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REVIEW ARTICLE

Pitfalls in Cutaneous Melanoma Diagnosis and the Need for New Reliable Markers

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Abstract
Cutaneous melanoma is one of the most aggressive forms of skin cancer, with the development of advanced stage disease resulting in a high rate of patient mortality. Accurate diagnosis of melanoma at an early stage is essential to improve patient outcomes, as this enables treatment before the cancer has metastasised. Histopathologic analysis is the current gold standard for melanoma diagnosis, but this can be subjective due to discordance in interpreting the morphological heterogeneity in melanoma and other skin lesions. Immunohistochemistry (IHC) is sometimes employed as an adjunct to conventional histology, but it remains occasionally difficult to distinguish some benign melanocytic lesions and melanoma. Importantly, the complex morphology and lack of specific biomarkers that identify key elements of melanoma pathogenesis can make an accurate confirmation of diagnosis challenging. We review the diagnostic constraints of melanoma heterogeneity and discuss issues with interpreting routine histology and problems with current melanoma markers. Innovative approaches are required to find effective biomarkers to enhance patient management.

1 Introduction

Cutaneous melanoma is a morphologically heterogeneous malignancy with high metastatic potential that accounts for the majority of skin cancer-related mortalities [1]. Globally, in 2020 there were 324,635 new cases of melanoma diagnosed and more than 57,000 melanoma-related patient deaths [2]. Australia has one of the highest incidences of this cancer in the world (36 cases diagnosed each year per 100,000 people) [2]. Despite the improvements in diagnostic approaches, misdiagnosed cases of melanoma are still reported, resulting in delayed treatment and worse prognosis (5-year survival rates significantly decrease to 61 and 26% for stage III and IV melanoma, respectively) [3]. Conversely,

Key Points

Routine histology in conjunction with ancillary tests, such as IHC, are frequently used for the diagnosis of melanoma.

Specific IHC markers that define melanoma pathogenesis would enhance diagnostic accuracy and improve patient outcomes.

We review the utility of histology and current markers for melanoma diagnosis and suggest a rationale for current/future biologically significant biomarker development to enable accurate melanoma diagnosis.

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overdiagnosis can result in over-management with implications for both patients and healthcare systems; therefore, an accurate melanoma diagnosis is crucial.

Melanomas exhibit a wide range of sizes, shapes and architectural growth patterns, which can resemble numerous benign and other malignant skin lesions [4–6]. The inherent heterogeneity of melanoma may make accurate recognition difficult both clinically and/or histologically, even for expert dermatologists and pathologists. Ambiguous lesions include subsets of dysplastic naevi, atypical spitzoid neoplasms, atypical blue naevi and melanocytomas; when such lesions demonstrate atypical features but have insufficient criteria for a melanoma diagnosis, they are often categorized as 'borderline' lesions to recognize the uncertainty [7–9]. Immunohistochemistry (IHC) has been employed as an adjunct to routine staining to refine the diagnosis of ambiguous lesions, but to date no IHC stain has been found to be entirely sensitive and specific [6]. The current IHC markers used in clinical practice (e.g. S100, Human melanoma black (HMB)-45, Melan-A, microphthalmia transcription factor (MITF) and SRY-related HMG-box gene 10 (SOX10)) can determine melanocytic lineage, but do not define the biological potential or primary pathogenesis for melanoma [10]. Preferentially expressed antigen in melanoma (PRAME) has been recently touted as useful in the diagnostic work-up of melanoma but has limited specificity [11]. Interpretation of these differentiation markers is sometimes inconclusive, thus there is a need for new sensitive and specific melanoma markers.

This review highlights some of the main challenges in melanoma diagnosis and evaluates the utility of current melanoma biomarkers, discussing their shortcomings in sensitivity and specificity, which can lead to less-than-optimal precision and accuracy. We also provide a future perspective on melanoma biomarker discovery, highlighting the need for a new strategy that enables the development of effective biomarkers to achieve accurate melanoma diagnosis.

2 Enduring Challenges in Cutaneous Melanoma Diagnosis

There are many subtypes of melanocytic lesions, for which epidemiological, clinical and histological features are well defined. Genomic data are being increasingly understood for a range of melanocytic lesions, including ultraviolet-driven cutaneous melanomas, acral melanomas, mucosal melanomas, spitzoid lesions and blue naevus-spectrum lesions [8, 12]. The gold standard of diagnosis for benign and malignant melanocytic lesions currently relies on a combination of clinical and histologic assessment, but a subset of challenging lesions remains diagnostically ambiguous with recognized interobserver subjectivity in the interpretation of histological features [13, 14]. The wide range of atypical features occurring in benign melanocytic lesions that mimic melanoma, coupled with the paucity of reliable diagnostic biomarkers for melanoma, can make a definitive diagnosis very challenging. Ultimately, challenges in the diagnosis of melanocytic lesions concern both the overdiagnosis of benign lesions and the underdiagnosis of melanoma, and the resultant impacts of over- and under-treatment on patients and healthcare systems.

2.1 Complications in Clinical Diagnosis of Melanocytic Lesions

Visual inspection of pigmented lesions for suspicious features is the cornerstone of clinical assessment. The A-F rubrik (Asymmetry, irregular Borders, Colour variation, Diameter/size, Evolution (change in size/colour/shape) and "Funny looking" (surface shape/structure/integrity)) is helpful in ascertaining whether a pigmented cutaneous lesion warrants a biopsy/excision [15]. Dermoscopy can be used to aid in the assessment of clinically suspicious lesions and has been shown to improve the clinical accuracy of melanoma diagnosis [16, 17]. While visual detection is the primary tool for clinically recognizing melanomas, it does have limitations. Clinical skin examination can be time-consuming and lesions on non-exposed skin (such as soles of the feet and genital regions) may be less frequently examined than other surfaces such as sun-exposed skin, therefore increasing the risk of missed or delayed diagnosis [18]. As the visual approach primarily relies on patterns of melanin distribution and vascular patterns, amelanotic or hypopigmented melanomas may be more difficult to recognize; indeed, these are often more likely to be brought to clinical attention at a more advanced stage due to other signs such as bleeding or ulceration [19–22]. Furthermore, some nodular melanomas can appear as small, symmetric, uniformly colored lesions at diagnosis, despite already having metastasized [22, 23].

New non-invasive technologies have been developed to facilitate melanoma diagnosis, with varying clinical applications and limitations. Sequential digital dermoscopy imaging (SDDI) has utility in diagnosing incipient melanomas by periodically detecting suspicious changes of the lesion [24, 25]. This technique can be applied for short-term monitoring (median interval of 3 months) to assess single equivocal melanocytic lesions that lack dermoscopic evidence of malignancy to warrant excision at baseline visit or for long-term monitoring (usually at intervals of 6–12 months) in high-risk patients with multiple melanocytic lesions [25]. An observational study showed that using SDDI can improve the sensitivity and specificity of melanoma diagnosis, and reduce unnecessary excisions, compared to using dermoscopy alone [26]. SDDI also aided in the detection of small-diameter melanomas, which usually

have suboptimal diagnostic accuracy [27]. Similarly, total body photography (TBP) monitors the patient's entire skin surface and can reveal new lesions or interval changes in pre-existing lesions, which might not otherwise be noted as suspicious for melanoma [28]. This technique is particularly beneficial for patients at high risk of developing melanoma [28]. Combining TBP and SDDI allowed the detection of thinner melanomas compared to those detected by traditional means from the New Zealand Cancer Registry data [29]. However, the utility of SDDI and TBP in monitoring lower risk populations remains uncertain [30]. Reflectance confocal microscopy can assist with the identification of melanoma for suspicious lesions located on certain chronic sun-exposed areas, regression and amelanotic lesions, but requires technical training and double reader concordance evaluation for better diagnostic accuracy [31, 32]. At present, there is no clear consensus regarding the widespread adoption of these techniques for melanoma diagnosis, and their utility in the clinical setting is the subject of ongoing evaluation.

2.2 Complications with the Histological Diagnosis of Melanoma

Histological assessment is the gold standard for confirming the diagnosis and staging of melanoma. The diagnosis is made in specialist pathology and dermatopathology settings by evaluating a range of cytological and architectural features, by light microscopy, of a given lesion. Features that are commonly in favor of a melanoma include poor circumscription of the intraepidermal melanocytic component, confluence and pagetoid spreading (i.e., upward migration above the basal layer) of the component in dermal-epidermal junction, prominence of cytologic atypia, lack of maturation (i.e., the change in morphology to smaller, less apparent cytoplasm, and less vesicular nuclei as melanocytes descend into the dermis) of the dermal component, infiltrative or expansile growth pattern, and elevated mitotic activity of dermal melanocytes [6, 33]. However, these histologic features are not pathognomonic for melanoma and can also be present, albeit to limited degrees, in a wide spectrum of benign naevi, resulting in substantial interobserver variability of assessment for some difficult melanocytic lesions [6, 7]. Histological differential diagnoses of melanoma include, but are not limited to, dysplastic naevi, blue naevi, spindle and epithelioid cell naevi (Spitz naevi), pigmented spindle cell naevi (Reed naevi), deep penetrating naevi, recurrent naevi, lentiginous junctional naevi and BRACA1-associated protein (BAP1)-inactivated melanocytic tumors (BIMTs) (some of the most common entities that closely resemble melanoma histopathologically are shown in Fig. 1, and other details of less common histologic mimics are described elsewhere [6, 34]).

Criteria for diagnosing a dysplastic naevus are broader than Clark's original description, but these lesions are generally characterized by epidermal involvement by a basal-predominant population of melanocytes, with irregular nested pattern and bridging across adjacent rete ridges (i.e., epithelial downward projections of the epidermis into the dermis), limited lentiginous proliferations and scattered nuclear atypia such as pleomorphism and prominent nucleoli [6, 34] (Fig. 1). The dermal components can be associated with eosinophilic lamellar fibroplasia and lymphohistiocytic inflammatory infiltrate, but generally demonstrate maturation [34] (Fig. 1). The World Health Organization (2018) recommends classifying dysplastic naevi as either 'low-grade dysplastic naevus' or 'high-grade dysplastic naevus' depending on the severity of atypia; however, consistent interobserver concordance for the upper and lower ends of the spectrum remains imperfect [35]. Although individual dysplastic naevi rarely progress to melanoma, their importance lies in that they may indicate a higher risk of melanoma for the patient (particularly when there are multiple lesions) and may be at risk of underdiagnosis when only partially sampled (e.g., for punch or shave biopsy) [36].

Blue naevi usually exhibit deep dermal or even subcutaneous involvement by spindle-shaped melanocytes with abundant melanin pigment [6] (Fig. 1). Blue naevi sometimes closely simulate metastatic melanoma, while blue naevus-like melanoma (BNLM) can often arise in the background of a blue naevus and have overlapping morphologic features [6, 34]. Spitz naevi comprise a characteristic population of spindle cells with large nuclei and prominent nucleoli (Fig. 1), epithelioid cells with polygonal cytoplasm and distinct borders, or a mixture of both [6]. Junctional nests are typically vertically oriented with artifactual clefting around nests. Maturation with depth is often not well developed or complete, and this may be a confusing feature [34]. Reed naevi, a Spitz naevi variant, are characterized by junctional or compound nests of uniform and pigmented spindle melanocytes with variable lymphocytic infiltrate [34]. Both Spitz naevi and Reed naevi can show alarming features that raise concern for melanoma. Deep penetrating naevi classically have a wedge-shaped dermal component with mild cytologic atypia, epithelioid/spindle-cell melanocytic nests, and heavily pigmented melanophages that extend deeply into the reticular dermis and the subcutaneous layer, while the junctional component is often well demarcated and inconspicuous [6, 37] (Fig. 1). Interestingly, atypical deep penetrating naevi (and non-malignant spitzoid neoplasms) are rarely associated with lymph node deposits that do not portend the same risk of progression as bona fide melanomas, but may nonetheless be difficult to distinguish from true melanoma [37].

Recurrent naevi re-emerging at the site of a previously excised pigmented lesion can cause a significant diagnostic

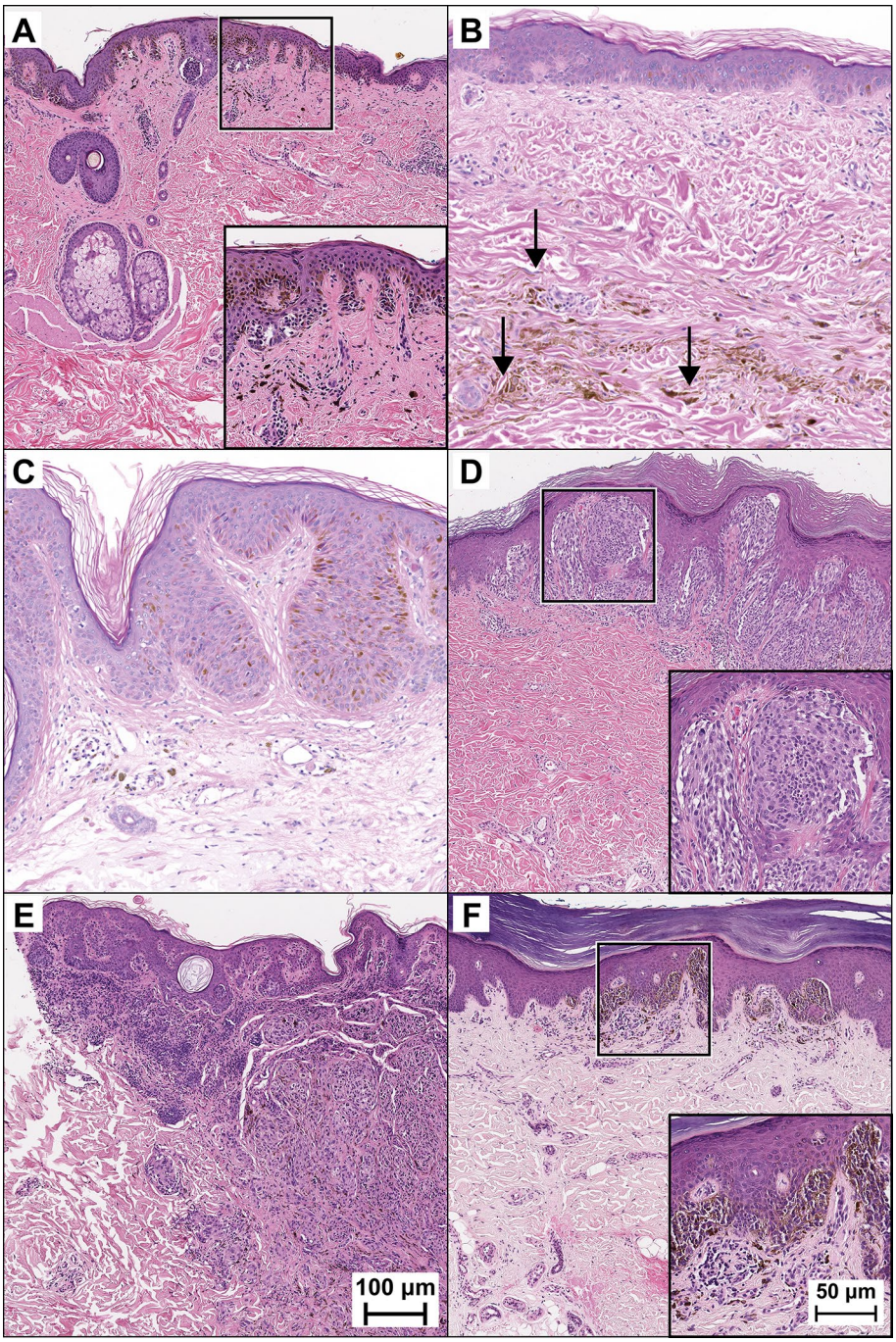

Fig. 1 Histological mimics of melanoma. Haematoxylin and eosin staining of histological mimics. **A** Low-grade dysplastic nevus, showing junctional proliferation of melanocytes with nesting, bridging and lamellar fibroplasia (zoomed-in inset). **B** Benign blue nevus, showing pigmented spindle cells within the reticular dermis (Arrowhead). **C** Pigmented seborrheic keratosis, showing acanthosis, hyperkeratosis, hyperpigmentation and horn cysts. **D** Spitz naevus, showing plump Spitzoid cells with prominent nucleoli (zoomed-in inset). **E** Combined naevus with components of deep penetrating naevus (right) and conventional compound naevus (left). **F** Acral junctional naevus, showing architectural features that mimic melanoma in situ along the junctions (zoomed-in inset)

dilemma by sharing similar histologic features with regressing or traumatized melanoma, including suprabasal movement of melanocytes, greatly variable melanocytic nested and single cell growth patterns overlying the dermal scar and cytologic atypia [34, 38]. Lentiginous junctional naevi typically show proliferation of solitary units and nests of melanocytes predominantly clustered around the tips of elongated and pigmented epidermal rete ridges, with variable chronic inflammatory infiltrate in the dermis; they may resemble lentiginous forms of melanoma if cytological atypia is present (but not severe) and significant pagetoid spreading is not acceptable [39]. Cutaneous BIMTs are characterized by loss of BAP1 protein in epithelioid dermal melanocytes and can appear histologically either as a sheet-like growth pattern of uniformly large epithelioid cells or biphasic pattern of small nevoid cells and large epithelioid cells [40]. Due to a spitzoid histopathological appearance with a similar degree of cytologic atypia, differential diagnosis of BIMTs can range from benign Spitz naevi to nevoid melanoma [40].

The WHO defined nine distinct subtypes or 'pathways' of melanoma based on their clinical, histologic, epidemiologic, and genomic characteristics [12, 35]. Recent advances in genetics reveal that melanoma pathogenesis does not simply follow a linear biological progression, but can occur through multiple pathways (in which some intermediate steps may be bypassed), or through other non-linear biopathways [8]. The neoplastic proliferation is initially triggered by gain-of-function mutations of growth-promoting genes (e.g., *BRAF* and *NRAS*) and usually followed by loss-of-function mutations in tumor suppressor genes (e.g., *CDKN2A*, *TP53*, and *NF1*) to fully transform into malignant melanoma [8, 12]. 'Borderline' lesions harbor the same mutations or fusions of single driver oncogenes as melanoma, but lack or have insufficient subsequently critical genomic alterations to establish the malignant clinical behaviour of melanoma [8, 12]. Many atypical variants of the lesions described above fall into this subset, and although this classification is imprecise, it recognizes that diagnosis is difficult and biological behaviour is therefore uncertain. The overall mutation burden as well as the malignant potential of such lesions lies intermediate between that of overtly benign nevi and unequivocally malignant melanoma [8]. A range of descriptive diagnostic terms have been proposed for problematic cases in which clinical and histological features of the lesions are not immediately clear to render a definitive diagnosis. These include atypical intraepidermal melanocytic proliferation (AIMP), melanocytic tumours of uncertain malignant potential (MELTUMP), superficial atypical melanocytic proliferation of uncertain significance (SAMPUS), and intraepidermal/dermal borderline melanocytic tumours (BMT)) [7]. For example, the term AIMP is used to refer to a high-grade intraepidermal melanocytic dysplasia that cannot be determined as benign, or is already at an early evolving melanoma stage designated as in situ; and the term MELTUMP is used to refer to an atypical melanocytic lesion that has a biological behaviour suggesting an intermediate metastatic potential between nevus and malignant melanoma [7, 41]. These terms still suffer from subjectivity, are not widely adopted in regular pathology practice, and are not WHO-recognized diagnostic entities.

On occasion, ancillary techniques such as fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), IHC and next generation sequencing (NGS) may be utilized to aide histopathological diagnosis; however, these ancillary tests currently have a limited role [9, 42]. Although FISH tests can assess unbalanced genomic aberrations in melanocytic lesions, they can only evaluate a limited number of chromosomal loci, and discordant results have been reported in a subset of cases [43]. While CGH has the advantage of detecting copy number aberrations by interrogating the entire genome, it requires large amounts of tissue and can be impaired by tumour cytogenetic heterogeneity [44]. In contrast, IHC is readily accessible, relatively inexpensive, and is reproducible, provided that the biomarker being used is both sensitive and specific [45–47]. A recent study recommended the combination of specific IHC-labelling and NGS analysis on differential mutational landscapes between spitzoid neoplasms and melanoma to provide better insight on lesion biology [48]. Nevertheless, further studies are still required to evaluate feasibility and clinical relevance of this approach for routine diagnostic work-up of ambiguous lesions.

3 Applications and Potential Pitfalls of IHC Markers in Melanoma Diagnosis

Proliferative and cellular markers have a limited capacity to differentiate melanomas from benign melanocytic lesions. The number of Ki-67-positive cells in a melanoma (10–30%) is usually higher than in benign naevi (< 5%), but these cut-offs remain imprecise and unreliable [49–54]. For instance, there are notable exceptions where mitotically active naevi can exhibit a high Ki-67 labeling index, while some slow-growing melanomas may display negative Ki-67 immunolabelling [42]. An increase of non-histone proliferating cell nuclear antigen (PCNA) labelling cells tends to correlate with the malignant potential of the lesions [55, 56]. The cell cycle inhibitor $p16^{INK4a}$ protein, a negative discriminator of melanoma, is unable to reliably distinguish Spitz naevi and melanoma [57]. Severely atypical cellular blue naevi can also exhibit p16 loss, while some melanomas retain p16 expression, indicating an additional pitfall in diagnosis [42, 58]. It has been recommended to use proliferative markers in combination with one or more melanocytic differentiation biomarkers to ensure only mitotic melanocytes are counted, rather than other cellular constituents such as lymphocytes, to avoid overestimation of proliferation [42].

Other non-melanocytic cutaneous lesions can occasionally present with histological features that raise the possibility of a melanoma in routine histology assessment. Some of these include pigmented and non-pigmented forms of actinic keratosis and poorly differentiated forms of malignancies such as squamous cell carcinoma, basal cell carcinoma and Merkel cell carcinoma [6, 59, 60]. In such cases, melanocytic and other appropriate lineage biomarkers are extremely useful, and oftentimes essential, to differentiate these lesions [42]. Most of the frequently used IHC markers in melanoma diagnosis are used for this purpose, and are proteins typically involved in melanosome biogenesis or melanocyte differentiation, including S100, HMB-45, Melan-A, MITF, SOX10 and PRAME [42, 61].

The S100 protein was one of the first biomarkers used to aid in melanoma diagnosis and remains the most sensitive marker for melanoma (97–100% sensitivity) [61] with

a strong, diffuse nuclear and cytoplasmic labelling pattern (Fig. 2). However, S100 immunoreactivity can be weak and focal in some melanomas (Fig. 3). Despite its high sensitivity, S100 is not a specific marker for melanoma (75–87% specificity) [61] and can be detected in nerve sheath cells, myoepithelial cells, adipocytes, chondrocytes and Langerhans cells as well as cancer derivatives of these cell types.

HMB-45 is a biomarker that recognizes the melanosome structural glycoprotein gp100 and exhibits high specificity (91–100%) for melanoma [61], but there is a wide range of reported sensitivity (69–93%); notably, HMB-45 is sensitive for primary melanoma, but its immunoreactivity is reduced in metastatic melanoma [62, 63] (Fig. 4). It has been observed that HMB-45 immunolabelling is confined to the superficial dermis and intraepidermal melanocytes with sparing of deeper melanocytes in many benign naevi, while in early melanoma it usually displays isolated distribution or clusters of cells throughout the dermis [64, 65] (Fig. 2). However, some melanomas do not have HMB-45 labelling (Figs. 3, 4) and uniform immunolabelling with HMB-45 is a hallmark of blue naevi and deep penetrating naevi [5, 42].

Melan-A, also known as melanoma-associated antigen recognized by T cells (MART-1), is an essential protein for melanogenesis that has also been used to confirm a diagnosis of melanoma [66]. Melan-A is less sensitive than S100 (75–92%), but has higher specificity for melanocytic neoplasms (> 95%) [61] (Figs. 2, 3). However, negative immunolabelling with Melan-A is occasionally observed in melanoma (Fig. 4), and vigilance is required as Melan-A immunolabelling of melanocyte dendritic processes along the dermal-epidermal junction in sun-damaged skin can result in false-positive diagnosis of melanoma in situ, due to overestimating melanocyte concentration and confluence [67]. Melan-A is controlled by MITF, which plays a key role in melanocyte differentiation and pigmentation by upregulating the machinery involved in melanosome biogenesis [68]. The most outstanding characteristic of MITF is its clear nuclear expression, which assists in the quantification of melanocytes without the interference from cytoplasmic pigment [69]. Furthermore, MITF does not label dendritic processes of melanocytes, and thus can precisely label melanocytes within atypical intraepidermal melanocytic lesions [69]. Despite its sensitivity (81–100%) [61], MITF immunolabelling has been found in an unexpectedly large number of non-melanocytic cell types and their tumour derivatives (e.g., macrophages, lymphocytes, fibroblasts, Schwann cells, smooth muscle cells, some breast carcinomas and renal cell carcinomas) [70].

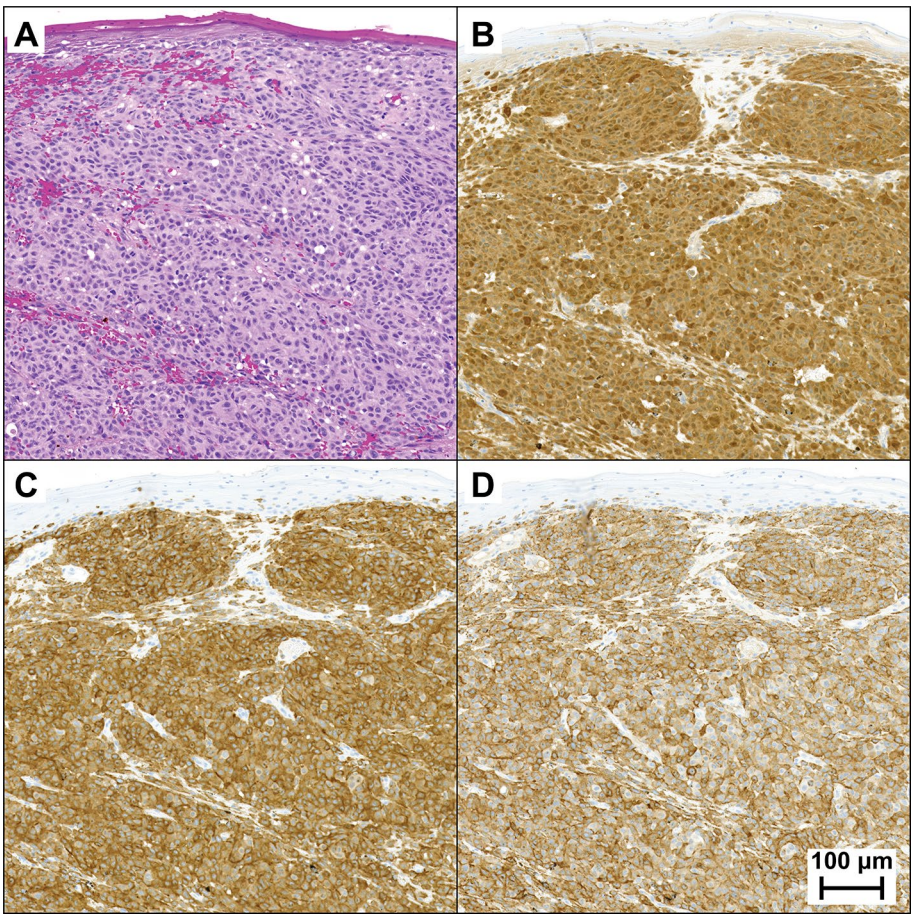

Fig. 2 Cutaneous melanoma of the anterior neck region. **A** Haematoxylin and eosin staining of malignant melanoma with tumor invading into the dermis. **B** Strong cytoplasmic and nuclear S100 immunolabelling. **C** Strong cytoplasmic and membranous Melan-A immunolabelling. **D** Membranous and some cytoplasmic HMB-45 immunolabelling

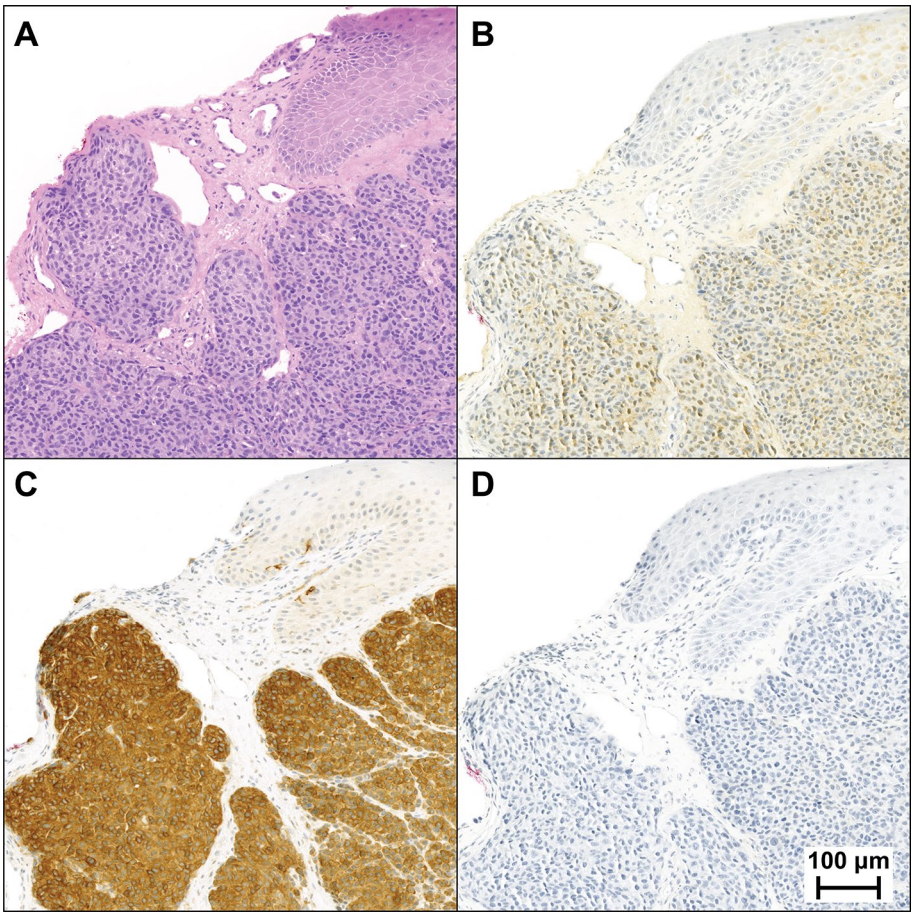

Fig. 3 Skin punch biopsy of malignant melanoma from the left foot. **A** Haematoxylin and eosin staining, showing nodular and nested proliferation of naevoid cells within the dermis. **B** Weak cytoplasmic and membranous S100 immunolabelling. **C** Strong positive cytoplasmic Melan-A immunolabelling. **D** Negative HMB-45 immunolabelling

MITF also regulates the expression of SOX10, a nucleocytoplasmic protein critical for melanocytic cell development, which is a nuclear melanocytic marker with relatively high sensitivity (81–100%) and specificity (88–100%) for melanoma [71, 72]. SOX10 can be particularly useful for the purpose of delineating subtle melanocytic proliferations (e.g., lentigo maligna, desmoplastic melanoma and subtle invasion in fibrosis or inflammation underlying melanoma in situ), but its labeling of scattered cells within scar tissues should be carefully considered to avoid confusion [73, 74].

PRAME has recently received significant attention as an emerging melanoma biomarker [11, 75]. In a study involving 400 melanocytic tumours, focal immunoreactivity of PRAME only presented in a minor population of melanocytic nevi (13.6%), whereas it was diffusely expressed in the majority of primary (83.2%) and metastatic melanomas (87%) [11]. However, junctional melanocytes in solar lentigines or chronically sun-damaged skin can also display nuclear labelling for PRAME, indicating possible pitfalls in the distinction of lentigo maligna from melanocytic hyperplasia [11]. Further studies support the potential of PRAME IHC in the assessment of ambiguous atypical melanocytic proliferations, but also acknowledge its limitations, especially in evaluating desmoplastic and spitzoid neoplasms [76, 77].

The individual markers discussed above lack the ability to consistently provide a definitive diagnosis on their own; panels of antibodies are often advocated to achieve a better chance of reliability in melanoma diagnosis but still suffer limitations [52, 78–80].

Some melanoma variants display low sensitivity labelling with current markers. Spindle cell melanoma and desmoplastic melanoma are relatively rare variants of melanoma, which are characterized by atypical spindled malignant melanocytes with the involvement of collagenous stroma [6, 81]. Spindle cell melanoma and desmoplastic melanoma can be difficult to recognize by clinical visual screening as these variants of melanoma can be deeply invasive and are frequently amelanotic, lacking specific or recognizable clinical features [81, 82]. Histologically, spindle cell melanoma and desmoplastic melanoma usually lack features of conventional melanoma and may be incorrectly diagnosed as hypertrophic scars or Spitz naevi [6, 83]. Moreover, they display a different immunophenotype to other subtypes of melanoma that typically label with S100 and SOX10, but are negative for MHB-45, Melan-A and MITF [6]. Although p75 neurotrophin receptor, nestin and vimentin are highly expressed in spindle cell melanoma and desmoplastic melanoma, they are not specific for melanoma and can be expressed in a wide

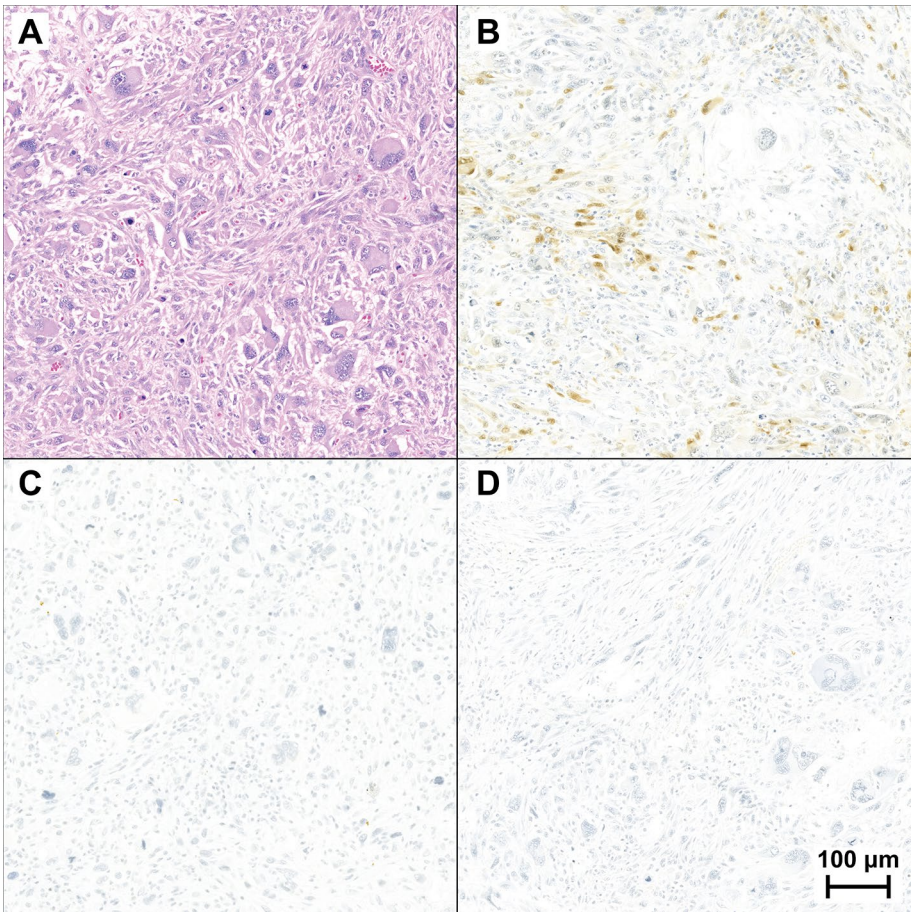

Fig. 4 Metastatic amelanotic malignant melanoma of the chest wall, which presented following the excision of a primary cutaneous melanoma. **A** Haematoxylin and Eosin staining of melanoma with spindled appearance and sarcomatoid differentiation. **B** Focal positive S100 immunolabelling. **C** Negative Melan-A immunolabelling. **D** Negative HMB-45 immunolabelling

range of mesenchymal-derived tumours and malignant spindle cell tumours [61, 81, 82]. Thus, spindle cell melanoma and desmoplastic melanoma remain as challenging variants in melanoma diagnosis.

While a wide range of IHC markers have been utilized in clinical practice for melanoma pathology assessment, this technology is still subject to significant problems with sensitivity and specificity. A range of IHC markers are supportive for melanocytic differentiation but do not reliably determine biological behaviour. The choice of which marker to use is debatable, and utilization of the various markers varies from one histopathology laboratory to another [10]. The potential diagnostic pitfalls of each biomarker should be acknowledged to help avoid both misdiagnosis and overdiagnosis, but until reliable biomarkers are discovered and properly validated this will continue to be a problem in clinical practice.

4 Conclusions and Future Perspectives

The high level of heterogeneity across naevi, melanomas and a range of other skin lesions can make the diagnosis of melanocytic lesions by both clinical and histological means difficult, and reliable diagnosis is an enduring challenge. IHC has an ancillary role to light microscopic assessment by haematoxylin and eosin staining, but its current strength lies with differentiating melanocytic lesions from non-melanocytic tumours, rather than determining the biological potential of an individual melanocytic lesion. Dedifferentiation of the cell of origin can occasionally occur during melanoma progression, and the loss of some or all of the melanocytic markers is a potential diagnostic pitfall [84]. None of the IHC markers currently used in regular pathology practice demonstrate sufficient sensitivity and specificity to consistently differentiate melanomas from benign melanocytic lesions, and their adoption across pathology practice is not standardized [10].

The recognition of ‘borderline lesions’ reflects not only the practical limitations of current diagnostic approaches, but also the complications within evolutionary stages of melanoma pathogenesis. These lesions serve as precursors of malignant transformation, although they are biologically heterogeneous and have a variable, usually low, risk of progression [12]. Ancillary genetic studies have the potential to supplement histological diagnosis, but many aspects of borderline lesions remain to be elucidated. This subset of lesions should always be investigated as parts of a validation

cohort for future melanoma biomarker development and any changes in mutational landscape should be carefully considered as it can inform on the risk of aggressive behaviour for these ambiguous lesions. Furthermore, the patterns and rates of progression of melanoma between individual patients is not uniform, with some tumours developing the potential to spread early in their course versus others that remain localized or as microscopic metastases for long periods before proliferating and spreading [85]. Adding to this complexity, when an initial presentation of melanoma is as metastatic disease, it may be difficult to determine the primary melanoma because of either spontaneous regression or a history of a previous lesion that has been incorrectly interpreted as a benign neoplasm and/or excised without adequate clinicalpathological analysis [86]. Identifying the alterations during initial events of melanocyte malignant transformation and assessing melanoma dormancy may pave the way for earlier melanoma diagnosis and better melanoma patient management.

A common approach for melanoma marker discovery is analyzing gene expression by microarray analysis using RT-PCR/RNA seq, or protein expression by proteomics using mass spectrometry, to seek differentially expressed genes/proteins in melanoma compared to normal tissue. While these omics analyses have extremely high technical validity, they may encounter numerous challenges in specimen collection, data processing and result interpretation [87]. Tissues selected for evaluation can be partially normal/non-malignant, or contain inflammatory cells and other tumour microenvironment constituents in addition to the tumour, and the tumour microheterogeneity and metabolic variability can be underestimated. Most of the potential markers yielded from these high-throughput omics approaches are composite sets of markers that individually lack sensitivity or specificity, markers of secondary pathogenesis, or indicators of downstream pathogenesis such as inflammation, and consequently fail to report on the primary pathogenesis of the melanoma.

The need for highly sensitive/specific melanoma markers necessitates a change in thinking and approach to identify reliable markers that have been properly validated for optimum clinical utility. While bioinformatics yields a very large set of potential markers, it is important to view this in the context of a biological pathway(s) that connects directly to the primary pathogenesis. Connecting a set of markers to a defined cell biological pathway enables mechanistic studies on molecular machinery that is involved in the cancer pathogenic process. However, this still does not connect the mechanistic biology directly to the primary cancer pathogenesis. To achieve this important outcome, the potential markers must be analyzed on cancer tissue in a test cohort and shortlisted candidates are only selected for further evaluation based on their capacity to identify target pathology. These candidate markers must then be properly validated and cross-validated on large independent patient cohorts of melanoma tissue from highly annotated biobanks. It is time for an innovative approach to melanoma marker discovery that incorporates all of the key parameters described above. High-sensitivity, high-specificity biomarkers will improve clinical pathology practice and patient management to achieve better outcomes for patients with melanoma.

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