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Case report

Cytopathology in the diagnostic appraisal of uncommon malignant neoplastic lesions

A. KALOGERAKI[1], ,S. DERDAS[2], I. KARVELA-KALOGERAKI[1], M. KARVELAS-KALOGERAKIS[1], G. SEGREDAKIS[1], K. STATHIAS[1], V. SINATKAS[1], D. TAMIOLAKIS[1]
[1] Department of Cytopathology, Medical School University of Crete, Heraklion, Crete, Greece and [2] Laboratory of Virology, Medical School University of Crete, Heraklion Crete, Greece

Key words

Myoepithelial carcinoma • Diffuse large B-cell lymphoma • Eccrine porocarcinoma • Primary effusion lymphoma • FNAC • FNAB • Cytology

Summary

Cytology and fine needle aspiration (FNA) cytology are accepted means of diagnosing and typing of common forms of malignant tumors. However, their usefulness for diagnosing less common neoplasms is not clearly established and this study was designed to examine this. We report four unusual cases of patients with malignant neoplasms in which cytology and fine needle aspiration cytology or aspiration biopsy (FNAC, FNAB) contributed significantly in establishing the diagnosis. These cases facilitate the diagnostic capabilities of cytology over a wide spectrum of neoplasms including rare lymphoproliferative disorders and carcinomas.

Introduction

Several tumours have been considered to pose some of the greatest diagnostic challenges in surgical pathology. Cytology and FNAC permit multiple sampling and/or sampling of different parts of an uncommon tumour to evaluate its heterogeneity.
Eccrine porocarcinoma (EP) is an uncommon adnexal skin tumour which recurs locally and metastasises to lymph nodes.Myoepithelial carcinomas arising in the salivary glands have a wide morphologic heterogeneity, resulting in easy confusion with other tumours.Lymphomas are a diverse group of neoplasms affecting the lympho-reticular system.They have been traditionally divided into Hodgkin's and non-Hodgkin's lymphoma. Non Hodgkin's lymphoma (NHL) may develop extranodally and can occur in the stomach, salivary glands and rarely in the oral cavity and jaw bones. Diffuse large B-cell Lymphoma (DLBCL) involves the oral cavity in 0.1% of cases and occasionally appears in the maxilla. Primary effusion lymphoma (PEL) is a human herpes virus 8 (HHV-8) – associated and very rare type of NHL usually confined to the body cavities without other tumor masses.
Our aim is to demonstrate the role of cytology in interpretating these uncommon neoplasms and to record the cytological criteria for the diagnosis.

Case studies

Case 1. Eccrine porocarcinoma

A 76-year-old woman, presented at hospital with a painless tumour in the right inguinal region.On physical examination, the skin overlying the tumour was yellowish in colour and polypoid in appearance.
A fine needle aspiration (FNA) biopsy was performed using a 25-gauge needle. The cytologic diagnosis was of an eccrine porocarcinoma (EP) that was confirmed histologically after surgical resection of the tumour as an eccrine porocarcinoma of Bowenoid type (Bowenoid porocarcinoma).
The cytology slides showed abundant material that comprised clusters, sheets and isolated neoplastic cells with

Correspondence
Alexandra Kalogeraki, Department of Pathology-Cytology,University of Crete, Medical Faculty, PO Box 1393, Heraklion, 71110 Crete, Greece - Tel. 00302810394692 - Fax 00302810394694 - E-mail: kalogerakimed@yahoo.gr

Fig. 1. Porocarcinoma. Giemsa stain X 400 (FNA).Neoplastic cells with basophilic cytoplasm, oval, hyperchromatic nuclei, and prominent nucleoli.

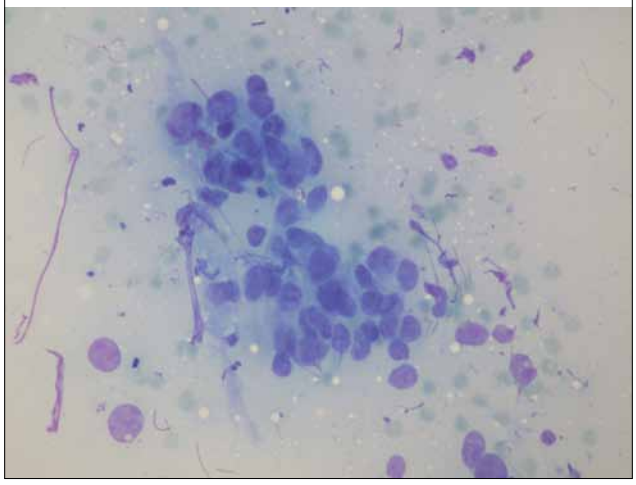


Fig. 2. Porocarcinoma.Hematoxylin & Eosin stain X 40 (Tissue section). Massive dermal infiltration by the tumor is demonstrated.

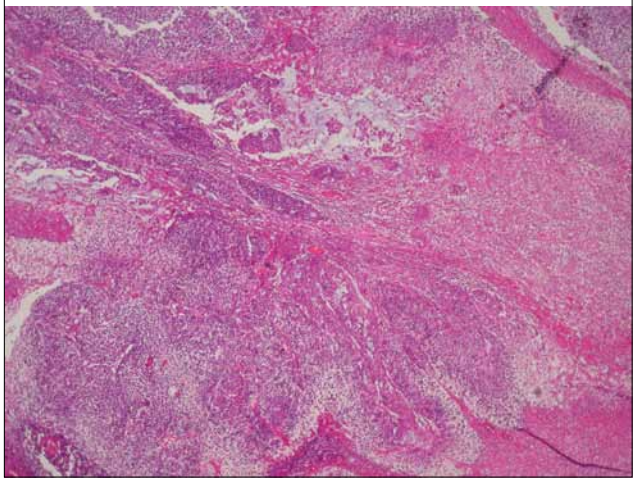
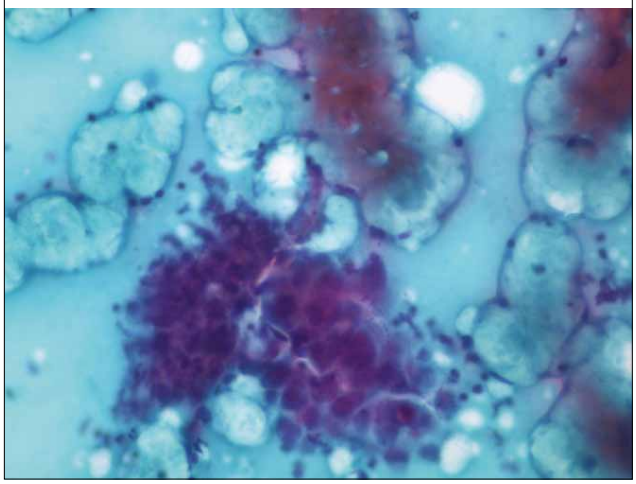


Fig. 3. Myoepithelial carcinoma.Papanikolaou stain X 400 (FNA). Hypercellularity, three-dimensional clusters with considerable overlapping and crowding ofneoplastic cells, which consisted predominantly of spindlecells with oval or elongated to spindle shaped nuclei, with considerable variation in size.

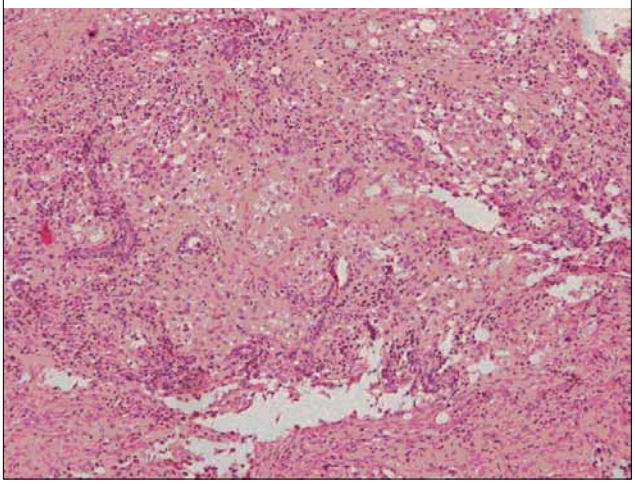


basophilic cytoplasm, oval, hyperchromatic nuclei, and prominent nucleoli (Fig. 1).The cells exhibited marked pleomorphism. Mitotic figures were not found and a background of necrotic debris was observed.Eccrine porocarcinoma cells may form and exhibit intracytoplasmic vacuoles, but in our case, they were not present.
Massive dermal infiltration by the tumour was demonstrated in tissue sections (Fig. 2). Solid cords and rosettes of neoplastic cells were observed. The majority of tumour cells were small with prominent nucleoli and nuclear pleomorphism.The cytoplasm was clear. Mitotic figures with atypia were found. There were areas with squamous differentiation. Two histopathological patterns were found with areas of eccrine porocarcinoma and Bowen disease.
The majority of neoplastic cells expressed strongly EMA and CK5/CK6 markers. The tumour cells did not express HMB45, chromogranin, CD56, CEA, and vimentin but were focally positive for MNF116 and involucrine.

Case 2. Myoepithelial carcinoma of the parotid gland

A 74-year-old female presented at hospital, having a 4.7cm painless solid mass in the right parotid gland.A CT guided FNAB was performed using a 21-gauge needle and the cytologic diagnosis was myoepithelial carcinoma (MCA) of the parotid gland, that was confirmed histologically after surgical resection of the neoplasm.
Cytologic examination revealed hypercellularity, three-dimensional clusters with considerable overlapping and crowding ofneoplastic cells, pleomorphism of tumor cells, which consisted predominantly of spindlecells, oval or elongated to spindle shaped nuclei, with considerable variation in size (Fig. 3).The chromatin was coarsely granular and the nucleoli were evident.Neoplastic cells were found to be weakly positive for S-100 protein and GFAP.By histology, the tumour was solid, with cells arranged in trabeculae,nests and cords. Tumour cells were mixed epithelioid and spindle with eosinophilic or clear cytoplasm, with eccentric nuclei (Fig. 4).Neoplastic cells

Fig. 4. Myoepithelial carcinoma.Hematoxylin & Eosin stain X 200. Cells arranged in trabeculae, nests and cords (tissue section).



were found in blood vessels, in the skin and facial nerve. Tumour cells were positive for PAS, PAS-D, S-100 protein, GFAP, P63, CK5/CK6, CK7, and CK14.

Case 3. Extra-nodal primary diffuse large B-cell lymphoma of the maxilla

A 76-year old female without a significant medical history, presented in September 2011 at University Hospital of Crete, with a large, painless tumour in the region of the left maxilla. A fine needle aspiration (FNA) biopsy of the tumour was performed using a 25-gauge needle.
The observation of aspiration smears revealed the massive presence of large atypical lymphocytes with visible multiple nucleoli. Among these large cells, mature lymphocytes were observed (Fig. 5).
An immunocytochemical study was carried out in air dried aspiration smears, showing that these large lymphocytes were of B origin: CD20 and PAX-5 were strongly positive and OPD4, CD15 and CD30 negative. The cytological diagnosis was of a diffuse large B-cell lymphoma of the maxilla.
A biopsy of the tumour was performed and histology confirmed the cytological diagnosis. Histology revealed large atypical lymphoid cells, centroblasts and immunoblasts with multiple and visible nucleoli and a population of mature lymphoid cells.Immunohistochemically the tumour cells were positive for CD20 PAX-5 (Fig. 6), CD79a, bcl-6, bcl-2 and MUM-1 and negative for CD10, CD30, CD5, and LMP-1negative. Ki-67 was positive in 40% of neoplastic cells.

Case 4. Primary effusion lymphoma

A 49-year-old male who was iatrogenically immunosuppressed for 29 years due to renal transplantation presented at University Hospital of Heraklion Crete with ascites and fever. No abnormal mass was revealed by computed tomography.
Because blood tests and chest and abdomen scans could not interpret the patient's symptoms, an exploratory laparotomy was performed. Three litres of ascetic fluid was removed, but no other abnormalities were found except for a slightly thickened peritoneum from which biopsy samples were obtained.
Cytologic preparations included conventional smears and Thin-Prep preparations. Both ethanol fixed, Papanicolaou's-stained and air-dried, Giemsa-stained smears were prepared. The smears contained numerous large isolated lymphoid cells with high nuclear/cytoplasmic ratios,large pleomorphic, vesicular, eccentric and lobular nuclei, occasional prominent nucleoli and amphophylic cytoplasm (Fig. 7). Mitotic figures were also present.
The neoplastic cells expressed CD3, CD138 (cytoplasmic expression) (Fig. 8), PAX-5 and CD30.CD45 was focally positive.Cytologic diagnosis was indicative of primary effusion lymphoma (PEL) with an aberrant T-cell immunophenotype.
Tissue samples from the peritoneum with maximum diameter of 1.7 cm were routinely processed for histologic examination.Many neoplastic cells were observed with

Fig. 5. DLBCL Papanikolaou stain X 400 (FNA).Large atypical lymphocytes with visible multiple nucleoli. Among these large cells, mature lymphocytes are observed.

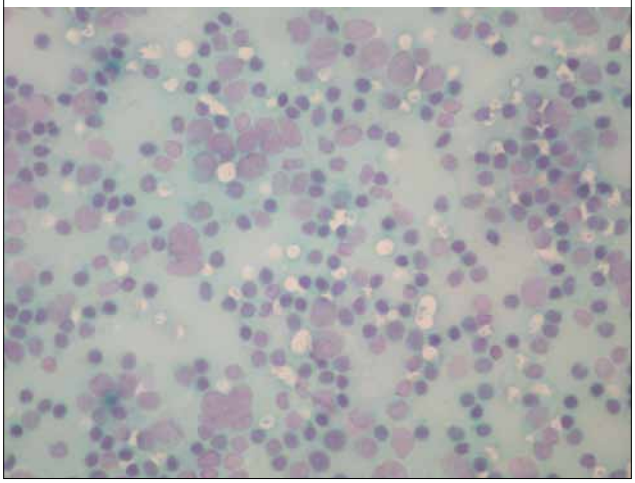


Fig. 6. DLBCL. PAX-5 immunostain X 400 (Tissue section). CD20 positive cells.

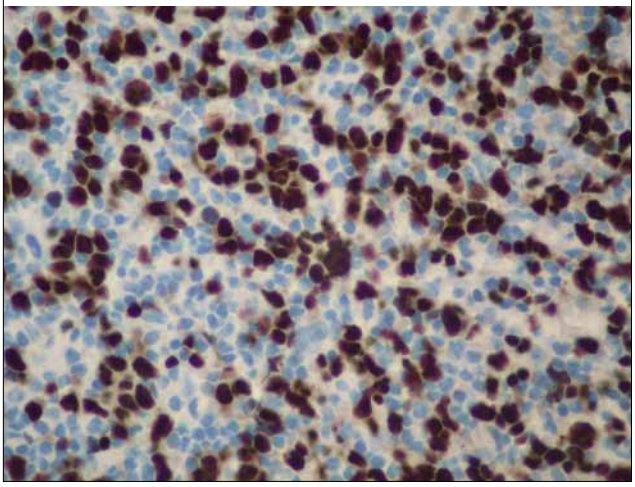


Fig. 7. Peritoneal PEL.Papanikolaou's stain X 400 (Cytologic preparation).Large isolated lymphoid cells with high nuclear/cytoplasmic ratio, large pleomorphic, vesicular, eccentric and lobular nuclei, occasional prominent nucleoli and amphophylic cytoplasm.

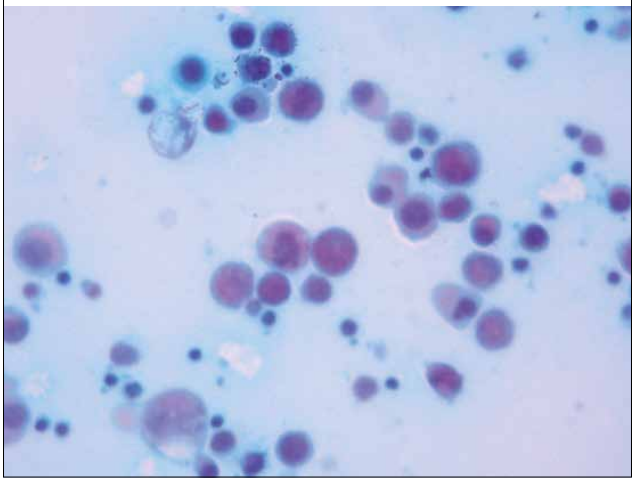


Fig. 8.Peritoneal PEL CD138 (cytoplasmic expression) immunostain X400 Cytologic preparation).

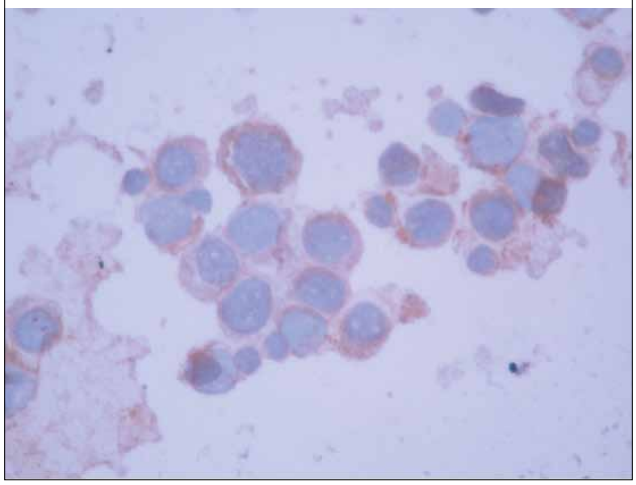
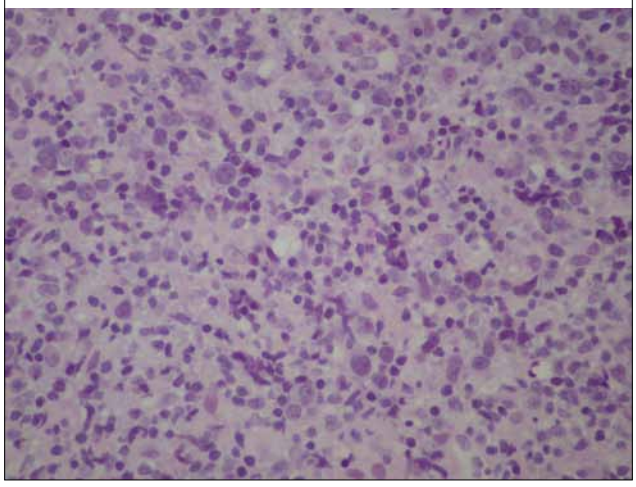


Fig. 9. Peritoneal PEHematoxylin & Eosin stain X 400 (tissue section). Neoplastic cells with amphophylic cytoplasm.



amphophylic cytoplasm (Fig. 9).They exhibited large lobular and anaplastic nuclei and numerous mitotic figures.Immunohistochemistry was performed and the neoplastic lymphoid cells expressed CD138, CD3 (cytoplasmic expression), CD43 and EMA.Using *in-situ* hybridization,thebiopsy specimens were positive for HHV-8 and negative for EBV.The patient was found to be seronegative for HIV.

Flow cytometry, a more desirable means of determining immunophenotype in suspected lymphomas, was not performed because of the scarcity of the specimen.

DNA was extracted from tissue samples and PCR analysis was performed.Results disclosed clonal rearrangements for immunoglobulin heavy chain gene (IgH), a finding strongly suggestive of B clonal population, and also rearrangements for T-cell receptor γ chain gene.

The patientwas treated withCHOP (cyclophosphamide, doxorubicin, prednisone, vincristine) chemotherapy and is still alive 10 months after diagnosis.

Discussion

Eccrine porocarcinoma represents only 0.005% of epithelial cutaneous neoplasms [1].

The clinical differential diagnosis is difficult, including seborrheic keratosis, pyogenic granuloma, poroma, amelanotic melanoma, SCC, BCC, verruca vulgaris, metastatic adenocarcinoma, and Bowen's disease. In our case the histologic diagnosis was of an eccrine porocarcinoma with a "Bowenoid " pattern.

In a series reported by Robson et al [2], the clinical diagnosis was never correct. The reported difficulty with accurate clinical diagnosis emphasises the need for ancillary studies, a role that FNA cytology could serve. Certainly, aspiration cytology could be expected to help distinguish an epithelial from a primary skin tumour.

Eccrine porocarcinoma has distinctive cytological features including presence of:

- two cell types, irregularly dispersed or forming nests or cords, sometimes harboring central necrosis.
- Abundant clear granular cytoplasm with small to large, occasionally multinucleated nuclei and small nucleoli in larger cells.
- granular chromatin with smooth nuclear membranes.
- Scanty cytoplasm and a round to oval nuclei with small but prominent nucleoli in smaller cells.

Aggregates of parakeratotic squamous cells and necrotic debris

In our case, slides showed clusters, sheets and isolated neoplastic cells with basophilic cytoplasm, oval, hyperchromatic nuclei, and prominent nucleoli The cells exhibited marked pleomorphism. Mitotic figureswere not found and a background of necrotic debris was observed.

The cytologic differential diagnoses includes: non-keratinizing squamous cell carcinoma (SCC), basal cell carcinoma (BCC), metastatic adenocarcinoma and malignant meelanoma (MM).

It is felt that cytology still can play a triage role in the evaluation of such tumours since it will provide an initial clue to the possibility of a primary cutaneous malignant epithelial neoplasm. A cytologic diagnosis of cutaneous carcinoma, regardless of subtypes, sometimes is sufficient for directing clinical management. Histologic confirmation is always warranted for a definitive diagnosis.Rege and Shet cautioned that a percentage of skin adnexal tumors is difficult to diagnose with aspirates and thus requires a core biopsy [3].This was also the case in our recently published relevant work [4].

Myoepithelial carcinomas have been underrecognized in the past, primarily by being lumped under a broader category of "malignant mixed tumor". Histologically, myoepithelial carcinomas are composed of one or several cell types: spindle, plasmacytoid, epithelioid, and clear cells. Frequently, one of the cell types predominates. The neoplastic cells grow either as multiple nodules or as large solid sheets separated by variable amounts of intervening hyaline or myxoid stroma.

The cytologic features in FNABs [5, 6],generally reflects

the histology. The cytologic smear can show spindle, epithelioid, or plasmacytoid cells. Scant fragments of metachromatic stroma intermixed with the neoplastic cells might be observed in the cytologic specimens of malignant myoepithelioma, regardless of the composition of the cell types.

Non-Hodgkin lymphoma of bone represents 2% of primary bone tumors and 5% of all primary extra-nodal non Hodgkin's lymphomas. Cytologic diagnostic accuracy in FNAB smears is intermediate in DLBCL. The cytologic differential diagnosis of DLBCL includes Hodgkin's disease, T-cell lymphoma, anaplastic large cell lymphoma but can be distinguished from these entities with application of appropriate immunocytochemical panel [7, 8].

PEL occurs predominantly in men with acquired immunodeficiency syndrome (AIDS), but can be seen in iatrogenically immunosuppressed transplant patients and elderly individuals. In addition to HIV and HHV-8 positivity, many patients also have seropositive results for Epstein-Barr virus (EBV) [9, 10].

PEL usually exhibits a complex karyotype without a characteristic aberration. This lymphoma frequently has an intermediate immunophenotype that is not clearly B cell or T cell in origin, but it is often positive for CD45, CD30, and epithelial membrane antigen (EMA) [11]. The differential diagnosis typically includes other high-grade lymphomas that occur in HIV-infected patients, immunosuppressed patients and elderly individuals.

The effusions show a dispersed popularity of large pleomorphic cells with eccentrically located nuclei, one or more prominent nucleoli, a perinuclear pallor and moderately abundant cytoplasm. Binucleated, multinucleated cells and mitotic figures are seen commonly. As far as cytomorphology is concerned, PEL shows features bridging immunoblastic and anaplastic large-cell lymphomas, with a frequent demonstration of plasma cell differentiation [12].

PEL cells in our case showed positive staining for B cell lineage (PAX-5), displayed markers of lymphocyte activation (CD30) and plasma cell differentiation (CD138), and showed positive staining for the leukocyte common antigen (CD45). Our case also showed the relative infrequent immunocytochemical expression of T-cell associated antigens. (CD3, CD43). Our case was also positive for HHV-8 by in situ hybridization, a finding that along with the particular cytomorphological and immunocytochemical findings confirmed that the lymphomatous effusion was a case ofPEL.

T-cell immunophenotype in PEL is a relatively rare event and only a few cases have been published until now [13].

The differential diagnosis of PEL, on a cytomorphological ground, includes other large cell lymphomas, such as, anaplastic large cell lymphoma (ALCL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL), with plasmacytoid differentiation, and pyothorax-associated lymphoma (PAL). The aberrant expression of T-cell antigens, the absence of EBVas well as the strong positivity for HHV-8, rendered the diagnosis of PEL as the most plausible.

In conclusion cytomorphology in conjunction with ancillary studies, especially immunohistochemistry, along with the clinical and/or radiographic data can approach a high diagnostic accuracy for the diagnosis ofuncommon tumours in our settings.

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