Ascitic fluid cytology and flow cytometry in the primary diagnosis of lymphoma – a case report

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Abstract Primary diagnosis of lymphomas from ascitic fluid is rare. We report a case in which a patient being worked up as a case of carcinoma head of pancreas turned out to be a lymphoma on routine ascitic fluid examination and was further sub-classified as a CD 10+ B-cell lymphoma on flow cytometric analysis.

Keywords Ascitic fluid · Lymphoma · Flow cytometry · Immuno-phenotyping · Scattergrams

Introduction

Patients often develop pleural effusions and ascites in advanced cases of non-Hodgkin lymphoma (NHL). However, primary presentation of a patient with serous effusions is uncommon in a case of lymphoma.

Case report

Clinical history

This 71-year-old patient presented with complaints of pain abdomen and progressive jaundice for 3 weeks. She also developed abdominal distension 2 weeks later. On examination, she was deeply icteric and had ascites, however, no lump was palpable. Liver function tests showed a total bilirubin of 6.00 mg/dl (direct bilirubin 3.5 mg/dl), SGOT - 84 IU/L, SGPT - 52 IU/L, alkaline phosphatase - 612 IU/L and GGT -568 IU/L. CT scan showed the presence of a mass in the head of pancreas with peripancreatic lymph nodes. Ascitic fluid tap was done and the patient was taken up for endoscopic retrograde cholangiopancreatography (ERCP) for palliation of jaundice. Provisional diagnosis based on the clinical lab and imaging parameters was carcinoma head of pancreas.

Cytopathological findings

Clear pale yellow ascitic fluid was received in the clinical pathology lab for examination. Smears made from the fluid were highly cellular and showed a pleomorphic population of intermediate to large sized cells lymphoid in morphology. The cells had a high nuclear: cytoplasmic ratio, moderate amount of basophilic cytoplasm and cytoplasmic

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vacuolations with some cells showing nuclear cleaving. Features were suggestive of a large cell lymphoma (based on Working Group Classification of Lymphomas) (Fig. 1). Flow-cytometric immunophenotyping was suggested for confirming the diagnosis of a lymphoma and its further sub-grouping according to WHO classification.

Fig. 1 Smear from ascitic fluid showing large abnormal and pleomorphic lymphoid cells (Wright Giemsa – 100 X)

Peripheral smear examination did not reveal any abnormal cells. Neutrophilic leucocytosis was noted with a total leucocyte count of 18,400/ul, hemoglobin was 9.6 g/dl and platelet count was 2, 27,000/ul. However, a bone marrow examination was not done.

Flowcytometric analysis

Three-color flow cytometry was performed on Beckman Coulter Epics XL flowcytometer. Gating was done using forward-side scatter and CD45-side scatter analysis. The ascitic fluid specimen was abnormal showing a single cell cluster corresponding to the lymphocyte region on the SSC/CD45 dot plot with low SSC and moderately bright CD45. These cells comprised 95% of the cells in the sample and displayed variable FSC implying a small to medium size. The abnormal cells were monoclonal B cells which showed kappa light chain restriction and expressed dim to moderately bright CD19, moderately bright CD20 and dim CD10. Dim expression of FMC7 and CD38 was also seen. These cells lacked CD5, CD34, CD23, CD11c, CD103, CD56, HLA-DR, and lambda light chain expression. The immunophenotypic profile was, therefore diagnostic of a CD10 positive mature B-Cell lymphoma (Figs. 2 and 3).

Fig. 2 Flow cytometry scattergrams: Showing the kappa light chain restricted lymphoid cells positive for CD45, CD19 and CD10.
A: Gate on all mononuclear cells based upon forward versus side scatter
B: Gate on mature lymphoid population based upon CD45 versus side scatter

Fig. 3 Flow cytometry scattergrams: Showing the CD19 positive B- lymphoid cells positive for CD20, CD38 and FMC7.

A: Gate on all cells shown in the first scattergram

B: Gate on CD19 positive B cells based upon CD19 versus side scatter

The possibilities considered were those of CD10 + diffuse large B-cell lymphoma (DLBCL) of follicular center cell origin, or a follicular lymphoma. However, as the morphology was suggestive of a high-grade lymphoma the former possibility of a DLBCL arising from a follicular lymphoma was more probable.

Discussion

Serous effusions are a common complication of lymphomas when extensive involvement is present, but patients with malignant lymphoma seldom have effusions as a primary presentation. Impaired lymphatic drainage appears to be the primary mechanism for pathogenesis.

Pleural effusions are seen in 20–30% of NHL and Hodgkin's disease (HD), however, the involvement of peritoneal and pericardial cavities is uncommon [1]. T-cell neoplasms, especially the lymphoblastic lymphomas, more frequently involve the serous cavities. Das, et al. reported pleural effusion in 41.6% of lymphoblastic lymphomas as opposed to 3.8% in other NHL types [2]. Of the B-cell lymphomas, follicular center cell lymphoma, diffuse large B-cell lymphoma, burkitt lymphoma, marginal zone

lymphoma, MALT lymphoma and lympho-plasmacytoid lymphoma, have been described in the literature as other lymphomas giving rise to effusions [1, 3]. Morphologic and flowcytometric evaluation of the lymphoma in our case helped us characterize the neoplasm as a diffuse large B-cell lymphoma, possibly, arising from a follicular lymphoma.

Cyto-morphologically, we had observed large pleomorphic cells suggestive of a high-grade lymphoma, but for confirmation of the presence of a lymphoma and for further characterization, flowcytometry on the ascitic fluid was required. Flowcytometry is well established as an aid in the cytopathologic diagnosis of lymphoid tissue on fine-needle aspiration (FNA); however, reports evaluating its usefulness in serous effusions are few [4]. It is of value in cases with atypical/suspicious cytomorphology as in our case, especially when prior diagnosis of lymphoma is not available. Further sub-typing of B-cell lymphomas is also facilitated by the use of flowcytometry [5]. Flowcytometric evaluation helped us confirm the lymphoma and further sub-classify it as a CD 10+ B-cell lymphoma [6, 7].

Unlike pleural effusion, ascites is a rare primary presentation of malignant lymphomas [1]. Das, et al. reported a frequency of pleural effusion in 8.6% of NHL, but pericardial effusion and ascites were seen in only 0.8% of

the cases [1, 8]. This case was interesting as the primary diagnosis of lymphoma was made on flow-cytometric immunophenotyping of ascitic fluid, since the clinical work up was being done primarily for the diagnosis of carcinoma head of pancreas.

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