Role of Ancillary Techniques in Diagnosis of Challenging Common Hematological Malignancies

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Abstract

Background: Usually, hematolymphoid malignancies present as generalized lymphadenopathy, splenomegaly, and cytopenias. However, sometimes, this may not be the scenario, and that is where the challenge arises. These unusual scenarios are present either in extranodal lymphoma or extramedullary acute leukemia, especially occurring at unusual sites and these often lays down a diagnostic dilemma. Aims: This study aims to evaluate the utility of extensive workup with amalgamation of radiological imaging, immunohistochemical panel, and flow-cytometry to diagnose these unusual hematolymphoid malignancies. Materials and Methods: This is a retrospective observational study of results obtained from a series of nine patients with various unusual manifestations of extranodal lymphoma or acute leukemia at tertiary center of Northern India since the past 18 months. Results: All nine cases which showed variable and unusual clinical presentations were studied involving all available ancillary techniques in the form of immunohistochemistry (IHC), flow cytometry (FCM). These cases because of their unusual presentation in the form of recurrent pericardial/peritoneal effusion, abdominopelvic masses, chronic cervicitis, and subacute intestinal obstruction mimicked various neoplastic and nonneoplastic lesions. IHC and/or FCM evaluating CD34, MPO, CD117 along with Tdt and B/T-cell markers played a vital role for a definitive diagnosis of aleukemic myeloid sarcoma primarily involving intestine, T-cell lymphoblastic lymphoma (LBL) in ovary, diffuse large B-cell lymphoma in ovary, cervix, and intestine with peritoneal carcinomatosis. Two cases of mediastinal masses revealed primary thymic B-cell lymphoma and T-cell LBL, respectively. Conclusion: Cases have highlighted the unusual clinical presentation of hematolymphoid malignancies and elicit the importance of providing a definitive opinion, which is mandatory to give appropriate effective and timely management for the best outcome.

Keywords: Colon, lymphoblastic lymphoma, mediastinum, myeloid sarcoma, pericardial effusion, pleural effusion, subacute intestinal obstruction, tuberculosis

INTRODUCTION

Generalized lymphadenopathy and bone marrow involvement are usual manifestations of hematolymphoid malignancies; however, some extranodal lymphoma and extramedullary acute leukemia involving the unusual sites may lead to diagnostic dilemma due to their overlapping morphological features. Myeloid sarcoma (MS) is seen most commonly in patients with acute myeloid leukemia (AML) and less frequently in chronic myeloid leukemia and is composed of myeloid blasts which in most of the cases may tend to get misdiagnosed morhologically as non-Hodgkin’s lymphoma (NHL).[11]

NHLs affect the extranodal sites in one-third of cases with the most common being gastrointestinal tract and skin. Hence, extranodal lymphoma manifesting at unusual sites/presenting with an unexpected presentation may again constitute a diagnostic predicament. High index of clinical suspicion, appropriate immunohistochemical markers and flow-cytometry helps in arriving at a correct diagnosis. In this study, we present a case series of an unusual presentation in the form of recurrent pericardial/peritoneal effusion, abdominopelvic masses, chronic cervicitis, and subacute intestinal obstruction mimicked various neoplastic and nonneoplastic lesions. IHC and/or FCM evaluating CD34, MPO, CD117 along with Tdt and B/T-cell markers played a vital role for a definitive diagnosis of aleukemic myeloid sarcoma primarily involving intestine, T-cell lymphoblastic lymphoma (LBL) in ovary, diffuse large B-cell lymphoma in ovary, cervix, and intestine with peritoneal carcinomatosis. Two cases of mediastinal masses revealed primary thymic B-cell lymphoma and T-cell LBL, respectively. Conclusion: Cases have highlighted the unusual clinical presentation of hematolymphoid malignancies and elicit the importance of providing a definitive opinion, which is mandatory to give appropriate effective and timely management for the best outcome.

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diseases with unusual presentation as the timely diagnosis lead to appropriate management and good outcome.

**Materials and Methods**

Nine patients of hematolymphoid malignancies with unusual presentations were studied both prospectively and retrospectively. These cases were worked up and studied at Departments of Laboratory Sciences and Molecular Medicine, Medical Oncology and Haematology at a tertiary care hospital of North India from July 2016 to October 2018. The cases were approached after peripheral blood smear, bone marrow examination morphologically after which flow cytometry (FCM) panel was decided and were evaluated to reach for diagnosis. Further, they were correlated with IHC whenever required. All relevant hematological (peripheral blood examination, bone marrow aspiration, biopsies, and FCM analysis) and biochemical investigations were tabulated and reviewed. FCM analysis was performed on Beckman FC 500 on the bone marrow aspirates in all relevant cases and relevant body fluids as has been mentioned in Table 1. All FCM samples were processed within 24 h and were processed as per institution protocol. Since the body fluids usually have low cell yields the target cell count was kept to as low as 25,000–50,000 cells/tube and only priority tubes were run from a given panel. Optimal acceptable percentage of viable cells is about 80%, which was measured using 7-AAD. For suspected cases of acute leukemia entire panel of antibodies

**Table 1: Clinical details, demographic data and laboratory investigations (n=9)**

| Case | Age/sex | Presenting signs and symptoms | Radiological impression | Provisional clinical diagnosis | Initial management | Salient lab findings | Positive markers FCM | Positive IHC | Final diagnosis |
|------|---------|-------------------------------|------------------------|------------------------------|-------------------|---------------------|---------------------|----------------|-----------------|
| 1    | 45/female | Pain abdomen and weight loss | Intestinal obstruction with stricture at ileocaecal junction | Tuberculosis | Right hemicolectomy and antitubercular treatment | Raised TLC, blasts 21% | Peripheral blood - Dim CD45, CD34, MPO, CD117, CD13, CD33, Ki67; 95% | Lymph node - CD34, Tdt | AML with myeloid sarcoma |
| 2    | 27/male | Pain abdomen and weight loss | Mesenteric lymphadenopathy | Tuberculosis | Antitubercular treatment | High TLC with blasts | Peripheral Blood CD11c, CD34, MPO | Pleural fluid: Bright CD 45 with CD 20, CD, κ, CD 19 | AML with monocytic differentiation |
| 3    | 37/male | Pain abdomen and weight loss | Loculated ascites with cecal mass | Tuberculosis | Right hemicolecotomy and antitubercular treatment | Mild anemia with normal TLC, platelet counts, pleural fluid positive for atypical lymphoid cells | Ascitic fluid: Dim CD45, SS, CD34, CD3, CD8, TdT, CD7 | Mediastinal Bx - CD3, CD8, Tdt | High Grade B-cell NHL |
| 4    | 22/male | Fever, right sided chest pain | Anterior mediastinal mass Pleural and pericardial effusion | Tuberculosis Lymphoma | Antitubercular treatment | Mild anemia with normal TLC, platelet counts, ascitic fluid - raised ADA and LDH levels | Medial Lymph node - Bcl-2, Mum1, PAX5, CD20 | Mediastinal Bx - CD3, CD8, Tdt | T-cell acute lymphoblastic lymphoma |
| 5    | 17/male | Neck swelling, breathlessness | Anterior mediastinal mass Pleural and pericardial effusion | None | None | Mild anemia with normal TLC, platelet counts, pericardial fluid positive for lymphoid cell infiltration | Pleural fluid: FCM - cCD3, CD34, CD8, CD7, TdT, CD1a | Mediastinal Bx - CD3, CD8, Tdt | T-cell acute lymphoblastic lymphoma |
| 6    | 22/male | Swelling right side neck, weight loss | Anterior mediastinal mass Pleural and pericardial effusion | Tuberculosis Lymphoma | Antitubercular treatment | Mild anemia with normal TLC, platelet counts, ascitic fluid smear show atypical lymphoid cell infiltration | FCM ascitic fluid - bright CD45/SSC, CD10, CD 20 | Mediastinal Bx - CD45, CD20 | Primary thymic B cell NHL |
| 7    | 61/female | Left sided lower abdominal pain | Ovarian malignancy | Sex cord stromal tumour | Surgical resection | Normal hematological profile None | | | | Diffuse large B-cell lymphoma |
| 8    | 15/female | Loss of appetite, mass per abdomen | Abdominopelvic mass, mesenteric lymphadenopathy | Dysgerminoma | Surgical resection | Borderline hematological profile with Striated mesenchymal tumour | Bone marrow aspiration: FCM - cCD3, CD34, CD8, TdT, CD1a | | | T-cell acute lymphoblastic lymphoma |
| 9    | 31/female | Bleeding per vaginum | Bulky cervix | Chronic cervicitis | Surgical resection | Normal hematological profile None | | | | Diffuse large B-cell lymphoma |

FCM: Flow cytometry; NA: Not available; AML: Acute myeloid leukemia; NHL: Non-Hodgkin’s lymphoma; IHC: Immunohistochemistry; TLC: Total leukocyte count; ADA: Adenosine deaminase; LDH: Lactate dehydrogenase
CD34, CD117, HLADR, TdT, CD19, CD10, CD79a, CD15, CD13, CD33, MPO, CD11c, CD14, CD3, CD4, CD8, CD7, cCD3, were done after examining peripheral blood/bone marrow slides. For suspected cases of NHL entire panel such as CD5, CD23, CD22, CD19, CD20, CD3, FMC 7, SMIG, CD38, CD138, CD3, CD4, CD8, CD10, CD34, CD200, kappa, lambda, and Ki67. Formalin-fixed paraffin-embedded tissue sections obtained from cell blocks and other relevant tissues stained with hematoxylin and eosin stain and other special stains were re-assessed. The IHC was performed using indirect technique with labeled streptavidin by requisite primary antibodies depending on the morphological differential diagnosis [Figure 1 (1a-4h)] IHC markers (DAKO antibody) used included CD19, CD20, CD3, CD4, CD8, Ki67, Bcl-2, Bcl-6, CD10, cyclin D1, CD5, and CD23. The clinicoradiological profile was correlated with hematological parameters, FCM analysis and the histopathology to obtain the final diagnosis [Figure 2 (5a-5e)].

RESULTS

Among nine cases, there were five males and four females with wide variation in the age group ranging between 15 and 61 years (median 31 years and mean of 38 years). All the cases are being tabulated in Table 1. Three patients presented with pain abdomen, and in view of clinicoradiological features and near-normal hematological profile, possibility of tuberculosis was considered. However, the histopathological and immunohistochemical features of surgical resected specimen led to the diagnosis of high-grade hematolymphoid malignancy. Two of the three cases with mediastinal mass having pleural/pericardial effusion were treated with anti-tubercular treatment which on biopsy was found to be of T-cell acute lymphoblastic lymphoma (LBL) whereas remaining one as primary thymic B-cell NHL. Among two of the ovarian malignancies with preoperative diagnosis of sex cord-stromal tumor and dysgerminoma each was found to have diffuse large B-cell lymphoma (DLBCL) and T-cell LBL, respectively. The patient presented with bleeding per vagina clinically diagnosed as chronic cervicitis was found to have DLBCL on cervical biopsy.

DISCUSSION

Hematolymphoid malignancies especially lymphomas, AML have varied clinical presentations especially in the form of extranodal and extramedullary presentations (MS), respectively. Although majority of the patients with systemic effusions especially ascites have peritoneal carcinomatosis; while lymphomatous infiltration of the peritoneum is far less common.
In this study, we have presented series of cases pertaining to the above scenarios which were either diagnostic dilemmas or required urgent diagnosis due to deteriorating patient condition.

There were four cases who presented with recurrent and massive effusions with pleural fluid showing tumor cells and with the use of FCM early diagnosis was made, and early management led to the prevention of any mortality. DLBCL presenting with ascites and three cases of T-cell LBL with pericardial and pleural effusions. Peritoneal “lymphomatosis” is rarer in literature than peritoneal carcinomatosis. The patterns of peritoneal involvement may occur without intestinal obstruction or as nonloculated exudative ascites. DLBCL presenting with ascites or as peritoneal infiltration without solid tumor component is rare. Peritoneal infiltration is usually associated with high-grade lymphoma with aggressive histological subtypes. One of our cases had loculated effusion with ileocaecal growth, but the biopsy was repeatedly inconclusive. The diagnosis was obtained by cell block and FCM, which enabled the commencement of treatment for the patient. These cases have furthermore importance as the protocol of doing FCM in body fluids is not only very stringent but also carries some meticulous steps. Right from the transport of sample which is in sterile bottle to processing of sample, which should be as early as possible preferably within 2 h to taking care of avoidance of degeneration of samples. Since usually, the cell counts in fluids are low, gating also becomes difficult. Since cells rapidly degenerate in body fluids due to lack of any anticoagulants, it is essential to quantify the viability of the cells and the accepted percentage of viable cells is 80%.

T-cell LBL comprises approximately 85%–90% of all LBL. Pleural and pericardial effusions along with mediastinal mass are common characteristics of T-LBL. In many clinical scenarios, these patients may come with acute respiratory distress and a rapid diagnosis is required for initiating the management. Accurate diagnosis is often a challenge because differentiating lymphoma cells from reactive lymphoid cells on cytological examinations may be difficult. Hence, ancillary techniques such as FCM and cell blocks are increasingly being utilized in the diagnosis, especially in cases of undiagnosed pleural effusions. We were able to provide rapid diagnosis in three such cases utilizing these minimally invasive ancillary techniques. In two of the three cases, body fluid manifested blasts which are otherwise not a usual phenomenon. FCM helped in reaching an early diagnosis and therefore an early management. Later on, both mediastinal and bone marrow aspiration and biopsy diagnosis of T-cell LBL was confirmed. Pleural effusion in lymphoma can occur by a variety of mechanisms, such as impaired lymphatic drainage owing to mediastinal lymph nodes or thoracic duct obstruction, pleural or pulmonary infiltration by tumor or venous obstruction. Pleural effusion secondary to leukemic cell infiltration is not a common phenomenon and infective causes of pleural effusion outnumber malignant causes. A retrospective analysis of pleural fluid cytological samples was performed by Awasthi et al. where out of 898 samples of pleural fluid, only 164 cases were found positive for malignancy, the remainder being of infective etiologies. Further, subcategorization confirmed that only 30 cases were of hematological origin with the most common etiology being NHL (20 cases) followed by acute lymphoblastic leukemia (four cases). He XL et al. and Ambrosio et al. reported 256 serous effusions associated with lymphoma of which 197 were pleural effusions. Third case who was diagnosed T-cell LBL was also unusual wherein a 15-year-old girl both clinically and radiological findings suggested of bilateral dysgerminoma and was taken to operation table. Her one ovary was resected and was sent for frozen section which revealed lymphoid blasts and therefore her other was stopped for resection. Ovarian biopsy along with wide panel of antibodies and FCM in bone marrow aspiration revealed the case to be of T-cell LBL. Interestingly, her hematological profile was apparently normal with no blasts in peripheral blood smear. The patient presently is in remission. Lymphomas presenting as ovarian tumors are uncommon and may occur as de novo or secondary as a part of systemic disease. Primary ovarian NHLs are extremely rare, accounting for 0.5% of all NHLs and 1.5% of all malignant ovarian neoplasms. It is postulated that primary ovarian NHL arises from hilar lymphoid tissue in the ovary. Fox et al. have suggested three criteria for the diagnosis of primary ovarian lymphoma: (1) tumor should be confined to the ovary and its regional lymph nodes, (2) bone marrow and peripheral blood have not contain any abnormal cells, and (3) if extraxvian disease appear later, there must be a few months between the time of ovarian and extraovarian lesions. Of the two cases; one case of DLBCL satisfied the above criteria and qualified for primary ovarian NHL. The second case of young female was extramedullary manifestation of T-cell LBL [Figure 1ia-ivh].

Primary malignant lymphoma of the uterine cervix and upper vagina is an extremely rare tumor of the female genital tract. In one series, cervix was involved in 1 out of 730 cases of NHL and 1 out of 176 cases of extranodal lymphoma. Clinical symptoms usually include vaginal bleeding (70%), perineal discomfort (40%), and persistent vaginal discharge (20%). As cervical lymphomas arise from the stroma rather than the mucosa, hence cervical cytology is not very sensitive, and deep biopsy is required for diagnosis. In literature, DLBCL accounted for 80% and 50% of cases followed by follicular lymphoma, small lymphocytic lymphoma, marginal zone B-cell lymphoma, and precursor T-cell LBL.

MS can exist with AML/myelodysplastic syndrome in 15%–35% or as harbinger of AML in 50% or rarely in isolated forms or as relapse in a treated patient. In 25%–30% cases, its aleukemic/isolated form also exists with the variable interval weeks to months between diagnosis of MS into the development of AML. Lymph nodes, bone, skin, orbit, and soft tissue are the most common sites of involvement by MS. In the gastrointestinal tract, MS commonly involves small bowel with stomach whereas, large intestine and appendix involvements being rare.
thickening, polyposidal mass, exophytic mass, or ulcerated lesions with clinical symptoms of abdominal discomfort, vomiting, and pain depending on its range from 2 to 20 cm.\[13\] Both of our cases involved small intestine and presented with pain abdomen and intestinal obstruction. 6.5% of MS occur in the gastrointestinal tract with extremely rare occurrence of isolated aleukemic cases as noted in one of our cases. Complications of gastrointestinal MS are intestinal obstruction, perforation or intussusception. MS can be subclassified into granulocytic/monoblastic/myelomonocytic types depending on maturation and cell types.\[7\] MS has no specific imaging features and hence is indistinguishable from lymphoma, undifferentiated cancer, Burkitt’s lymphoma, Ewing’s sarcoma, rhabdomyosarcoma, neuroblastoma, extramedullary hematopoiesis, and inflammation as happened in two of our cases of MS.\[2\] Multiple modalities including immunohistochemical stains, conventional cytogenetics, fluorescent in situ hybridization cytogenetics, and FCM assist to finalize the diagnosis of aleukemic isolated MS. IHC for CD34, MPO, CD117, CD13, CD33, Ki67 are common markers in tumors with myeloid differentiation whereas CD14, CD11c, and CD163 favors monoblastic differentiation [Figure 1a-ivh]. In addition, pancytokeratin, vimentin, S100, Tdt, CD3, CD20, CD79a, CD10, Bcl6, MUM1, lysozyme, CD56, CD61, CD30, and glycoprophin should be added to exclude other differential diagnoses. Accurate initial diagnosis of isolated aleukemic MS was offered only in 53% cases, others being misdiagnosed as NHL.\[16\] Definitive diagnosis of MS has great prognostic significance as most patients may develop full-blown AML.

**Conclusion**

Our series of cases highlight the unusual clinical presentations of hematolymphoid malignancies and importance of keeping them in the differential diagnosis while managing these patients. It also elicits the importance of providing a definitive opinion utilizing multidisciplinary approach, minimally invasive techniques, and ancillary diagnostic measures. This enables appropriate, effective, and timely management of such cases for the best clinical outcome.

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**Informed consent**

Informed consent was obtained from all individual participants included in the study.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the legal guardian has given his consent for images and other clinical information to be reported in the journal. The guardian understands that names and initials will not be published, and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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