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**Case Report**

# Role of flowcytometry in atypical lymphocytosis

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## ABSTRACT

Infectious mononucleosis (IM) is a benign condition defined by lymphomononuclear cell proliferation. Symptoms range from a vague constitutional syndrome to fever, rash, jaundice, hepatosplenomegaly, lymphadenopathy, and in rare cases, autoimmune hemolytic anemia. The viruses responsible for this syndrome are EBV (the most prevalent), CMV, HIV, Hepatitis virus, Adenovirus, and others. Because the virus mostly impacts lymphomononuclear cells and the reticuloendothelial system, it may trigger intense lymphoproliferation, leading to uncommon manifestations in peripheral smear. These can be misdiagnoses as leukemia/lymphoma. The clinical profile of a brief history, mild organomegaly, and peripheral smear morphology commonly overlaps. This pitfall in the diagnosis can lead to ineffective treatment. Immunophenotyping by flowcytometry or immunohistochemistry, a mono-spot test, and specific viral ag/ab assays are required for a correct diagnosis. Flowcytometry profiles are not always adequate to rule out neoplastic proliferations. We provide a case study of a young teenage boy who arrived with an abrupt history of low-grade fever, hepatosplenomegaly, cervical lymphadenopathy, elevated leukocyte count, and atypical lymphoid cells on peripheral smear. What was previously thought to be a hematological malignancy was discovered to be a self-limiting acute IM (CMV caused), and the patient was discharged after a brief course of treatment.

**Keywords:** Acute IM, CMV, Immunophenotyping, T-lymphoproliferative disorder

## INTRODUCTION

Infectious mononucleosis (IM) is a disease that affects teenagers and young adults. Symptoms range from a vague constitutional syndrome to fever, rash, jaundice, hepatosplenomegaly, lymphadenopathy, and, in rare cases, autoimmune hemolytic anemia.[1] In most cases, the sickness is self-limiting. The viruses involved in this syndrome include EBV (the most prevalent), CMV, HIV, Hepatitis virus, HHV 6, adenovirus, and others. Because the virus primarily affects lymphomononuclear cells and the reticuloendothelial system, it can cause intense lymphoproliferation with unusual forms in a few cases, which can be misdiagnosed as leukemia/ lymphoma.[2] The clinical profile of a brief history, mild organomegaly, and peripheral smear morphology frequently overlaps. In rare situations, this very common error might lead to excessive inquiries and incorrect treatment.

Absolute lymphocytosis on a peripheral smear with a variable high leukocyte count is typically the defining feature. Atypical lymphocytes, also known as Downey-type cells, make up at least 10% of the total lymphocyte population.[3] These cells resemble neoplastic atypical lymphoid cells in appearance. It is typically challenging to distinguish this proliferation from neoplastic T-lymphoproliferative diseases, particularly hepatosplenic lymphoma, because it primarily involves T cells. Immunophenotyping, mono-spot tests and particular viral ag/ab tests should be performed to get the proper diagnosis clinical presentation. This will prevent incorrect labeling or additional invasive testing such as bone marrow examination or lymph node biopsy.[4] The case report of a young teenage boy who had a low-grade fever, hepatosplenomegaly, cervical lymphadenopathy, elevated leukocyte count, and unusual lymphoid cells on a peripheral smear is presented here. After a brief period of

treatment, the patient who was diagnosed with a hematological malignancy had been discharged from the hospital as the condition had been identified to be a self-limiting acute IM.

## CASE REPORT

A 17-year-old boy presented with short history of generalized weakness, low appetite, headache for past 10 days. He gave history of low-grade fever. On clinical examination he had cervical lymphadenopathy level II, III (largest measuring 1.5 cm) and mild hepatosplenomegaly. Rest of general and systemic examination was within normal limits. Complete blood count revealed WBC count of 26×109/L (which rose to 32×109/L the next day) hemoglobin 13.6 gm/dL and platelet count 305×109/L and 75% lymphoid cells on differential count. Peripheral blood smear morphology showed absolute lymphocytosis with 65% atypical lymphoid cells mimicking neoplastic lymphoblasts/ lymphoma cells. The cells were large with 4-6 times the diameter of adjacent small lymphocytes and had a moderate amount of deep to pale blue cytoplasm with large nuclei and few showing visible nucleoli and cytoplasmic granules (Figure 1).

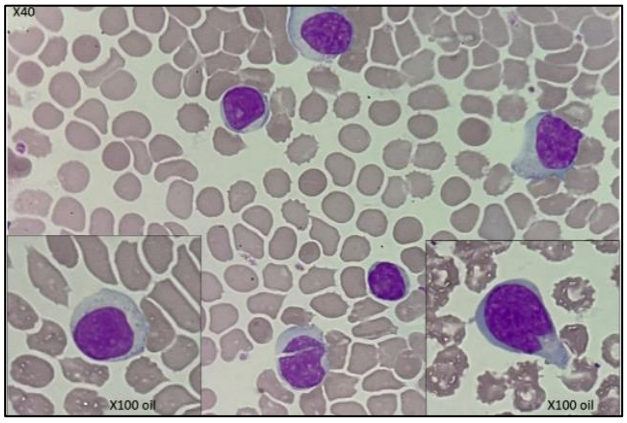


**Figure 1: Leishman-stained smears shows lymphocytosis with large atypical lymphocytes (x40) inset shows blastoid appearance of few lymphocytes (x100 oil).**

Biochemistry investigation showed urea-10 mg/dl, creatinine-0.77 mg/dl, uric acid-8.5 mg/dl, total bilirubin-0.45 mg/dl, total proteins-7.9 gm/dl, albumin-4.1 gm/dl, AST-360 IU/L, ALT-274 IU/L and LDH-827 IU/L. With such acute clinical history and high leukocyte count and deranged biochemical profile, the patient was referred to our center as a case of hematolymphoid malignancy. At our center after repeating basic investigations, we decided to go ahead with Immunophenotyping to characterize the atypical cells encountered in peripheral smears. Flowcytometry was performed on Navios Ex 10 colour flow cytometer (Beckman Coulter, USA) and analysis was done using Kaluza software on CD45/SSC plot. Peripheral blood cells were stained with a selected panel of antibodies customized to rule out possible leukemia or lymphoma. Technique used was stain-lyse- wash technique.[5] Majority (99%) of gated lymphoid cells showed strong but variable sCD3 expression, out of which 8% cells were CD4 and 85% cells were CD8 T cells (reversed CD4 /CD8 ratio, 1:8, normal CD 4:8 ratio-2:1) indicating proliferation of cytotoxic CD8 T cells. These cells on further analysis showed strong CD5, HLA-DR, cyto CD3, TCR alpha/beta as well as CD38 expression (Figure 2). The cells were negative for CD10, CD19, CD20, CD34, CD56, CD64, CD117, CD13, CD14, CD33, CD41, cyto MPO, cyto CD79a, CD11c, TCR gamma/delta*. We identified 6% of double negative cells of TCR alpha/beta/gamma/delta. We did not have facility for identifying V-beta TCR gene rearrangement on flow or by PCR. Considering preserved clinical profile and above immunophenotype, we suggested work up for EBV/CMV serology and heterophile Ab test to rule out IM. The test detected significantly high titer of IgM antibodies against the cytomegalovirus (IgM index-5.38 AU/mL {normal<1.00}, IgG-0.70 AU/mL {normal<6.00}) which confirmed the diagnosis of CMV induced IM. Patient was given supportive treatment and recovered completely after a week. Leukocyte count normalized after about 2-3 week with normalization of liver enzymes and uric acid.

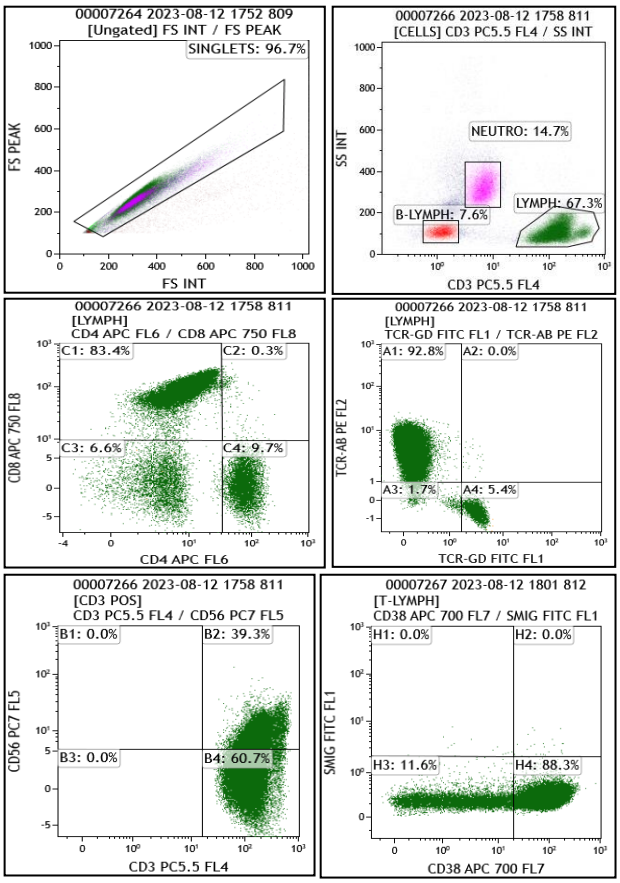


**Figure 2: Flow plots showing 67.6% CD 3 positive lymphocytes which show reversed CD 4: CD 8 ratio in peripheral blood suggestive of marked cytotoxic T cell hyperplasia secondary to CMV virus. A small population (6.6%) of double negative T cells also noted secondary to reactive T lymphocytosis.**

## DISCUSSION

Acute CMV infection causes a strong CD8 T cell hyperplasia, which is thought to limit/control for viral replication and further for latency establishment.[5,6] According to Callan et al the majority of CMV-specific T lymphocytes have an activated/memory phenotype and hence exhibit activation markers such as HLA-DR and CD38.[7] This expansion occurs during the latent subclinical period, allowing for clonotypic amplification and selection. Increased numbers of CD8 cytotoxic-suppressor T cells have also been observed in various viremias, including HIV, EBV, and hepatitis C.[8] Since, clinical profile of patients usually overlap with lymphoproliferative disorder, characterizing these cells on flowcytometry is essential. To differentiate from neoplastic T cell proliferations some markers establishing a mature profile and monoclonality are useful. Immaturity markers like CD34, Tdt, CD 1a, CD99 helps ruling out acute leukemia (T-ALL). T lymphomas, on the other hand, are difficult to distinguish due to a lack of accurate monoclonality markers. Further Flowcytometry V beta TCR gene marker and TRBC profiling are being studied to establish clonality. TCR gene re-arrangement using PCR is possible, however it may result in a perplexing monotypic profile in a few cases of IM.[9] In a rare circumstance, loss of any T-specific marker (CD3, CD5, CD2, CD7) may be beneficial. T-cell antigenic aberrancy, like nonneoplastic T-cell diseases, should be read with caution. CD7 expression on T-cells can be down-regulated or absent in a range of reactive diseases, including inflammatory dermatoses and rheumatoid arthritis.[9] T cell clones are found in many cases of acute IM and other viremias. The fact that peripheral T cell neoplasms frequently test negative for the activation antigen HLA-DR, which can provide clue to the right diagnosis, it may be heterogeneously expressed in variety of T-cell lymphoproliferative diseases.[10] If the HLA-DR T-cell population exhibits antigenic downregulation of CD7 and CD5, it is critical to connect these immunophenotyping findings with the findings of peripheral blood smears and suitable viral serologic markers to rule out the likelihood of IM.

## CONCLUSION

The patients of IM many times may not require any specific treatment, however diagnosis if not made in time may lead to unnecessary investigations and stress to patient. Thus, careful clinicopathological correlation is warranted in the interpretation of immunophenotyping and clonality data in T cell proliferation in association with IM to avoid erroneous diagnosis of neoplastic T-cell disorders and hence exposure to hazardous as well as expensive chemotherapy.

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