

CASE REPORT

Histopathological, immunophenotypic and clinical particularities and evolution of a case of hepatosplenic T-cell lymphoma in transformation to leukemia

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Abstract

We present the possibilities of diagnosis correlating the pathological, immunophenotyping and clinical aspects of a rare case of T-cell lymphoma in a 23-year-old patient with leukemic transformation. In our consideration, it is very important to describe this case because in the literature there are very few cases presented and the treatment of this type of lymphoma does not present optimal results, the evolution of the patients being from three months to two years. The treatment modality that gives the possibility to prolong survival and cure is hematopoietic stem cell transplantation.

Keywords: hepatosplenic T-cell lymphoma/leukemia, immunophenotyping, hematopoietic stem cell transplantation.

Introduction

Hepatosplenic T-cell lymphoma (HSTCL) is a rare form of peripheral T-cell lymphoma. This disease represents only 1.4% of T-cell lymphomas, in the international T-cell non-Hodgkin lymphoma study [1] being considered an entirely separate entity by *World Health Organization Classification (WHO 2008)* [2]. There are very few cases described in the literature and the optimal management of the cases is not yet established.

HSTCL can occur at any age but it is most often diagnosed in teenagers or young adults with strong male predominance [3]. In several cases, it has been described to appear in patients that are immunocompromised (e.g., solid organ transplant recipients, patients treated with Azathioprine, etc.) [4]. The tumor cells infiltrate sinusoids in the liver, spleen and bone marrow, without lymph node extension. Patients present with hepatosplenomegaly without peripheral adenopathy or significant lymphocytosis. Cytopenia is usually present especially thrombocytopenia. Due to the disappointing results of Anthracycline-based regimens, these patients are usually switched to Platinum-based salvage regimens followed by collection of stem cells and autologous hematopoietic stem cell transplantation [5] and in case of relapse by allogeneic stem cell transplantation [6].

Patient, Methods and Results

For positive diagnosis of the presented case, we analyzed the morphology, the immunophenotyping of the bone marrow, the pathological finding of the spleen in correlation with the clinical features of the patient.

Clinical and paraclinical aspects

The patient is a 23-year-old male with an evolution of nine months from diagnosis. The disease started with organomegaly respectively the enlargement of the liver and spleen and the presence of B-symptoms (fever, night sweats, fatigue). Peripheral adenopathy was not present.

At the time of the diagnosis, the patient presented the following paraclinical results (Table 1).

Table 1 – Laboratory data of the patient at the diagnosis

Parameter, units	Patient	Reference values
WBC, $\times 10^9/L$	11.5	3.6–10
Hematological panel		
Neutrophils [%]	61	42.2–75.2
Lymphocytes [%]	19.7	20.5–45
Monocytes [%]	17.9	2.2–8
RBC, $\times 10^9/L$	5.2	3.5–6
Hb [g/dL]	14.8	12–17
MCV, fL	88.8	78–95
Platelets, $\times 10^9/L$	140	150–450

Parameter, units	Patient	Reference values
Bone marrow aspirate	16% atypical lymphocytes	
Biochemistry		
AST [U/L]	40.3	5–38
ALT [U/L]	50.8	0–41
GGT [U/L]	62	8–61
Urea [mmol/L]	5.3	2.8–7.5
Creatinine [mg/dL]	1.09	0.5–1.2
LDH [U/L]	547	135–225
Serological tests		
Antibody anti HBc – negative		
Antigen HBs – negative		
Antibody anti HCV – negative		
Antibody anti HIV1,2 –negative		
Antibody anti Toxoplasmosis IgG – negative		
Antibody anti EBV IgG positive >170 AU/mL (NV>22 AU/mL)		
Antibody anti EBV IgM negative		
WBC – White Blood Cells; RBC – Red Blood Cells; Hb – Hemoglobin; MCV – Mean Cell Volume; AST – Aspartate Aminotransferase; ALT – Alanine Aminotransferase; GGT – γ -Glutamyl Transpeptidase; LDH – Lactate Dehydrogenase; HBc – Hepatitis B core; HBs – Hepatitis B surface; HCV – Hepatitis C Virus; HIV – Human Immunodeficiency Virus; IgG – Immunoglobulin G; EBV – Epstein–Barr Virus; NV – Normal value; IgM – Immunoglobulin M.		

Morphology and immunohistochemistry

Quantitatively sufficient, formalin-fixed bone marrow biopsy material obtained from the right posterior iliac crest was decalcified in EDTA, embedded in paraffin and cut at 4–5 μ m for HE, Giemsa and Gömöri staining. Histopathologically, the tissue sample showed hypercellularity with presence of the trilineage hematopoiesis and mostly non-paratrabeular, interstitial and intrasinusoidal monotonous cell proliferation, characterized by irregular, medium-sized nuclei and rim of pale basophilic cytoplasm, loosely condensed nuclear chromatin with small inconspicuous nucleoli, admixed with eosinophils and histiocytes (Figure 1). Stroma was not interested by reticulin fibers proliferation.

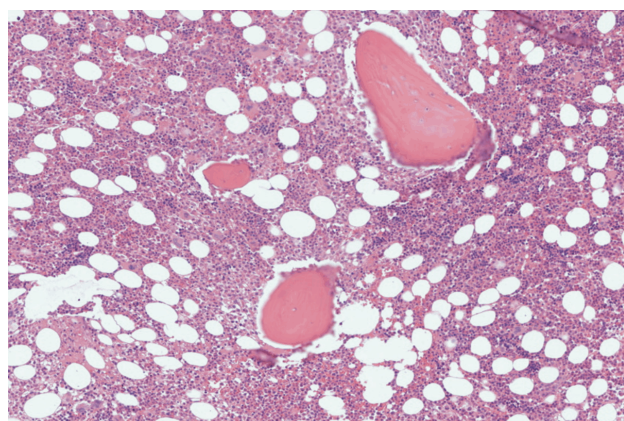


Figure 1 – Bone marrow involvement in HSTCL (intra-sinusoidal and interstitial pattern), visualized by HE staining (ob. 10 \times).

Immunophenotype

The immunophenotyping by flow cytometry performed from the bone marrow shows 20% atypical T-cell with the following immunophenotype: CD45+ (bright), CD3+ (downregulated), CD7+, CD2+, CD4-, CD8-, CD5-, CD1a-, CD45RO+, CD45RA-, CD25-. Several natural killer cell-associated antigens had variable expression CD56+ (50%), CD16+ (51%), CD11c+ (40%), CD11b+ (85%)

The interstitial and intrasinusoidal pattern of the multiplied cells, their morphological appearance, on a background maintained hematopoiesis, we focused to a lymphoproliferative process, confirmed by cell immunophenotyping.

Immunohistochemical stains was performed with LabVision Novocastra and Dako reagents, using: lineage restricted antigens identifying hematopoietic and lymphoid cells: MPO, CD61 (LabVision, 1:25, clone 2F2), Glycophorin A (LabVision, 1:300, clone JC159), CD20cy (Dako 1:400 clone L26), CD3 (LabVision, 1:200, clone SP7), CD4 (Dako, 1:80, clone 4B12), CD8 (Novocastra, 1:40, clone 295), CD56 (Novocastra, 1:75, clone 1B6), CD5 (LabVision, 1:50, clone Sp19), CD68 (Dako, 1:600, clone KP.1), and markers indicating cell differentiation stages: TdT (Dako, 1:40), CD99 (LabVision, 1:300, clone O13), CD34 (LabVision, 1:200, QBEnd/10) and CD117 (Dako, 1:500, clone c-kit). Bound antibodies were visualized using heat induced epitope retrieval method by the UltraVision LP Large Volume Detection System HRP Polymer detection system, DAB chromogen, followed by Hematoxylin counterstaining.

MPO, CD61, Glycophorin A highlighted the preserved hematopoiesis with CD117 and CD34 positive blasts less 1%. The interstitium involved non-hematopoietic cells showed a CD3 (Figure 2) and CD7 positivity, representing about 20% of all cells infiltrate that was only partially positive for CD56. Most of the cells were double negative for CD4/CD8 and CD5. Endothelial view by CD34 emphasized the intrasinusoidal component of the infiltrate. Absence of the TdT and CD99 positive cells excludes the precursor lymphoid cell origin. Stroma contains CD68 positive macrophages and reactive CD4 positive T-cells. Immunostain for Granzyme B was negative.

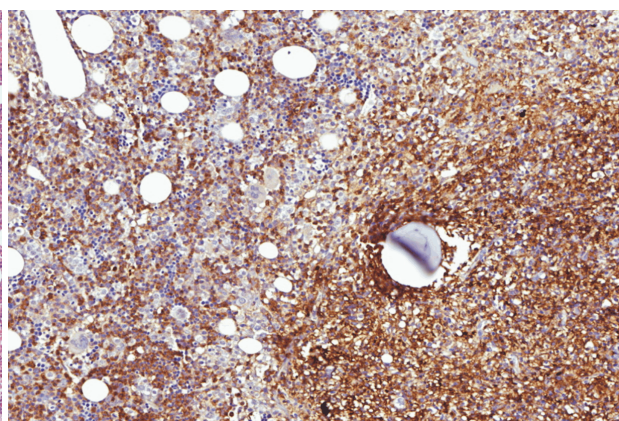


Figure 2 – Bone marrow: tumor cells express CD3 (DAB chromogen, ob. 10 \times).

and CD57-. The immature cell antigens were negative: CD34-, TdT-, CD99-. HLA-DR was negative, CD38- (Figures 3–6).

The results of immunohistochemistry and immunophenotyping pleaded for the diagnosis of mature T-cell lymphoma, hepatosplenic T-cell lymphoma, the cells being positive for CD3, CD2, CD7 and negative for CD8, CD4, CD5 with variable expression of CD56 antigen.

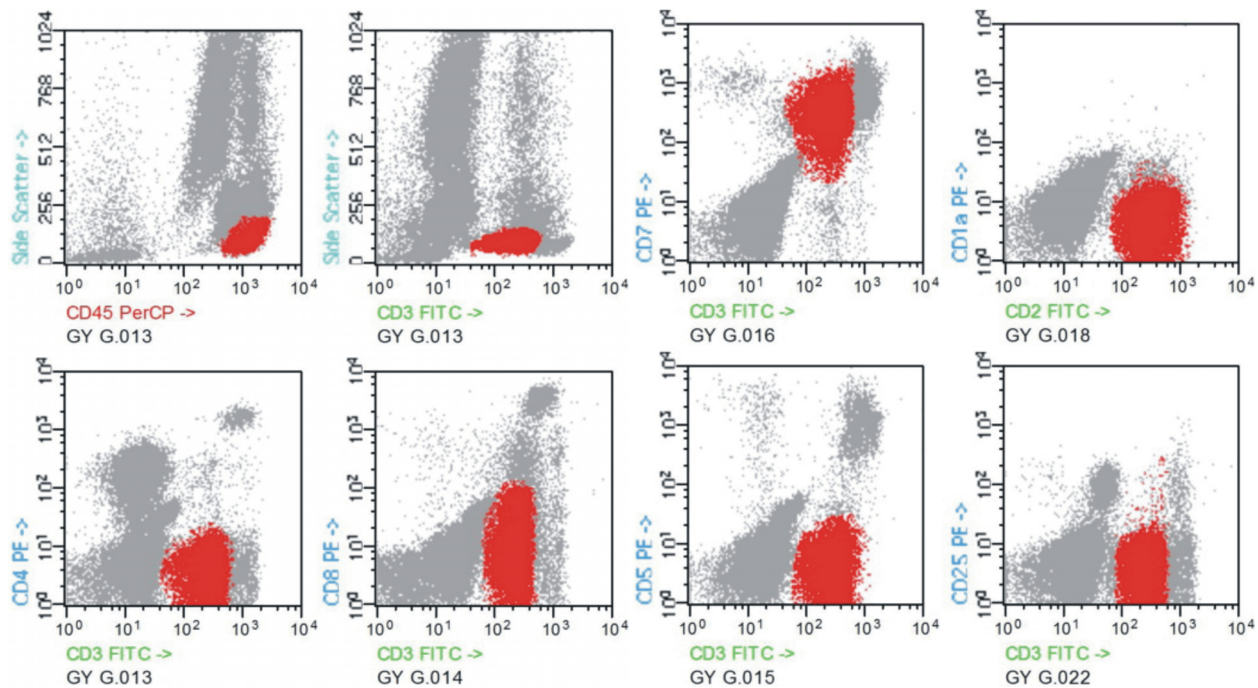


Figure 3 – HSTCL: neoplastic cells (red) are located in lymphocytes region on side scatter/CD45 dot plot. CD3 is downregulated compared with normal lymphocytes. CD4 and CD8 are negative.

Figure 4 – HSTCL: neoplastic cells (red) are CD7+, CD2+, CD5-, CD1a-, CD25-.

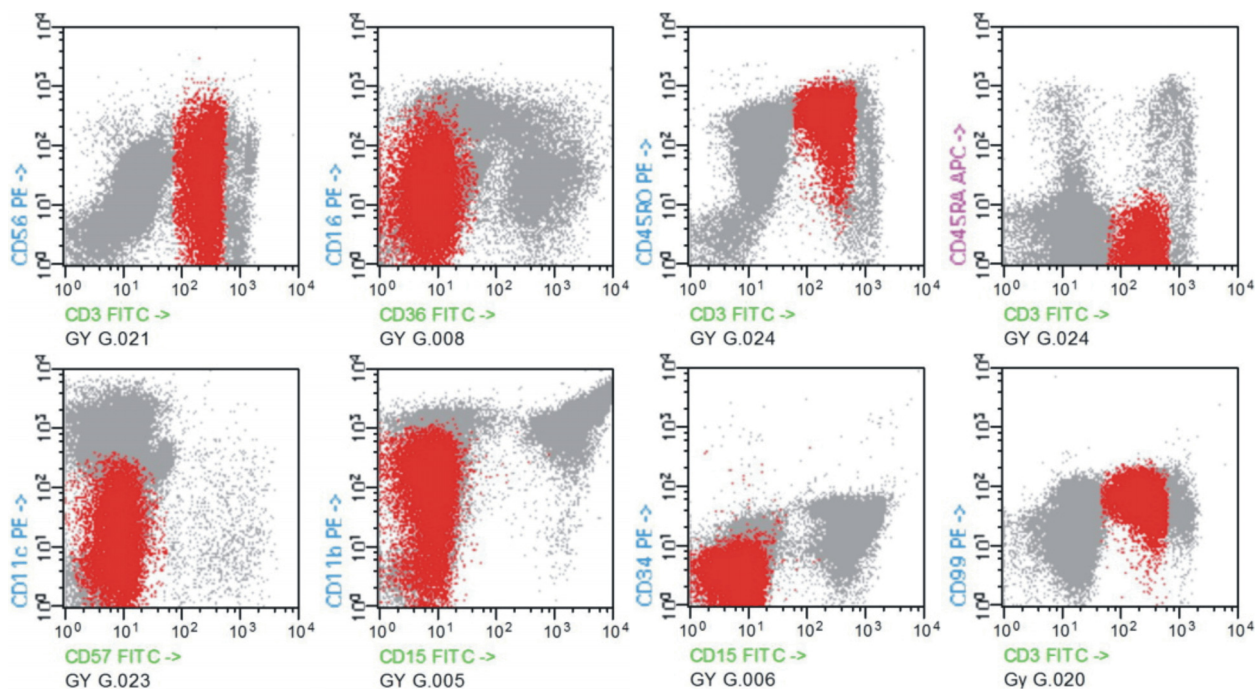


Figure 5 – HSTCL: neoplastic cells (red). Several natural killer cell-associated antigens had variable expression CD56+ (50%), CD16+ (51%), CD11c+ (40%), CD11b+ (85%) and CD57-.

Figure 6 – HSTCL: neoplastic cells (red) are CD45RO+, CD45RA-. The immature cell antigens were negative: CD34-, CD99-, TdT- (not shown).

Treatment and evolution

The treatment of the patient was started with CHOP regimen at 28-day intervals. The evolution was not favorable due to fact that after two weeks following the CHOP regimen the spleen enlargement reappeared and became quite invalidating to the patient. Fever and B-symptoms disappeared.

After four courses, the patient was referred to the

Bone Marrow Transplantation Unit in Tîrgu Mureș, Romania, for reevaluation and mobilization of stem cells for autologous transplantation.

At presentation to our clinic, the patient was febrile presenting leukocytosis, thrombocytopenia and anemia and important splenomegaly with a tumoral size spleen causing important abdominal discomfort. We administered the DHAP regimen (Cisplatin, Cytosar, Dexamethasone)

followed by administration of G-CSF (granulocyte colony stimulating factor) 10 µg/kg. The evolution after the regimen was clinically very favorable with important involution of the spleen size and the disappearance of the B-symptoms. The mobilization of the hematopoietic stem cells was late. The CD34+ cell count raise on day 14–16 post-DHAP + G-CSF regimen instead of day 10–12.

We obtained a number of 4.7×10^6 CD34+ cells/kg that permitted us to perform autologous stem cell transplantation. We decided to perform the pre-transplant

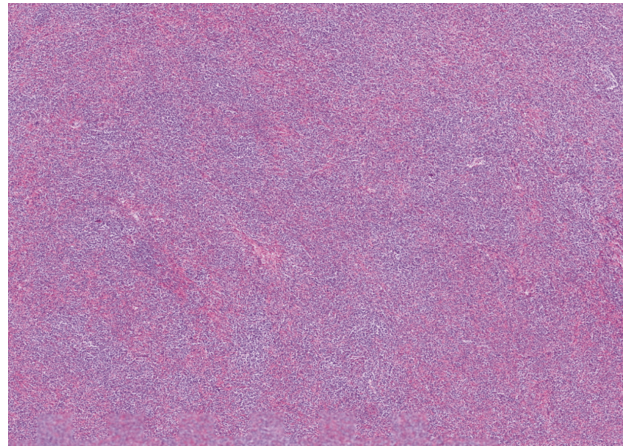


Figure 7 – Spleen with normal white pulp and expanded red pulp. The tumor cells involve cords and sinuses (HE staining, ob. 4×).

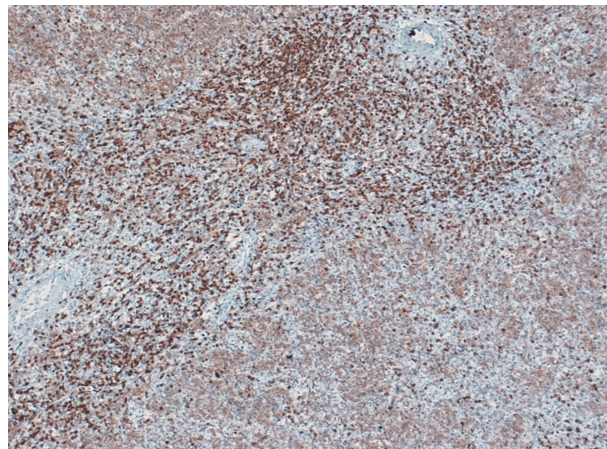


Figure 9 – The tumor cells express CD7 (DAB chromogen, ob. 10×).

The postoperative evolution was favorable with no fever or infections but one-month post-surgery fever reappeared and the hematological examination revealed a high white blood count $65\,000 \times 10^6/L$ with low platelet count and anemia. The examination of the peripheral blood immunophenotyping revealed a percent of 25% of malignant T-cells, which lead us to the conclusion of leukemic transformation.

The strategy of treatment had to be changed from performing autologous stem cell transplantation preceded by standard BEAM conditioning (Busulfan, Cytosar, Etoposide, Melphalan) [7] to treatment of the leukemic transformation before performing the autologous transplant. He received the induction treatment for T-cell leukemia. The therapeutic response was partial with the 4% of atypical cells.

splenectomy because three weeks following this “salvage” DHAP protocol the spleen enlarged again and the thrombocytopenia persisted.

The morphological examination of the enlarged spleen (600 g) showed normal white pulp and expanded red pulp, without any gross lesions. The tumor cells involve cords and sinuses (Figure 7). Microscopic findings were similar to bone marrow involvement, showing the characteristic pattern of hepatosplenic T-cell lymphoma (Figures 8 and 9).

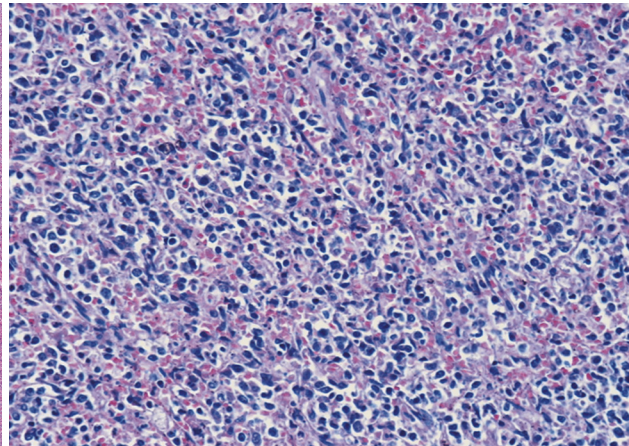


Figure 8 – Spleen: monomorphic infiltrate composed of medium to large lymphocytes with slightly irregular nuclei and partially dispersed chromatin (HE staining, ob. 20×).

For the purpose of conditioning and due to the lack of a compatible HLA identical sibling donor, we decided to do the standard Bu–Cy high dose conditioning regimen (Busulfan 16 mg/kg, Cyclophosphamide 200 mg/kg) [7] and perform the autologous stem cell transplant.

The transplantation was performed four weeks ago with a very difficult evolution due to grade IV mucositis, enteral infection, repeated episodes of melena due to the very late engraftment for platelets (on day 21). The first bone marrow aspirate control four weeks post transplant showed only 1.5% atypical T-cells from total number of nucleated cells. In present, the patient is in a stable clinical condition but he needs a very through follow up because of the very high rate of relapse. We start search for unrelated donor for allogeneic stem cell transplant in case of relapse this is being the only way of treatment that can save the patient.

Discussion

Positive diagnosis is based on the combined evaluation of the clinical aspects, morphological and immunophenotypic results.

The HE staining based morphological aspect of the bone marrow is not suggestive for diagnosis, because intrasinusoidal and interstitial pattern characterized splenic B-cell marginal zone lymphoma too. After determining T-cell origin, HSTCL must be distinguished from T-lineage lymphoblastic lymphoma (T-LBL) with frequent extramedullary involvement for spleen and liver. T-LBL can be excluded based on pattern of distribution and staining (lack of TdT and CD99). Differential diagnosis may be difficult without immunohistochemistry and flow

cytometry [8]. Immunostaining for CD3, CD7, CD56 and Granzyme B is useful in highlighting the interstitial and focal intrasinusoidal infiltration of tumor cells in the bone marrow biopsy [9].

Immunophenotyping is essential for diagnosis of HSTCL. The determination of clonality of mature T-cell lymphoproliferative disorders (T-cell LPD) is based on detection of aberrant antigenic profile. The main abnormality is the lack and/or aberrant expression (down- or up-regulated) of one or more pan-T-cell antigens, CD3, CD5, CD2, CD7. [10] The most frequently phenotype of HSTCL is CD3+, CD4-, CD8-, CD2+, CD5-, CD7+. In some cases, CD8 may be positive. Several natural killer cell-associated antigens CD56, CD16, CD11c, CD11b are frequently positive, but CD57 is negative [11]. In our case, the immunophenotype of the malignant cells had a similar pattern described in literature. The morphology of the neoplastic cells and the CD3 positivity excluded NK-cell lymphoma. In spite of the early diagnosis and therapy, the patient presented leukemic transformation. In disease progression, the blastic form of lymphoma cells may appear in the peripheral blood [10]. The immature cell antigens CD34, TdT, CD99 are useful to distinguish T-cell LPD in leukemic transformation from T-cell acute lymphoblastic leukemia (T-cell ALL). Another helpful feature is the location of malignant population on the side scatter/CD45 dot plot. In T-cell ALL, the neoplastic cells are in the blast region with lower expression of CD45, in T-cell LPD the abnormal cells are in the lymphocyte region with brighter expression of CD45 [11]. In our case, the malignant cells were located in the lymphocyte region and was negative for CD34, TdT, CD99 confirmed also by immunohistochemistry.

Hepatosplenic T-cell lymphoma is knowingly a very aggressive disease. We intended to present this difficult case because of its complexity and because of fast and unfavorable evolution. The partial and short response to CHOP (Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) chemotherapy showed the necessity of more aggressive chemotherapy. This is the reason why we administered DHAP salvage chemotherapy followed by stem cell collection. Splenectomy was necessary for the recovery of platelets and for histopathological examination for adequate positive diagnosis to exclude other types of lymphoma. Due to the particular evolution of the case with leukemic transformation, we administrated T-cell leukemia protocol to obtain remission.

In lymphomas, we usually use BEAM protocol for conditioning [6]. In this case, due to the leukemic transformation we administered the Bu-Cy high-dose protocol this being the conditioning treatment for acute

leukemia to obtain a durable response. In case of relapse, the only way to rescue this patient is to perform the unrelated allogeneic hematopoietic stem cell transplantation [5].

Conclusions

In the presented case, we could observe positive correlation between morphological, immunophenotypic and clinical features of the rare T-cell lymphoma that had a quick, unfavorable leukemic evolution rescued until the present time by autologous stem cell transplantation.

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