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# Cytologic features of small cell melanoma

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## Abstract

Small cell melanoma (SCM) is an aggressive variant of malignant melanoma (MM), which has been rarely described in the cytology literature. The aim of this study was to describe the clinical and cytologic features of a series of cases of metastatic SCM with discussion of the differential diagnosis of metastatic SCM diagnosed by fine-needle aspiration (FNA). A retrospective review of cases was performed, identifying two FNA cases and one core biopsy with touch preparation of metastatic SCM. Clinical presentation, cytomorphology features, ancillary tests, and final diagnoses were documented and analyzed. Patients ranged in age from 69 to 85 years-old. Cytomorphologic features included the presence of a monomorphic population of dispersed small round blue cells, with scant cytoplasm, high nuclear to cytoplasmic ratios, dense nuclear chromatin, and inconspicuous nucleoli. Acinar like arrangement (n = 2) and nuclear molding (n = 1) were also present. All cases showed diffuse positivity for the melanocytic markers SOX10 and Melan A by immunohistochemistry (IHC). Expression of neuroendocrine markers was variable. Diagnosing metastatic SCM at unusual anatomic sites by FNA cytology is a challenging task, especially in patients without known prior history of melanoma. Cytomorphology of SCM is unique, differing from conventional MM in many aspects, including the presence of acinar formations and a lack of typical melanoma features, such as large cells, intracytoplasmic melanin, and macronucleoli. IHC is critical for establishing the diagnosis of SCM.

KEYWORDS
cytomorphology, fine-needle aspiration, malignant melanoma, metastatic melanoma, small cell melanoma, touch preparation

## 1 | INTRODUCTION

Fine-needle aspiration (FNA) has a limited role in the diagnosis of primary cutaneous malignant melanoma (MM), as these lesions are usually diagnosed by punch biopsy, shave biopsy, or elliptical excision. However, MM frequently presents as metastatic disease at superficial cutaneous and non-cutaneous sites. As a rapid, safe, and inexpensive method, FNA has become a test of choice for diagnosing such metastatic disease and procuring material for ancillary/molecular testing in patients with a known history of melanoma. Several studies have

shown that the sensitivity and specificity of FNA for diagnosing metastatic melanoma is >95%.[1–5] Diagnosis of metastatic melanoma remains challenging in patients without prior history of melanoma. The situation is further complicated when unusual variants of MM present as metastatic disease.[6]

MM is a great mimicker with diverse morphology. Hence, when interpreting FNA cytology the consideration of MM in the differential diagnosis is commonplace.[7] The diagnosis of MM on FNA is typically verified with immunohistochemistry (IHC), which can essentially confirm the diagnosis in most cases. Different histologic variants of

melanoma include (i) common variants such as spindle cell, nodular, and desmoplastic melanoma, and (ii) unusual variants such as balloon cell, rhabdoid, signet cell, myxoid, choroid, metaplastic, and small cell melanoma (SCM).[8,9] The cytology features of the usual and many unusual variants of MM in FNA specimens are well-described in the literature. However, there is a sparsity of literature describing the cytomorphology of more unusual subtypes such as SCM.[10] The aim of this study is to thus review the clinical presentation, cytomorphologic spectrum, and immunophennotypic features in a case series of SCM.

## 2 | MATERIALS AND METHODS

A retrospective review of three FNA cases of metastatic SCM was performed. The cases were collected from the University of Pittsburgh Medical Center, Pittsburgh, PA, USA (n = 1) and University of Michigan, Ann Arbor (n = 2), including FNA samples (n = 2) and a core needle biopsy with touch preparation (n = 1). All cases were performed by radiologists under ultrasound or computerized tomography (CT) guidance. Rapid onsite evaluation (ROSE) was performed in all cases. For two cases evaluated by FNA, air-dried Diff-Quik stained and alcohol-fixed Papanicolaou stained direct smears were prepared from each pass and cellblocks were prepared. For the one case evaluated by core biopsy, air-dried Diff-Quik stained imprint slides were prepared and the core submitted in 10% buffered formalin. Aspirated material was collected for ancillary studies including flow cytometry and molecular profiling. IHC was performed on cellblock sections and the core biopsy in all three cases with appropriate controls.

The available data collected included clinical presentation, patient demographics, radiology findings, cytology and surgical pathology diagnoses, and follow-up information. Cytomorphology details including specimen cellularity, pattern, cell size/shape, presence of melanin pigment, cytoplasm and nuclear features, and background findings were recorded. All cases were rereviewed to confirm the diagnoses.

Flow cytometry was not performed for any case. The results of available ancillary tests (IHC and molecular) were also documented.

## 3 | RESULTS

### 3.1 | Clinical findings

All patients were elderly with an average age of 77 years (range of 69–85 years). The clinical presentation, radiologic, and demographic findings are summarized in Table 1. A clinical diagnosis of melanoma was not suspected in two of the three cases. Clinical presentations were as follows:

*Case* A. A 77-year-old, previously healthy man with no known history of malignancy presented to the emergency room with a chief complaint of abdominal pain. A CT scan of his abdomen showed a large lesion in the left lower quadrant and a pancreatic mass. Ultrasound-guided FNA of the left lower quadrant mass was performed.

*Case* B. An 85-year-old woman presented with a palpable posterolateral abdominal wall mass and no known history of malignancy. A needle core biopsy with touch preparations was performed.

*Case* C. A 69-year-old woman with a remote history of smoking, and a prior history of a right arm MM with epithelioid and nevoid features 10 years ago, presented with a 6 month history of cough and hemoptysis. Bronchoscopy evaluation at that time revealed *Aspergillus fumigatus* in bronchial washing cultures, and a CT scan of her chest revealed a large right lower lobe lung mass, which was initially suspected to be an aspergilloma.

TABLE 1 Demographics, clinical presentation, and imaging of cases diagnosed as small cell melanoma

| Case | Age/gender | Clinical presentation | Anatomic site of tumor | Imaging findings | Prior diagnosis of melanoma | Multicentric presentation | Other findings |
|---|---|---|---|---|---|---|---|
| A | 77 year/male | Abdominal pain | Left lower quadrant of abdominal mass | CT abdomen: Large mass in left lower quadrant of abdomen and pancreatic mass | No | Yes | None |
| B | 85 year/female | Palpable postero-lateral abdominal wall mass | Abdominal wall mass | Not available | No | No | None |
| C | 69 year/female | Hemoptysis | Lung mass | CT chest: Large right lower lobe lung mass | Yes, 10 year prior melanoma with epithelioid and nevoid features of right arm skin, Clark level IV, depth of invasion 2.3 mm | No | None |

Abbreviation: CT, computerized tomography.

**TABLE 2**  Spectrum of cytomorphologic findings in small cell melanoma cases

| Cytomorphology | Case A | Case B | Case C |
| --- | --- | --- | --- |
| Cellularity | Highly cellular | Highly cellular | Highly cellular |
| Cell arrangement | Small dispersed isolated cells | Small dispersed isolated cells | Predominantly dispersed isolated cells |
| Cell clusters | Occasional loose clusters | Occasional loose clusters | Occasional loose clusters |
| Cell size | Mostly small to medium | Mostly small to medium | Medium to small |
| Cell shape | Round to oval | Round | Round |
| Cell cytoplasm | Scant to absent | Absent to minimal | Scant |
| Cytoplasmic borders | Indistinct | Indistinct | Distinct |
| Cytoplasmic vacuoles | − | − | + |
| Bizarre cells | − | − | − |
| N/C ratio | High | High | High |
| Nuclear contour | Smooth to focal irregular | Smooth to focal irregular | Irregular to smooth |
| Chromatin pattern | Condensed | Condensed | Condensed |
| Nucleoli | Inconspicuous to occasional small | Inconspicuous to occasional small | Inconspicuous to occasional small |
| Intranuclear pseudo-inclusions | Rare | − | − |
| Binucleation/multinucleation | − | − | − |
| Nuclear placement | Central | Central | Central to eccentric |
| Nuclear molding | − | − | + (focal) |
| Mitotic figures | + | + | + |
| Melanin pigment | Rare, extracellular | − | − |
| Melanophages | − | − | − |
| Apoptotic debris | − | − | − |
| Peritheliomatous pattern | + (focal) | − | + (focal) |
| Acinar formation | ++ | + | − |
| Other | Occasional spindle to plasmacytoid cells | Occasional spindle to plasmacytoid cells | Epithelioid cells |

However, the lesion grew from 8.1 to 13.7 cm over a period of 5 months by CT imaging, and the patient accordingly underwent an endobronchial ultrasound-guided FNA of the lung mass.

## 3.2 | Cytopathology findings

### 3.2.1 | ROSE diagnoses

Case A: Adequate: positive for neoplasm, favor neuroendocrine neoplasm. Case B: Adequate: positive for small round blue cell tumor (SRBCT). Case C: Adequate: atypical cells present.

### 3.2.2 | Cytomorphology

The cytology aspirate smears (n = 2) and touch preparation (n = 1) were highly cellular. The cytomorphologic spectrum of findings in the cases is summarized in Table 2. Cytologic features of the aspirate from Case A and touch preparation of Case B were similar (Figures 1 and 2), both showing a monomorphic population of dispersed small round cells with scant to absent cytoplasm and a high nuclear: cytoplasmic ratio. A rosette-like or acinar-like arrangement of the tumor cells was notable in Case A. In addition, Case A demonstrated more typical melanoma features focally, including rare intranuclear pseudo-inclusions and a peritheliomatous growth pattern. Both cases showed occasional spindle and plasmacytoid cells. The needle core biopsy of Case B contained solid sheets of non-cohesive small round cells with scattered small blood vessels and prominent mitotic figures. Case C (Figure 3) was composed of small- to medium-sized cells with scant cytoplasm and eccentric to centrally located nuclei. Focal peritheliomatous growth pattern was noted on the cellblock section from Case C. Rare cells had cytoplasmic vacuoles. In both FNA cases, cellblock material showed a dispersed population of medium to small cells (Cases A and C). Lymphoglandular bodies, necrosis, or a tigroid background was not present in any of these cases.

## 3.3 | Ancillary test findings

As the diagnosis of melanoma was not suspected clinically in two of the cases and because of the small round cell morphology appreciated
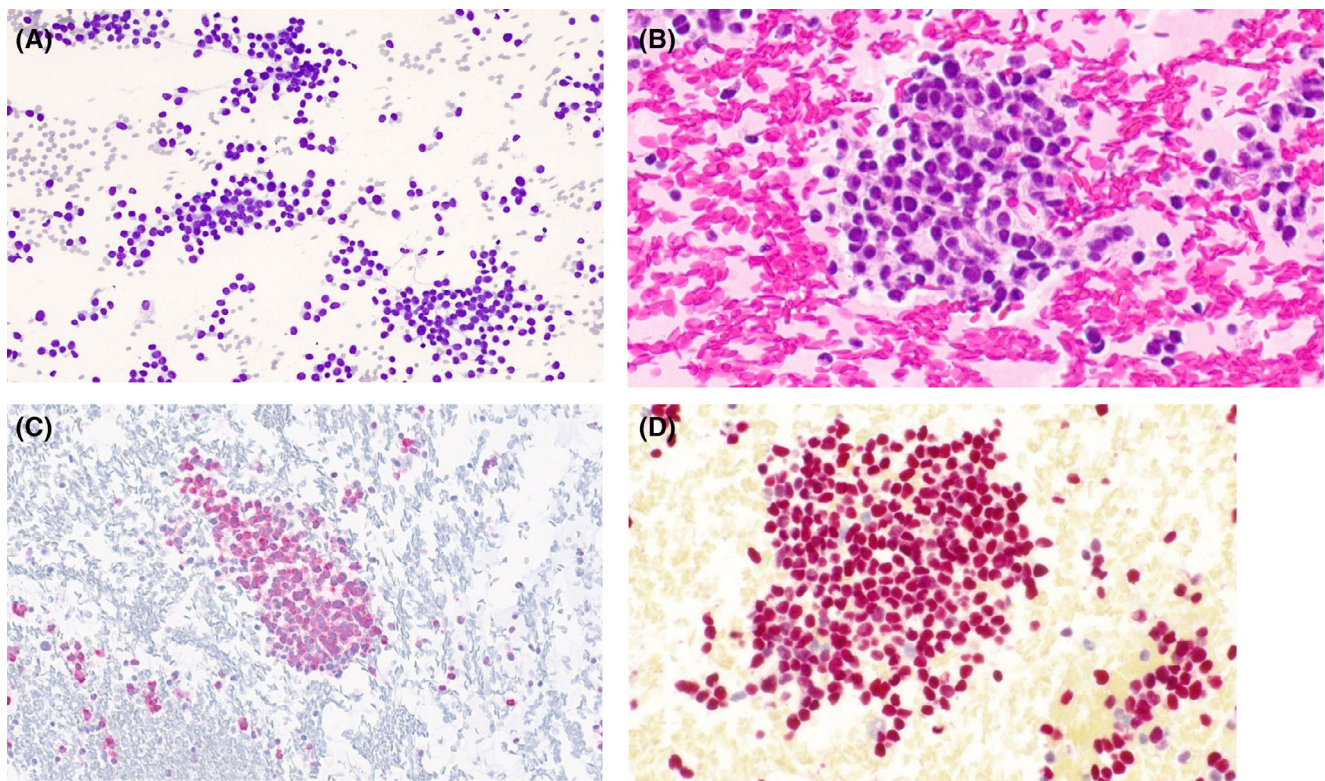
**FIGURE 1** Case A (A) Cellular aspirate showing a dispersed population of small round blue cells with high nuclear: cytoplasmic ratio and a bloody background. Note the rosette-like or acinar arrangement in some of the cell clusters. (Diff-Quik stain, magnification ×200). (B) Cellblock showing a similar dispersed population of small round tumor cells with hyperchromatic nuclei (Hematoxylin and Eosin stain, magnification ×400) with diffuse cytoplasmic positivity for S-100 (C) and nuclear SOX-10 expression (D)

at ROSE, ancillary studies were required for definitive establishment of the diagnosis of SCM. A final diagnosis of melanoma with small cell features or small round cell morphology was given after confirmatory IHC performed in each case. All cases showed diffuse positivity for multiple melanocytic markers including SOX10 (3/3), Melan A (3/3), and HMB45 (2/2). S-100 was expressed in 1/2 cases. Neuroendocrine markers were equivocal where CD56 was interpreted to be positive in 2/2 cases examined, while synatophysin was weakly expressed in 1/2 cases and chromogranin was expressed in 0/2 cases. TTF-1 was negative in both cases where this stain was performed (0/2). Pancytokeratin was not expressed in any case (0/3). An extended panel of IHC markers used in working up the differential diagnoses of SRBCTs was also performed including CD99 (1/2), desmin (0/2), myogenin (0/2), WT1 (0/2), and CD45 (0/2).

Fluorescent in situ hybridization studies were performed for Case A and were negative for SYT and EWSR1 translocation. Molecular testing performed on the cellblock material showed a NRAS p. Q61R mutation (Case A) and BRAF c1799T > A V600E (Case C). Molecular studies were not performed for Case B.

## 3.4 | Patient outcomes

None of the patient had follow-up surgical resection due to presence of metastatic disease.

*Case* A. This patient underwent an endoscopic ultrasound-guided FNA of their pancreatic mass 2 weeks later that showed similar cytology features of SCM. One-month later, the patient was found to have widespread metastatic disease involving bilateral thighs, liver, and lung. A primary site for this patient's MM was not established. This patient was alive at 8 months follow-up and being treated with a combination of anti-PD1and tyrosinase kinase inhibitor therapy.

*Case* B. This patient was unfortunately lost to follow-up.

*Case* C. This patient underwent treatment for metastatic melanoma with one cycle of ipilimumab plus nivolumab, before transitioning to palliative radiation. She died of MM after 6 months.

## 4 | DISCUSSION

SCM is a rare variant of MM with a vertical growth pattern that is characterized by sheets of small monomorphic cells with round to oval nuclei and high nuclear: cytoplasmic ratio, mimicking other SRBCT. This variant was first described in 1965 by Reed et al., who described
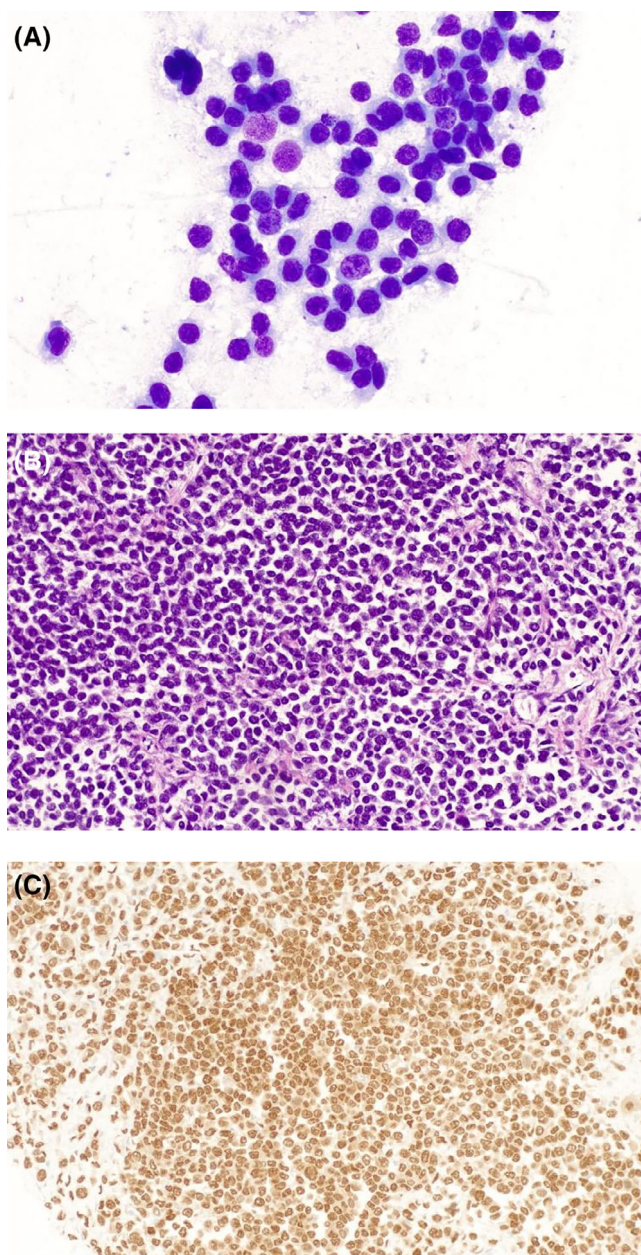
**FIGURE 2** Case B (A) Touch preparation showing a dispersed population of small round cells with minimal cytoplasm and a high nuclear: cytoplasmic ratio. Note the presence of occasional plasmacytoid cells. (Diff-Quik stain, magnification ×400). (B) Corresponding core biopsy showing SCM comprised of sheets of discohesive small round blue cells (Hematoxylin and Eosin stain, magnification ×400) and (C) showing diffuse nuclear positivity for SOX-10. SCM, small cell melanoma

12 cases of MM with undifferentiated lymphoblastic morphology arising from giant congenital nevi.[11] In children, SCM are believed to arise from either congenital nevi or de novo.[12] In adults, the pathogenesis of this variant is largely unknown. SCM affects the trunk area and has been described at both mucosal and cutaneous sites as well.[13-16] In addition, a few case reports have described individual cases of metastatic SCM involving visceral sites such as the ovary, stomach, and pleura.[17-20]

The features of SCM have been infrequently described in the cytology literature. To the best of our knowledge, there is only one case report in the cytology literature specifically describing a case of metastatic SCM, which presented as multicentric disease involving the anterior mediastinum, lung, and pleura.[10] An FNA of the anterior mediastinal mass showed discohesive small round cells with aberrant expression of CD43. Based this finding this case was initially mistaken for lymphoma on cytology. That patient required an open lung biopsy procedure for a definitive diagnosis. Only a few other cytology studies have described some degree of small cell morphology in metastatic MM cases.[5,21-23]

The differential diagnosis for SCM is similar to that of other SRBCTs encountered in cytologic preparations, including lymphoma, alveolar rhabdomyosarcoma, Ewing sarcoma, high-grade synovial sarcoma, and neuroblastoma in pediatric to adolescent patients, and high-grade malignant peripheral nerve sheath tumor, Ewing-like sarcomas (*CIC-DUX4*, *BCOR* associated sarcomas), small cell neuroendocrine carcinoma, Merkel cell carcinoma, and germ cell tumors in addition to others in adults. That being said, a complete discussion of cytologic differential of SRBCTs is beyond the scope of this article. Table 3 summarizes an IHC panel that can be useful for working up a suspected SCM case.[24] Based on the anatomic location, the differential diagnosis for our cases included metastatic pancreatic neuroendocrine tumor (NET) (Case A), metastatic carcinoma versus lymphoma (Case B), and small cell neuroendocrine carcinoma (Case C), as well as other mesenchymal SRBCTs (e.g., Ewing sarcoma). IHC performed on cellblock material was crucial for establishing the correct diagnoses. For each case, tumor cells expressed at least two melanocytic biomarkers including SOX10 and Melan A in all three cases. Since the patient in Case A had a prior history of pancreatic mass and a left lower quadrant abdominal mass showing small round cells with prominent acinar-like or rosette-like architecture and rare plasmacytoid cells to exclude a NET a panel of neuroendocrine markers was performed. The tumor cells in this case were diffusely positive for CD56, and rare cells were weakly positive for synaptophysin, but were completely negative for chromogranin. For Case B, lymphoid IHC markers were negative. In Case C, the given the tumor location was lung and the patient had a remote history of smoking, this raised the possibility of small cell carcinoma. However, for this Case C the tumor cells were all negative by IHC for pan-cytokeratin, TTF-1, synaptophysin, and chromogranin.

None of the three cases showed classic cytologic features diagnostic of a conventional melanoma, such as dispersed polygonal to epithelioid cells with eccentric nuclei, conspicuous macro-nucleoli, intranuclear pseudo-inclusions, intracellular cytoplasmic melanin, binucleated and/or multinucleated cells, or associated melanophages. Case A did show a rare intranuclear inclusion. A peritheliomatous growth pattern,[25] a reported cytologic clue to metastatic melanomas was identified in two cases. The most common differential diagnosis in prior cytology studies of SCM was lymphoma. However, lymphoglandular bodies were not present in any of the cases examined. Molecular studies performed on cytology material in our case series showed that BRAF c1799T > A V600E mutation (Case C) and
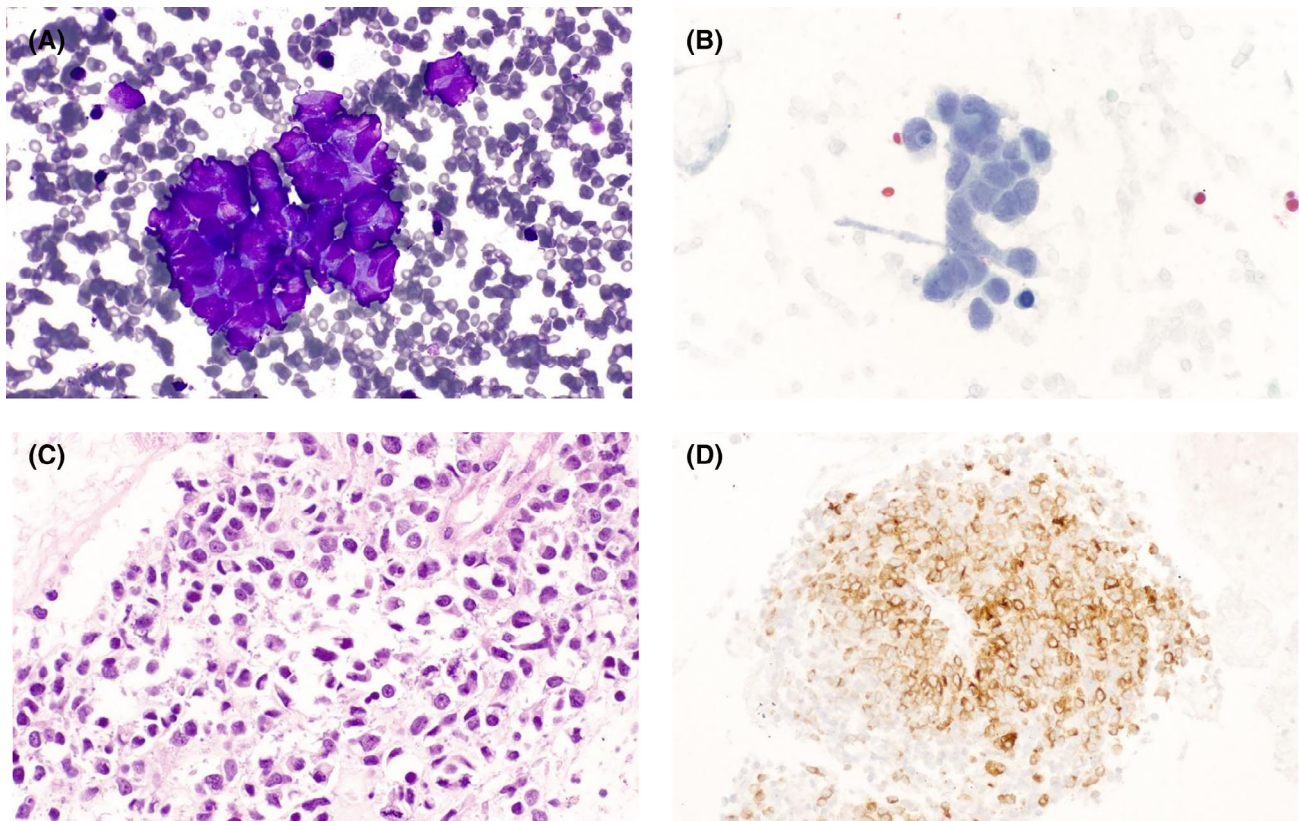
**FIGURE 3** Case C (A) FNA aspirate showing a cohesive cluster of medium-sized tumor cells with scant to moderate cytoplasm, occasional cytoplasmic vacuoles, and an eccentric to central nucleus. (Diff-Quik stain, magnification ×400). (B) Note the smooth nuclear contours of tumor cells with prominent molding (Papanicolaou stain, magnification ×400). (C) Cellblock section showing a dispersed population of round to epithelioid melanoma cells (Hematoxylin and Eosin stain, magnification ×400) with diffuse cytoplasmic positivity for Melan-A (D). FNA, fine-needle aspiration

**TABLE 3** Immunohistochemistry work-up in the differential diagnosis small cell melanoma

| | Cytokeratin and/ or epithelial membrane antigen | CD-45 | SOX-10 | CD-99 | Neuroendocrine markers | Desmin | WT-1 | Other |
|---|---|---|---|---|---|---|---|---|
| Small cell melanoma | − | − | + | − | −/+ | − | − | HMB-45, S-100, MART-1, Tyrosinase |
| Lymphoma | − | + | − | +/− | − | − | − | B or T-cell markers |
| Small cell neuroendocrine carcinoma | + | − | − | − | + | − | − | RB loss, p53 overexpression, TTF-1 |
| Merkel cell carcinoma | + (dot-like) | − | − | − | + | − | − | Neurofilament+, Merkel cell polyomavirus antibody |
| Small cell osteosarcoma | − | − | − | +/− | − | − | − | SATB2, osteocalcin |
| Mesenchymal chondrosarcoma | − | − | − | + | − | − | − | SOX-9, S-100 (chondrocytes only) |
| Solid alveolar rhabdomyosarcoma | − | − | − | − | +/− | + | − | Myogenin, MyoD1 |
| Desmoplastic small round cell tumor | + | − | − | − (few +) | − | + (dot-like) | + | − |
| Ewing sarcoma | − (rare+) | − | − | + (diffuse membranous) | +/− | − | − | NKX2.2 |
| *CIC*-rearranged sarcoma | − (rare+) | − | − | −/+ (20%) | − | − | + | ETV4 |

**TABLE 3** (Continued)

| | Cytokeratin and/or epithelial membrane antigen | CD-45 | SOX-10 | CD-99 | Neuroendocrine markers | Desmin | WT-1 | Other |
|---|---|---|---|---|---|---|---|---|
| *BCOR*-rearranged sarcoma | − (rare+) | − | − | +/− | − | − | − | BCOR, TLE, SATB2 |
| Neuroblastoma | − | − | − | − | + (synaptophysin) | − | − | PHOX2B |
| Blastemal predominant Wilm's tumor | + | − | − | − | − | + | + | |

NRAS p.Q61R mutation (Case A) were present, two of the most common molecular alterations reported in conventional MM.[26] The patient in Case A was treated with targeted therapy using a combination of anti-PD1 and a tyrosine kinase inhibitor. There are too few cases in this limited series to comment on the biologic behavior of these SCMs.

Literature review shows conflicting evidence in terms of prognosis of SCM. Barnhill et al. concluded that a melanoma showing a vertical growth pattern and small cell morphology was an independent poor prognostic factor in their study of pediatric melanomas, whereas Karkham et al. found a similar prognosis to conventional MM.[27,28] A study by Cuellar FA et al. demonstrated that SCM morphology is more likely to be associated with sentinel node involvement.[29]

In conclusion, diagnosing metastatic SCM at unusual sites by cytology is a challenging task, especially in patients without a prior history of melanoma. In our case series, two of the three patients presented with metastatic disease at the time of diagnosis and an unknown primary. Our study highlights the cytomorphology of SCM that differs from conventional MM in many aspects. This rare variant of melanoma is characterized by small round blue cells lacking intracytoplasmic melanin, macronucleoli, binucleation, and multinucleation. Previously undescribed cytologic features outlined herein is a prominent acinar-like or rosette like morphology, mimicking NETs in two of our cases. In addition, SCM can show nuclear molding mimicking small cell neuroendocrine carcinoma. Achieving a correct cytologic diagnosis of SCM requires an integrated clinicopathologic approach utilizing extensive histologic analysis, carefully selected ancillary studies, such as IHC and molecular diagnostics and a high index of suspicion.

## AUTHOR CONTRIBUTION

All authors were equally involved in the conceptualization, review, writing and editing of this manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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