



Original Article

Body cavity fluid flowcytometry in the diagnosis of haematolymphoid neoplasms

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Abstract

Introduction: Flow cytometry provides a rapid and accurate analysis of haematopoietic tumour cells in serous fluids.

Material & Methods: This is a study of body cavity fluids in which flow cytometry was used to characterize the haematopoietic cells. Period of study is from January 2012 to May 2018.

Results: 39 patients were included in the study, out of which, there were 8 ascitic fluid, 2 CSF (cerebrospinal fluid) and 29 pleural fluid samples. Among the ascitic fluid samples, most common cases were of Burkitt lymphoma. In the 2 CSF cases, one was a rare case of leptomeningeal Diffuse Large B cell lymphoma and the other was a reactive lymphoid population. Among the pleural fluids, most common was T- Lymphoblastic Lymphoma. Among the pleural fluid samples there were 4 reactive lymphocytosis (3 associated with Classical Hodgkin Lymphoma & 1 with a Primary Mediastinal Large B cell Lymphoma). The 3 benign effusions associated with Classical Hodgkin Lymphoma showed an abnormal increased CD4:CD8 ratio.

In addition to flow cytometry, in cases of DLBCL/ Burkitt lymphoma IHC markers like bcl2, bcl6, SIgM, TdT and MIB were done on cell block preparation to corroborate the diagnosis.

Conclusion: Flow cytometry helps rapidly and accurately characterize benign and malignant haematolymphoid effusions and in conjugation with immunohistochemistry on cell block preparation obviates the need for a tissue biopsy. The increased CD4 T cell subset in pleural fluid samples of Classical Hodgkin Lymphoma probably corresponds to the increase in T regulatory cells with a CD4, CD25, CD152, and FoxP3 immunophenotype which has been identified in the background T cell population of Classical Hodgkin Lymphoma.

Keywords: Body fluid, flow cytometry.

Introduction

The accumulation of fluid in a body cavity indicates local or systemic disease. Flow cytometry allows a rapid and accurate analysis of haematopoietic cells in body fluids like cerebrospinal fluid (CSF), pleural fluid and ascitic fluid. Flow cytometry in combination with immunohistochemistry on cell block preparation provides a huge advantage as it often obviates the need for a more invasive procedure like a tissue biopsy from a mediastinal mass or a bowel mass. This study is a simple retrospective analysis of our institutes experience with body fluid flow cytometry in haematopoietic neoplasms over a period of 6.5 years.

Materials and Methods

This is a retrospective study of six and a half years duration from Jan 2012 to May 2018. A total of 39 cases of body cavity fluids in which flow cytometry was the primary technique used for diagnosis were selected. In all the cases, cytomorphology was studied by Giemsa staining and the flow cytometry panel was decided based on morphology. In cases where we were undecided, between T cell versus B cell, an initial screening panel comprising CD20, CD5 and CD45 was employed before deciding on the complete panel. The body cavity fluid samples received were cerebrospinal fluid, ascitic fluid and pleural fluid.

Flow cytometry panel: The fluid sample was sent in ethylenediaminetetraacetic acid and was processed for immunophenotyping by FCM. The cells were prepared by whole bloodstain, lyse and wash technique. In samples where the amount of fluid obtained was less than 2 ml as it often happens with CSF, the wash step was omitted to avoid loss of cells. It is suspended in sheath fluid and then acquired. Six-colour immunophenotyping was performed using a FACS Verse (Becton Dickinson, San Jose, CA, USA). A minimum of 10,000 events were acquired using side scatter versus leukocyte common antigen gating. Data were analysed with Cell Questpro

software (Becton Dickinson). Fluorochromes used were Fluorescein isothiocyanate (FITC), Phycoerythrin (PE), Peridinin chlorophyll protein Complex: CY5.5 (PerCP-Cy5.5), Streptavidin-Phycoerythrin Cy7 (PE-Cy7), Allophycocyanin (APC) and Allophycocyanin H7 (APC H7. A panel of directly conjugated monoclonal antibodies, comprising of CD2,CD3,CD5, CD7,CD4,CD8, CD10,CD34,HLADR,CD19, CD20,CD13,CD33,CD117,CD64,CD11c,CD14,c yMPO,cyCD79a, cyCD3 and cyCD22 were used. In select cases, CD38, kappa& lambda were done in addition.

IHC on cell block/tissue biopsy: In all cases, the remaining sample was sent for preparing a cell block for during further IHC if required. In cases of T lymphoblastic leukaemia or reactive lymphocytosis TdT was done on the cell block or available tissue. In all cases of Burkitt lymphoma/ Diffuse Large B cell lymphoma bcl2, bcl6, SIgM, TdT and MIB 1 labeling index was done on the cell block or tissue biopsy.

Results

39 patients (19 females, 20 males) were included in the study. of the 39 cases, there were 8 asciticfluid, 2 CSF and 29 pleural fluid samples. (Table1)

Nature of sample	Malignant effusions	Benign effusions
Cerebrospinal fluid	1 primary leptomenigeal Diffuse Large B cell lymphoma	1 reactive T lymphocytosis
Ascitic Fluid	5 Burkitt lymphoma 2 Diffuse Large B cell lymphoma 1 Plasma cell myeloma	Nil
Pleural Fluid	20 T-Lymphoblastic Lymphoma 1 Mixed Phenotype Acute Lymphoma B/T. 2 Burkitt Lymphoma 1 Acute MonocyticLeukaemia(A MLM5B) 1 Adult T cell lymphoma/leukaemia	4 reactive lymphocytosis (3 associated with Classical Hodgkin Lymphoma & 1 with a Primary Mediastinal Large B cell Lymphoma).

T cell lineage lymphomas: There were twenty cases of T Lymphoblastic lymphoma with 12

cases showing predominantly similar immunophenotype CD4+,CD8+, CD2+, CD3dim+, CD7+,CD34-,HLADR-,CD10- & cyCD3+. Four cases were CD10+ and four cases were HLADR+. Two cases in addition showed aberrant expression of CD13 and one case of CD117. In all twenty cases, the patient had a mediastinal mass and there was no peripheral blood or bone marrow involvement. Of these twenty cases, in nine cases, Tdt was done on the cell block preparation and was positive in eight cases. There was 1 case of Adult T cell lymphoma/leukaemia in pleural fluid with classical CD4+, loss of CD7 immunophenotype.

Mixed phenotype: There was 1 case of Mixed phenotype Acute Lymphoblastic Lymphoma B/T which was diagnosed by flow cytometry in pleural fluid of a 24 year old doctor. He presented with complaints of exertional dyspnoea with cough and expectoration of 3 weeks duration. On examination he had left sided pleural effusion with trachea shifted to right. He had no lymph node enlargement or hepatosplenomegaly. His CT scan thorax revealed an 8.5 x 4.8 cm soft tissue density mass in prevascular region between great vessels along with enlarged right paratracheal, subcarinal, paracardiac, right and left hilar lymph nodes. Flow cytometry of his pleural fluid was done and the immunophenotype was as follows. CD4+,CD8-,CD3-,CD2+,CD5+,CD7+, HLADR+, CD19+,CD10+,CD34-,CD13-,CD33-,CD117-, CD64-, cyCD3+,cyCD79a+,cyCD22+ and cyMPO-. A mediastinal lymph node trucut biopsy of this patient was done which was positive for PAX5 and CD7 by immunohistochemistry. His peripheral smear and bone marrow was negative for blasts. This patient was started on Hyper CVAD regimen and is currently in remission as on his last follow up.

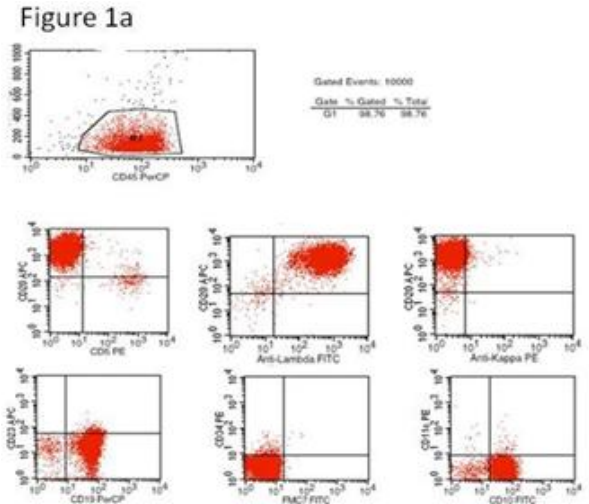
B cell lineage lymphomas: There were seven cases of Burkitt lymphoma, diagnosed on flow cytometry of ascitic fluid and pleural fluid. In the two cases there was involvement of peripheral blood and bone marrow. All cases

showed a similar immunophenotype with CD19+,CD10+,CD34-,HLADR+,TdT-,CD20 bright + with lambda light chain restriction and no aberrant marker expression. In one of the cases, there was an intestinal mass biopsy in which immunohistochemistry was done which was bcl2, bcl6+, SIgM+ with MIB1 labeling index of 100%. In two cases, immunohistochemistry was done on the bone marrow biopsy which was bcl2-, bcl6+ and SIgM+. In the remaining four cases IHC was done on the cell block in the remaining 4 cases. There were 2 cases of DLBCL diagnosed on ascitic fluid and 1 extremely unusual case of Primary Leptomeningeal Diffuse Large B cell lymphoma diagnosed on flow cytometry of CSF sample. He was a 60 year old factory worker who presented with complaints of headache, mild fever and altered sensorium of 15 days duration. On examination he was emaciated, afebrile and stuporous. Neck stiffness was present. He had no lymph node enlargement or hepatosplenomegaly. His blood counts were normal. His CT scan brain and MRI which were done from outside were reported as normal. His CSF sample showed a high count with large atypical lymphoid cells which had moderate cytoplasm, large nuclei with prominent nucleoli. Flow cytometry of his CSF sample was done which showed the following immunophenotype, CD20+,CD10+,CD19+, CD34-,TdT-, CD3-,CD2-, CD5-,CD7-,CD13-, CD33-,CD117-,HLADR-, cyMPO-,cyCD3- with lambda light chain restriction. Cell block was prepared from the remaining CSF sample which was bcl2+, bcl6- and SIgM positive. An MRI brain was repeated at our centre. Axial T1W, T2 W, diffusion and post contrast sequences of the brain, showed diffuse leptomeningeal enhancement involving bilateral cerebral and cerebellar hemispheres. Bilateral trigeminal and facial nerves are thickened with homogeneous post contrast enhancement and restricted diffusion. His CT scan neck, chest, abdomen, pelvis were normal. Taking into consideration the radiological findings, a diagnosis of Primary

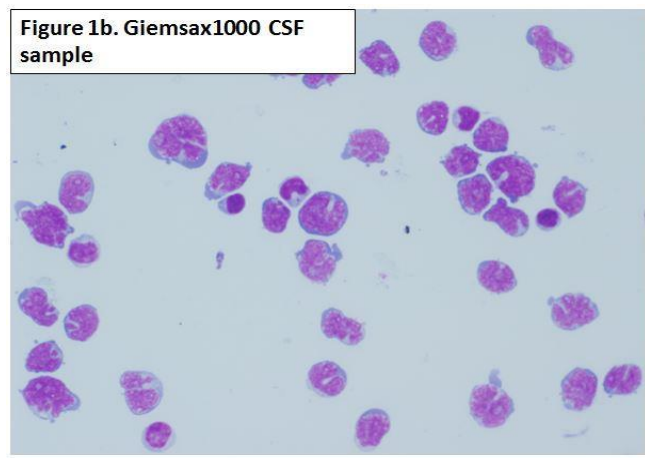
Leptomeningeal Diffuse large B cell lymphoma was arrived at.

Benign lymphocytic effusions: There were 5 cases of reactive benign lymphocytosis. 1 in CSF and 4 in pleural fluid. The CSF sample showed a reactive T cell lymphocytosis. Among the 4 pleural fluid samples, 3 were associated with Classical Hodgkin Lymphoma & 1 with a Primary Mediastinal Large B cell Lymphoma. In the one case of Primary Mediastinal Large B cell Lymphoma, the pleural fluid sample had a CD4:CD8 ratio of 1:2. The 3 benign effusions associated with Classical Hodgkin Lymphoma showed an abnormally increased CD4:CD8 ratio. 2 cases had a CD4:CD8 ratio of 7:1 while one case had a CD4:CD8 ratio of 9:1. Fig 2. Case is of a 27 year old man, who presented with dyspnoea, cough, fever of 2 months duration. On examination, he has extensive cervical lymph node enlargement. His CT chest showed a large anterior mediastinal mass with massive right pleural effusion & lung collapse. His peripheral smear & bone marrow were normal. His HIV status was negative. His pleural fluid sample showed lymphoid cells which were? malignant/benign. Flow cytometry immunophenotype favoured a benign effusion (CD2+,CD3+,CD5+, CD7+, HLADR-,CD10-,CD34-, Tdt- with no aberrant antigen expression) ,except for an abnormal CD4:CD8 ratio of 7:1. The same day, FNAC of his cervical lymph node was done which showed Classical HRS cells. The lymph node biopsy was reported as Mixed Cellularity Classical Hodgkin Lymphoma (HRS cells were CD30+,CD15+,CD20-, PAX5 weak+) .The abnormal CD4:CD8 ratio seen in the pleural fluid probably represents the background activated CD4+ T cell population.

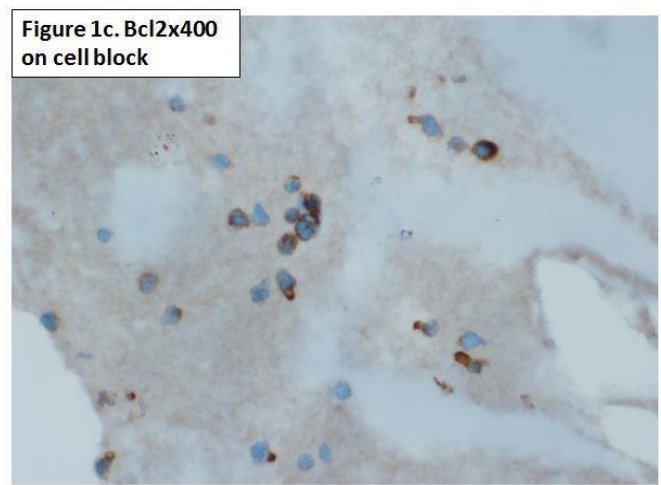
Figure 1 Case of Primary Diffuse Leptomeningeal Lymphoma



1a Flow cytometric dot plots showing tumour cells are CD20 +, CD10+ with lambda light chain restriction.

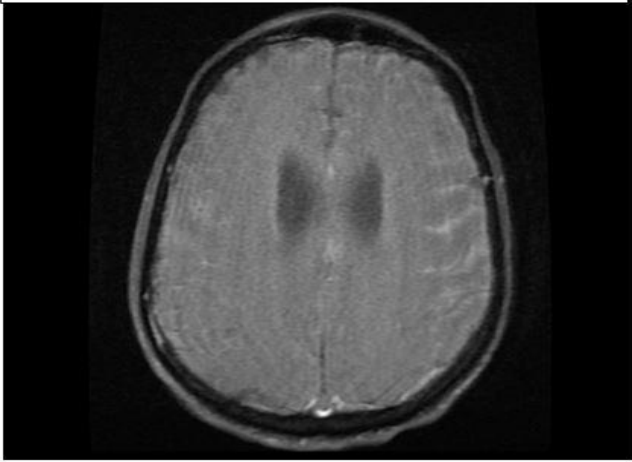


1b. Giemsa 1000 Tumour cells have moderate cytoplasm, large nuclei with prominent nucleoli



1c. bcl2x400 Cell block preparation with tumour cells showing bcl2 positivity.

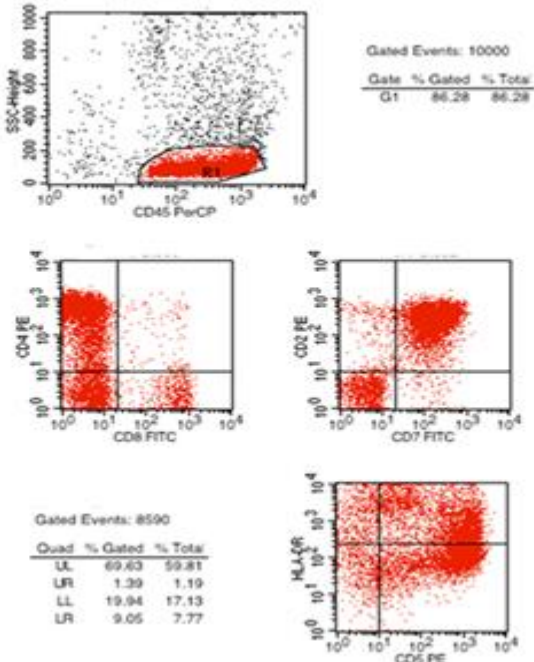
Figure 1d. MRI showing diffuse leptomenigeal enhancement



1d. Axial T1W, T2 W, Diffusion and post contrast sequences of the brain, show: Diffuse leptomenigeal enhancement involving bilateral cerebral and cerebellar hemisphere. Bilateral trigeminal and facial nerves are thickened with homogeneous post contrast enhancement and restricted diffusion.

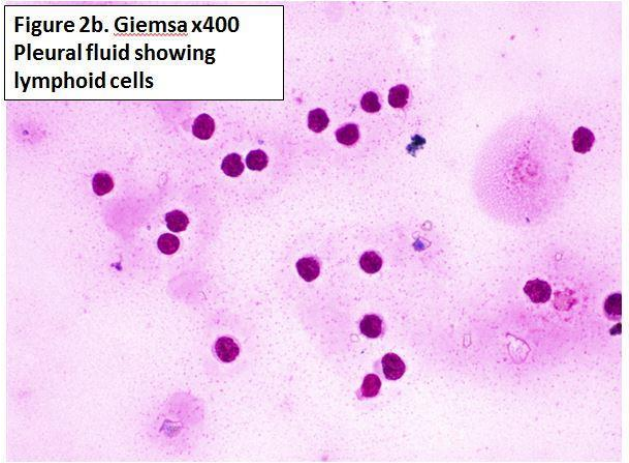
Figure 2 Case of Classical Hodgkin Lymphoma with pleural fluid showing lymphocytosis with increased CD4:CD8 ratio.

Figure 2a



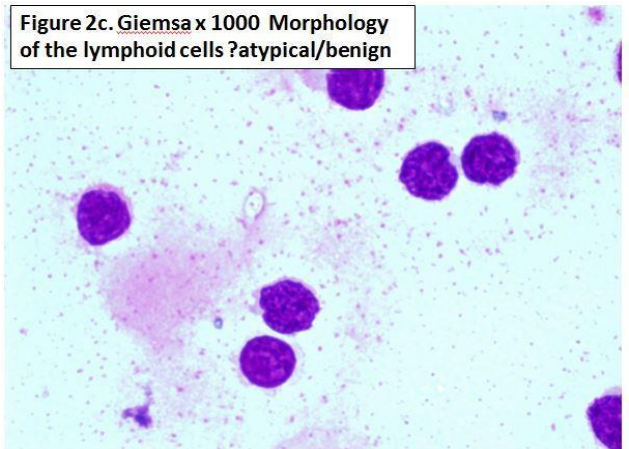
2a. Flow cytometric dot plots showing increased CD4:CD8 ratio of 9:1.

Figure 2b. Giemsa x400 Pleural fluid showing lymphoid cells



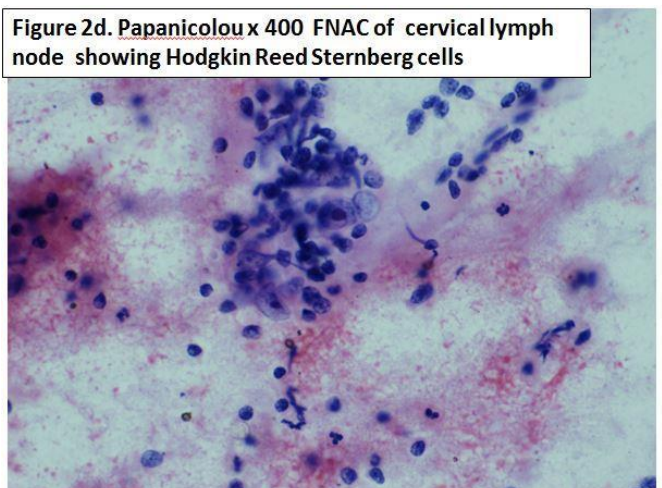
2b Giemsa x400 Pleural fluid showing lymphoid cells

Figure 2c. Giemsa x 1000 Morphology of the lymphoid cells?atypical/benign



2c Giemsa x1000 Lymphoid cells?atypical/benign

Figure 2d. Papanicolou x 400 FNAC of cervical lymph node showing Hodgkin Reed Sternberg cells



2d Papanicoloux400 Hodgkin Reed Sternberg cells seen in fine needle aspiration cytology of lymph node

Discussion

Potential pitfalls: In fluids, care has to be taken not to rely heavily on the morphology of cells as

they can appear to be more pleomorphic than they actually are.

Malignant lymphoid effusions: Among the 35 malignant effusions, two stand out for their unusual nature and rare occurrence.

1. **Mixed phenotype Acute Lymphoblastic Lymphoma B/T-** is a very rare subtype of Acute Leukaemia/Lymphoma. This case is rare and unusual because the patient had no evidence of peripheral blood or bone marrow involvement at the time of presentation or throughout the course of the disease. Mixed Phenotype Acute Leukaemia comprises 2-5% of all acute leukemias^[1]. The 2008 World Health Organisation classification established strict criteria for diagnosis of mixed phenotype acute leukemia, emphasizing myeloperoxidase for myeloid lineage, cytoplasmic CD3 for T lineage and CD 19 with other B markers for B lineage assignment^[1, 2]. MPAL are associated with poor outcome as compared to other acute leukemias and clinically presents challenges in diagnosis and treatment^[3, 4]. However, the true incidence is difficult to establish due to problems with definition, inter-laboratory variations and non-availability of flow cytometry in most laboratories.

2. **Primary Leptomeningeal Diffuse Large B cell lymphoma** is a known, exceedingly rare subtype of Primary Central Nervous System Lymphoma (PCNSL), and it represents less than 0.1% of all Non-Hodgkin's Lymphomas (NHL)^[5]. PLML is primarily a disease that originates from the meninges without any brain or systemic extension and/or involvement. It usually presents with nonspecific neurologic symptoms and signs such as headache, meningeal signs, and cranial nerve involvement with poor prognosis^[6]. In our case, no evidence of parenchymal central nervous system or systemic tumor was identified either at the time of presentation. (Figure 1) This unusual form of neurologic lymphoma must be

differentiated from the more common clinical situations of primary parenchymal lymphoma with meningeal involvement and systemic lymphoma complicated by lymphomatous meningitis.

Benign lymphocytic effusions These benign lymphocytic effusions can be encountered as a response to solid tumors or as a reactive response in inflammatory and reactive conditions. In majority of cases, a reactive T cell population is seen regardless of underlying pathology. Normally, in the pleural fluid, the proportion of CD4+ T cells and CD8 + T cells roughly reflect that seen in the peripheral blood which is 2:1. CD8+ T cells with an activated immunophenotype, HLADR+, CD57+, CD25+ and CD28- are common in pleural reactions. In cases of sarcoidosis and tuberculosis, the CD4: CD8 ratio is raised and can go upto 10:1 or 20:1, reflecting the protective role of CD4+ T helper lymphocytes against mycobacterial infections^(7,8). Classical Hodgkin lymphoma is a B cell neoplasm where the neoplastic population represents less than 1% and frequently less than 0.01% of the total cells^[1]. The neoplastic population, referred to as Hodgkin or Reed-Sternberg (HRS) cells, can often be recognized morphologically by large cell size, sometimes multilobated nuclei, and characteristically prominent nucleoli. Until recently, the diagnosis relied exclusively on the morphologic appearance of the tumor in fixed paraffin-embedded tissue sections supplemented by immunohistochemistry. Recently, a reliable flow cytometric assay for direct detection and isolation of the HRS cells in this disease has been developed. The increased CD4 T cell subset observed in three pleural fluid samples of Classical Hodgkin Lymphoma in our study probably corresponds to the increase in T regulatory cells with a CD4, CD25, CD152, and FoxP3 immunophenotype which has been identified in the background T cell population of Classical Hodgkin Lymphoma.⁽⁹⁾ (Figure 2)

Conclusion

The relatively rapid analytical time makes flow cytometry an attractive initial diagnostic modality. This analysis can guide further morphologic and molecular workup leading to savings in cost and time of the total evaluation. In many cases, the immunophenotype obtained by flow cytometry alone can be diagnostic for specific hematologic neoplasms in a correct clinical and morphologic context. Because of the ability to analyze a large number of cells, flow cytometry is ideally suited for the detection of relatively rare, antigenically distinct populations within complex cell mixtures. Though immunohistochemistry remains the gold standard of lymphoma diagnosis, it is a tool which may involve difficulties, related to inadequate samples, differential diagnostic problems, or critical delays, before the definitive diagnosis is reached.⁽¹⁰⁾ Through the cases described, we have tried to highlight the potential of Flow cytometry, combined with morphology and immunohistochemistry on cell block specimens, in select cases, as a valuable, non invasive, informative tool for the early assessment of lymphomas. We recommend that it should be considered as an initial test in all undiagnosed cases of atypical haematolymphoid cells in pleural effusion, in order to assess the possibility of an underlying hematolymphoid neoplasm.

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