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Fine Needle Aspiration Cytology of Hematolymphoid Malignancy

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I. INTRODUCTION

The possible use of fine needle aspiration(FNA) cytology in the final diagnosis of various hematolymphoid malignancy has been questioned by several authors.[1,2] There are some recent articles in which malignant lymphoma can be correctly diagnosed with cytology smears.[3-8] Some types of lymphoma, such as follicular center cell lymphomas, are notoriously difficult to separate conclusively from the reactive hyperplasia by cytology. With the use of immunologic analysis, it is possible to differentiate malignant lymphoma from reactive hyperplasia and to characterize fully the lymphoid population. Immunocytochemical staining has made it possible to reach the same diagnostic accuracy on FNA material as in studies on frozen sections. In fact, the results are so promising that analysis of surgical biopsy material is not always required for final diagnosis unless the growth pattern of the lymphoma, nodular or diffuse, is of clinical importance.

This paper describes cytologic features of various kinds of hematolymphoid malignancy. In addition, the reliability and usefulness of immunologic analysis of fine needle aspiration material as an aid in cytomorphologic diagnosis of hematolymphoid malignancy are discussed.

II. MATERIALS AND METHODS

1. Patients

Nine patients were aged from 20 to 65 years. All of them were male patients. They were referred to the Department of Pathology, Asan Medical Center between May and October 1992 for FNAC. In 5 patients, there were no previous diagnoses of malignant disease. The remaining 4 patients had been previously diagnosed as having non-Hodgkin's malignant lymphoma(2 cases), multiple myeloma(1 case) , or acute lymphoblastic leukemia(1 case).

In every case, a percutaneous fine needle aspiration biopsy was performed with a 23-gauze needle using the procedure described by Zajicek.[9] In two patients with malignant lymphoma, a lymph node was removed and analyzed by histopathology and immunohistochemistry, if available.

2. Slide and Cytospin preparations.

The aspirated material was used to make both smears for cytologic diagnosis and cytospin preparations for immunocytochemistry, if available. Air-dried smears were stained by Giemsa technique, and ethanol-fixed smears were stained by the Papanicolaou method or hematoxylin-eosin staining. The cytologic diagnosis of lymphoma was based on Working formulation[10].

Cytospin preparation of aspirated cells suspended

in 1.5ml of ice-cold phophate buffered saline(PBS) were found to have little or no background staining than the use of smears of aspirated cells for immunocytochemistry. The cell viability in the suspensions was analyzed by trypan blue exclusion. The total concentration of cells was adjusted to 1-2x10^6/ml. Within 30minutes, cytospin preparations were done in a cytocentrifuge(600 rpm for 3 minutes). The cell preparations were air dried and stored at−70℃ until use. Before immunocytochemical staining, the cells were fixed in cold acetone(−20℃) for ten minutes, air dried and rinsed in tris-buffered saline(TBS) for five minutes. A three-step alkaline phosphatase immunostaining was used.[11] Two anti-human monoclonal antibodies, pan B(MB2) and pan T(MT1) cell markers (Bio Genex Co., CA) were used.

3. Surgical Biopsies

Biopsy specimens were obtained fresh and unfixed. Parts of each specimen were fixed in both 10% buffered formaline and B5 solution, and processed for morphologic study. Histopathologic diagnosis followed Working formulation.[10] Immunologic analysis were performed on cryosections using an ABC(avidin-biotin peroxidase complex) method.[12] The monoclonal antibodies used in immunohistochemistry were the same with those used in immunocytochemistry, pan B and pan T cell markers.

Ⅲ. RESULTS

Cases selected in this study were summarized in Table 1.

Table 1. Morphologic Diagnosis & Immunolgic findings in FNA and Sugical Biopsy.

	Age/Sex	Site	FNA Diagnosis	Surgical Biopsy Diagnosis
Case 1	44/M	Cervical LN	ML,intermediate	ML, diffuse, mixed
Case 2	50/M	Cervical LN	ML.intermediate	ML, diffuse, mixed
Case 3	60/M	Cervical LN	ML, large	ML, diffuse large
Case 4	45/M	Inguinal LN	ML, large	ML, immunoblastic
Case 5	65/M	Flank	ML, large(B cell)	ML, diffuse, large
Case 6	49/M	G−I tract	ML, large(T cell)	ML, diffuse, mixed in cervical LN
Case 7	63/M	SCL LN	HD	HD, mixed
Case 8	33/M	Axilla	Multiple myeloma	N−D
Case 9	20/M	Chin	ALL	ALL

LN : Lymph node ML : Malignant lymphoma
HD : Hodgkin's disease SCL : Supraclavicular
ALL : Acute lymphoblastic leukemia
G−I : Gastrointestinal
N−D : Not−done

Case 1 to 4:

Four patients had enlarged cervical or inguinal lymph nodes. FNA cytology revealed large atypical lymphoid cells with inconspicuous nucleoli. Also were scattered small mature lymphocytes. These features were suggestive of malignant lymphoma(Fig.1a). The surgical biopsy specimens showed diffuse malignant lymphoma. They were either large cell type, mixed or immunoblastic type(Fig.1b).

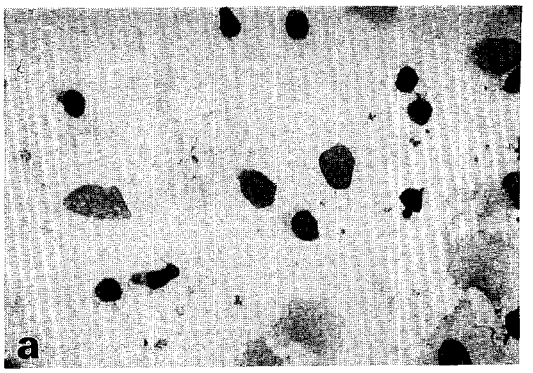

Fig. 1a : FNA smear of malignant lymphoma (case 4) ; Large tumor cells are admixed with mature lymphocytes (Giemsa stain).

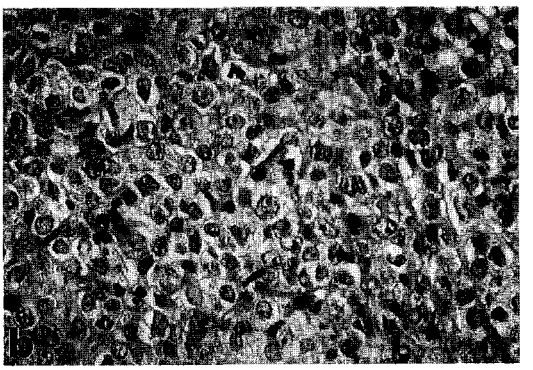

Fig. 1b : Histopathology of the lymph node ; Diffusely proliferating large immunoblasts show prominent nucleoli and variable amount of cytoplsm (H& E stain).

Case 5:

This patient was previously diagnosed as having intestinal non-Hodgkin's lymphoma by endoscopic biopsy. He had received chemotherapy, however flank mass was recently detected. The mass was diagnosed as malignant lymphoma by FNA cytology. Immunophenotyping was also done on cytospin preparation, which showed B cell monoclonality.

Case 6:

FNA smears of the lower abdominal mass were highly cellular, consisting of monotonous round lymphoid cells. They have scanty cytoplasm and occasionally prominent nucleoli(Fig.2a). The immunocytochemical results showed diffuse membranous pattern for pan T cells(Fig. 2b). The surgical biopsy specimen obtained from cervical lymph node showed malignant lymphoma , diffuse , mixed type.

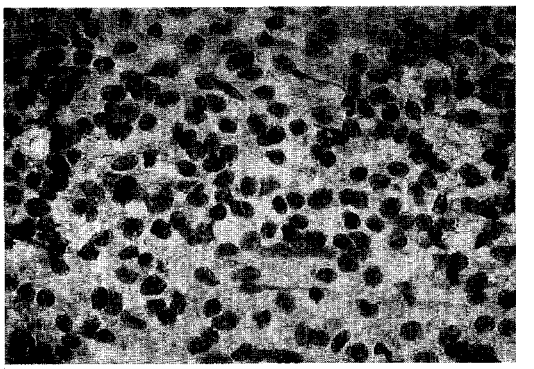

Fig. 2a : FNA smear of abdominal mass in case 6 ; Highly cellular smear shows monotonous tumor cells with occasional nucleoli and coarse chromatin (H& E stain).

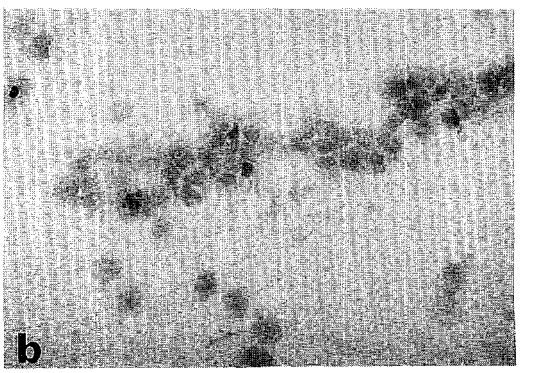

Fig. 2b : Immunocytochemical stain for phenotyping ; Tumor cells are diffusely and strongly positive for pan T cell marker.

Case 7:

This patient had enlarged supraclavicular lymph nodes and mediastinal mass. Lymph node aspirates revealed large binucleated cells and small lymphocytes which was diagnosed as Hodgkin's disease (Fig.3a). On lymph node biopsy sections, many

Reed-Sternberg cells were also seen. It was Hodgkin's disease, mixed celluarity(Fig. 3b)

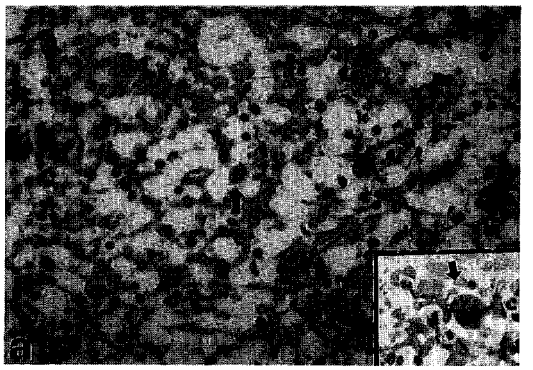

Fig. 3a : FNA smear of Hodgkin's disease;There are large binucleated or mononuclear tumor cells (inset) in the background of hetergeneous cells (H & E stain)

Fig. 3b : Lymph node reveals many diagnostic Reed-Sternberg cells(arrow) in the mixture of lymphocytes (H & E stain).

Case 8:

This patient was previously diagnosed as having multiple myeloma. After chemotherapy, axillary and buttock masses were palpated. Aspirate taken from axillary mass showed tumor cells with plump cytoplasm and eccentrically located atypical nuclei (Fig.4), which confirmed recurrence of multiple myeloma.

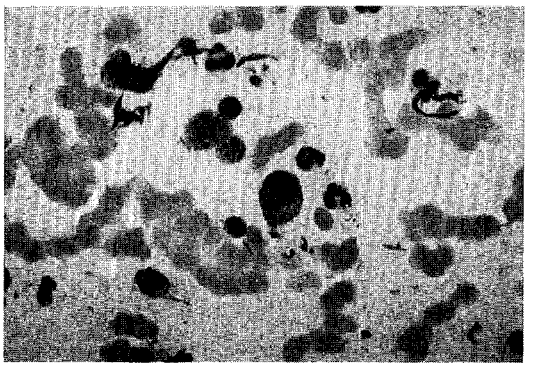

Fig. 4 : FNA smear of multiple myeloma ; Note atypical plasmacytoid cells with eccentric nuclei and perinuclear cytoplasmic clearing(Giemsa stain).

Case 9:

This patient had received chemotherapy after he was diagnosed as having acute lymphoblastic leukemia. During follow-up, a subcutaneous mass of chin was detected. Cytology smears of the mass showed medium-sized lymphoblastic leukemic cells. They have variable amount of cytoplasm and fine nuclear chromatin pattern(Fig.5).

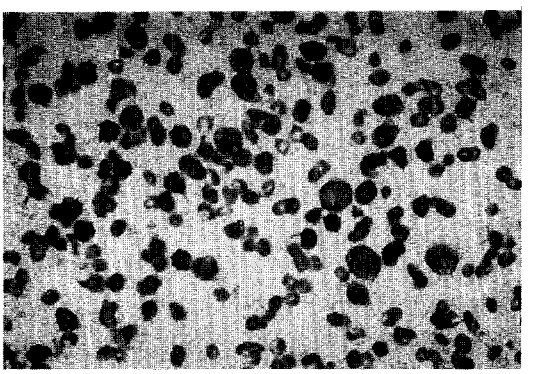

Fig. 5 : FNA smear of acute lymphoblastic leukemia ; Many lymphoblastic leukemic cells are variable in size and show round nuclei and small amount of cytoplasm(Giemsa stain).

Ⅳ. DISCUSSION

Fine needle aspiration(FNA) cytology has for

many years been accepted as an accurate technique for diagnosis of both primary and metastatic epithelial neoplasms. However, its usefulness in diagnosing hematolymphoid malignancy, has been questioned even in the most pro-FNA countries (e.g. Sweden). This scepticism still seems to be shared and fostered even by many cytopathologists. One of the FNA pioneers, Tosef Zajicek, wrote that "if the cell population is of a monotonous but benign apperance, histologic examination should always be done, since about 20% of cases of well-differentiaed lymphocytic lymphoma cannot at present be recognized in smears of aspirate".[9] This statement has wrongfully been interpreted as a position against the use of FNA cytology in diagnosing non-Hodgkin's lymphoma. Therefore in our country, FNA cytology has not been actively applied in diagnosis of hematolymphoid malignancy. In a few studies, cytologic features were described especially in Hodgkin's disease.[13] This negative attitude was not shared by other authors.[14,15] Koss et al. expressed the following opinion : "The diagnosis of lymphoma, although often possible and accurate, requires considerable caution and experience".[15] It was also predicted that immunocytochemistry could be used to corroborate the diagnosis and classification of lymphomas on FNA material akin to the situation in histopathlogy. During the last several years, this assumption has proven to be correct, and there is a rapidly growing body of documentaion that FNA material can be used both for diagnosis and subclassification of hematolymphoid malignancy. In most cases of this study, cytologic features were compatible with those of histopathology. Case 6 with an abdominal lymphoma showed discrepancy with surgical biopsy in subclassification. However, FNA was obtained from the different site. Both findings were maligant lymphoma, intermediate grade. Additionally, the result of immunologic stain was better than that of surgical biopsy. We summarize some of these results which are so promising that they are bound to have an impact on the future diagnostic work-up of patients with hematolymphoid malignancy. Cytomorphology in conjunction with immunocytochemistry will lead to an increased accuracy in the diagnosis of malignant lymphoma. In addition, it will also allow phenotyping and subtyping of lymphoma cells.

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=국문초록=

혈림프계 악성종양의 세침흡인 세포검사

울산대학교 의과대학 서울중앙병원 병리학교실
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과거 수 십년간 세침흡인 세포검사는 상피성 종양을 진단하는데 유용한 방법으로 이용되어 왔으나, 혈림프계 악성종양에 있어서는 그 유용성에 대한 의문이 제기되어 왔다. 그러나 최근 수년간, 세포학적 검색뿐만 아니라 면역세포학적 염색을 통해 혈림프계 악성종양을 진단하고 세분화하는 것이 가능하다는 보고들이 늘고 있다. 국내에서는 세침흡인 세포검사를 통해 혈림프계 악성종양을 진단하는 보고가 활발하지 않고, 면역세포학적 염색을 실제 진단에 이용하고 있는 기관은 아직 없는 것 같다. 저자들은 1992년 5월부터 10월까지 6개월간 본원 해부병리과에 의뢰된 환자에서 행한 세침흡인 세포검사중 9예의 림프증식성 질환을 경험하였는데 그 중 6예가 비Hodgkin 림프종양, 1예가 Hodgkin 림프종양, 1예가 다발성 골수종 그리고 1예는 급성 림프모구성 백혈병이었다. 악성림프종양중 2예는 세포면역학적 염색을 통해 세포형에 대한 분류까지 가능하였다. 따라서 혈림프계 악성종양에서도 세침흡인 검사물로 세포학적 진단이 가능할 뿐아니라, 면역세포화학 염색을 이용하면 이들 혈림프계 악성종양의 진단이 더욱 정확해 질 뿐 아니라 진단의 세분류가 가능하다고 사료된다.

Key Words : Fine needle aspiration cytology, Hematolymphoid malignancy