Introduction

Intravascular large-B cell lymphoma (IVBL) [1], as per World Health Organization (WHO) classification, is a diffuse large B-cell lymphoma featuring by the presence of lymphoma cells exclusively within intravascular spaces. The histopathologic findings are subtle due to the rarity of the neoplastic cells in blood vessels. Clinical presentations are non-specific and focal space-occupying lesions or lymphadenopathy are always lacking. It is a diagnostic challenge. Secondary hemophagocytic syndrome is uncommon and is typically associated with infection, malignancy, and suppressed immune states. Intravascular lymphoma has a strong association with hemophagocytic syndrome in Asian patients, the so-called "Asian variant", but not in Western patients. We report a case of intravascular B-cell lymphoma in a Caucasian patient associated with secondary hemophagocytic syndrome. The patient was diagnosed by core liver biopsy and successfully treated. This case demonstrates the importance of high index of suspicion and astute histopathologic examination in recognition of this unusual clinical and pathologic combination.

Keywords: Intravascular lymphoma, hemophagocytic syndrome, liver, Asian variant, hepatosplenomegaly

Case Report

Intravascular large B-cell lymphoma with hemophagocytic syndrome (Asian variant) in a Caucasian Patient

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Abstract: Intravascular lymphoma is an aggressive and extremely rare extranodal lymphoma with neoplastic lymphoid cells confined exclusively within intravascular spaces. The histopathologic findings are subtle due to the rarity of the neoplastic cells in blood vessels. Clinical presentations are non-specific and focal space-occupying lesions or lymphadenopathy are always lacking. It is a diagnostic challenge. Secondary hemophagocytic syndrome is uncommon and is typically associated with infection, malignancy, and suppressed immune states. Intravascular lymphoma has a strong association with hemophagocytic syndrome in Asian patients, the so-called "Asian variant", but not in Western patients. We report a case of intravascular B-cell lymphoma in a Caucasian patient associated with secondary hemophagocytic syndrome. The patient was diagnosed by core liver biopsy and successfully treated. This case demonstrates the importance of high index of suspicion and astute histopathologic examination in recognition of this unusual clinical and pathologic combination.

Keywords: Intravascular lymphoma, hemophagocytic syndrome, liver, Asian variant, hepatosplenomegaly
not Western patients (the so-called "Asian variant"). Interestingly, our patient is a Caucasian. The combination of features in this case made it diagnostically challenging.

Clinical history

The patient was a 51 year-old Caucasian male who has no Asian or Eskimo lineage but a small fraction of Choctaw lineage. He presented with night sweats, daily fevers up to 103°F, 20 lb weight loss, and progressive fatigue. During hospitalization, his transaminases elevated to the range between 400-500 U/L (AST 453 U/L, normal 7-40 U/L; ALT 410 U/L, normal 10-45 U/L). He also developed progressive anemia requiring weekly blood transfusion and also thrombocytopenia. Lymphocytes and polymorphonuclear leukocytes were not increased in his peripheral blood. The patient also had markedly elevated ferritin to 5,977 ng/mL (normal 10-240 ng/mL), elevated lactose dehydrogenase, triglycerides, and total bilirubin. He underwent an exhaustive infectious disease work-up including viral hepatitis, cytomegalovirus, Herpes Simplex Virus 1 and 2, human immunodeficiency virus, parvovirus B19, mycoplasma, Lyme disease, Ehrlichiosis and Rocky Mountain spotted fever, and fungal infections. All were negative. Blood cultures for bacteria were negative and no significant findings were noted on chest imaging studies. No evidence of active tuberculosis was discovered by culture and chest x-ray. His IgM level for Epstein-Barr virus was negative but polymerase chain reaction for Epstein-Barr virus was minimally positive (DNA quantitative study result of 100 copies/mL; normal is undetectable). Serum protein electrophoresis did not show evidence of an M-spike. Arsenic, lead, and mercury levels were all within normal limits. Imaging studies for evaluation of malignancy did not reveal any focal lesions. During the later period of his first admission, the patient began to develop arthralgias and swollen joints, prompting an extensive rheumatologic workup that was noncontributory.

On his second admission 5 weeks after his initial presentation, repeated imaging revealed massive hepatosplenomegaly. Positron emission tomography-computerized tomography (PET-CT) demonstrated diffuse hypermetabolic activity with significant fluorodeoxyglucose-18F (FDG) uptake extending from the level of the left diaphragm through the subdiaphragmatic space and continuous into the spleen. The spleen was massively enlarged (14.8 x 10.4 x 23.0 cm) with a peak SUV of 9.8. There were also small amounts of pleural and pericardial fluid collections without elevated FDG uptake. Computerized tomogram (CT) with contrast enhancement (Figure 1) demonstrated hepatosplenomegaly with massively enlarged spleen and multiple well demarcated lesions with the largest one measuring 3.1 cm in greatest dimension. These lesions were hypometabolic and interpreted as splenic infarcts. Imaging studies did not demonstrate enlarged lymph nodes or evidence suggestive of mass forming occupying neoplastic diseases.

A diagnosis of secondary HPS was made with uncertain underlying etiology. His clinical condition continued to deteriorate. His serum ammonia level elevated to 47 µmol/L (normal 11-32 µmol/L) suggesting decline of liver functions and he developed mental impairment suggesting hepatomecephalopathy. A bone marrow and percutaneous core liver biopsy were performed as diagnostic procedures.

Pathology

Studies performed

Hematoxylin and eosin stain was performed on both bone marrow and liver biopsy; reticulin stain, Prussian blue stain, periodic acid Schiff (PAS) stain with and without diastase digestion,
and Masson's trichrome stain were performed on liver biopsy. Immunohistochemistry was performed with a Ventana Benchmark automated staining machine (Ventana, Tucson, AZ) with antibodies for CD3 (Clone 2G6, Ventana), CD5 (Clone SP19, Ventana), CD20 (Clone 265, Ventana), CD163 (Clone 10D6, Thermo Scientific, Fremont, CA), and ALK-1 (Clone ALK1, Dako, Glostrup, Denmark). Diaminobenzidine was used as chromogen with light hematoxylin counter stain. Flow cytometry was performed on both liver and bone marrow biopsies. Cytogenetic study for karyotype was performed in the bone marrow biopsy.

Liver biopsy

Wide spread expansion of sinusoid by Kupffer cells was present throughout every core with little variation among different zones of the hepatic lobules (Figure 2A). Large atypical cells histologically suggestive of neoplastic lymphoid cells in the form of scant and scattered single cells or small clusters were present within the sinusoid randomly distributed in all the biopsy cores (Figure 2A). While some of these atypical lymphoid cells appeared attached to the sinusoid (Figure 2B), others appeared free floating within the sinusoid (Figure 2C). These atypical lymphoid cells demonstrated a range of pleomorphic changes (Figure 2B, inset). A small subset of these cells, typically as scattered single cells, was multinucleated. There were also occasional foci of neutrophilic infiltration (Figure 2B). No granulomas were identified. Trichrome stain demonstrated mild increase in collagenous fibers in the central vein with short extension into the surrounding sinusoid (Figure 2D) but neither bridging fibrosis nor cirrhosis was identified. The extent of sinusoidal involvement and expansion by Kupffer cells was best demonstrated by PAS stain (Figure 2E) and immunohistochemistry for CD163 (Figure 2F). Some Kupffer cells demonstrated hemophagocytosis with engulfed red blood cells (Figure 2D, inset) and small pyknotic cells consistent with degenerative polymorphonuclear leukocytes (Figure 2E, inset). Some of the atypical multinucleated giant cells were surrounded by Kupffer cells (Figure 2F, inset). Immunohistochemistry for CD20 was strongly positive in the atypical lymphoid cells (Figure 2G) and the multinucleated bizarre cells (Figure 2G, inset). Immunohistochemistry for CD3 demonstrated a small number of scattered positive cells without atypia or enlarged nuclei consistent with reactive T-lymphocytes. Only scant lymphocytes were positive for CD5 and no definitive positive immunoreactivity was noted in the atypical lymphoid cells. The atypical lymphoid cells were not immunoreactive for ALK-1 protein. Prussian blue stain demonstrated substantial increase in iron storage in Kupffer cells and mild (grade 2/4) increase in iron content in hepatocytes. The reticulin network was preserved and no abnormal deposition or abnormal glycogen storage was demonstrated by PAS stain with and without diastase digestion.

Bone marrow biopsy

The bone marrow was hypercellular. There was erythroid hyperplasia, dyserythropoiesis, and dysmegakaryopoiesis (Figure 2H and 2I). Although no definitive atypical cells could be definitively recognized as per histologic criteria, scant atypical cells were demonstrated by immunohistochemistry for CD20 (Figure 2I, inset). No acid fast bacilli were identified by acid fast stain. No granuloma formation was noted.

Cytogenetics

Cytogenetic study of the bone marrow showed consistent chromosomal anomalies in 2 out of 21 cells as follow: 49,X,-Y,der(2),-3,der (4),+5,der(16),+mar x4 [2]/46, XY [19].

Flow cytometry

No increase in B-cells was demonstrated in either the liver or bone marrow biopsy to suggest a B-cell based lymphoproliferative disorder.

Clinical development

Clinically, the patient’s condition met 5 out of the 8 diagnostic criteria for HPS [2]. While biopsy was being evaluated, the patient received intravenous methylprednisolone 1 mg/kg/day for three days. Once the diagnosis of IVBL with HPS was established, the patient received one cycle of rituximab, cyclophosphamide, and vincristine. Following the start of therapy the patient developed severe hyperbilirubinemia (15 mg/dL, normal is 0.3-1.2 mg/dL), and significant hepatitis which resolved within 21 days. The patient received his second and third cycle of chemotherapy with R-EPOCH, which involved rituximab, etoposide, cyclophosphamide, doxorubicin, prednisone and vincristine. PET-CT revealed full remission of abnormal uptake of FDP after his third cycle of chemotherapy. His
anemia as well as elevated transaminases also resolved. The patient underwent autologous bone marrow transplantation in CR1 and remained in remission for a year at the time when this report was prepared.

Discussion

Demonstration of neoplastic lymphoid cells in blood vessels without associated mass or lymphadenopathy is the *conditio sine qua non* for diagnosis of IVBL. Histologically, the small clusters and scattered single atypical lymphoid cells can be rather subtle. The small clusters of atypical lymphoid cells with low level of pleomorphism often mimic hepatitis. The larger atypical lymphoid cells, particularly the multinucleated ones, and those that appear "floating" in the
Intravascular B-cell lymphoma and hemophagocytic syndrome

sinusoid are pathognomonic for IVBL. Immuno-

histochemistry for CD20 highlighted the B-cell

lineage of these atypical cells and confirmed

the diagnosis of IVBL. A high index of suspicion

and astute identification of the intravascular

atypical lymphoid cells are the key to successful
diagnosis of IVBL in this case. The clinical mani-

festations of this patient met 5 (fever, splenomegaly, anemia and thrombocytopenia, hypertriglyceridemia, hyperferritinemia) out of the 8 diagnostic criteria established by Henter et al. [2] (fever, splenomegaly, cytopenias affecting at least two of three lineages in the peripheral blood, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis in bone marrow, spleen, or lymph nodes, low or absent NK-cell activity, hyperferritinemia, high levels of soluble interleukin 2 receptor). Hemophagocytosis was histologically confirmed in Kupffer cells that expanded the sinusoid (Inset in Figure 2D and 2E). With all evidence considered, this is a case of IVBL with secondary HPS.

HPS is a rare clinical syndrome that is often fatal if left untreated. It can be subdivided into a primary (familial) form and a secondary form [2, 3]. Separating the primary from the secondary form is not always possible. The primary form is a rare, genetically heterogeneous disorder that typically presents by 1-2 years of age. Mutations of several genes including the perforin (PFR) [4, 5], hMunc13-4 (UNC13D) [6, 7], PRF1 [8], FHL [9], and Syntaxin 11 [10]. The various involved mutations produce immunologic dysregulation; a viral infection often triggers HPS via T-cell and macrophage activation [2]. Although still rare, the secondary form is far more common and may occur in any age. The secondary form comprises about one-fourth of pediatric HPS and almost all adult cases [2]. It is often associated with infection (most commonly viral) or malignancy, sometimes in the setting of immunodeficiency or immunosuppressive therapy. The prognosis of secondary HPS is strongly influenced by the underlying etiology and appropriate treatment of the underlying etiology. Lymphomas associated secondary HPS have poor prognosis in general [11].

Henter et al. have established 8 criteria for the diagnosis of HPS when the criteria based on molecular diagnosis cannot be fulfilled [2]. Manifestations include fever and other constitutional symptoms, cytopenias, lymphadenopathy, hepatosplenomegaly, abnormal liver function tests and coagulopathies. Hemophagocytosis is one of the diagnostic criteria but it is neither required nor by itself alone sufficient for the diagnosis [2]. Mechanistically, HPS is not a single entity but an altered immune status featured by hyperinflammatory phenotype with hypercytokinemia accompanied by excessive activation of lymphocytes and macrophages. Histologically, there is an excessive proliferation of macrophage and phagocytic vacuoles containing red blood cells or degenerative cell debris are characteristically present.

IVBL is a rare extranodal B-cell lymphoma without a mass or nodal enlargement. The majority are B-cell lymphomas with a mature phenotype (i.e., not precursor cell phenotype). Rare cases with T-cell or NK-cell phenotype [12-14] have been reported. The intravascular growth pattern is postulated to be resulted from defects in surface receptors for extravascular migration, lack of CD29 and CD54 adhesion molecules, and lack of leukocyte surface glycoprotein CD18 [15]. IVBL is almost always a diagnostic challenge, both clinically and pathologically, and post mortem diagnoses are not uncommon.

The clinical presentations of IVBL are diverse. In addition to the constitutional symptoms, the thromboembolic nature of the intravascular neoplastic lymphoid cells contributes to most of the focal clinical manifestations in the form of focal necrosis, infarction, focal neurologic deficits, and strokes. The splenic infarcts in this case are most likely caused by IVBL thromboemboli. The initial presentations of IVBL often suggest a disseminated pathologic process pointing to infections. Skin and central nervous system are the most commonly affected organs, followed by liver, spleen, kidney, and bone marrow. Almost any other organs can be involved but with lower frequency [12]. Manifestations are non-specific with constitutional symptoms, dermatological manifestations, neurological manifestations, and pain as the most common ones [12]. Patients may also have organomegaly, abnormal increase in uptake of FDP, "B-symptoms", and other less common ones such as respiratory distress [1, 12, 16]. Although IVBL is an aggressive malignancy, the cutaneous variant of IVBL limited to the skin is a favorable presentation with distinct clinical characteristics [12].

The manifestations of intravascular lymphoma
also vary geographically. IVBL occurring in patients of Western and Asian origins have different pattern of clinical presentation. While constitutional symptoms such as general fatigue and edema as well as involvement of the central nervous systems are common in all patients, involvement of skin is more common in patients in Western countries and involvement of the gastrointestinal tract is more common in Asian countries [16]. IVBL has a high tendency to be associated with HPS occurring in patients in Asia, particularly Japan, comprising the so-called "Asian Variant" of IVBL but is not associated with HPS in Western countries [12, 16, 17]. In one study [12, 16, 17], HPS occurred in 44% of Japanese patients and 18% in patients from other Asian countries but no association in patients from Western countries. Clinical features in patients with IVBL-associated HPS are different from those without HPS. Patients with HPS associated IVBL are more likely to have stage IV disease, fever, hepatosplenic involvement, bone marrow infiltration, dyspnea, anemia, and thrombocytopenia but they rarely exhibit cutaneous or central nervous system involvement. The "Asian variant" also has a high tendency to develop inappropriate anti-diuretic syndrome [18, 19]. Interestingly, this patient in this case was a Caucasian.

Histologically, the lymphoma cells are found exclusively within the lumen of small blood vessels and vascular spaces such as hepatic sinusoid, and capillaries which makes recognition of the neoplastic cells in the lung and bone marrow particularly difficult. Random skin biopsies has been suggested as a minimally invasive diagnostic procedure [19]. Bone marrow biopsy, a common procedure in the diagnosis and work up of lymphoma, is not the most efficient method for the diagnosis of IVBL [20]. A negative bone marrow biopsy does not rule out the diagnosis of IVBL. When clinical manifestations are suggesting intravascular lymphoma, identification of atypical B-cells by immunohistochemistry in bone marrow would strongly suggest IVBL but would not confirm the diagnosis. Although demonstration of intravascular lymphoma cells in tissue is required for diagnosis, intravascular lymphoid cells in bone marrow can be encountered in several conditions other than IVBL. These entities include persistent polyclonal B-cell lymphocytosis, hairy cell leukemia, B-cell chronic lymphocytic leukemia, mantle zone lymphoma, splenic marginal zone lymphoma, hepatosplenic gamma-delta lymphoma, and some diffuse large B-cell lymphoma [12, 15, 21, 22].

In conclusion, this Caucasian patient has all the clinical and pathologic features of IVBL combined with HPS that are characteristically seen in the "Asian variant" of IVBL. This case illustrated an important point that the "Asian variant" is not restricted only to Asian patients and a high index of suspicion is essential for the diagnosis of this rare combination.

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