Case Report

An unusual case of Primary Effusion Lymphoma with aberrant T-cell phenotype in a HIV-negative, HBV-positive, cirrhotic patient, and review of the literature

Charitini Nepka, MD, PhD1, Dimitrios Kanakis, MD, PhD1,2, Maria Samara, PhD1, Andreas Kapsoritakis, MD, PhD3, Spyridon Potamianos, MD, PhD3, Maria Karantana, MD, PhD1, Georgios Koukoulis, MD, PhD1

Address: 1Department of Pathology and Cytology, University-Hospital of Larissa, 41110 Larissa, Greece; 2Department of Pathology, Democritus University of Thrace, University-Hospital of Alexandroupolis, 68100 Alexandroupolis, Greece; 3Department of Gastroenterology, University-Hospital of Larissa, 41110 Larissa, Greece

E-mail: Charitini Nepka - cnepka@yahoo.gr; Dimitrios Kanakis* - aristeotes_stageira@yahoo.com; Maria Samara - msamar@med.uth.gr; Andreas Kapsoritakis - kapsoritakis@med.uth.gr; Spyridon Potamianos - spotam@med.uth.gr; Maria Karantana - mairikarantana@gmail.com; Georgios Koukoulis - gkouk@med.uth.gr

*Corresponding author

Published: 29 June 2012
Received: 3 February 2012
CytoJournal 2012, 9:16
Accepted: 24 May 2012

This article is available from: http://www.cytojournal.com/content/9/1/16
© 2012 Nepka, et al.; licensee Cytopathology Foundation Inc.

Access this article online

Quick Response Code: Website: www.cytojournal.com DOI: 10.4103/1742-6413.97766

Abstract

Primary effusion lymphoma (PEL) is an unusual, human herpes virus-8 (HHV-8) – associated type of lymphoma, presenting as lymphomatous effusion in body cavities, without a detectable tumor mass. It primarily affects human immunodeficiency virus (HIV)-infected patients, but has also been described in other immunocompromised individuals. Although PEL is a B-cell lymphoma, the neoplastic cells are usually of the 'null' phenotype by immunocytochemistry. This report describes a case of PEL with T-cell phenotype in a HIV-negative patient and reviews all the relevant cases published until now. Our patient suffered from cirrhosis associated with Hepatitis B virus (HBV) infection and presented with a large ascitic effusion, in the absence of peripheral lymphadenopathy or solid mass within either the abdomen or the thorax. Paracentesis disclosed large lymphoma cells with anaplastic features consisting of moderate cytoplasm and single or occasionally multiple irregular nuclei. Immunocytochemically, these cells were negative for both CD3 and CD20, but showed a positive reaction for T-cell markers CD43 and CD45RO (VCHL-1). Furthermore, the neoplastic cells revealed strong positivity for EMA and CD30, but they lacked expression of ALK-1, TIA-1, and Perforin. The immune status for both HHV-8 and Epstein-Barr virus (EBV) was evaluated and showed positive immunostaining only for the former. The combination of the immunohistochemistry results with the existence of a clonal rearrangement in the immunoglobulin heavy chain gene (identified by PCR), were compatible with the diagnosis of PEL. The presence of T-cell markers was consistent with the diagnosis of PEL with an aberrant T-cell phenotype.

Key words: Cirrhosis, HHV-8, HIV, HBV, primary effusion lymphoma
INTRODUCTION

It was Cesarman and colleagues, in 1995, who first identified KSHV DNA sequences within a distinct subgroup of AIDS-related non-Hodgkin lymphomas (NHL) localized in body cavities, presenting as lymphomatous effusions.[1] Subsequently, Nador et al. (1996) introduced the term ‘primary effusion lymphoma’ (PEL) in order to describe this particular type of lymphoma, which was lacking a tumor mass, but was accompanied by HHV-8 infection.[2] According to the definition provided by the World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues (issue 2008), the primary effusion lymphoma is a large B-cell neoplasm that usually presents as a serous effusion without a detectable tumor mass and is universally associated with HHV-8. Some patients with PEL, secondarily develop solid tumors in the adjacent structures, such as, the pleura, whereas, rare cases of HHV-8-positive lymphomas (indistinguishable from PEL) present as solid tumor masses, and have acquired the designation of extracavitary PEL.[3] Furthermore, PEL is included in the category of HIV-associated lymphomas, having been referred in the literature as 'lymphomas occurring more specifically in HIV-positive patients',[4] although it has been almost simultaneously reported in HIV-negative patients also.[5-7] In this setting PEL has been linked to other immunodeficiency conditions, such as, following an organ transplantation,[6-10] cancer,[11,12] old age,[13,14] or even cirrhosis.[15,16] There are also published reports that describe PEL associated with liver cirrhosis and as a simultaneous infection with either Hepatitis B or C virus.[17,18]

As far as cytomorphology is concerned, PEL shows features bridging immunoblastic and anaplastic large-cell lymphomas, with a frequent demonstration of plasma cell differentiation. Interestingly, PEL exhibits a ‘null’ immunophenotype, as it lacks expression of both B- and T-cell associated antigens.[5]

We herein report a case of HIV-negative PEL, with underlying Hepatitis B virus (HBV)-related liver cirrhosis, which additionally showed the relative infrequent immunocytochemical expression of T-cell associated antigens. Our case was also positive for HHV-8, a finding that along with the particular cytomorphological and immunocytochemical findings, confirmed that the lymphomatous effusion was a case of PEL.

CASE REPORT

Clinical summary

The patient was an 88-year-old man of Mediterranean descent, with a history of cirrhosis and underlying HBV infection. At the time of presentation in the clinic, he complained of generalized weakness and fatigue over the last month. Physical examination revealed icterus of the skin and sclera, as well as abdominal distention, owing to the presence of ascitic fluid without any detectable hepatosplenomegaly.

The peripheral blood count showed macrocytic anemia (Hb: 11.6 g / dL, MCV: 108.0 fl), thrombocytopenia (PLT: 113 × 10^3 / μL) and normal WBC count and differential. In particular, the exact values were as follows: WBC: 4.0 × 10^3 / μL, Neut.: 72%, Lymph.: 18%, Mono.: 9.0%, Eosino.: 1.0%, RBC: 3.09 × 10^6 / μL, Hb: 11.6 g / dL, MCV: 108.0 fl, MCH: 37.6 pg, and PLT: 113 × 10^3 / μL. His serum profile provided the following results: Gluc: 105.0 mg / dl, UREA: 32.0 mg / dl, CREAT: 0.88 mg / dL, SGOT: 223.0 U / L, SGPT: 155 U / L, T-Bil.: 7.0 mg / dl, Bl-Bil.: 3.0 mg / dl, γ-GTP: 50 U / L, ALP: 165 U / L, LDH: 241 U / L, Prot: 7.4 g / dl, Alb: 2.1 g / dl, CK: 183 U / L. The biochemical examination of the collected peritoneal fluid revealed: Gluc 111 mg / dl, Prot: 2.2 g / dl, Alb: 0.65 g / dl, LDH: 1590 U / L, Chol: 7.0 mg / dl, and Trigl: 16 mg / dl.

Abdominal Ultrasound-Sonography (US) demonstrated liver cirrhosis and massive ascites. Computed-Tomography (CT), which was carried out later confirmed the ascites, although with no visible peritoneal implants. Furthermore, no pathological lymphadenopathy was detected by the obtained CT-scans.

Paracentesis was performed, yielding a large amount (800 ml) of ascitic fluid that was subsequently sent for cytological evaluation. After centrifugation of the sample, the sediment was used for ThinPrep preparation as well as for routine preparation, with direct smearing stained with both Pap and Giemsa. The remaining sediment was used for cell block preparation, using the plasma-thrombin method and standard H and E staining.

Cytological findings

The Thin Prep material consisted of abundant large-sized malignant cells, with a moderate amount of basophilic cytoplasm, which were arranged singly. Most of them had a single nucleus, but occasionally, bi-nucleated or multinucleated cells were also seen. The nucleus had coarse chromatin pattern, irregular nuclear outlines, and a single or multiple prominent nucleoli. A high mitotic activity, with abnormal mitoses was present, and a large number of apoptotic bodies as well as nuclear debris were additionally seen [Figures 1a and 1b].

Immunocytochemistry

The immunophenotypic profile of the tumor was determined by standard immunoperoxidase methods using paraffin sections from cell block material. The primary antibodies used were: AE1 / AE3, EMA, Vimentin,
Melan A, MPO, Fascin, Perforin, Bcl-6, CD3, CD4, CD5, CD8, CD10, CD15, CD20, CD30, CD34, CD43, CD45 (LCA), CD45RO, and CD138 antigens. In addition, anaplastic lymphoma kinase 1 (ALK-1) protein, TIA-1, LMP-1, and HHV-8 (Latent Nuclear Antigen-1; LANA-1) were also investigated. Antigen retrieval was performed using ethylenediamine tetraacetic acid (EDTA)-based solution (CC1). The primary antibody, secondary antibody, and avidin-enzyme conjugate were then visualized using the precipitating enzyme diaminobenzidine (DAB). The detailed immunocytochemical list of the antibodies used in our case is depicted in Table 1.

Molecular analysis
A possible Epstein–Barr virus (EBV) infection was excluded using the EBV-encoded RNA (EBER) in situ hybridization test in cell block material. On the contrary, the infection with the Hepatitis B virus was further confirmed with the identification of the HBV-DNA. Moreover, sediment from the ascitic fluid was examined using the polymerase chain reaction (PCR) method to detect any clonal rearrangement of the immunoglobulin heavy chain gene (IgH). Moreover, we checked for gene rearrangement in the T-cell receptor.

In particular, and in more detail, the methods used are described below and the obtained results are shown in the attached figures.

Molecular analysis was performed using the IdentiClone IgH Gene Clonality Assay kit (CE-IVD, InVivoScribe Technologies, USA). The DNA was extracted and amplified by primers that target the conserved framework of the variable (V) regions, the conserved joining (J) regions, as well as the diversity and joining regions, according to the manufacturer’s instructions. The DNA quality was tested by the Specimen Control Size Ladder Mix, which contains multiple oligonucleotides targeting the housekeeping genes [supplied with the Kit, Figure 3].

The presence or absence of clonal T-cell Receptor Gamma chain gene rearrangements was evaluated using the IdentiClone TCRG Gene Clonality Assay (CE-IVD, InVivoScribe Technologies, USA) according to the manufacturer’s instructions, as mentioned earlier.

The PCR products were analyzed in agarose gel electrophoresis, followed by non-denaturing polyacrylamide gel electrophoresis (PAGE), in the presence of positive (clonal) and negative (polyclonal) controls [Figures 4–6].

Other laboratory tests
A bone-marrow aspirate showed no evidence of lymphoma. The serological investigation revealed the following results: HbsAg: (+), Anti-Hbc: IgG(+), Anti-Hbe: (-), HbeAg: (+), Anti-Hbs: (-), Anti-HCV: (-), and Anti-HIV: (-).

Final cytological diagnosis
The final diagnosis that was based on both cytological and immunocytochemical findings, in conjunction with the appropriate molecular workup, was indicative of primary effusion lymphoma with aberrant T-cell expression. The patient unfortunately died one week after the final diagnosis.

DISCUSSION

Dissemination of Non-Hodgkin’s lymphomas (NHL) in serous cavity fluids has been reported in approximately 10% of all malignant effusions. The term PEL was initially introduced by Nador et al., in 1996, in order to describe a novel type of lymphoma, presenting exclusively as a lymphomatous effusion in the absence of a detectable solid mass. In a majority of lymphoma cases other than PEL, a precise subtyping of the neoplasm in cytological specimens is not required, as the actual diagnosis will have been already established in the solid parts of the tumor, before the acquisition of the cytological sample. However, on some occasions where

Table 1: Immunohistochemistry-results

| Positive markers | Negative markers |
|------------------|------------------|
| CD4 (ca. 10% of the cells) | CD3 |
| CD5 (ca. 3% of the cells) | CD10 |
| CD8 (ca. 5% of the cells) | CD15 |
| CD30 (ca. 60% of the cells) (Figure 2a) | CD20 |
| CD43 (ca. 15% of the cells) (Figure 2b) | CD34 |
| CD45 [LCA] (ca. 30% of the cells) (Figure 2c) | CD138 |
| CD45RO (ca. 90% of the cells) (Figure 2d) | Bcl-6 |
| EMA (ca. 90% of the cells) (Figure 2e) | AE1 / AE3 |
| HHV-8 [LANA-1] (nuclear expression in ca. 90% of the cells) (Figure 2f) | Vimentin |
| Fascin (ca. 4% of cells) | Melan A |
| | MPO |
| | ALK-1 |
| | TIA-1 |
| | Perforin |
| | LMP-1 |
| | EBER (in situ hybridization) |

Figure 1: (a) Cell block of FNA material showing a striking nuclear pleomorphism as well as a high mitotic index with irregular mitoses and numerous apoptotic bodies (H and E stain, magnification x200). (b) Higher magnification of the figure 1a, with particular emphasis on the details noted above (H and E stain, magnification x400)
the serous cavity effusion is more accessible relative to the primary site of involvement, the cytological and immunocytochemical interpretation is, without doubt, of utmost importance, especially when a PEL diagnosis is under consideration.

As we have already mentioned above, primary effusion lymphoma has a strong association with HHV-8 infection, but it also seems to have a relative ‘preference’ for occurrence in HIV-positive individuals. Indeed, this unique type of lymphoma has been estimated to account for approximately 4% of NHL in HIV-positive patients, but only for 0.3% of NHL in HIV-negative patients. In these cases, where the patients are HIV negative (as in our case; see also review of all published so far [HHV-8(+) and HIV(-)] cases in Table 2), other immunodeficient conditions may exist. Our patient, although negative for HIV infection, presented with liver cirrhosis associated with Hepatitis B virus. Cirrhosis, which is an established condition of immunodeficiency, has been described in association with PEL; specifically there are a few reports that demonstrate the presence of PEL together with liver cirrhosis. Although a majority of PEL-cases referred to in literature are HHV-8 positive, there are several examples in which the absence of HHV-8, with or without a simultaneous absence of HIV infection, has been observed. These particular cases have been further referred in the literature as ‘HHV-8-unrelated PEL-like lymphomas’, in order to differentiate them from ‘true’ PEL. Therefore, the presence of HHV-8 has been established almost as a criterion, to consider a lymphoma as a PEL, as the former is considered to play a crucial role in the pathogenesis of the latter. In this context, it has been proposed that the name PEL should be assigned...
### Table 2: Table of all yet published [HHV-8(+) and HIV(-)] cases of PEL

| Reference                          | Age | Sex | Localization         | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies                                                                 | Detection of viral infection with different methods                                                                 |
|-----------------------------------|-----|-----|-----------------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| Nador RG, et al., N Engl J Med, 1995[22] | 85  | M   | Pleura                | Congestive heart failure. Coronary artery disease. Chronic renal failure. Aortic-valve replacement | Immunophenotyping showed CD45 positivity of all neoplastic cells, expression of CD20 by some neoplastic cells, and the lack of T-cell-lineage associated antigens | Presence of clonal rearrangements of the immunoglobulin heavy chain gene by the polymerase chain reaction (PCR) with VH-FR111 / JH consensus primers | The previously described amplification product of 233 base pairs was identified, confirming the presence of KSHV. No evidence of Epstein–Barr virus (EBV) by immunohistochemical staining for the latent membrane protein of EBV by in situ hybridization for EBER, or by PCR amplification of the EBNA-2, EBNA-3C, and EBER regions |
| Carbone A, et al, Br J Haematol, 1996[15] | 69  | F   | Peritoneum            | Cirrhosis (variceal bleeding)                                                    | B-cell immunophenotype. LCA(+), CD15(-), CD30(+), EMA(+)                         | by the ISH technique, monotypic κ mRNA was detected. K-RAS detection by PCR analysis. C-MYC alterations were absent. No rearrangements of BCL-2 with the IgH locus were found by a nested PCR assay | EBER negative. PCR analysis demonstrated KSHV positivity |
| Nador RG, et al., Blood, 1996[2]    | 78  | M   | Peritoneum            | Results of the particular case in the performed immunocytochemistry cannot be clearly acquired | Identification of clonal IgH gene and the clonal lambda light-chain gene rearrangements |                                                                                                                                    |                                                                                                                  |
| Said JW, et al, Blood, 1996[23]    | 85  | F   | Pleura (bilateral)   | Kaposi’s Sarcomas of both legs. Gangrene with bilateral above-knee amputation    | Positive for CD20, CD45, and CD30, but negative for Ig light chains, CD3, CD5, CD79a, CD43, CD15, and EMA | A clonal band indicating Ig gene rearrangements was detected with the JH probe                                               | The examined case exhibited a distinct 233-bp band similar to the KS lesion used as a positive control, indicating the presence of KSHV. The presence of EBV was identified by Southern blot hybridization of BamH1 digested DNAs to a probe specifically for the EBV genomic termini, which indeed gave the expected band. EBV infection was further confirmed by PCR amplification of the EBNA-2, EBNA-3C, and EBER regions. PCR analysis failed to detect HIV sequences in the neoplastic cells. Serology for HIV was also negative |

(Continued)
Table 2: Contd-

| Reference                  | Age | Sex | Localization               | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies                                                                 | Detection of viral infection with different methods                                                                 |
|---------------------------|-----|-----|----------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| Said JW, et al., Blood,   | 46  | F   | Around a breast implant    | Surgery for bilateral silicone implants (five years before presentation of PEL) | Strongly positive for CD30. Membrane staining for CD43. Negative for Ig κ and λ light chains and for CD3, CD20, CD45, CD79a, T-cell receptor βF1 and CD15   | A clonal band was detected in the Southern blot with the Ig JH probe. The JK light chain probe showed no rearranged bands   | The results of serum ELISA for antibodies to HIV and of polymerase chain reaction (PCR) for HIV RNA were negative. The cells from the ascitic fluid were positive for Kaposi sarcoma-associated herpes virus by PCR. They were negative for several other herpes viruses tested, including EBV, cytomegalovirus, and human herpes viruses 6 and 7. High numbers of copies of Kaposi sarcoma-associated herpes virus DNA sequences were present in the ascitic fluid cells (as determined by Southern blot hybridization) when compared with similar amounts of DNA from the BC-1 cell line, which has approximately 40 to 80 copies of viral genome per cell. Negative for HIV and Epstein-Barr virus (EBV). By reverse transcriptase-PCR, HHV-8-related transcripts, including vG-coupled protein receptor, vBcl2, vCyclin D, vIL-6, vMIPI, and vMIPII, were detected in the PEL from the pleural cavity and the gastric lymphoma, whereas, these transcripts, except for vIL-6, were not detected in a lymph node biopsy with MCD. Expression of hIL-10 was weak in the PEL from the pleural cavity, and expression of hIL-6 was undetectable in all three lesions. |
| 1996[23]                  |     |     |                            |                                                                              |                                                                                                                                   |                                                                                                                                                                         |                                                                                                                                                                               |
| Strauchen JA, et al.,     | 94  | M   | Pleura (left side),        | Stage II adenocarcinoma of the colon (1981), Kaposi’s sarcoma (1988)         | The lymphoma cells in the ascitic fluid cell block and in the autopsy tissue showed a 'null cell' immunophenotype with loss of B-cell antigens (CD45 focally positive, CD20-negative, CD45RO-negative, CD30-negative, HLA-DR-negative, CD68-negative, epithelial membrane antigen-positive, cytokeratin negative, κ and λ immunoglobulin light chain-negative) | Clonal rearrangement of the immunoglobulin JH and JK genes was present (confirming the presence of a clonal B-cell proliferation). No abnormally migrating band indicative of c-myc rearrangement was seen                                  |                                                                                                                                                                               |
| Ann Intern Med,           |     |     | Peritoneum                 |                                                                              |                                                                                                                                   |                                                                                                                                                                         |                                                                                                                                                                               |
| 1996[11]                  |     |     |                            |                                                                              |                                                                                                                                   |                                                                                                                                                                         |                                                                                                                                                                               |
| Teruya-Feldstein J, et al.| M   | Pleura | Multicentric Castleman’s disease (MCD), Gastric large cell lymphoma |                                                                              |                                                                                                                                   |                                                                                                                                                                         |                                                                                                                                                                               |
| Lab Invest, 1998[24]      |     |     |                            |                                                                              |                                                                                                                                   |                                                                                                                                                                         |                                                                                                                                                                               |

(Continued)
| Reference                  | Age | Sex | Localization          | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies                                                                 | Detection of viral infection with different methods |
|----------------------------|-----|-----|-----------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|---------------------------------------------------|
| Vu HN, et al., Surgery, 1998[25] | 85  | M   | Pleura (bilateral)    | Recurrent inguinal hernia. Arthritis. New onset heart failure. Systemic Castleman’s disease. | Immunostains of paraffin sections revealed the neoplastic cells to be positive for CD45 (leukocyte common antigen) and CD30 (Ki-1), but almost no CD20 (L26) positive B cells | Analysis for the heavy chain gene, showed that virtually all cells from the effusion belonged to a single neoplastic B-cell clone caused by demonstration of di-allelic gene rearrangements instead of a germline band | Herpes zoster virus test was negative. EBER in situ hybridization for Epstein-Barr virus (EBV) was also negative. Southern blot results: Cells from the pleural effusion showed a single clonal EBV band at a level somewhat less than one gene copy per cell. When probed against HHV-8, the pleural effusion cells demonstrated multiple genomic copies |
| Jones D, et al., N Engl J Med, 1998[8] | 59  | M   | Pleura                | Orthotopic heart transplantation because of end-stage congestive heart disease. A biopsy of an inguinal lymph node (eight years before; 1990) confirmed the presence of Kaposi’s sarcoma, which appeared on the skin one year after (1991). Furthermore, refractory hypertension, chronic renal insufficiency and atrial fibrillation | Immunohistochemical and flow-cytometric analyses revealed that neoplastic cells bound antibodies against the activation markers CD30, epithelial membrane antigen, and CD38, and failed to react with antibodies against B-cell markers (CD10, CD19, CD20, and CD79a), T-cell markers (CD3, CD5, and CD45RO), myelomonocytic markers (CD1b, CD13, CD14, and CD33), CD45, cytokeratin, CD30, EMA, CD38 (+) B-cell markers (CD10, CD19, CD20, and CD79a), T-cell markers (CD3, CD5, and CD45RO), myelomonocytic markers (CD1b, CD13, CD14, and CD33), CD45, cytokeratin, Igμ(+), Igδ(-), Igκ and Igλ undetermined | Southern blot analysis performed with DNA prepared from the pleural fluid revealed two non-germline immunoglobulin heavy-chain bands of approximately equal intensity, a finding compatible with the presence of a single dominant lymphoid clone that had rearranged both immunoglobulin heavy-chain alleles. Cytogenetic analysis of 10 cells in metaphase from the pleural fluid revealed the following clonal chromosomal aberrations: 47–50,XY;der(2)add(2)(p23)add(2)(q37);add(4)(q25);add(4)(q35);add(4)(q25);add(10)del(7)(q12);del(9)(p12);add(11)(q25);add(12)(q24);add(14)(q24);add(17)(p12);add(18)(q23);+19;+20;+21;+22;+mar1;+2–5mars | Enzyme-linked immunosorbent assays for serum HIV antibodies, a polymerase-chain-reaction (PCR) assay for HIV sequences in serum, and viral cultures, were all negative. Neoplastic cells failed to react with antibodies against EBV latent membrane protein 1. The tumor cells also failed to express EBV encoded RNA transcripts, as assessed by in situ hybridization. No hybridizing bands were observed with a probe specific for EBV genomic sequences. With the use of a primer set that was specific for HHV-8, abundant product of the expected size (233 bp) was noted after only 15 cycles of amplification of tumor DNA, a result that could be explained by the presence of a large number of HHV-8 copies in the tumor cell population due to active viral replication |
| Okada T, et al., Int J Hematol, 1998[26] | 101 | M   | Pleura (bilateral)    | Congestive heart failure. Chronic renal failure. Anteroseptal myocardial infarction | CD45(+), CD20(+), Igμ(+), CD3(-), CD4(-), CD5(-), CD8(-), CD45RO(-), and CD79a(-). Also, Igκ(+), Igλ(-), Igκ and Igλ; undetermined | KSHV was demonstrated in large numbers in the neoplastic cells using semiquantitative PCR analysis. The presence of the EBV on the tumor cells was also studied by PCR. On the contrary, the patient was anti-HIV antibody-negative | (Continued) |
| Reference                  | Age | Sex | Localization | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies                                                                 | Detection of viral infection with different methods |
|----------------------------|-----|-----|--------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-----------------------------------------------|
| Cobo F, et al., J Pathol, 1999[16] | 58  | M   | Peritoneum   | Two-month history of weight-loss, pruritus and abdominal discomfort. In addition there was a history of liver cirrhosis | The neoplastic cells expressed the activation markers CD30, CD38, and EMA, and lacked lineage-specific antigens, including B-cell (CD19, CD20, CD22, heavy and light chain immunoglobulins), T-cell (CD2, CD3, CD5, CD7, CD4, CD8, CD43), and myelomonocytic markers (CD13, CD33, CD14, CD11c, myeloperoxidase). A proportion of the neoplastic cells expressed CD45 as well as the CD45RO isoform | Diagnostic molecular procedures yielded a clonal immunoglobulin heavy chain rearrangement, a T-cell receptor chain gene in the germline, c-MYC in the germline, and the lack of NPM-ALK rearrangement | ELISA and western blot tests for HIV were negative. Serology for Hepatitis C virus and Hepatitis B virus surface antigen was also negative. Both immunohistochemical analysis for LMP-1 as well as in situ hybridization for EBER-1 were negative. PCR analysis for EBV was further negative, but showed amplification of the KS330233 HHV-8 sequence. Moreover, the presence of a high copy number of HHV-8 sequences was demonstrated by Southern blot using BamHI and HindIII restriction enzymes. RNA transcripts from all seven HHV-8 ORFs examined (vCyD, vbcl-2, vGPCR, vIL-6, STP-like, vIRF, and vFLIP) were identified with the expected size |
| Ascoli V, et al., Eur Respir J, 1999[12] | 89  | M   | Pleura       | Remote history of malaria (1936), systemic hypertension, surgery for lumbar intervertebral disk herniation (1984) and large bowel adenocarcinoma (1994) | Tumor cells were CD45-positive or negative, B-cell-associated antigen (CD20, CD19, CD22), T-cell-associated antigen (CD5, CD45R0), cytokeratin, CD68, CD15-negative EMA (focal), - and CD30 (focal)-positive. Small lymphocytes were mostly T-cells (CD5+ / CD45R0+); other reactive cells were macrophages (CD68+) and mesothelial cells (cytokeratin-positive). In the first case, a few lymphoma cells were vimentin-positive | Clonal rearrangement of the IgH gene was detected. No variation from the germline configuration of control DNA was found by analyzing the c-myc, Bcl-2 and Bcl-6 genes | Both patients were seronegative for HIV, Hepatitis B, and C virus. The HHV-8 DNA sequences were detected in DNA extracts from lymphoma cells. The EBV genome was absent in both cases |
| Ascoli V, et al., Eur Respir J, 1999[12] | 75  | M   | Pleura       | Systemic hypertension, Chronic obstructive airways disease. Dilated cardiomyopathy. Surgery for lumbar intervertebral disk herniation | Clonal rearrangement of the IgH gene was detected | | |
| Reference                          | Age | Sex | Localization          | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies                                                                 | Detection of viral infection with different methods                                                                 |
|-----------------------------------|-----|-----|-----------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| Dotti G, et al., Leukemia, 1999⁷    | 56  | M   | Peritoneum            | Cardiac transplantation for dilative cardiomyopathy (five years before PEL presentation) | Flow cytometry showed the following immunophenotype: CD45(+), HLA-DR(+), CD38(+), CD1138(+), CD34(-), CD13(-), CD33(-), CD3(-), CD2(-), CD5(-), CD7(-), CD10(-), CD20(-), CD19(-), SmIg(-), CD56(-). CD30 antigen positive expression into the cytoplasm was determined by immunocytochemistry. Ig expression (κ and λ chains) was found negative both within the cytoplasm and on the cell surface | The karyotype showed abnormalities in 13 of 15 metaphases analyzed: 48, XY, +inv(3)(p21q12) or der(4)t(4;) (qter:qter), +del11(q23.3)[11] / 49, idem, +mar[2] / 46, XY[2]. Southern blotting demonstrated a clonal rearrangement of the IgH gene. As the karyotypic analysis showed deletion at 11q23, further investigation for the presence of genetic rearrangement of the ALL-1 gene was carried out, but a germline configuration was finally demonstrated. Similarly, no rearrangements were found within the BCL-6 and c-myc genes. PCR detection of Bcl-2 / IgH translocation showed negative results | Southern blotting demonstrated integration of the EBV genome. PCR amplification of the EBNA-3C and LMP-1 genes supported the involvement of the EBV-type A strain and demonstrated the wild-type sequence of the LMP-1 gene. Furthermore, PCR analysis was performed by the use of KS330233 primers derived from the KSHV DNA sequence. The expected 233 bp HHV-8 fragment was detected in both the PEL cells and bone marrow-derived mononuclear cells, obtained from the patient at diagnosis |
| San Miguel P. et al., Acta Cytol, 1999²⁷ | 83  | M   | Pleura (right side)  | Ischemic myocardiopathy. Chronic gastric ulcer. Prostate hyperplasia              | CD45(+), CD30(+), CD38(+), EMA(+), HMB-45(-), S-100(-), LMP-1(-). Neoplastic cells did not express any lineage-associated T- or B-lymphocyte antigens | Polymerase chain reaction analyses on genomic DNA from the pleural effusion, demonstrated the presence of HHV-8 sequences in the absence of the Epstein-Barr virus | (Continued)                                                                                                    |
Table 2: Contd/

| Reference | Age | Sex | Localization | Clinical history | Immunophenotype | Various molecular and cytogenetic studies | Detection of viral infection with different methods |
|-----------|-----|-----|--------------|------------------|-----------------|------------------------------------------|---------------------------------------------------|
| Codish S, et al., Am J Hematol, 2000[28] | 73  | F   | Peritoneum and Lymph Nodes | Multicentric Castleman's disease and multiple KS lesions | Positive for CD30, EMA but negative for CD3, CD79a and ALK1 | Various molecular and cytogenetic studies | Immunohistochemistry for HHV-8 by staining with an antibody against the latent nuclear antigen 1 (LNA-1) showed that HHV-8 positive immunoblastic cells were localized in the mantle zone of the lymph node follicle, with up to 30% of the cells positive for LNA-1. Furthermore, the neoplastic cells were negative for EBV latent-membrane antigen (LMP-1). A high titer of anti-HHV-8 antibodies (1:25,600) was detected in a serum sample and in a sample from the ascitic fluid by an indirect immunofluorescent antibody assay (IFA). Polymerase chain reaction (PCR) for HHV-8 DNA sequences was positive in the KS lesion, lymph node with MCD, and cells from the peritoneal fluid. ELISA serologies for HIV, hepatitis B surface antigen, and hepatitis C were negative. |
| Ariad S, et al., Arch Pathol Lab Med, 2000[29] | 68  | M   | Pleura (left side) and Peritoneum, Lymph Node (left Axilla) | Coronary bypass surgery (eight years before) | Neoplastic cells were positive for CD30 and EMA, but lacked reactivity for CD3, CD20, and the ALK protein (ALK1) | Various molecular and cytogenetic studies | Enzyme-linked immunosorbent assay serology for HIV, Hepatitis B surface antigen, and Hepatitis C, were normal. The results of both immunohistochemistry for EBV latent-membrane antigen and PCR for EBV were negative. On the contrary, the results of the HHV-8 polymerase chain reaction (PCR) on the lymph node specimen were positive. |
| Polskj JM, et al., Leuk Lymphoma, 2000[30] | 80  | M   | Pleura (bilateral) | Hypertension. Ischemic heart disease. Chronic obstructive pulmonary disease. Peripheral vascular disease. Renal insufficiency. Status-post cerebrovascular accident with a residual left-sided weakness | By flow cytometry, at least 60% of the lymphoma cells expressed CD7, CD45, CD56, HLA-DR, selective kappa surface light chains, and CD38, whereas, in the remaining activation or differentiation markers studied, CD30 and CD138, were not expressed | Various molecular and cytogenetic studies | A single band, corresponding to HHV-8 was detected in both the original PCR reaction and the nested PCR. Serum HIV testing was negative. |

(Continued)
| Reference | Age | Sex | Localization | Clinical history | Immunophenotype | Various molecular and cytogenetic studies | Detection of viral infection with different methods |
|-----------|-----|-----|---------------|------------------|----------------|------------------------------------------|--------------------------------------------------|
| Perez CL, et al., Clin Diagn Lab Immunol, 2001[31] | 72 | M | Pericardium, Pleura (bilateral), Peritoneum, Lymph Node | Two years earlier lymphoproliferative disease, and biopsy of lymph node showed a B- [CD19(+), CD20(+), CD22(+)] immunophenotype with co-expression of CD10 and CD23 | The B-cell population expressed CD19, CD20, and CD22 with co-expression of CD10 and CD23 antigens and kappa light chain restriction. The T-cell population consisted of 21% CD4 cells and 12% CD8 cells | Clonal kappa light chain rearrangement | Results of enzyme-linked immunosorbent assay serology for HIV, HTLV 1 and 2, hepatitis B virus surface antigen, and hepatitis C virus were negative. PCR was positive for HHV-8 and negative for EBV in both a lymph node biopsy specimen and liquid effusion |
| Klepfish A, et al., Leuk Lymphoma, 2001[32] | 78 | M | Pleura (left side) | Longstanding hypertension, Cerebrovascular accident, Tuberculosis (55 years previously) | LCA(CD45RB)(+), CD20(+), CD3(-) | | Serology for HIV was negative. Through the use of nested PCR the patient’s tumor cells clearly demonstrated the presence of HHV-8, but no evidence for EBV could be noticed |
| Lechapt-Zalcman E., et al., Arch Pathol Lab Med, 2001[33] | 87 | M | Pleura (right side) | Strong positivity for CD30 and CD3, but weak staining for CD45. Negative staining for CD19, CD20, and CD79a (B-cell antigens). Negativity also for CD138, CD2, CD5, ALK1, TIA-1, and Cytokeratin (KL1, MNF116) | The PCR–denaturing gradient gel electrophoresis analysis of the T-cell receptor γ chain gene rearrangement showed the presence of a predominant T-cell clone within an oligoclonal T-cell expansion in the pleural lymphocyte population, whereas, no clonal population was detected in the peripheral blood lymphocytes. No clonal rearrangement of the IgH chain gene was found in the pleural effusion lymphocytes | | |
| Ascoli V, et al., Haematologica, 2002[34] | 92 | M | Pleura (bilateral) | Congestive heart failure | Negative B- and T-cell markers, but positive CD138 | Monoclonal rearrangement of the immunoglobulin heavy chain genes. PAX-5 gene mutations | HHV-8 (+). HIV (-). EBV (+) |
| Buonaiuto D, et al., Ann Ital Med Int, 2002[35] | 87 | M | Pleura | Congestive heart failure | Positive B- and T-cell markers, but positive CD138 | Tumor cell exhibited an indeterminate (non-B non-T) phenotype | HHV-8 (+). HIV (-). EBV (+) |
| Klein U, et al., Blood, 2003[36] | 70 | M | Peritoneum and Pleura | Kaposi's Sarcoma | Negative B- and T-cell markers, but positive CD138 | Tumor cell exhibited an indeterminate (non-B non-T) phenotype | HHV-8 (+). HIV (-). EBV (+) |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

(Continued)
| Reference          | Age | Sex | Localization       | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies                                                                                           | Detection of viral infection with different methods                                                                 |
|--------------------|-----|-----|--------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Boulanger E, et al., Am J Hematol, 2004[37] | 78  | M   | Peritoneum         | Congestive heart failure                                                         | CD45(+), CD30(+), CD19(-) and CD20(-) CD45(+), CD30(+), CD138(+), and CD7(+) but CD19(-), CD20(-), CD2(-) and CD3(-) | Southern blot hybridization performed on BamHI / HindIII and BglII digests, and the IgHJ6 probe detected the presence of monoclonal rearrangements of immunoglobulin heavy chain genes in both cases | Serology for HIV was negative. The PCR analysis demonstrated the presence of KSHV / HHV-8 sequences, but the absence of detectable EBV sequences |
| Munichor M, et al., Acta Cytol, 2004[38]    | 74  | M   | Pleura (right side) | Ischemic heart disease                                                           | In the performed immunocytochemical staining, most of the malignant lymphoid cells showed positive staining for CD30, CD45, and CD138 / Syndecan-1. CD3 | Rearrangements of immunoglobulin heavy and kappa light chain genes                                                                 | Serologic studies showed no detectable HIV or EBV antibodies. Strong nuclear positivity in the anti-HHV-8 monoclonal antibody latent nuclear antigen-1 (LNA-1) stain, in the majority of the malignant cells. In the nested PCR amplification, the patient’s tumor cells clearly contained KSHV, but no evidence of EBV could be demonstrated |
| Danese C, et al., Clin Ter, 2004[39]        |     | M   | Pleura (left side) |                                                                                 |                                                                                                                                   |                                                                                                                                   | Immunohistochemical and molecular assays performed on the pleural fluid, demonstrated the presence of HHV-8. Serologic test for HIV (ELISA) was negative. EBV negative                                                                 |
| Reference                        | Age | Sex | Localization                  | Clinical history                                                                 | Immunophenotype                                                                                                      | Various molecular and cytogenetic studies                                                                 | Detection of viral infection with different methods                                                                 |
|---------------------------------|-----|-----|--------------------------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| Luppi M, et al., Leukemia, 2005 | 96  | M   | Pleura (bilateral)            |                                                                                  | HHV-8 and EBV positive. HIV, Hepatitis B and C viruses negative                                                   |
|                                 | 70  | M   | Peritoneum and Pleura         | Kaposi's Sarcoma                                                                 | HHV-8 positive. HIV, Hepatitis B, and C viruses negative                                                        |
|                                 | 77  | M   | Pleura (bilateral)            | Heart failure. After presentation of PEL. The following complications were diagnosed: bilateral uveitis, renal insufficiency, and myocardial infarction | HHV-8 positive. HIV, Hepatitis B, and C viruses negative                                                       |
| Halfdanarson TR, et al., Ann Oncol, 2006 | 78  | M   | Pleura (bilateral) & Lymph Nodes | Five-year history of fluctuating anemia and mild lymphadenopathy                  | Serology of HIV was negative. but there was evidence of past infections with human herpes virus 8 (HHV-8) and Epstein–Barr virus |
| Hsieh PY, et al., J Formos Med Assoc, 2007 | 54  | M   | Pleura and Peritoneum         | Chronic Hepatitis B (25-year history) with Liver cirrhosis (last three years)  | The immunophenotyping of the lymphoma cells was positive for CD38, negative for CD19, cytoplasmic immunoglobulin (both κ and λ), and cytoplasmic CD3. The cell block of the ascites showed MUM-1(-), VS38C(+), and EBER(-). The nuclei of the lymphoma cells were positive by immunohistochemistry for the HHV-8-associated latent protein | All of the examined mitotic cells (13 in number) showed clonal aberrations                                      | Hepatitis B surface antigen (HBsAg) was positive. Hepatitis B e antigen (HBeAg) was negative, and antibody to HBeAg (anti-HBeAg) was positive. Antibody to HCV (anti-HCV) was negative. HHV-8 DNA could be detected in the lymphoma cells of the pleural effusions. Serology of HIV was negative |

(Continued)
Table 2: Contd/-

| Reference                | Age | Sex | Localization                  | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies                                                                 | Detection of viral infection with different methods                                                                 |
|--------------------------|-----|-----|--------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|
| Su YC, et al., Kaohsiung J Med Sci, 2008 [42] | 69  | M   | Pleura (right side), Peritoneum | Oral cancer (squamous cell carcinoma) five years previously. Chronic renal disease. Mild fatty liver | A majority of the atypical lymphoid cells were positive for vimentin, CD30, CD138, multiple myeloma oncogene I (MUM-1), and negative for cytokeratin, calretinin, CD3, CD20, CD10, CD45, CD79a, Bcl-2, Bcl-6, Pax-5, and latent membrane protein I (LMP-1) | A gene rearrangement study using primers directed against the framework III region of immunoglobulin heavy chain (\(\text{IgH}\)) gene using polymerase chain reaction showed monoclonal results | Patient's serum was negative for HIV and human T lymphotropic viruses (HTLV) I / II, when using enzyme-linked immunosorbent assay. Immunocytochemical positivity for HHV-8 latency-associated nuclear antigen was observed. In situ hybridization for Epstein-Barr virus was negative |
| Siddiqi T, et al., Clin Lymphoma Myeloma, 2008[43] | 78  | M   | Pleura (right side)            | Hypertension. Coronary artery disease. Stroke. Aortic valve repair after aneurysm of the ascending aorta | Immunohistochemistry revealed the following results: CD3(-), CD20(-), CD79a(-), CD30(+), CD45(+), and CD138(+) | Polymerase chain reaction (PCR) for immunoglobulin heavy chain (\(\text{IgH}\)) gene rearrangement did not detect an amplification | Latent membrane protein-1 stain for EBV was negative. EBV-encoded RNA in situ hybridization was negative as well, but the HHV-8 nuclear immunostain was strongly positive in the atypical cells. HIV testing was negative |
| Boulanger E, et al., Am J Transplant, 2008[10] | 57  | M   | Pleura (right side)            | Kidney transplantation. Disseminated Kaposi's Sarcoma                               | The results of immunohistochemistry were as follows: CD3(-), CD20(-), CD38(+), and CD138(+) | Monoclonal rearrangement of the immunoglobulin heavy chain (\(\text{IGH}\)) gene was detected by PCR in the pleural effusion mononuclear cells collected from the patient. Monoclonal rearrangement of T-cell receptor gamma genes was not detected | Both patients were HIV negative. Evidence for HHV-8 infection of tumor cells was provided by a positive immunostaining reaction for latency-associated nuclear antigen-I (LANA-1) and high levels of HHV-8 DNA loads in a quantitative polymerase chain reaction (PCR) assay. Southern blot analysis of HHV-8 clonality showed oligoclonal HHV-8 episomes in the two cases. Both patients were found to be HHV-8 seropositive before renal transplantation. In situ hybridization for detection of EBV-encoded RNAs (EBER) was negative in both cases |
| 63  | M   | Peritoneum, Pleura (bilateral) and Pericardium | Kidney transplantation          | Kidney transplantation. Disseminated Kaposi's Sarcoma                               | The results of immunohistochemistry were as follows: CD3(-), CD20(-), CD30(+), and CD138(+) | No evidence for expansion of a B-cell clonal population was found in the PEL of the patient. Monoclonal rearrangement of T-cell receptor gamma genes was not detected |  | (Continued) |
### Table 2: Contd...

| Reference                  | Age | Sex | Localization                  | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies | Detection of viral infection with different methods |
|----------------------------|-----|-----|--------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|-------------------------------------------|-----------------------------------------------------|
| Brimo F. et al., Cytojournal, 2009[44] | 69  | M   | Pericardium, Pleura (bilateral) and Peritoneum | Schizophrenia, Diabetes mellitus, Hypertension, Renal insufficiency, Atrial fibrillation | Immunohistochemical studies showed positive staining of the neoplastic cells for CD45, and cytoplasmic staining for the T-cell marker CD3, in all specimens; staining for CD2, CD4, CD5, CD7, and CD43 was negative. Immunostains for the B-cell markers CD20, CD79a, and PAX-5 were negative, as were ALK-1, bcl 2, bcl 6, CD10, CD138, and keratin AE1/3. Ki67 positivity was present in 80% of the nuclei. | Normal immunoglobulin levels and absence of a serum monoclonal protein | Using an antibody directed against HHV8 latent nuclear antigen (LNA), there was positive staining in 25 – 30% of the neoplastic lymphocytes. In addition, in situ hybridization for Epstein-Barr virus-encoded RNA (EBER) done on one of the cell blocks showed positivity in about 50 – 60% of the nuclei. Further investigations confirmed the HIV-negative status and established that the patient did not have hepatitis B or C infection |
| Stingaciu S, et al., Clin Adv Hematol Oncol 2010[45] | 73  | F   | Pleura (bilateral)             | Stage I Breast Cancer (seventeen years before). Diabetes. Systemic Hypertension | Immunophenotyping showed that the neoplastic cells were positive for leukocyte common antigens CD45, human leukocyte antigen (HLA)-DR-positive (85%), CD38-positive (75%), CD138-positive (33%), and CD30-positive (20%), as well as the absence of B-cell and T-cell markers. | Serologic tests showed that Hepatitis B virus (HBV) surface antigen, Hepatitis C virus (HCV) antibody, and HIV antibody were all negative, but there was evidence of past infections by HHV-8 with immunoglobulin antibodies at 1,280 IU (N < 160). The polymerase chain reaction for HHV-8 was positive in the blood and pleural fluid. EBV was positive |
| Yakounis X, et al., Anticancer Res, 2010[47] | 73  | F   | Pleura (left side), esophagus and skin | Type 2 diabetes mellitus | The immunophenotypic study showed the following results: CD3(-), CD20(-), CD45(+), CD30(+), EMA(+), CD138(-), and CD79a(-) | The patients serum was negative for HIV antibody (ELISA) and positive for HHV-8 DNA by PCR |

(Continued)
| Reference                          | Age | Sex | Localization                  | Clinical history                                                                 | Immunophenotype                                                                 | Various molecular and cytogenetic studies | Detection of viral infection with different methods |
|-----------------------------------|-----|-----|-------------------------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------|---------------------------------------------------|
| Yiakoumis X, et al., Anticancer Res, 2010[7] | 43  | M   | Pleura (left side) and Lymph Nodes (cervical and inguinal) | Hodgkin’s lymphoma (25 years prior to PEL-presentation). Hepatitis C infection (18 years before) | The immunophenotypic study of the cells in a pleuritic effusion showed the following results: CD30 (ki-1)(+), CD138(+), EMA(+),and vimentin (+), but CD15(-), CD20(-), LCA(-), CD3(-), CD79a(-), CD45RO(-), S100(-), myeloperoxidase(-), and CD68(-). The immunophenotypic study of the cells in the biopsied lymphnodes showed the following results: CD138(+), LMP1 (+), CD3(- ), LCA(-), and CD79a(-) | Various molecular and cytogenetic studies by multiplex polymerase chain reaction confirmed a monoclonal IGH@ rearrangement with a polyclonal T-cell receptor pattern | The serology for HIV was negative. Positivity was found for HHV-8 infection with PCR, both in the patient's serum and pleuritic fluid |
| Makis W, et al., Clin Nucl Med, 2010[18] | 65  | M   | Pleura and Peritoneum         | Hepatitis virus (HCV)-related liver cirrhosis                                   | CD45(+), HLA-DR(+), CD38(+), CD30(+), EMA(+), CD7(+), MUM1 / IRF4               | Gene rearrangement studies by multiplex polymerase chain reaction confirmed a monoclonal IGH@ rearrangement with a polyclonal T-cell receptor pattern | Serological screening for HIV, Hepatitis B and Hepatitis C was negative. There was strong nuclear expression of human Herpes virus 8 (HHV8) latent protein (LANA), and Epstein-Barr virus-encoded RNA (EBER) by in situ hybridization |
| Gandhi SA, et al., Br J Haematol, 2011[46] | 55  | M   | Peritoneum, Pleura            | Cryptogenic cirrhosis of the liver. Refractory ascites                           | CD45(+), HLA-DR(+), CD38(+), CD30(+), EMA(+), CD7(+), MUM1 / IRF4               | Gene rearrangement studies by multiplex polymerase chain reaction confirmed a monoclonal IGH@ rearrangement with a polyclonal T-cell receptor pattern | Serological screening for HIV, Hepatitis B and Hepatitis C was negative. There was strong nuclear expression of human Herpes virus 8 (HHV8) latent protein (LANA), and Epstein-Barr virus-encoded RNA (EBER) by in situ hybridization |

(Continued)
### Table 2: Contd-

| Reference | Age | Sex | Localization       | Clinical history                                                                 | Immunophenotype                        | Various molecular and cytogenetic studies                                                                 | Detection of viral infection with different methods |
|-----------|-----|-----|---------------------|----------------------------------------------------------------------------------|----------------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------|
| Nakayama-Ichiyama S, et al., Ann Hematol, 2011 [47] | 67  | M   | Peritoneum          | Chronic Hepatitis C Infection, Liver Cirrhosis, Hepatocellular Carcinoma        | CD45RO(+), CD79a(-) and CD20(-)        | Southern blotting revealed a clonal rearrangement of the T cell receptor Jγ chain gene. No clonal rearrangement of the immunoglobulin heavy chain gene was found by Southern blotting. Cytogenetic analysis of GTG banding was performed and the results were 46, XY, t(7;8)(q32;q13) [3] / 46, XY [17] | Hepatitis C virus (HCV) viremia was confirmed by a quantitative assay of viral load of 7.8 log IU / mL in the plasma. The results of serological tests for human immunodeficiency virus (HIV), Epstein–Barr virus, and human T-lymphotropic virus 1 were negative. DNA of human herpes virus (HHV) 6 (> 2.0 × 10 copies / 106 cells) and HHV8 DNA (>2.0× 10 copies / 106 cells) were detected in the peripheral blood leukocytes. The nucleoli of lymphoma cells were positive for latent HHV6 and HHV8 |
| Ganzel C, et al., Am J Hematol, 2011 [48] | 81  | M   | Pleura (right side) | Ischemic heart disease. Recurrent cerebrovascular accidents. Chronic renal failure. Kaposi's sarcoma on the legs (about three years before) | Immunophenotypic analysis revealed the neoplastic cells to be CD45(+), CD38(+), HLA-DR(+), CD138(-). All B- and T-cell markers were negative, except for CD79a, which was positive. Also neoplastic cells demonstrated positive immune reaction for the HUM1 gene (indicates clonality of the disease) | On cytological examination, neoplastic cells showed nuclear immunostaining for LANA-1 (a gene of Kaposi Sarcoma Herpes Virus / Human herpes Virus 8 [KSHV / HHV8]), LMP immunostaining for Eppstein-Barr virus (EBV) and EBV-polymerase chain reaction (PCR) of the fluid were negative |
only to this primary lymphomatous effusion that shows evidence of HHV-8 infection, along with the supportive morphological and immunophenotypical criteria. The standard assay to detect HHV-8 infection in tissues is the immunocytochemical staining for LANA-1, whereas, EBV infection is most reliably identified by in situ hybridization for EBV-RNA, as immunocytochemical staining for EBV latent membrane protein (LMP-1) is almost always negative. Accordingly, after completion of the relative tests, our case has shown the presence of HHV-8 and the absence of EBV infection. Previous molecular studies have approved the occurrence of immunoglobulin gene rearrangements (this is also true in our case) and somatic hypermutation in PEL cells, indicating that the cell of origin is a post-germinal center B-cell.

Similarly, the experiments carried out by Fais et al., further suggested that PEL originated from mature antigen-experienced B-cells. Specifically, this group performed sequencing-analysis in immunoglobulin (Ig) genes from seven AIDS-related PEL. The obtained results showed that most of the samples used lambda light chain genes, two cases expressed mu chains, whereas, gamma chains were also found in two cases. In all cases, significant deviations from the presumed germline counterpart were found in both the expressed VH and VL genes. Statistical evidence for antigen selection was evident in four out of the seven samples studied. Evidence for selection was more frequent in the light chain genes than in the heavy chain genes. Gene expression profile analysis, which had already been performed in cases of AIDS-related PEL, had also confirmed a plasmablastic derivation. The cells of PEL immunocytochemically exhibited an indeterminate or ‘null’ lymphocyte phenotype, a they showed positive staining for the leukocyte common antigen (LCA; CD45), but negative staining for both routine B-cell- (including surface and cytoplasmic immunoglobulin, CD19, CD20, CD79a) and T-cell-markers (CD3, CD4, CD8). Instead, various markers of lymphocyte activation (CD30, CD38, CD71, human leukocyte antigen DR) and plasma cell differentiation (CD138) are usually displayed. Unlike the majority of reported cases, which show—as noted before—a ‘null’ immunophenotype, our case expressed, to a variable extend, T-cell markers (such as CD4, CD8, CD43; Figure 2b) and CD45RO (Figure 2d), but showed no expression of the B-cell marker CD20.

The occurrence of a T-cell immunophenotype in PEL is a relatively rare event and only a few cases have been published until now. The differential diagnosis of PEL, on a cytomorphological ground, includes other large cell lymphomas, such as, anaplastic large cell lymphoma (ALCL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL), with plasmacytoid differentiation, and pyothorax-associated lymphoma (PAL). Among them, the Anaplastic Large Cell Lymphoma (ALCL) and PEL share similar morphological and immunocytochemical characteristics. Morphologically, they are both high-grade lymphomas, composed of large cells with pleomorphic nuclei and prominent single- to multiple nucleoli. However, the typical ‘hallmark cells’ with the eccentric kidney- or horseshoe-shaped nuclei and a prominent Golgi zone, which have been previously described in ALCL, are only seldom seen in PEL. Immunocytochemically both are positive for CD30 and EMA. Furthermore, ALCL demonstrates a T-cell immunophenotype. With regard to all the arguments raised herewith, while keeping in mind that our case also showed a T-cell profile, it was particularly difficult to rule out an ALCL. However, the negative immunostaining for ALK-1, in conjunction with the strong positivity for HHV-8, as it was a fact in our case, rendered the diagnosis of PEL as the most plausible. The exclusion of the rest of the lymphoma subtypes from the final diagnosis was more obvious. As far as Diffuse large B-cell lymphoma (DLBCL) and in particular the immunoblastic variant is concerned, confusion may arise, as this may also show morphological features similar to PEL. However, both the immunophenotype and HHV-8 detection are helpful in distinguishing these two entities, as DLBCL expresses B-cell markers and is HHV-8 negative. Unlike the classic Burkitt lymphoma (BL), the variant of BL with plasmacytoid differentiation, often seen in AIDS patients, may demonstrate common morphological characteristics with PEL. In these cases, immunophenotyping usually shows a B-cell lineage in BL compared with the common ‘null’-phenotype of PEL or the rarer T-cell immunophenotype of our case. In addition, the negative staining for both CD10 and Bcl-6 as well as the simultaneous presence of HHV-8, all observed in the present case, did not correlate with the diagnosis of a BL. Finally, pyothorax-associated lymphoma (PAL) is, as the term implies, a pleural EBV-associated NHL that develops after longstanding chronic pleural inflammation. Cytologically, PAL may be indistinguishable from PEL, thus immunophenotyping and HHV-8 testing are essential in this distinction. Indeed, PAL expresses B-cell markers, is HHV-8 negative, and even more it goes hand in hand with the presence of EBV.

**CONCLUSION**

All in all, the diagnosis of PEL should be kept in mind whenever lymphomatous effusions, without a detectable primary solid mass, are present and the immunodeficiency status is either evident or is speculated. In such cases, a concrete panel of immunohistochemical markers together with specific molecular studies should be carried out. However, the hallmark of this particular entity remains the identification of HHV-8 infection.
COMPETING INTEREST STATEMENT BY ALL AUTHORS

No competing interest to declare by any of the authors.

AUTHORSHIP STATEMENT MADE BY ALL AUTHORS

Each author acknowledges that this final version was read and approved. According to the International Committee of Medical Journal Editors (ICMJE http://www.icmje.org) an ‘author’ is generally considered to be someone who has made substantive intellectual contributions to a published study. Authorship credit should be based on (1) substantial contribution to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions (1), (2), and (3). Other contributors, who do not meet these criteria for authorship, are listed in the ‘acknowledgements’ section. All authors of this article declare that they qualify for authorship as defined by ICMJE (http://www.icmje.org/#author). Each author has participated sufficiently in the study and takes public responsibility for the appropriate portions of the content of this article.

ETHICS STATEMENT BY ALL AUTHORS

As this is a case report without patient identifiers, approval from Institutional Review Board (IRB) is not required at our institution.

REFERENCES

1. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences are present in AIDS-related body-cavity-based lymphoma. N Engl J Med 1995;332:1186-91.
2. Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Said J, et al. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's-sarcoma-associated herpes virus. Blood 1996;88:645-56.
3. Said J, Cesarman E. Primary effusion lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, eds. World Health Organization Classification of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2008. p. 260-1.
4. Raphael M, Said J, Borisch B, Cesarman E, Harris NL. Lymphomas associated with HIV infection. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al, editors. World Health Organization Classification of Tumors of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2008. p. 340-2.
5. Carbone A, Gloghini A. KSHV / HHV8-associated lymphomas. Br J Haematol 2007;140:13-24.
6. Brimo F, Michel RP, Khetani K, Auger M. Primary effusion lymphoma: a series of 4 cases and review of the literature with emphasis on cytomorphic and immunocytochemical differential diagnosis. Cancer 2007;111:224-33.
7. Yiakoumis X, Pangalis GA, Krztonic MC, Vassilakopoulos TP, Kourtidou FN, Kalpadakos C, et al. Primary Effusion Lymphoma in Two HIV-negative patients successfully treated with pleurodesis as first-line therapy. Anticancer Res 2010;30:271-6.
8. Jones D, Ballestas ME, Kaye KM, Guitia JM, Winters GL, Fletcher J, et al. Primary effusion lymphoma and Kaposi's sarcoma in a cardiac-transplant recipient. N Engl J Med 1998;339:444-9.
9. Dotti G, Fiocchi R, Motta T, Fachinetti B, Chiodini B, Borleri GM, et al. Primary effusion lymphoma after heart transplantation: a new entity associated with human herpesvirus-8. Leukemia 1999;13:664-70.
10. Boulanger E, Alfonso PV, Yahiaoui Y, Adle-Biassette H, Gabarre J, Agbalika F. Human herpesvirus-8 (HHV-8)-associated primary effusion lymphoma in two renal transplant recipients receiving rapamycin. Am J Transplant 2008;8:707-10.
11. Strauschen JA, Hauser AD, Burstein D, Jimenez R, Moore PS, Chang Y. Body cavity-based malignant lymphoma containing Kaposi sarcoma-associated herpes virus in an HIV-negative man with previous Kaposi sarcoma. Ann Intern Med 1996;125:822-5.
12. Ascoli V, Scalzo CC, Danese C, Vaccia K, Pistilli A, Lo Coco F. Human herpes virus-8 associated primary effusion lymphoma of the pleural cavity in HIV-negative elderly men. Eur Respir J 1999;14:1231-4.
13. Schulz TF. Epidemiology of Kaposi's sarcoma-associated herpes virus / human herpesvirus. Adv Cancer Res 1999;76:121-60.
14. Carbone A, Gloghini A. PEL and HHV8-unrelated effusion lymphomas: classification and differentiation. Cancer 2008;114:225-7.
15. Carbone A, Gloghini A, Vaccher E, Zagonel V, Pastore C, Dalla Palma P, et al. Kaposi's sarcoma-associated herpesvirus DNA sequences in AIDS-related and AIDS-unrelated lymphomatous effusions. Br J Haematol
lymphoma (PEL) suggests a plasmablastic derivation and identifies PEL-specific transcripts. Blood 2003;101:415-21.

37. Boulanger E, Hermine O, Fernandez JP, Radford-Weiss L, Brousse N, Meignin V, et al. Human herpesvirus 8 (HHV-8)-associated peritoneal primary effusion lymphoma (PEL) in two HIV-negative elderly patients. Am J Hematol 2004;76:88-91.

38. Munichor M, Cohen H, Sarid R, Manov I, Iancu TC. Human herpesvirus 8 in primary effusion lymphoma in an HIV-negative male. A case report. Acta Cytol 2004;48:425-30.

39. Danese C, Angrisani L, Colotto M, Clarice A, Ferranti E A five year follow-up of an HHV-8 related lymphoma in a HIV-negative elderly patient. Clin Ter 2004;155:543-6.

40. Luppi M, Trovato R, Barozzi P, Vallisa D, Rossi G, Re A, et al. Treatment of herpesvirus associated primary effusion lymphoma with intracavitary cidofovir. Leukemia 2005;19:473-6.

41. Halfdansdottir TR, Markovic SN, Kalokhe U, Luppi M A. Non-chemotherapy treatment of a primary effusion lymphoma: durable remission after intracavitary cidofovir in HIV negative refractory to chemotherapy. Ann Oncol 2006;17:1849-50.

42. Su YC, Chai CY, Chuang SS, Liao YL, Kang WW. Cytologic diagnosis of primary effusion lymphoma in a HIV-negative patient. Kaohsiung J Med Sci 2008;24:548-51.

43. Siddiqi T, Joyce RM. A case of HIV-negative primary effusion lymphoma treated with bortezomib, pegylated liposomal doxorubicin, and rituximab. Clin Lymphoma Myeloma 2008;8:300-4.

44. Brimo F, Popradi G, Michel RP, Auger M. Primary effusion lymphoma involving three body cavities. Cytojournal 2009;6:21.

45. Songcuciu T, Tichioni M, Sudaka I, Haudebourg J, Mounier N. Intracavitary cidofovir for human herpesvirus-8-associated primary effusion lymphoma in an HIV-negative patient. Clin Adv Hematol Oncol 2010;8:367-74.

46. Gandhi SA, Mufi G, Devereux S, Ireland R. Primary effusion lymphoma in an HIV-negative patient associated with hypogammaglobulinemia. Am J Hematol 2011;86:777-81.

47. Navassa M, Rimola A, Rodes J. Bacterial infections in liver disease. Semin Liver Dis 1997;17:323-32.

48. Ohshima K, Ishiguro M, Yamasaki S, Miyagi J, Okamura S, Sugio Y, et al. Chromosomal and comparative genomic analyses of HHV-8-positive primary effusion lymphoma in five HIV-negative Japanese patients. Leuk Lymphoma 2002;43:595-601.

49. Van den Bosch K, Van der Merwe B, De Vylder V, Van Driel H, Van den Bossche B, et al. CD7 and CD56-positive primary effusion lymphoma in a human immunodeficiency virus-negative patient. Leuk Lymphoma 2000;39:633-9.

50. Perez CL, Rudoy S. Anti-CD20 monoclonal antibody treatment of human herpesvirus-8-associated, body-cavity-based lymphoma with an unusual phenotype in a human immunodeficiency virus-negative patient. Clin Diagn Lab Immunol 1999;6:893-6.

51. Klefpish A, Sarid R, Shalird M, Shividel L, Berrebi A, Schattner A. Primary effusion lymphoma (PEL) in HIV-negative patients--a distinct clinical entity. Leuk Lymphoma 2001;41:439-43.

52. Lechap-Szalanczky E, Challine D, Delfau-Larue MH, Haouzi C, Desvaux D, Gaulard P. Association of primary pleural effusion lymphoma of T-cell origin and human herpesvirus 8 in a human immunodeficiency virus-negative man. Arch Pathol Lab Med 2000;124:753-5.

53. Polski JM, Evans HL, Grosso LE, Popovic WJ, Taylor L, Dunphy CH. CD7 and CD56-positive primary effusion lymphoma in a human immunodeficiency virus-negative host. Leuk Lymphoma 2000;39:633-9.

54. Klein U, Gloghini A, Gaidano G, Chadburn A, Cesarman E, Dalla-Favera R, et al. Gene expression profile analysis of AIDS-related primary effusion
58. Fais F, Gaidano G, Capello D, Gloghini A, Ghiootto F, Roncella S, et al. Immunoglobulin V region gene use and structure suggest antigen selection in AIDS-related primary effusion lymphomas. Leukemia 1999;13:1093-9.

59. Gaidano G, Gloghini A, Gattei V, Rossi MF, Cilia AM, Godeas C, et al. Association of Kaposi’s sarcoma-associated herpesvirus-positive primary effusion lymphoma with expression of the CD138 / syndecan-1 antigen. Blood 1997;90:4894-900.

60. Carbone A, Gloghini A, Larocca LM, Capello D, Pierconti F, Canzonieri V, et al. Expression profile of MUM1 / IRF4, BCL-6, and CD138 / syndecan-1 defines novel histogenetic subsets of human immunodeficiency virus-related lymphomas. Blood 2001;97:744-51.

61. Said JW, Shintaku IP, Asou H, deVos S, Baker J, Hanson G, et al. Herpesvirus 8 inclusions in primary effusion lymphoma. report of a unique case with T-cell phenotype. Arch Pathol Lab Med 1999;123:257-60.

62. Boulanger E, Agbalika F, Maarek O, Daniel MT, Grollet L, Molina JM, et al. A clinical, molecular and cytogenetic study of 12 cases of human herpesvirus 8 associated primary effusion lymphoma in HIV-infected patients. Hematol J 2001;2:172-9.

63. Chan AC, Chan JK, Yan KW, Kwong YL. Anaplastic large cell lymphoma presenting as a pleural effusion and mimicking primary effusion lymphoma. A report of 2 cases. Acta Cytol 2003;47:809-16.

64. Brimo F, Michel RP, Khetani K, Auger M. Primary effusion lymphoma: a series of 4 cases and review of the literature with emphasis on cytomorphicologic and immunocytotoxic differential diagnosis. Cancer 2007;111:224-33.

65. Nakatsuka S, Yao M, Hoshiba Y, Yamagato S, Iuchi K, Aozasa K. Pyothorax-associated lymphoma: a review of 106 cases. J Clin Oncol 2002;20:4255-60.

EDITORIAL / PEER-REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a double blind model (authors are blinded for reviewers and vice versa) through an automatic online system.