Utility of Fine Needle Aspiration Cytology and Immunophenotyping in the Diagnosis of Lymphoid Lesions

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Abstract

Context: Fine needle aspiration cytology, in conjunction with flow cytometry is now widely used as a reliable and accurate method for the assessment of various lymphoid lesions. Recent changes in the classification of non-Hodgkin's lymphoma emphasized cytomorphologic features and immunophenotyping in the classification of lymphomas that are both addressed in cytologic evaluation and adjunctive flow cytometry.

Objective: The objective of this study is to determine the utility of fine needle aspiration and immunophenotyping in the assessment of lymphoid lesions, and whether flow cytometry is more useful in the evaluation and sub classification of the small cell morphology group of lymphomas than of the large cell morphology group of lymphomas.

Design: A retrospective analysis of 175 fine needle aspirations in 167 patients was obtained during the period between 1995 –2000, with a histologic and/or clinical follow-up was performed. A comparison of the utility of flow cytometry in the diagnosis of small cell morphology lymphomas to large cell morphology lymphomas was performed.

Results: Flow cytometry was performed on 72 of 175 (41%) of fine needle aspiration specimens clinically suspicious of lymphoma. Excisional follow-up biopsy was obtained in 78 of 175 (44.5%) cases. Based on cytomorphologic evaluation, 82 cases (47%) were considered negative, 34 cases (19%) were considered atypical, 32 cases (18%) were positive for non-Hodgkin's lymphoma-small cell morphology, 21 cases (12%) were positive for non-Hodgkin's lymphoma-large cell morphology, 3 cases (2%) were positive

for Hodgkin's lymphoma, and 3 cases (2%) were nondiagnositic. Immunophenotyping utilizing flow cytometry was the diagnostic parameter in 28 of 32 cases (88%) of non-Hodgkin's lymphoma-small cell morphology group and in 11 of 24 cases (46 %) of the non-Hodgkin's lymphoma-large cell morphology/Hodgkin's lymphoma group.

Conclusion: Immunophenotyping by flow cytometry is more essential for the accurate evaluation and classification of small cell morphology than large cell morphology lymphoid lesions in fine needle aspiration cytology.

Introduction

Fine needle aspiration (FNA) cytology is a widely used diagnostic method for the assessment of various benign and malignant lymphoid lesions. It has been proven to be an accurate, reliable, and cost effective method of diagnosing and sub classifying malignant lymphoma. It is routinely performed as the primary diagnostic work-up for patients with lymphadenopathy, documenting recurrent disease, for staging, and for obtaining material for ancillary studies (1-12).

The recent changes in the classification of non-Hodgkin's lymphoma (NHL) emphasized the diagnostic importance of cytomorphology, immunophenotyping, and molecular findings, in addition to histology (2,7-9,10,13,15-17). Previous studies have addressed the usefulness and accuracy of immunophenotyping using flow cytometry (FC) in the FNA diagnosis of lymphoid lesions (2,13,14). There has not been an emphasis on the usefulness of FC when comparing small cell morphology and large cell morphology lymphoid neoplasia.

Material and Methods

We retrospectively reviewed the cytologic features of FNA cytology and FC results in a total of 175 FNA cases in 167 patients conducted at Scott & White Hospital, Temple, Texas, U.S.A., during the period between 1995-2000. Additionally, correlation with histologic and/or clinical follow-up was carried out. FNA cases with no histologic or clinical follow-up were excluded from our study.

All cases were diagnosed using the Revised European American Lymphoma/World Health Organization criteria by pathologists board certified in surgical pathology. Not all pathologists were board certified in cytopathology. The small cell morphology group included small cell lymphocytic, follicular (grade 1), follicular (grade 2), marginal zone, mantle cell, and small cell (not otherwise specified) lymphomas. The large cell morphology group included follicular lymphoma (grade 3), large cell lymphoma, and Hodgkin's lymphoma.

FC was performed in 72 of 175 cases (41%). FNA specimens received for FC are filtered and washed as required. A cytospin is prepared for each specimen, which is reviewed by a pathologist, prior to selecting the panel of antibodies for analyzing the cells from the FNA specimen. The above panel was tailored to the special requirements such as patient's history and/or number of viable cells available in the specimen. The instrument used was the Becton Dickinson's FACScan (BD Biosciences, San Jose, CA), 3 color staining/analysis. Approximately 0.5 million cells are allocated to each tube to which the appropriate antibodies are added. Prior to staining the kappa/lambda tubes with antibodies, these cells are washed 2-3 times with phosphate buffered saline (PBS) to remove any free floating kappa or lambda that may be present. The tubes are incubated for 15-30 minutes at room temperature and protected from light. After initial incubation, red blood cells are lysed in Becton Deckinson's FACS Lysin Solution (BD Biosciences, San Jose, CA). After a 5-10 minute room temperature incubation to lyse red cells, each tube is centrifuged to pellet the cells. The lyse solution is decanted and the cells are washed with PBS. The PBS is decanted and the cells are fixed in 0.5 ml of a 0.5%

formaldehyde fixative solution. The panel of tubes is then collected on the FACScan. Ten thousand events are collected from each tube, after a threshold is established to exclude debris. The data collected is then analyzed using Becton Dickson's Cell-Quest software (BD Biosciences, San Jose, CA) The CD45 data is used to establish all populations in the specimen by gating from a CD45 fluorescein isothiocyanate conjugated (FITC) versus side scatter (SCC) dot plot. The data from the remaining tubes (excluding propidium iodine) is analyzed by gating CD45 prep versus SCC dot Plot. Analyzing at least ten thousand ungated events derives the viability.

Results

The aspiration material was obtained either from superficial locations in139 of 175 cases (79%) or from deep locations in 36 of 175 cases (21%). An excisional follow-up biopsy was obtained in 78 of 175 cases (44.5%). Review of clinical follow-up treatment of all positive cases with FNA results with or without the follow-up biopsy was performed. FNA diagnoses are reported in (Table 1) as follows: Non diagnostic (n=3), negative (n=82), atypical (n=34), positive for NHL-small cell morphology (small cell group) (n=32), positive for NHL-large cell morphology (large cell group)(n=21), and Hodgkin's lymphoma (HL)(n=3).

The patients ranged in age from 6 to 98 years of age. There was a 1.4: 1 female: male ratio. The primary site was nodal (n=148) and extra nodal (n=27). Among the (n=175) FNAs, a history of a previous diagnosis of a malignant lymphoma was available in (n=40) cases. There were 16 of 32 in the small cell group that had a previous diagnosis of

lymphoma. There were 10 of 24 in the large cell group that had a previous diagnosis of lymphoma. Over half of the cases (29 out of 56) did not have a previous diagnosis of lymphoma. FC was performed in 72 of 175 cases (42%). It was diagnostic in 28 of 32 cases (88 %) of the small cell group and in 11of 24 cases (45 %) of the large cell/Hodgkin's group.

As reported above, a follow-up excisional biopsy was done in 78 of 175 (44.5%) of the cases and is shown in Table 2. For the small cell group (n=32), follow-up biopsy was performed in 13 cases. For the large cell group (n=21), follow-up biopsy was obtained in 5 cases. Four cases reported by FNA as negative were determined by the follow-up biopsy to be false negative FNA results. Three of the false negatives were from the large cell group and the fourth was a HL group. Most of the atypical diagnostic category cases (30 of 34) were followed by open biopsies, which revealed a positive diagnosis of malignant lymphoma in 23 of 30 cases (75%). All three cases with non-diagnostic FNA results were followed by open biopsies, two resulting in a final diagnosis of small cell group lymphoma and one resulting in a final diagnosis of negative for neoplasia.

The small cell group included 32 cases (Table3). There was a previous diagnosis of lymphoma in 16 cases. In 19 cases there was no follow-up biopsy. In six cases there had been neither a previous diagnosis of lymphoma nor follow-up biopsies and the treatment depended on FNA results. The 13 follow-up biopsies in this group confirmed the FNA diagnosis and sub classification of NHL in 12 of 13 cases as follows: small lymphocytic lymphoma (n=2), follicular lymphoma, grade 1 (n=6), follicular lymphoma, grade 2

(n=1), mantle cell lymphoma (n=3), small cell, not otherwise stated (n=1). In one case there was a discrepancy in the sub classification of the case where the FNA result was small cell group follicular lymphoma, grade 2, while the biopsy result was follicular lymphoma, grade 3.

The large cell group included 21 cases. There was follow-up biopsy in 5 of 21 cases. In all of the cases the biopsy result confirmed the FNA diagnosis. In 16 of 21 cases there was no follow-up biopsy, but there was a previous diagnosis of lymphoma in 8 of 16 cases. In 8 cases, which had neither a previous diagnosis nor follow-up biopsies, the treatment depended solely on FNA results. There were 3 cases of Hodgkin's lymphoma diagnosed by FNA cases. One of these cases had a previous diagnosis of Hodgkin's lymphoma. FNA results of Hodgkin's lymphoma in the remaining two cases were confirmed by follow-up biopsy.

The atypical included 34 cases. There was follow-up biopsy in 30 of 34 cases. The follow-up biopsies revealed the following: benign (n=7); small cell group (n=9), follicular lymphoma, grade 1 (n=3), follicular lymphoma, grade 2 (n=3), marginal zone lymphoma (n=1), small cell group, not otherwise stated (n=2); large cell group (n=8); and Hodgkin's lymphoma (n=6).

Sensitivity for NHL (with positive or atypical results considered as true positives) was 81% and sensitivity for NHL and Hodgkin's lymphoma combined was 83%. The false negative rate was 7.6%. Specificity for NHL was 100% (calculated from positive or

negative biopsies, with atypical or non-diagnostic biopsies not included). The false positive rate in our study was 0%. Accuracy for NHL in our study was 87% (Confidence Interval 76%-95%) and accuracy for NHL and Hodgkin's lymphoma combined was 87% (Confidence Interval 77%-94%).

Comments

The initial diagnosis of lymphoma by FNA is challenging and, in the past, has been considered controversial (18). The combination of FNA and immunophenotyping by FC has been increasingly accepted as a method for primary diagnosis of NHL (19). The new REAL classification places greater emphasis on individual cell morphology combined with immunophenotypic classification, whereas, earlier classification schemes emphasized histologic architecture (18). Review of the literature reveals the usefulness of this approach in studies, which have correlated histology and clinical follow-up with initial FNA diagnoses, supplemented with FC. In one study, 41 FNA samples were obtained and classified as lymphoma, with 25 cases confirmed as lymphoma on follow-up biopsy and 22 of 25 confirmed to match the sub classification. The discrepancies were due to grading in follicular lymphoma, Hodgkin's disease, and T-cell lymphoma (7). Another prospective study of image guided percutaneous biopsies for the primary diagnosis of lymphoma showed that FC was used in 10 of 43 cases (23%) and immunohistochemistry was used on core biopsies in 33 of 43 cases (77%). A diagnostic result was obtained in 37 of 43 cases (86%) and 36 of 43 cases (84%) were treated according to the diagnosis rendered (20). It is clear that FC plays a crucial role in the FNA diagnosis and sub classifying of lymphoma, especially the small cell group

(2,9,12,14,21). The utility of flow cytometry according to cell size has not been emphasized in these previous reports.

Immunophenotyping of lymphomas utilizing FC has long been recommended (18). Immunophenotyping of lymphomas can be performed in a variety of manners, including FC and immunohistochemistry of cytospin preparation from FNA material, immunochistochemistry of a cellblock preparation from FNA material or immunohistochemistry of core needle biopsies. Immunophenotyping of FNA specimens by FC and immunohistochemistry on cytospin preparations have been compared and, in the past, were felt to be equally reliable. The disadvantage of flow cytometry was felt to be the lack of direct correlation of cell morphology to the immunophenotyping results. FC was felt to have the advantages of rapid results, automation, objective statistics and precision, and multiple parameter evaluation. Cytospin preparations were felt to allow better evaluation of cell morphology with the correlation with the immunophenotype (15).

FC is very useful in the diagnosis of the small cell group with well-defined phenotypes used in their classification scheme (17). It has been reported that FC was useful and, in many instances, necessary for the diagnosis of indolent lymphomas (which are primarily a NHL with small cell morphology appearance) in 23 out of 23 cases. Two indolent small cell morphology lymphomas, that were not sub typed correctly, were diagnosed as mantle cell lymphomas, when followed-up with lymph node biopsy (16). The diagnosis of mantle cell lymphoma, based on morphology alone is known to be difficult, but recent

studies have reported that mantle cell lymphoma can be separated from chronic lymphocytic leukemia (CLL) by FC, since CD23 negative expression is seen in only mantle cell lymphoma. A small proportion (5%) of mantle cell lymphomas was reported to have an overlap with CLL and display a weak expression of CD23 (21).

Diagnosis of large cell lymphoma by flow cytometry can be difficult due to frequent apoptosis, cell fragility, necrosis, high mitotic rates, and difficulty in gating on appropriate cell populations. There is also the possibility that the lymphoma may not show a diagnostic immunoglobulin light chain restriction phenotypic expression by FC. One study showed, on review of 51 cases of large cell lymphoma with an adequate submitted sample, that 4 cases (8%) failed to show monoclonality (22). A recent study showed that flow cytometry was useful or necessary in 11 of 15 large cell morphology lymphomas and Hodgkin's lymphoma in FNA. FC was not considered necessary in HL, was considered misleading in two large B-cell lymphomas and only considered necessary in three cases of large B-cell lymphoma. They reported no false positives and 3 false negatives. Therapy for both indolent and large cell morphology lymphomas was instituted in 32 out of 36 of the patients on whom follow-up was available, based on FNA and FC only (16). Immunohistochemical stains on cytospin material, cellblock material, or core needle biopsy may be useful in special cases of large cell morphology hematolymyphoid lesions, such as Anaplastic large cell lymphoma with an immunophenotype of (CD30 +,CD15-,S100-,cytokeratin -)(23).

The drawbacks of FC only on FNA samples for lymphoma have been listed previously as loss of architectural features (such as proliferative centers in small lymphocytic lymphoma), few diagnostic cells with Hodgkin's lymphoma, lack of sufficient cells for FC without on-site evaluation of specimen as it is being acquired for determining sufficient cell viability, and sampling problems inherent to any small biopsy technique (16).

Our study demonstrates that FNA continues to be a highly sensitive technique for the diagnosis of lymphoma. It is highly definitive and specific for sub classification of lymphoma when FC is performed and considered diagnostic. All atypical diagnosis by FNA should have careful follow-up, as 68% of the atypical group in this study were eventually diagnosed as lymphoma. Review of clinical follow-up confirmed that all 20 cases of positive FNA results for neoplasia with follow-up biopsy, which were positive for neoplasia, would have received the same treatment for their hematolymphoid neoplasia if only the FNA diagnosis had been used (As performed by Dr. Mark H. Holguin, Division of Hematology/Oncology). In 14 of 26 positive cases of NHL, the FNA was the only material available to make the primary diagnosis and provide treatment for the patient. This corresponds with previous reports of FC becoming accepted as the definitive diagnosis of lymphoma (6). FC is essential for the accurate evaluation of small cell morphology lymphoma with FC being the diagnostic parameter in 28 of 32 cases (88%). FC is useful for large cell morphology/Hodgkin's lymphoma being the diagnostic parameter in 11 of 24 case (46%), but additional studies (immunohistochemistry on cellblock, cytospins, or core needle and molecular biology)

may be required to increase the diagnostic accuracy of this group. This may require an on-site evaluation of the material at the time of procurement to triage the material efficiently. Whenever possible, preparation of cytospins from FNA material for immunostaining would be helpful in supporting the cytomorphologic diagnosis in cases when flow cytometry is not definitively diagnostic.

Additionally, FNA results of lymphoid lesions, as in all body sites, should be interpreted in correlation with the clinical findings. This is demonstrated by the performance of follow-up excisional biopsies in four cases reported as negative on FNA. Sampling error and/or partially involved lymph nodes may be a contributing factor to false negative results.

Acknowledgement

A debt of gratitude is owed to Wayne Bryant (senior flow tech) (MT, ASCP) for his technical assistance in flow cytometry.

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Table 1. Fine-needle aspiration (FNA) diagnosis by flow cytometry and/or cytomorphology.

FNA Diagnosis Categories	Number of Cases
Non-diagnostic	3 (2%)
Negative	82 (47%)
Atypical	34 (19%)
Positive Small cell	32 (18%)
Positive Large cell	21 (12%)
Hodgkin's Disease	3 (2%)
Total	175 (100%)

Table 2. Follow-up biopsy results of previous fine needle aspiration (FNA) diagnoses.

	Follow-up Biopsy Diagnosis	Not Done	Negative	Atypical	Small Cell morphology lymphoma Positive Low Grade	Large Cell morphology lymphoma Positive High Grade	Hodgkin's Lymphoma
FNA Diagnosis							
Non-diagnostic		0 (0%)	1 (33%)	0 (0%)	2 (67%)	0 (0%)	0 (0%)
Negative		57 (70%)	18 (22%)	3 (4%)	0 (0%)	3 (4%)	1 (1%)
Atypical		4 (12%)	7 (21%)	0 (0%)	9 (26%)	8 (24%)	6 (18%)
Positive Small Cell Morphology Lymphoma		19 (58%)	0 (0%)	0 (0%)	13 (39%)*	0 (0%)	0 (0%)
Positive Large Cell Morphology Lymphoma		16 (76%)	0 (0%)	0 (0%)	0 (0%)	5 (24%)	0 (0%)
Hodgkin's Disease		1 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (67%)

*One case of follicular lymphoma (Grade 2) by FNA was upgraded to follicular lymphoma (Grade 3) on the larger biopsy, due to sampling differences.

Table 3. Fine needle aspiration (FNA) diagnosis by flow cytometry (FC) and cytomorphology.

FNA Diagnostic Categories	**FC Positive**	**FC Negative**	**FC Not Performed**
Non-diagnostic	0 (0%)	1 (33%)	2 (67%)
Negative	0 (0%)	10 (12%)	72 (88%)
Atypical	0 (0%)	18 (53%)	16 (47%)
Positive Small Cell Morphology Lymphoma	28 (88%)	1 (3%)	3 (9%)
Positive Large Cell Morphology Lymphoma	11 (52%)	3 (14%)	7 (33%)
Hodgkin's Lymphoma	0 (0%)	0 (0%)	3 (100%)