# HHV8 and EBV negative primary effusion-based lymphoma: a case report of a new provisional entity and review of literature.

Marta Canelo-Vilaseca[1], Gustavo Tapia Melendo[1], Elisa Orna[1], Marc Sorigué[1], Isabel Granada[1], Azahara Palomar-Muñoz[2], Ana Castillo[1], Javier Grau[1], Alba Mesa[1], Josep-Maria Ribera[3], Juan Manuel Sancho[3], and Jose-Tomas Navarro[4]

[1]Hospital Germans Trias i Pujol
[2]Hospital Universitario de Bellvitge
[3]Catalan Institute of Oncology-Josep Carreras Leukaemia Research Institute
[4]Catalan Institute of oncology-Josep Carreras Leukaemia research Institute

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**Abstract**

Primary effusion lymphoma (PEL) is a rare non-Hodgkin lymphoma confined exclusively to body cavities without detectable tumor masses. The term PEL-like is an entity similar to PEL in clinical presentation but without relation to human herpesvirus 8 (HHV8). We report a case of HHV8- and EBV-negative primary effusion-based lymphoma.

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Marta Canelo-Vilaseca[1], Gustavo Tapia[2], Elisa Orna[1], Marc Sorigué[1], Isabel Granada[1], Azahara Palomar-Muñoz[3], Ana Castillo[2], Javier Grau[1], Alba Mesa[1], Josep-Maria Ribera[1], Juan-Manuel Sancho[1], José-Tomás Navarro[1].

[1]Hematology Department, ICO-Hospital Germans Trias i Pujol, Josep Carreras Leukemia Research Institute, Universitat Autònoma de Barcelona-Badalona, Spain.

[2]Pathology Department, Hospital Germans Trias i Pujol, Badalona, Spain.

[3]Nuclear Medicine-PET (IDI) Department, University Hospital of Bellvitge-IDIBELL, Barcelona, Spain.

**Correspondence** : José-Tomás Navarro. Hematology Department, ICO-Hospital Germans Trias i Pujol, Josep Carreras Leukemia Research Institute, Universitat Autònoma de Barcelona. Carretera de Canyert s/n. CP 08916. Badalona. Spain. E-mail: jnavarro@iconcologia.net. Telephone number: 34-934978868.

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## INTRODUCTION

Primary effusion lymphoma (PEL) is a rare aggressive B-cell neoplasm that presents with serous malignant effusions without detectable tumor associated mass.[1] It is related with HHV8 infection and mainly affects immunocompromised patients such as HIV-infected individuals, recipients of solid-organ transplantation or

elderly patients.[3] Moreover, the coinfection with Epstein-Barr virus (EBV) is frequent. The term "primary effusion lymphoma" was first described in the literature in 1996 by Nador et al.[3] to define malignant lymphomatous effusions related to HHV8 infection. In 2001, the World Health Organization (WHO) introduced the category of PEL only for HHV8-related primary lymphomatous effusions. In 1996 Hermine et al.[4]reported the first HHV8 negative lymphomatous effusion in an HIV-seronegative patient. Since then, more than 60 HHV8-unrelated effusion-based lymphomas (EBL) have been reported in the literature, most of them in Japan, and terms as EBL and PEL-like lymphomas have been used. Recently, the ICC of Mature Lymphoid Neoplasms has recognized these cases as a new provisional entity named HHV8 and EBV-negative primary effusion-based lymphoma.[2] Moreover, WHO Classification, 5th edition, has also recently recognized this entity as fluid overload-associated large B-cell lymphoma.[5]

We report a 72-year-old patient that presented with pericardial effusion with no evidence of tumor mass. Morphologic, immunophenotypic and molecular studies of the tumor cells of the pericardial effusion were performed. Cytological analysis showed large cells with irregular nuclei, prominent nucleoli and vacuolized cytoplasm. Immunophenotype showed expression of CD45, CD19, CD20 without detection of surface immunoglobulin. The tumor cells were negative for LANA-1 and EBER. Finally, PEL-like was diagnosed. To date, clinical and pathologic features of HHV8-unrelated, effusion-based lymphomas (EBL) are poorly understood. We report herein a case of the new entity HHV8- and EBV-negative primary effusion-based lymphoma, presenting in a Caucasian woman and review the literature.

## CASE REPORT

We report the case of a 72-year-old Caucasian woman, with a medical history of hypertension, hypothyroidism and mild obesity, who was admitted to hospital because of progressive dyspnea and appearance of bilateral leg oedemas above the ankles. No weight loss or concomitant fever were reported. Physical examination revealed distant heart sounds. The liver, spleen and peripheral lymph nodes were not palpable. Laboratory data on admission was remarkable for B-type natriuretic peptide level of 1117 pg/ml (normal value: <250 pg/ml). Complete blood count, renal and hepatic function were within normal values and the serum lactate dehydrogenase (LDH) was not elevated. A chest X-ray showed cardiomegaly (figure 1a) and transthoracic echocardiogram revealed pericardial effusion. The patient was admitted to the cardiac care unit, where pericardiocentesis was successfully carried out, yielding 800 mL of serohematic fluid. The pericardial liquid analysis showed features of exudative effusion (glucose <5mg/dl, red blood cells 15871 cell/μL, white blood cells 850 cell/μL with 55% of atypical cells, protein 49.6 g/L, LDH 4034 U/L, ADA 84,2 U/L). Malignant-appearing cells were detected in the cytospin preparation. Microbiological tests were negative including mycobacteria culture and multiplex polymerase chain reaction (PCR) for herpesvirus (VHS1, VHS2, VVZ, CMV and VH6). A whole-body positron emission tomography (PET) scan showed moderately hypermetabolic pericardial and pleural layers and right tonsillar enlargement (figure 1b). Tonsil excisional biopsy showed reactive follicular hyperplasia and a bone marrow biopsy showed no evidence of involvement by lymphoma. Serologic tests were negative for HIV, hepatitis C virus (HCV) and hepatitis B virus (HBV); whereas immunoglobulin G for EBV was positive, indicating a prior infection.

Cytological evaluation demonstrated large atypical cells variable in size and nuclei, ranging from round to more irregular shape with prominent nucleoli and abundant basophilic cytoplasm with vacuoles (figure 2a). Moreover, some mitotic figures were seen (figure 2b). In flow cytometric analysis, these cells were positive for CD45, CD19, CD10, CD20 and CD38 and lacked surface immunoglobulin expression. A formalin-fixed paraffine-embedded cell block was performed for morphology, immunocytochemistry (IHC) and fluorescence in situ hybridization (FISH) evaluation. IHC study showed positivity for CD20 (figure 3a) and PAX5 and negativity for CD3, CD10, CD30, MUM-1, BCL-6, BCL-2 and c-myc. Neither BCL-6, BCL-2 nor C-MYC gene translocation were detected by FISH. Moreover, EBV-encoded RNA (EBER) was negative (figure 3b) and protein latency-associated nuclear antigen 1 (LANA-1) was negative on IHC (figure 3c). High Ki67 expression was observed (>80%). Cytogenetic findings showed a complex karyotype.

All these findings pointed towards a diagnosis of PEL-like or HHV8 and EBV-negative primary EBL according to the ICC.[2] The patient was treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin,

vincristine and prednisone) for six cycles achieving complete remission evaluated by PET scan. At the time of this report, the patient remains in complete response for 16 months.

## DISCUSSION

PEL-like or HHV8 and EBV-negative PEBL is a rare B-cell neoplasm that typically presents as a malignant serous effusion in non-immunocompromised patients. In the last decade, several reports of HHV8-negative effusion lymphomas have been published demonstrating remarkably similar cytomorphologic characteristics to PEL but differences in immunophenotype, demographics, response to treatment and clinical outcome; suggesting that PEL-like is a distinct entity. As mentioned before, to date only HHV8-positive PEL is a recognized entity in the current WHO classification[1]; but HHV8 and EBV-negative PEBL has been recently suggested as a provisional entity by the International Consensus Classification of Mature Lymphoid Neoplasms.[2]

Our case is of particular interest because of the Caucasian origin of the patient as most of the published data are case series reported from Japan[6]. Despite its different origin, the other characteristics are similar to those described in the Japanese cases. Its prevalence is unknown and its diagnosis is challenging (especially because it has not been described in the current WHO classification).

HHV8 and EBV-negative PEL is considered to have a distinct etiopathogenic mechanism than HHV8-positive PEL. Different theories have emerged with no conclusive data to date. Kobashi et al[7] suggested in 2007 that immunosenescence related to age could play a major role, as most of the patients were older (median age of 70 years) compared with PEL individuals. In the same line, Ohshima et al[8] concluded that multi-step genomic abnormalities found in PEL-like cases could be related to age. In their study, most of the patients were elderly men with all cases showing complex karyotypes but without identification of a driver genomic abnormality. Suggested mechanisms of lymphomagenesis in HHV8 and EBV-negative PEL include impaired immune surveillance and dysregulatory haemopoietic pathway. Unlike PEL, in which HHV8 acts as an exogenous stimulus for local B-cell expansion, no viral mechanism has yet been identified in PEL-like lymphomas. In our case, Epstein-Barr virus-encoded small RNA in situ hybridization was negative but the patient had seropositivity for EBV. In the literature, around one third of cases of PEL-like lymphomas are infected with EBV.[9]However, whether EBV is simply a prior infection or a trigger of PEL-like lymphoma remains unclear and further investigation is needed. Regarding HCV infection in PEL-like lymphomas, there is a slightly higher prevalence of its infection among these patients (30% to 40% of patients). Association between HCV and B-cell non-Hodgkin lymphoma is well reported in the literature. Thus, it has been suggested that fluid overload related to cirrhosis due to HCV infection plays a role in PEL-like lymphomas. Moreover, underlying medical condition leading to fluid overload has been observed too in almost one-half of the patients.[7] The ICC also recognizes chronic serosal stimulation by fluid overload in its pathogenesis.[2]Although this last hypothesis needs further confirmation and not all the authors agree on that.[6]

Tumor cells in HHV8 and EBV-negative PEL express pan-B-cell markers and they are negative for plasma cell markers in contrast with PEL. CD45 antigen expression is present in both neoplasms indicating hematological derivation of the effusion cells.[10]

According to the reported series, the site of the effusion varies but the most common location was pleural, followed by ascites and being the pericardial one the less common.[7] Nonetheless, approximately 40% of the cases showed multiple body cavity lesions including pericardial in all of them.[6] In our case, probably both pericardial and pleural effusions had a neoplastic involvement according to images from PET scan (figure 2b). Nonetheless, the reason why this B-cell neoplasm is exclusively confined to serous membranes without developing tumor masses is still non-explained.

Diffuse Large B-cell lymphoma associated with chronic inflammation is another B-cell neoplasm which also arises in the serous membranes but unlike our case, the pathogenic mechanism and localization of this entity is well understood. Chronic inflammation causes local immunosuppression permitting EBV-infected memory B-cells evade immunosurveillance, accumulate genetic alterations and evolve to lymphoma.[11]

There is no standard treatment for this entity due to the small number of cases although the majority of them are treated with chemotherapy including anthracycline and rituximab (as tumor cells express CD20). In a retrospective study conducted by Kaji et al, 86% of patients received CHOP-like regimens.[6] Other systemic treatments consisted of rituximab in monotherapy or in combination with etoposide. Perhaps, due to the introduction of rituximab, the prognosis seems to be better in PEL-like lymphomas.[12] In this study conducted in Japan[6], the 2-year overall survival was 84.7% (95% confidence interval, 71.3-92.1) compared to 1-year overall survival of 30% in PEL. Moreover, there are some cases that were successfully treated only with drainage of the effusion.[13] Its survival might be conditioned by the advanced age at diagnosis and patients' comorbidity.[14]

## CONCLUSION

In summary, HHV8 and EBV-negative PEL is an extremely rare neoplasm involving body cavities without detectable tumor mass, characterized by atypical blastic cells expressing pan-B-cell markers. Unlike PEL, HHV8 and EBV-negative PEL usually presents in elderly patients without known immunodeficiency. Distinguishing PEL-like from PEL is important because better response to treatment and outcomes are achieved with standard therapies. Further investigation is needed to better understand this entity. To our knowledge, few cases of this new recognized entity have been described in Caucasians.

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