

# Early Diagnosis of an Aggressive Childhood Malignancy with Flow-cytometric Immunophenotyping: A Case Report

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## Abstract

Serous effusions may occur as complications in different types of lymphomas. Among these, pleural effusions are commonly observed; however, involvement of peritoneal or pericardial cavity is rare. Different diagnostic modalities like cytological examination, immunocytochemistry, cytogenetics have been used to diagnose various malignancy, thereby attaining a fast and accurate diagnosis. As effusion in lymphoma is associated with poor outcome, an early diagnosis is very much important. We describe a case of aggressive Non-Hodgkin lymphoma, i.e., Burkitt lymphoma in a previously healthy 7-year-old male child, where the diagnostic ascitic & pleural fluid cytomorphology in conjunction with flow-cytometric immuno-phenotypic analysis of the effusion helped in clinching an early diagnosis.

**Keywords:** Burkitt lymphoma; FNAC, Flow-cytometry; Immuno-competent host; Serous effusion

## Introduction

Burkitt lymphoma (BL) is a highly aggressive tumor with a rapid cell turnover. It is a rare B-cell lymphoma with varied clinical presentations.<sup>[1,2]</sup> This tumor was first reported in Africans having strong association with Epstein-Barr virus (EBV) in its causation. It accounts for only 0.76% of solid malignant tumors in Indian children.<sup>[1-3]</sup> Though it is the fastest growing aggressive lymphoma, it is potentially curable with highly intensive multidrug chemotherapy along with central nervous system prophylaxis. The three distinct forms of BL, i.e., endemic, sporadic and immunodeficiency-associated have different etiologies, presentations and prognosis. Though, a pleural effusion is commonly noted in these patients, rarely peritoneal & pericardial fluid can be the initial presentation along with abdominal mass.<sup>[1-5]</sup>

Here in, we report a rare case of BL in an immunocompetent previously healthy 7-year-old child, who presented with moderate ascites followed by bilateral pleural effusion. On further investigations, cervical lymph node and a large conglomerate of intrabdominal & retroperitoneal nodes were noted on ultrasound and contrast enhanced computed tomography (CECT). A diagnostic fluid cytomorphology along with application of flow-cytometry on ascitic fluid helped in making an early diagnosis of Burkitt lymphoma which guided to an immediate intervention in the patient. The patient was stable till the last follow up.

## Case Report

A 7-year-old male child presented to hospital with complaints of swelling on the right side of neck for last one month along

with pain abdomen, fever and abdominal distension for last 15 days. The patient was apparently well one month back when the parents of child noted a painless swelling on right side of neck which progressively increased in size reaching up to 2.5 cm × 2.5 cm. Fine needle aspiration cytology (FNAC) done outside was reported as reactive lymphoid hyperplasia. Ultrasound abdomen showed evidence of bilateral pleural effusion with basal lung atelectasis and diffusely bulky and edematous pancreas suggestive of pancreatitis.

However; the child suddenly started having dull aching pain in the epigastric region for last 15 days along with mild fever and increasing neck swelling following which he was admitted to our hospital. There was no history of jaundice, puffiness of face, hematuria, oliguria, anuria, etc. The condition of child started deteriorating within a day after admission with increased abdominal pain & distension along with high grade fever, pedal edema and fast breathing. He also had few episodes of loose stools with greenish sticky mucus.

There was no history of similar illness, any hospitalization, bleeding disease, recurrent infection, blood transfusion, leukemia, etc. A repeat ultrasound abdomen showed moderate ascites with thick echogenic debris and omental thickening. Further, CECT abdomen showed conglomerating soft tissue

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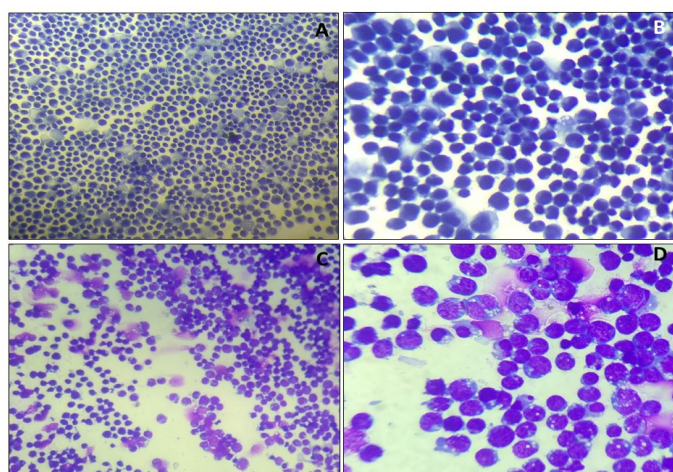
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density predominantly in retroperitoneal, peripancreatic, aortocaval, preaortic, left paraaortic and mesenteric region causing displacement of nearby major arteries like celiac, superior mesenteric arteries, etc. Also noted was moderate ascites & bilateral pleural effusion causing passive atelectasis of lungs with ill-defined hypodense areas in splenic parenchyma.

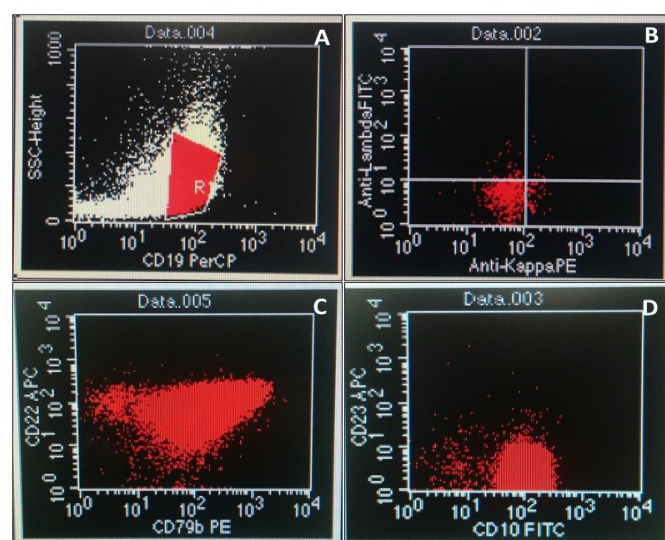
His serum amylase and serum lipase were markedly raised. However, his complete blood counts and other biochemical parameters were within normal limits. The patient was non-reactive to HIV-I and II and showed negative mantoux test.

The patient was managed aggressively with appropriate antibiotics, steroids, continuous pulmonary airway pressure (CPAP), etc., and subsequently his ascitic fluid and pleural fluid were sent for cytological examination. Papanicolaou and giemsa stained smears were highly cellular and showed discretely lying atypical lymphoid cells which were intermediate in size with granular chromatin, evident nucleoli and scant to moderate amount of dense blue cytoplasm; many of them showing cytoplasmic vacuolations [Figures 1A-1D]. The background showed mitotic figures, histiocytes and lipid vacuoles. A repeat FNAC from the cervical lymph node swelling was done which showed similar cytomorphology as noted in ascitic and pleural fluid examination. A differential diagnosis of non-hodgkin lymphoma with possibility of Burkitt lymphoma was considered based on clinico-radiological and cytological findings.

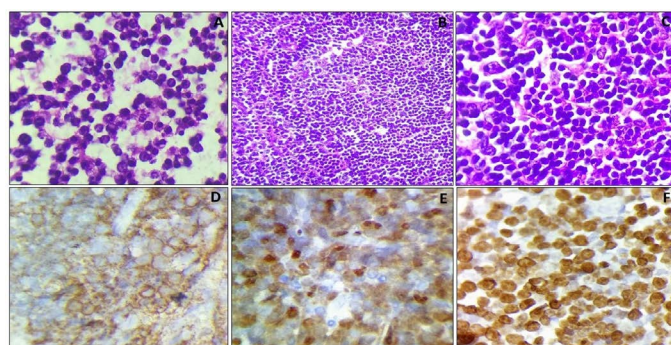
Further, flowcytometric immunophenotypic analysis of ascitic fluid was done which revealed immunoreactivity to B-cell markers, CD 19, CD79b, CD22; germinal centre marker, CD10 [Figures 2A-2D]. Cell block from ascitic and pleural fluid was also made which showed cellular tumor comprising of malignant cells as described above along with interspersed tingible body macrophages [Figure 3A]. An early diagnosis of Burkitt Lymphoma was given based on effusion cytology and flowcytometric immunophenotypic analysis; thereby helping the clinicians in initiating the specific chemotherapy. Subsequently, lymph node biopsy was also sent which showed similar features



**Figure 1:** Highly cellular smears showing intermediate sized atypical lymphoid cells with granular chromatin, evident nucleoli and scant to moderate amount of dense blue cytoplasm; many of them showing cytoplasmic vacuolations [Papanicolaou, A- 100x, B- 200x; Giemsa, C-100x, D-400x].



**Figure 2:** Flow-cytometric immunophenotypic analysis of ascitic fluid from the patients revealing immunoreactivity for CD 19, kappa-restriction, CD22, CD79b, CD10.



**Figure 3:** Cell block section showing the tumor cells [H&E, 200x]; B&C-Section from lymph node biopsy showing evidence of atypical lymphoid population with interspersed tingible body macrophages [H&E, A- 100x & 200x]; D-F: IHC images showing positivity for CD20, Bcl6 & Ki67 (98-100%), respectively.

and IHC panel showed positivity for CD10, CD20, bcl6, Ki67 (98-100%) [Figures 3B-3F]. Bone marrow aspiration and biopsy did not show evidence of non-hodgkin lymphoma.

A final diagnosis of Burkitt lymphoma was made based on the clinico-radiological and cyto-histological findings in conjunction with flowcytometric immunophenotypic analysis and immunohistochemistry. This comprehensive and systematic approach helped in making an early diagnosis and providing an immediate treatment to the patient.

## Discussion

Serous effusions occur in different underlying pathologies like malignancies, tuberculosis, renal disease.<sup>[1]</sup> In a study by Johnston et al. among 584 patients with serous effusion, 15% had underlying lymphoma.<sup>[2]</sup> Usually, T-cell lymphoma was associated with serous effusion while only rare B-cell lymphoma developed pleural or peritoneal involvement.<sup>[1-5]</sup>

The pathogenesis behind the serous effusion in lymphoma includes impaired lymphatic drainage due to the obstruction in mediastinal lymph nodes or thoracic duct, venous obstruction, pulmonary infection, radiotherapy, or involvement of pleura or

peritoneum by the tumor.<sup>[2-6]</sup> Rarely, the lymphoma cells may block the lymph tunnels thereby causing obstruction and further impairment of these channels. These fluids may be studied to look for presence of malignant cells. The combined approach of different diagnostic modalities like cytological examination of fluid in conjunction with immunocytochemistry and flow-cytometry aid in obtaining a fast and accurate diagnosis. Serous effusion in lymphoma is generally associated with adverse prognosis.<sup>[5-8]</sup> In the present case, the child presented with ascites and pleural effusion along with abdominal and cervical lymphadenopathy. Cytological examination of fluid and flow-cytometry aided in an early diagnosis of Burkitt lymphoma.

Burkitt lymphoma is an aggressive B-cell lymphoma composed of intermediate sized non-cleaved, diffuse, undifferentiated malignant cells of lymphoid origin. It is the fastest growing tumor in human with doubling time of less than 24-hours. It accounts for approximately 50% of all childhood cancers; however, outside African subcontinent, it accounts for less than 2% cases of NHL.<sup>[2-4]</sup> In study by Pramanik et al.<sup>[3]</sup> in India, among 263 patients over a 10-year period, only 2 cases of BL were noted.

The role of EBV pathogenesis in its causation is not fully understood, however, it affects B-cells via C3d complement receptor CD21. Other factors like chromosomal instability, immune defects, protein deficiency, etc have also been implicated.<sup>[6-8]</sup> There are three main types of Burkitt's lymphoma. The endemic forms mainly occur in equatorial Africa. It is the most common malignancy of children in this area and is associated with chronic malaria and Epstein-Barr virus (EBV). It particularly affects the jaw, other facial bone, distal ileum, caecum, ovaries, kidney or breast. Secondly, the sporadic form occurs outside Africa. It is also associated with EBV but to a lesser degree. This type most often affects the ileocecal region and the jaw is less often involved. Immunodeficiency-associated BL is usually associated with HIV infection or the use of immunosuppressive drugs. Different staging methods are used in prognosis of BL.<sup>[9-12]</sup> However, the involvement of pleural effusion, ascites or involvement of CNS or bone marrow group it as Stage IV, as noted in our case with presence of ascites and pleural effusion.

BL cells are generally monotonous medium sized cells with round nuclei, and a basophilic cytoplasm along with vacuolations. Cytologically the cells are the small non-cleaved cells with normal germinal centres of the secondary lymphoid follicles. These cells differ from cells of lymphoblastic lymphoma cells which have intermediate sized non-convoluted nuclei with coarse chromatin and with more abundant cytoplasm.

In our case characteristic cytomorphology of BL cells were noted on fluid cytology. Effusion cytology shows uniform population of non-cohesive lymphoid cells with non-cleaved nuclei, prominent multiple nucleoli and scant to moderate basophilic cytoplasm, few showing cytoplasmic vacuolation. This case demonstrates the importance of recognizing the cytologic features of BL in serous fluid and can be an initial clue in diagnosing BL.

Cytologic diagnosis of BL along with application of use of proliferation marker on cell block made from the effusion sample can help in an early diagnosis of a high-grade lymphoma and in providing early intervention in the patient; as noted in our case. However, a definitive diagnosis requires histopathology along with other immuno-histochemical markers. This is because cytologic smears can be deceptive at times due to air-drying effect or other technical errors. But histopathology might take 2-3 days along with IHC, we can go ahead flow-cytometry to achieve an early diagnosis.

It has been noted in literature that the percentage of positive cytological findings may vary from 22.4-94.1% in cases of various non-Hodgkin lymphoma (NHL).<sup>[10-14]</sup> However, in order to achieve an accurate diagnosis, a combined approach including immunocytochemistry (ICC), flowcytometric immunophenotyping, histology and cytogenetics is required.

In the present case, the flow-cytometric panel aided in an early diagnosis with immunoreactivity for CD10, CD 19, CD79b, CD 22 along with kappa-light chain restriction. But still flow-cytometry does not help in getting the Ki67; hence we must go for Ki67 marker on cell block or biopsy for further confirmation of BL. A high proliferation rate was also noted (98-100% Ki67). Three specific translocations, t(8;14), t(8;22) and t(2;8), having in common 8q24 band involvement, are thought to be present in the overwhelming majority of cases.<sup>[5,13-14]</sup> These stereotyped translocations have been shown in many cases to be related to DNA molecular rearrangements of the immunoglobulin genes and the c-myc oncogene.<sup>[5,13-14]</sup>

Burkitt's lymphoma is a fast-growing tumour but also very sensitive to chemotherapy. However, sporadic and immunodeficiency-associated Burkitt's lymphoma can be less sensitive than the endemic variant to chemotherapy. Different regimens have been used with varied success rates. The development of brief but very intensive chemotherapy has led to a very high cure rate in children with Burkitt's lymphoma. The use of these regimens in adults, often in combination with the monoclonal antibody rituximab, can also be very effective.

While managing a patient of BL, a complication, i.e., tumor lysis syndrome should always be kept in mind which show(s) elevated levels of serum BUN and creatinine which occur due to high rate of spontaneous cell death in the disease causing potentially fatal electrolyte imbalances, like hyperkalemia, hyperuricemia, hyperphosphatemia, hypocalcemia, etc.<sup>[4,5,12-14]</sup> Allopurinol should be started at diagnosis with hydration & alkalinisation and close monitoring of electrolytes. Renal dialysis may be needed, as oliguric renal failure can also occur in case of uric acid nephropathy.

## Conclusion

To conclude, BL is a highly aggressive non-Hodgkin lymphoma with a very rapid growth rate and varied presentations. A proper cytological examination of serous fluids along with application of flow-cytometric immunophenotypic analysis aid in giving an early and accurate diagnosis in a young child, following which

an immediate, highly intensive multidrug chemotherapy can be started in these patients.

### Conflict of Interest

The authors disclose that they have no conflicts of interest.

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