



Review Article

Classic KSHV/HHV-8-positive Primary Effusion Lymphoma (PEL): A Systematic Review and Meta-Analysis of Case Reports

Ilaria Cozzi, Giovanni Rossi, Emma Rullo and Valeria Ascoli.

Department of Radiological, Oncological and Pathological Sciences, Sapienza University, Rome.

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Abstract. Primary effusion lymphoma (PEL) is a large B-cell lymphoma growing within body-cavities caused by the Kaposi sarcoma-associated herpesvirus (KSHV)/human herpesvirus-8 (KSHV/HHV-8). It is mainly reported in HIV-infected patients. The uncommon occurrence in the elderly supports a form paralleling classic Kaposi sarcoma (KS), i.e. classic PEL, whose characteristics are relatively underexplored. To better understand the diagnostic modalities and clinical-epidemiological features of classic PEL, articles reporting cases of PEL were identified through MEDLINE/EMBASE databases (January 1998-July 2020) and screened according to PRISMA guidelines to extract individual-level data. A comparison was also performed between classic PEL and classic KS to evaluate similarities and differences. We identified 105 subjects (median age 77 years; 86% males), mainly from Mediterranean countries (52%, first Italy) and Eastern Europe (7%). Common comorbidities were heart failure (32%), cirrhosis (16%), and malignancy (20%) including lymphoid neoplasms. Pleural cavity was the commonest site (67%). PEL diagnosis was based on cytomorphology (89%), evidence of KSHV/HHV-8 infection (94%), EBV co-infection (28%) and clonality of IGH (59%), IGK (14%), TRG (9%) alone or in multiple combinations. Compared to KS, age ($P<.001$), gender-ratio ($P=.08$) and mortality ($P<.001$) were significantly higher in PEL, whereas the frequency of PEL as a second primary was similar ($P=.44$). This is the first systematic review of classic PEL case reports highlighting heterogeneity and lack of a uniform multidisciplinary approach at diagnosis, in the absence of specific guidelines as it happens for rare cancers. It is conceivable that classic PEL is still underdiagnosed in Mediterranean countries wherein KSHV/HHV-8 is endemic.

Keywords: Primary effusion lymphoma; Human herpesvirus 8; KSHV; Kaposi's sarcoma; elderly; B lymphocyte gene rearrangement.

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Correspondence to: Valeria Ascoli - Dipartimento di Scienze Radiologiche, Oncologiche e Anatomico-Patologiche, Università Sapienza, Viale Regina Elena, 324 - 00161, Roma. Tel: +39-06-49973397. E-mail: valeria.ascoli@uniroma1.it

Introduction. Primary effusion lymphoma (PEL) is a rare form of large B-cell lymphoma growing in liquid phase within body cavities covered by the serous membranes (pleura, pericardium, peritoneum), typically without solid tumor lesions. PEL cells lack B-cell markers, present Ig gene rearrangements, and a gene expression profile consistent with an

immunoblastic/plasmablastic derivation. The tumor clone is always infected by the Kaposi sarcoma-associated herpesvirus (KSHV)/human herpesvirus-8 (KSHV/HHV-8, gamma-herpesvirus) and may be coinfecting with Epstein-Barr virus (EBV). KSHV/HHV-8 was first detected in AIDS-related Kaposi sarcoma (KS)¹ and found thereafter in the other forms of KS:

sporadic/classic KS, endemic/sub-Saharan African KS, and iatrogenic KS. KSHV/HHV-8 was then discovered in AIDS-related body cavity-based lymphoma,² designated as a distinct clinicopathologic entity as PEL in 1996³ and then included in the WHO classification of neoplasms of the hematopoietic and lymphoid tissues published in 2001 and updated in 2008 (ICD-O-3 code 9678/3).⁴ As KS, PEL has been described in HIV-infected individuals with severe immunodeficiency (AIDS-related PEL) and HIV-uninfected patients such as solid organ transplant recipients (iatrogenic PEL) and elderly patients from areas with moderate/high prevalence for KSHV/HHV-8 infection. The latter supports a distinct clinico-epidemiological form of PEL resembling classic KS, i.e., classic PEL.^{5,6} As of yet, cases of PEL outside HIV infection in Africa have not been formally reported.

Given the rarity of KSHV/HHV-8-positive PEL, the epidemiology is poorly defined. Available Cancer Registry data such as the SEER database in the United States refer to PEL in the HIV-positive setting,⁷ as most published studies. PEL cases in the HIV-negative are mainly presented as isolated case reports supplemented by nonsystematic reviews of the literature, and more rarely in small case series. However, no studies are investigating the diagnostic work-up flow or providing specific strategies to reach a final diagnosis, considering the challenge of a lymphoma growing in effusions outside the HIV infection. In addition, data regarding the patient origin, comorbidities associated with the clinical presentation, other primary neoplasms, laboratory results have been scarce.

We have performed a systematic review of the case reports of classic PEL to analyze all the available data recorded in the literature. The aims include providing insights into clinicopathological and epidemiological features of classic PEL and determining the diagnostic modalities, focusing on KSHV/HHV-8 status ascertainment and clonality analysis. For the purpose of this study, we defined 'classic PEL' all the KSHV/HHV-8-positive PEL outside HIV-infection that were also non-iatrogenic and non-associated with primary immunodeficiency. To better understand this form of PEL, we compared classic PEL with classic KS, the most common and better studied HHV-8-associated disease.⁸

Methods.

Data sources and search strategy. We carried out a computerized search of the Ovid MEDLINE (PubMed) and EMBASE (Ovid) databases for potentially relevant studies in the English language published from January 1995 to July 2020: full articles with abstract, letters, and brief articles as images. Given the rarity of the disease, we also considered congress abstract indexed in search databases and non-English studies with at least an English abstract providing sufficient information for

inclusion. The electronic search strategy was as follows: KSHV AND lymphoma* [title] or PEL [title]; KSHV AND PEL [title]; KSHV/HHV8 AND lymphoma* [title]; KSHV-positive AND lymphoma*[title]; KSHV-associated AND lymphoma*[title]; Primary AND Effusion AND lymphoma* [title]; Body AND Cavity AND lymphoma* [title]; KSHV/HHV-8-associated AND lymphoma* [title]; KSHV/HHV-8-related AND lymphoma* [title]; Body AND cavity-based AND lymphoma*[title]; Primary AND Effusion AND lymphoma* [title] or PEL [title](((lymphoma, primary effusion[MeSH Terms]) OR (primary effusion lymphomas[MeSH Terms])) OR (primary effusion lymphoma[MeSH Terms])) OR (lymphomas, primary effusion[MeSH Terms]) OR (KSHV AND primary effusion lymphoma* [MeSH Terms])).

Inclusion and exclusion criteria. The eligibility criteria were: i) primary localization of PEL in a serous cavity covered by mesothelium (effusion-based disease without solid nodal/extranodal tissue involvement); ii) morphologic features and immunophenotype consistent with PEL; iii) demonstration of KSHV/HHV-8 infection of the tumor clone. For inclusion, the published cases must have documentation of diagnosis method(s) and individual patient data. Criteria for exclusion were: i) negative/unrelated or unknown KSHV/HHV-8 status; ii) HIV-infection; iii) solid organ transplant or immunosuppressive therapy; iv) primary extracavitary localization of PEL; v) primary immunodeficiency or idiopathic lymphocytopenia. Further exclusion criteria were either non-relevant titles or biomolecular and preclinical studies related to KSHV/HHV-8, and narrative reviews. Case series that had their analysis pooled without the description of individual patient data were also excluded.

Screening of literature. The screening of eligible publications was carried out independently by three reviewers (ER, GR and VA), first by screening titles and abstracts and then reviewing the full text. All references meeting the inclusion criteria, including those with scarce details, were considered. Disagreements were resolved by consensus, and the final decision was made by the author VA. In addition, IC undertook an extensive updated literature search. All authors have contributed to the data presentation and manuscript text. The whole review process was performed according to PRISMA guidelines.⁹

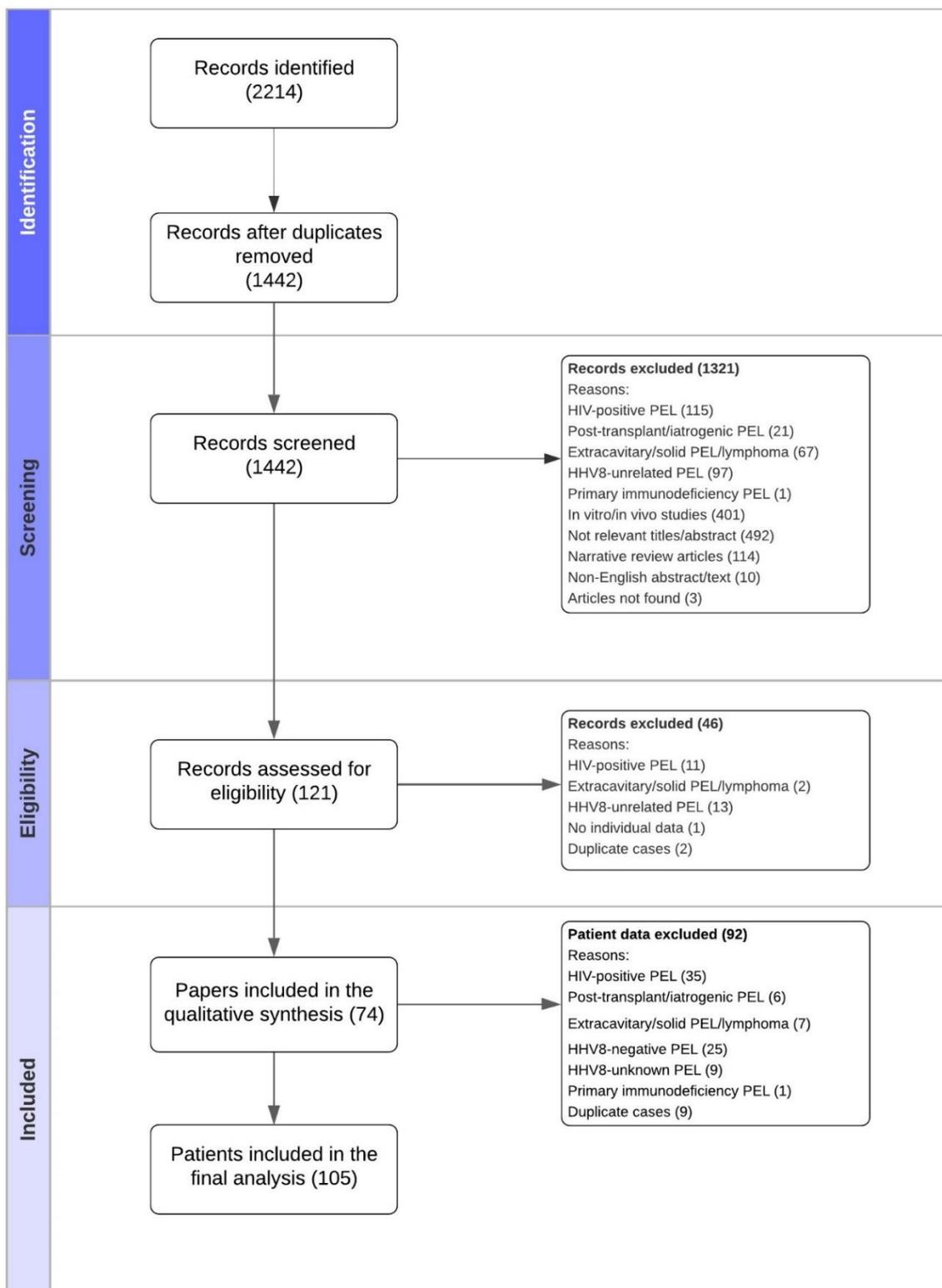
Data extraction. We extracted data on patient and PEL characteristics. Briefly, the following aspects were considered: age, gender, country-of-origin, the institution of diagnosis, comorbidities [Multicentric Castleman Disease (MCD), KS, heart failure, cirrhosis and cause, other primary cancers], laboratory data

including blood count, LDH, markers of inflammation, site of PEL, immunophenotype, immunoglobulins and T cell receptor genes clonality evaluation, search for KSHV/HHV-8 and EBV viral genomes, and outcomes.

Data synthesis and analysis. Descriptive statistics were used to summarize data, with median and interquartile range for continuous variables and frequencies and

percentages for dichotomous variables. The nonparametric Wilcoxon signed-rank test was used to compare the median, the t-test to compare the mean and the Fisher's exact test to determine nonrandom associations between two categorical variables. Stata version 14.2 (StataCorp, College Station, TX, USA). $P < 0.05$ was considered statistically significant.

Figure 1. PRISMA flow diagram describing the case selection process.



Comparison between classic PEL and classic KS. A PubMed search strategy including the terms Kapos*[Title] AND classic*[Title] OR Mediterranean [Title] was carried out (January 1994-June 2020) to perform a comparison of the clinic-epidemiological characteristics between classic PEL and classic KS. Since the classic variant of KS is described as primarily occurring in individuals in ethnic groups from Middle East, Eastern Europe, and the Mediterranean regions,⁸ the comparison was limited to classic PEL and classic KS cases from these geographical areas. We decided arbitrarily to focus on large case series/studies reporting at least 30 cases of classic KS who have presented to referral centers for KS over a certain period, from countries that in turn had reported cases of PEL. For inclusion, the published cases must have had reviewers (VA, GR) screened all titles and abstracts for eligibility.

Results.

Selection and description of studies. The database searches identified 2214 studies (**Figure 1**). After removing duplicate publications and papers that did not meet our inclusion criteria, the remaining 121 studies were assessed for eligibility. Forty-six studies were excluded following a more detailed review. We included 74 studies in the final analysis, accounting for 105 individual patient cases. Over the 23-year period of publications, 32 studies were published during the first 12 years (1995-2008)^{3,6,10-39} and 42 during the second 11 years (2009-2020)⁴⁰⁻⁸¹ as full articles (n=58), letters (n=7), images (n=5), and conference papers/abstracts (n=4). Sixty-eight (91.9%) articles reported one or two cases.

Patient demographics, comorbidities and PEL site. Demographics for 105 patients and clinical characteristics are listed in **Table 1**. The median (interquartile range, IQR) age was 77 (69-85) with no gender difference ($P>.05$). The male-to-female ratio was 6:1. The country-of-origin was documented in 46 cases. For publications by institutes and centers where this information was unreported (42 cases), we used the country affiliation of co-authors as a substitute for patients' country-of-origin; however, this strategy did not apply to 16 studies (17 cases) because of countries with a high rate of immigration (USA, Canada, England). Combining reported and presumed country-of-origin, the majority of cases were from the Mediterranean basin (52.3%) and Eastern Europe (6.9%), followed by East Asia (35.2%). The most represented countries were Italy (21 cases), Taiwan (16 cases), Japan (8 cases), South Korea (6 cases), and Greece (6 cases).

The clinical data were not consistently described. Of the underlying conditions known in 95 patients (90.5%), heart failure was the most common (31.5%); comparing patients with heart failure versus those without, there

Table 1. Demographic and clinical characteristics of 105 patients with classic KSHV/HHV-positive PEL

	Number (%)
Demographic characteristics	
Male	90 (85.7)
Female	15 (14.3)
Median age, years	77
Country of origin (n = 88)	
Italy	21 (23.9)
Greece	6 (6.9)
Spain, Portugal	5 (5.7)
France	3 (3.4)
Turkey	2 (2.3)
Israel, Lebanon	4 (4.5)
Algeria, Morocco	5 (5.7)
Eastern Europe ^a	6 (6.9)
Taiwan	16 (18.2)
Japan	8 (9.1)
South Korea	6 (6.9)
Others ^b	6 (6.9)
Clinical characteristics	
Comorbidities (n = 95)	
Heart failure	30 (31.6)
Liver cirrhosis	15 (15.8)
KS and/or MCD	11 (10.5)
Malignancy (n = 93)	23 ^c
Colon	4
Lymphoid neoplasms ^d	4
Stomach ^e	3
Breast	3
Prostate	3
Lung	2
Oral cavity	2
Others ^f	2
Number of cavities involved	
One	61 (59.1)
Pleural	41 (67.2)
Peritoneal	16 (26.2)
Pericardial	4 (6.6)
Two or more	44 (41.2)

n, number of patients with available data; KS, Kaposi sarcoma; MCD, multicentric Castleman disease; ^aRussia (n=4), Eastern Europe, not otherwise specified (n=2); ^bHong Kong, Argentina, Colombia, Peru, Philippines, Mali; ^cReferring to events not patients; ^dHodgkin's lymphoma (n=3), chronic lymphocytic leukaemia (n=1); ^eGastrointestinal stromal tumor (n=1); ^fLiver (n=1), ovary (n=1).

was no difference in terms of age and sex ($P>.05$). Fifteen patients (15.8%) had liver cirrhosis related to HBV and/or HCV (n=9), alcohol (n=3), cryptogenic (n=3); the median age of cirrhotic patients was significantly lower with respect to that of non-cirrhotic patients ($P<.001$); there were also two HBV and two HCV mono-infected patients without clinical evidence of cirrhosis. Overall, a history of prior or concomitant malignancy was reported in 19/93 cases (20%), for a total of 23 cancers. Three subjects had triple multiple primary cancers. The co-occurrence of other KSHV/HHV-8-related neoplasms was reported in 11 cases: KS (n=6), MCD (n=3), KS and MCD (n=2). Laboratory parameters were described in a minority of the cases reviewed (49 patients, data not shown in Tables), of which 35 (71.4%) were reported anemic, 3 (6.1%) leukopenic, and 14 (28.6%) as thrombocytopenic. The median (IQR)

hemoglobin count was 11 (9.4;12.4) in 36 patients whose absolute value was reported. The CD4 count resulted below the normal range in 10 out of 14 cases (71.4%), with a median (IQR) value of 241.5 (240;540). LDH and markers of inflammation (ESR and/or CRP) were elevated in >70% of the patients tested (23/32 and 22/28, respectively). Elevated serum beta2-microglobulin was found in 8 out of 9 patients tested.

Effusion in a single cavity was the most commonly observed manifestation. Except for three cases involving both the peritoneal and pericardial cavities, effusions in multiple sites constantly involved the pleural cavity as either bilateral effusion [pleural only (n=15), plus pericardial (n=5), plus peritoneal (n=3), plus pericardial & peritoneal (n=3)] or unilateral effusion [plus peritoneal (n=11), plus pericardial (n=4)]. The investigation of a possible association between underlying pathologies and site of PEL revealed statistically significant relationships between peritoneal site and cirrhosis (P<.001) and bilateral pleural site and heart failure (P<.001).

Methods of diagnosis, the sample of diagnosis, immunophenotype, virology, and clonality. **Table 2** summarizes the integration of morphology with immunophenotype and molecular techniques employed to diagnose PEL. Pathologic diagnosis was performed primarily by cytology of effusions. The immunophenotype was determined by flow cytometry (n=7) and immunohistochemistry (IHC) on smears and/or cell blocks obtained from effusions (n=85) or other specimens (n=4) for a total of 96 cases. The majority of the cases lacked B/T cell antigens; yet, there were both CD3 positive (n=6) and CD20 positive PELs (n=8). Details of morphology and immunophenotype were not specified in 9 cases. The majority of the cases reviewed (99/105; 94.3%) had proven KSHV/HHV-8 infection within PEL cells via IHC and/or molecular analysis; expression of LANA-1 alone was the most common approach (53/99; 53.5%), followed by DNA extraction and PCR amplification of viral sequences alone (32/99; 32.3%), or through both methods (14/99, 14.1%). Serum antibodies against KSHV/HHV-8^{33,61} serum/effusion fluid⁴⁸ and lymph node KSHV/HHV-8 DNA¹⁹ and unspecified method/sample⁷² were used as evidence of KSHV/HHV-8 etiologic involvement in a marginal fraction of cases (6/105; 5.7%). Tumor EBER status (EBV coinfection) was determined in 46/105 (43.8%) cases revealing mostly EBV-negative tumors (71.7%).

Clonality investigation was performed in 50 cases (47.6%); yet, the technique of clonality detection was unspecified for 5 and reported for 45 samples, respectively: PCR (n=27), Southern blot (n=8), FISH (n=7), immunonephelometry, Northern blot, and chromosomal aberrations study (n=1 for each method). The tracking of antigen-receptor gene rearrangements

Table 2. Pathological characteristics of 105 KSHV/HHV-8-positive PEL samples.

Samples used for the diagnosis	Number (%)
Effusion cytology	93 (88.7)
Lymph node fine-needle aspirate	1 (0.9)
Serous membrane biopsy	2 (1.9)
Unspecified	9 (8.6)
Immunophenotype	
CD20 negative	78/86 (90.7)
CD3 negative	67/73 (91.8)
CD138 positive	46/63 (73.0)
CD38 positive	24/25 (96.0)
CD30 positive	55/71 (77.5)
EMA positive	23/26 (95.6)
LANA-1 positive	67/67 (100.0)
EBV-LMP-1 negative	22/22 (100.0)
B/T cell clonality	
Monoclonal	44/50 (88.0)
IGH	26
IGH+IGK	2
IGH+TRG	2
IGH+IGK+TRG	2
IGK	6
TRG	4
Unspecified	2
Polyclonal/no clonal amplification	6/50 (12.0)
Virology	
KSHV/HHV-8-DNA positive	46/46 (100.0)
EBV-DNA positive ^a	10/31 (32.3)
EBER-ish positive ^b	13/46 (28.3)

^aLMP-1 was absent in 4/10 cases tested; ^bNo case was tested for LMP-1

for clonality analysis was successful in 44 out of 50 cases. Most PELs harbored rearrangements of the IGH locus alone or in multiple combinations with IGK and/or TRG and of the IGK locus alone, identifying a B-cell lineage; some cases had rearrangement of TRG alone (genotypic infidelity); the residual cases were reported as clonal without any reference to a specific gene rearrangement. Some cases were described as 'polyclonal' or 'negative for clonal rearrangement of the IGH genes'. Clonality was unavailable, not ascertained, or 'failed' in the residual 55 cases (52.4%).

Finally, considering two periods in comparison (1995-2009 vs. 2010-2020), the analysis of clonality and proof of KSHV/HHV-8 infection based on the search of the viral genome were reported more often in articles published during the first phase (P=0.009 and P<.001, respectively). On the other hand, LANA immunostaining was performed more frequently in the subsequent ten-year interval (P<.001).

Comparison between classic PEL and classic KS. The database search for classic KS identified 348 articles and a single systematic review referring to the treatment of classic KS.⁸² Overall, 308 studies did not meet the inclusion criteria for the following reasons: case reports or series less than 30 cases (n=208); unavailable clinical/demographical data (n=24); narrative reviews (n=6); data from non-Mediterranean areas (n=20);

registry data (n=13); non-English language studies (n=27); other studies (n=10). After excluding articles with duplicate data (n=14) and lower-quality studies with insufficient information (n=14), the remaining 12 publications were used for the comparison.^{83–94} **Table 3** shows a concise comparison of demographics, clinical and laboratory characteristics between classic variant of PEL and classic variant of KS from countries of the Mediterranean regions, Middle East, and eastern Europe. In the group of patients with PEL as compared to KS, the mean age was significantly higher ($P<.001$), and the male-to-female ratio was higher, though non-significant ($P=.08$). The percentage of patients with malignancy was similar in the two cohorts ($P=.44$). The number of deaths for the disease was significantly higher in PEL ($P<.001$). Only 2 KS studies reported detailed laboratory data;^{93–94} compared to KS, PEL cases had significantly lower hemoglobin level ($P<.001$), lymphocyte and CD4 counts ($P<.001$) while there was no significant difference in the white blood cell counts ($P=.08$).

Table 3. Comparison between classic PEL and classic Kaposi sarcoma.

	Classic PEL n=46 (%)	Classic KS n=1593 (%)
Demographic characteristics		
Age, mean ^a , years	76.8	68.8
Male/Female	5.57:1	2.76:1
Country of origin ^b		
Italy	21 (45.6)	636 (39.9)
Greece	6 (13)	68 (4.3)
Spain	4 (8.7)	53 (3.3)
Portugal	1 (2.2)	-
France	3 (4.3)	133 (8.3)
Morocco ^c , Algeria ^d	5 (10.8)	56 (3.5)
Israel	3 (6.5)	248 (15.6)
Turkey	2 (4.3)	248 (15.6)
Lebanon	1 (2.2)	-
Clinical characteristics^b		
Malignancies	8/41 (19.5)	78/519 (15)
Laboratory parameters, mean		
Hemoglobin, g/dL	11.68 (22)	13.35 (53)
White blood cells, $\times 10^3/\mu\text{L}$	6.78 (21)	7.04 (53)
Lymphocytes/ μL	1236 (15)	2017 (121)
CD4 cells/ μL	255 (9)	839 (68)
Death for disease	27/37 (72.9)	40/633 (6.3)

^aTo compare age at diagnosis, we calculated the weighted mean;

^bNumber of patients (%); ^cMorocco (2 cases); ^dAlgeria (3 cases).

Discussion. There was high heterogeneity in describing patient and PEL data across different studies. We present here a systematic review of published cases of classic PEL providing clinical and pathological insight into rare disease, excluding cases developing in the HIV setting or any immunosuppressive state (post-transplant, iatrogenic, and rare cases associated with primitive immunodeficiencies).^{79,95} The review was undertaken and reported using the PRISMA guidelines, including only cases of PEL caused by KSHV/HHV-8. The similar epidemiological background shared by classic PEL and

classic KS is why we compared the two diseases.

One major finding is that a significant proportion of the cases were observed in the elderly (more men than women) over age 75 from Mediterranean countries (Italy first) and Eastern Europe, and East Asia (Taiwan, Japan). Asia and Europe are home to some of the world's oldest populations (ages 65 and above). At the top is Japan at 28 percent, followed by Italy at 23 percent.⁹⁶

We observed three recurring underlying conditions in comorbidity with classic PEL: heart failure, cirrhosis, and malignancy. Bilateral pleural effusions are commonly seen in patients with congestive heart failure; ascites is the most common complication of liver cirrhosis. Considering the great exchange of lymphocytes between pleural and peritoneal cavities and secondary lymphoid organs, the presence of cirrhosis and heart failure in PEL patients raises interest in the potential pathogenetic role of associated effusions as an exogenous stimulus for local clonal expansion of a subset of KSHV/HHV-8-infected B-cells homing to body cavities.⁹⁷ It is conceivable that these conditions play a role in the development of PEL. Furthermore, patients with heart failure have an increased significant risk of hematologic malignancies, with an incidence rate ratio of 1.45 (95% CI 1.14–1.85, $P=0.0027$).⁹⁸ Liver cirrhosis might be considered a condition with immunological disturbances associated with a higher risk of developing nodal/extranodal lymphoproliferative disorders, even as primary effusion lymphoma in a body cavity.⁹⁹ Interestingly, we found a statistical association between the bilateral pleural site of PEL and congestive heart failure, and the peritoneal site of PEL and cirrhosis. Regarding malignancy, the 20% frequency of multiple primaries among PEL patients is slightly higher than expected in literature (in the range of 2–17%).¹⁰⁰ Interestingly, PEL patients aged ≥ 70 years versus younger ones have the same frequency of multiple primaries (19.1% vs. 20.5%, respectively) at variance with the described higher prevalence in the elderly compared with younger patients (15% vs. 6%).¹⁰¹ It is impossible to further comment on classic PEL and associated malignancies for the small number of cancers and the lack and/or incompleteness of oncological data in several articles. However, the finding of prior Hodgkin's lymphoma in three males (2 HCV-positive; ages 43–44; 1 with colon cancer: age 68) is somewhat peculiar for the rarity of this cancer. A further intriguing result is that of squamous cell carcinomas of the oral cavity in two subjects (a man, age 69, from Taiwan where the oral cancer incidence rate is the highest in the world; a woman, age 77, from Italy with also breast cancer and alcohol-related liver cirrhosis). We are not aware of data about the risk of PEL secondary to a primary cancer; yet, a cancer registry study has reported a significantly elevated risk of classic KS as a second primary neoplasm following Hodgkin's lymphoma with

an odds ratio (OR) of 7.5, chronic leukemia (OR=10), and breast cancer (OR=2.2) and a non-significant risk after oral cavity malignancy (OR=1.9).¹⁰² In more than half of the PEL cases, no laboratory data were reported thus preventing the description of shared clinical features, with the exception of anemia (low hemoglobin), decreased immunity (low CD4 count) together with low-grade chronic inflammation (elevated markers of inflammation) in the few patients who were tested; the latter can be referred to as inflammaging,¹⁰³ which contributes to the pathogenesis of age-related PEL.

In the current review, heterogeneity emerges in the diagnostic process of PEL. Cytological examination of effusions and identification of KSHV/HHV-8 infection in tumor cells by LANA positive immunostaining and/or by molecular search of KSHV/HHV-8 genome contributed to the diagnosis in 100% of instances where the testing was performed. However, sporadic cases were diagnosed by histology of the serosal membranes rather than by cytology of the intracavitary fluid,⁷⁷ with proof of KSHV/HHV-8 involvement by finding viral genome in extra-cavitary tissue samples¹⁹ or by serology testing.^{33,48,61} The presence of KSHV/HHV-8 in lymphoma cells should be considered an absolute requirement for diagnosing KSHV/HHV-8-associated PEL, whereas the detection of serum antibodies against KSHV/HHV-8 is only a marker of infection, not of disease.

Genetics of PEL typically include finding clonal rearrangements of IG genes or, more rarely, of T-cell receptor genes. Indeed, most reported cases of PEL harbor rearrangements of the IGH locus alone or in multiple combinations with IGK and/or TRG, but also of the IGK locus alone (identifying a B-cell lineage); some cases had rearrangement of TRG alone (genotypic infidelity). Nevertheless, clonality is not being systematically investigated in PEL (50 cases, 47.61%), and six cases resulted polyclonal (n=2) or 'negative for clonal rearrangement of the IGH genes' (n=4), all of them tested only for IGH locus by PCR. Therefore, considering the heterogeneity of rearrangements, the best way to ascertain clonality in PEL would be to investigate IGH and IGK and TCR. Failure to demonstrate clonality could be ascribed to: (i) impaired primers annealing; (ii) genetic alterations involving the IG loci; (iii) lack to investigation of IGK locus or TRG rearrangements. A polyclonal pattern may be the expression of an effusion that mimics PEL described as pseudo-PEL;¹⁰⁴ alternatively, it may suggest the possibility of polyclonal PEL or emerging PEL.¹⁰⁵ The question of why clonality assessments are not available in many cases (>50%) is not commented on by different authors, and regrettably, these rare PELs have not been better analyzed. Clonality testing represents a qualitative improvement to characterize lymphoproliferative diseases and clarify the lineage of a null-phenotype lymphoma such as PEL.

Although the epidemic (HIV-related) and iatrogenic (post-transplant) variants of PEL easily find a match regarding KS in these settings, less known is the comparison between classic PEL in the elderly and classic KS for the lack of ad hoc studies. The comparison between the two diseases was not straightforward. We considered only 12 studies out of more than three hundred, to collect fragmentary data; excluded articles focused on the pathogenesis and the molecular landscape of KS, and therapeutic options. Nevertheless, we attempted to build up a control group of KS patients to compare it with classic PEL patients. It must be pointed out that this comparison has limits linked to the difference in the size of the two groups, that may affect the statistical power. Our comparison indicates remarkable similarities between PEL and KS in the elderly population, supporting a clinical variant of PEL paralleling classic KS but has put in light some differences. In classic-PEL there is a slightly higher male prevalence, and patients are significantly older. Other important differences are found comparing the laboratory data: PEL patients have lower lymphocytes count, CD4 count, and hemoglobin levels. Classic PEL is far more lethal compared to classic KS. These divergent aspects raise up some questions: (i) if the classic variants of PEL and KS have a distinct biologic behavior (very aggressive, PEL; indolent, KS) that would explain the clinical and laboratory differences or (ii) if these diseases provoke a different outcome because occurring in two different groups of hosts. Many preclinical studies support the first hypothesis. KS spindle cells do not behave like typical cancer cells; they do not form tumors in nude mice, and the majority of KS tumors are polyclonal; all KS tumor-derived cells to date have lost viral genomes upon ex vivo cultivation. By contrast, PEL cell lines exhibit monoclonality, easily grow when implanted in nude mice and maintain KSHV/HHV-8 indefinitely. A few clinical observations support the second hypothesis: the host status may influence the different clinical course between classic KS and classic PEL. PEL patients are older than KS patients; their cellular immunity decrease because of the immunosenescence process that leads to reduced lymphocytes and CD4 counts, thus favouring KSHV/HHV-8 virulence, PEL cells survival, and worse prognosis and lethality. To note, a minority of very elderly PEL patients (median age=85) had concomitant KS in advanced stages with progressive disease. Nevertheless, the finding of indolent cases of classic PEL and aggressive and lethal classic KS cases may also be related to the host. Finally, the worse prognosis of PEL patients may be explained because this lymphoma is hidden in a body cavity effusion, differently from KS lesions that arise on the skin and can be detected in their very early phases.

The present review carries inherent limits associated

with the quality and completeness of the published studies. Considering the rare occurrence of PEL outside immunodeficiency settings, we preferred to include rather than exclude all available cases reported as abstracts, short reports, images, in addition to full articles. Another controversial issue might be the *a priori* exclusion of extracavitary PEL cases. KSHV/HHV-8 has been associated with a wide and heterogeneous group of lymphoproliferative processes including liquid-based PEL and tissue-based extracavitary/solid lymphomas with significant clinicopathological overlap.¹⁰⁶ Although extracavitary/solid HHV8-positive lymphoma can precede or follow a typical case of cavity effusion-based PEL, our study aimed indeed to focus on the effusion-only PEL subgroup. This choice was based on our case-series study on PEL with effusion-only disease in the elderly, in the HIV-negative context.⁷⁹ Remarkably, a recent multi-institutional case-series study has reported that 7 out of 8 HIV-negative PEL patients (median age >75 years) had effusion-only disease; by contrast,

patients with extracavitary PEL were younger and more likely HIV-positive.¹⁰⁷ The comparison between classic PEL and classic KS was hampered by the lack of uniformity in KS studies that often report aggregated data rather than highlighting individual patient's profile, so data availability bias is a potential concern of our analysis. A higher power study with more cases is warranted to further investigate the differences between classic PEL and classic KS.

Conclusions. Our study is the first systematic review of the literature focusing on classic PEL, a clinicopathological variant of KSHV/HHV-8-related effusion-only PEL probably underrecognized in the elderly population of KSHV/HHV-8 endemic areas.

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