyperplasia by surgical biopsy. From the cytomorphological perspective, as Mendon deduced [18], if the aspiration of the reactive node is derived from the large germinal center, the proportion of large cells (centroblasts and dendritic cells) and number of mitoses might be impressive enough to suggest malignant lymphoma. From the cell block structure perspective, small tissues aspirated in the cell blocks may be part of the enlarged germinal center, thus mantle zones usually could not be seen. During cell block preparation, these small tissues are artificially gathered, leading to easily misdiagnosed lymphoma. As 10-15% of FL cases, especially those of high grade, are BCL-2 negative [19-21], it is difficult to distinguish between florid follicular hyperplasia and FL based on morphological and immunophenotypical findings. Therefore, the best way to confirm florid follicular lesions indicated by FNAB is via surgical biopsy.

It is worthy of note that except for ALCL, NK/T, and T-cell LBL, which have characteristic immunophenotypes, other T-cell lymphomas present with significant morphologic and immunophenotypic variation. Although FNAB combined with cell block immunophenotyping helps the diagnosis of T-cell lymphoma, further classification remains difficult.

The cell block technique is not new [22] and has been used by many researchers in the past 20 years. However, its utilization is far from universal, probably owing to insufficient sample collection and complicated preparation procedure. There are two important technical aspects in our study. First, the aspirator was modified. We used our patented aspirator in a pencil-grip operation manner, which offers considerable benefits [23]. One of such advantages is the maintenance of sufficient negative pressure for the collection of more cells and tissue fragments, allowing for not only routine cytological smear but also cell block preparation. The other aspect is our cell block preparation procedure. Traditional cell block preparation methods include agar embedding, HistoGel, and plasma thrombin techniques [24]. However, these are relatively complicated procedures, requiring reagents that are inconvenient to prepare and preserve. We have attempted to simplify the cell block preparation procedures and reagents since 2008, and such an effort has resulted in our current ethanol coagulation and formaldehyde fixation method.

In the United States, 22-27G (0.5-0.65 mm) aspirators are usually used in FNAB, whereas 20-23G (0.6-0.9 mm) aspirators are commonly used in Sweden (Northern Europe) and our country. In this study, we used modified 21G (0.8 mm) aspirators, which are relatively thicker than those used in the United States, in a pencil-grip operation manner. One of the concerns was whether patients could tolerate the procedure without excessive bleeding in the absence of anesthesia. Our observations showed that all patients could tolerate the pain, especially those with lymphadenopathy, who only felt subtle pain when needles were inserted into the skin. Not too much pain was felt when needles were inserted into the lymph nodes, probably due to the absence of nerves there. FNAB is a minimally invasive procedure, usually with a trace amount of bleeding during aspiration with almost no effect on the human body. Koss et al. [13] suggested that the procedure should be terminated once bleeding started for optimal results. However, based on our experience, quick insertion of the needle several times when a small amount of bleeding appears may allow more specimens to reach the shank and the tube, thus ensuring the collection of sufficient amount of sample. Furthermore, traces of blood are very important for lymphocyte smear because it ensures appropriate smear thickness, uniform cell distribution, and clear cytomorphology.

Another concern of FNAB is whether it has any impact on the pathological diagnosis of subsequent surgical biopsy (such as infarction, hemorrhage, and structural damages, etc.). In our

study, none of the cases with surgical biopsy data were affected by FNAB or had any difficulties in subsequent pathological diagnosis. Therefore, as shown in a prior study [25], such complications are rare in clinical practice.

Flow cytometry is currently the most common and effective adjunct to FNAB for establishing lymphoma diagnosis. Compared with flow cytometry, cell block immunohistochemistry has several advantages. First, it is relatively easy and inexpensive to prepare cell blocks and perform immunohistochemistry studies without extensive training. Second, histological morphology could be directly observed in cell block immunohistological staining slides. Furthermore, cell block immunophenotyping offers higher diagnostic accuracy for Hodgkin's lymphoma and some T-cell lymphomas, which could not be diagnosed by flow cytometry. Finally, an issue with flow cytometry is the loss of archival tissue for complementary analyses, reclassification, and research purposes, whereas cell block specimens allow for preservation and further study in the future.

In summary, our retrospective analysis suggested that the use of FNAB morphology analysis and cell block immunohistochemistry offered high diagnostic accuracy for primary and recurrent lymphoma and enables further classification in many cases. Achieving a definitive diagnosis and classification using this method could help avoid more invasive and expensive surgical biopsy procedures. Despite its limitation and pitfalls, cell blocks should be considered as a reliable and useful adjunct to FNAB for establishing a definitive lymphoma diagnosis. Besides immunohistochemistry, cell blocks could also be used for cytogenetic and molecular biology detection, and samples can be preserved for any future correlative scientific studies.

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Disclosure of conflict of interest

None.

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References

[1] Ensani F, Mehravaran S, Irvanlou G, Aghaipoor M, Vaeli S, Hajati E, Khorgami Z, Nasiri S. Fine-needle aspiration cytology and flow cytometric immunophenotyping in diagnosis and classification of non-Hodgkin lymphoma in comparison to histopathology. Diagn Cytopathol 2012; 40: 305-310.

[2] Zeppa P, Vigliar E, Cozzolino I, Troncone G, Picardi M, De Renzo A, Grimaldi F, Pane F, Vetrani A, Palombini L. Fine needle aspiration cytology and flow cytometry immunophenotyping of non-Hodgkin lymphoma: can we do better? Cytopathology 2010; 21: 300-310.

[3] Mathiot C, Decaudin D, Klijanienko J, Couturier J, Salomon A, Dumont J, Vielh P. Fine-needle aspiration cytology combined with flow cytometry immunophenotyping is a rapid and accurate approach for the evaluation of suspicious superficial lymphoid lesions. Diagn Cytopathol 2006; 34: 472-478.

[4] Zeppa P, Marino G, Troncone G, Fulciniti F, De Renzo A, Picardi M, Benincasa G, Rotoli B, Vetrani A, Palombini L. Fine-needle cytology and flow cytometry immunophenotyping and subclassification of non-Hodgkin lymphoma: a critical review of 307 cases with technical suggestions. Cancer 2004; 102: 55-65.

[5] Mourad WA, Tulbah A, Shoukri M, Al Dayel F, Akhtar M, Ali MA, Hainau B, Martin J. Primary diagnosis and REAL/WHO classification of non-Hodgkin's lymphoma by fine-needle aspiration: cytomorphologic and immunophenotypic approach. Diagn Cytopathol 2003; 28: 191-195.

[6] Liu K, Stern RC, Rogers RT, Dodd LG, Mann KP. Diagnosis of hematopoietic processes by fine-needle aspiration in conjunction with flow cytometry: A review of 127 cases. Diagn Cytopathol 2001; 24: 1-10.

[7] Nicol TL, Silberman M, Rosenthal DL, Borowitz MJ. The accuracy of combined cytopathologic and flow cytometric analysis of fine-needle aspirates of lymph nodes. Am J Clin Pathol 2000; 114: 18-28.

[8] Dong HY, Harris NL, Preffer FI, Pitman MB. Fine-needle aspiration biopsy in the diagnosis and classification of primary and recurrent lymphoma: a retrospective analysis of the utility of cytomorphology and flow cytometry. Mod Pathol 2001; 14: 472-481.

[9] Siebert JD, Weeks LM, List LW, Kugler JW, Knost JA, Fishkin PA, Goergen MH. Utility of flow cytometry immunophenotyping for the di-

agnosis and classification of lymphoma in community hospital clinical needle aspiration/biopsies. Arch Pathol Lab Med 2000; 124: 1792-1799.
[10] Jeffers MD, Milton J, Herriot R, McKean M. Fine needle aspiration cytology in the investigation on non-Hodgkin's lymphoma. J Clin Pathol 1998; 51: 189-196.
[11] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumors of hemotopoietic and lymphoid tissues. Lyon: IARC; 2008.
[12] Cheng GY, Yu XM, Liu J, You QH, Zhou XG. A novel cell block technique for fine needle aspiration biopsy samples. Chin J Pathol 2011; 40: 558-559.
[13] Koss LG, Melamed MR. Koss diagnostic cytology and its histopathologic bases. 4th edition. Philadelphia, PA: Lippincott Williams & Wilkins; 2006.
[14] Austin RM, Birdsong GG, Sidawy MK, Kaminsky DB. Fine needle aspiration is a feasible and accurate technique in the diagnosis of lymphoma. J Clin Oncol 2005; 23: 9029-9030.
[15] Qizilbash AH, Elavathil LJ, Chen V, Young JE, Archibald SD. Aspiration biopsy cytology of lymph nodes in malignant lymphoma. Diagn Cytopathol 1985; 1: 18-22.
[16] Das DK, Gupta SK. Fine needle aspiration cytodiagnosis of Hodgkin's disease and its subtypes. II. Subtyping by differential cell counts. Acta Cytol 1990; 34: 337-341.
[17] Friedman M, Kim U, Shimaoka K, Panahon A, Han T, Stutzman L. Appraisal of aspiration cytology in management of Hodgkin's disease. Cancer 1980; 45: 1653-1663.
[18] Mendon ME. Fine needle aspiration cytology of lymph nodes. Prog Diagn Cytol 1999; 32: 453-456.
[19] Gelb AB, Rouse RV, Dorfman RF, Warnke RA. Detection of immunophenotypic abnormalities in paraffin-embedded B-lineage non-Hodgkin's lymphomas. Am J Clin Pathol 1994; 102: 825-834.
[20] Lai R, Arber DA, Chang KL, Wilson CS, Weiss LM. Frequency of bcl-2 expression in non-Hodgkin's lymphoma: a study of 778 cases with comparison of marginal zone lymphoma and monocytoid B-cell hyperplasia. Mod Pathol 1998; 11: 864-869.
[21] Utz GL, Swerdlow SH. Distinction of follicular hyperplasia from follicular lymphoma in B5-fixed tissues: comparison of MT2 and bcl-2 antibodies. Hum Pathol 1993; 24: 1155-1158.
[22] Karnauchow PN, Bonin RE. "Cell-block" technique for fine needle aspiration biopsy. J Clin Pathol 1982; 35: 688.
[23] Wang P, Yu XM. A primary nipple lymphoma diagnosed by a modified fine-needle aspiration method. Diagn Cytopathol 2012; 40: 719-723.
[24] Kulkarni MB, Desai SB, Ajit D, Chinoy RF. Utility of the thromboplastin-plasma cell-block technique for fine-needle aspiration and serous effusions. Diagn Cytopathol 2009; 37: 86-90.
[25] Lioe TF, Elliott H, Allen DC, Spence RA. The role of fine needle aspiration cytology (FNAC) in the investigation of superficial lymphadenopathy; uses and limitations of the technique. Cytopathology 1999; 10: 291-297.