

Hepatosplenic T Cell Lymphoma: Diagnostic Conundrum

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ABSTRACT

Hepatosplenic T cell lymphoma (HSTCL) is a very rare and aggressive peripheral T cell lymphoma that comprises less than 1% of Non-Hodgkin lymphomas (NHL). It is derived from cytotoxic T-cells, usually of $\gamma\delta$ T cell receptor type, and is characterized by primary extranodal disease with typical sinusoidal infiltration of the liver, spleen and bone marrow by medium-sized lymphoid cells. HSTCL occurs more frequently in immunocompromised patients, especially in those receiving long-term immunosuppressive therapy. The differential diagnosis is varied, and the clinical course is dismal with a poor response to currently available therapies. Herein we report a case of HSTCL in a 20-year-old immunocompetent male who presented with fever, pallor, weight loss, bicytopenia, hepatomegaly, and massive splenomegaly, highlighting the diagnostic conundrum and pointers towards an accurate diagnosis. The key role for diagnosis was the combination of morphologic finding of atypical lymphoid cells in the bone marrow, typical immunophenotypic profile on flow cytometry and the pattern of involvement of the liver and the spleen, even in the absence of full-fledged diagnostic panels and tools. The report of this case is an endeavor to emphasize the high index of suspicion for timely detection of such a rare entity.

Keywords: Hepatosplenic T-cell lymphoma; Immunophenotyping; Immunocompetent; Hepatosplenomegaly; Bicytopenia

INTRODUCTION

Hepatosplenic T-cell lymphoma (HSTCL) was first described as a distinct clinicopathologic entity in 1990¹. HSTCL is a rare peripheral T-cell lymphoma characterized by hepatosplenic sinusoidal infiltration of monotonous, medium-sized, nonactivated cytotoxic T cells, usually of γ/δ T-cell receptor type. HSTCL comprises less than 1% of Non-Hodgkin lymphomas (NHL), and has been estimated to contribute only 1.4% of all T-cell lymphomas^{2,3}. HSTCL is more common among young males in their teenage years and in young

adulthood^{2,3}. It exhibits a rapidly progressive clinical course and a poor response to currently available therapies, with a 5 year survival rate of 7%⁴. Herein, we report a case of this rare disease from our institute, the first from North East part of India highlighting the clinicopathologic features of this uncommon T cell Non-Hodgkin Lymphoma (NHL) and emphasizing a combination of clinical findings, histologic features, flowcytometry and immunohistochemistry (IHC) in detecting this unique entity.

Case presentation

A 20-year-old male presented with fever, weakness, generalised rashes, and abdominal discomfort since 4 weeks. On examination, pallor, moderate hepatomegaly, marked splenomegaly and mildly enlarged right axillary lymph node were discerned. Complete hemogram showed anaemia (Hemoglobin 10g/dl) with thrombocytopenia (Platelet count 80000/cmm). Total leucocyte count was within normal range (6200/ μ l); however, the peripheral blood smear showed around 8% atypical cells. The other abnormal findings were moderately raised liver enzymes and increased C-reactive protein. Coagulation profile, renal function tests, viral markers (for hepatitis B, hepatitis C and Human Immunodeficiency Virus) and autoimmune profile [Antinuclear antibodies (ANA) and Anti-neutrophil cytoplasmic antibodies (ANCA)] were unremarkable. Abdominal ultrasonography confirmed massive splenomegaly and moderate hepatomegaly. Fine needle aspiration cytology (FNAC) of the axillary lymph node, performed as a screening procedure revealed reactive lymphoid hyperplasia. In view of the bicytopenia, bone marrow aspiration and biopsy were performed. Bone marrow aspiration showed a cellular marrow infiltrated by abnormal medium-sized lymphoid cells (60%), having a high N: C ratio, round to oval nuclei, clumped chromatin and scant to moderate cytoplasm. Some of these cells resembled blasts, thus arousing a dilemma between acute leukemia or lymphoma infiltration [Figure 1]. Cytochemistry with Myeloperoxidase (MPO) stain and Periodic acid Schiff (PAS) stain on the marrow aspirate did not show positivity in the abnormal cells. Meanwhile flow cytometric analysis was performed on the marrow aspirate sample using a comprehensive panel.

The sample was processed by standard lyse-wash procedure and data was analyzed using the CellQuest Pro Software in the four-color Becton Dickinson (BD) FACS Calibur flow cytometer. The abnormal cell cluster was gated (R1) on CD45, Forward Scatter and Side Scatter which comprised 71.37 % of total leukocyte population. The gated cells revealed positivity for both Surface and Cytoplasmic CD 3, CD 8, CD 7 and CD 56, and

negativity for CD 34, CD 4, CD 5, and terminal deoxynucleotidyl transferase (TdT). B cell markers (CD 19, CD 20, CD 10, CD 79a) and myeloid markers (CD 33, HLA-DR, CD 13, MPO) were negative. [All antibodies from BD Biosciences, San Jose, CA, USA]. The T cell receptor analysis ($\alpha\beta$ and $\gamma\delta$) could not be performed due to the unavailability of the markers. The immunophenotype suggested a T cell NHL infiltrating the marrow, with the possibility of HSTCL, in light of the clinical and the radiological findings. Negativity of the atypical cells for TdT and CD 34 and positivity for surface CD 3 did not favor a leukemic disease [Figure 2].

The marrow biopsy showed interstitial infiltration by medium sized atypical lymphoid cells having similar morphology as was seen in the aspirate study. IHC demonstrated positivity of the infiltrating lymphoid cells for CD 3 and negativity for CD 20, CD 34 and TdT, thus confirming involvement by a mature/peripheral T cell lymphoma [Figure 1]. Whole body Computed tomography scan did not reveal any other abnormal lesion. The case was subsequently discussed in the tumour board, and a decision was taken to undertake biopsy/FNAC from the liver and the spleen for diagnostic confirmation of HSTCL. FNAC from the spleen divulged infiltration by abnormal lymphoid cells having similar morphology as had been seen on the bone marrow study. Trucut biopsy from the liver revealed distinctive sinusoidal dilatation as well as infiltration by abnormal T lymphoid cells as identified by CD 3 on IHC; CD 1a was negative [Figure 3]. All these findings thus corroborated and confirmed the diagnosis of HSTCL, involving the liver, spleen and bone marrow. Further ancillary tests such as cytogenetics/molecular analysis could not be done. The patient was treated with modified CHOP [cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulphate (oncovin) and prednisone] regimen, and was doing well after having completed six cycles of chemotherapy.

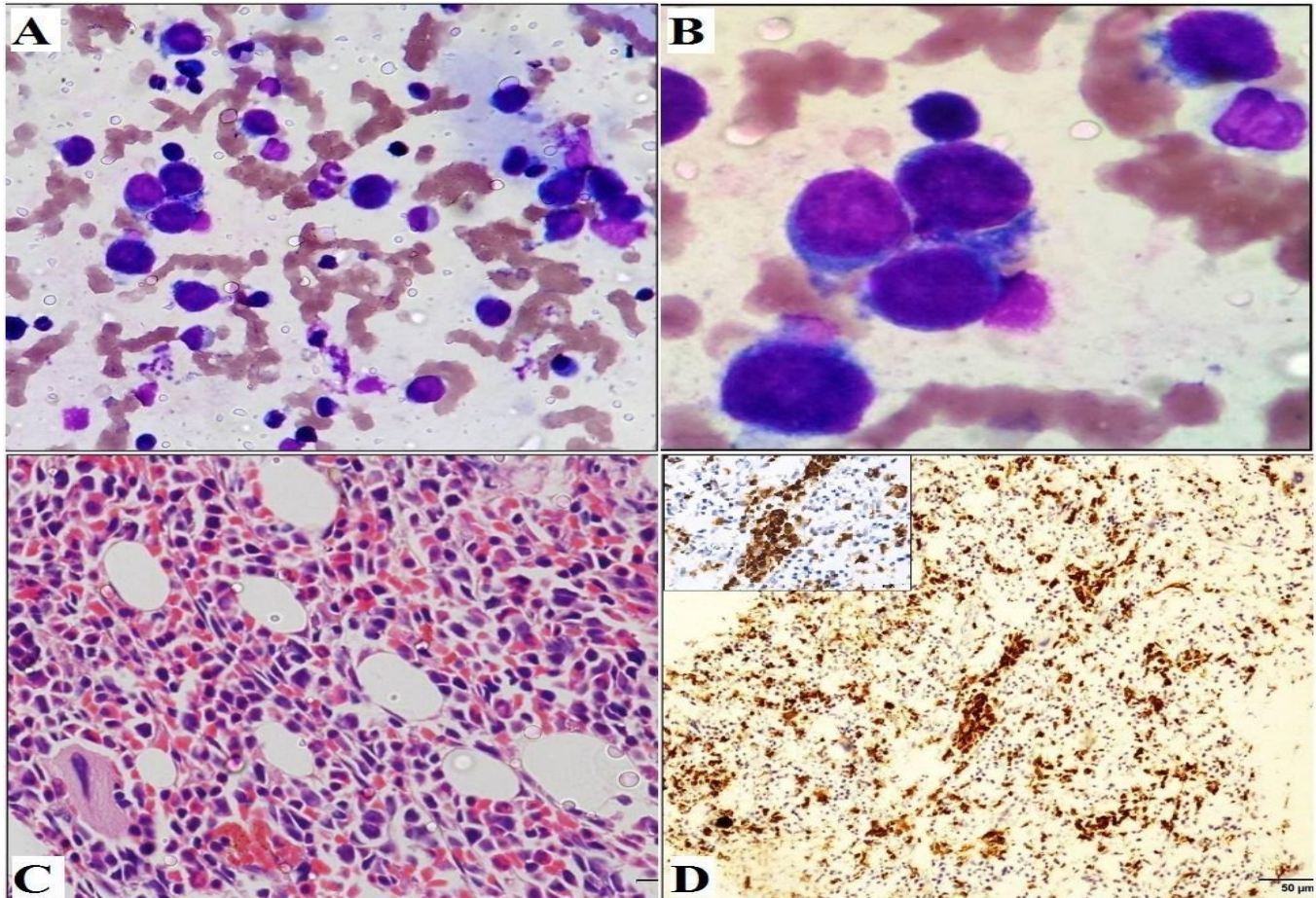


Figure 1. Bone marrow aspirate (A, B) and biopsy (C, D) showing infiltration by atypical lymphoid cells, which are positive for CD3 on IHC (D, inset) [Leishman, (A) 400X & (B) Oil immersion; H & E (C) 400X; IHC (D) 200X, (inset) 400X].

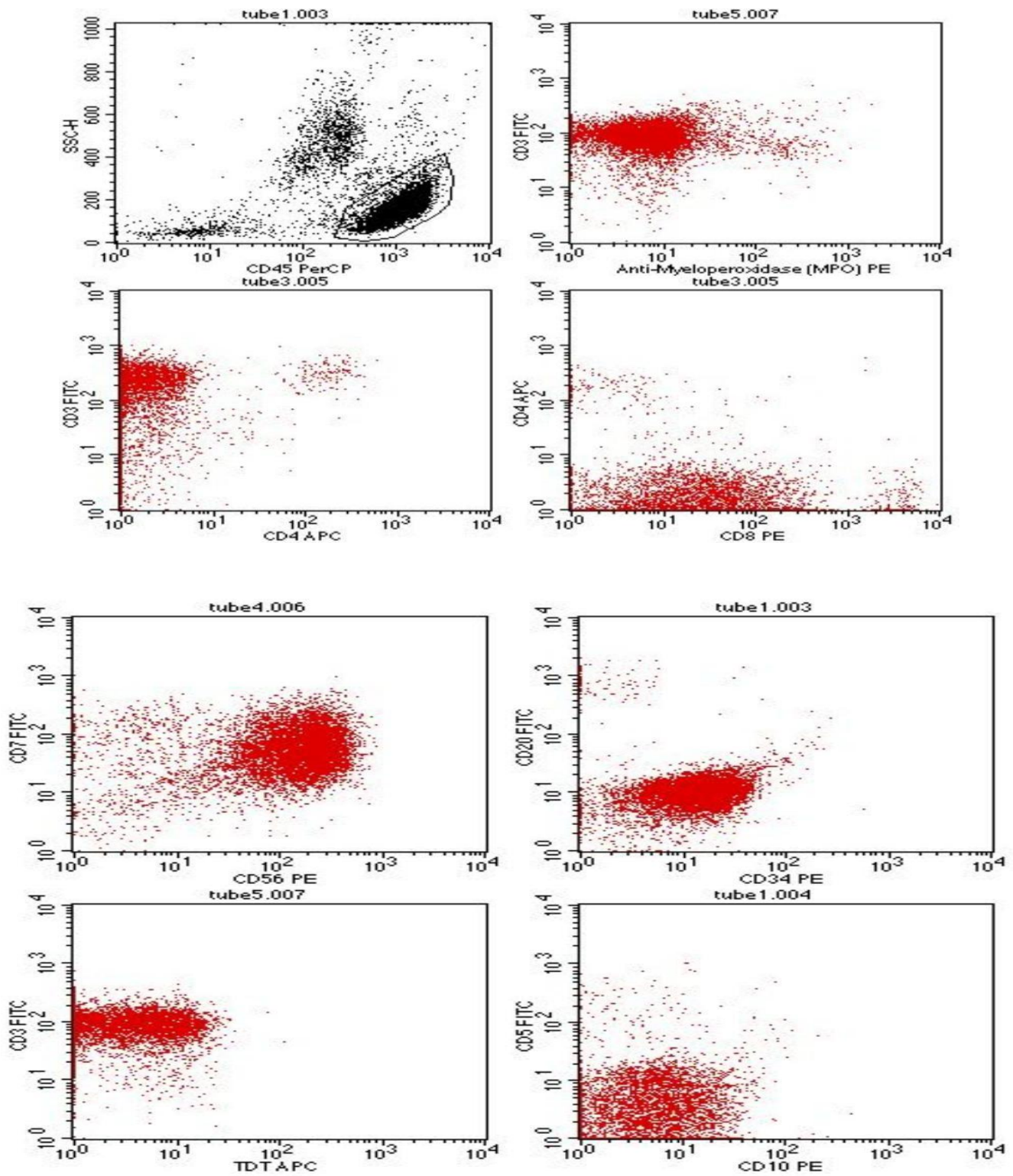


Figure 2. Flow cytometric analysis exhibiting predominance of the lymphoid population which was gated on the CD45 vs Side Scatter plot. The gated events demonstrate positivity for cytoplasmic CD3, surface CD3, CD8, CD7 and CD56, while negativity for MPO, CD4, CD34, CD20, TdT, CD10, CD5 and CD19 (not shown).

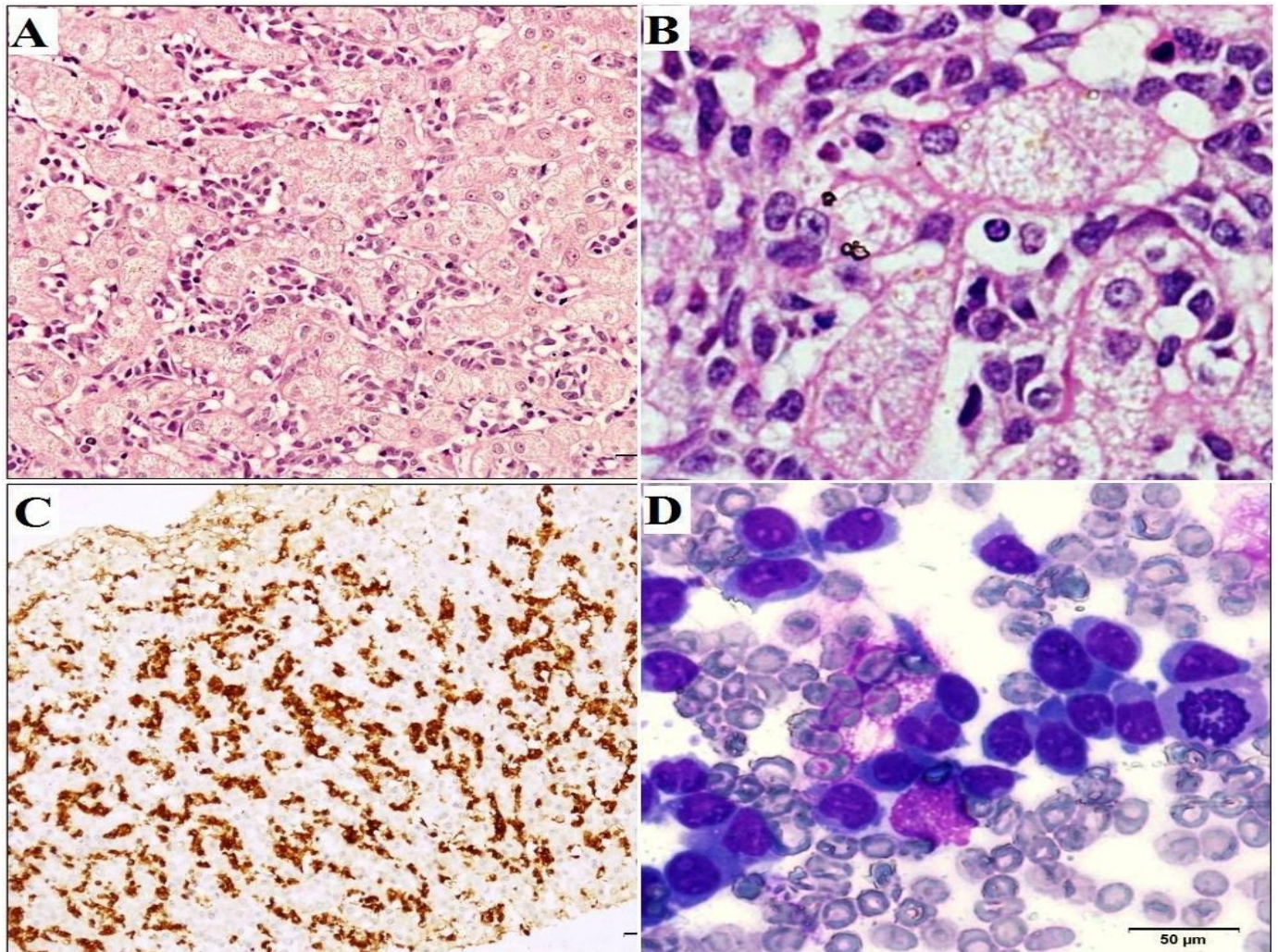


Figure 3. Liver biopsy (A, B, C) and splenic FNAC (D) revealing sinusoidal infiltration by atypical lymphoid cells. IHC on the liver biopsy demonstrates reactivity of the cells for CD3 [H & E, (A) 200X (B) 400X; IHC (C) 200X; MGG (D) 400X].

DISCUSSION

Hepatosplenic T-cell lymphoma (HSTCL) is a rare and clinically aggressive type of T-cell lymphoma that arises most often in adolescents and young adults with a median age ranging from 29-38 years in various studies²⁻³. This entity was first described by Farcet et al., in 1990 and since then only a limited number of cases of this entity have been reported¹. It is believed that HSTCL arises from peripheral γ/δ (or less commonly α/β) cytotoxic memory T cells of the innate immune system, especially from the Vd1 subset that shows a predilection for homing to the splenic red pulp⁵. Although the pathogenesis of HSTCL is poorly understood, it has been postulated that chronic antigen stimulation in the setting of immune deficiency or dysregulation might be important. Approximately 20% of cases occur in young patients with immune suppression, including organ transplant recipients and patients with leukemia receiving chemotherapy⁶.

The male to female ratio is about 9:1⁴. Patients often present with fever, fatigue, weight loss, and abdominal discomfort from hepatosplenomegaly, and sometimes, with jaundice because of liver involvement. Hepatomegaly and splenomegaly, more commonly massive is the most common finding on physical and radiographic examination. Lymphadenopathy is uncommon, reported in less than 25% of patients³. The bone marrow is almost always involved. Patients usually manifest thrombocytopenia, along with leucopenia and anemia². The above mentioned symptoms and the findings of hepatosplenomegaly, anemia, thrombocytopenia and marrow involvement pertained to the patient in our study. Although absolute peripheral lymphocytosis is uncommon, a small population of circulating neoplastic lymphocytes may be seen at initial presentation in approximately 50% of patients with HSTL⁷, as was observed in our case too. HSTL usually show mild to moderate elevation of liver enzymes which is due to the liver cell damage and cases with even fulminant hepatic failure have been reported⁸. The elevated bilirubin levels are considered to be due to

periportal adenopathy, which could be the cause in the current case too⁹. Thus, such a clinicopathologic picture may suggest a diagnosis of acute leukemia initially. The diagnosis of HSTCL requires a high index of suspicion. A characteristic feature is the preferential localization of the neoplastic cells within the sinusoids of the liver and spleen. Different patterns of bone marrow involvement have been reported, including exclusively sinusoidal, interstitial, and mixed sinusoidal and interstitial. The cytologic features of the neoplastic cells can be varied; small, small to medium, medium, or medium to large cells. The medium and/or large sized cells can resemble blasts, with more open chromatin and frequently small but conspicuous nucleoli¹⁰. Such medium sized blastoid cells were discerned infiltrating the marrow, liver and spleen in our case, raising the possibilities of leukemic picture vis-à-vis lymphoma infiltration. Cases of gamma delta origin have rearranged TRG genes and show a biallelic rearrangement of TRD genes. TRB genes are rearranged in alpha beta cases. Isochromosome 7q is present in most cases, and with disease progression, a variety of FISH patterns equivalent to 2-5 copies of $i(7)(q10)$ or numerical and structural aberrations of the second chromosome 7 have been detected².

The diagnosis of HSTCL is usually established using a combination of clinical findings, morphology on liver and/or splenic biopsy, bone marrow biopsy, and peripheral blood smear, immunophenotypic profile, and cytogenetic/molecular findings. Among those, the morphologic and immunophenotypic findings are most crucial for diagnosis. As discussed above, a prominent sinusoidal infiltrate in the liver, spleen, and/or bone marrow of medium-sized lymphoid cells with the characteristic immunophenotype is sufficient for diagnosis of HSTL, if the clinical picture is appropriate⁷. The morphologic finding of infiltration of atypical lymphoid cells and not blasts in the bone marrow in the absence of significant lymphadenopathy should alert the pathologist that one might be dealing with this lymphoma entity rather than an acute leukemia or a lymphoblastic lymphoma, especially in a young

adult. The neoplastic cells are CD 3+ and usually TCR $\gamma\delta$ +, TCR $\alpha\beta$ -, CD 56+/-, CD 4-, CD 8-/+ , CD 5- and TIA1+. A minority of cases appear to be of $\alpha\beta$ type. Thus, delineation of the T cell receptor type is not mandatory for the diagnosis, especially in the event of a resource limited setting like ours where markers for T cell receptor may not be available. The differential diagnosis of HSTCL includes other lymphoproliferative processes that may show sinusoidal bone marrow involvement, such as intravascular lymphoma and splenic marginal zone B-cell lymphoma; the B-cell lineage of the neoplastic cells should easily distinguish these processes from HSTCL¹⁰. The main differential diagnosis of CD 8+ HSTCL (as in our case) on flow cytometric analysis is T-cell large granular lymphocyte leukemia (T-LGLL). T-LGLL usually affects elderly patients who have a history of autoimmune disorders, and these patients have an indolent clinical course. T-LGL lymphocytes have cytoplasmic azurophilic granules and a characteristic immunophenotype with expression of TIA-1, granzyme B, and $\alpha\beta$ TCR³. Also, HSTCLs unlike T-LGLs express CD 56, lack CD 57 and CD 5, are more often TCR $\gamma\delta$ +, and commonly demonstrate isochromosome 7q. Aggressive NK cell leukemia/lymphoma, lymphoblastic lymphoma/leukemia (as mentioned earlier), other peripheral T cell lymphomas and myelodysplastic syndrome also need to be excluded^{7,10}. Although aggressive NK-cell leukemia/lymphoma is typically negative for surface CD3, the epsilon chain of CD3 is expressed. So, IHC staining for CD3 cannot reliably distinguish between NK cells and T cells, due to the fact that CD3 antibody detects only the epsilon component of the T cell receptor complex. Also, determining the localization of CD3 staining (surface or cytoplasmic) by IHC is subjective. Therefore, flowcytometric immunophenotyping, by way of detection of surface and cytoplasmic CD3 separately and also surface TCR, is more useful than IHC for distinguishing aggressive NK-cell leukemia/lymphoma from HSTL¹¹. In contrast to HSTL, T cell acute lymphoblastic leukemia/lymphoma should have dim to negative CD45, express TdT uniformly, CD34 (30% of cases) and CD10 (15-40% of cases)^{7,12}, and be negative for

surface CD3. The neoplastic cells of other peripheral T-cell lymphomas akin to HSTCL also commonly have loss of pan-T cell antigens, including CD5, but are not usually distributed in the sinusoids of the spleen, liver, and bone marrow and, frequently express CD4 and are often restricted to α/β TCR. In HSTCL the sinusoidal lymphoid infiltrate can be subtle on the bone marrow biopsy and, sometimes, entirely obscured by a reactive proliferation of hematopoietic cells. In these cases, the bone marrow may appear hyperplastic, and in the context of peripheral cytopenias, myelodysplastic syndrome or myelodysplastic/myeloproliferative neoplasm may be considered at initial evaluation, which could mislead in the diagnostic workup⁷. Thus, a meticulous flowcytometric evaluation in every bone marrow aspirate taken for peripheral cytopenias in such a clinical setting should be emphasised.

HSTCLs manifests rapidly progressive course. Because of its rarity, no specific therapy for HSTCL has been formulated. To date, the most common therapeutic strategies have been CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) and HyperCVAD (fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with high-dose methotrexate and cytarabine), followed by autologous or allogeneic stem cell transplantation. Although some patients initially respond to chemotherapy, relapse is seen in most cases, and the overall prognosis of HSTCL is extremely poor with the median survival being less than 2 years^{2,3,7,13}.

CONCLUSION

Despite a classical clinical presentation of bi/pancytopenia with organomegaly and lack of significant lymphadenopathy, this disorder is usually not the initial clinical suspicion. The present study is the first reported case of HSTCL from the northeastern part of India and stresses the need of considering the diagnosis of HSTCL in the clinical setting of cytopenias with the morphologic findings of atypical lymphoid cells and not blasts in the bone marrow, massive hepatosplenomegaly and absence of significant lymphadenopathy, especially in young

males. A better awareness of this disorder is warranted amongst pathologists and physicians to stimulate research and hasten the progress in understanding the pathogenesis of HSTCL, which hopefully will lead to development of novel and promising therapeutic strategies.

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