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*Case Report*

# Hepatosplenic γδ T-cell lymphoma presenting with Coombs'-negative hemolytic anemia: a case report

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**Abstract:** Hepatosplenic T-cell lymphoma (HSTCL) is a rare, highly aggressive T-cell lymphoma which is characterized by cytotoxic T cell proliferation in the sinusoids of liver, red pulp of the spleen, and sinuses of the bone marrow. HSTCL patients are mainly young men, commonly present with B-symptoms, hepatosplenomegaly and cytopenia, but hemolytic anemia is rare. Here we present a case of hepatosplenic γδ T cell lymphoma in a 32-year-old man who was presented with Coombs'-negative hemolytic anemia. $^{18}$FDG PET/CT demonstrated diffusely increased bone marrow uptake and hepatosplenomegaly with increased FDG uptake. Bone marrow morphological examination showed diffuse proliferation of CD7+CD3+CD16+TCRγδ+, CD5-CD4-CD8- neoplastic T cells, detected by flow cytometry. Tumor lymphocytes also histologically seep into the sinus or sinus of the bone marrow and spleen. The patient underwent a variety of strong chemotherapy, but the condition quickly deteriorated and he eventually died of cerebral hemorrhage. The patient's diagnosis and treatment procedure are described here, including a literature review.

**Keywords:** Hepatosplenic γδ T cell lymphoma, flow cytometric immunophenotyping, hemolytic anemia, bone marrow morphology

## Introduction

Hepatosplenic T cell lymphoma (HSTCL) is a rare and highly aggressive peripheral T cell lymphoma, accounting for about 1.4% of all peripheral T-cell and natural killer/T-cell lymphomas [1]. This neoplasm results from a proliferation of cytotoxic T cells, and majority of cases harbor the γδ T-cell receptor (TCR), whereas a few cases have been shown to express αβ-TCR [2]. For people with immunomodulatory disorders, about 20% of HSTCL cases are associated with organ transplantation and immunosuppressive therapy, most commonly Crohn disease [3, 4].

Clinically, patients commonly present with B symptoms and splenomegaly [5, 6]. Anemia and thrombocytopenia are the most common laboratory abnormalities in patients with HSTCL [5]. However, autoimmune hemolytic anemia (AIHA) is rare and the exact pathogenesis is not well defined. The objective of this study is to report the clinical and pathological features of a 32-year-old male who was diagnosed with HSTCL that was complicated with Coombs' negative hemolytic anemia.

## Case presentation

A 32-year-old male was referred to our hospital because of fatigue lasting one-month, abdominal distention and ten-days of dark-colored urine. His medical history was not significant. Physical examination showed a yellow tinge to the whites of the eyes, and splenomegaly was noticed, palpable at 15 cm below the costal margin. Liver and peripheral lymph nodes were impalpable. Laboratory tests demonstrated: (1) White blood cell count $8.3\times10^9$/l Neu 4.49 (54.2%), Ly 2.09 (25.5%), Mono 1.53 (18.4%), platelet count $97\times10^9$/l, red blood cells $2.53\times10^{12}$/l, hemoglobin level 73 g/l. Erythrocyte mean corpuscular volume (MCV) was 85fl and reticulocyte rate was raised to 6.0%. (2) Liver function tests were normal. Direct bilirubin was 10 umol/L, but indirect bilirubin was raised to 31 umol/L. (3) Lactate dehydrogenase (LDH) was raised to 624 IU/L. (4) Serology for HBV showed anti-Hbs and anti-Hbc were reactive, whereas anti-HAV, anti-HCV, EB, CMV were negative. (5) The direct and indirect Coombs' tests were negative. Peripheral blood smears showed multicolor and nucleated red

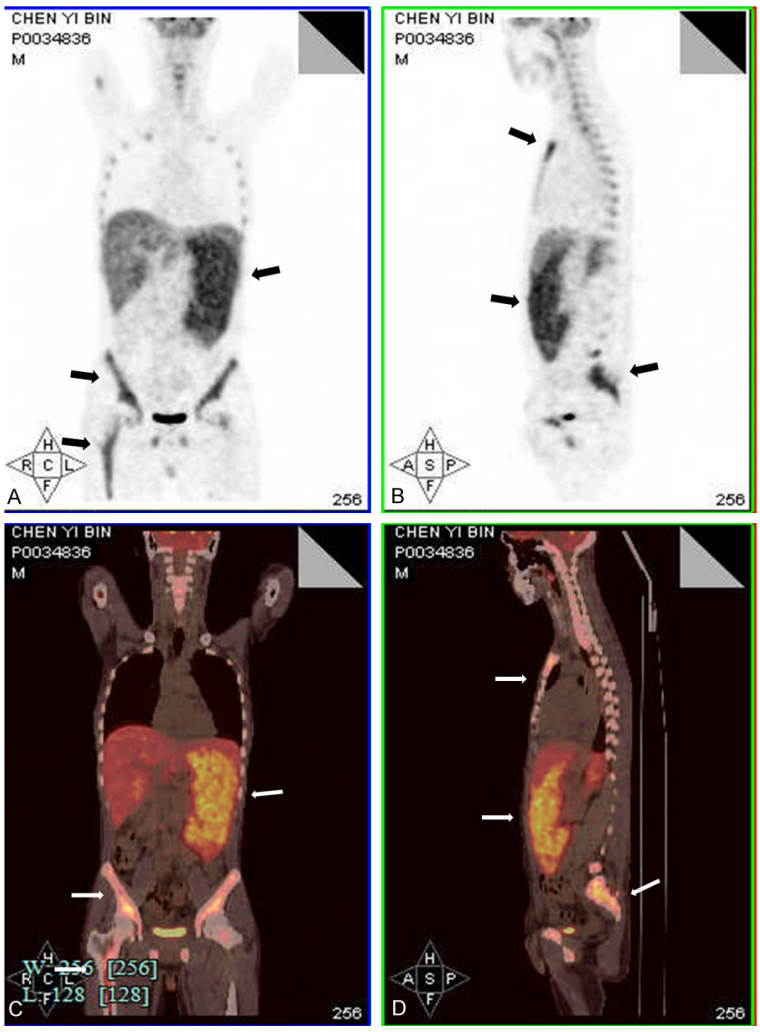


**Figure 1.** 32-year-old man with γδHSTCL at initial presentation. Coronal $^{18}$F-FDG PET (A) and $^{18}$F-FDG PET/CT fusion (B) demonstrating splenomegaly with high FDG uptake in the spleen, and skeletal medullary cavity (the arrows: spleen, left femur and pelvis). Lateral $^{18}$F-FDG PET (C) and $^{18}$F-FDG PET/CT fusion (D) demonstrating splenomegaly and high FDG uptake in the spleen and skeletal medullary cavity (the arrows: spleen, sacral and sternum).

blood cells. According to these laboratory results, he was initially diagnosed with hemolytic anemia.

### Radiological findings

The patient was noted to have massive splenomegaly on computed tomography (CT) scan of the abdomen. Due to concern for the presence of malignancy causing hemolysis anemia, positron emission-computed tomography (PET-CT) was performed, which revealed diffuse fluorodeoxyglucose (FDG) activity in the spleen and skeletal medullary cavity, but showed no evidence of lymphadenopathy (**Figure 1**).

### Morphological findings

The patient underwent a bone marrow aspirate examination. Giemsa-stained smears of bone marrow showed the presence of hypercellular marrow with erythroid hyperplasia (polychromatic erythrocytes were visible), hypoplastic granulocytopoiesis, and reduced megakaryocytes. Bone marrow infiltration was up to 47.5% with abnormal medium size polymorphic cells, medium large amounts of cavitation cytoplasm, mostly showing round or polymorphic nuclei with condensed chromatin and conspicuous nucleoli (**Figure 2**). Flow cytometric analysis of bone marrow revealed a population of abnormal monoclonal T-cells (51.45%) expressing CD7, CD3, CD16, TCRγδ, but negative for CD5, CD4, CD8, CD56, CD57 (**Figure 3**).

### Pathological findings

Bone marrow biopsy revealed hypercellular bone marrow with a scattered intrasinusoidal infiltration of medium sized lymphoma cells, many with irregular nuclear contours (**Figure 4A**). Immunohistochemistry showed that these tumor cells were positive for CD3 (**Figure 4B**).

Spleen resection was performed in the patient. Under the microscope, the spleen structure was almost unrecognizable and the white pulp shrunk. The spleen sinus of the red medulla was clearly extended and diffuse into the homogeneous medium-sized tumor cells, having sparse cytoplasm, circular to elliptical nuclei, slightly dispersed chromatin, and no clear nucleoli. Cells in mitosis were rarely detected. These neoplastic cells were positive for CD3, TIA-1 and negative for CD20, CD56, CD4, CD8, Perforin, granzyme B and EBER, immunohistochemically (**Figure 5**).

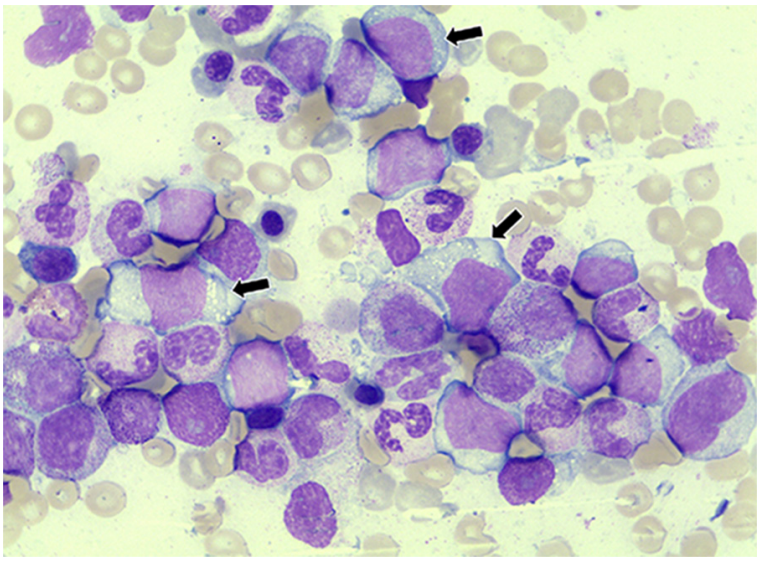

**Figure 2.** HSTCL cells in bone marrow aspirate (Giemsa staining 400×). Cells are in medium size with large amounts of cavitation cytoplasm, mostly showing round or polymorphic nuclei with condensed chromatin and conspicuous nucleoli (the arrows: tumor cells).

### Cytogenetic findings

Conventional cytogenetic analysis of bone marrow cells was performed with G-banding method, and the karyotype is normal.

### Follow up

The patient received intensive combination chemotherapy. The effect of treatment was evaluated by minimal residual disease (MRD) in the bone marrow. After the first two courses of chemotherapy regimen with ECHOP+LASP (etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone, L-asparaginase) and Hyper-CVAD-A (cyclophosphamide, vincristine, doxorubicin, dexamethasone), there was still 11% MRD remaining in bone marrow detected by flow cytometry. Therefore the regimen changed to a multidrug combination chemotherapy (isocyclophosphamide, vincristine, methylprednisolone, mitoxantrone, cisplatin tumor necrosis factor-α) and obtained encouraging effects (MRD: 0.55%). However, HSCTL was refractory to the treatment after two more courses of the therapy and the patient's health deteriorated rapidly. The patient eventually died of intracerebral hemorrhage, 6 months after the initial diagnosis.

## Discussion

HSTCL is a rare subtype of the peripheral T cell lymphomas, first described as a distinct clinicopathological entity by Farcet in 1990, which has been recognized in the Revised European-American Lymphoma (REAL) classification in 1994 as well as the subsequent World Health Organization (WHO) classification [7]. The peak accidence of this malignancy is in young males with a median age of 34, and with a male to female ratio of 9:1 [5, 6, 8, 9].

As exhibited in this patient, HSTCL has a rapidly progressive course with poor outcome and high mortality; therefore, there is still a lack of evidence-based treatment recommendations due to its low prevalence [8]. The median overall survival of HSTCL patients ranges from 3 to 28 months, with various treatment regimens including bone marrow transplantation being used without much success [5-7, 10].

Patients with HSTCL typically present with B symptoms, such as fever, fatigue, and weight loss [5, 6]. Splenomegaly is the most consistent physical examination finding observed in virtually all patients, and hepatomegaly can be found in approximately 40%-88% of patients [5, 6, 11], but substantial lymphadenopathy is rarely presented. Anemia and thrombocytopenia are the most common laboratory abnormalities in HSTCL patients, with more than 80% of patients presenting, which may be due to spleen isolation, bone marrow infiltration, cytokine-mediated phage cell syndrome, alone or in combination [5, 7, 8]. In our report, the patient initially presented with moderate anemia, and further laboratory examinations confirmed the diagnosis of Coomb'-negative hemolytic anemia. There are only seven prior cases associated with AIHA reported in the literature [12-18], and two of them presented with Coomb'-negative hemolytic anemia [12, 18]. The pathogenesis of autoimmune hemolytic anemia in

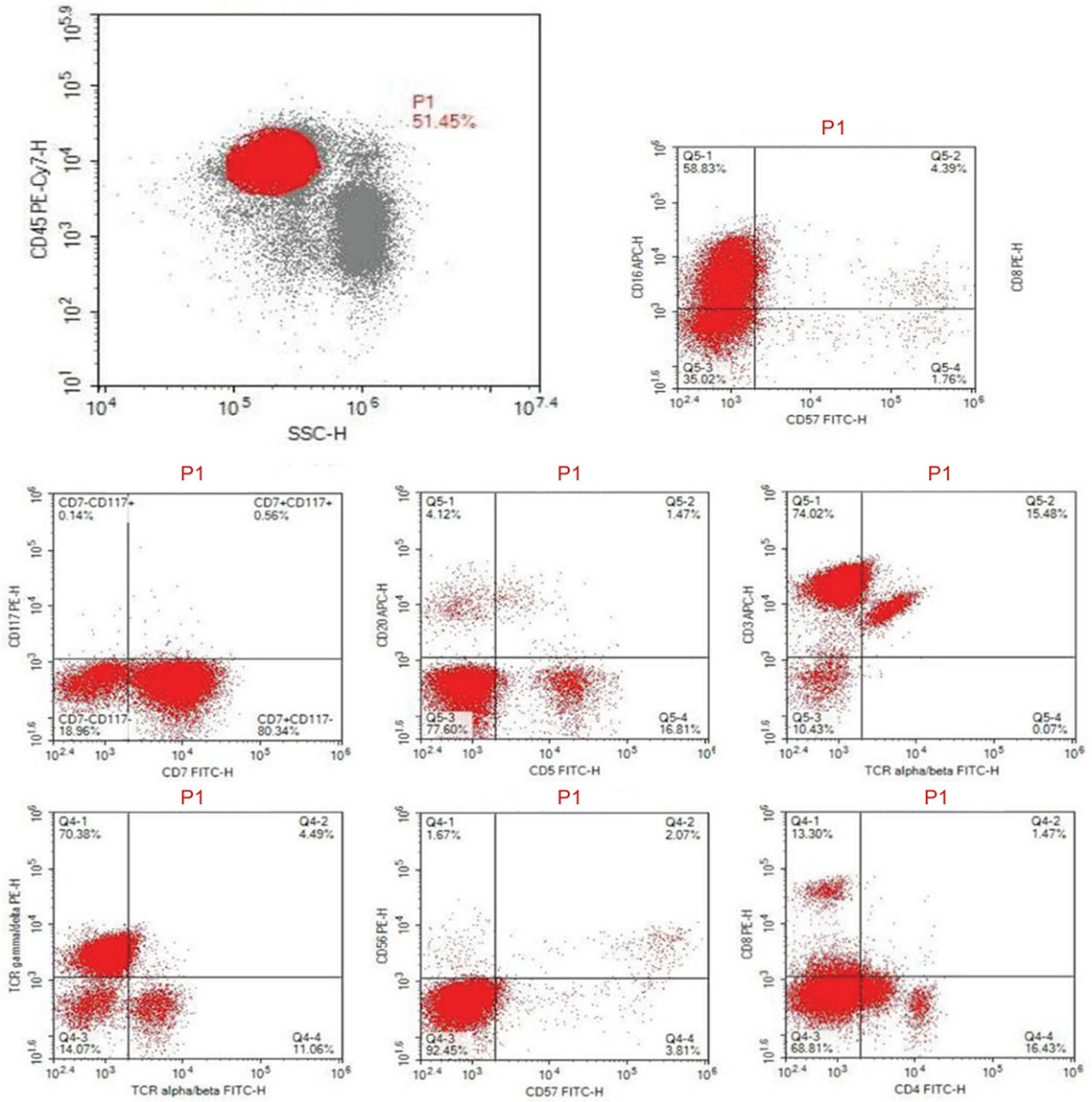


**Figure 3.** Flow cytometry of HSTCL in bone marrow. The figure showed that tumor cells account for about 51.45% of non-erythroid cells in bone marrow.

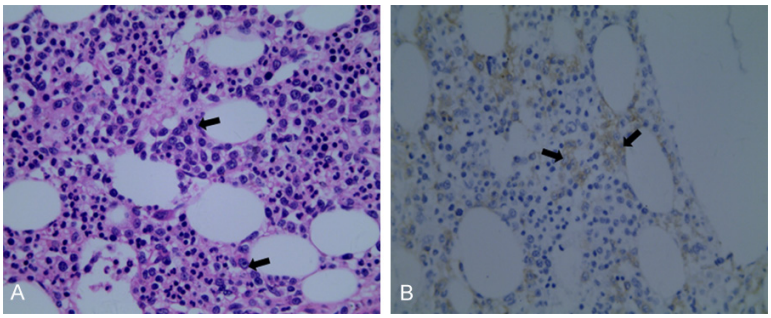


**Figure 4.** Histologic feature and immunohistological finding of HSTCL in bone marrow (the arrows: tumor cells). A. Intrasinusoidal infiltration of HSTCL cells. H&E 400×. B. CD3 IHC 400×.

HSTCL is not well defined, although neoplastic γδ T cells mediated a direct cytotoxic effect to erythrocyte destruction have been reported as a potential mechanism [15]. It is noteworthy that approximately5-10%ofautoimmunehemolytic anemias are Coomb'-negative, and several reasons account for the absence of a positive antiglobulin test in these cases, which includes

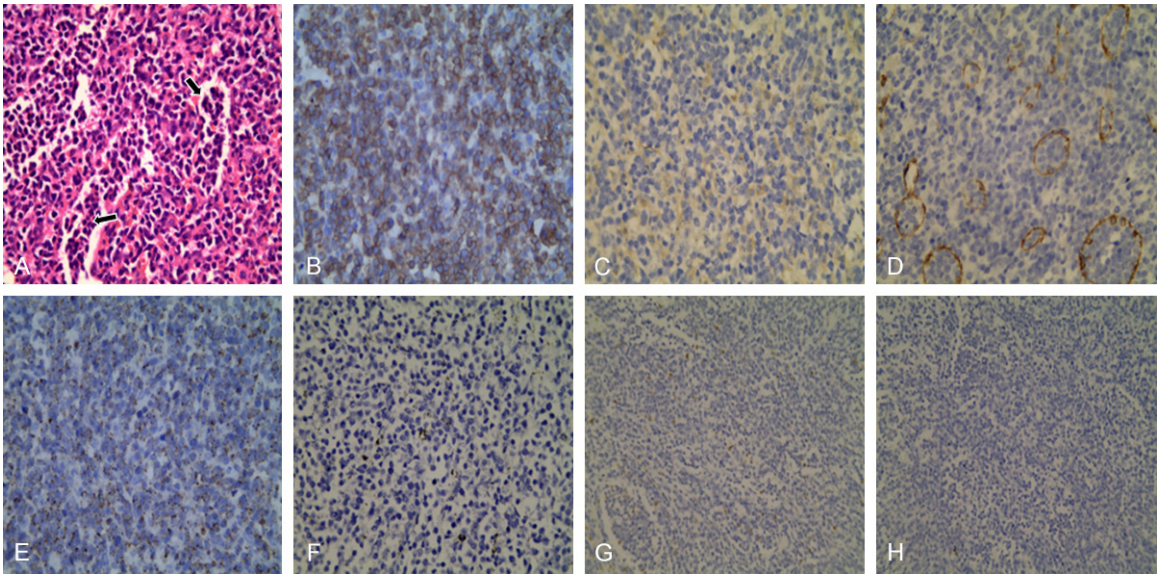


**Figure 5.** Spleen involvement by HSTCL. A. HSTCL infiltrates the sinusoids of the red pulp of spleen (the arrows: tumor cells). H&E 400×. B-H. Expression of marker proteins determined by immunohistochemical staining. B. CD3 IHC 400×. C. CD4 IHC 400×. D. CD8 IHC 400×. E. TIA-1 IHC 400×. F. EBER CISH 400×. G. granzyme B IHC 200×. H. Perforin IHC 200×.

low affinity IgG, IgA or IgM autoantibodies, and NK cell mediated hemolysis independent of antibody [19]. Thrombocytopenia is commonly seen in HSTCL and its severity has been shown to associate with disease progression [5]. Furthermore, its reappearance indicates the relapse in patients who achieved complete remission [5].

Diffuse infiltration by neoplastic lymphoid cells in the spleen is a typical pathological feature of HSTCL. Histologically, malignant cells involve and expand the sinusoids of the red pulp of spleen, and the white pulp is always atrophic or absent [20]. Although there are characteristic morphologic changes in the spleen, it is still a huge challenge diagnosing a HSTCL. Splenectomy is not a routine procedure for preliminary diagnosis, and peripheral lymph nodes are rarely enlarged, and these restrictions make it difficult to obtain qualified pathological specimens. It is worth noting that two-thirds of patients found bone marrow involvement at the time of diagnosis [21], which can be considered a constant feature of presentation, and this is a simple and feasible procedure for obtaining sufficient and qualified ring drill biopsy specimens for histological and immunohistochemical analysis. The improvement of discrete bone marrow infiltration using immunophenotype analysis led to the change of HSTCL diagnostic strategy [5]. Tumor cells infiltrate different bone marrow patterns, including sinus gaps, interstitial and mixed sinus gaps and interstitial, which are difficult to observe in H&E staining sections, but can be aggravated by immunohistochemical staining [11]. On the basis of the typical morphologic finding and immunophenotypic CD3 expression, the diagnosis can be established [11].

Flow cytometrical and immunohistochemistical analyses of biopsy specimens are crucial for diagnosis. The lymphoma cells typically have the following phenotype: CD2+, CD3+, CD7+, CD5-, CD4-, CD8-, CD56+/-, CD57-, TdT-, and TCRαβ/γδ+ [12, 22]. CD8 is expressed in a minority cases [12]. As reported in previous cases [7], neoplastic cells are positive for TIA-1 and negative for perforin and granzyme B in our patient, manifest as a mature, non-activated cytotoxic T-cell phenotype. Differentiation according to the TCR, the majority of HSTCL are the γδ subtype, approximately 20% of them express αβ TCR [7]. Both of these two subtypes have similar clinical, morphological, and cytogenetic findings, but αβ subtype appears to have a worse prognosis [23].

The characteristic chromosomal abnormality in HSTCL is isochromosome 7q ([i7q]), and trisomy

8 is also frequently detected in some patients [2, 5]. Interestingly, these anomalies can then be detected when a relapse or disease progresses, which does not exist at the time of initial diagnosis. However, our patient did not have genetic abnormalities, which may be related to the sensitivity of the G banding.

## Conclusion

In summary, we present a rare case of HSTCL complicated with Coombs' negative hemolytic anemia. Rapid and accurate diagnosis of HSTCL is a challenge, especially in the absence of pathological specimens of the spleen or liver. In this case, a bone marrow biopsy provides a new diagnostic strategy.

## Disclosure of conflict of interest

None.

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