A case report-application of pericardial effusion cytology and next-generation sequencing technology: quick and secure diagnosis of primary effusion lymphoma

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Background
Primary effusion lymphoma (PEL) is an uncommon subtype of non-Hodgkin lymphoma (NHL) that usually involves the pleura, pericardium, and peritoneum without an obvious tumour mass, with multiple plasma effusions as its main clinical feature. We report a case of a massive pericardial effusion in an elderly male with a final diagnosis of PEL.

Case summary
A 70-year-old male patient was admitted to hospital with symptoms of chest tightness, shortness of breath, fatigue, loss of appetite, and cough with phlegm after a pericardial effusion had been found for 5 months. The next-generation sequencing of pericardial effusion found human herpesvirus type 8 (HHV-8) infection, and further cytomorphological and immunohistochemical examination were done. According to the patient’s HHV-8 infection, the pathological features of heterogeneous B cells with plasmablastic differentiation and the immunohistochemical characteristics of PEL, the final diagnosis was made as human immunodeficiency virus-negative PEL.

Discussion
The diversity and non-specificity of PEL symptoms, as well as its rarity, make it difficult to diagnose. In this case, we used the next-generation sequencing technology to screen the pathogen of the patient’s pericardial effusion and carried out morphological and immunohistochemical examination of the cells in the pericardial effusion, which provided a clinically operable diagnosis for an uncommon disease, enabling us to make a clear diagnosis faster and start treatment in time.

Introduction
Human immunodeficiency virus (HIV)-associated primary effusion lymphoma (PEL) is a rare and aggressive subtype of B-cell non-Hodgkin lymphoma (NHL).1 All lymphoma cells display the viral genome of Kaposi sarcoma (KS) associated herpesvirus/human herpesvirus 8 (KSHV/HHV-8), with 70%–80% of patients complicated with Epstein-Barr virus (EBV) infection.2 The main clinical feature is malignant effusions in multiple body cavities including pleural, pericardial, and abdominal cavities, and most of them are not accompanied by masses. However, there are several reports of HHV-8-related HIV-negative cases, mainly in immunodeficient and elderly patients.3,4 PEL is typically diagnosed based on cytological evidence of effusion fluid, and tumour cells show significant changes in size, from large immune cells or plasma cells to cells with acellular morphology. The tumour cells usually express CD45, but lacking pan–B-cell antigens, including CD19, CD20, and CD79a. In association with the tumour
cells with plasmablastic differentiation, the lymphoma cells express plasma cell-related markers such as CD38, CD138, and VS38c. These cells usually lack T-cell/natural killer-cell antigens.1 PEL has no established therapy, but it is sometimes similar to the treatment of other aggressive NHLs. Even in the era of effective combination antiretroviral therapy, its published median overall survival remains less than 1 year.2

### Timeline

| Date       | Events                                                                 |
|------------|------------------------------------------------------------------------|
| 2021.01    | Patient’s cardiac ultrasound revealed a small amount of pericardial effusion. |
| 2021.03    | Patient experienced chest tightness, shortness of breath, fatigue, loss of appetite, and productive cough. |
| 2021.06.21 | Patient admitted to hospital with massive pericardial effusion.         |
| 2021.06.23 | Pericardiocentesis was done through apical approach                    |
| 2021.06.29 | The next-generation sequencing of pericardial effusion found human herpesvirus type 8 infection, fluorodeoxyglucose (FDG)-positron emission tomography showed patchy FDG metabolism significantly elevated at the right auricle. |
| 2021.07.09 | Morphological and immunohistochemical findings of the pericardial effusion cells were consistent with the diagnosis of primary effusion lymphoma. |
| 2021.07.09 | Patient received chemotherapy with a VRD regimen (bortezomib 2.4 mg d1, d4, d8, d11 + dexamethasone 20 mg d1-2, d4-5, d8-9, d11-12 + lenalidomide d1-21). |
| 2021.07.20 | Patient discharged from hospital to continue oral medication (prednisone 65 mg twice daily, lenalidomide 25 mg once daily, calcium carbonate 600 mg once daily, and rabeprazole 10 mg once daily) and was advised to be admitted to hospital for the next chemotherapy treatment 3 weeks later. |
| 2021.08.30 | Patient died of respiratory and circulatory failure at the local hospital 1 month after discharge, the exact cause of death and the data at the time of death were unknown. |

### Case presentation

A 70-year-old male patient was admitted to our hospital with pericardial effusion found for 5 months. He was presented with chest tightness, shortness of breath, fatigue, loss of appetite, and productive cough. Three years ago, he underwent a ventriculoperitoneal shunt for a traumatic brain injury and has been receiving anti-epileptic treatment. He also had a history of chronic bronchitis and hypertension.

Physical examination revealed a heart rate of 56 beats per minute and 23 breaths per minute, blood pressure of 130/78 mmHg, and coarse breath sounds and audible rales in both lungs. The patient had no jugular venous distention, no bilateral lower limb edema, and no superficial lymph node enlargement or hepatosplenomegaly. Laboratory findings were significant for the elevated C-reactive protein 44.5 mg/L (reference range 0–8.0 mg/L), lactate dehydrogenase (LDH) 346 U/L (reference range 120–250 U/L), CA125 408.4 U/mL (reference range 0–35.0 U/mL), free light chain kappa 54.7 mg/L (reference range 6.7–22.4 mg/L), free light chain lambda 57.1 mg/L (reference range 8.3–27.0 mg/L), and D-dimer 3205 μg/L (reference range 0–700 μg/L). Antinuclear antibody test showed positive for SSA25. Tests for hepatitis B, hepatitis C, syphilis, HIV, or tuberculosis were all negative. Electrocardiogram showed low voltage in the extremity leads. Echocardiogram revealed a large amount of pericardial fluid in the posterior wall (depth 2.3 cm) and inferior pericardium (depth 2.4 cm) (Figure 1). There were no signs of cardiac tamponade such as cardiac sway, right atrial, or right ventricular wall collapse. Computed tomography (CT) and ultrasound revealed a small amount of bilateral pleural effusion, but no abdominal effusion, no mass, lymph node enlargement, or hepatosplenomegaly was detected. Fluorodeoxyglucose (FDG)-positron emission tomography (PET) showed a slight increase of FDG metabolism in the pericardial cavity and in the left pleural effusion, as well as a significant patchy FDG metabolism near the right auricle (Figure 2). Pericardiocentesis was done through apical approach, and a total of 1550 mL of bloody pericardial fluid was drained over a period of 1 week. Laboratory exam of the effusion showed red blood cells 350,000/μL, nucleated cells 80/μL, LDH 3181 U/L, protein 45.024 g/L, glucose 3.10 mg/dL, adenosine deaminase 114.80 U/L, ferritin >3351.12 ng/mL (reference range 7.0–323.0 ng/mL), and CA125 109.9 U/mL (reference range 0–35.0 U/mL). Cytology of the pericardial fluid was repeatedly sent for examination, but no tumour cells were found. Bacterial cultures of the pericardial fluid as well as smear microscopy of Mycobacterium tuberculosis and GeneXpert examination were negative.

In order to identify a possible infectious cause of the patient’s pericardial effusion, next-generation sequencing (NGS) of pericardial fluid was used to screen for presence of pertinent pathogens. Results showed that common microflora and HHV-8 infection were identified during DNA detection process with 345 sequences detected and 99.0% confidence level, and the RNA detection process also showed HHV-8 infection with 18 sequences detected and 99.0% confidence level. Torque teno virus, a common human opportunistic infection virus, was also detected. Neither DNA nor RNA detection process showed evidence of fungal or parasitic infection. The detection of HHV-8 suggested the possibility of lymphoproliferative disorders such as KS, PEL, multicentric Castleman disease (MCD), MCD-associated plasmablastic lymphoma and HHV-8+, and EBV+ germinotropic lymphoproliferative disorder.5 Immunophenotyping of pericardial effusion cells suggested that the abnormal plasma cell population accounted for approximately 66.7% of non-erythroid cells, expressing CD38, CD138, cyclappa, but lacking CD19, CD20, CD27, CD28, CD56, and cykappa. Next the sediment from the centrifugation of the pericardial effusion was made into paraffin-embedded (FFPE) cell blocks, and differentiated cells with a heteromorphic plasmablastic morphology were found in the specimens. Immunohistochemical (IHC) staining of FFPE cell blocks showed CK (pan) (mesothelial +), EMA (partial +), CD20 (−), CD3 (lymphocyte +), CD38 (+), CD138 (minority +), ALK (−), CD30 (+), CD43 (partial +), c-Myc (++, 50%), HHV-8 (+), MUM1 (+), EBER (−), Ki-67 (+95%+), Bcl-2 (+), Bcl-6 (+).
(+), and CD10 (−) (Figure 3). Morphological and IHC findings of the patient’s pericardial effusion cells were consistent with the diagnosis of HIV-negative PEL. In addition, the patient’s bone marrow puncture results showed a slightly elevated percentage of mature plasma cells at 4%.

After definitive diagnosis, the patient received chemotherapy with a Bortezomib, Lenalidomide, and Dexamethasone (VRD) regimen (bortezomib 2.4 mg d1, d4, d8, d11 + dexamethasone 20 mg d1-2, d4-5, d8-9, d11-12 + lenalidomide d1-21). However, the patient died of respiratory and circulatory failure at the local hospital 1 month after discharge, the exact cause of death and the data at the time of death are unknown.

Discussion

Owing to diverse clinical manifestations and etiologies of pericardial diseases, the cause of some pericardial effusion is difficult to establish by routine examination, so accurate and individualized examination are needed. In this case, without pericardial effusion NGS test and pericardial effusion cytology, which confirmed the diagnosis of PEL through typical evidence of HHV-8 infection and lymphoma cells, we would not be able to distinguish the nature of the disease and specific types of tumour cells.

Traditional methods for identifying pathogens include microbial culture, smear staining, antigen detection, and serological testing.
which can help to identify pathogens to some extent, but NGS technologies can provide more comprehensive and unbiased identification of pathogens and their variants, as well as their resistance genes, allowing for individualized treatment options. Since the advent of NGS, there has been a rapid uptake of this technology in biologic research, which has resulted in significant advancements across multiple fields, including the understanding of the human microbiome associated with healthy normal flora and disease states in multiple body sites. NGS technology allows multiple DNA sequence reactions to be generated in parallel using a template library generated from selected genomic regions by using capture probes or polymerase chain reaction (PCR) amplification or unselected total genomic nucleic acids. This generates a large amount of sequence data, which is then assembled, mapped to a reference genome, and interrogated for nucleotide sequence differences from the reference genome using sophisticated bioinformatics solutions.\textsuperscript{7}

On the other hand, cytomorphology is the fundamental basis for cytological diagnosis, where ancillary studies play an increasing role in the work with body fluid specimens, and IHC staining of FFPE cell block is the most popular and readily available method and also played an important role in the diagnosis of this case.\textsuperscript{8}

In conclusion, PEL is a highly malignant form of lymphoma, and it often involves the pleura, pericardium, and peritoneum, with multiple plasma cavity effusions as the main symptom. The diagnosis of PEL relies on the clinical presentation of a large number of malignant effusion and associated imaging evidence, as well as the type and characteristics of cells in the effusion confirmed by cytological examination. The limitations of this case are the lack of post-discharge follow-up and follow-up assessment of the patient's physical condition, and the lack of clarity as to whether the patient's death was due to progression of the primary disease or a side effect of chemotherapy or a pre-existing condition.

This case highlights the importance of effective pathogen screening with NGS and precise cytological examinations for establishing a quick and secure diagnosis for cardiac primary tumour in patients with suspected malignant pericardial effusion.

**Consent:** The authors confirm that written consent for submission and publication of this case report including images and associated text has been obtained from the patient in line with COPE guidelines.

**Conflict of interest:** None declared.

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