

CYTOLOGIC DIAGNOSIS OF PRIMARY EFFUSION LYMPHOMA IN AN HIV-NEGATIVE PATIENT

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Primary effusion lymphoma (PEL) is an unusual and rare type of non-Hodgkin's lymphoma characterized by lymphomatous effusion of pleural, pericardial or peritoneal cavities without lymphadenopathy or organomegaly. It is associated with human herpes virus-8 (HHV-8) and occurs most often in immunodeficient patients. We present a case of PEL in a 69-year-old male presenting with pleural effusion and ascites. Fluid aspiration showed a monomorphic population of atypical lymphoid cells, which were medium- to large-sized, with mono- or binucleated hyperchromatic nuclei and a small to moderate amount of basophilic cytoplasm containing cytoplasmic vesicles. Immunohistochemically, the lymphoid cells expressed CD138 and multiple myeloma oncogene 1, were positive for HHV-8, and were monoclonal for immunoglobulin heavy chain gene rearrangement. They were negative for Epstein-Barr virus by *in situ* hybridization. Unfortunately, the patient died during the first course of chemotherapy with cyclophosphamide, vincristine and prednisone.

Key Words: human herpes virus-8, human immunodeficiency virus, primary effusion lymphoma
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Primary effusion lymphoma (PEL) is an unusual and rare type of non-Hodgkin's lymphoma (NHL), and is characterized by lymphomatous effusion of pleural, pericardial or peritoneal cavities without lymphadenopathy or organomegaly [1,2]. It was documented to be associated with human herpes virus-8 (HHV-8) infection and most often occurs in immunocompromised patients, particularly in patients with acquired immunodeficiency syndrome (AIDS) [3]. Human immunodeficiency virus (HIV)-negative, HHV-8 positive cases have been rarely reported, usually in areas of high HHV-8 prevalence, such as the Mediterranean

region [3–8]. We recently encountered a patient with PEL who was HHV-8 positive but HIV negative. In this study, we present our experience for cytologic diagnosis of PEL.

CASE PRESENTATION

A 69-year-old male patient had a history of oral cancer (squamous cell carcinoma) treated by intra-arterial chemotherapy 5 years previously, chronic renal disease, and mild fatty liver (HBsAg and anti-HCV negative). In July 2006, he presented with progressive bilateral lower leg edema and distended abdomen and he was hospitalized for further examination. Chest X-ray revealed pleural effusion on the right side and abdominal ultrasound revealed marked ascites. Lymph node enlargement and organomegaly were not observed. Hemogram revealed mild anemia



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(hemoglobin, 11.6 g/dL) with normal white blood cell and platelet counts. His renal function was poor with blood urea nitrogen (BUN) level of 75 mg/dL, serum creatinine (Cr) level of 1.8 mg/dL, and creatinine clearance rate (Ccr) of 37.7 mL/minute. Blood biochemistry was normal except for elevated lactic dehydrogenase. His serum was negative for HIV and human T lymphotropic viruses (HTLV) I/II using enzyme-linked immunosorbent assay.

The tapped ascites had a turbid appearance. Cytologic examination with Papanicolaou stain revealed numerous atypical lymphoid cells of varying morphology. Most of the tumor cells were medium- to large-sized, some with plasmacytoid features and

cytoplasmic microvacuoles. The remaining sediment was fixed in 10% buffered formalin, embedded in paraffin, and processed using a routine cell block procedure. Microscopically, hematoxylin and eosin staining revealed a monomorphic population of atypical lymphoid cells, which were medium- to large-sized, and were single- or binucleated with hyperchromatic nuclei. There was a small to moderate amount of basophilic cytoplasm containing cytoplasmic vesicles (Figure A). In addition, microorganisms were not identified by acid-fast staining.

Immunohistochemical stains were performed on 4- μ m-thick sections from a cell block prepared from ascites. The majority of the atypical lymphoid cells

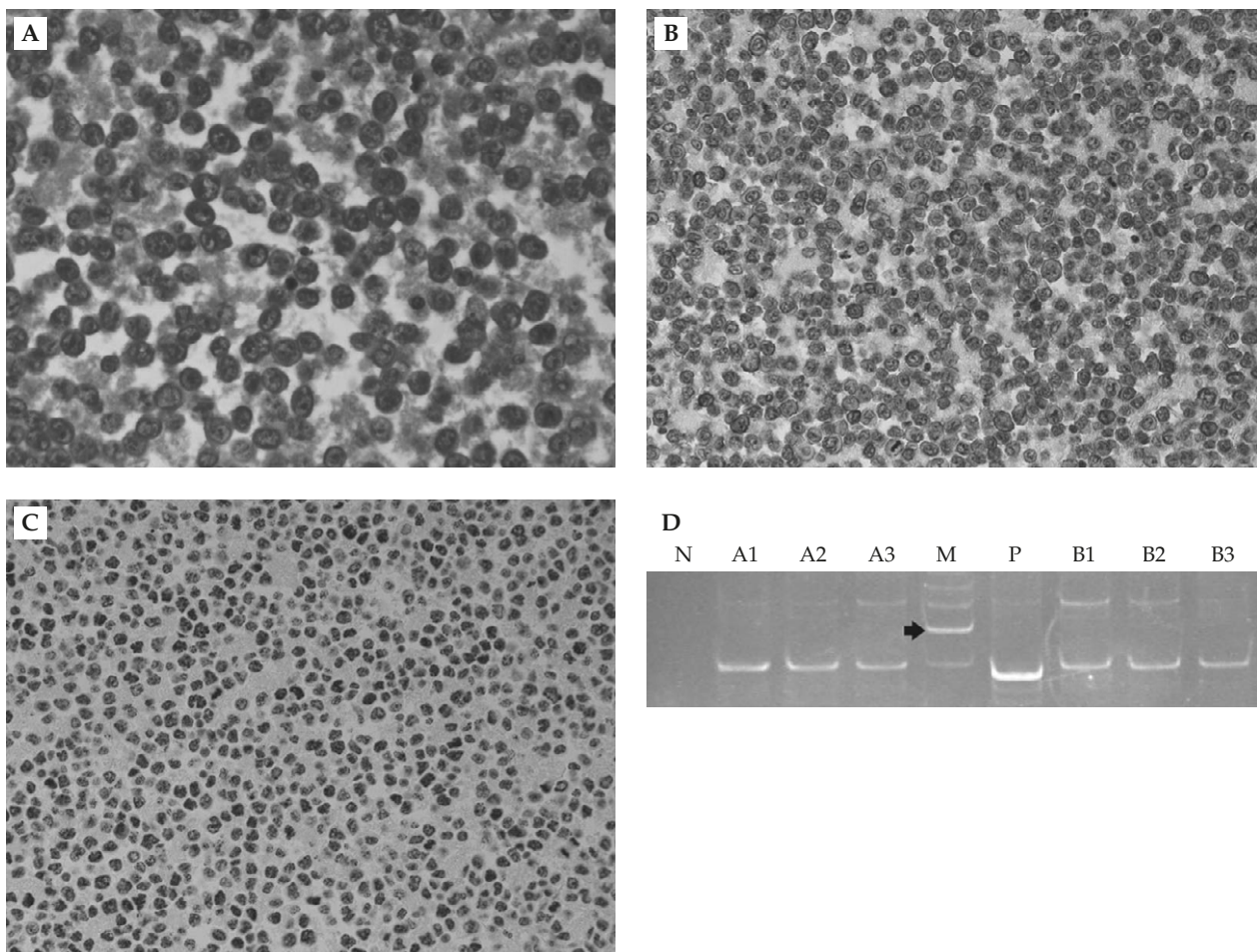


Figure. (A) Photomicrograph of a cell block section prepared from ascites shows medium- to large-sized tumor cells with single- or binucleated, hyperchromatic nuclei and a small to moderate amount of basophilic cytoplasm (hematoxylin & eosin). In situ hybridization shows that the tumor cells were positive for: (B) CD138; and (C) human herpes virus-8 latency-associated nuclear antigen but not for Epstein-Barr virus (data not shown). (D) Gel electrophoresis of the PCR product using primers for the framework III region of the immunoglobulin heavy chain gene revealed single bands. Lane N = negative control; Lane M = 25-bp DNA size marker with an arrow indicating the brightest band at 125 bp; Lane P = positive control (human Burkitt lymphoma cell line, BJAB); Lanes A1–A3 and B1–B3 = triplicates using 1 μ L and 2 μ L of DNA sample for experiments, respectively.

were positive for vimentin, CD30, CD138 (Figure B), multiple myeloma oncogene 1 (MUM-1), and HHV-8 latency-associated nuclear antigen (Figure C), and negative for cytokeratin, calretinin, CD3, CD20, CD10, CD45, CD79a, Bcl-2, Bcl-6, Pax-5, and latent membrane protein 1 (LMP-1). *In situ* hybridization for Epstein-Barr virus (EBV) was negative. A gene rearrangement study using primers (Ig-FR3, ACA CGG C(C/T)(G/C) TGT ATT ACT GT and Ig-LJH, TGA GGA GAC GGT GAC C) directed against the framework III region of immunoglobulin heavy chain (IgH) gene using polymerase chain reaction showed monoclonal results (Figure D).

The patient received one cycle of COP (cyclophosphamide, vincristine and prednisone) chemotherapy, but subsequently died due to neutropenic fever 3 months after diagnosis.

DISCUSSION

PEL was first reported in 1995, and was classified as a specific entity by the World Health Organization Classification of Tumors in 1999 [3,9]. PEL is a neoplasm of large B-cells, and HHV-8 infection constitutes the genetic hallmark of the disease, which was assumed to be essential for PEL diagnosis. In addition to infection by HHV-8/Kaposi's sarcoma-associated herpes virus, PEL is also frequently positive for EBV, particularly in AIDS-related cases, whereas most PELs of HIV-negative hosts are EBV negative [3]. However, the pathogenesis of PELs remained unknown and is currently under active investigation. The clinical, morphologic and molecular features of HIV-related and HIV-unrelated PELs are similar, but PELs appear to occur at a substantially older age in immunocompetent hosts than in HIV-infected individuals [2,10].

PELs tend to present as serous effusions, usually only affecting one area, without a contiguous solid tumor mass. Rare cases involve more than one serous cavity, which may result in PEL progression [11]. The most common site of involvement is the pleural space, but the peritoneal and pericardial cavity can also be affected. Involvement of the gastrointestinal tract, soft tissue and other extranodal sites has also been reported [3,12–14].

Diagnosis of PEL is normally based on cytologic evaluation of the fluid [15,16]. The morphology of the

affected fluid shows a variety of lymphoid cells, which bridge the features of immunoblastic large cell lymphoma and anaplastic large cell lymphoma. They tend to be multinucleated or multilobulated large cells with prominent nucleoli. The basophilic cytoplasm frequently contains small clear vacuoles. The mitotic activity is usually high. Differential diagnosis includes anaplastic large cell lymphoma, myeloma and undifferentiated carcinoma.

Typically, PELs exhibit a non-B, non-T, indeterminate immunophenotype, but they are positive for the CD45, CD138/syndecan-1 and MUM-1 molecule, which indicates that the lymphoma has an advanced stage of B-cell differentiation, close to plasma cells [17,18]. The expression of CD30, CD38, CD71, and epithelial membrane antigen is associated with their activation [19]. At the molecular level, clonal rearrangement of the IgH gene has been seen in the majority of cases [20,21]. In addition, PEL is devoid of the chromosomal translocations associated with other aggressive B-cell NHLs, such as translocations of c-MYC, BCL-1, BCL-2 and BCL-6 [3,4,22,23].

The prognosis of malignant effusion lymphoma is very poor, although chemotherapy such as high-dose methotrexate combined with a CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone)-derived regimen has resulted in transient remissions [10,20, 24–26]. In previously published series, PEL patients had a median survival of less than 6 months after diagnosis [3,25]. The use of highly active antiretroviral therapy has been suggested as a novel therapy for PEL patients. The duration of remission can last several months [26,27].

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以細胞學診斷人類免疫缺陷病毒陰性之 原發性積液淋巴瘤

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原發性積液淋巴瘤是一種罕見的非何杰金淋巴瘤。不同於其他淋巴瘤多以器官或淋巴結腫大來表現，其特徵是含有淋巴瘤的體液在體腔內（如腹腔、胸腔、心包膜腔）異常聚積，而患者多有 8 型人類疱疹病毒 (HHV-8) 感染相關或好發在免疫不全的人身上。我們報告一位 69 歲男性以腹腔、胸腔積水作表現的原發性積液淋巴瘤的病例。原發性積液淋巴瘤的細胞型態為單形性非典型性淋巴組織增生，具有中型到大型的單核或雙核及少量到中量有空泡的嗜鹼性細胞質。免疫染色方面，這些非典型性淋巴球表現 CD138、MUM1 及 HHV-8。並作 PCR 檢定其為單株細胞免疫蛋白重鏈基因重組而原位雜交顯示為 Epstein-Barr 病毒陰性。不幸，此患者於確診後在接受第一次化學治療的過程中死亡。

關鍵詞：8 型人類疱疹病毒，人類免疫缺陷病毒，原發性積液淋巴瘤
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