Fine needle aspiration (FNA) cytology of Merkel cell carcinoma (MCC) - a review of 69 cases.

Running Title: FNAC of Merkel cell carcinoma

Paul W Shield PhD[1,2]

Heinrich Crous B App Sci[2]

1. School of Biomedical Sciences, Queensland University of Technology
2. Cytology Department, Sullivan Nicolaides Pathology

Address for correspondence: Associate Professor Paul Shield, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, GPO Box 2434, Brisbane 4001 AUSTRALIA. Ph: 61 731384157; Fax: 07 38712937 Email: pw.shield@qut.edu.au

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Abstract

Background: This study reviewed the clinical presentation, cytologic findings and the immunophenotype of 69 Merkel Cell Carcinoma (MCC) cases sampled by FNA.

Methods: Demographic and clinical data, the cytology findings and results of ancillary testing were reviewed.

Results: Median patient age was 78 years (37 – 104) with a 1:1.8 female to male ratio. The most common FNA sites sampled included lymph nodes in the neck, the axillary region, the inguinal region and the parotid gland. Most patients had a history of MCC (68%) &/or non-MCC malignancy (70%).

The common cytologic pattern was a cellular smear with malignant cells arranged in a dispersed pattern with variable numbers of disorganised groups of cells. Cytoplasm was scant or absent and nuclei showed mild to moderate anisokaryosis, stippled chromatin, inconspicuous nucleoli and nuclear molding. Numerous apoptotic bodies were often present.

Cell block samples (28 cases) were usually positive for cytokeratins in a perinuclear dot pattern, including 88% of cases with CK20 positivity. CD56 was the most sensitive (95%) neuroendocrine marker on cell blocks and was also positive with flow cytometry in 9 cases tested.

Conclusions: MCC is most commonly seen in FNA specimens from the head and neck of elderly patients, often with a history of previous skin lesions. Occasional cases present in younger patients and some may be mistaken for other round blue cell tumors, such as lymphoma. CD 56 may be a useful marker in cell block preparations and in flow cytometric analysis of MCC.

Keywords: Fine Needle Aspiration; Merkel cell carcinoma; Cytology; CD56

Introduction

Merkel cell carcinoma (MCC) is a rare aggressive neuroendocrine tumor of skin that commonly metastasises to lymph nodes and may be a target for fine needle aspiration (FNA). The tumor occurs primarily in elderly Caucasian patients and is more common in immunosuppressed individuals[1]. It also occurs relatively commonly in patients with a history of another neoplasm. Clinically, MCC usually appears as a small red-purple hard nodule, most often on sun exposed areas such as the head, neck or extremities, and is often indistinguishable from other skin cancers[1]. It carries a poor prognosis, with mortality rates exceeding those of melanoma. Metastases to local lymph nodes occurs in about 2/3 of patients and distant metastases and death in about 1/3[1]. In 10-20% of cases with metastatic disease the primary tumor is not identified[2], possibly due to spontaneous regression of the cutaneous primary in some cases[3].

Most published studies of the cytologic findings of MCC are single case reports or small series. Queensland, 'the Sunshine State' of Australia, has high rates of skin cancer and MCC is not uncommonly encountered in our FNA practice. Although most cases have a characteristic morphology and a known history, the diagnosis of MCC can be problematic when other small cell tumors are a consideration in the differential diagnosis, and particularly when a primary tumor has not been identified or the clinical history is not typical. We reviewed the clinical and cytological presentation and the immunophenotype of a large series of confirmed MCC cases reported on FNA to highlight the potential pitfalls posed by MCC in FNA specimens.

Methods

Sixty-nine FNA cases from 64 patients reported as suspicious for or consistent with Merkel Cell Carcinoma (MCC) over an 8 year period were reviewed. All cases had a clinical history of MCC provided and/or had a histologic report of MCC on file or had subsequent histologic confirmation. Demographic and clinical data were collated and the slides were reviewed to summarize cytologic features. Five cases were prepared as air-dried Romanowsky stained smears only and in 64 cases

fixed Papanicolaou stained smears were also available. Results of ancillary testing performed on needle rinses were also summarised.

Results:

Patient age ranged from 37 to 104 years (median age 78) with a 1:1.8 female to male ratio. Only four (6%) patients were aged less than 60 years. The specimens were collected from a range of sites (Table 1). Most were in the head/neck region, particularly lymph nodes in the cervical region (30 cases; 43.5%), the parotid gland (8; 11%) or other lymph nodes in the axillary (20%) or inguinal region (11%). Metastatic lesions in the pancreas (2) and breast (1) were also reported. Three (4%) of the lesions sampled were primary cutaneous tumors. Most patients (45/64; 70%) had a history of non-MCC neoplasm: SCC (33; 48%), BCC (33; 48%), melanoma in 7 cases (10%), and in two cases NHL (3%). A history of multiple neoplastic skin lesion biopsies was present in 68% (44/64).

The most frequently observed cytologic features are summarised in Table 2. The common cytologic pattern was a highly cellular smear in which malignant cells predominated. The cells were usually arranged in a dispersed pattern with most cases (88%) also showing at least some cohesive disorganised groups of cells. Cell grouping was, however, highly variable, with some cases presenting in a totally dispersed, lymphoma-like, pattern (Fig. 1) while a few cases were composed predominantly of loosely cohesive malignant cells (Fig. 2). A careful search was required in some cases to identify small cell groups composed of tightly packed molded nuclei with little or no cytoplasm (Fig. 3). Single cells were usually stripped of cytoplasm but, when present, it was delicate and pale with ill-defined cell borders (Fig. 4). Cytoplasmic intermediate filament buttons, as described by some authors[4] were noted in only 3/69 (4%) of cases. Nuclei showed mild to moderate anisokaryosis, stippled chromatin and inconspicuous nucleoli. Nuclear molding was usually focally apparent (Fig. 5). Two cases contained malignant cells with marked anisokaryosis and some cells with enlarged nuclei with irregular nuclear membranes. Numerous apoptotic bodies were present in 60% of cases (Fig. 6) but necrosis was rarely seen (13%). Lymphoglandular bodies were identified in two

cases (3%) only, both of which contained moderate numbers of lymphocytes. Cell block samples were immunostained in 28 cases using a variety of stain panels. Results are summarised in Table 3. Most cases tested showed perinuclear dot positivity for cytokeratins, with 88% (22/28) positive for CK20 (Fig. 7). CD56 was the most sensitive neuroendocrine marker for MCC, with positive staining in 20/21 (95%) cell block cases (Fig. 8). CD56 was also detected by flow cytometry in 9/9 cases, giving an overall sensitivity of 97% (29/30).

Discussion

Data from the US and Australia indicates that the incidence of MCC is increasing.[5,6] Factors implicated in the development of MCC include infection with Merkel cell polyomavirus,[7] immunosuppression,[8] sun exposure[9] and altered function of tumor suppressor genes.[10]

The tumor has a characteristic presentation in FNA and should be suspected when small round blue cell tumors are present in specimens collected from the head and neck of elderly patients, particularly those with a history of previous skin lesions. Almost 70% of cases in this series had a history of MCC provided. A similar proportion had a history of other malignancy, including prior melanoma in 10% of patients and one patient each with B-cell lymphoma and chronic lymphocytic leukemia (CLL). MCC often occurs in patients with another neoplasm, most frequently with SCC, CLL or B-cell lymphoma.[2,11] This may complicate the FNA diagnosis, particularly as some of the cases reviewed showed a primarily dispersed population of small to medium sized malignant cells with only mild anisokaryosis.

Lymphoid cells pose the most common differential diagnosis for MCC in FNA. Lymph nodes are the most common site in which MCC is sampled by FNA and the malignant cells may be misinterpreted as benign or malignant lymphoid cells. A dispersed population of malignant cells with mild anisokaryosis may be overlooked in a lymphoid-rich background. Alternatively, MCC may be mistaken for lymphoma.[2] In the current series an initial impression of lymphoma led to the performance of cell surface markers by flow cytometry rather than cell block preparation. In these

cases, as others have reported,[12] we have found CD56 to be a valuable marker that works well with flow cytometry and is a useful addition to the surface marker panel if the predominant cell population is found to be CD45 negative. It must remembered however that some T-cell lymphomas express CD56[13] and that plasma cell neoplasms may be CD45- / CD56+. Table 4 lists the morphologic features that are useful in discriminating MCC from lymphoma. The use of both Romanowsky and Papanicolaou stains on cytology smears is valuable as they highlight different features: lymphoglandular bodies and nuclear molding are best appreciated on the former, while the stippled chromatin pattern and the presence and size of nucleoli are more obvious on Pap stained smears. An absence of lymphoglandular bodies is a useful feature of MCC, however assessment of their presence is difficult when abundant necrotic material is present and they may be present in smears containing benign lymphocytes.

Cytoplasmic and free intermediate filament buttons, so-called ‘blue blobs’ have been described as a feature of MCC,[4] however although cytokeratin perinuclear dot positivity was seen with IHC, we found it uncommon in conventional cytology stains. Several studies of MCC have not identified this feature and it has been suggested they are more commonly seen in haematoxylin and eosin stained specimens.[14]

Small cell carcinoma, metastatic from both lung and non-pulmonary sites, may also enter into the differential diagnosis. Morphologically MCC is less likely to show marked anisokaryosis (only 7% in this series) and necrosis (13%). Clinical history and immunohistochemistry may also be helpful. Small cell carcinoma, both lung and non-pulmonary, usually express TTF1 and rarely express CK20.[15,16] Cytogenetic anlaysis for trisomy of chromosome 6 & 8, using FISH on cytology slides, rarely present in SCLC but common in MCC, may also assist.[17] Small cell carcinoma of salivary gland, a rare neoplasm, is virtually indistinguishable from metastatic MCC if a primary tumor is not identified, showing a similar morphology and immunophenotype. Eight cases in the current series were metastatic MCC presenting as parotid lesions from primary tumors mostly on the face (7/8).

Basaloid tumors must also be excluded, particularly in head and neck sites. Basal cell carcinoma typically presents as cohesive groups of uniform cells, sometimes with pallisading. Pilomatrixoma is another consideration, particularly as it may be mistaken clinically for a lymph node in the head/neck region. It usually occurs in younger patients and also usually contains squamous cells, ghost cells and multinucleate histiocytes. A range of salivary gland neoplasms may also have a basaloid pattern on FNA,[18] however the cells from these lesions generally show more cohesion, less anisokaryosis, nuclear molding and lack the stippled chromatin of MCC. In addition, other features such as metachromatic stromal elements may help in identifying salivary gland neoplasms. A history of melanoma was not uncommon in this series, occurring in 10% of cases. It is notoriously varied in appearance and may present as a small round cell tumor. Nucleoli are usually prominent in melanoma but IHC may be very helpful in these cases. Although melanoma may express CD56,[13] MCC is usually cytokertain positive and negative for melanoma markers.

In summary MCC is a rare but increasingly encountered tumor that may be reliably diagnosed by FNA if the clinical, morphologic and immunophenotypic information is avaliable. FNA may be a useful diagnostic modality to confirm metastasis, aid with treatment planning and exclude other tumors and conditions.

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Fig. 1. Lymphoma-like presentation of dispersed malignant cells with little or no cytoplasm. (FNA Axilla; Quick Dip stain; x100).

Fig. 2. Malignant cells predominantly arranged in cohesive disorganised groups. (FNA neck lymph node; Papanicolaou stain; x100).

Fig. 3. Tight group of molded nuclei with little or no cytoplasm. (FNA groin lymph node; Papanicolaou stain; x400).

Fig. 4. Malignant cells with finely stippled chromatin and delicate cytoplasm. (FNA neck lymph node; Papanicolaou stain; x400).

Fig. 5. Malignant cells with scant cytoplasm and nuclear molding. (FNA groin lymph node; Quick Dip stain; x400).

Fig. 6. Malignant cells with numerous apoptotic bodies. (FNA neck lymph node; Papanicolaou stain; x200).

Fig. 7. Cell block preparation showing perinuclear dot positivity for Cytoketarin 20. (FNA neck lymph node; Haematoxylin counterstain; x400).

Fig. 8. Cell block preparation showing membrane staining with CD56. (FNA axilla; Haematoxylin counterstain; x400).

Table 1. Sites of FNA for the 69 cases of Merkel cell carcinoma reviewed. LN= lymph node.

Site	N	%
Lymph node, cervical	30	43.5
Lymph node, axillary region	14	20.3
Parotid gland	9	11.6
Lymph node, inguinal region	8	11.6
Upper arm	2	2.9
Pancreas	2	2.9
Lymph node, celiac	1	1.4
Breast	1	1.4
Chest	1	1.4
Thigh	1	1.4

Table 2. Most common cytomorphologic features observed in 69 FNA cases of Merkel Cell Carcinoma.

Cytological Feature	%
Moderate to high cellularity	98
Nuclear molding	96
Stippled chromatin on Pap	95
Inconspicuous nucleoli	94
Mild to moderate anisokaryosis	93
Mainly dispersed	89
Cohesive groups	59
Apoptotic bodies	62
Necrotic debris	13

Table 3. Immunohistochemical staining results on cell blocks prepared from FNA specimens of Merkel Cell Carcinoma.

Stain	N	% +ve
Cytokeratins		
CK20	28	88%
Cam 5.2	17	94%
AE1/AE3	10	100%
MNF113	6	83%
Neuroendocrine markers		
CD56	21	95%*
Synaptophysin	13	84%
Chromogranin A	11	36%
Melanoma markers		
S100	9	0%
HMB45	5	0%
Melan A	5	0%
CD45	12	0%**

*An additional 9/9 cases positive for CD56 with flow cytometry.

**An additional 9/9 cases negative for CD45 by flow cytometry.

Table 4. Cytomorphologic features of value in discriminating Merkel Cell Carcinoma from lymphoma.

Feature	Merkel Cell Carcinoma	Lymphoid cells/lymphoma
Arrangement	Variable but often at least some small cohesive groups	Dispersed
Lymphoglandular bodies	Only if significant lymphoid population present	Usually common
Apoptosis	Often present	Variable, often absent
Cytoplasm	Absent or minimal; delicate, pale and ill-defined	Thin rim, cyanophilic
Nuclei	Usually mild to moderate anisokaryosis	Usually mild anisokaryosis
Nucleoli	Absent/Inconspicuous & small	Variable; may be prominent
Chromatin	Fine, stippled	'chunky'
Nuclear molding	Usually at least some present	Usually absent