Fine needle aspiration cytology of unsuspected metastatic primitive neuroectodermal tumour (PNET): a case report

Sharda Lallu, Sarla Naran, Diane Kenwright and Peter Bethwaite

Abstract
We report a case of primitive neuroectodermal tumour (PNET) of the tongue that metastasized to a left cervical lymph node in a 47 year old female with a short history of sore throat and swelling in the left upper neck. At presentation a left hypoglossal nerve palsy was evident along with masses in the posterior third of her tongue and the upper region of the sternocleidomastoid muscle. Fine needle aspiration (FNA) of the neck mass revealed a cellular population of spindle cells arranged as syncytial aggregates with indistinct cell borders. Tumour cells were oval, polygonal and spindled in appearance with scant fragile cytoplasm, a high nuclear/cytoplasmic ratios, nuclear hyperchromasia, irregular and molded nuclei, granular chromat in inconspicuous nucleoli. Histologic appearance, ultrastructural and immunohistochemical findings confirmed the diagnosis of PNET.

Key words: fine needle aspiration cytology, metastasis, left neck, peripheral primitive neuroectodermal tumour (PNET), tongue


Introduction
Peripheral primitive neuroectodermal tumour (PNET) is a rare malignant small round cell tumour of neuroectodermal origin with variable cellular differentiation (1,2). The term was first used for a group of embryonal tumours located in the central nervous system (cPNET) and then expanded to include similar peripherally located tumours (pPNET) (3). Peripheral PNETs affect mainly children and young adults, commonly involving the thoracopulmonary region (Askin tumour), pelvis, abdominal region and extremities. These tumours are aggressive, with a tendency to recur and to metastasize especially to bone marrow, brain, lungs and lymph nodes (4).

FNA cytology of PNET in various locations has been reported sporadically (5). The occurrence of these tumours in the head and neck is rare (6,7) and we report the fine needle aspiration (FNA) cytology of a PNET arising in the posterior tongue, that had metastasized to cervical lymph nodes in a 47 year old woman, who had dysphagia with evidence of relapse in the posterior tongue with a 60 mm mass in the base of tongue on CT examination, with evidence of multiple pulmonary metastases. The patient died due to the progression of distant metastasis.

Material and methods
FNA of the left neck mass was performed with a 25 gauge needle and smears were prepared on site and fixed in 95% ethanol and stained with Papanicolaou stain. The remaining material from the needle was washed in 30% ethyl alcohol in physiologic saline. From half of this material a filter preparation was made on size 5 μm Sartorius AG-Cellulose Acetate filter (Germany) using the cytosieve method and stained by Papanicolaou method. The remainder of the aspirate sample was spun down and from the sediment a cell block was made and fixed in 10% formalin, routinely processed and stained with Hematoxylin-Eosin (H & E). A portion of the repeat FNA sample of the neck mass was sent for electron microscopy which was fixed in phosphate-buffered glutaraldehyde, postfixed in osmium tetroxide, embedded in Epon and sections were cut and stained with uranyl acetate / lead citrate. For cytogenetic analysis the part of sample was fixed in cytogenetic transport medium.

Immunohistochemistry was undertaken using the Strep-Avidin Biotin method (Ventana ES). Sections from the cell block of FNA neck and tongue biopsy were stained with Vimentin (1:4000 Dako), CD99 (1:100 Dako), Bcl-2 (1:30 Dako), NSE (1:2000 Dako), Cytokeratin (AE1/AE3) [1:1000 Dakocytomation], CAM 5.2 (1:100 Dickinson), EMA (1:750 Dako), LCA (1:300 Dako), S100 (1:4000 Dako), HMB 45 (1:500 Dako), Synaptophysin (1:80 Dakocytomation), Actin (1:2500 Dako), Desmin (1:200 Dako) and Myoglobin (1:8000 Dakocytomation). PAS histochemical staining was also performed on cell block and biopsy sections.

Results

Cytologic findings
FNA cytology smears (Figure 1) and clot preparations (Figure 2A) were highly cellular and composed of syncytial aggregates of tumour cells with indistinct cell borders and scanty stroma. The tumour cells were oval, polygonal and spindled in appearances. The cells ranged in size with scant and fragile cytoplasm, high nuclear/cytoplasmic ratios, hyperchromatic, angulated nuclei showing nuclear molding, focal pseudorosette formations, granular chromat in inconspicuous nucleoli and some mitotic figures. The background contained free lying nuclei, blood, apoptotic bodies and necrotic debris.
Figure 1. Filter preparations from FNA showing syncytial aggregates of tumour cells with oval to polygonal and spindly appearances, hyperchromatic nuclei, high N/C ratio and indistinct cell border (Papanicolaou stain X 400).

Figure 2A. Cell block preparations of FNA showing syncytial aggregates of tumour cells with oval, polygonal, spindly appearances, very high N/C ratio, hyperchromatic, angulated nuclei with scant cytoplasm (Hematoxylin-eosin stained X 400).

Histologic findings of the tongue biopsy
The tumour comprised sheets of tumour cells surviving around delicate blood vessels. The tumour cells contained small mildly hyperchromatic angulated nuclei together with a small amount of eosinophilic to clear cytoplasm (Figure 2B). There was a suggestion of a myxoid quality to the background stroma and ill-defined rosette formations. Numerous mitotic figures were identified including abnormal figures and necrotic tumour debris.

Figure 2B. Tongue biopsy section showing sheets of tumour cells contained mildly hyperchromatic, angulated nuclei, high N/C ratio together with a small amount of eosinophilic to clear cytoplasm (Hematoxylin-eosin Stain X 400).

Immunohistochemical findings
Immunohistochemical staining on cell block from FNA neck and histologic sections of tongue biopsy showed positive membranous staining within tumour cells for CD99 (Figure 3), Vimentin, Bcl-2 and focal positive staining was seen with NSE. Immunohistochemical stains for Cytokeratin AE1/AE3, CAM 5.2, EMA, LCA, S100, HMB45, Chromogranin, Synaptophysin, Smooth Muscle Actin, Desmin and Myoglobin were all negative. The PAS stain demonstrated moderate amounts of glycogen within the cytoplasm of the tumour cells.

Figure 3. Immunohistochemical stained on cell block section showing strong positivity for CD99 (CD 99 Stain X 400).

Electron microscopy
Electron microscopy showed primitive, undifferentiated cells with few cytoplasmic organelles but abundant glycogen. Non-specific intermediate filaments were present but no cytokeratin filaments or myofilaments. Cell junctions were infrequent and rudimentary. Occasional small, dense core granules were noted, but interdigitating cell processes were absent.

Cytogenetic analysis
Cytogenetic analysis failed to demonstrate metaphases for analysis. Paraffin sections analysed with Fluorescence In situ Hybridization (FISH) studies of the EWS gene showed a normal signal pattern (two yellow signals) with EWSR1 (Zymed laboratories locus 22q12/q13) break apart probe in the majority of sites examined consistent with a normal result.

Discussion
Primitive neuroectodermal tumours are members of the Ewing’s sarcoma family composed of small round cells normally lacking morphologic evidence of neuroblastic differentiation in the form of neuropil or ganglion cell formation. PNET’s account for 1% of soft tissue tumours most commonly involving the thoracopulmonary region (Askin tumour), pelvis, abdominal region and extremities; its presence in the chest wall, posterior mediastinum, myocardium, kidney, vagina, bladder, parotid and orbit has been reported (8). There is propensity for rapid metastatic spread to the distal sites especially the lung, liver, bone marrow, lymph node, pleura and diaphragm. Primary lesions are rare in the head and neck region. The literature reports 43 patients with PNET in the head and neck region, with a mean age of 21 years (9). The prognosis of PNET in this site is also generally poor.

PNET, Ewing’s sarcoma, and Askin tumour of the thoracopulmonary region are now considered to be part of the PNET-Ewing’s sarcoma family as cytogenetic studies show similar abnormalities; namely the t(11;22) (q24;q12) translocation. The EWS-FLI1 fusion transcript can be detected in 80-90% of the PNET-Ewing’s sarcoma family by reverse transcriptase polymerase chain reaction (RT-PCR) (8). FISH did not show EWS gene rearrangement in our case. A few isolated cases have been reported of Ewings sarcoma with no detectable rearrangement using an EWSR1 break apart probe. Abnormalities were subsequently detected in these cases using FISH probes for
the specific fusion product (10). Therefore, in some rare cases, even if a normal result is reported, a diagnosis of Ewing sarcoma or PNET cannot be completely excluded. In 5 to 10 per cent of EWS, peripheral primitive neuroectodermal tumours, it is not possible to demonstrate rearrangements of EWS on 22q12 and ETS related oncogenes. Some studies suggest that the type of EWS chimeric fusion transcript has prognostic importance (11).

The cytologic diagnosis of PNET poses a diagnostic challenge due to overlapping cytomorphic features with those of other small round cell tumours. The potential for misclassification is accentuated when PNETs occur in unusual locations and in an adult group, as demonstrated in our case. The diagnosis of PNET requires a combination of ancillary studies such as immunohistochemistry, cytogenetic, molecular genetics and electron microscopy.

Immunohistochemical staining shows strong dot like or perinuclear vimentin staining and diffuse membranous staining for CD99 in all members of the Ewing/PNET group, although weaker CD99 staining may be seen in other “round blue cell tumours” including rhabdomyosarcoma, T cell lymphomas, synovial sarcoma and small cell neuroendocrine tumours (11). There is variable expression of other neuroectodermal antigens including NSE (positive in this case), synaptophysin, neurofilament, GFAP and PGP9.5. Although not evident in the current case, 20% of cases may show dot-like cytokeratin positivity (11). In most cases the combination of vimentin and strong membranous CD99 and NSE positivity with negative expression of cytokeratins, muscle and haematologic antibodies should exclude neuroblastoma, rhabdomyosarcoma, desmoplastic round cell tumour and leukaemia-lymphoma (12). Ultrastructural features vary depending on the degree of differentiation along the Ewings/PNET spectrum.

The cytologic differential diagnosis of PNET includes lymphoma, neuroblastoma, embryonal rhabdomyosarcoma, small cell carcinoma, synovial sarcoma, small cell variant of melanoma, basaloid squamous cell carcinoma and poorly differentiated non keratinized squamous cell carcinoma (13-18). In this case the absence of lymphoglandular bodies and the presence of large clusters of cohesive cells and a negative stain for LCA ruled out a lymphoma. The presence of neurophil matrix in the background, unipolar cytoplasmic tags (neurites) of the cells forming frequent Homer-Wright rosettes and ganglion cells seen in neuroblastoma were not identified. In addition, neuroblastomas are uniformly negative for CD99 and vimentin.

Rhabdomyosarcoma often shows cellular aggregates, single dispersed cells varying in size and shape, prominent nucleoli, dense chromatin, multinucleation and cytoplasmic vacuoles that were not seen in this material. Rhabdomyosarcomas are positive for desmin, actin and myoglobin. Small cell carcinoma usually shows smearing artefacts, nuclear molding, stippled chromatin with inconspicuous nucleoli, necrotic debris and karyorrhexis. These features are also seen in PNET but negative staining with AE1/AE3, EMA and CD56 normally exclude small cell carcinoma.

Poorly differentiated synovial sarcoma can have the morphologic appearance of a “small round blue cell tumour” and overlapping cytologic features with PNET. The cytologic mimicy makes them unlikely to be diagnosed solely on morphologic grounds. The immortal primitive-tenchical markers CD99, Bcl-2, AE1/AE3, cytokeratin and EMA markers are normally positive in synovial sarcoma. In this case, EMA, AE1/AE3 and CAM 5.2 were negative. Bcl-2, although usually positive in synovial sarcoma, is not a very helpful marker in the differential diagnosis as it is positive in other soft tissue tumours (15), and was positive in our case. The small cell variant of melanoma consists of small crowded cells with hyperchromatic nuclei, scant cytoplasm, small cystic spaces, necrosis and prominent hialnosis and are positive for CAM 5.2 and AE1/AE3. Non keratinizing squamous cell carcinoma consists of oval or polygonal, syncytial aggregates of tumour cells with indistinct cell borders, scanty cytoplasm, hyperchromatic nuclei, irregularly distributed coarse chromatin, mitoses and necrosis. Immunohistochemical staining may be helpful as these tumours lacks immunoreactivity for CD99, NSE and are positive with epithelial markers (13-18).

In summary, FNA cytology along with the ancillary studies such as immunohistochemistry, molecular analysis, cytogenetics, FISH and EM on the aspirated material, will enable a correct diagnosis of primary or metastatic PNET and exclude the other small round cell malignancies that enter into the differential diagnosis as we have experienced in this case.

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