Cytologic Diagnosis of Metastatic Melanoma by FNA: A Practical Review

Andrea Ronchi, MD[1]; Marco Montella, MD[1]; Federica Zito Marino, BD, PhD[1]; Giuseppe Argenziano, MD, PhD[2]; Elvira Moscarella, MD[2]; Gabriella Brancaccio, MD[2]; Giuseppe Ferraro, MD[3]; Giovanni Francesco Nicoletti, MD[3]; Teresa Troiani, MD[4]; Renato Franco, MD, PhD [1]; and Immacolata Cozzolino, MD, PhD[1]

Malignant melanoma (MM) is a highly aggressive neoplasm with a growing worldwide incidence. It is not uncommon that the disease is already metastatic at the time of the first diagnosis. Regional lymph nodes and skin are the first and most common metastatic sites, followed by distant visceral sites (lungs, liver, and central nervous system) and bone. In this clinical setting, fine-needle aspiration (FNA) often represents the first diagnostic approach. FNA is a useful tool to obtain a rapid and accurate diagnosis, in conjunction with ancillary techniques and molecular analysis, as recommended by recent guidelines. The aim of this review was to describe the cytomorphology, immunocytochemical tools, and molecular tools used for the diagnosis of MM metastases on FNA. ***Cancer Cytopathol* 2022;130:18-29**.

KEY WORDS: *BRAF*; fine-needle aspiration; immunocytochemistry; melanoma; sentinel node.

INTRODUCTION

Malignant melanoma (MM) is a highly aggressive neoplasm with a high rate of metastasis.[1] In addition to its prevalence in adults, the disease also affects younger patients, and the general worldwide incidence of MM has increased 8-fold among young women and 4-fold among young men over the past 40 years,[1] with an average age at diagnosis of 57 years.[2] The mean age at diagnosis varies according to sex because there is a female preponderance in young patients (male-to-female ratio, 4:10 in patients aged 20-24 years) and a male preponderance in patients aged >55 years (male-to-female ratio, 16:10 in patients aged >55 years).[3] Despite the improved awareness of the risk factors for prevention of MM and the development of targeted and immune therapies, the mortality attributable to MM remains high, and metastases are relatively frequent.[4] Regional lymph nodes (LNs) and skin are the first and the most common metastatic sites, followed by distant visceral sites (lungs, liver, and central nervous system), and bones.[5] Currently, the therapeutic options for patients with stage III (clinically positive LNs or clinical satellite/in-transit metastases) and stage IV (distant metastases) MM include surgery and/or systemic therapy.[6,7] Moreover, the choice of systemic therapy depends on the presence of BRAF-activating mutations.[6] In these clinical settings, fine-needle aspiration (FNA) is a useful tool to obtain neoplastic cells for rapid and accurate diagnosis and for molecular evaluations as recommended

Corresponding Author: Renato Franco, MD, PhD, Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, Università degli Studi della Campania "Luigi Vanvitelli," via Luciano Armanni 5, 80138, Naples, Italy (renato.franco@unicampania.it).

[1]Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy; [2]Dermatology Unit, Department of Mental and Physical Health and Preventive Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy; [3]Plastic Surgery Unit, Multidisciplinary Department of Medical, Surgical, and Odontological Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy; [4]Oncology Unit, Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy

Received: May 6, 2021; **Accepted:** June 2, 2021

Published online July 26, 2021 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cncy.22488, wileyonlinelibrary.com

by relevant guidelines.[6] FNA offers several advantages: it offers the possibility of repeat sampling even for anatomic sites not easily biopsied by other sampling techniques. Furthermore, cytopathologists or cytotechnicians can perform rapid on-site evaluation (ROSE) to ensure adequate and representative sampling of the neoplasm and proper triage of the cytologic samples, allowing the use of ancillary techniques.[8]

The objective of this review was to describe the morphologic, immunocytochemical, and molecular tools for the diagnosis of MM metastases using FNA.

FNA IN THE DIAGNOSIS OF METASTATIC MM

Evidence for the diagnostic performance of FNA in the evaluation of metastatic MM has accumulated over recent decades, FNA is highly effective and accurate, with reported sensitivity and specificity ranging between 86.5% and 100%[8-15] and 92.3% and 100%, respectively.[9-17] The false positive (FP) rate ranges from 0.6% to 2%,[10-11,13,14,17] whereas the reported false negative (FN) range is from 0% to 8%.[10-17] A summary of data pertaining to the diagnostic performance of FNA in the diagnosis of metastatic MM is provided in Table 1.[10-17]

One of the most frequent causes of an FP FNA diagnosis is the misinterpretation of macrophages with eccentric nuclei, distinct nucleoli, and dispersed, granular chromatin or of fibroblasts with plump nuclei in benign lesions as MM cells. This occurs more often in hypocellular FNA samples,[10] A good practice in these situations is to classify such cases as inconclusive, with a recommendation for repeat FNA or tissue sampling such as core biopsy for further evaluation. Some data suggest that the diagnostic performance of FNA also depends on the experience of the operator, with an experienced operator defined as an operator who performs at least 100 FNAs per year.[18] Rodrigues et al correlated the diagnostic accuracy of FNA with the operator's experience, showing that the diagnostic performance of FNA is higher if the procedure is performed by experienced operators.[15] Furthermore, high levels of operator experience also correlated with lower rates of indeterminate FNA diagnoses and nondiagnostic samples. Those authors demonstrated high diagnostic FNA accuracy among experienced operators, with sensitivity, specificity, positive predictive value, FP rates, and FN rates of 100%, 92.3%, 99%, 1%, and 0%, respectively.[15]

TABLE 1. Diagnostic Performance of Cytology for Detecting Metastatic Malignant Melanoma

Reference	No. of Cases	Palpable/Guided	Site	Performer	ROSE	ICC (Technical Support)	SE, %	SP, %	PPV, %	FP, %	FN, %
Basler 1997[14]	56	Palpable	LN	NS	Yes	No	100.0	100.0	100.0	0.0	0.0
Cangiarella 2000[12]	115	Palpable	LN	Cytopathologist	Yes	Yes (air-dried smears)	97.2	100.0	100.0	0.0	2.7
Dalle 2006[16]	120	US	LN	Radiologist	NS	No	98.2	96.1	96.5	2.0	1.0
Hafstrom 1980[13]	87	Palpable	Variable	Cytopathologist	Yes	No	94.0	100.0	100.0	0.0	6.0
Murali 2007[11]	2204	Both	Variable	Cytopathologist, radiologist	Yes	Yes (cytospins/CB)	92.1	99.2	99.4	0.6	6.7
Perry 1986[10]	298	NS	Variable	NS	NS	No	86.5	95.6	98.0	2.0	8.0
Rodrigues 2000[15]	99	Both	Variable	Cytopathologist	Yes	Yes (CB)	100.0	92.3	99.0	1.0	0.0
Voit 2000[17]	739	Both	Variable	NS	No	No	98.0	100.0	100.0	0.0	2.0

Abbreviations: CB, cell block; FN, false negative; FP, false positive; ICC, immunocytochemistry; LN, lymph node; NS, not specified; PPV, positive predictive value; ROSE, rapid on-site evaluation; SE, sensitivity; SP, specificity; US, ultrasound.

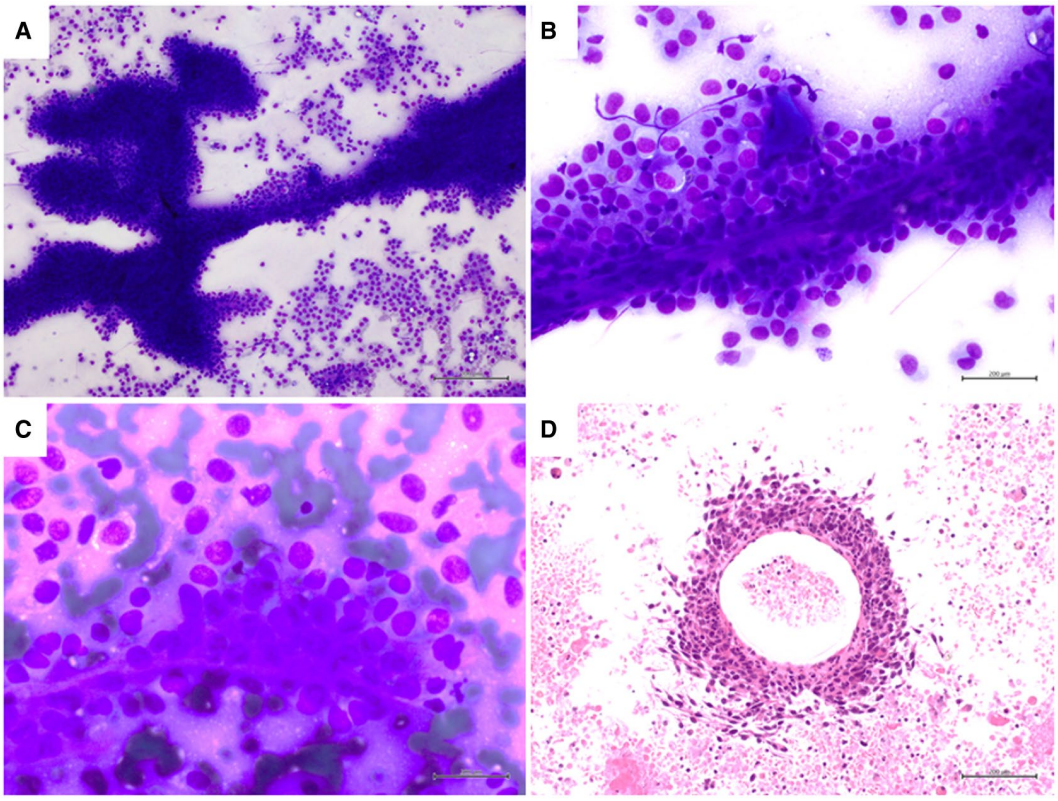

Figure 1. Fine-needle aspiration smears of metastatic melanoma are shown. (A) A direct smear shows a branching blood vessel surrounded by numerous melanoma cells. Several diagnostic, dispersed malignant cells are present in the background (May-Grunwald-Giemsa stain, original magnification ×100). (B,C) Viable melanoma cells are shown closely clinging to the blood vessel wall (May-Grunwald-Giemsa stain; original magnification ×400 in B, ×600 in C). (D) A cell-block section shows numerous melanoma cells surrounding a central blood vessel (H&E stain, original magnification ×200).

Interestingly, the only FP FNA case reported in that study involved subcutaneous localization of squamous cell carcinoma with unusual cytologic features, which was misdiagnosed as MM based on cytomorphology alone. This case highlights the importance of confirmatory immunocytochemistry (ICC) in the diagnosis of MM. Basler et al retrospectively evaluated 56 FNA procedures performed on palpable LNs with metastatic disease and reported that both sensitivity and specificity were 100%.[14] However, FNA is technically easier in cases of palpable LNs because the metastases are generally large when the LN is clinically palpable. In both studies, the use of ROSE at the time of collection positively influenced the diagnostic adequacy and accuracy. Perry et al retrospectively evaluated a series of 298 FNA procedures performed in patients with suspected metastatic MM and reported a specificity of 86.5%, an FN rate of 8%, and an FP rate of 2%.[10] These data show that diagnostic accuracy may be affected more by the quality of the FNA sample than by interpretation errors.[11,13]

BASIC CONCEPTS

An FNA diagnosis of metastatic MM can be challenging, and the differential diagnosis may include a broad range of lesions.[11] The cellularity of smears is usually moderate to high, but cellularity may be markedly reduced in cases with desmoplastic variants and small metastases. The cellular population is organized as single cells, occasional aggregates of a few cells, loose clusters, or 3-dimensional groups with a peritheliomatous pattern. The peritheliomatous pattern is a histologic feature characterized by neoplastic cells closely surrounding a central blood vessel, which has recently been described in MM and in other neoplasms[19] (Fig. 1). The discohesive pattern is mainly seen in FNA cases of MM with an epithelioid or plasmacytoid morphology, whereas syncytial or

3-dimensional groups and fragments are more frequently observed in cases with spindle or mixed morphology.[20] Epithelioid, plasmacytoid, and spindle cells are the most frequently encountered cell morphologies. Other rare cytomorphologic variants of MM include small, rhabdoid, signet-ring, myxoid, and balloon cells. MMs with an epithelioid cytomorphology represent >70% of all cases and are characterized by the presence of cells with a polygonal shape, moderate-to-abundant granular or clear cytoplasm, indistinct cytoplasmatic borders, and mildly or moderately hyperchromatic large nuclei with granular and clumped chromatin[10,21] (Fig. 2A). Plasmacytoid cells show eccentric nuclei, thickened and irregular nuclear membranes, basophilic cytoplasm, clearly defined borders, prominent and usually a single nucleolus, and frequent nuclear pseudoinclusions (Fig. 2B). Spindle cells have a basophilic and elongated cytoplasm with bipolar tapering cytoplasmic ends and enlarged, centrally located, and spindle-shaped nuclei with membrane indentations and small nucleoli (Fig. 2C). Small cells are characterized by scant basophilic cytoplasm and round or oval, eccentrically placed nuclei (Fig. 2D). Pleomorphic and multinucleated cells are also frequent findings (Fig. 2E). Mitotic figures are usually readily identified. Cytoplasmic vacuoles are often observed in air-dried smears and are a characteristic cytoplasmic feature of melanoma cells.[22] The vacuolated cells may be considered intermediate forms between nonvacuolated cells and hypervacuolated balloon cells.[21] Cytoplasmic melanin pigment appears yellow-brown with Papanicolaou stain and brown-black with May-Grunwald-Giemsa stain. Although the occurrence of the melanin pigment has been reported to vary across different series, overall, it is observed in <50% of cases.[10,21,23] Melanin pigmentation may sometimes be observed macroscopically in the aspirated material (Fig. 3). When present in the appropriate morphologic background, melanin pigmentation is an important cytomorphologic clue for the diagnosis of MM. However,

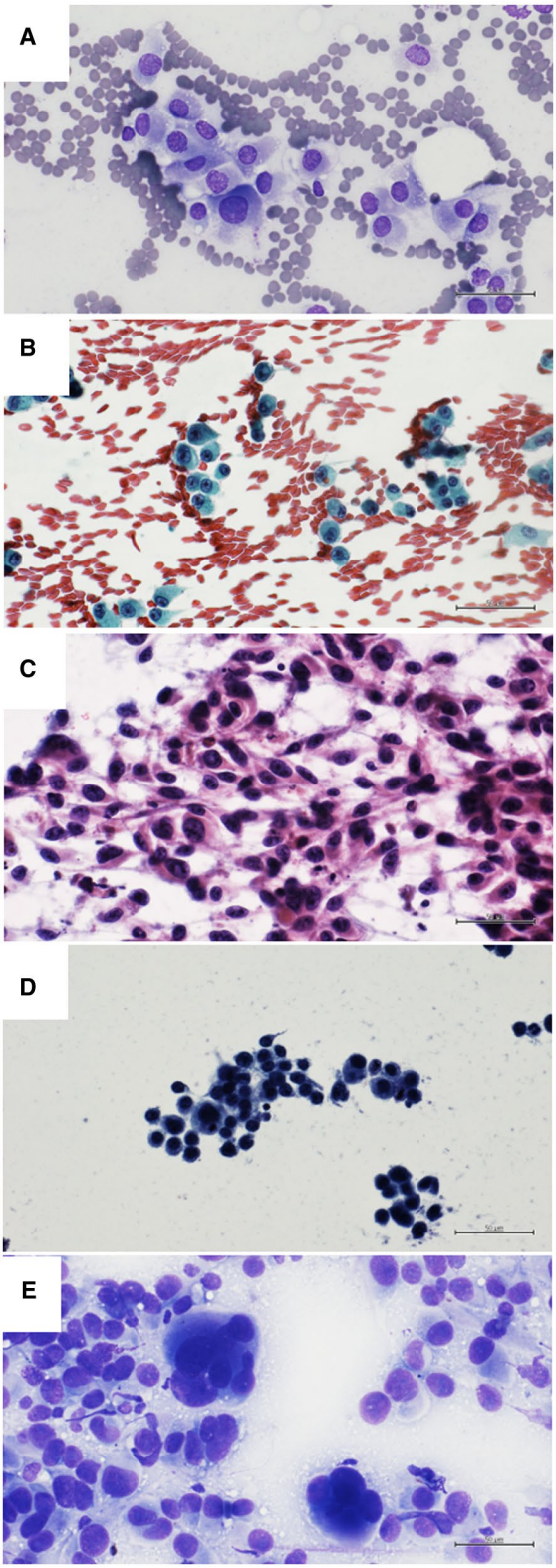

Figure 2. Morphologic features of malignant melanoma metastasis on direct smears are shown, including (A) large epithelioid cells with round-to-oval nuclei and abundant, sometimes microvacuolized cytoplasm (May-Grunwald-Giemsa stain, original magnification ×400); (B) discohesive plasmocytoid cells with round nuclei and dense cytoplasm, well defined borders, and evident nucleoli (Papanicolaou stain, original magnification ×400); (C) numerous spindle-shaped cells with oval-to-fusiform nuclei and evident cytoplasmatic projections (Papanicolaou stain, original magnification ×400); (D) a small cell component with inconspicuous nucleoli and scarce cytoplasm mixed with a larger cellular component (Papanicolaou stain, original magnification ×400); and (E) malignant multinucleated cells mixed with an epithelioid and plasmocytoid cell population (May-Grunwald-Giemsa stain, original magnification ×400).

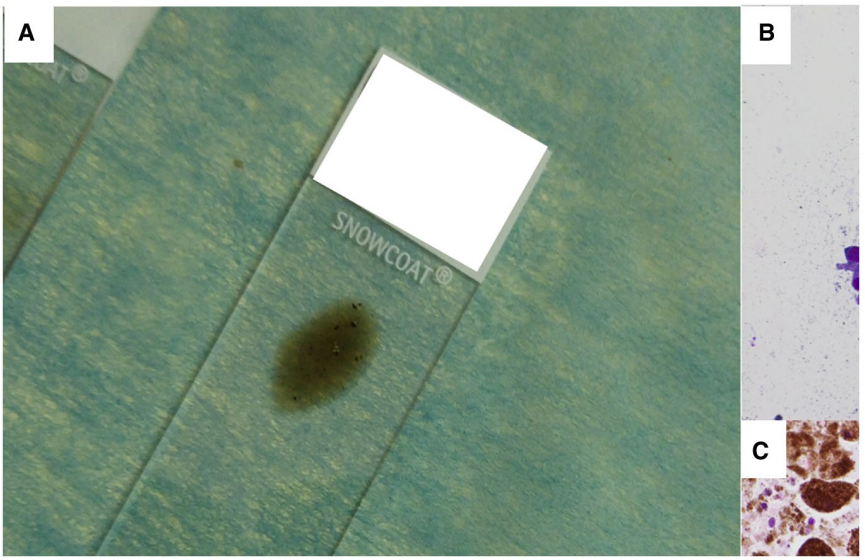

Figure 3. Melanin pigment is shown in a cytologic sample of malignant melanoma. (A) Melanin pigment may be macroscopically evident on the slide, particularly when abundant. The pigment is abundant in (B) the corresponding smear (May-Grunwald-Giemsa stain, original magnification ×400) and (C) cell-block section (H&E stain, original magnification 400x). Some epithelioid cells are recognized in both cytopreparations, allowing the diagnosis.

macrophages may engulf melanin or melanin-like pigments even in the absence of MM, mainly in samples aspirated from the pulmonary hilum in mediastinal LNs or from dermatopathic lymphadenopathies. Attention must be paid to distinguish melanin from other pigments, such as hemosiderin and lipofuscin. Pigmentation can also be observed in the background, which is usually hemorrhagic and, in some cases (usually <20%) contains necrosis.[10,24] In our experience, necrosis was observed in approximately 4% (3.85%) of MM FNA cases.

Targeted therapy and immunotherapy are known to have the potential to induce significant modifications in tumor masses, which are characterized by regression of the neoplastic cells, accumulation of macrophages, and an inflammatory infiltrate.[25] These posttherapy changes may cause an FP diagnosis of MM in FNA examinations. FNA of these masses after targeted therapy shows aggregates of melanophages, coarse extracellular melanin pigment deposition, and foci without a defined population of MM cells. The absence of reactivity in a panel of melanocyte immunocytochemical markers can be a useful finding in the evaluation of these samples.

The epithelioid pattern of MM is a potential diagnostic pitfall because it can be confused with carcinoma. Although a dispersed cell pattern tends to indicate MM, cellular discohesion can also be seen in some carcinomas. The pleomorphic and spindle cell forms of MM involve a differential diagnosis that includes sarcoma as well as benign fibroblastic and other mesenchymal proliferations. Although dispersed spindle cells and the presence of melanin pigment are useful clues to the diagnosis of MM, ancillary studies would almost certainly need to be performed.[26] Aspirates of MM with a plasmacytoid morphology can be confused with certain other neoplasms, including lobular breast carcinoma, multiple myeloma, neuroendocrine neoplasms, and myoepithelial neoplasms of the salivary gland. For small cell forms of MM, in which the dispersed cell pattern combined with the small size of the cells can be a major diagnostic pitfall, non-Hodgkin lymphoma and neuroendocrine carcinoma are the main conditions identified in the differential diagnosis. Ultimately, in all potential cases of metastatic MM assessed by FNA, correlation with the clinical setting (sex, age, site of lesion), clinical history, and effective use of ancillary studies is mandatory.

ADVANCES IN THE CYTOLOGIC DIAGNOSIS OF MM

Immunocytochemical Findings

The cytomorphology of MM is remarkably heterogeneous; therefore, demonstration of melanocytic differentiation

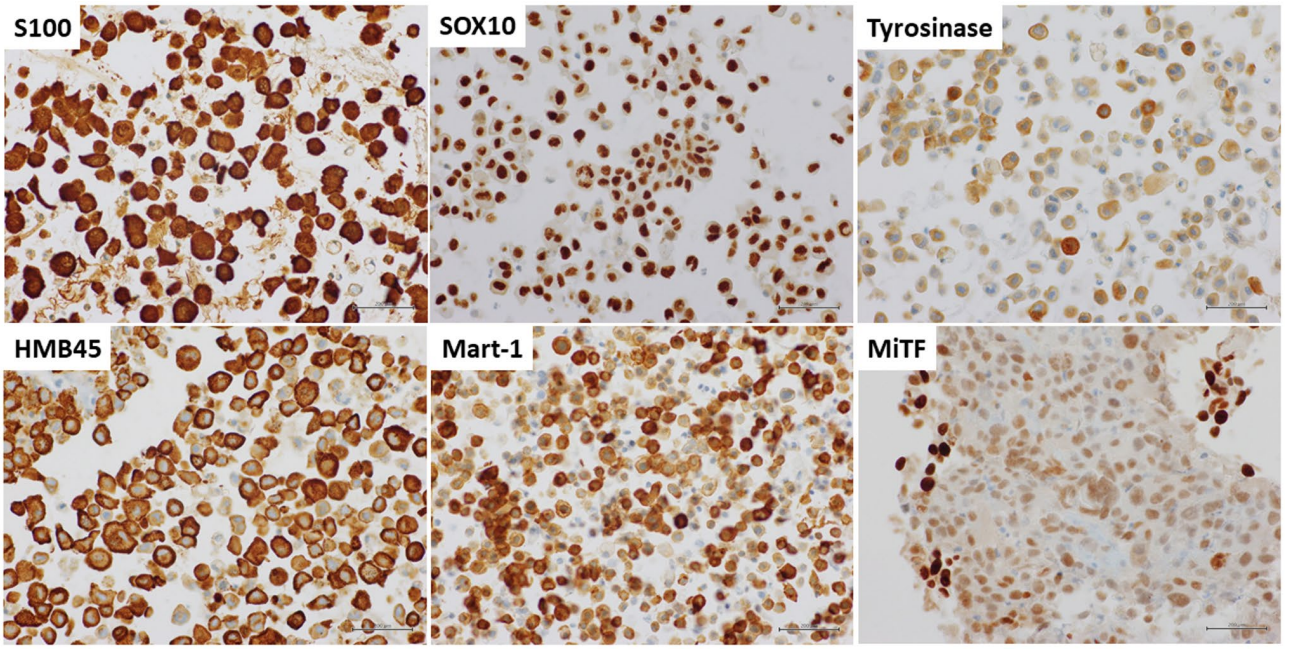

Figure 4. This is an overview of malignant melanoma immunocytochemistry on cell-block sections showing cytoplasmic and nuclear positivity for S100, nuclear positivity for Sry-related HMg-box gene 10 (SOX10), cytoplasmic positivity for tyrosinase, cytoplasmic positivity for the melanoma marker antibody HMB45, cytoplasmic positivity for melanoma-associated antigen recognized by T cells (MART-1), and nuclear positivity for microphthalmia transcription factor (MiTF) (immunocytochemical stains, original magnification ×400).

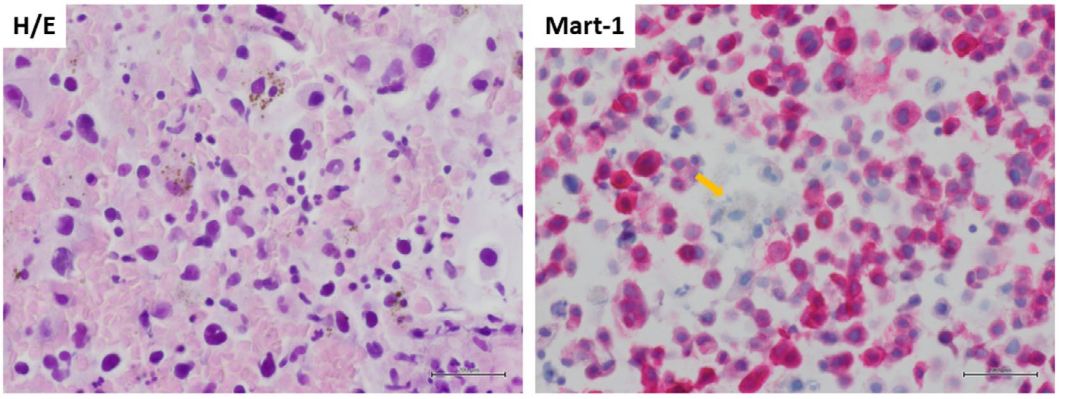

Figure 5. The interpretation of immunocytochemistry may be challenging in pigmented cases because the melanin pigmentation may be misinterpreted as viable tumor. In this circumstance, Fast Red immunocytochemical staining may be useful. (*Left*) In this illustrative case, large cells containing melanin pigment in the cytoplasm are observed in the cell-block section (H&E stain, original magnification ×600). (*Right*) Immunocytochemical Melan-A evaluation with Fast Red shows positive staining in the neoplastic cells; note the negative histiocyte (yellow arrow; immunocytochemical stain, original magnification ×600).

by ICC is essential for a definitive diagnosis in most cases. In this setting, ROSE is particularly important to ensure that the aspirated material is adequate for both morphologic and immunocytochemical evaluations.[27] Moreover, a cell block (CB) is recommended because it allows for the preparation of multiple slides dedicated to different MM ICC markers (Fig. 4). In cases with abundant pigmentation, in which the melanin may make ICC interpretation difficult, Fast Red immunocytochemical staining may be helpful (Fig. 5).

The most widely used melanocytic markers include S100 (cytoplasmic and nuclear staining), Melan-A

(cytoplasmic staining), HMB45 (a melanoma marker antibody; cytoplasmic staining), and Sry-related HMg-box gene 10 (SOX10) (nuclear staining). Melanoma-associated antigen recognized by T cells (MART-1), HMB45, and tyrosinase are commercialized together as a *pan-melanoma cocktail*. Although S100 is classically considered the most sensitive marker and HMB45 is considered the most specific marker, few studies have evaluated the diagnostic performance of melanocytic markers for the diagnosis of metastatic MM in cytologic samples (Table 2).[28-39] More recently, SOX10 has emerged as a sensitive marker for melanocytic differentiation. Clevenger et al tested the diagnostic performance of a pan-melanoma cocktail and SOX10 on direct smears in a series of 50 MM cases, and SOX10 showed positive immunoreactivity in all cases, with a sensitivity of 100%, whereas the pan-melanoma cocktail showed a sensitivity of 86%.[29] We recently evaluated the yield of the most frequently used melanocytic markers (S100, MART-1, HMB45, and SOX10) on CB sections.[28] In our study, SOX10 and S100 were the most sensitive markers, with a sensitivity of 100%, which was higher than the sensitivity of MART-1 (sensitivity, 97%) and HMB45 (sensitivity, 95%).[28] Moreover, SOX10 appeared to be superior to all other melanocytic markers in terms of staining performance because, as a nuclear marker, it yielded easily interpretable pigmented neoplastic cells. Furthermore, unspecific background staining was sometimes observed in S100-stained sections.[28]

Almost all cytologic ICC studies evaluated the sensitivity, but not the specificity, of the markers. Consequently, data for the specificity of melanocytic markers have been obtained from histologic studies. Furthermore, different cytologic preparations have been used in different studies, including air-dried direct smears, alcohol-fixed direct smears, alcohol-fixed cytospins, destained alcohol-fixed slides, and CB sections. The details are provided in Table 2.

Although S100 and SOX10 are the most sensitive markers of melanocytic differentiation, they are not highly specific because they show reactivity in several other neoplasms of neuroectodermal origin. Indeed, S100 may be expressed by some carcinomas, some mesenchymal tumors, and Langerhans cell histiocytosis.[40-42] SOX10 is more specific than S100 as a melanocytic marker, but it is not entirely specific, because it shows positive findings in some mesenchymal tumors, diffuse astrocytomas, and pleomorphic undifferentiated sarcomas.[41,43-44] A differential diagnosis involving malignant nerve sheath tumor and clear cell sarcoma may be particularly challenging, because the immunophenotypes of these neoplasms may overlap with MM.

HMB45 is the most specific marker, but a subset of MM cases are negative for HMB45, mainly in metastatic settings, and the marker is not entirely specific.[45] Indeed, HMB45 may be expressed by clear cell sarcoma, some pigmented neuroectodermal tumors, perivascular epithelioid cell tumors, and some renal cell carcinomas.[46,47] Conversely, MM cells may aberrantly express nonmelanocytic markers. MM cells often express markers of mesenchymal phenotypes, including vimentin, and this could confuse the differential diagnosis with mesenchymal neoplasms.[39,48] The aberrant expression of cytokeratins is a well known event in MM, which complicates the differential diagnosis with poorly differentiated carcinomas.[31,49]

Predictive Evaluations on Cytologic Samples

In addition to morphologic and immunocytochemical evaluations for diagnostic purposes, additional ancillary testing may be needed for clinical management. Current therapy for patients with advanced MM often depends on the mutation status of the *BRAF* gene. BRAF inhibitors may be used as first-line therapy in patients who have *BRAF* mutations, which are present in >50% of MM cases.[50,51] Among the activating *BRAF* mutations, >90% are present in codon 600, and >90% of these are single-nucleotide mutations that result in the replacement of a valine residue with a glutamic acid residue (BRAF V600E GTG>GAG). The second most common mutation is BRAF V600K GTG>AAG, in which valine is replaced by lysine, which accounts for 5% to 6% of cases.[52]

Although molecular techniques like polymerase chain reaction and next-generation sequencing are used for evaluation of the mutational status of the *BRAF* gene, a mutation-specific antibody for the BRAF V600E mutation is also available for ICC evaluation (Fig. 6). Although this test has shown high diagnostic performance in terms of both sensitivity and specificity on histologic samples, there is limited information about its application to cytology specimens. Bernacki et al tested *BRAF* mutation status on DNA extracted from cells manually microdissected from the slides of 37 consecutive FNA cases and correlated the results with mutational data from the

TABLE 2. Literature Review of Immunocytochemistry Metastatic Malignant Melanoma Cytologic Samples

Reference	No. of Melanoma Cases	ICC Cytopreparation Type(s)	Diagnostic Performance of Immunocytochemical Markers							
			S100	HMB45	MART1	SOX10	Pan-Melanoma Cocktail	SOX10/Keratin Dual-Color	KBA.62	MiTF	Tyrosinase
Ronchi 2021[28]	38	Cell block	SE, 100%	SE, 95%	SE, 97%	SE, 100%					
Clevenger 2014[29]	50	Direct smears				SE, 100%	SE, 86%			SE, 90%	
Perrino 2015[30]	298	Destained, Papanicolaou-fixed, alcohol-fixed smears								SE, 95%	
Mito 2018[31]	34	Cell block						SE, 94%; SP, 95%			
Erdag 2013[32]	60	Acetone-fixed cytospins and cell block slides	SE, 86.7%	SE, 43.3%	SE, 56.7%				SE, 75%		
Hookim 2012[33]	17	Direct smears	SE, 100%	SE, 81%	SE, 88%						
Fetsch 1999[34]	207	NA		SE, 80%	SE, 90%						
Fetsch 2000[35]	62	Air-dried–fixed, acetone-fixed cytospins; cell block									SE, 61%; SE, 92%
Simmons & Martin 1991[36]	15	Cell block	SE, 33.3% (polyclonal antibody)	SE, 86.7%							
Nasiell 1991[37]	81	Cytospins	SE, 100%	SE, 80%							
Kapila 1991[38]	19	Ethanol-fixed smears	SE, 100%								
Domagała 1991[39]	14	NA	SE, 42.9%								

Abbreviations: HMB45, melanoma marker antibody; ICC, immunocytochemistry; Kba.62, melanoma marker monoclonal; MART1, melanoma-associated antigen recognized by T cells; MiTF, microphthalmia transcription factor; NA, not applicable; SE, sensitivity; SOX10, Sry-related HMg-box gene 10; SP, specificity.

corresponding excision samples. The molecular analysis was performed on Diff-Quik–stained FNA smears and tissue blocks, and *BRAF* mutation status was obtained in 92% of cases.[53] Similar results were obtained by Chen et al, which determined the *BRAF* mutation status in 93% of 30 FNA cases.[54]

In both studies, the concordance between molecular analyses performed on cytologic and histologic specimens was 100%. Although it is not possible to predict *BRAF* mutation status based on morphologic assessments in FNA specimens, some morphologic findings seem to be more frequent in *BRAF*-mutated MMs. A statistically significant association has been observed between spindle cell morphology and BRAF V600 mutations.[55]

Other potential biomarkers with prognostic and/or predictive utility include PD-L1, NRAS, and KIT. NRAS mutations are particularly common in sinonasal and vaginal MMs.[56,57] KIT and PDGFRA mutations occur in a significant subset of mucosal and acral MMs. In particular, KIT mutations may predict response to treatment with imatinib and may necessarily correlate with KIT copy number or CD117 immunocytochemical expression.[58,59] Inactivation of BRCA1-associated protein (BAP1) characterizes some MMs, which morphologically overlap with Spitz nevi and nevoid MM.[60,61]

DIAGNOSIS OF MM METASTASES IN PATIENTS WITH UNKNOWN PRIMARY MM

In 3% of cases, the primary site of the MM may be unknown (MUP). The first complete definition of MUP was established by Das Gupta in 1963.[62] It is defined by the presence of MM in the subcutaneous tissue or LN or with visceral localization in the absence of a known primary skin, ocular, or mucosal lesion.[62] There are 2 possible hypotheses used to explain the pathogenesis of MUP. The first hypothesis suggests that the primary neoplasm undergoes a process of regression under the influence of immune surveillance, whereas a clonal *motile* cellular component may cause metastatic dissemination.[63] The other hypothesis suggests that ectopic melanocytes located in the LN might undergo a malignant transformation leading to metastatic MM.[63] MUP is classified by the American Joint Committee on Cancer as stage III or IV, depending on whether it was initially diagnosed within an LN/subcutaneous tissue or visceral organ, respectively.[64] As for the diagnosis, a multidisciplinary

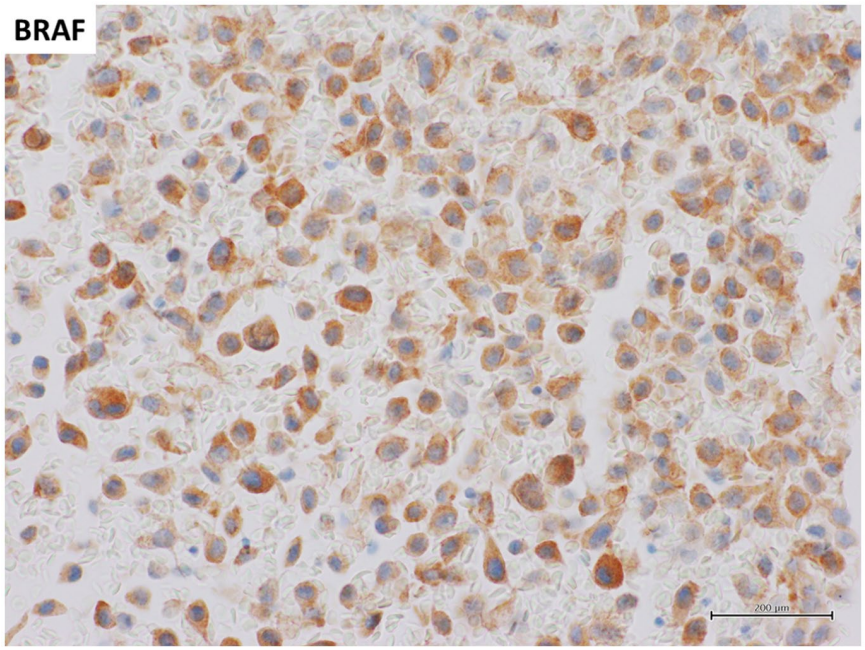

Figure 6. On BRAF immunocytochemistry, granular cytoplasmatic staining is observed in the neoplastic cells (immunocytochemical stain, original magnification ×400).

approach is recommended. The diagnosis of MUP is based on medical history, physical examination, dermatoscopy, cytologic/histologic examination, and imaging. The most common form of MUP is involvement of an LN, where up to 60% of all cases occur.[64] The involved LN site should guide exploration and indicate the possible primary site of origin. In addition, clinical evaluation should always include ophthalmologic and anogenital evaluations. The morphologic, immunophenotypic, and molecular findings are comparable to those observed in metastatic MM with a known primary lesion.[65]

THE ROLE OF FNA IN SENTINEL LN EVALUATION

Sentinel LN (SLN) evaluation is recommended for staging in all MMs that are pathologic stage ≥T1b.[66] There are conflicting opinions regarding the use of FNA in SLN evaluation, and the diagnostic performance of FNA in SLN evaluation is highly variable. Indeed, the reported sensitivity ranged from 4.7% to 82%, and specificity ranged from 72% and 99%.[67-71] The updated European Organization for Research and Treatment of Cancer protocol for pathologic evaluation of SLNs for MM provides for surgical excision of SLN and histologic examination.[72] Nevertheless, FNA may play a role in SLN evaluation in specific clinical settings. Indeed, FNA could be performed as a presurgical screening, especially in a subgroup of patients with larger MM metastases, so that patients with positive FNA results could be directly referred for LN dissection. This may be important, because SLN biopsy is a procedure with potential concomitant morbidity and an FN rate ranging between 9% and 21%.[73] Oude Ophuis et al evaluated a series of 1000 patients and proposed a step-wise approach for SLN of patients with MM based on ultrasound (US) and FNA.[74] In particular, among patients with suspected US and negative FNA findings, SNL biopsy is recommended to detect microscopic occult disease. Patients with negative US and FNA findings may only require follow-up assessments and US surveillance.[75]

SUMMARY

FNA may be applied to the diagnosis of metastatic MM in different clinical settings, including suspicious enlarged LNs or visceral metastases in patients with a known previous MM, metastases of unknown primary origin, or SLN evaluation. The diagnosis of MM may be challenging, mainly because of the heterogeneity of cytomorphologic findings and architectural patterns observed on smears. Therefore, knowledge of the morphologic clues and pitfalls is mandatory. ICC plays an important ancillary role

in confirming the diagnosis and in resolving the differential diagnosis, which can include a range of other neoplasms. Furthermore, cytologic samples may be used to evaluate BRAF mutations for predictive purposes in cases of tumor progression. Because the amount of neoplastic cells is limited in cytologic samples, cytologists have to ensure optimal triage of the sample to allow morphologic evaluation and, eventually, ICC and predictive molecular tests.

FUNDING SUPPORT

No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Andrea Ronchi: Contributed to conceptual interpretation of the data, reviewed the international literature, and contributed to writing of the article. **Marco Montella:** Contributed to conceptual interpretation of the data and reviewed the international literature. **Federica Zito Marino:** Contributed to conceptual interpretation of the data and reviewed the international literature. **Giuseppe Argenziano:** Contributed to conceptual interpretation of the data and editing of the article. **Elvira Moscarella:** Contributed to conceptual interpretation of the data and reviewed the international literature. **Gabriella Brancaccio:** Contributed to conceptual interpretation of the data and reviewed the international literature. **Giuseppe Ferraro:** Contributed to conceptual interpretation of the data and editing of the article. **Giovanni Francesco Nicoletti:** Contributed to conceptual interpretation of the data and editing of the article. **Teresa Troiani:** Contributed to conceptual interpretation of the data and editing of the article. **Renato Franco:** Contributed to conceptual interpretation of the data and editing of the article. **Immacolata Cozzolino:** Contributed to conceptual interpretation of the data, reviewed the international literature, and contributed to writing of the article.

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