SOLVING CLINICAL PROBLEMS IN BLOOD DISEASES

A physician or group of physicians considers presentation and evolution of a real clinical case, reacting to clinical information and data (boldface type). This is followed by a discussion/commentary.

Positive HIV ELISA test, autoimmune hemolytic anemia, and generalized lymphadenopathy: A unifying diagnosis

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A 41-year-old Caucasian man with a medical history of hypertension and an Eastern Cooperative Oncology Group performance status of 0 presented with abdominal pain, fatigue, and 10-pound weight loss for 2 months. He denied fevers or night sweats. On physical exam, the patient had bilateral inguinal lymphadenopathy up to 2 cm in diameter. Computed tomography (CT) scans revealed lymphadenopathy above and below the diaphragm and mild splenomegaly. Hemoglobin level was 13 g dL^{-1} (normal range: 13.5–16.0 g dL^{-1}). The patient underwent an excisional biopsy of a left cervical lymph node and was discharged in stable condition.

At this time, the differential diagnosis in this mildly anemic young man with weight loss and lymphadenopathy is very broad but includes infections and autoimmune processes. Oncologic conditions, such as lymphomas, should also be evaluated.

He represented 1 week later with severe fatigue and hypotension. The patient had a hemoglobin of 5.5 g dL^{-1}, white blood cell count of 23.7 $\times$ 10^9 L^{-1} (normal range: 3.5–11.0 $\times$ 10^9 L^{-1}), and platelet count of 123 $\times$ 10^9 L^{-1} (normal range: 150–400 $\times$ 10^9 L^{-1}). The differential showed 55% neutrophils, 6% bands, 20% lymphocytes, 11% monocytes, 4% eosinophils, and 2% metamyelocytes. Patient denied hemoptysis, melena, or bright red blood per rectum. Two units of packed red blood cells (RBCs) were transfused with minimal increase in his hemoglobin levels.

The hemoglobin level went from 13 to 5.5 g dL^{-1} in 1 week; this is an acute process. The differential includes acute bleeding or hemolysis. His blood counts also show leukocytosis and thrombocytopenia. Given the lack of response to transfusions, this could be an immune-mediated hemolytic phenomenon either primary or associated with an infectious process, such as acute EBV or *Mycoplasma* infection. The patient should also be worked up for gastrointestinal bleeding, although it is less likely the cause of such a marked decrease in his hemoglobin without evidence of frank hematochezia or melena.

Additonal workup showed an elevated lactate dehydrogenase (LDH) level (663 IU L^{-1}), reticulocytosis (8.2%), and undetectable haptoglobin (<5.8 mg dL^{-1}). A peripheral blood smear did not show schistocytosis or spherocytosis but revealed circulating myelocytes, metamyelocytes, nucleated RBCs, and RBC agglutination. Fecal occult blood test was negative.

This presentation is consistent with an acute acquired hemolytic process. The age of the patient makes congenital causes of hemolysis unlikely. The differential diagnosis includes autoimmune or drug-induced hemolysis and cryoglobulinemia. The presence of RBC agglutination supports the possibility of cryoglobulinemia. The lack of schistocytes does not support a microangiopathic process such as thrombotic thrombocytopenic purpura or disseminated intravascular coagulation. The concurrent presence of myeloid and erythroid precursors suggests a myelophthisic anemia, which could be associated with intramedullary hemolysis.

Direct Coombs test was positive for immunoglobulin G (IgG) and complement. A strong IgM cold autoantibody was also identified. These findings were consistent with warm and cold autoimmune hemolytic anemia (AIHA). A Human Immunodeficiency virus (HIV) ELISA drawn previously was reported as positive; however, his wife and he denied any behavioral risks. Serologic tests for *Mycoplasma*, hepatitis A, hepatitis B, hepatitis C, Cytomegalovirus, Herpes Simplex virus, Varicella zoster virus and toxoplasma were nonreactive. Epstein Barr virus (EBV) IgM was negative but IgG was elevated consistent with a past infection. His Ig levels were IgG 2,190 mg dL^{-1} (normal range: 562–1,585 mg dL^{-1}), IgM 855 mg dL^{-1} (normal range: 30–246 mg dL^{-1}), and IgA 509 mg dL^{-1} (normal range: 72–372 mg dL^{-1}), but no M-spike was detected by serum and urine protein electrophoresis. The patient was started on a plasmapheresis and steroids to treat cold and warm AIHA, respectively.

These laboratory data support a diagnosis of a cold and warm AIHA in this patient with a positive HIV ELISA test. Several studies have shown an association between HIV infection and an abnormal Coombs test [1]; however, the relationship with overt AIHA is unclear and only a handful of cases have been reported [2]. As a sensitive test, the HIV ELISA is used for screening purposes but a Western blot test should be ordered to confirm a current HIV infection as the ELISA can be falsely positive. In fact, lymphomas and systemic lupus erythematosus, among others, can be associated with false-positive HIV ELISA tests. In patients with AIHA, warm autoantibody production can be idiopathic; however, cold antibodies are usually associated with other processes, including lymphoproliferative disor-

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ders, which should be evaluated in this patient with a positive HIV ELISA test and lymphadenopathy, as the risk of developing aggressive B-cell lymphomas (BCLs) is higher in HIV-infected individuals. The differential diagnosis for this patient's hypergammaglobulinemia also includes lymphoma, HIV infection, and autoimmune conditions. I believe the patient's lymph node excisional biopsy will be helpful on evaluating this case further.

Pathologic findings from the excisional lymph node biopsy showed a preserved nodal architecture with scattered reactive cortical lymphoid follicles, occasionally surrounded by clear monocytoid cells (Fig. 1A,B). There was focal expansion of the paracortex by a mixed population of small lymphocytes, histiocytes, plasma cells, and immunoblasts (Fig. 1C). Paracortical vascular proliferation was also identified. Immunostains for CD20 highlighted germinal center B-cells and few paracortical immunoblasts (Fig. 1D). Numerous CD10-positive cells were seen in a perifollicular distribution (Fig. 1E) that coexpressed CD3 (Fig. 1F) and CD4 (Fig. 1G), but were negative for CD5, CD7, CD8 (Fig. 1H), BCL6, and MUM1/IRF4. The paracortical regions demonstrated a moderately high proliferation index (Ki-67 $\sim$ 60%; Fig. 1I). Anaplastic lymphoma kinase and EBV latent membrane protein-1 were negative. Flow cytometry detected an aberrant T-cell population with loss of CD7 and coexpression of CD10 and CD25. Polymerase chain reaction (PCR) studies revealed two minor T-cell receptor (TCR) beta bands and one TCR gamma band as well as genomic EBV. PCR for IgH gene rearrangements was negative. The morphologic, immunophenotypic, and molecular findings were consistent with angioimmunoblastic T-cell lymphoma (AITL).

AITL is indeed a unifying diagnosis in this challenging case. AITL can present with protean symptoms. In this case, this patient's lymphadenopathy, weight loss, hypergammaglobulinemia, and AIHA can be fully explained by AITL. From the pathological perspective, AITL is a T-cell lymphoma that frequently develops with a coincident population of EBV-positive, CD20-positive B-cells that can be clonal, posing a diagnostic difficulty in which AITL is confused with a T-cell-rich BCL. An additional concern is the possibility of a concurrent diffuse large BCL (DLBCL) occasionally seen in patients with AITL; however, in this case, there was an absence of sheets of large CD20-positive cells and clonal Ig heavy chain gene rearrangements were not identified by PCR testing. Additionally, the coexpression of CD3 and CD4 with loss of CD7 and the presence of TCR gene rearrangements in the malignant cells strongly support a T-cell lymphoma. The presence of CD10 and the morphological features such as paracortical vascular proliferation are consistent with AITL. The relationship between AITL and a positive HIV ELISA in this case is intriguing.

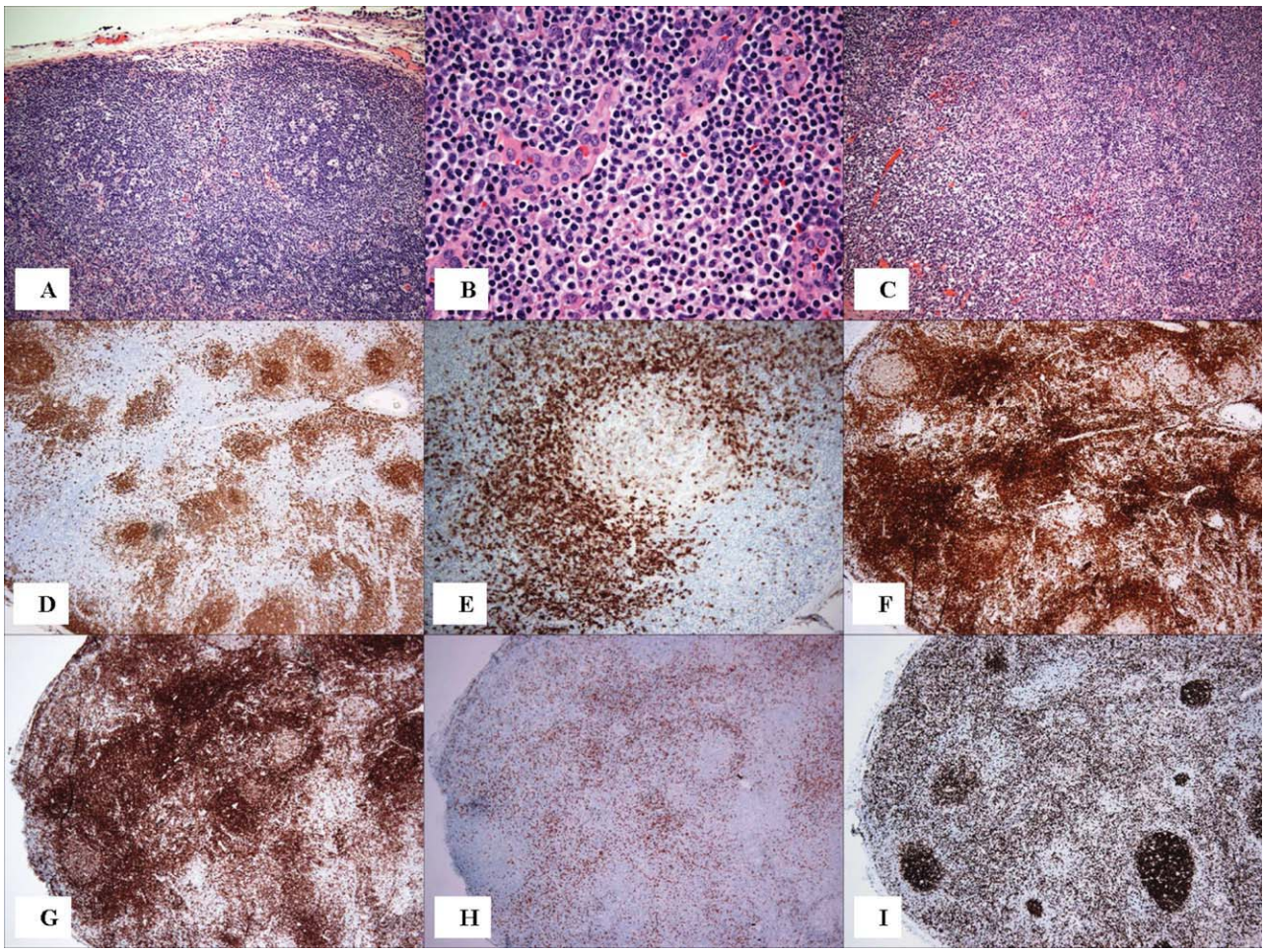

Figure 1. Pathological characteristics of a representative case of angioimmunoblastic T-cell lymphoma [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Although HIV infection has been mostly associated to the development of aggressive BCLs, there may be up to a 15-fold increase in the risk of T-cell lymphomas [3]. Nonetheless, T-cell lymphomas are a rare occurrence in HIV-infected individuals with less than a 100 cases reported in the literature. Furthermore, in a recent series of 51 patients with HIV-associated T-cell lymphoma, only two patients had AITL [4]. Based on this patient's diagnosis, staging and risk-stratification should include CT scans of the chest, abdomen, and pelvis, bone marrow biopsy and aspirate, LDH levels and an evaluation of cardiac function.

A bone marrow biopsy revealed marked hypercellularity (95%) with trilineage hyperplasia. Few interstitial lymphoid aggregates were noted consisting of a mixture of CD20+ B-cells and CD3+/CD5+ T-cells with partial loss of CD7 in T-cell clusters. Molecular studies performed on bone marrow biopsy were negative for TCR gene rearrangement. The patient was considered stage IV due to a likely bone marrow involvement by AITL. His International Prognostic Index (IPI) score was high-intermediate at 3. His Prognostic Index for Peripheral T-cell Lymphoma (PIT) score was 2. CT scans done previously showed generalized adenopathy without apparent extranodal involvement. His LDH levels had decreased to 412 IU L^{-1}. The HIV Western blot came back negative. His $CD4^+$ cell count and $CD4^+/CD8^+$ ratio were normal, and his HIV viral load was undetectable.

Bone marrow involvement by lymphoma affects staging and risk-stratification. In this case, the results from the bone marrow biopsy were not definitive; however, the degree of hypercellularity and the loss of CD7 expression in the lymphoid aggregates make a strong case for lymphomatous involvement. The IPI score does not use bone marrow involvement per se as a prognostic factor but bone marrow involvement is considered an extranodal site and would categorize a patient as stage IV, an adverse prognostic factor in the IPI [5]. On the other hand, the PIT score includes bone marrow involvement as one of the prognostic factors [6]. In DLBCL, bone marrow involvement has been associated with central nervous system affection and prophylactic intrathecal chemotherapy could be administered; however, this is not a common practice for T-cell lymphomas. Interestingly, his HIV Western blot was negative, and there was no additional evidence of HIV infection. To the best of our knowledge, only one case in the literature had reported a false-positive HIV ELISA test in the setting of AITL [7]. Nonetheless, HIV testing should be routinely done in any patient with a diagnosis of lymphoma.

Because of the presence of a heavy background of CD20-positive B-cells and ongoing AIHA, the patient was started therapy with rituximab cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), recovering dramatically after one cycle, and being discharged in stable condition. He completed six cycles of R-CHOP as an outpatient. His only complication was a grade 2 peripheral neuropathy. A possitron emission tomography (PET)/CT scan done after finishing R-CHOP showed no evidence of 18-F fluorodeoxyglucose-avid disease. His HIV ELISA was negative and bone marrow biopsy demonstrated no abnormalities. The patient was then referred for autologous stem cell transplantation (ASCT) in first remission. He was mobilized with subcutaneous filgrastim and plerixafor. The conditioning regimen consisted of cyclophosphamide, carmustine, and etoposide, without total body irradiation (TBI). He experienced no major complications during his hospital course and has since done well without evidence of recurrent disease 24 months after diagnosis.

Discussion

AITL was initially described in 1974 by Frizzera et al. [8] and Lukes et al. [9]. The 2008 WHO classification recognizes AITL as a distinct subtype of PTCL, accounting for approximately 20% of the PTCL cases [10]. The incidence of AITL varies depending on the geographic location and it seems to be more frequent in Europe than in North America [11]. Clinically, AITL usually presents with extranodal involvement, rash, arthralgias, polyclonal hypergammaglobulinemia, and autoimmune phenomena [10]. The cell of origin seems to be a follicular helper T-cell (TFH) [10]. TFHs overexpress CXCR5 and CXCL13 mediating B-cell recruitment into the lymph node [10]. These B-cells expand and become activated and can undergo EBV-mediated transformation [10]. Importantly, there is no standard approach to AITL. In fact, the National Comprehensive Cancer Network recommends a clinical trial for the therapy of AITL and other PTCLs [12]. Complete response (CR) rates to chemotherapy have ranged between 20 and 60% with a median survival of approximately 30 months [11,13].

Our patient presented with several concurrent autoimmune phenomena, likely related to his AITL. Our patient was diagnosed with AIHA, mediated by both warm and cold autoantibodies, and a false positive HIV ELISA test. The HIV ELISA and direct Coombs tests became negative after four cycles of chemotherapy, presumably associated to the achievement of a CR after chemotherapy.

Because of the presence of an autoimmune hemolytic process and an extensive CD20-positive background in the pathological sample, the patient was treated with the combination of rituximab and CHOP. The use of rituximab in AITL is rather controversial. Although preliminary studies suggested a potential benefit [14,15], a recent multicenter phase II study including 25 patients with AITL treated with R-CHOP did not confirm these findings [16]. However, only six patients (24%) in that study had a CD20-positive background, as our case did. Also, it is unclear how many patients presented with concurrent autoimmune conditions. The authors concluded R-CHOP did not improve the response rates in patients with AITL but further studies to delineate biological subgroups are ongoing.

The role of ASCT in PTCL is an evolving field. Because of a high-risk disease reflected by a PIT score of 2 and an IPI score of 3, our patient was referred for an ASCT in first remission. We found three retrospective studies looking at the role of ASCT in AITL patients, accounting for a total of 130 patients receiving ASCT in first remission [17–19]. Although retrospective studies carry inherent biases, the existing data appear to support the use of high-dose chemotherapy followed by ASCT in first remission with 5-year overall survival (OS) rates of 50–60%. Furthermore, in a recent prospective study, which included 27 patients with AITL, the authors concluded that ASCT in first remission should be considered in patients with aggressive PTCL [20]. The study reported a 3-year OS rate of 48%. Of note, only two thirds of the patients underwent ASCT in this study. The main reason for not undergoing ASCT was disease progression. The achievement of a CR before ASCT is a strong predictor of outcome across studies and primary refractory patients seem to derive little benefit, if any. A recent study suggests that allogeneic stem cell transplantation could be of value in patients with AITL with a 3-year OS rate of 64% [21]. However, the optimal type of transplantation for AITL remains undefined. A multicenter randomized controlled trial comparing allogeneic versus ASCT in patients with PTCL in first remission is ongoing. Of note, TBI was not used as part of the conditioning regi-

men in this patient. Although TBI was included in many of the studies evaluating ASCT in the management of PTCL, it is not without an increased risk; a retrospective study reported a 20% 10-year incidence of secondary cancers with an associated mortality of 10% [22].

In conclusion, AITL can present with concurrent autoimmune processes, in this case, AIHA and a false positive HIV ELISA test. However, these can improve with lymphoma therapy. Although there is not a standard of care for patients with AITL, this patient achieved a CR with R-CHOP, which was followed by high-dose chemotherapy without TBI and ASCT in first remission, and continues without evidence of disease 24 months after diagnosis. Further research is needed to delineate guidelines to treat patients with this challenging lymphoma.

References

1. Saif MW. HIV-associated autoimmune hemolytic anemia: An update. AIDS Patient Care STDS 2001;15:217–224.
2. Koduri PR, Singa P, Nikolinakos P. Autoimmune hemolytic anemia in patients infected with human immunodeficiency virus-1. Am J Hematol 2002;70:174–176.
3. Biggar RJ, Engels EA, Frisch M, et al. Risk of T-cell lymphomas in persons with AIDS. J Acquir Immune Defic Syndr 2001;26:371–376.
4. Castillo JJ, Beltran BE, Bibas M, et al. Prognostic factors in patients with HIV-associated peripheral T-cell lymphoma: A multicenter study. Am J Hematol 2011;86:256–261.
5. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993;329:987–994.
6. Gallamini A, Stelitano C, Calvi R, et al. Peripheral T-cell lymphoma unspecified (PTCL-U): A new prognostic model from a retrospective multicentric clinical study. Blood 2004;103:2474–2479.
7. Muta T, Yamano Y. Angioimmunoblastic T-cell lymphoma associated with an antibody to human immunodeficiency virus protein. Int J Hematol 2003;78:160–162.
8. Frizzera G, Moran EM, Rappaport H. Angio-immunoblastic lymphadenopathy with dysproteinaemia. Lancet 1974;1:1070–1073.
9. Lukes RJ, Tindle BH. Immunoblastic lymphadenopathy. A hyperimmune entity resembling Hodgkin's disease. N Engl J Med 1975;292:1–8.
10. Dogan A Gaulard P, Jaffe ES, et al. Angioimmunoblastic T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC; 2008:309–311.
11. Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: Pathology findings and clinical outcomes. J Clin Oncol 2008;26:4124–4130.
12. National Comprehensive Cancer Network. NCCN Guidelines Version 1.2011. Peripheral T-cell Lymphoma. TCEL-3. Available at http://www.nccn.org/professionals/physician_gls/PDF/nhl.pdf, accessed September 3, 2011.
13. Alizadeh AA, Advani RH. Evaluation and management of angioimmunoblastic T-cell lymphoma: A review of current approaches and future strategies. Clin Adv Hematol Oncol 2008;6:899–909.
14. Joly B, Frenkel V, Belhadj K, et al. Rituximab in combination with CHOP regimen in T-cell angioimmunoblastic lymphoma (AILD-TL) rich in large B cells. Favourable results in four patients. ASCO Meeting Abstracts 2004;22(Suppl 14):6694.
15. Joly B, Frenkel V, Gaulard P, et al. Rituximab in Combination with CHOP regimen in angioimmunoblastic T-Cell lymphoma (AITL). Preliminary results in 9 patients treated in a single institution. ASH Annual Meeting Abstracts 2005;106:2686.
16. Joly B, Plonquet A, Grare M, et al. Rituximab in combination with CHOP regimen in angioimmunoblastic T-cell lymphoma: Results of the phase II RAIL trial—A prospective study of the Groupe d'Etude des Lymphomes de l'Adulte (GELA). ASCO Meeting Abstracts 2010;28(Suppl 15):8049.
17. Schetelig J, Fetscher S, Reichle A, et al. Long-term disease-free survival in patients with angioimmunoblastic T-cell lymphoma after high-dose chemotherapy and autologous stem cell transplantation. Haematologica 2003;88:1272–1278.
18. Rodriguez J, Conde E, Gutierrez A, et al. Prolonged survival of patients with angioimmunoblastic T-cell lymphoma after high-dose chemotherapy and autologous stem cell transplantation: The GELTAMO experience. Eur J Haematol 2007;78:290–296.
19. Kyriakou C, Canals C, Goldstone A, et al. High-dose therapy and autologous stem-cell transplantation in angioimmunoblastic lymphoma: Complete remission at transplantation is the major determinant of Outcome-Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol 2008;26:218–224.
20. Reimer P, Rudiger T, Geissinger E, et al. Autologous stem-cell transplantation as first-line therapy in peripheral T-cell lymphomas: Results of a prospective multicenter study. J Clin Oncol 2009;27:106–113.
21. Kyriakou C, Canals C, Finke J, et al. Allogeneic stem cell transplantation is able to induce long-term remissions in angioimmunoblastic T-cell lymphoma: A retrospective study from the lymphoma working party of the European group for blood and marrow transplantation. J Clin Oncol 2009;27:3951–3958.
22. Brown JR, Yeckes H, Friedberg JW, et al. Increasing incidence of late second malignancies after conditioning with cyclophosphamide and total-body irradiation and autologous bone marrow transplantation for non-Hodgkin's lymphoma. J Clin Oncol 2005;23:2208–2214.