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Clinical Cytology in the Diagnosis and Management of Melanoma

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1. Introduction

Melanoma, an aggressive tumor of the melanocytes is more prevalent among the white-skinned individuals with the highest incidence reported from Australia and New Zealand (Elwood M,2002). Primary melanomas usually involve the skin, mucosa, retina and leptomeninges. They are known to metastasize to lymph nodes, lung, liver and virtually any part of the body (Basler et al., 1997; Saqi et al., 2002; Ustün et al., 2002; De Las Casas et al., 2004; Parwani et al., 2004; Mohr et al., 2009). Surgical excision with adequate margins is the treatment of choice for cutaneous melanoma. The prognosis of melanoma varies with the site and stage of the disease (Damala et al., 2004; Mohr et al., 2009). Advanced disease with local recurrence, or distant metastasis carries a grave prognosis (Saqi et al., 2004). A variety of imaging techniques such as the chest and abdominal computed tomography (CT) scan, magnetic resonance imaging (MRI) of the brain and positron emission tomography (PET) are used in the staging work-up of patients with cutaneous melanoma, chiefly, for evaluating the potential metastatic sites such as the lungs, lymph nodes, liver and brain. An imaging work-up is generally not recommended in patients with a <1.0 mm thick lesion, as the cure rate in such cases is >90%. It is also not essential in patients with primary melanoma thicker than 1.0 mm, wherein a sentinel lymph node biopsy (SLNB) serves as a better staging tool (Mohr et al., 2009).

Justifiably, histologic examination of the surgical biopsies plays a major role in the diagnosis of primary and metastatic melanomas. Nonetheless, role of non-invasive/ minimally invasive cytologic techniques has also been adequately addressed (Rai, 2007). The cytologic techniques vary with the nature and location of the lesion and these include fine needle aspiration cytology (FNAC), imprint cytology (IC), scrape cytology, as well as, the examination of exfoliative samples such as the serous body fluids, sputum, cerebrospinal fluid (CSF) and synovial fluid (Paridaens et al., 1992; Deshpande et al., 2001; Saqi et al., 2002; Ali et al., 2005). Of these, FNAC is widely employed and its primary role is to confirm the metastatic or recurrent melanoma lesions (Saqi et al., 2002; Mágori, 2003; Siddaraju et al., 2007). By cytologic means, various authors have been able to detect metastatic melanoma deposits in a wide variety of sites inclusive of adrenal, kidney, omentum, pancreas, parotid, soft tissue, spine, breast, thyroid and brain (Saqi et al., 2002; Artal et al., 2004; Hernandez et al., 2004; Gombos et al., 2004; Bozbora et al., 2005; Kung et al., 2009; Kaneko et al., 2009). Not infrequently, cytology is used for diagnosing primary melanoma as well (Layfield et al., 1993; Dey et al., 1996; Gupta et al., 2003; Mágori, 2003; Kotoulas et al., 2007). A significant

literature is also available with regard to the cytologic diagnosis of ocular melanomas (Paridaens et al., 1992; Kashyap et al., 2002; Augsburger et al., 2002; Char et al., 2006; Young et al., 2007, 2008; Solo et al., 2009). Ultrasound (US) or computed tomography (CT) guided FNAC is particularly valuable for visceral metastases. More recently, lymph node ultrasound (US) with fine needle aspiration cytology (FNAC) is considered a potential cost effective alternative for an SLNB (Voit et al., 2006; van Akkooi et al., 2010). Although, rare studies have reported very low sensitivity which is attributed to tiny and necrotic lesions (Voit et al., 1999; van Rijk et al., 2006); most authors have emphasized the importance of US-guided FNAC in the detection of sentinel lymph node metastasis (Voit et al., 1999, 2002, 2006 & 2010). With a positive cytology of the sentinel node, surgeons can proceed with the direct radical lymph node dissection in patients with melanoma. Significantly, attempts have also been made to apply molecular- based techniques on the material obtained by FNA, as well as on exfoliative samples (Angeletti et al., 2004; Kalogeraki 2006; Maat et al., 2007; Savoia et al., 2008; McCannel et al., 2010).

The present chapter deals chiefly with the role of cytology in the clinical management of melanoma patients. A careful evaluation of cytologic samples is of critical importance for a variety of reasons, going to be addressed subsequently in this chapter. Despite the considerable, current knowledge about the cytomorphology of melanoma, owing to its inherent histomorphologic variation; a cytologic diagnosis is often difficult, especially, when the lesions are encountered in unusual locations, or with amelanotic presentation. Apparently, such situations necessitate the need for ancillary tests like immunocytochemistry (ICC) and ultrastructural examination (Mágori, 2003; Rosai, 2005). The importance of clinical history need not be overemphasized when interpreting cytologic specimens; for example, a known history of melanoma can significantly contribute to a cytodiagnosis of recurrent and metastatic melanoma (Mágori, 2003).

2. Histopathology of melanoma

Melanoma is well-known for its widely varied histomorphologic pattern. The major histologic forms include superficial spreading melanoma, nodular melanoma, acral lentigenous melanoma and desmoplastic melanoma (Piris et al., 2010). The histologic patterns include pseudo-glandular, pseudo-papillary, peritheliomatous, hemangiopericytoma-like, trabecular and alveolar. The tumor may be associated with fibroblastic response, formation of metaplastic or neoplastic cartilage, osteoclast-like giant cells and pseudo-epitheliomatous hyperplasia of the overlying dermis (Rosai, 2005). Also described are the cases of psammomatous, myxoid, balloon cell and signet ring cell melanomas (Eckert et al., 1992; Mowat et al., 1994; Hitchcock et al., 1999; Monteagudo et al., 2001). Individual melanoma cells can be epithelioid, spindle shaped or extremely bizarre with their size ranging from that of a small lymphocyte to giant multinucleate forms. The cytoplasm may be eosinophilic, basophilic, foamy, rhabdoid, oncocytic, or completely clear. Melanin can be abundant or absent (amelanotic). Malignant melanoma is readily diagnosed by the presence of melanin granules. Although amelanotic melanoma can also contain a few melanin granules, it is often difficult to differentiate it from non-melanotic tumors (Rosai, 2005). Currently, the criteria considered for the histologic diagnosis of cutaneous melanoma include the asymmetry and poor circumscription of the lesion; predominance of single melanocytes; irregular confluent nests; suprabasal melanocytes; hair follicle involvement and absence of maturation; cytologic atypia; dermal lymphocytic infiltrate and necrosis.

However, the diagnostic process should not just involve the mechanical use of these histologic parameters; as a study by Urso et al. has clearly established that, one or more of these histologic features are often present in benign melanocytic nevi as well. Of these, absence of maturation, mitoses and necrosis are never encountered in benign nevi (Urso et al., 2005). The versatile histologic patterns and responses of melanoma are apparently reflected on the cytologic materials as well.

3. Cytologic evaluation of melanoma

3.1 Technical aspects

The most commonly used cytologic technique is the fine needle aspiration (FNA); however, exfoliative and imprint cytology are also used depending on the clinical situation (Paridaens et al., 1992; Deshpande et al., 2001; Saqi et al., 2002). The technique of FNAC is as per that described in any of the standard text books (Orell & Vielh, 1999). Orbital FNA is performed via transcleral, transcorneal or transvitreal approach using 25-30guage needle (Augsburger et al., 2002; Char et al., 2006; Young et al., 2007, 2008). For tiny and vascular lesions, non-aspiration technique is preferable (Solo et al., 2009). In case of suspected conjunctival melanoma, imprint cytology using cellulose acetate strips has been found to be very useful (Paridaens et al., 1992). Imprint cytology of sentinel node can give a quicker and reliable result (Badgwell et al., 2011). Touch imprints taken from the edge of the primary cutaneous/ mucosal ulcerative lesion can often assist in distinguishing a carcinoma from melanoma. Figure 1 is that of a touch imprint taken from the ulcer edge in one of our cases of melanoma.

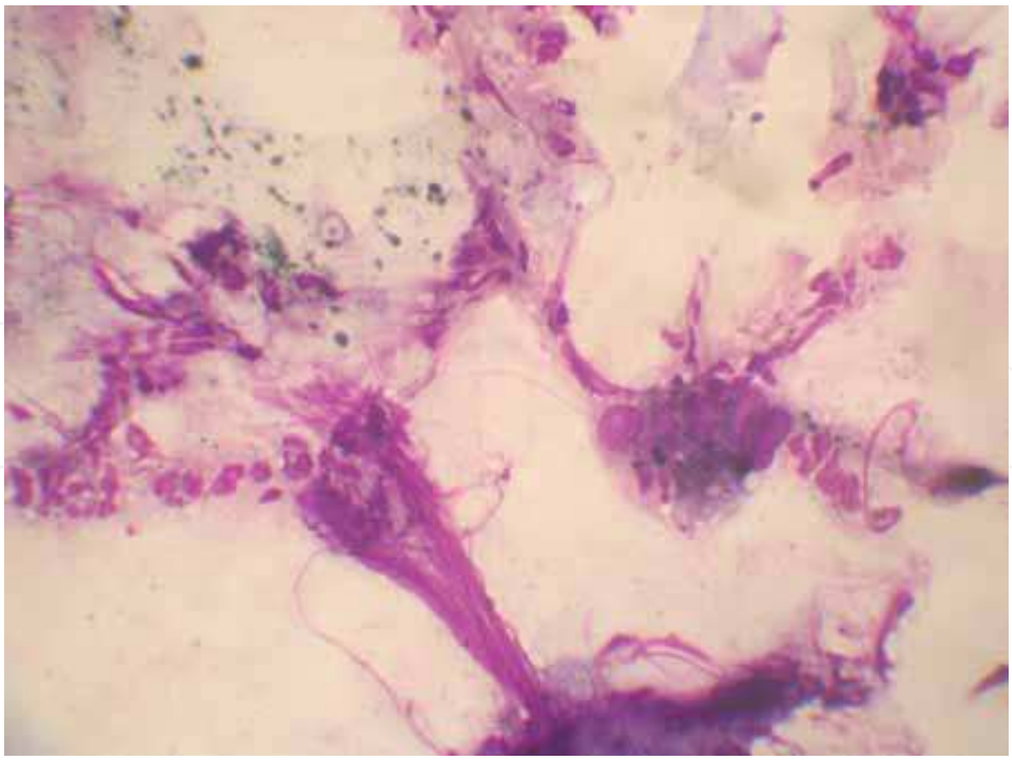

Fig. 1. An imprint taken from the ulcer edge showing pigmented cells; a few characteristic extracellular melanin granules are also appreciated (MGG stain, X400)

3.2 Naked eye examination of cytologic samples

Gross nature of the cytologic material in cases of pigmented melanoma is usually blackish and often fluid-like (Artal et al., 2004). With respect to melanotic melanomas, this is our experience as well. Rare cases of metastatic melanoma manifesting with blackish pleural effusion have been documented. Naked eye examination of such fluid material should serve as a 'clue' to the metastatic involvement of the thorax by melanoma. However, clinicians, as well as, the pathologists should be aware of the fact that a blackish effusion is often related to bacterial and fungal infections, or to hemorrhage (Liao et al., 2010).

3.3 Microscopic examination of cytologic samples

As for the microscopic picture, irrespective of primary, recurrent, metastatic, melanotic or amelanotic melanomas; a striking cytomorphologic feature documented by most authors is its high cell-yield with a predominant population of dissociated cells (Woyke et al., 1980; Perry et al., 1986; Nasiell et al., 1991 Deshpande et al., 2001; Saqi et al., 2002; De Las Casas et al., 2004; Parwani et al., 2004; Damala et al., 2004; Siddaraju et al., 2007). Marked cellularity and nuclear pleomorphism; with plasmacytoid (cells with eccentrically placed nuclei), polygonal or spindle cells; inclusion-like prominent nucleoli; intra-nuclear cytoplasmic inclusions; increased mitotic activity; and variable number of bi- and multinucleated cells are the other features frequently observed in melanoma (Woyke et al., 1980; Perry et al., 1986; Kapila et al., 1991; Layfield et al., 1993; Dey et al., 1996; Deshpande et al., 2001; Saqi et al., 2002; Kashyap et al., 2002; Gupta et al., 2003; Artal et al., 2004; Gombos et al., 2004; Siddaraju et al., 2007). Melanotic melanomas show a variable amount of pigment. Even the cases of amelanotic melanomas often exhibit coarse or fine pigment granules in rare or, a few cells which can be picked up on careful cytologic examination (Perry et al., 1986; Kapila et al., 1991; Deshpande et al., 2001). The cytologic diagnosis of amelanotic melanoma is challenging; because, in the absence of pigment, the tumor cells may mimic those of carcinoma or sarcoma in cytologic samples, particularly those obtained by fine needle aspiration. Irregular nuclear outline and coarse chromatin are the features common to any of the malignant lesions, which is also applicable for melanoma cells (Woyke et al., 1980; Layfileld et al., 1993; Deshpande et al., 2001).

Of the general cytomorphologic features elaborated above, intranuclear cytoplasmic inclusions (INCIs) are of diagnostic importance, only in conjunction with other cytomorphologic and clinical features. Their occurrence in papillary (PTC), medullary (MTC) and anaplastic thyroid carcinomas (ATC); bronchioloalveolar carcinoma (BAC), hepatocellular carcinoma (HCC) and renal cell carcinoma (RCC) is well known (Orell, Silverman, Leiman, 1999). Interestingly, we have also documented a rare case of embryonal rhabdomyosarcoma manifesting with frequent INCIs on FNAC (Kumar et al., 2009). Often, we have observed them as artifactual change in the cervicovaginal smears (perhaps due to air drying artefact) and in degenerated cells on FNAC. Uncommonly, they have also been noticed in certain benign lesions such as pigmented villonodular synovitis and inflammatory psedotumors (Gangane et al., 2003; Hosler et al., 2004). The table1 highlights some of the common, as well as, rare conditions that manifest with INCIs along with the clinical and cytologic features that assist in their distinction from each other. This aspect is of importance, particularly, while dealing with the metastatic lesions.

Serial no.	Condition	Incidence	Major cytologic / clinical features assisting in accurate diagnosis
1	Melanoma	Frequent	High cell-yield, dissociated pleomorphic cells, melanin pigment, inclusion-like nucleoli, intranuclear cytoplasmic inclusions (INCIs), expression of melanoma markers
2	Papillary thyroid carcinoma (PTC)	Frequent	Papillary pattern, metaplastic epithelial cells, nuclear grooves and other associated features
3	Medullary thyroid carcinoma (MTC)	Less frequent than PTC	Amyloid, cytoplasmic red granules
4	Anaplastic thyroid carcinoma (ATC)	Less frequent than PTC	Rapid clinical course, absent expression of melanoma markers
5	Bronchioloalveolar carcinoma (BAC)	Common	Prominent clustering, papillary and adeno pattern
6	Hepatocellular carcinoma (HCC)	Common	Sinusoidal pattern, cords of cells, naked nuclei, intracellular bile pigment (well differentiated), Elevated serum alpha-feto protein (AFP)
7	Renal cell carcinoma (RCC)	Common	Single cells, cohesive fragments, sheet-like pattern, finely vacuolated/ granular cytoplasm, bland nuclei in low grade RCC
8	Embryonal rhabdomyosarcoma (ERMS)	Extremely unusual	Small round cell tumor, strap cells, expression of muscle markers
9	Artifactual (our unpublished observation)	Due to processing defect or degenerative change	Poor quality smears
Benign lesions			
1	Inflammatory pseudotumor (Hosler et al., 2004)	Uncommon	Prominent inflammatory component, features identifiable as reactive changes
2	Pigmented villonodular synovitis (Gangane et al., 2003)	Uncommon	Joint involvement, demonstration of hemosiderin pigment by Perl's stain
3	Epithelioid angiomyolipoma (orell, 1999)	Well known	Associated adipocyte component

Table 1. Showing differential diagnosis of intranuclear cytoplasmic inclusions (INCIs)

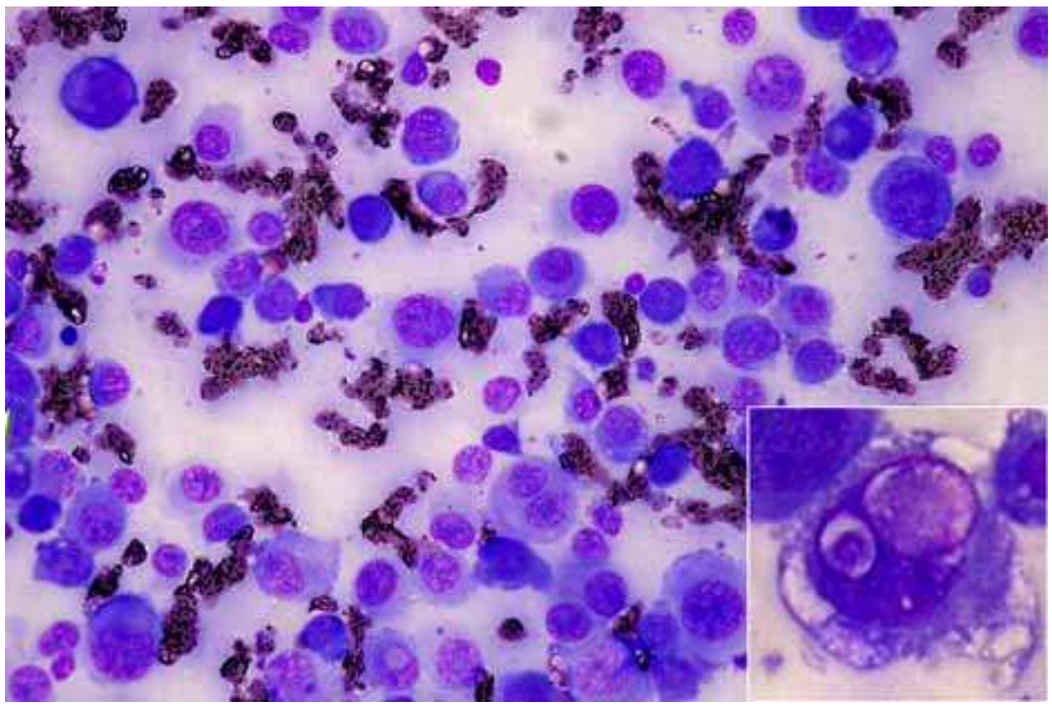

Fig. 2. Our case of recurrent amelanotic melanoma showing plasmacytoid cells; inset highlights distinct INCIs (MGG stain, X400)

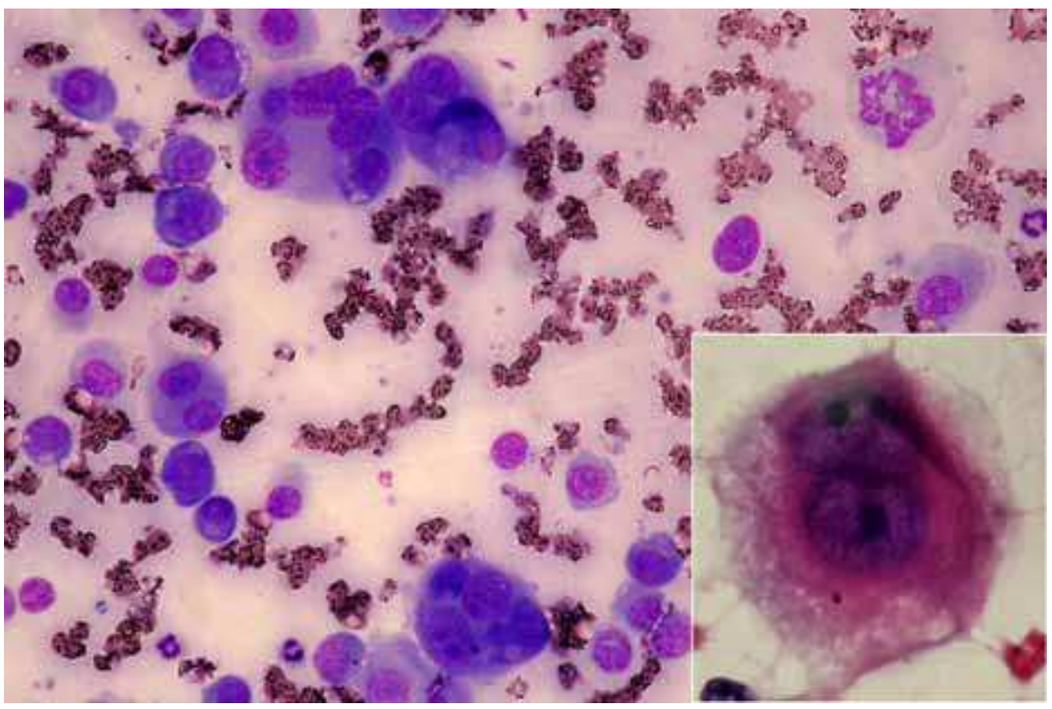

Fig. 3. Another microscopic field from the same case showing multinucleated giant cells with a mitosis (MGG stain, X400); inset shows a digitally magnified cell with an ecto-endocytoplasmic differentiation and prominent inclusion-like nucleoli (PAP stain, x400)

Reviewing further literature on cytology of melanoma is perhaps; of immense help to the clinicians in understanding the diagnostic difficulties encountered, especially by the cytopathologists. In fact, there is a genuine need for the close interaction between the clinicians and pathologists for the proper management of patients with melanoma. Naseill et al. (1991) categorized 81 metastatic melanomas on cytology into classic, carcinoma-like, spindle cell, lymphoma-like, myxoid, clear cell and undifferentiated melanomas. This clearly explains why melanoma has a varied differential diagnosis consisting of a variety of carcinomas, sarcomas and hematolymphoid malignancies that often comprise also of non-Hodgkin's lymphoma (NHL) (Naseill et al., 1991) and plasmacytoma/ myeloma (Siddaraju et al., 2007). In this context, we like to share one of our interesting cases of a recurrent melanoma that manifested with 3 different swellings (axilla, chest wall and back) exhibiting features of a pleomorphic malignant tumor on FNAC. At presentation, the patient's requisition carried a diagnosis of recurrent soft tissue sarcoma, with which the cytologic findings did not match. Cytomorphology varied so widely, with most of the cells displaying plasmacytoid appearance (figure 2). At one stage, owing to the elderly age of the patient, we had a suspicion of plasmacytoma/ myeloma, although, cytologic and clinical features were not very typical. Numerous bizarre bi-nucleate and multi-nucleate giant cells (figure 3); variable chromatin pattern; prominent inclusion-like nucleoli; frequent intra-nuclear cytoplasmic inclusions (figure 2: inset); varied cytoplasmic features (figure 4) such as intensely eosinophilic, as well as, basophilic cytoplasm; cytoplasmic vacuolations; rare signet ring cells and cells with ecto-endoplasmic differentiation (figure 3: inset) were noted. There were also a few strands of fibrocollagenous tissue, to which the tumor cells were adherent (figure 4: inset); this corresponded with the distinct alveolar pattern observed on the later review of the histologic sections. Some of the cytologic features suggested an

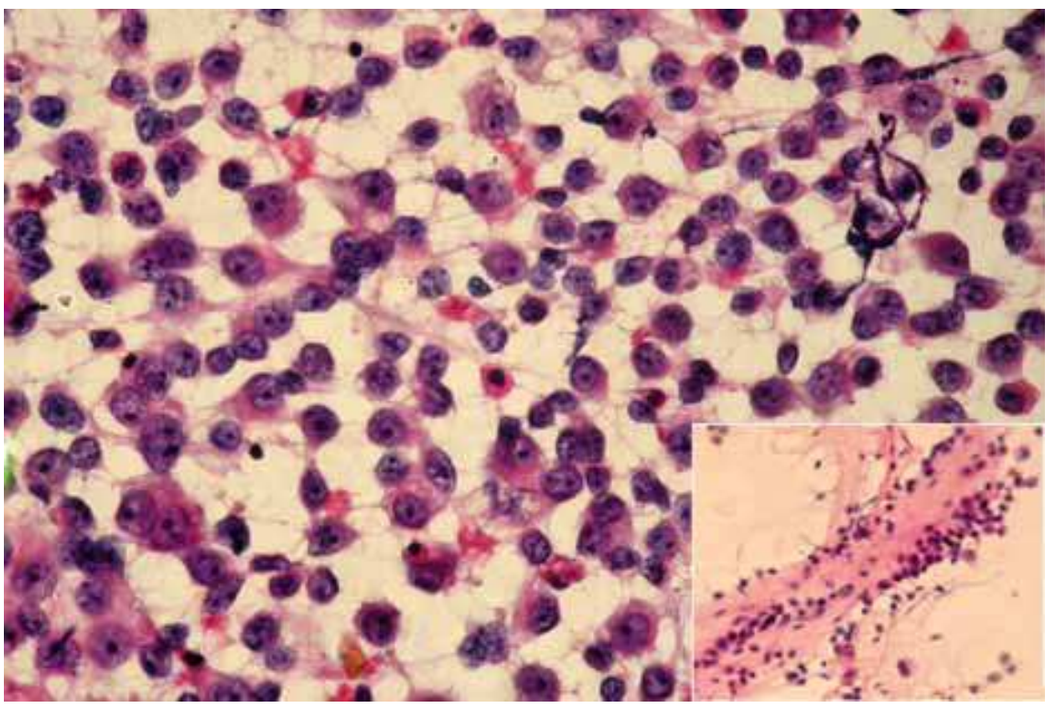

Fig. 4. A smear showing cells with varied cytoplasmic features; inset shows a fibrocollagenous strand with adherent neoplastic cells (PAP stain, x400)

amelanotic melanoma; however, in view of the clinical diagnosis of recurrent soft tissue sarcoma, we offered a diagnosis of an undifferentiated malignant tumor with a possibility of amelanotic melanoma. Subsequently, we learnt that it was diagnosed elsewhere, as a malignant peripheral nerve sheath tumor (MPNST), based on its immunoexpression of S-100. Histologically, the sections revealed a prominent alveolar pattern, vaguely simulating an alveolar rhabdomyosarcoma with a tiny focus displaying junctional activity. We procured the paraffin tissue blocks of this case and performed IHC with desmin, S100, and Melan-A, of which the latter two markers were strongly positive (figures 5&6), confirming our cytologic suspicion of melanoma (Siddaraju et al., 2007).

Other unusual cytologic features described by the various authors are the papillary fragments with fibrovascular cores (Baloch et al., 1999), myxoid stroma (Elliot et al., 2001) ballon cells (Friedman et al., 1982), signet ring cells (Siddaraju et al., 2007) with pseudolipoblastic appearance (Holck et al., 2002), cells with rhabdoid features (Slagel et al., 1997) and cells arranged in alveolar pattern (Gupta et al., 2003). Although rare, a divergent differentiation such as chondroid, neural, myofibroblastic, and osteocartilagenous differentiation is well known in melanomas; and the lack of awareness of this fact can create serious diagnostic problems to the evaluator. Such an instance is well exemplified by a rare case of anal melanoma that also manifested with a mass in the groin. The FNA of the groin mass in that particular case revealed a neoplasm rich in chondroid matrix, raising the possibility of a second primary mesenchymal tumor. However, a review of the histologic slides from the primary growth showed divergent chondroid differentiation in the anal melanoma, establishing the metastatic nature of the groin mass (Hanley et al., 2009). Some of the variants of melanoma are discussed separately.

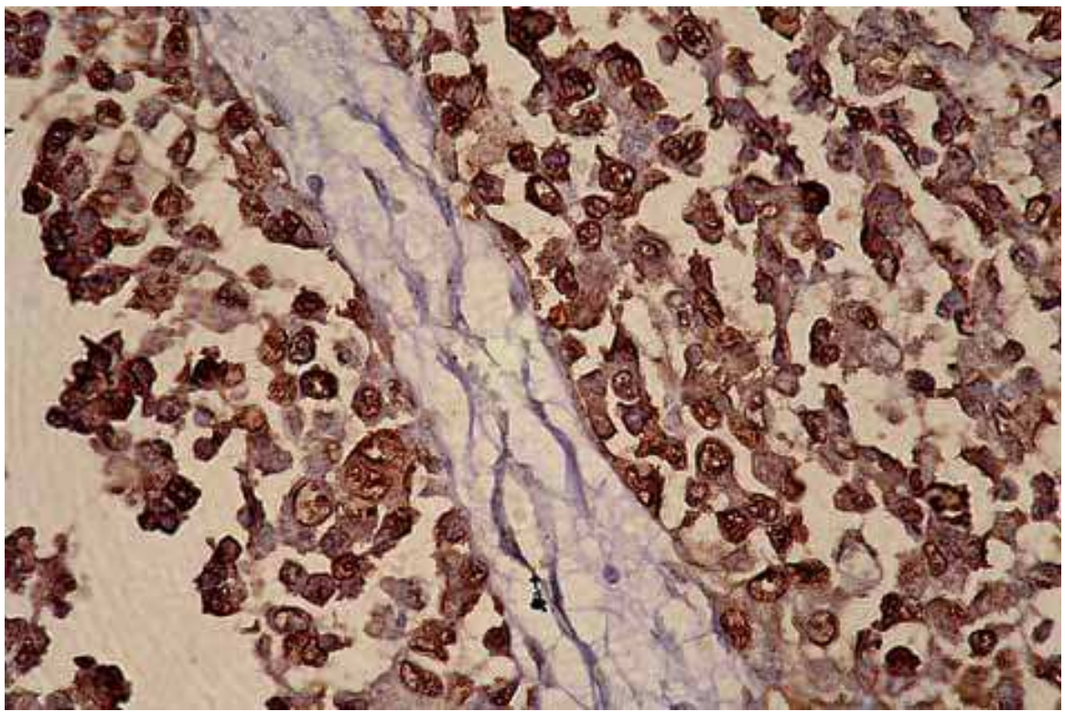

Fig. 5. Melanoma cells exhibiting cytoplasmic and nuclear expression of S-100 on histologic section (IHC stain, X400)

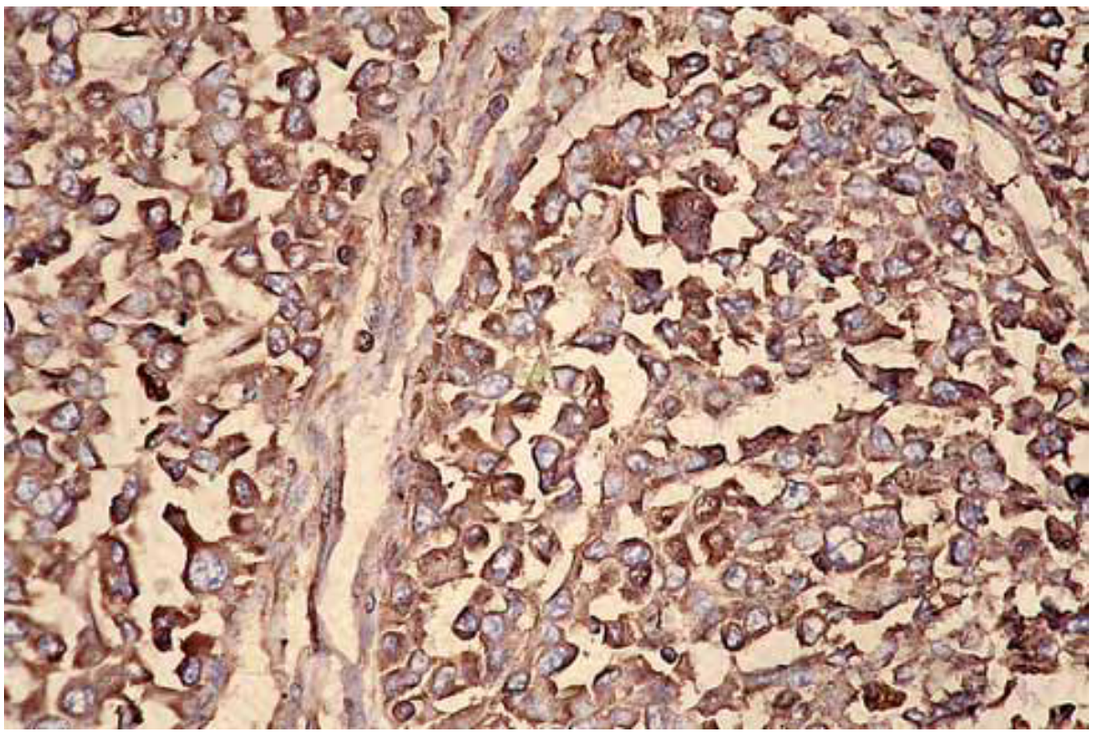

Fig. 6. Neoplastic cells with cytoplasmic Melan-A expression on histologic section (IHC stain, X400)

3.3.1 Spindle cell melanoma

Spindle cell melanoma is a morphologic variant of melanoma which is difficult to diagnose on specimens obtained by fine-needle aspiration (FNA). Piao et al. (2008) studied a large series (67 patients) of metastatic spindle cell melanomas on FNAC (all primaries were histologically proven). The smears were cellular and comprised predominantly of spindle cells that frequently formed cohesive fascicles, or whorls intermingled with scattered epithelioid tumor cells. Significantly, the common features of melanoma such as dishesive cellular distribution, cytoplasmic melanin pigment, intranuclear cytoplasmic inclusions, macronucleoli, and binucleation or multinucleation were infrequent in these cases. When present, they were found in cells with epithelioid morphology. Remarkably, 9% of the cases totally lacked the classic characteristics of melanoma. Spindle cells displayed variable cytologic atypia that ranged from deceptively bland cells resembling the reactive fibroblasts to those indistinguishable from pleomorphic high-grade sarcomas. When the morphologic features were compared with those of the primary tumor, the discrepant cell type was observed in 20% of the cases with the previous primary lesions displaying the epithelioid morphology. Interestingly, the spindle cells also tended to lose immunoexpression of melanoma markers. In such cases, familiarity with cytologic features combined with clinical and immunoperoxidase findings are required to avoid misinterpretation (Piao et al., 2008). Prominent inclusion-like nucleoli are highly characteristic of melanomas; however, we have seen rare cases of pure spindle cell melanoma metastasizing to inguinal nodes that almost completely lacked nucleoli. The classic clinical history of pigmented lesion in the sole of foot, with cells exhibiting melanin pigment facilitated an easy diagnosis in such instances.

On FNAC, in the absence of a reliable clinical history, an amelanotic spindle cell melanoma is likely to be mistaken for spindle cell sarcomas, or, in a location like inguinal region, for a metastatic squamous cell carcinoma with spindle cell morphology.

3.3.2 Desmoplastic melanoma

Desmoplastic melanoma (DM) is an unusual, non-pigmented, sclerosing variant of spindle cell malignant melanoma that can range in appearance from sarcomatoid to scar-like lesion. Cytomorphologic features described on FNA include a moderate cellularity with pleomorphic spindle cells occurring singly and in small aggregates. The spindle cells exhibit plump nuclei with deep grooves and folds, coarse and clumped chromatin, and inconspicuous to multiple, prominent nucleoli, along with naked spindly nuclei. Intranuclear cytoplasmic inclusions are rare. Generally, cytoplasm is scanty to moderate in amount with a fine wispy character, clinging to the nuclear poles. Often, a variable amount of dense stromal material associated with a few spindle cells is seen. Point to be remembered is that immunohistochemically, most desmoplastic melanomas are positive for S-100 and negative for specific melanoma markers (van Ells et al., 2007).

3.3.3 Balloon cell melanoma

Balloon cell melanoma is a histologic variant composed predominantly, or entirely of large cells with abundant, vacuolated cytoplasm. It shares the common cytologic features of melanoma, such as discohesion, nuclear pleomorphism and intranuclear cytoplasmic pseudoinclusions, but generally lacks melanin pigment; however, it is positive for S-100, HMB-45 and Melan-A (Baehner et al., 2005). The entity needs to be distinguished from metastatic adenocarcinoma, liposarcoma and other clear cell tumors.

3.3.4 Signet ring cell melanoma

Although rare, cytology of signet ring cell melanoma has been on record. Tsang et al. (1993) documented a case that manifested as a groin mass in a patient who had undergone below knee amputation for superficial spreading melanoma of the right foot. FNA smears in this case showed poorly cohesive, large cells with eccentric nuclei, most of which exhibited signet ring morphology. Only a small proportion of cells displayed melanin pigment clinching the right diagnosis. In the absence of melanin pigment and the clinical history, such cases are likely to be misdiagnosed as metastatic signet ring cell adenocarcinoma. Appropriate history and application of ICC can avoid misinterpretation of such cases. The case reported by Tsang et al was histologically proven to be a 'metastatic signet ring cell melanoma' with positive expression of HMB-45 and S-100, and negativity for cytokeratin.

3.3.5 Myxoid melanoma

Myxoid melanoma is an extremely rare variant with only a limited number of cases documented. Initial FNA cytologic description of this distinct variant of melanoma was given by Rocomora et al. in 1988. The lesion needs to be distinguished from other myxoid tumors such as myxoid liposarcoma (myxoid LPS), extraskeletal myxoid chondrosarcoma (extraskeletal MCS), myxoid malignant fibrous histiocytoma (myxoid MFH), chordoma and malignant peripheral nerve sheath tumor (MPNST). Although certain cytomorphologic features assist distinguishing these lesions from each other, a cytopathologist may have to

depend on ICC in difficult situations. The table 2 shows the salient clinical, cytologic and immunocytochemical features that help distinguishing these lesions. It's worth noting that myxoid melanoma is usually HMB-45 negative, but strongly positive for S-100, along with a positive melanoma marker like NK1/ C3 (Elliot et al., 2001).

Tumor	Average age (yrs)	Commonest site	Salient cytology	ICC
Myxoid LPS	46	Deep seated tissues- thigh, buttock, extremities	Round to spindle cells with lipoblasts and chicken-wire capillary network; lacy myxoid background	S-100 + Vimentin + Keratin – HMB45 –
Extra skeletal MCS	65	Deep seated soft tissue of extremities	Small, round to oval cells, pleomorphic mesenchymal cells in an abundant loose myxoid stroma	S-100 + Vimentin + Keratin + HMB45 –
Myxoid MFH	65	Thigh, trunk, head and neck	Large, pleomorphic, bizarre cells in a myxoid background	S-100 + Vimentin + Keratin + HMB45 –
Chordoma	Any age for spheno-palatine site	Sacrococcygeal, spheno-palatine	Small round to oval cells and and physaliphorous cells in a loose myxoid stroma	S-100 + Vimentin + Keratin + EMA + HMB45 –
MPNST	3-35		Epithelioid variant: large pleomorphic cells, INCI +/ -, melanin +/ -	S-100+ (focal) Vimentin + Keratin – Desmin – HMB45 +/ –

Table 2. Cytologic differential diagnosis of myxoid melanoma

3.4 Location-based cytologic interpretation of melanoma

Differential diagnosis of melanoma depends not only on the overlapping cytomorphologic features or patterns, but also on the site of primary or metastatic involvement. For example, an amelanotic melanoma of the cervix may have a differential diagnosis of rhabdomyosarcoma, mixed malignant mullerian tumor (MMMT), adenocarcinoma and poorly differentiated squamous cell carcinoma (Deshpande et al., 2001). An amelanotic melanoma (epithelioid type) involving the glandular organs like salivary gland and breast is likely to be misinterpreted as an adenocarcinoma (Cangiarella et al., 1998; Bangerter, 2009). Similarly, a primary or metastatic spindle cell (amelanotic) melanoma of the breast may have a differential diagnosis of a variety of sarcomas such as leiomyosarcoma, malignant fibrous histiocytoma (MFH), neurogenic sarcoma, or dermatofibrosarcoma protuberans (DFSP) (Artal et al., 2004) and also a malignant phylloides, when epithelial component is

absent. There also has been a rare case report of mediastinal spindle cell melanoma which was misinterpreted as 'spindle cell thymoma' owing to its location in the anterior mediastinum. Cytologic smears of this case were hemorrhaghic with a loosely dispersed population of spindle cells having prominent nucleoli. A rare situation as this, stresses the need for cytopathologists to be cautious when interpreting mediastinal spindle cell lesions. Melanoma in the mediastinum is extremely rare and both physicians and pathologists should be aware of this remote possibility (Bavi et al. 2005).

With the fact that melanoma can mimic a wide variety of neoplasms, one also needs to be careful while dealing with the aspirates from multiple sites; particularly, in the absence of a reliable history, as it is not infrequent to find a coexisting second lesion with melanoma. In this context, we share the experience of one of our recent cases with a previous history of melanoma of foot. This patient presented with inguinal lymphadenopathy, as well as, an intra-abdominal mass. FNA from the inguinal node yielded blackish material, which on microscopic examination showed abundant melanin pigment that obscured the morphology of neoplastic cells (figure 7); while, the aspirate from the abdominal mass showed well preserved cellular elements, with loosely clustered and dissociated population of pleomorphic malignant cells (figure 8). Although, variation in cytomorphology of the two different aspirates raised suspicion of a dual malignancy, considering the clinical history, a thorough search for melanin pigment was made; and rare pigmented malignant cells, as well as, a few melanophages were detected, confirming the diagnosis of metastatic melanoma in both the sites.

3.5 Interpretation of melanotic melanoma and its differentiation from other pigmented lesions

Often a cytopathologist needs to be cautious, when interpreting melanotic melanoma as well. Appearance of melanin pigment in cytologic smears varies according the stain used. Most cytology laboratories use one of the Romanowsky stains (in our laboratory it is May-Grünwald- Giemsa) along with the Papanicolou (PAP) and haematoxylin-eosin (H-E) stains. Various authors describe melanin pigment to be yellow to brown-black, and bluish-black in the Papanicolaou/ H-E stained, and dark-brown to black on the May-Grünwald-Giemsa (MGG) stained smears (Saqi et al., 2002; Kashyap et al., 2002). In our experience, melanin pigment takes up shades of brown colour with the Papanicolaou (figure 10), and bottle-green hue with MGG stain (figure 7). The intensity of color varies with the amount of intra or extracellular melanin pigment. An abundant pigment obscures the cytomorphologic details, necessitating the need for a careful search for cells with preserved morphology (figure 7). Owing to the overlapping shades of color, the melanin pigment needs to be differentiated from other endogenous pigments such as lipofuscin, hemosiderin and bile; special stains such as Perl's, periodic-acid-Schiff (PAS), Fontana-Masson and Ziehl-Neelsen (Z-N) stains are useful in this regard (Perry et al., 1986). Melanin is positive with Fontana-Masson; lipofuscin with PAS and Z-N stains; and hemosiderin with Perl's stain. Bile pigment is generally identified by its greenish-black and yellowish-green appearance in MGG and Papanicolaou stains respectively (Orell, 1999). Gathering appropriate history is also of relevance in differentiating melanin pigment from that of tattooing. Tattooing (intradermal injection of an exogenous pigment) is known to cause discoloration of the draining lymph nodes resulting in so called 'tattoo lymphadenitis' (Bordea et al., 2009). Anthracotic pigment, the other exogenous pigment, generally does not cause diagnostic difficulty, as it is confined to the alveolar macrophages of the respiratory cytologic samples;

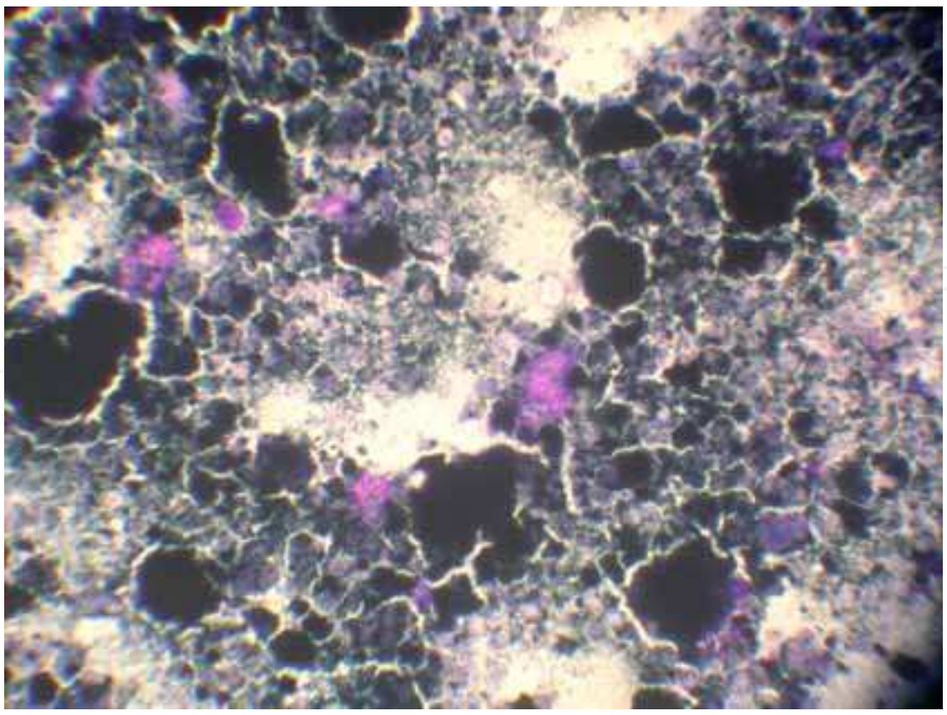

Fig. 7. FNA smear from inguinal node showing an abundant bottle-greenish pigment obscuring the cell morphology (MGG stain, x400)

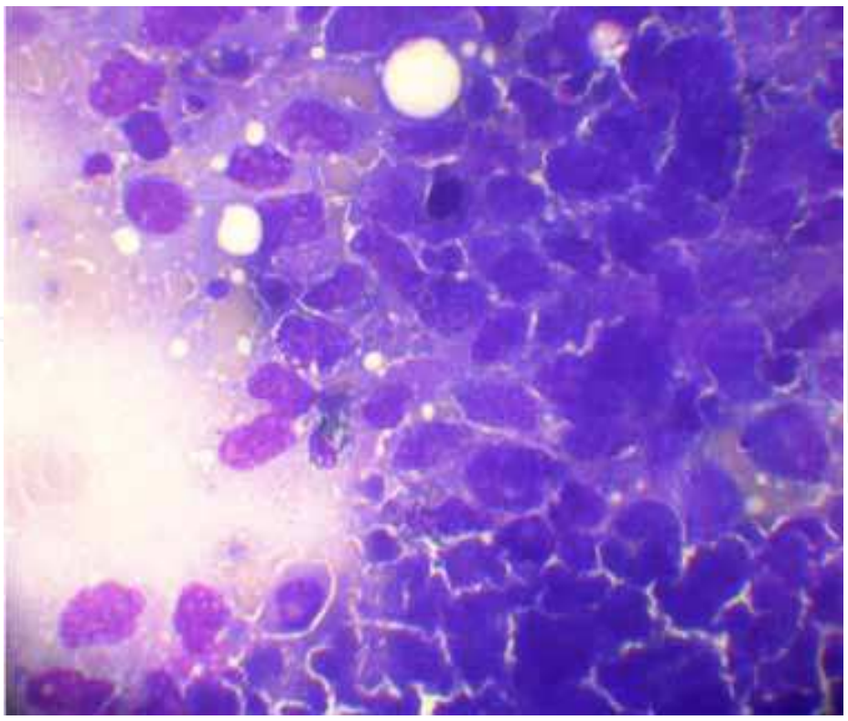

Fig. 8. Aspirate from the intra-abdominal mass of the same case showing pleomorphic melanoma cells with a rare cell displaying melanin pigment (MGG stain, x400)

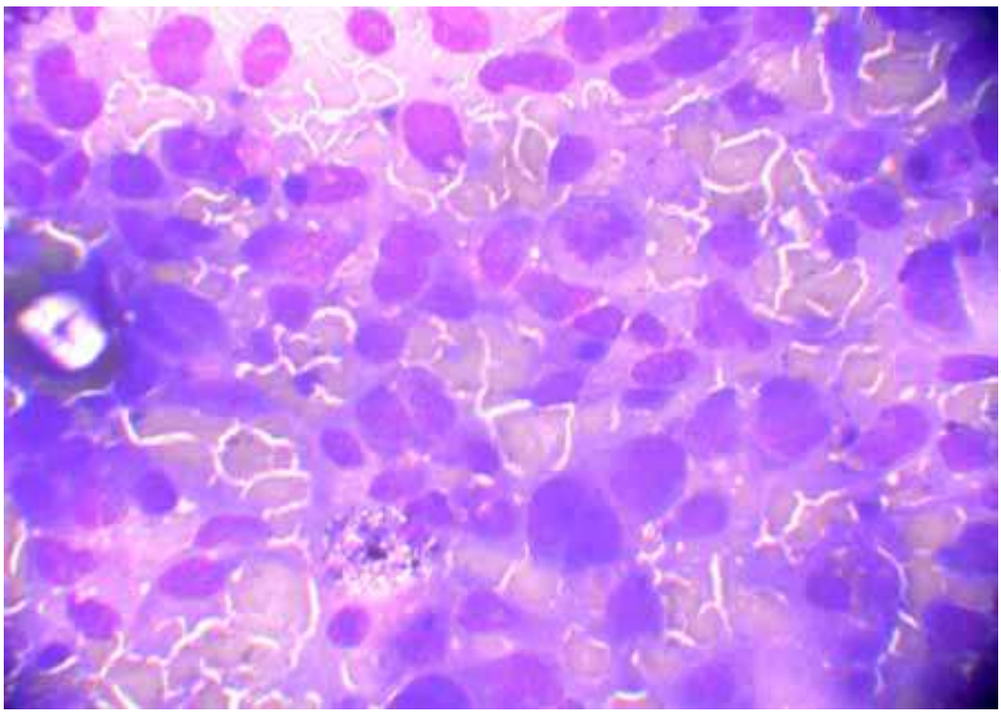

Fig. 9. Another microscopic field from the abdominal aspirate showing dissociated, pleomorphic, non-pigmented melanoma cells

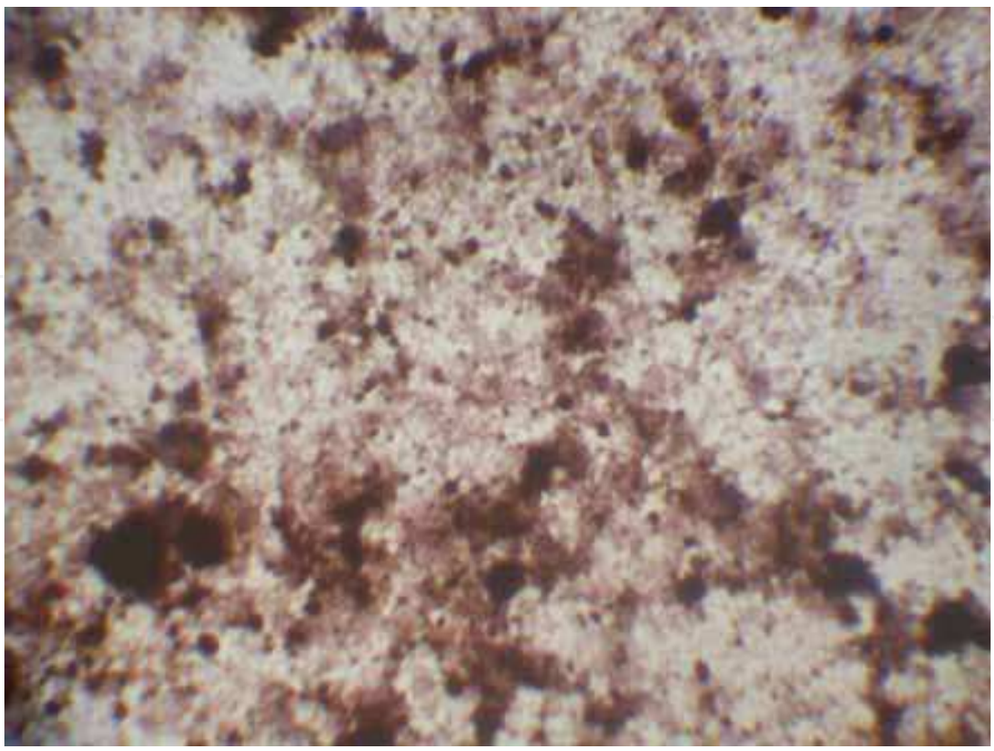

Fig. 10. Abundant melanin pigment with shades of browinish color (PAP stain, X400)

their absence in epithelial/ epithelial-like cells is of help in excluding a melanoma, or, other pigmented neoplasms. Although, the pigment can also be seen in an anthracotic node, it is rarely sampled for cytologic examination. There has been a case report of exaggerated pigmented granulomatous reaction to the artificial joint implant in a 72-year-old man who had undergone bilateral total hip arthroplasty, in whom, aspirate from the inguinal region was misinterpreted as metastatic melanoma from an unknown primary. Microscopically, the smears of this patient showed both intracellular and extracellular black pigment with obscured cytomorphology; although, appreciable cells were reported to be pleomorphic with prominent nucleoli. The patient had no evidence of primary cutaneous melanoma. On surgical exploration and histologic examination, what was presumed to be the bulky enlarged lymph nodes was found to be the soft tissue with pigment-laden macrophages and granulomatous reaction that occurred as a response to metallic prosthetic material. This rare case further emphasizes the need for critical attention to the clinical details and also a careful cytologic evaluation in the presence of pigmented debris obscuring the cytomorphology (Xin et al., 2004). Table 3 shows various types of pigments and their distinguishing features.

Pigment	History, location and microscopic appearance	Fontana-Masson	Perl's stain	PAS stain	Z-N stain
Melanin	Shades of brown on Papanicolau & bottle greenish-blackish on MGG stains	Positive	Negative	Negative	Negative
Hemosiderin	Golden-brown on Papanicolaou & greenish-brown/ blackish on MGG	Negative	Positive	Negative	Negative
Lipofuscin	Golden-yellow to brown, autofluorescence	Negative	Negative	Positive	Positive
Blie	Cytologic samples from liver, greenish black with MGG and yellowish green with PAP stain	Negative	Negative	Negative	Negative
Carbon (anthracotic)	Alveolar macrophages in the respiratory samples	Negative	Negative	Negative	Negative
Tattoo	History of tattooing	Negative	Negative	Negative	Negative

Table 3. Showing features distinguishing various pigments

In the context of the pigmented lesions, we would like to share our experience of a pigmented pulmonary neoplasm in which we suggested the possibility of pulmonary melanoma. Unfortunately, due to the patient's refusal to undergo further FNAC / biopsy,

work-up of this case was incomplete; however, based on the available clinical data and a careful cytomorphologic evaluation of the intra-thoracic FNA material from the lung, we could suggest a diagnosis of 'primary pulmonary melanoma'. A solitary lung lesion in the absence of swelling or lesion elsewhere (skin, GIT and eye); and a predominant population of dissociated or loosely clustered cells (figs 11 & 12), made us consider the possibility of a primary pulmonary melanoma, rather than the metastatic deposit. Here, the point noteworthy is that a pigmented neoplasm in the intra-thoracic location need not necessarily be a melanoma and should be differentiated from other rare pigmented tumors like melanocytic carcinoid, melanotic paraganglioma and melanotic schwannoma (Dountsis et al., 2003). Morphologically our case did not fit into any of these lesions. To our knowledge, melanocytic carcinoid and melanotic paraganglioma have not been documented on cytology, but there have been rare case reports of melanotic schwannoma diagnosed on FNAC, as well as on exfoliative cytology. In all these cases the authors found it highly difficult to distinguish melanoma vs. schwannoma, not only due to the morphologic overlap, but also due to its immunophenotypic and ultrastructural resemblance (Jaffer et al., 2000; Schmitz et al., 2005). Melanotic schwannoma most frequently occurs in the paraspinal region and less commonly at locations such as the intestines, heart, bronchus, liver, skin, soft tissue and bone (Jaffer et al., 2000). The table 4 shows the differential diagnosis of an intra-thoracic melanotic lesion.

Pigmented lesion	*Number /location*	*Evidence of extra-primary site*	*Cytology*
Primary melanoma	Usually solitary/ lung	Nil	High cell yield; dispersed/ loosely clustered, pleomorphic malignant cells; inclusion-like nucleoli; intra-nuclear cytoplasmic inclusions
Metstatic melanoma	Usually multiple/ lung	Detectable on further work-up: eg. Skin, GIT or eye	Same as that of primary melanoma
Melanocytic carcinoid	Usually solitary/ lung	Nil	Depends on the type of carcinoid (classic, atypical, spindle cell or, adenocarcinoid)
Melanotic schwannoma	Usually solitary/ mediastinal		Usually, a spindle cell population with other features such as Verocay bodies
Melanotic paraganglioma	Usually solitary/ mediastinal		Evidence of cells with red cytoplasmic granularity

Table 4. Differential diagnosis of an intra-thoracic melanotic lesion on FNAC

Melanoma of lung is extremely rare accounting for 0.01% of all lung tumors. Clinically, it is confused with other conventional types of lung cancer (Wilson et al., 1997). The tumor is frequently endobronchial in origin and 30% of the cases are diagnosed incidentally on chest radiography (Ost et al., 1999). A preoperative computed tomography guided FNAC is of great help in its detection; this was how it was detected in our patient as well. The proposed criteria for diagnosing the primary pulmonary melanoma comprise clinical detection of a solitary lung tumor; absence of melanoma of the skin, mucous membrane and eye or, any other detectable tumor at the time of diagnosis; histologically demonstrable junctional changes like "dropping off" or "nesting" of melanoma cells just beneath the bronchial

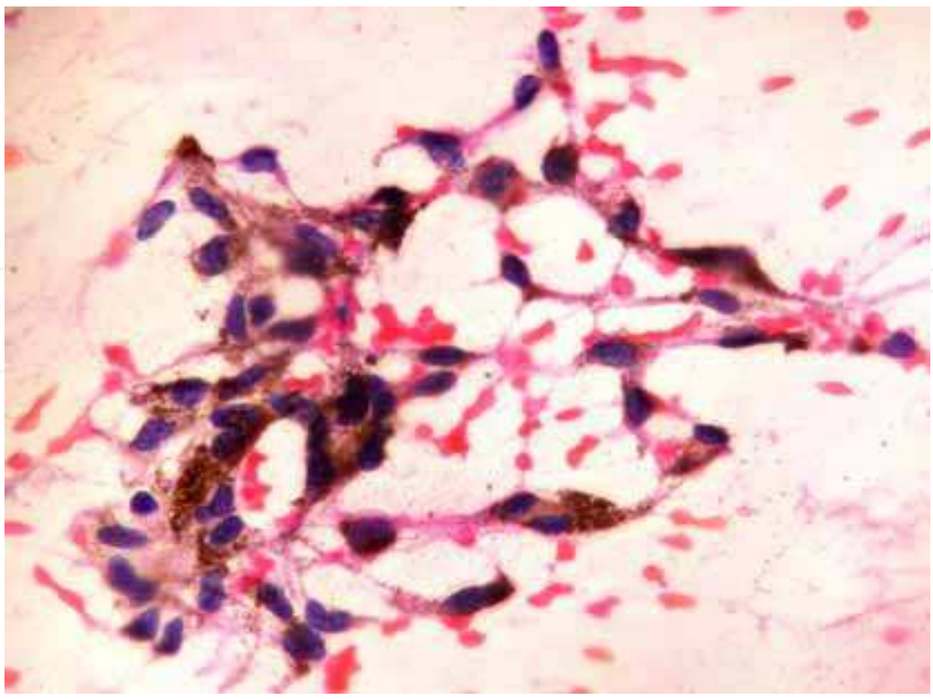

Fig. 11. The aspirate smear from primary pulmonary melanoma showing a microscopic field with a predominant spindle cell population (PAP stain, X400)

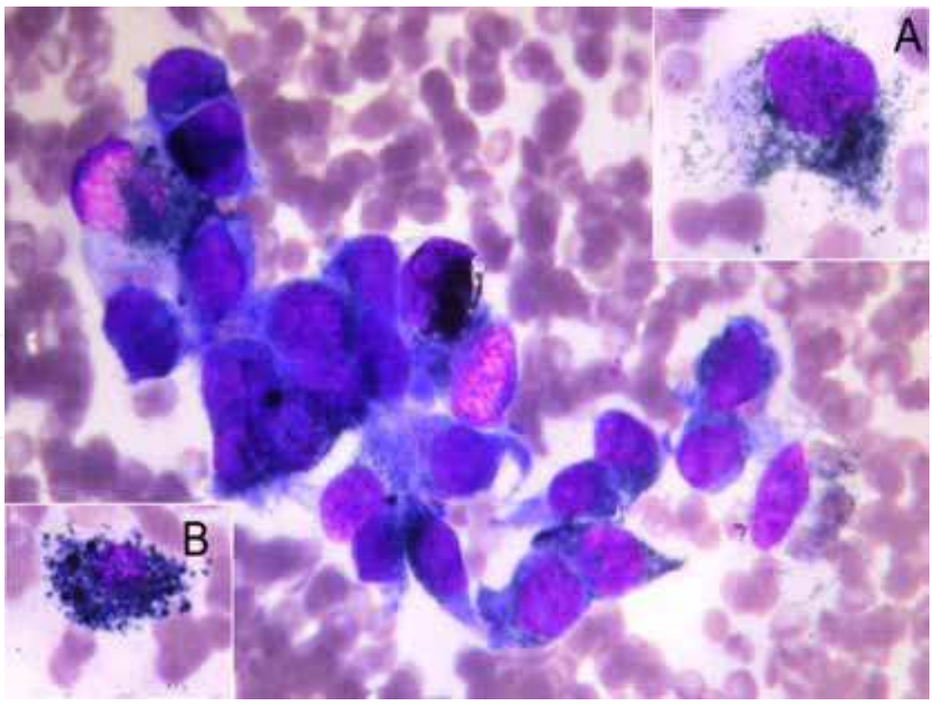

Fig. 12. Another microscopic field from the same case showing loosely cohesive pigmented melanoma cells with epithelioid morphology; Inset A- a pigmented neoplastic cell (MGG stain, X400); Inset B- a melanophage (MGG stain, X400)

epithelium; and invasion of the bronchial epithelium by melanoma cells. Although, more than 30 cases of the primary pulmonary melanomas are documented, not all of them have met the required criteria. Due to the small number of cases reported, experience with respect to the management and the prognosis of the disease is also limited (Dountsis et al., 2003). However, the surgical treatment includes resection of the tumor with an oncologically adequate margin; lobectomy, or pneumonectomy (Rosai, 2001). As for the occurrence of pulmonary melanoma, one of the hypotheses says that melanocytes exist throughout the body as cells of a dispersed neuroendocrine system. Normally, these melanocytes migrate to the epidermis and the dermoepidermal junction of the skin, but they may also migrate to the viscera during embryogenesis. This hypothesis suggested for locations like esophagus and larynx may also be applicable for lung (Pomeranz et al., 1995).

The other melanotic lesions to be considered by the cytopathologists are dermatopathic lymphadenopathy, pigmented basal cell carcinoma (BCC) and Bednar tumor. Dermatopathic lymphadenopathy is easily differentiated from metastatic melanoma not only by the clinical history, but also by the distinct cytomorphologic features. Smears from a case of dermatopathic lymphadenitis show a spectrum of reactive lymphoid cells along with cohesive (usually) clusters of melanin-laden histiocytes, in contrast to characteristically discohesive melanoma cells. We have documented a case of pigmented BCC which displayed basaloid, as well as, spindle cell population seen as cohesive fragments, and clusters on cytologic smears. Cytologically, the absence of cell-dissociation easily excluded a melanoma, while a combination of basaloid and spindle cells excluded a Bednar tumor. Cohesive clusters of basaloid cells at places exhibiting peripheral palisading favored a definitive diagnosis of pigmented BCC. (Siddaraju et al., 2008)

The incidence of melanoma in India is low as compared to that of the western population (Nair et al., 1998). Most cases of melanoma we encounter in our institute (JIPMER, which is a referral center) are those involving the soles of the feet. Many of these patents are males who present with inguinal lymphadenopathy. In the last two years, we encountered 27 metastatic melanomas, most of them with primaries in the soles of the feet. The morphologic aspects we recorded were similar to those already described in the indexed literature. Personally, I have not come across the variants such as pure balloon cell, myxoid and signet ring cell melanomas. In our experience, diagnosing pigmented melanoma is not much of a problem (although, care needs to be taken with the pigmented lesions as well); but diagnostic difficulties are common with amelanotic melanomas and the problem should be resolved with an extreme attention to the clinical details, as well as, by the application of ancillary tests. In my personal experience, the cytomorphologic features of great assistance in the accurate cytodiagnosis of amelanotic melanoma are a high cell-yield; dispersed, pleomorphic cell population; inclusion-like prominent nucleoli and the presence of intranuclear cytoplasmic inclusions.

3.6 Ancillary tests for melanoma

Immunocytochemistry is the most useful ancillary test in difficult situations. Specific immunomarkers for melanoma include HMB-45, Melan-A, tyrosinase and microphthalmia transcription factor. S-100, although least specific, is of great practical importance, as it is more consistently positive in melanoma than any other specific markers. Also, the entities that enter the differential diagnosis of melanoma are generally negative for S-100. Melanoma antigen recognized by T cells (MART-1) is another sensitive as well as specific marker for melanoma. Between melan A and MART-1, Melan A has a tendency to stain more cells (Fetsch et al., 2001). Not just the melanoma markers, but also a panel of markers need to be used depending on the differential diagnosis. Thus, one may have to use various

epithelial (such as cytokeratin), mesenchymal (such as vimentin) and hematolymphoid (B and T, as well as CD68) markers depending on the clinical and cytomorphologic manifestations (Pelayes et al., 2010). Some authors have shown liquid-based cytology (LBC) preparations to be superior to conventional smears for ICC (Pelayes et al., 2010). Very rarely ultrastructural features need to be evaluated for diagnosing melanomas and the features include the presence of premelanosomes and melanosomes. Melanoma of soft parts or Clear cell sarcoma of soft parts (CCSSP) is phenotypically and immunohistochemically indistinguishable from cutaneous melanoma. Although, clinical features and certain cytomorphologic findings are of some value in distinguishing between the two, a definitive diagnosis may require the cytogenetic evaluation. This may be of particular importance with metastatic/ recurrent lesions with inadequate history (Tong et al., 2002; Kumar et al., 2003; Salem Shabb et al., 2003).

3.7 Role of cytology in ocular melanomas

Clinical diagnosis of ocular melanoma is difficult, especially, when the lesion is amelanotic. Ocular cytology, in particular FNAC can assist in its accurate diagnosis, especially with the ancillary aids (Kashyap et al., 2002; Augsburger et al., 2002; Char et al., 2006; Young et al., 2007 & 2008; Solo et al., 2009). FNAC, when done with essential precaution under the supervision of an ophthalmologist and under CT/ US guidance, provides highly accurate results (Solo et al., 2009). Studies have found no evidence of local tumor dissemination or recurrence following FNA (Faulkner-Jones et al., 2005; Pelayes et al., 2010). Young et al. in their study of 25 patients with macular choroidal melanoma, could obtain diagnostic material in 74% of the cases. Although, complications such as retinal perforation, submacular/ vitreous hemorrhage occurred in some cases, none of them required treatment. Submacular and vitreous hemorrhages cleared spontaneously within a month's period (Young et al., 2008). Augsburger et al. (2002) reported their experience of transvitreal FNAC of 34 small, non-invasive, melanocytic choroidal tumors. The cell-yield was diagnostic in 64.7% the cases. Of these, 47.1% were diagnosed as melanoma and the rest were interpreted as intermediate lesions (11.8%) and benign nevi (5.9%) respectively. The cases with insufficient FNA material (12 lesions) were designated as "nevus versus melanoma" for facilitating a further FNA follow-up. Four of these "nevus versus melanoma" cases were eventually reclassified as small choroidal melanomas and treated; while the others turned out to be benign nevi. Thus, considerable numbers of small melanocytic choroidal tumors that were likely to be categorized as 'choroidal melanoma' were shown to be benign nevi or intermediate lesions on FNA follow-up (Augsburger et al., 2002).

Iris ring melanomas are rare. In the initial stage, either they are not diagnosed or misdiagnosed as 'pigmentary glaucoma'. The patients with iris ring melanomas may not have an obvious mass lesion and, present usually with a subtle heterochromia or marked pigmentation, visible only in the trabecular meshwork. Most series have described an iris ring melanoma as a tumor involving the 360 degrees of the anterior chamber angle, although it can sometimes present as an apparently focal iris mass. Char et al. (2006) could accurately diagnose 11 of 16 cases (69%) on FNAC, performed using a 25 gauge needle going through a transcorneal route into the iris - corneal angle 180 degrees away from the main tumour mass. In cases of heterochromia without a distinct mass, an area of angle pigmentation was aspirated in the same manner. Morphologically, most cases were either of spindle cell, or, mixed spindle/ epithelioid morphology. The limitation of FNAB in

diagnosing iris ring melanomas is that, even in an optimal setting, the specimens are paucicellular, often leaving the cytopathologists in a diagnostic dilemma (Char et al., 2006).

Cytology has a significant role in distinguishing pigmented lesions of the conjunctiva which can be benign, pre-malignant, or frankly malignant (melanoma). Conjunctival melanomas are potentially lethal and they arise in association with pre-existing primary acquired melanosis (PAM). It is important to recognise and monitor this precursor lesion owing to its malignant potential. Follow-up of suspicious conjunctival lesions by repeated biopsies may cause symblepharon, lid deformities, and discomfort to the patient. Conjunctival impression cytology using cellulose acetate paper has been in use for diagnosing various ocular surface disorders. Being less distressing to the patients, this quick, simple, non-invasive and inexpensive technique has proven useful for neoplastic lesions as well. The technique involves pressing a 2 x 6 mm cellulose acetate filter paper (millipore) held by forceps onto the conjunctival surface with the aid of a small solid rod for 3 to 5 seconds. The cellulose acetate imprint strip (obtained without using topical anaesthesia) is placed in a fixative containing glacial acetic acid, 37% formaldehyde, absolute ethyl alcohol and distilled water. Subsequently, the specimen is stained with PAS/ Papanicolaou stains and mounted on glass slides.

The topographic distribution and relative proportion of atypical melanocytes are better appreciated on impression cytology. Benign conditions such as conjunctival nevus and primary acquired melanosis (PAM) without atypia are not associated with the ascent of atypical melanocytes through the conjunctival epithelium; while the late stages of PAM and obvious melanomas are associated with the ascent. This is the basis for the interpretation of conjunctival impression cytology (Paridaens., 1992). Paridaens et al. (1992) evaluated conjunctival imprint cytology in differentiating melanocytic tumours of the bulbar conjunctiva in 24 patients. Impression cytology predicted the histologic diagnosis by detection of superficial atypical melanocytes and their proportion relative to benign epithelial cells in 73% of the cases (Paridaens et al., 1992). One should remember that the benign nevi, PAM without atypia, and early stages of PAM with atypia can only be diagnosed by histology, with cytology being 'negative' in such instances. Repeated examinations may increase the sensitivity of impression cytology. A disadvantage of touch imprint cytology, in comparison to exfoliative cytology, is the difficulty in focusing due to the thickness of the filter paper, which affects the cytologic assessment. Another limitation is its inability to sample the lesions located in the fornix and tarsal conjunctiva; in these cases, one can opt for exfoliative cytology with the use of cotton wool swab after topical anaesthesia. In general, although a diagnostic biopsy remains necessary for determination of the origin and extent of the lesions; the use of imprint cytology can minimise the frequency of biopsy in recurrent tumors and suspicious areas (Paridaens et al., 1992).

Our experience with ocular melanomas is limited. In one of our studies that evaluated the use of fine needle cytology in orbital and eyelid lesions, we had a single case of recurrent choroidal melanoma, which was diagnosed with the non-aspiration needling technique. Although grossly, material obtained appeared scanty, presence of obvious malignant cells with melanin pigment facilitated an easy diagnosis (Solo et al., 2009).

3.8 Melanoma of soft parts

"Melanoma of the soft parts (MSP)" or "clear cell sarcoma of the soft parts (CCSSP)" is a distinct entity with unique genetic rearrangement (t12;22) (q13;q12). Its morphologic,

immunohistochemical and ultrastructural resemblance to cutaneous melanoma is well established. This phenotypic resemblance between the two necessitates the need for distinguishing CCSSP from the metastatic deposits of cutaneous melanoma (Kumar et al., 2003). Morphologhic features shared by these tumors on FNA smears include high cell yield; a predominant population of dissociated cells with plasmacytoid, polygonal and rarely spindle shapes; hyperchromasia (in some cases); prominent, round nucleoli and intranuclear cytoplasmic inclusions (INCIs). Mitoses are rare or variable and intracellular melanin is extremely rare (Tong et al., 2002; Kumar et al., 2003; Salem Shabb et al., 2003). Features such as 3-D clusters with microacinar pattern (Tong et al., 2002; Kumar et al., 2003); pale, blue, fragile or vacuolated and clear cytoplasm; a few bi- and multinucleated cells, vesicular nuclei (Tong et al., 2002; Kumar et al., 2003); extreme pleomorphism with bizarre giant cells and frequent mitoses have also been described in CCSSP (Tong et al., 2002; Kumar et al., 2003; Salem Shabb et al., 2003). As in melanoma, tumor cells exhibit positivity with histochemIcal stains such as Fontana-Masson and Schmorl's stains (Tong et al., 2002; Kumar et al., 2003) and immunohistochemically express melanoma markers such as S-100, HMB-45 and melan-A (Tong et al., 2002; Kumar et al., 2003). Ultrastructurally, often they show melanocytic differentiation with melanosomes (Tong et al., 2002; Kumar et al., 2003). However, clinically, the tumor differs from cutaneous melanoma by its younger age group (median age 30), female predilection and a general tendency to involve the tendons and fascia of the upper and lower extremities. Prognostically, in comparison to cutaneous melanoma, it runs an indolent course, although bigger tumors are likely to show a rapid deterioration (Tong et al., 2002) with multiple local recurrences, distant metastasis and poorer prognosis (Kumar et al.2003). Owing to the considerable morphologic overlap, a variety of cytologic differential diagnoses are considered, of which the important ones comprise alveolar soft part sarcoma, carcinomas, synovial sarcoma and epithelioid sarcoma (Kumar et al., 2003). Chong et al. (1997) documented a case of CCSSP of the gluteal region in a 12-year-old child, manifesting as a small round cell sarcoma on FNAC. The case exhibited cohesive cell balls, rossettes and dissociated cells in a clean background. Many cells had eccentric nuclei with irregularly dispersed chromatin, occasional intranuclear vacuoles and eosinophilic nucleoli; the later features in an aspirate from the deep seated mass, in a child should suggest the possibility of CCSSP and prompt an ICC demonstration of melanoma markers (Chong et al., 1997).

3.9 Further advances in cytology of melanoma

In situations where routine cytomorphology and immunomarkers fail to contribute to the diagnosis of melanoma, certain other ancillary studies on cytologic samples (some of which are molecular-based) have shown to provide useful diagnostic and prognostic information. Angeletti et al (2004) performed a novel tyramide-based tyrosinase assay (a simple in-situ biochemical test) on FNA material obtained from a small series of melanoma patients. For standardization, the authors used YUGEN8 melanoma cell lines and the HeLa cells as positive and negative controls respectively. They showed this simple, quick and inexpensive assay to be a sensitive and specific method for diagnosing melanoma on cytologic samples. At molecular level, HSP-70 protein, C-myc oncogene, and HLA-DR antigen are said to play a significant role in the metastasis and prognosis of cutaneous melanomas. Kalogeraki et al. (2006) studied immunoexpression of these proteins on cytologic material by ICC, and found it to be significantly associated with 'Clark levels'. In particular, HSP-70 expression is believed to be of value in the identification of melanoma patients with poor prognosis

(Kalogeraki et al., 2006). Studies also have shown tyrosinase reverse transcription polymerase chain reaction (RT-PCR) on fine-needle aspirates (FNA-PCR) to increase the diagnostic sensitivity of cytology, when US/ FNAC fails to detect the sentinel node metastasis in melanoma (Voit et al., 1999). Such studies have been performed on exfoliative samples as well. In a study involving 79 urine samples from patients with metastatic melanoma, Savoia et al. (2008) demonstrated that RT-PCR studies on exfoliative samples increased the sensitivity of cytology. The authors emphasized its utility as an additional tool in cases of negative or suspicious conventional urinary cytology.

Also being performed is the cytogenetic analysis on FNA material for detection of monosomy 3, an abnormality, associated with the adverse outcome in patients with uveal melanomas. Young et al. (2008) successfully carried out fluorescent-in-situ-hybridization (FISH) and/ or GeneChip 500k NspI Mapping array analysis on cytologic samples from 24 patients with macular choroidal melanoma and based on their results, emphasized its importance in the detection of chromosomal aberrations on cytologic material. However, Maat et al. (2007) demonstrated the heterogeneity for copy of chromosome 3 on FISH analysis performed on paraffin sections and felt that assigning patients to risk categories based on FNA samples for cytogenetic studies (for monosomy3) may be subject to error. Another significant study on FNA material by McCannel et al. (2010) demonstrated 2 cytogenetically distinct groups of choroidal melanomas characterized by chromosome 3 loss, or chromosome 6p gain. These findings may provide new insight into the biologic nature of choroidal melanoma and are likely to contribute to the development of targeted therapies (McCannel et al., 2010).

4. Conclusion

A variety of cytologic techniques are available to aid clinical evaluation of patients with melanoma, of which fine needle aspiration cytology (FNAC) is the frontrunner. The techniques have a significant role, particularly in the detection of potential metastatic sites and the recurrent lesions. They have a major role in the pre-therapeutic diagnosis of primary ocular melanomas and their recurrence. Though, specifically not indicated, it is often useful for incidental detection of primary cutaneous melanomas and also, the primary melanomas occurring elsewhere. With adequate and appropriate clinical details, an experienced cytopathologist, acquainted with the varied cytomorphologic spectrum, in particular of amelanotic melanomas can provide a reasonable diagnosis in most cases. The use of ancillary aids such as immunocytochemistry (ICC) and electron microscopy (EM) are of critical value in the diagnostically challenging cases. The care also needs to be taken when interpreting the pigmented lesions, to exclude the rare lesions simulating melanotic melanomas. Currently, the ultrasound (US) examination of sentinel node with fine needle aspiration cytology (FNAC) is under consideration as a potential, cost-effective alternative to sentinel lymph node biopsy (SLNB). Also, being attempted is the use of cytologic material for the molecular and cytogenetic evaluation to provide useful prognostic information with the possible therapeutic implication. Clinicians should make judicious use of the simple, non-invasive/ minimally invasive cytologic techniques in the management of melanoma patients.

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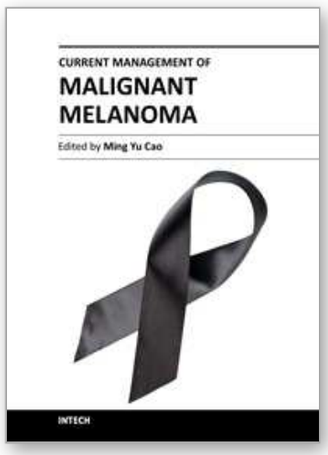

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Management of melanoma is challenging, especially for the late stage of the disease. Development of new therapies and optimizing current treatments are being pursued in attempt to further improve the survival rate. The book provides up-to-date knowledge and experience in early diagnosis, prevention and treatment of melanoma as well as current ongoing clinical studies on melanoma. The book also provides the most recent perspectives of research on the molecular basis of melanoma, such as melanoma associated genes and a possible link between stress and melanoma.

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