Article

The Utility of Flow Cytometry in the Diagnosis of Pulmonary Hematolymphoid Neoplasms: Single Institution Experience

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Abstract: Multiparameter flow cytometry is a great tool for rapid identification and immunophenotypic characterization of hematolymphoid disease. This study aims to define the diagnostic utility of flow cytometric analysis in hematolymphoid disease of the lung and pleural space. Cases were retrospectively identified based on diagnosis with hematolymphoid malignancy including leukemias and non-Hodgkin lymphomas, and non-hematolymphoid entities. Pathology slides were reviewed. Flow cytometry list mode data were reanalyzed. Among 510 cases, 124 cases are positive by flow cytometry, of which 123 cases are pulmonary hematolymphoid malignancies including 53 newly diagnosed cases. The newly diagnosed cases include 17 pleural fluids, 34 tissue biopsies, and 2 bronchoalveolar lavages and are classified as either primary (2/53, or 4%) or secondary (51/53, or 96%). For the diagnosis of pulmonary hematolymphoid malignancy, the flow cytometry assay has a sensitivity of 97.6%, and specificity of 99.7%. In conclusions, flow cytometry is an essential diagnostic tool for rapid detection and categorization of pulmonary lymphoid neoplasms. Flow cytometry can improve diagnostic certainty for hematolymphoid malignancy in the pulmonary location.

Keywords: Flow cytometry, hematolymphoid malignancy, immunophenotype, diagnosis

Introduction

The lung can be involved by hematolymphoid malignancy, often by direct spread from adjacent structures, or as a manifestation of disseminated disease. Lymphoproliferative disorders detected as primary lesions in the lung are rare, representing only 0.3% of all primary pulmonary malignancies, 1.0% of all the cases of non-Hodgkin lymphoma and 3.0Ð4.0% of all extra nodal manifestations of non-Hodgkin lymphoma [1-5].

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Flow cytometry is a proven powerful tool in the pathological diagnosis of hematolymphoid disease. It can routinely detect very small populations of malignant cells. Multiparameter flow cytometry has become an essential diagnostic tool for rapid detection and categorization of lymphoid neoplasms. In this regard, the primary roles of flow cytometry are to assign lineage to cell populations, distinguish between reactive and neoplastic cell populations, and establish a differential diagnosis for neoplastic populations based on their immunophenotypic features. Lineage determination is aided through identification of lineage specific and/or associated markers, e.g., CD19 for B-lineage cells and CD3 for T-lineage cells. Distinguishing reactive from neoplastic popu-

lations is accomplished through recognizing clonality and/or aberrant patterns of marker expression in the abnormal cells [6]. In the current study, we aim to assess the utility of flow cytometry in the diagnosis of patients with pulmonary hematolymphoid malignancy in our institute.

Materials and Methods

The Health Insurance Portability and Accountability Act (HIPAA) waiver of individual authorization was obtained from the University of Texas Southwestern Medical Center Institutional Review Board (Protocol No. STU 122013-023). We retrospectively reviewed the flow cytometry analyses of pulmonary specimens from our institutional database between 2016-2019. The specimens are comprised of pleural fluids, bronchoalveolar lavages, bronchoscopy or CT guided fine needle aspirations (FNAs), and video-assisted thoracoscopic surgery (VATS) or surgical biopsies of lung mass. In our institution, samples are triaged for flow cytometry analysis if a hematolymphoid malignancy is suspected especially with documented clinical history. Pulmonary hematolymphoid neoplasms (PHLNs) were detected by 10-color panel flow cytometry by identification of clonal or immunophenotypic aberrant hematolymphoid populations, which is accompanied by morphologic evaluation, immunohistochemical tests, molecular tests as needed, and clinical correlation to make final diagnosis.

Pulmonary tissue samples often have low cellularity. This is particularly true of bronchoalveolar lavage samples, pleural fluid samples and fine needle aspiration samples of solid tissue. Such low cellularity only allows limited panel of flow cytometry analysis. Therefore, triage of these low cellular samples is an essential step to ensure an optimal diagnostic approach and is based on patient demographics, clinical history and disease suspicion [7] along with a brief cytologic evaluation. For an example, when B-cell lymphoma is one of the major diagnostic considerations, surface immunoglobulin light chain evaluation will be an indispensable part of the assay.

Primary pulmonary hematolymphoid neoplasms are classified if no extrapulmonary lesions were detected by clinical radiological work up at the time of diagnosis or within three months of diagnosis [4]. Lymphoproliferative diseases may also secondarily involve the lung and most cases in this study fall into this category. The application of flow cytometry in the diagnosis of CD45 negative hematolymphoid disease such as classical Hodgkin lymphoma (CHL) is technically challenging [7]. Our study does not include CHL analysis as Hodgkin cells and Reed-Sternberg cells of CHL are also often identified cytologically on a cytospin preparation and therefore not submitted for flow cytometry analysis in our institution.

Results

We retrieved 510 pulmonary specimens submitted for flow cytometry with either relevant clinical history or cytological evaluation suspicious for hematolymphoid malignancy. 123 out of 124 cases with positive flow cytometry result are positive for pulmonary hematolymphoid neoplasms (PHLNs), which include 53 new diagnosis and 70 follow-up cases of previously diagnosed lymphoproliferative disease (Figure 1). Median age of all cases was 54 years with M:F ratio of 1.05:1. The percentage of aberrant cells detected range between 0.01% to 89%.

Among the 53 specimens of newly diagnosed cases, there are 17 pleural fluids, 34 tissue biopsies, and 2 bronchoalveolar lavages. All flow cytometry positive cases were confirmed by tissue examination either concurrently or previously for cases with established diagnosis.

These newly diagnosed cases were classified as either primary (2/53, or 4%) or secondary (51/53, or 96%). These data are consistent with previous reports on the low incidence of PHLNs[1-4]. All the newly diagnosed PHLNs fall into the category of B-cell neoplasms, primary effusion lym-

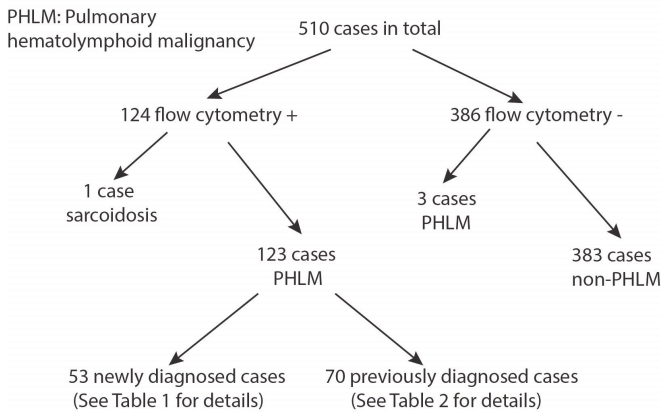

Figure 1: Cohort flow chart

phoma (PEL), plasma cell neoplasms, T-cell neoplasms, and myeloid neoplasms (Table 1). Of the B-cell neoplasms, diffuse large B-cell lymphoma (DLBCL) was the most common diagnosis (11/53 (20.8%)). Of the T-cell neoplasms, T-lymphoblastic leukemia/lymphoma is the most common entity (12/53 (22.6%). Among the 70 follow-up cases DLBCL is the most common diagnosis (16/70 (22.9%) (Table 2). We also have 386 cases with negative flow cytometry test result, among which 96 cases have hematolymphoid malignancy either in remission or concurrently.

Among all the positive cases, the percentage of aberrant cells detected ranges from 0.01% to 89%. There is only one positive case for which 0.01% aberrant cells was detected in a paucicellular pleural fluid sample. This patient has a previously established diagnosis of B-ALL, and a higher population of aberrant cells (0.44%) were detected in a following more cellular pleural fluid sample 7 days later (Table 2). In this case flow cytometry analysis was used to monitor disease progression. For the one case of chronic myeloid leukemia (CML) (Table 2), our flow cytometry detected a population of CD34+ myeloblasts that are CD7(partial +), CD13(partial +), CD36(partial +), CD38(variably +) and CD56(few +) in the pleural fluid samples of a patient with history of treatment resistant CML. The results were also confirmed by FISH or PCR to demonstrate t (9;22) translocation. This population of aberrant cells were detected in 3 consecutive pleural samples from the same patient within 17 days. There is one false positive case with a final diagnosis of sarcoidosis (Table

Table 1: Newly diagnosed hematolymphoid malignancy by flow cytometry

Hematolymphoid malignancy	Case number	Percentage
Diffuse large B cell lymphoma	11	20.8%
mediastinal large B cell lymphoma	5	9.4%
Follicular lymphoma	4	7.5%
Marginal zone lymphoma	3	5.7%
Multiple myeloma	3	5.7%
Plasmablastic lymphoma	2	3.8%
B cell lymphoma	2	3.8%
Mantle cell lymphoma	1	1.9%
Lymphoplasmacytic lymphoma	1	1.9%
Primary effusion lymphoma	1	1.9%
Chronic lymphocytic leukemia / small lymphocytic lymphoma	1	1.9%
T-lymphoblastic leukemia/lymphoma	12	22.6%
Anaplastic large cell lymphoma	1	1.9%
Angioimmunoblastic T cell lymphoma	1	1.9%
T cell leukemia/lymphoma	1	1.9%
Peripheral T cell lymphoma	1	1.9%
Mixed phenotype acute leukemia	1	1.9%
Acute myeloid leukemia	2	3.8%

Table 2: Follow-up hematolymphoid malignancy diagnosis by flow cytometry

Hematolymphoid malignancy	Case number	Percentage
Diffuse large B cell lymphoma	16	22.9%
Multiple myeloma	9	12.9%
Chronic lymphocytic leukemia /small lymphocytic lymphoma	6	8.6%
Marginal zone lymphoma	4	5.7%
B-lymphoblastic leukemia/lymphoma	4	5.7%
Follicular lymphoma	3	4.3%
Primary mediastinal large B cell lymphoma	1	1.4%
B cell lymphoma	1	1.4%
Burkitt lymphoma	1	1.4%
Mucosa associated lymphoid tissue lymphoma	1	1.4%
T cell leukemia/lymphoma	7	10.0%
T -lymphoblastic leukemia/lymphoma	6	8.6%
Acute myeloid leukemia	10	14.3%
Chronic myeloid leukemia	1	1.4%

3). For this case flow cytometry assay identified a minute population of cells of large cell size with evidence to suspect B-lineage non-Hodgkin lymphoma with plasmablastic or immunoblastic differentiation. PCR to evaluate for clonal immunoglobulin heavy chain gene rearrangement is therefore suggested. The diagnosis of sarcoidosis is eventually made in combination with morphological and clinical evaluation without performing the suggested PCR test.

Among 386 cases with negative flow cytometry results (Figure 1), 96 cases have history of hematolymphoid malignancy, 287 are proved to have other pulmonary disease later (Table 3). Flow cytometry tests play a role in helping rule out pleural or pulmonary involvement by hematolymphoid malignancy. There are 3 false negative cases. Further tests from biopsy or resection specimen showed that 3 cases have pulmonary involvement by hematolymphoid malignancy. These include one pleural specimen diffuse large B cell lymphoma case and two bronchoalveolar lavage specimens of T cell lymphoma cases. For all these 3 cases extreme pauci-cellularity has limited the ability of flow cytometry test to detect malignant cells, concurrent cytology evaluation was also negative for hematolymphoid malignancy. The flow cytometry assay has a sensitivity of 97.6%, and specificity of 99.7% (Table 3)

Discussion

With advancements in technologies such as CT scan-guided biopsy, micro coil insertion followed by video-assisted thoracoscopic surgery (VATS) resection of lung nodules, and the endobronchial ultra-

Table 3: Summary of all flow cytometry cases

Flow cytometry	HLM involving lung/pleural space	HLM not involving lung/pleural space	Other
+	123	0	1
-	3	96	287

HLM: hematolymphoid malignancy, Other: non-hematolymphoid malignancy or inflammatory diseases

sound/electromagnetic navigation bronchoscopy of peripheral lung nodules, more pulmonary biopsy specimens of high quality are available for pathological evaluation of lymphoproliferative disorders in the lung. Lymphoma, leukemia and plasma cell neoplasms display a variety of patterns of infiltration of the lung parenchyma and pleura. Non-Hodgkin lymphoma is significantly more frequent than Hodgkin lymphoma in the lung, and marginal zone lymphoma is the most frequent primary pulmonary lymphoma, all other subtypes of non-Hodgkin lymphoma may affect the lung at some point during their course [1-5].

The spectrum of entities reported here provides helpful information for the diagnosis of pulmonary hematolymphoid diseases. This demonstrates that flow cytometry is a powerful tool in aiding the diagnosis of pulmonary hematolymphoid malignancy in pulmonary samples from patient with relevant history and clinical suspicion for malignancy and played a significant role in the timely assessment of disease status. It is worth mentioning that flow cytometry is an ancillary test, not a final diagnosis. The interpretation of flow cytometry results also requires morphologic, genetic and clinical correlation.

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