Primary Effusion Lymphoma

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ABSTRACT

Primary effusion lymphoma (PEL) is a rare HIV-associated non-Hodgkin’s lymphoma (NHL) that accounts for approximately 4% of all HIV-associated NHL. PEL has a unique clinical presentation in having a predilection for arising in body cavities such as the pleural space, pericardium, and peritoneum. PEL cells are morphologically variable with a null lymphocyte immunophenotype and evidence of human herpesvirus (HHV)-8 infection. The exact oncogenic mechanisms of HHV-8 have not been clearly defined. Treatment is usually with combination CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy and antiretroviral therapy (if HIV positive). The prognosis for PEL is poor, with a median survival time of around 6 months. As the exact molecular steps in HHV-8–driven oncogenesis are unraveled, it is hoped that more specific therapeutic targets will be revealed.

Disclosure of potential conflicts of interest is found at the end of this article.

EPIDEMIOLOGY/OVERVIEW

Primary effusion lymphoma (PEL), formerly known as body cavity lymphoma, accounts for about 4% of all HIV-associated non-Hodgkin’s lymphomas (NHLs) [1]. The development of NHL in a patient infected with HIV was declared an AIDS-defining illness in 1985 [2]. Since then, estimates have suggested that anywhere from 5% to 20% of HIV-positive patients will develop NHL at some point during their life, with a relative risk of 60–200 times that of the general population, and NHL is the AIDS-defining illness in about 3%–4% of all HIV-positive patients [3].

The introduction of highly active antiretroviral therapy (HAART) in the late 1990s has decreased the overall incidence of AIDS-related NHL (ARL). The most significant impact of HAART has been a dramatic decrease in the incidence of primary central nervous system lymphoma (PCNSL) [4].

The most common histological types of ARL include Burkitt’s or Burkitt’s-like lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), which includes the subtypes immunoblastic (including most cases of PCNSL) and centroblastic lymphoma, plasmablastic lymphoma (including oral cavity involved and multicentric Castleman’s disease [MCD] associated), and PEL (Table 1) [5]. PEL was first described in 1989 [6], and since then much has been learned about its unique pathogenesis, specifically the role of human herpesvirus-8 (HHV-8). Given its rarity, however, there are very few longitudinal observational series of patients with PEL, and no large prospective trials have ever defined optimal treatment strategies. The vast majority of the observed and published series on ARL have all been comprised of more common histological types, such as BL or DLBCL, and it is not clear that their findings and observations apply to PEL given its unique clinical presentation and pathogenesis. We review here the current state of knowledge on PEL, its pathogenesis, treatment, and potential implications for future therapy.
**PATHOGENESIS**

By definition, cases of PEL must display evidence of infection by Kaposi’s sarcoma–associated herpesvirus, otherwise known as HHV-8. In addition, most HIV-positive cases (>90%) also show evidence of Epstein–Barr virus (EBV) infection. Many other histologic subtypes of NHL can present with a primary neoplastic effusion [7, 8], but these cases, many of which are extranodal DLBCL or BL, should not be classified as PEL without evidence of HHV-8 infection. By definition, cases of PEL must display evidence of infection by Kaposi’s sarcoma–associated herpesvirus, otherwise known as HHV-8. In addition, most HIV-positive cases (>90%) also show evidence of Epstein–Barr virus (EBV) infection. Many other histologic subtypes of NHL can present with a primary neoplastic effusion [7, 8], but these cases, many of which are extranodal DLBCL or BL, should not be classified as PEL without evidence of HHV-8 infection and supportive morphological and immunophenotypic criteria.

HHV-8 is a member of the gamma herpes virus family, which includes EBV. It was first characterized in HIV-infected patients with Kaposi’s sarcoma in 1994 [9]. Subsequently, HHV-8 was found to be associated with other disorders, including PEL [10] and MCD [11]. HHV-8 is a linear double-stranded DNA virus that is not ubiquitous but has endemic areas of infection including sub-Saharan Africa (seroprevalence of 50%–70%) and the Mediterranean region (seroprevalence of 20%–30%), while North America exhibits only a 1%–3% infection rate among asymptomatic blood donors [12].

The exact mechanism by which HHV-8 promotes oncogenesis in PEL is an area of active investigation. HHV-8 genomes exist in PEL cells as mono- or oligoclonal episomes. Most infected cells express a latent pattern of gene expression, while a very small percentage expresses genes characteristic of the lytic phase [13]. Even with the expression of latent genes, infected cells can undergo clonal expansion, eventually leading to neoplastic transformation through mechanisms of increased proliferation and impaired apoptosis. Three latent gene products that are thought to play significant roles are latency-associated nuclear antigen-1 (LANA-1), viral cyclin (v-Cyc), and viral FLICE inhibitory protein (vFLIP).

LANA-1 is essential for the segregation and maintenance of viral DNA during replication [14]. In addition, it has also been shown to bind and inhibit the human tumor suppressor genes p53 [15] and Rb [16], thereby impairing apoptosis of infected cells. v-Cyc is a viral homologue of cyclin D and binds to human cyclin-dependent kinase 6 (CDK6), resulting in resistance to CDK inhibitors, progression through the cell cycle, and uncontrolled cell division [17]. vFLIP seems to inhibit apoptosis by blocking Fas-mediated caspase activation as well as activating nuclear factor-κB (NF-κB) [18–20]. Other potential genes that may be playing a role include viral interleukin-6 (IL-6) and bcl-2 homologues, a viral interferon regulatory factor and a viral G-protein–coupled receptor [21–25].

While the majority of cases of PEL show evidence of infection with EBV in addition to HHV-8, EBV plays an unclear role in PEL oncogenesis. Early studies in PEL had shown that EBV displayed a restricted latency phenotype with very low expression of its known transforming genes, suggesting that EBV played a supportive role in cell transformation [26]. Gene-expression profiling has identified molecular signatures in EBV-positive and EBV-negative cases of PEL. Striking similarities between the two groups suggest that HHV-8 plays the dominant role in coinfected cases; however, PEL cases without EBV infection had significantly higher expression levels of several genes involved in the regulation of the mitogen-activated protein kinase pathway, suggesting that activation of this pathway may compensate for the lack of EBV infection [27].

**CLINICAL PRESENTATION**

Patients with PEL are usually HIV-positive men with a decreased CD4 T-cell count at diagnosis. Pre-existing AIDS is common. In addition, a significant portion of patients have pre-existing Kaposi’s sarcoma or MCD, reflecting the
other known manifestations of HHV-8 infection. PEL is clinically characterized by its unique predilection to arise as lymphomatous growth in a liquid phase in body cavities such as the pleural, pericardial, and peritoneal spaces, usually without associated extracavitary tumor masses. Symptoms are usually a result of mass effects from accumulation of the malignant effusion. Hence, patients commonly present with dyspnea (from pleural or pericardial disease) or abdominal distension (from peritoneal disease). Dissemination to distant sites is not uncommon and most patients have historically survived no more than several months after initial diagnosis. The most frequent causes of death are opportunistic infection, HIV-related complications, and progression of lymphoma.

As immunophenotypical and molecular methods have improved, cases of solid tumor masses exhibiting a similar morphology, immunophenotype, and gene expression profile as PEL have recently been described [28]. Interestingly, several of these case reports have shown that these extracavitary presentations tend to involve the gastrointestinal tract either initially or at relapse [28–32], and over half of such cases develop a malignant effusion later in their course. It is unclear if these patients have the same natural history as PEL, but they should most likely be included in a common classification of HHV-8–associated lymphoproliferative disease.

Several cases of PEL have also been described in patients who are HIV negative. Some cases have been described in patients after solid organ transplantation while patients are receiving systemic immunosuppression [33, 34]. Other patients tend to be elderly men of Mediterranean descent whose lymphoma cells show evidence of HHV-8 infection, but without EBV [35]. Not surprisingly, this is the same population at risk for HIV-negative classical Kaposi’s sarcoma [36].

**DIAGNOSIS/PATHOLOGY/DIFFERENTIAL DIAGNOSIS**

The diagnosis of PEL rests upon pathological analysis of involved tissue using morphologic, immunophenotypic, molecular, and virologic criteria. The diagnosis is usually made on a cytological preparation (e.g., liquid-based preparation, cytopsin, cell block) of the involved effusion fluid, but biopsies of body cavity–lining tissue may also show small numbers of neoplastic cells adherent to mesothelial-lined surfaces. Occasionally, there is underlying invasion of these surfaces by the malignant cells without formation of a substantial mass. Morphologically, the neoplastic cells are large, have round to irregular nuclei, prominent nucleoli, and varying amounts of cytoplasm that is occasionally vacuolated. The cells exhibit a range of appearances, from immunoblastic (round nuclei with central prominent nucleoli) to plasmablastic (eccentric nuclei with abundant cytoplasm, sometimes containing a perinuclear hof) to anaplastic (very large round or polygonal cells with bizarre, pleomorphic nuclei) (Fig. 1A, B) [37]. Anaplastic forms include multinucleated and Reed–Sternberg-like cells [38].

Detecting evidence of viral infection by HHV-8 in the neoplastic cells is essential for the diagnosis of PEL. While serology is the best way to assess if a patient has been previously infected with HHV-8, immunohistochemical staining for LANA-1 is the standard assay to detect evidence of HHV-8 infection in tissue (Fig. 1C). Quantitative polymerase chain reaction has been used to measure peripheral blood HHV-8 viral load, but assays have not been standardized, and studies have not yet demonstrated any prognostic value, such as correlation of viral load with response to therapy or survival [39, 40]. Evidence of EBV infection is most reliably detected by in situ hybridization for EBV RNA; immunohistochemical staining for EBV latent membrane protein (LMP-1) is negative [38].

Molecular studies have provided evidence that immunoglobulin gene rearrangements and somatic hypermutation have occurred in PEL cells [41], suggesting that the cell of origin is a post–germinal center B cell. Gene-expression profiling has suggested a phenotype intermediate between immunoblasts and plasma cells [42]. Immunophenotypically, PEL cells typically display a “null” lymphocyte phenotype, meaning that CD45 is expressed, but routine B-cell (including surface and cytoplasmic immunoglobulin, CD19, CD20, CD79a) and T-cell (CD3, CD4, CD8) markers are absent. Instead, various markers of lymphocyte activation (CD30, CD38, CD71, human leukocyte antigen DR) and plasma cell differentiation (CD138) are usually displayed [37, 43, 44]. Lastly, cytogenetic analysis has revealed complex karyotypes but no common chromosomal abnormality in PEL [45].

The most common differential diagnostic consideration in cases of PEL is a lymphomatous effusion associated with another type of NHL. Many subtypes of low-grade and high-grade NHL may be associated with lymphomatous effusions (Fig. 2A, B), but confirmation of the typical morphology and immunophenotype described previously and evidence of HHV-8 are required for the diagnosis of PEL [37]. Many of these case reports can be classified as variants of other extranodal NHLs through immunohistochemistry and genetic studies. Specifically, HIV-positive patients may have BL with plasmacytoid differentiation that rarely presents as a lymphomatous effusion; such cases may show morphologic overlap with PEL, but will be positive for the characteristic c-myc gene rearrangement and show no evidence of HHV-8 infection. Peripheral T-cell NHLs [46] presenting with an effusion can usually be dis-
tinguished by routine immunohistochemistry for T-cell markers or T-cell receptor (TCR) gene rearrangement studies. Because of similar morphology, T-cell anaplastic large cell lymphoma may also be confused for PEL in the appropriate clinical context [47]; immunohistochemical staining for anaplastic lymphoma kinase in addition to the TCR gene rearrangement studies may be helpful in these cases. Finally, pyothorax-associated lymphoma arising in the setting of long-standing pyothorax related to treatment of tuberculosis may enter into the differential diagnosis. This lymphoma is more common in Japan and usually arises in elderly men without HIV risk factors, but with a history of pulmonary tuberculosis or tuberculous pleuritis treated with pneumothorax, resulting in a pyothorax. In these patients, it is usually a large cell lymphoma of B-cell lineage, expressing B-cell markers and staining EBV positive [48].

The clinical and immunophenotypic characteristics of pyothorax-associated lymphoma and absence of evidence of HHV-8 infection make its distinction from PEL relatively straightforward.

EVALUATION/STAGING
Evaluation of a patient with a new diagnosis of PEL includes a complete blood count and comprehensive chemistry panel including a serum lactate dehydrogenase level. Although PEL is usually not present in distant sites, initial staging studies should routinely include a chest/abdomen/pelvis computed tomography scan with consideration of nuclear imaging, such as positron emission tomography. Bone marrow biopsy should be performed if there are coincident cytopenias. Lumbar puncture is not a part of routine staging (as the incidence of central nervous system involvement in PEL is rare), but should be undertaken if clinically indicated. While these staging studies may not influence the choice of therapy, they do serve as a baseline estimation of disease burden to allow assessment of response during and after treatment. Standard Ann Arbor staging for PEL is not relevant, as by definition, all cases of PEL are stage IV. Similarly, the International Prognostic Index, while demonstrating prognostic value in large series of NHL [49] and ARL [50], has never been validated in a cohort of patients with PEL.

TREATMENT
To date, the prognosis for patients with PEL has been poor, with very few long-term survivors. One recent single-center series of 11 patients had a median survival time of 6 months [1], while the most recent large multicenter series of 28 patients showed a median survival time of 6.2 months and a 1-year overall survival rate of 39.3% [51]. Given the rarity of this disease, there have been no large prospective clinical trials to define appropriate therapy for PEL. Four of the largest published series are shown in Table 2. Therefore, patients should be encouraged to enroll in well-designed clinical trials whenever possible.

The use of HAART appears to be associated with a better prognosis in a multivariate analysis [51] and in trials with other ARLs [52, 53]. In addition, there are case reports of patients with PEL achieving prolonged remission with antiretroviral therapy alone [54, 55], suggesting a role for immune reconstitution in control of this aggressive lymphoma. Thus, initiating or continuing HAART as part of supportive therapy is recommended when commencing treatment for HIV-positive patients with PEL. In addition, the routine use of growth factors such as G-CSF to avoid prolonged periods of neutropenia resulting from chemotherapy is standard practice for all ARL patients. Consider-
ation should also be given to prophylaxis against *Pneumocystis jirovecii* pneumonia (PCP) and routine monitoring of cytomegalovirus reactivation while on therapy.

Much like other NHLs, combination chemotherapy forms the backbone of therapy for PEL, and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)-like regimens are considered first-line therapy [1, 56]. In one series, modified CHOP-like regimens were able to achieve a 43% complete remission rate [1], whereas high-dose methotrexate was added to CHOP-like regimens in another report showing a similar rate of success [57]. We would caution against the use of high-dose methotrexate, especially in the initial cycles, given the accumulation of methotrexate in effusions with resultant delayed clearance and the increased risk for systemic toxicity [58].

Rituximab, an anti-CD20 monoclonal antibody that has been incorporated into the standard therapy for many B-cell NHLs, does not play a role in PEL therapy because CD20 is not usually expressed by PEL cells. High-dose chemotherapy with autologous stem cell transplant (ASCT) has been reported in only two individual case reports of PEL [59, 60], and currently, there is no evidence to support using ASCT as consolidation therapy in first remission. However, extrapolating from other studies in ARL [61], it can be considered for chemotherapy-responsive relapsed disease in patients who maintain a good performance status.

Novel approaches for PEL therapy outside traditional chemotherapy have been suggested as well. These include the addition of antiviral therapy as well as inhibition of specific cellular targets. Two recent reports have described treating four elderly patients with intrapleural injections of the antiviral drug cidofovir with durations of remissions lasting several months [62, 63]. The efficacy of antiviral treatment can potentially be improved if PEL cells can be induced to enter the lytic phase of viral replication, as the majority of antiviral drugs are most effective in the lytic phase. In vitro data have suggested that the combination of the antiseizure medication valproate, which induces lytic replication, with either ganciclovir or foscarnet can bring about apoptosis of infected cells [64].

Another approach may be to target NF-κB through the use of proteasome inhibition with drugs such as bortezomib, which induces apoptosis of PEL cell lines in vitro [65]. An additional mechanism to induce apoptosis is combination therapy with interferon-α and azidothymidine (AZT), which has been used with success in one reported patient [66]. A current trial headed by the National Cancer Institute is attempting to combine several of these approaches by using combination therapy with antivirals, bortezomib, and systemic chemotherapy. Other approaches that have been proposed include targeting latency phase genes such as LANA-1, using small RNA transcripts to si-

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**Table 2. Series of primary effusion lymphoma patients**

<table>
<thead>
<tr>
<th></th>
<th>Simonelli et al. [1]</th>
<th>Boulanger et al. [51]</th>
<th>Boulanger et al. [57]</th>
<th>Nador et al. [8]</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>28</td>
<td>7</td>
<td>15</td>
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<tr>
<td>HIV +</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>87%</td>
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<tr>
<td>Mean/median CD4</td>
<td>98/ul</td>
<td>133/ul</td>
<td>192/ul</td>
<td>84/ul</td>
</tr>
<tr>
<td>Male</td>
<td>90%</td>
<td>96%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>EBV infection</td>
<td>–</td>
<td>72%</td>
<td>57%</td>
<td>93%</td>
</tr>
<tr>
<td>Previous KS</td>
<td>27%</td>
<td>67%</td>
<td>71%</td>
<td>33%</td>
</tr>
<tr>
<td>Previous MCD</td>
<td>9%</td>
<td>33%</td>
<td>43%</td>
<td>n/a</td>
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<tr>
<td>Sites of disease</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Pleural</td>
<td>63%</td>
<td>89%</td>
<td>71%</td>
<td>60%</td>
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<tr>
<td>Pericardial</td>
<td>9%</td>
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<td>13%</td>
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<tr>
<td>Peritoneal</td>
<td>36%</td>
<td>61%</td>
<td>29%</td>
<td>27%</td>
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<tr>
<td>Extracavitary</td>
<td>45%</td>
<td>43%</td>
<td>71%</td>
<td>13%</td>
</tr>
<tr>
<td>HAART</td>
<td>72%</td>
<td>78%</td>
<td>71%</td>
<td>0%</td>
</tr>
<tr>
<td>CHOP-like chemotherapy</td>
<td>72%</td>
<td>79%</td>
<td>100%</td>
<td>n/a</td>
</tr>
<tr>
<td>CR rate to CHOP</td>
<td>43%</td>
<td>50%</td>
<td>57%</td>
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<tr>
<td>Median survival time</td>
<td>6 months</td>
<td>6.2 months</td>
<td>5–9 months</td>
<td>3–4 months</td>
</tr>
</tbody>
</table>

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete remission; EBV, Epstein–Barr virus; HAART, highly active antiretroviral therapy; KS, Kaposi’s sarcoma; MCD, multicentric Castleman’s disease.
ience essential viral genes, and augmenting host immunity against HHV-8 [67].

**CONCLUSIONS**

In summary, PEL is a rare NHL, usually occurring in the context of HIV infection. It most commonly arises in the pleural, pericardial, or peritoneal spaces and usually displays a null lymphocyte phenotype by immunohistochemistry with evidence of HHV-8 infection. Generally, it is considered an aggressive NHL, and even with combination chemotherapy, prognosis remains quite poor with median survival times of only about 6 months. However, with better understanding of the unique oncogenesis of HHV-8, it is hoped that rational and specific targets for therapy can become the basis for greater therapeutic success.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

E.H. has acted as a consultant for and has a financial interest in Genentech.

**REFERENCES**


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