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ORIGINAL ARTICLE

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Primary malignant melanoma of the gallbladder in dysplastic naevus syndrome

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Abstract A case of gallbladder involvement by malignant melanoma in a 57-year-old woman is reported. The gallbladder, resected for cholelithiasis, harboured a pedunculated polypoid dark mass, which histologically revealed sheets and nests of epithelioid cells with hyperchromatic nuclei in the lamina propria and at the junctional level. These cells were pigmented (with positive reaction with Schmorl's stain and bleaching with peroxide) and showed immunohistochemical positivity for S-100, gp 100 antigen (HMB-45 antibody) and vimentin. The patient, affected by dysplastic naevus syndrome, had a melanoma in situ excised from the scalp 8 years earlier. The features of the investigated lesion address towards a diagnosis of primary gallbladder melanoma. Furthermore, this is the first time that the existence of such a controversial entity is sustained by the ultrastructural investigation of melanosomes, demonstrating the presence of two melanocitary populations, a typical one exclusively junctional and an atypical one both at the junctional level and in the lamina propria.

Keywords Gallbladder · Primary malignant melanoma · Electron microscopy · Dysplastic naevus syndrome

Introduction

Gallbladder primary malignant melanoma is a rare and controversial entity; 28 cases have been reported, but many divergences in their evaluation do exist. As a matter of fact, widely accepted objective criteria of primitivity are lacking. The proposed requirements for primitivity assessment [13, 18] have been often criticised [14, 17], so that many of the reported cases of primary malignant melanoma of the gallbladder have been questioned [13, 20], and doubts about the possible occurrence of such a pathology have been raised [14, 20]. Having established that melanoma is the neoplastic type most frequently responsible for metastatic involvement of gallbladder, accounting for over 50% of such events [7, 20], and that about 15% of disseminated melanoma involve gallbladder [20], justifies this scepticism. The present paper is significant in this context, because it not only deals with a gallbladder melanoma that fulfils the main reported (although discussed) requirements for primitivity assessment, but also, for the first time, produces an objective evidence of primitivity, demonstrating at an ultrastructural level the presence of a double population of melanocytes: a typical one exclusively junctional and an atypical one both junctional and infiltrating the lamina propria. The previous excision of a scalp primary melanoma in the same patient does not contradict the primitivity of the present gallbladder involvement, since the former was in situ and, thus, had a negligible risk of metastases [9] and was separated from the present lesion by an 8-year time lapse; moreover, the patient is affected by dysplastic naevus syndrome, a condition which increases the risk of developing primary melanomas [16, 25].

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Clinical history

A 57-year-old Caucasian female complained of epigastric pain. An ultrasound scan documented a cholelithiasis. The patient, affected by dysplastic naevus syndrome, had a melanoma in situ excised from the scalp 8 years earlier. After cholecystectomy, a small dark polypoid lesion attached to the cholecystic body was found, together with gallstones. A malignant melanoma was diagnosed. No metastatic disease was evidenced. The patient's postoperative course was uneventful and, 13 months later, she remains alive and free of any significant symptoms.

Matherials and methods

The gallbladder was fixed in 4% buffered formalin, processed in the usual manner and paraffin embedded. Sections were stained with haematoxylin and eosin; the Schmorl's method and melanin

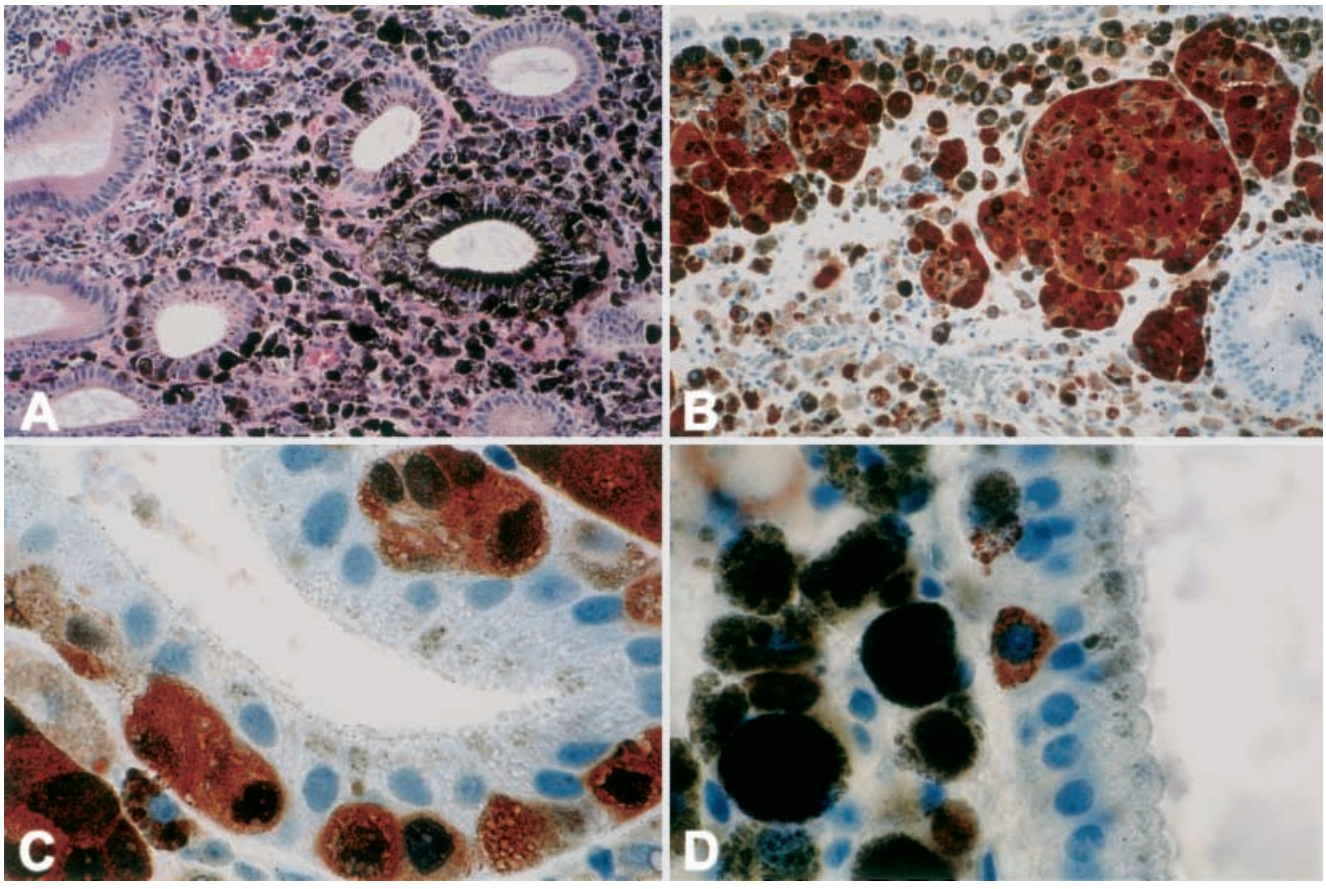

Fig. 1 **A** Sections of the tumour. Melanoma cells are both present at the junctional level and in the lamina propria together with dark melanophages (haematoxylin and eosin; original magnification ×200). S-100 (**B**, **C**) and gp 100 antigen (HMB-45 antibody) (**D**) tumour immunoreactivity. Melanoma cells exhibit red positive reaction with both antibodies [AEC (3-amino-9-ethil-carbazole)-immunoperoxidase; original magnification: ×200 (**B**); detail of the junctional component ×1000 (**C**, **D**)]

bleach with peroxide were performed. For immunohistochemical studies, sections were incubated with antibodies to S-100, gp 100 antigen (HMB-45 antibody) and vimentin (Ylem, Avezzano, AQ, Italy). Following incubation with avidin-biotin-peroxidase (ABC; Vector, Burlingame, Calif.), the reaction was revealed with 3-amino-9-ethil-carbazole (AEC). Specimens for electron microscopy, obtained from histology paraffin blocks (where the small lesion was entirely enclosed), were deparaffinized, post-fixed in 1% osmium tetroxide for 45 min, dehydrated in ethanol and embedded in Epon 812. Thin sections, stained with uranyl acetate and lead citrate, were examined under a Philips EM 400 transmission electron microscope.

Pathologic findings

The gallbladder measured 6×2.5×2.5 cm, with a 0.4-cm thick wall. There was a single dark peduncled polypoid lesion, measuring 9×3×3 mm, attached to the gallbladder body. A cystic duct lymph node was present. Histologically, the lesion was composed of sheets and large nests of epithelioid cells with hyperchromatic nuclei and low mitotic index, confined to lamina propria with a junctional component (that is, the presence of intraepithelial aggregates of melanoma cells; Fig. 1). Brown, granular pigment was abundant; it showed a positive reaction with Schmorl's stain and bleached with peroxide. Immunoperoxidase stain for S-100, gp 100 antigen (HMB-45 antibody; Fig. 1) and vimentin (not shown) was positive. Thus, the morphological and immunohistochemical features of the lesion were consistent with a diagnosis of melanoma. The cystic duct lymph node was free of metastasis. Ultrastructurally, two populations of melanocytes were identified. One population was exclusively junctional, with typical melanosomes that were ellipsoidal with parallel filaments and more or less dense deposits of melanin or their variants (namely spherical stage-II melanosomes, designed "normal" because they contain filamentous structures in parallel array [25]; Fig. 2A, B). The other population was present both at the junctional level and in the lamina propria, with an increased number of mostly abnormal melanosomes that were spherical with melanin present either as finely dispersed radiodense granules or as peripheral deposits partially obscuring the sub-membrane clear zone but sparing the melanosomal centre or with disordered nonparallel melanofilaments (type "granular" or "peripheral deposition of melanin" and "abortive" abnormal melanosomes, respectively [25]; Fig. 2C–E).

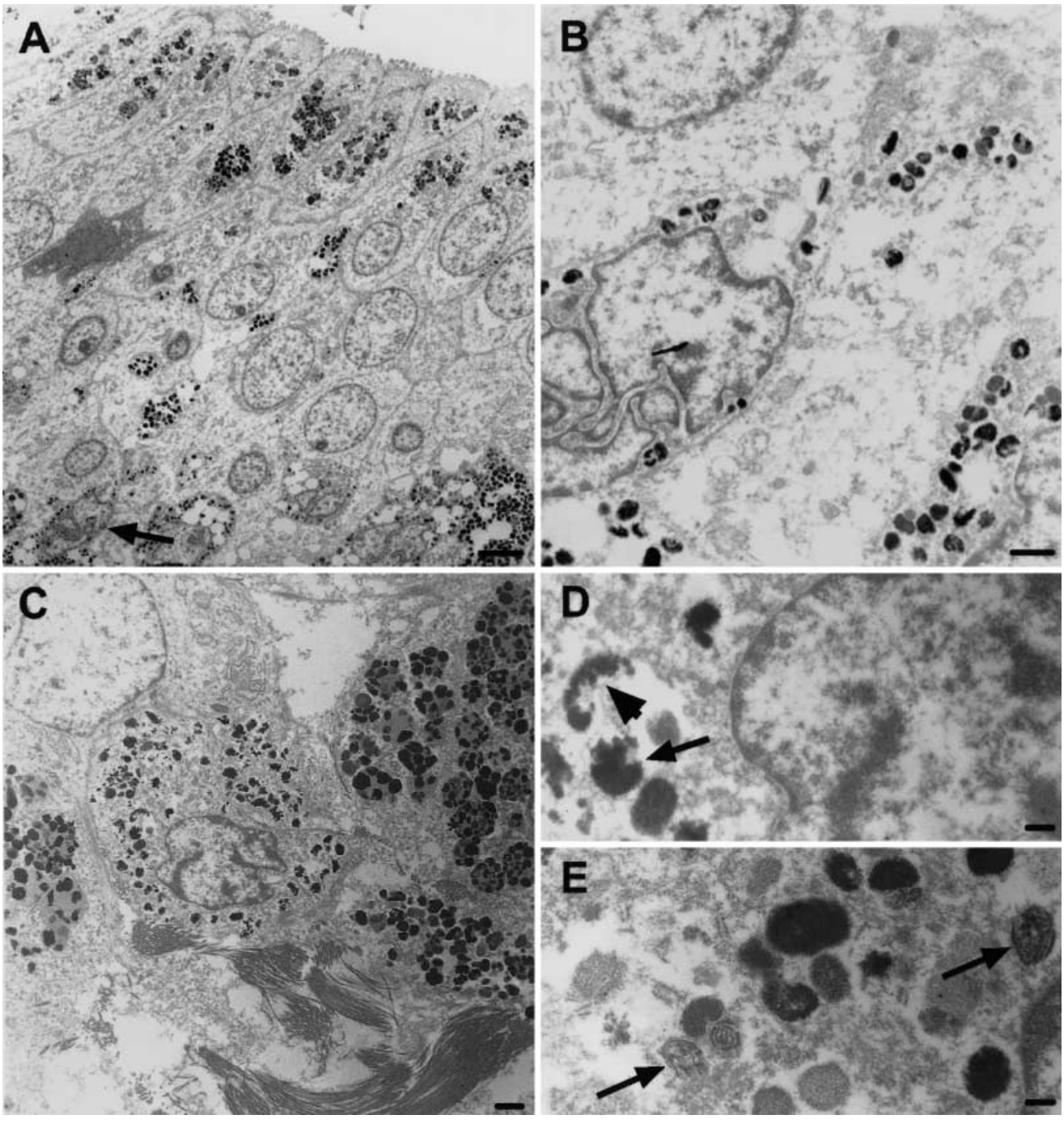

Fig. 2 Electron micrographs of a typical melanocyte found at the junctional level (**A**, **B**) and of melanoma cells (**C**–**E**). **A** Panoramic view shows a typical melanocyte (*arrow*) and the cholecystic epithelium (some cells of which contain melanosomes), together with some melanophages characterised by the presence of compound melanosomes (*scale bar* 4.5 μm; original magnification ×1000). **B** Closer view evidencing the relatively low amount of melanosomes in the same melanocyte (compare with **C**) and their normal morphology [ellipsoidal with parallel filaments and more or less dense deposits of melanin or their variants; namely, spherical stage-II melanosomes, designed "normal" because they contain filamentous structures in parallel array [25] (*scale bar* 1 μm; original magnification ×5000)]. **C** A melanoma cell in lamina propria (*centre*) showing an increased number of melanosomes (compare with **B**). The melanoma cell lies between two melanophages, rich in compound melanosomes (*left* and *right*). Collagen bundles were also present (*bottom*; *scale bar* 1 μm; original magnification ×3150). **D** Most of the melanosomes of the melanoma cell shown in **C** were abnormal [types "granular", spherical and with melanin present as finely dispersed radiodense granules (*arrow*) or "peripheral deposits of melanin", with melanin partially obscuring the sub-membrane clear zone [25] (*arrowhead*; *scale bar* 300 nm; original magnification ×16,000)]. **E** In other melanoma cells, "abortive" melanosomes, with disordered nonparallel melanofilaments, were found (*arrows*) along with granular melanosomes (*scale bar* 300 nm; original magnification ×16,000)

Table 1 Gallbladder primary malignant melanoma (GPMM; 28 cases) are reported. Diagnosis at necropsy is often associated with widespread neoplastic disease. Criteria for assessing GPMM: *I* exclusion of a previous primitive; *II* absence of involved sites other than gallbladder; *III* unicity of lesion; *IV* polypoid or papillary lesion; *V* presence of a junctional melanocitary component

Case no.	Reference	Age (years)	Gender	Time of diagnosis	Tumour size and shape	Junctional component	Metastases at the time of diagnosis	Reasons for considering the tumour as primary[a]	Criteria not satisfied
1	Wieting and Hamdi [35]	40	Female	Necropsy	Single polypoid with broad base, 2×1.2×1.1 cm	Not stated	Yes	No primary tumour elsewhere	II, V(?)[b]
2	Rosenthal [26]	48	Male	Necropsy	Polypoid with narrow pedicle, 4.5×4×2 cm + 3 satellite lesions	Not stated	Yes	No primary tumour elsewhere	II, III, V(?)
3	Pautler and Gallavan [22]	65	Male	Necropsy	Single polypoid, 4×3×3 cm	Not stated	Yes	Possible gall-bladder origin but meninges considered the more likely primary site	II, V(?)
4	Thayer et al. [31]	69	Female	Cholecyst-ectomy	Single papillary, size not stated	Not stated	Yes	Meninges considered the more likely primary site[c]	II, V(?)
5[e]	Walsh [34]	45	Male	Cholecyst-ectomy	Single trilobulated, 7.5×2.5×1 cm	Yes	Yes	Junctional changes	II
6	Jones [15]	72	Male	Necropsy	Polypoid with pedicle, 6.5×3.5×3 cm + a separate macular lesion	Not stated	Yes	Gross morphology + no primary tumour elsewhere	II, V(?)
7	Raffensperger [24]	46	Female	Cholecyst-ectomy	Polypoid, 1.5 cm + a separate macular lesion	Not stated	Yes	No tumour elsewhere (unique metastasis: biliary lymph node)	II, III, V(?)
8[e]	Debiec et al. [6]	71	Female	Cholecyst-ectomy	Single sessile, size not stated	Not stated	No	No tumour elsewhere	V(?)
9	Simmard [30]	74	Female	Cholecyst-ectomy	Multiple plaques	Not stated	Yes	No primary tumour elsewhere	II, III, IV, V(?)
10	Simmard [30]	71	Female	Necropsy	Multiple small polypoid	Not stated	Yes	No primary tumour elsewhere	II, III, V(?)
11	Peison and Rabin [23]	58	Male	Cholecyst-ectomy	Soft dark tissue studded diffusely over the mucosa	No	No	Gross morphology + no primary tumour elsewhere	III, IV, V
12[d]	Peison and Rabin [23]	54	Male	Cholecyst-ectomy	Single polypoid, 4×3×0.7 cm	Yes	No	Junctional component with melanocytes without nuclear atypia + no tumour elsewhere	None
13	Peison and Rabin [23]	50	Male	Cholecyst-ectomy	Single polypoid, 3×2×1.5 cm	No	Not stated	Gross morphology + no primary tumour elsewhere	II(?), V
14	Sierra-Callejas and Warecka [29]	44	Male	Necropsy	Two closely located polypoid, 2×1.5×1.3 cm + 2.2×1.4×1 cm	Not stated	Yes	No primary tumour elsewhere	II, III, V(?)
15	Hatae et al. [11]	72	Male	Cholecyst-ectomy	Single ill-defined solid, 2×2 cm	Not stated	No	No tumour elsewhere	IV, V(?)
16[e]	Carle et al. [4]	47	Female	Cholecyst-ectomy	Single polypoid, 1.5 cm	Yes	Yes	Junctional changes + no primary tumour elsewhere	II
17	Anderson et al. [1]	45	Female	Cholecyst-ectomy	Two separate h polypoid, 4 cm eac	Not stated	Yes	Gross morphology	II, III, V(?)

Table 1 (continued)

Case no.	Reference	Age (years)	Gender	Time of diagnosis	Tumour size and shape	Junctional component	Metastases at the time of diagnosis	Reasons for considering the tumour as primary[a]	Criteria not satisfied
18	Borja et al. [3]	31	Male	Cholecyst-ectomy	Polypoid with broad base, 2 cm + two separate nodules, 3 mm each	Yes	Yes	Gross morphology + no primary tumour elsewhere	II, III
19	Naguib and Aterman [21]	25	Male	Cholecyst-ectomy	Irregular papillary with broad base, 2.2×2.2×0.7 cm + papillary satellite lesion, 0.5 cm	Yes	No	Gross morphology + junctional change + no tumour elsewhere; but a skin biopsy disclosed the possibility of a gallbladder metastasis from a regressed, undiagnosed, malignant melanoma.	I, (?), III
20[d]	Seul [28]	48	Male	Cholecyst-ectomy	Single polypoid with broad base, 1.5×1×1 cm	Yes	No	Gross morphology + no tumour elsewhere + junctional component + initial invasiveness	None
21[e]	Rudolph [27]	51	Female	Cholecyst-ectomy	Single polypoid with broad base, 4.5×3 cm	No	No	Gross morphology + no primary tumour elsewhere	V
22[e]	Verbanck et al. [33]	52	Female	Cholecyst-ectomy	Single polypoid, 5×4×4 cm	Yes	Yes	Gross morphology + no primary tumour elsewhere + junctional component	II
23[e]	Dong et al. [7]	55	Male	Cholecyst-ectomy	Single polypoid with narrow base, 5×2.5×2.2 cm	Not stated	No	No tumour elsewhere	V(?)
24[d]	Heath and Womack [13]	29	Male	Cholecyst-ectomy	Single papillary, 2.5 cm	Yes	No	Gross morphology + no primary tumour elsewhere + junctional component	None
25	Guerini et al. [8]	73	Male	Seemingly cholecyst-ectomy	Single polypoid, 1.8 cm	No	Yes	Gross morphology + no primary tumour elsewhere	II, V
26	Habeck [10]	57	Male	Necropsy	Single polypoid, 4 cm	Not stated	Yes	No primary tumour elsewhere	II, V(?)
27[e]	Hatanaka et al. [12]	51	Male	Cholecyst-ectomy	Single polypoid, 3.5×2×0.8 cm	Yes	Yes	Gross morphology + no primary tumour elsewhere + junctional component	II
28[d]	Velez et al. [32]	62	Female	Cholecyst-ectomy	Single small polypoid	Yes	No	Gross morphology + no primary tumour elsewhere + junctional component	None
29[d]	Present case	57	Female	Cholecyst-ectomy	Single polypoid, 0.9×0.3×0.3 cm	Yes	No	Junctional component + typical melanosomes confined to the junctional melanocytes + Gross morphology	None (restricting principle I to previous invasive melanomas)

[a] As results from the cited papers
[b] Criterion likely not satisfied (lack of concerning data or explicit uncertainty in regard)
[c] The interpretation of the tumour as a possible primary neoplasm comes from later reviews of the literature
[d] Case which satisfies all five criteria listed for assessing GPMM
[e] Acceptable case admitting that criterion II can be escaped if there is simultaneous satisfaction of criteria III, IV and V or I

Discussion

Twenty-eight cases of gallbladder primary malignant melanoma (GPMM) have been reported (Table 1). However, the existing controversies on the subject, mainly because of the lack of definitive objective criteria of primitivity, resulted in the questioning of many of the reported GPMMs [13, 20], to the point that doubts about the possible occurrence of such a pathology have been repeatedly raised [14, 20]. The reported presence of normal melanocytes in the gallbladder [4, 13, 23, 29] supports the possibility of the existence of GPMMs [7]. Nevertheless, the acquisition of further evidence for or against the occurrence of such a pathology would be highly opportune.

The criteria proposed by the specific literature for distinguishing a GPMM from a secondary gallbladder melanomatous involvement are the following: (1) the exclusion of a previous primitive melanoma, (2) the absence of synchronous involvement of sites other than the considered one, (3) the unicity of the lesion, (4) its polypoid or papillary shape and (5) the presence of a junctional melanocitary component [4, 7, 12, 13, 16, 17, 18, 23]. These criteria are, however, very weak, especially if singly taken. In fact, secondary gallbladder melanomas can be polypoid [18] or produce single neoplastic masses [7, 17] or intraepithelial spread, which results in a "junctional component" [7, 20] and can be the only metastatic site [2]. Heath and Womack sustain the necessity of the simultaneous satisfaction of criteria 3–5, with the possible substitution of the latter with principle 1 [13]. However, it could be advisable to accept only cases fulfilling all of the five requirements in order to minimise the possibility of a false primitivity. This would reduce the number of acceptable GPMMs to four (Table 1). Therefore, we looked for further objective evidences for sustaining a primitive melanomatous involvement of gallbladder both in our case and in general terms. We performed an ultrastructural study to distinguish between benign melanocytes and melanoma cells [16], the latter bearing a higher number of melanosomes, largely atypical [25]. The ultrastructural atypical deviations of melanocytes have been studied so far, mainly with the aim of giving additional diagnostic clues in the gamut of primary cutaneous melanocitary borderline lesions [16, 19, 25].

In this case, we are facing a more clear-cut question. Do all of the melanocytes in the investigated lesion present atypical features, as reasonably expected in a metastasis [5], or are there both typical and atypical cells, as in primary cutaneous melanomas [19]. If so, is there any correlation between their features and their location (i.e. junctional or not), as reasonably expected in a primary neoplasm arising in a mucosa containing melanocytes? In our case, the electron microscopy showed the presence of two populations of melanocytes. One was exclusively junctional, with a relatively small amount of largely typical melanosomes (Fig. 2A and B), and the other was both junctional and invading the lamina propria, larger in size and had an increased number of mostly abnormal melanosomes (Fig. 2C–E). These data strongly suggest that the investigated lesion is a primitive one, composed of two melanocitary populations, one benign and junctional, the other malignant and invasive.

In the same patient, the occurrence of a scalp melanoma excised 8 years before cholecystectomy potentially favours a secondary cholecystic involvement [7]. However, this precedent is likely irrelevant since: (1) that melanoma was in situ and thus had a negligible risk of metastases [9],(2) the 8-year time lapse separating the two events, although not excluding a secondary localisation [5, 17, 20], is quite a long time span and (3) the patient's dysplastic naevus syndrome increases the risk of developing primary melanomas [16, 25].

In conclusion, we report a case of GPMM whose primitivity is sustained by a remarkable combination of diverse evidences. In particular, for the first time, an objective sign of primitivity, such as the ultrastructural evidence of a typical junctional melanocitary population is obtained (favouring the acceptance of the existence of GPMM as an entity). In addition, it is the first reported GPMM occurring in a dysplastic naevus syndrome.

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