Human herpesvirus 8-related primary effusion lymphoma in four HIV-uninfected patients without organ transplantation

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Keywords
Exudative effusion, human herpes virus 8, non-Hodgkin’s lymphoma.

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Abstract
Primary effusion lymphoma (PEL) is a rare subtype of non-Hodgkin’s lymphoma. PEL is closely related in pathogenesis to human herpesvirus 8 (HHV-8) and typically occurs in patients with significant immunodeficiency. Cases of PEL in patients without either HIV infection or transplantation have been reported from HHV-8-endemic areas, but very rarely from the Asia-Pacific region. In this case series, we describe the clinical and immunohistochemical presentations of four patients in southern Taiwan who were diagnosed with HHV-8-related PEL. All four patients were HIV-negative and had not received organ transplantation. To our knowledge, this is the first authentic report of HIV-unrelated PEL from the ethnically Chinese population. Considering the non-specific clinical manifestations, the pivotal roles of specific assays for correct diagnosis, and the dismal prognosis of PEL, it is important for clinicians to include PEL as one of the differential diagnosis when approaching HIV-uninfected patients with unexplained exudative body cavity effusion.

Introduction
Primary effusion lymphoma (PEL) is a rare subtype of non-Hodgkin’s lymphoma that was initially diagnosed in patients with advanced-stage infection by HIV, and had a typical predilection for body cavities without discernible tumorous masses. PEL is closely related to human herpesvirus 8 (HHV-8, also known as Kaposi’s sarcoma-associated herpesvirus). Over the last three decades, cases of PEL in HIV-uninfected patients have been described, and most of which were reported from regions with high prevalence of HHV-8-related infection and in patients who had received immunosuppressants post organ transplantation. PEL was very rarely reported from the Asia-Pacific region. In this case series, we describe the clinical, radiographical, cytological, and immunohistochemical presentations of PEL in four HIV-uninfected patients who lived in southern Taiwan and had no previous history of organ transplantation or immunosuppressive therapy. Their demographic and clinical characteristics are displayed in Table 1.

Case Series

Case 1
A 93-year-old man presented with progressive dyspnoea lasting for several weeks without fever in December 2016. He had histories of hypertension, dyslipidaemia, and chronic kidney disease. Initial surveys showed no oedema and no significant change in his renal or cardiac functions. Physical palpation did not detect enlarged lymph node, liver, or spleen. The chest radiograph revealed moderate left-sided pleural effusion (Fig. 1A). Upon thoracentesis, bloody fluid was aspirated. Analyses of the fluid reported an exudative nature with a very high level of lactate dehydrogenase (LDH), and the presence of many atypical cells exhibiting large pleomorphic nuclei, prominent nucleoli, and abundant
### Table 1. Demographic characteristics and pertinent clinical data of the four patients.

| Parameters                        | Case 1       | Case 2       | Case 3       | Case 4       | Normal range |
|-----------------------------------|--------------|--------------|--------------|--------------|--------------|
| Sex                               | Male         | Male         | Male         | Male         | NA           |
| Age of diagnosis (years)          | 93           | 77           | 69           | 78           | NA           |
| Body height (cm)                  | 162          | 157          | 153          | 159          | NA           |
| Body weight (kg)                  | 42.2         | 59.0         | 49.1         | 56.9         | NA           |
| Outcome                           | Died         | Complete remission | Died         | Ongoing treatment | NA           |
| **Haemogram**                     |              |              |              |              |              |
| WBC ($10^3/μL$)                   | 10.1         | 9.3          | 9.3          | 8.7          | 3.4–9.1      |
| Neutrophil (%)                    | 51.9         | 78.9         | 71.6         | 73.3         | 43.0–64.0    |
| Lymphocyte (%)                    | 11.0         | 12.3         | 13.5         | 10.6         | 27.0–47.0    |
| Monocyte (%)                      | 35.9         | 8.1          | 12.9         | 14.4         | 3.0–9.0      |
| Eosinophil (%)                    | 1.2          | 0.4          | 1.4          | 1.4          | 0–6.0        |
| Basophil (%)                      | 0            | 0.3          | 0.6          | 0.3          | 0–1.0        |
| Platelet ($10^3/μL$)              | 114          | 322          | 325          | 78           | 138–353      |
| Haemoglobin (g/dL)                | 8.6          | 13.6         | 9.4          | 12.3         | 13.5–17      |
| Haematocrit (%)                   | NA           | 40.7         | 28.5         | 36.7         | 39.1–48.9    |
| **Blood immununo-biochemistries** |              |              |              |              |              |
| LDH (U/L)                         | 271          | 322          | 150          | 179          | 135–225      |
| Total protein (g/dL)              | 5.2          | NA           | 7.3          | 7.3          | 6.4–8.3      |
| Albumin (g/dL)                    | 2.8          | NA           | 2.8          | 4.3          | 3.5–5.0      |
| Glucose (mg/dL)                   | 112          | NA           | NA           | NA           | 70–115       |
| IgG (mg/dL)                       | 1480         | NA           | 2270         | 1320         | 750–1690     |
| IgA (mg/dL)                       | 154          | NA           | 3.61         | 62.5         | 82–463       |
| IgM (mg/dL)                       | 53           | NA           | 67.9         | 80.3         | 46–304       |
| HIV screening                     | Negative     | Negative     | Negative     | Negative     | Negative     |
| HBs Ag                            | Negative     | Negative     | Positive     | Negative     | Negative     |
| HCV Ab                            | Negative     | Negative     | Negative     | Negative     | Negative     |
| EBV viral load (copies/mL)        | NA           | Undetectable | <204         | Undetectable | Undetectable |
| β2-Microglobulin (mg/L)           | 12.62        | 3.36         | 17.70        | 47.09        | 0.8–2.2      |
| **Effusion analyses**             |              |              |              |              |              |
| Site of effusion                  | Left pleural | Pericardial  | Right pleural| Right pleural| NA           |
| Appearance                        | Bloody       | Bloody       | Yellowish    | Bloody       | NA           |
| LDH (U/L)                         | 7450         | NA           | 172          | 3030         | NA           |
| Total protein (g/dL)              | 3.8          | NA           | 3.5          | 4.1          | NA           |
| Glucose (mg/dL)                   | 29           | NA           | 217          | 38           | NA           |
| WBC (count/mm$^3$)                | 11,400       | 22,090       | 250          | 3089         | NA           |
| **Immunohistochemical staining**  |              |              |              |              |              |
| of tumour cells                   |              |              |              |              |              |
| HHV-8                             | Positive     | Positive     | Positive     | Positive     | NA           |
| EBER in situ hybridization        | Negative     | Positive     | Positive     | Negative     | NA           |
| Positive cell markers             | CD30, CD138, | CD45, CD138, | CD138        | CD138        | NA           |
|                                  | kappa (focal)| MUM-1, BCL-6 |              |              |              |
|                                  |              | (weak)       |              |              |              |
| Negative cell markers             | CD3, CD20,   | CD3, CD10,   | CD3, CD20,   | CD3, CD20,   | NA           |
|                                  | lambda       | kappa, lambda| calretinin,  | kappa, lambda|              |
|                                  |              | CK, WBC      | CK, TTF-1    | CK, WBC      |              |

**Note:** BCL-6, B-cell lymphoma 6 protein; CD, cluster of differentiation; CK, cytokeratin; EBER, EBV-encoded small RNA; EBV, Epstein–Barr virus; HBs Ag, surface antigen of hepatitis B virus; HCV Ab, antibody against hepatitis C virus; HHV-8, human herpesvirus 8; Ig, immunoglobulin; LDH, lactate dehydrogenase; MUM-1, multiple myeloma oncogene 1; NA, not available; TTF-1, thyroid transcription factor-1; WBC, white blood cell.
cytoplasm (Fig. 2A, E). Immunophenotypically, these atypical cells were positive for cluster of differentiation (CD) 138, CD30, HHV-8 (Fig. 2F–H), and (focally) kappa chains, but negative for lambda chains and Epstein–Barr virus (EBV)-encoded small RNAs (EBER) in situ hybridization. Computed tomography (CT) scanning from the neck to the pelvis detected no lymphadenopathy, hepatosplenomegaly, or any solid tumour. Pathological and cytogenetic studies of the bone marrow reported hypocellularity without any malignant cell or monoclonal chromosomal change; immunostaining for HHV-8 was negative. Screening tests for HIV and chronic viral hepatitis were negative. The patient decided to receive only palliative treatments, and died five months later.

**Case 2**

A 77-year-old man came to our emergency room in May 2017 complaining about constipation and abdominal
fullness for one week without fever. His past medical history was remarkable for a left parasagittal meningioma that was resected in 2005 without recurrence. Initial examination detected no hypotension, cervicoaxillary lymphadenopathy, hepatosplenomegaly, or anorectal mass. However, the chest radiograph revealed an enlarged cardiac silhouette with a “water-bottle” shape (Fig. 1B). A large amount of pericardial effusion, with diastolic collapse of the right atrium, was confirmed by echocardiography (Fig. 1E). An emergent surgical window was created for drainage. Examination of the surgically sampled pericardial effusion and tissue observed atypical lymphoid cells (Fig. 2B) that immunohistochemically stained positive for CD138, CD45, multiple myeloma oncoprotein 1 (MUM-1), and HHV-8 (upper inset of Fig. 2B); equivocally for B-cell lymphoma 6 (BCL-6); and negative for CD3, CD20, both kappa and lambda chains, CD10, calretinin, and cytokeratins (CKs). Focally, some tumour cells also stained positive for EBER in situ hybridization (lower inset of Fig. 2B), and these cells were negative for HHV-8. A whole-body CT scan revealed a few mildly enlarged paratracheal lymph nodes (up to 1.8 cm in diameter), but no ascites, solid tumour, or abnormal thickening of the gastrointestinal walls. Examination of the biopsied bone marrow reported hypocellularity but normal cytogenetics and no neoplastic cells; HHV-8 immunostaining was negative. Screening tests for HIV and viral hepatitis were negative. The patient subsequently received six cycles of chemotherapy with cyclophosphamide, vincristine, and prednisone (the “COP-regimen”) and has remained in a state of complete remission for more than two years until present.

Case 3

A 69-year-old man with chronic hepatitis B, end-stage renal disease, and multiple metabolic comorbidities presented to our clinic of chest medicine in September 2018.
with progressively aggravating dyspnoea (without fever) for three months. Right-sided pleural effusion was detected on the initial chest radiograph (Fig. 1C). Analysis of the pleural fluid reported an exudative nature with a mildly elevated level of LDH. Diagnostic pleuroscopy was subsequently performed, during which diffuse thickening of the pleura (without nodularity), fibrin deposition, and multiple loculation of yellowish effusion were observed (Fig. 1F). Histological examination of the effusion and biopsied pleura revealed the presence of atypical lymphoid cells (Fig. 2C) that were immunohistochemically positive for CD138 and HHV-8 (lower inset of Fig. 2C), but negative for CD3, CD20, thyroid transcription factor 1 (TTF-1), and calretinin. Some cells were also positive for EBER in situ hybridization. The whole-body CT scan reported mildly enlarged lymph nodes (up to 2.3 cm) in bilateral supra-clavicular, mediastinal, and para-aortic regions, but neither hepatosplenomegaly nor solid tumour. Examination of the bone marrow reported normocellularity without clonal structural abnormality; HHV-8 immunostaining was negative. Screening tests for HIV and hepatitis C were negative. The patient then received chemotherapy with COP-regimen plus rituximab. The therapy was complicated with refractory sepsis, and the patient died about one month later.

**Discussion**

Ever since its first report in patients with advanced HIV infection [1–3], PEL has been subsequently diagnosed in HIV-uninfected patients. Many of these HIV-uninfected cases were identified in HHV-8-endemic regions, or were found in recipients of organ transplantation undergoing immunosuppressive therapy [4–6]. Sporadic cases have been reported in patients with neither HIV infection nor transplantation history from other regions including Japan [7] and South Korea [8]. Zhao et al. from China described four cases of HIV-unrelated PEL. However, this report (in Chinese) did not provide data on either the immunophenotypes or the status of HHV-8 infection of the tumour cells. Besides, nodules in the lungs and on the pleural surfaces were identified in all four patients. These drawbacks might have potentially rendered the diagnosis of PEL questionable [9]. To our knowledge, ours is the first comprehensive and diagnostically reliable case series of HIV-unrelated PEL from the ethnically Chinese region.

All four patients in our report presented with nonspecific symptoms that were relating to the accumulation of lymphomatous effusion. In particular, they all lacked typical “B-symptoms.” This feature highlights the importance for clinicians to maintain high vigilance when approaching unexplained exudative effusion of body cavities even in patients without HIV infection. In addition, all four patients in our series had advanced ages when PEL was diagnosed, which is similar to most of the previously reported HIV-uninfected cases, but contrasts with the relatively young age of diagnosis of HIV-related PEL. Moreover, tumour cells of all four patients were immunophenotypically positive for the post-germinatal centre CD138 and negative for typical B- or T-lineage markers, and exhibited evidence of HHV-8 infection. HHV-8 plays important roles in the oncogenesis and diagnosis of PEL [10,11]. Although cases of “PEL-like lymphoma” without identifiable HHV-8 infection have been reported that present similarly as primary lymphomatous effusion, the evidence of intracellular presence of HHV-8 is necessary to diagnose PEL [10,11]. PEL with a T-predominant immunophenotype has also been described [14]. Besides, tumour cells from two patients were also positive for EBER. The presence of EBV in PEL has been frequently documented in previous reports, and a potentially collaborative role of EBV with HHV-8 in the pathogenesis has been proposed [15]. Furthermore, patients in cases 2–4 exhibited mildly enlarged mediastinal lymph nodes that were not examined histologically. The possibility of concurrent HHV-8-related Castleman’s disease or...
infection- or neoplasm-related lymphadenopathy could not be completely excluded. Besides, cases of PEL manifesting chiefly as solid tumorous masses (the so-called "solid-variant PEL") have been reported [16]. Nevertheless, considering the relative predominance of effusion versus lymphadenopathy in our patients, and also considering the cytological and immunophenotypical findings and the fact that no other solid mass and no neoplastic cells in the bone marrow was identified, the diagnosis of PEL is reasonable and reliable. Cases of PEL with lymphadenopathy have also been reported previously [4,12]. Finally, two of the four patients died shortly after diagnosis, which reflected the current dilemma in managing PEL such that no standard therapy was available and that the overall prognosis still remains very poor [11].

Conclusion

PEL does not exclusively occur in HIV-infected or post-transplantation patients. The presentation of PEL can be very non-specific, and a high degree of clinical suspicion, particularly in HHV-8-non-endemic regions, is pivotal to initiate pertinent and targeted surveys to reach the correct diagnosis. Breakthroughs in, and consensus on, therapeutic strategies for PEL are still pending to improve the overall dismal outcome.

Disclosure Statement

Appropriate written informed consent was obtained for publication of this case series and accompanying images.

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