

CASE REPORT

Amelanotic vulvar melanoma: case report and review of the literature

FLAVIA BADERCA^{1,3)}, SILVIA COJOCARU³⁾, ELENA LAZĂR^{2,3)},
CODRUȚA LĂZUREANU^{2,3)}, RODICA LIGHEZAN¹⁾,
AURORA ALEXA¹⁾, M. RAICA¹⁾, T. NICOLA⁴⁾

¹⁾Department of Histology

²⁾Department of Pathology

"Victor Babeș" University of Medicine and Pharmacy, Timisoara

³⁾Service of Pathology

⁴⁾Department of Oncology

Emergency County Hospital, Timisoara

Abstract

A rare case of amelanotic vulvar melanoma is presented. The patient was a 71-year-old woman complaining of vulvar itching and yellowish vaginal discharge who underwent a complete gynecological evaluation during which a suspicious grey-whitish mass on her vulva was observed. The tumor presented superficial ulceration and was located in the upper half of the labia minora and clitoris. Initially it was suspected to be a vulvar carcinoma. A biopsy was taken and a histopathological suspicion of amelanotic melanoma was rendered. The mass was radically excised and the diagnosis was confirmed using HMB-45, Melan-A and anti-S-100 protein antibodies. Malignant melanoma is readily diagnosed by the presence of melanin granules. Although amelanotic melanoma contains a few melanin granules, it is often difficult to differentiate it from other non-epithelial malignant tumors. This report describes a case of amelanotic melanoma of the vulva, which was correctly diagnosed by immunohistochemical staining with the HMB-45, Melan-A antibody and for the S-100 protein.

Keywords: vulvar melanoma, mucosal melanoma, amelanotic melanoma, immunohistochemistry, HMB-45, Melan-A, S-100.

Introduction

Melanoma is a tumor originating from neuroectoderm. Vulvar melanoma may develop from preexisting junctional or compound nevi as well as de novo from melanocytes resting in the basal layer of squamous epithelium. The malign melanoma has three histologic subtypes: superficial spreading, nodular, and acral lentiginous [1], the second having the worst prognosis.

Vulvar malignant melanomas are extremely rare neoplasms, representing less than 3% of all cancers in women, 9% of all external genital tract malignancies and 9% of all primary vulvar malignancies. Because the vulva is covered by squamous epithelium, about 85% of primary vulvar malignancies are squamous cell carcinomas. However, a wide variety of other malignancies is found on the vulva, including malignant melanoma, carcinoma arising from the Bartholin's gland, basal-cell carcinoma, and soft-tissue sarcoma [2].

In the female genital tract, the vulva is the most common site of melanoma's occurrence, comprising 1.3% of all melanomas, followed by vaginal melanoma, which accounts for 0.3% [3].

Vulva presents interesting characteristics in melanoma development. Although vulva constitutes 2% of body surface, 5% of malign melanomas exist at its level. Vulvar melanoma is the second most frequent (9%) vulvar cancers [1].

The vulvar melanoma incidence peaks between the sixth and seventh decade of life and its prognosis is related to the tumor thickness and lymph node status. The overall five-year survival rate for vulvar melanoma ranges from 25% to 85%, depending on the extent of involvement [3].

In this paper, we present a case of amelanotic melanoma of the vulva, which was initially suspected to be a vulvar carcinoma, but was subsequently correctly diagnosed by HMB-45, Melan-A and S-100 protein immunohistochemistry.

Material and methods

We present a 71-year-old patient with a vulvar tumor. The patient was Caucasian and non-smoker and was admitted in the Department of Oncology from Emergency County Hospital of Timisoara. The tumor was a mucosal lesion, involving labia minora and clitoris.

She had a history of cholecystectomy in 1970 and colon surgery in 1980. The patient has no family history of melanoma or dysplastic nevi.

At the time of diagnosis the patient took oral antidiabetics and anti hypertensive drugs. Her general conditions were satisfactory and her routine blood and urine tests were normal except blood glucose (120 mg/ml). The thorax radiogram was normal.

Clinical observations came from the original records

of the patient, with emphasis on symptoms that brought her to doctor. The primary anatomic site of the tumor, the absence of the pigmentation, the configuration of the tumor and the absence of satellite lesion were noted. The pathologist complemented these data with the macroscopic and microscopic description.

Specimens

Samples were obtained from fresh surgical specimens of a vulvar mass after partial vulvectomy. Specimens were fixed in 4% w/v buffered formalin, embedded in paraffin. Histological sections, 4 μ m thick, were routinely stained with Hematoxylin–Eosin (HE).

Immunohistochemistry

The diagnosis of melanoma was confirmed using monoclonal antibodies against HMB-45, Melan-A, S-100 protein, vimentin and cytokeratin.

Immunohistochemistry was done on formalin-fixed, paraffin-embedded tissue specimens, using the three steps labelled streptavidin–biotin-immunoperoxidase technique (LSAB2, code K0673, DAKO, Denmark).

Sections were dewaxed, rehydrated, washed in distilled water, and then rinsed in PBS (pH 7.2).

Sections were incubated with 3% hydrogen peroxide solution for 5 minutes (step 1, peroxidase block), washed twice with PBS.

After endogenous peroxidase inhibition and antigen retrieval, the sections were incubated with the primary antibodies.

Anti-melanosome, HMB-45 (code N1545, ready-to-use) has been shown to react with a 10 kDa segment of a neuraminidase-sensitive sialylated glycoconjugate present in pre- and early-stage (immature) melanosomes, a glycoprotein thought to be part of the premelanosome complex.

The presence of this antigen indicates an active melanosome formation and thus melanocytic differentiation. It is not expressed in normal resting adult melanocytes, regardless of the degree of pigmentation. Only upon activation, adult melanocytes can re-express the HMB-45-defined antigen.

Applied on formalin-fixed, paraffin-embedded tissues, the monoclonal mouse anti-human HMB-45 needed 20 minutes heat-induced epitope retrieval (DakoCytomation Target Retrieval Solution, pH 9) and 30 minutes incubation at room temperature with the primary antibody. The negative control reagent used for LSAB2 immunohistochemical procedures was Universal Negative Control, Mouse (code N1698).

Melan-A is a transmembrane protein composed of 118 amino acids with unknown function. Anti-Melan-A (code N1622, ready-to-use) was shown to specifically recognize a 20–22 kDa doublet in Melan-A mRNA-positive melanoma cell lines. Melan-A immunolabelling needed a heat induced antigen retrieval, the rest of the techniques being similar to HMB-45. Anti-Melan-A has been reported to stain melanocytes of normal skin and melanomas, Leydig cells, occasionally Sertoli cells, the cortex of the adrenal gland, granulosa cells as well as hilus and stromal cells of the ovary. The cellular staining pattern for Melan-A is cytoplasmic.

Anti-S-100 antibody (polyclonal, code N1573, ready-to-use) identified S-100 positive cells from normal and neoplastic tissues and was used for the differential diagnosis of melanomas or nerve sheath tumors versus carcinomas. S-100 is a 20–30 kDa calcium binding protein which occurs in three dimeric forms: S-100ao, S-100a and S-100b composed of $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$ subunits. It is expressed by glial cells, neurons, Schwann cells, melanocytes, Langerhans cells and interdigitating reticulum cells.

Formalin-fixed, paraffin-embedded tissues were incubated for 10 minutes at room temperature with the primary S-100 antibody. The negative control reagent used for LSAB2 was Universal Negative Control, Rabbit (code N1699).

Anti-vimentin (clone V9) antibody was used as a marker of the optimal fixation and embedding procedures.

The pan-CK antibody was used to confirm the melanoma diagnosis.

After incubation with the primary antibody, the slides reacted with a labelled streptavidin-biotin system and then treated with 3,3'-diaminobenzidine-peroxidase substrate solution, as chromogen (DAB Tablets, S3000) until color was visualized.

Sections were washed twice in distilled water for 5 minutes to stop the reaction, then counterstained in haematoxylin for 5 minutes, washed, dehydrated, cleared in xylene, mounted with DPX, and glass coverslipped. All reagents for the immunohistochemical technique were supplied from Dako, Denmark.

Sections were examined under oil immersion with a $\times 100$ objective on a Nikon Eclipse E-400 microscope, and images were captured using a Coolpix 995 digital camera and a DN-100 digital imaging system.

Histological sections were reviewed independently by two pathologists, and then discussed for consensus.

Results

The patient, aged 71 (gravida 5, para 1, and in menopause for 20 years) presented to a private gynecological cabinet on November 2007.

Her main complaint was the presence of a mass on her vulva that had been increasing in size for two years, in addition to a yellowish discharge and a vaginal bleeding. On a routine examination, the gynecologist found a light grey tumor, involving the upper half of the left labia minora and the clitoral area (glabrous skin). The tumor did not involve the vagina or cervix, which were atrophic. The gynecologist observed that the lesion of the vulva was not pigmented. The lesion, 50 \times 20 \times 15 mm in size, was irregular, vegetative and ulcerated. Bleeding was seen from the ulcer in the centre of the tumor. The uterus was smaller than normal, ovaries were atrophic.

A clinical diagnostic hypothesis of vulvar carcinoma was put forward.

Small forceps biopsy fragments were taken, immediately fixed in 10% v/v buffered formalin and sent for histopathological examination.

On November 17, 2007, the biopsy fragments arrived to Pathology Laboratory. There were four small

tissue fragments, length between 2 to 3 mm and 1 mm thick, white greyish in color. The slides from all tissue fragments were stained with haematoxylin-eosin.

Histological examination of the biopsy specimens revealed a diffuse infiltration of the vulvar mucosa with large pleomorphic tumor cells. The cells were rounded in shape and were arranged in small clusters. The tumor cells had large oval or rounded pleomorphic nuclei, with irregular coarse chromatin and pale eosinophilic cytoplasm. Most of the cells had multiple nuclei with distinct nucleoli. Some of nuclei had large, eosinophilic nucleoli. Few cells presented an intranuclear chromophobe vacuole. These findings suggested a non-epithelial malignant tumor of the vulva.

The patient returned to her gynecologist and she sent her to the Oncology Department of the Emergency County Hospital of Timisoara.

In February 4, 2008, the physical examination detected a vulvar mass, 6×2×2 cm in size, with asymmetry and irregular borders on the left aspect of the vulva. The tumor was involving the left labia minora and clitoris. It was vegetative, ulcerated and infected, but not indurate or tender; no inguinal adenopathy was present. The tumor was bleeding when touched.

On February 7, 2008, the patient underwent partial vulvectomy, which removed the whole tumor mass with a 5 mm normal perineal area, in order to decide the specific treatment plan. The specimen was sent for histopathological examination.

After the operation, the clinical TNM staging according to UICC and AJCC was T4bN0M0, stage IIC.

The patient did not have any problem during the postoperative period except an infection in the partial vulvectomy incision, which was cured with an appropriate antibiotic treatment.

Visual inspection of the biopsied specimen, 6×4×4 cm in size, showed the presence of a grey-white vegetative vulvar mass, 55×20×15 mm in size. The mass was lined by an eroded oozy mucosa covered by a yellowish slime, had a solid, rather homogeneous grey to white cut surface, was lobulated and lined by a greyish pseudomembrane made up of fibrin and remains of the ulcerated squamous epithelium.

Scanning magnification discloses a raised, dome-shaped tumor, with asymmetry (Figure 1). The tumor showed a solid, infiltrating epithelioid cell proliferation with alveolar pattern and indistinct borders. It was confined to glabrous skin. Both compartments of the vulva (skin with hair follicles and glabrous skin) were visible on a few sections, in most cases, the two compartments being visible on different sections.

The overlying epithelium was thin, effaced, and ulcerated on large area. Cohesive nodules or small nests of tumor cells formed the connective component (Figure 2).

The tumor cells were large, amelanotic, round or oval, and displayed euchromatic kidney-shaped nuclei with prominent nucleoli and fine, even distributed chromatin (Figure 3).

Cytoplasm was abundant, pale and eosinophilic. Some of the tumor cells were spindle shaped, with ill defined, lacy cytoplasmic borders (Figure 4).

At first examination, the tumor cell population appeared monotone, but a closer examination revealed frequent cellular and nuclear enlargement, variation in nuclear size and shape, hyperchromasia and prominent nucleoli. Some cells presented an extreme cytologic atypia with very large, irregularly shaped and brightly eosinophilic nucleoli (Figure 5).

An important intraepithelial component was represented by a pagetoid intraepithelial spread consisting of atypical melanocytes that often had large nucleolated nuclei and abundant pale cytoplasm (Figure 6). The melanocytes were present single or in nests. The distribution was uneven and the nests had irregular shapes and showed confluence. Poor lateral circumscription was present, with single enlarged melanocytes found lateral to last nest.

The basal layer of epithelium was replaced by confluent single tumor cells (Figure 7). In other parts, a pagetoid scatter of tumor cells was present. Moreover, because of a large tumor cells amount present in the dermis, the overlying epithelium was thinned, the cuboidal basal epithelial cells being replaced by large, squamous cells, presenting vacuolar changes.

The stromal and inflammatory reaction tended to be inconspicuous. Because of the invasiveness of the lesion, the asymmetric outline was a major characteristic. Extensive and highly irregular junctional tumor nests were found at a variable distance to each other and merged. There was a lack of maturation, manifested by a failure of nests, cells, nuclei or nucleoli to become smaller towards the base of the lesion. These cells contain no pigment deposits.

The distribution pattern and the cytological characteristics of tumor cells were different from one side of lesion to the other. The secondary form of asymmetry was represented by a variable epithelial thickness.

Immunohistochemical findings

A weak and focal HMB-45 labelling was found in tumor cells, particularly in the epithelial component, in addition to superficial situated melanocytes, entrapped within sclerosis or fibrosis (Figure 8). However, HMB-45 staining was generally weaker at the lower edge of the tumor (Figure 9). The positive reaction of the isolated melanocytes present in the epithelium, demonstrated the activation of these cells.

The Melan-A immunolabelling of melanocytes had a cytoplasmic pattern and was more intense than the HMB-45 labelling. The immunoreaction was heterogeneous, some clusters of tumor cells being more positive than other is. The reactivity of Melan-A immunolabelling decreased from strongly to weakly positive or negative toward the same cluster cells. Some mature neoplastic melanocytes were strongly positive for Melan-A (Figure 10). Epithelioid and spindle cells also showed granular positive reactivity in the cytoplasm. Even the melanocytes from basal layer of the epithelium showed a clear positive reaction for Melan-A (Figure 11).

Immunohistochemical analysis revealed that the tumor cells were also positive for the S-100 protein.

All the tumor cells presented an intense, cytoplasmic and nuclear immunoreaction for S-100 protein (Figure 12). Even if the intensity of reaction was almost uniform in the tumor cells, there was some variation of intensity, due to the presence of some large polygonal tumor cells, which expressed an intense but diffuse cytoplasmic and nuclear immunoreaction for S-100 protein. The immunoreaction for vimentin was positive, with a weak and diffuse pattern of distribution. There was no evidence of positive immunoreaction for cytokeratin in tumor cells, with a consistent cytokeratin staining in the normal cells of underlying epithelium. All these findings supported the diagnosis of ulcerated amelanotic melanoma with a prevalent epithelioid cell component.

Tumor thickness

Because the tumor was exceeding 4 mm, we used a fine ruler to measure the tumor thickness according to Breslow. We measured the distance (in mm) between the outermost epithelial layer of the squamous mucosa and the deepest part of the tumor. Because of the ulceration, the upper reference point was the bottom of the ulcer crater.

Tumor invasion

Clark level I–V could be used only in the hairy skin of the labia majora, because the glabrous skin of the vulva has no clearly defined reticular layer in the subepithelial connective tissue. In such cases, tumor invasion must be classified according to Chung system.

The neoplasm was 11 mm-thick according to Breslow and stage IV according to Chung. The lateral and lower surgical margins were free of disease, with no evidence of local spread or metastasis. On the slides, no lymphovascular invasion or satellite lesion have been observed. The histopathological TNM stage was pT4bN0M0, stage IIC, concordant with the clinical stage, in both AJCC and UICC classification systems. Subsequent studies, magnetic resonance imaging or lympho-scintigraphy with technetium-99 colloid albumin, were not performed until the date of writing. The patient had no adjuvant treatment during the last four weeks since surgery, but careful follow-up will be required, at three months interval.

☐ Discussions

Anatomy

For brief review, the vulva's main features are the mons pubis, the labia majora and minora, the clitoris (with its prepuce), the vaginal vestibule delimited by the clitoris, the labia minora, and the fourchette and containing the orifices of the urethra and the vagina, perineal body and their supporting subcutaneous tissues [2]. Covering the outer, lateral part of the labia majora is skin with hair follicles, sebaceous, apocrine, and eccrine glands and subcutaneous fat. The inner (medial) part along with the labia minora is covered by glabrous skin with a stratified, thin, squamous epithelium containing numerous sebaceous, apocrine, and eccrine glands but no hair follicles or fat tissue. The

keratin of epithelium in the outer part of the vulva is gradually replaced with glycogen, so that the vestibule keratinization disappears. The large Bartholin and Skene glands and the small vestibular glands produce mucous in the vestibule, the epithelium of which gradually merges with that of the vagina. Accordingly, the vestibular membrane, strictly speaking, can be designated as a mucous membrane.

The density of melanocytes differs in various parts of the body but is greatest in the face, head, neck, and genital areas. In one study, vulvar autoptic biopsies, from 60 patients (without vulvar disease) revealed the presence of the melanocytes in the basal layer of the vulvar skin and mucosal epithelium in all cases accompanied by a patchy or linear pigmentation [4, 5].

Primary cancer of the vulva is uncommon, accounting for only 3–5% of all gynecologic malignancies and <1% of all cancers in women [2, 6–8]. Approximately 85% of invasive vulvar cancers arise from the squamous epithelium [4]. Malignant melanoma is the second most common vulvar malignancy accounting for 2% to 9% of cases [7]. Melanomas of female genitalia represent 2 to 3 percent of all melanomas [2, 7]. Melanoma has also been reported to occur in the vagina, uterus, cervix, and ovaries, but these instances are extremely rare and seldom curable [7]. Primary carcinoma of the Bartholin's gland accounts for about 5% of vulvar malignancies. Although basal cell carcinomas of the skin are extremely common, they represent only about 2% of vulvar cancers. Sarcomas can arise from any of the supporting mesenchymal tissues of the vulva, but they account for only 1% to 2% of primary vulvar malignancies. Soft-tissue sarcomas of the vulva include leiomyosarcoma, rhabdomyosarcoma, malignant fibrous histiocytoma, angiosarcoma, liposarcoma, and others. Leiomyosarcoma is the most common sarcoma involving the vulva [2]. Vulvar melanomas occur most commonly on the labia minora or the clitoris [1, 5, 6, 9, 10]. Melanoma of the vulva was originally described by Hewett in 1861. Melanoma is primarily a disease of less pigmented skin, with white women developing it more frequently than African American, Asian, or other more heavily pigmented races [2].

Vulvar melanoma has rarely been reported in young or teenage females. Like our patient, most patients were postmenopausal, with a mean age of 73 [6, 8, 9]. This contrasts with cutaneous melanoma whose mean age is 35, with one third of all patients diagnosed before 45 years of age. It could be expected that the frequency of vulvar melanoma would raise as the age of the population rises. In a study of 245 patients with vulvar melanoma over a 25-year period, Ragnarsson-Olding BK *et al.* [5] found that the incidence of cutaneous melanoma had increased but that of vulvar melanoma had not [7].

At the time of diagnosis, our patient had local disease only, in conformity with recent studies, which found that at the time of the initial diagnosis, 65% of the patients had local disease only, approximately one third of patients had regional lymph node involvement, and 5% had distant metastasis [3].

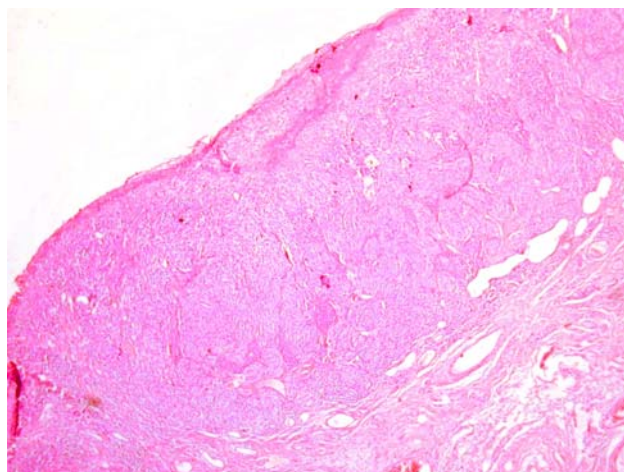


Figure 1 – Scanning magnification shows an asymmetric but well-demarcated melanocytic proliferation with ulceration in large areas (HE, ob. $\times 4$)

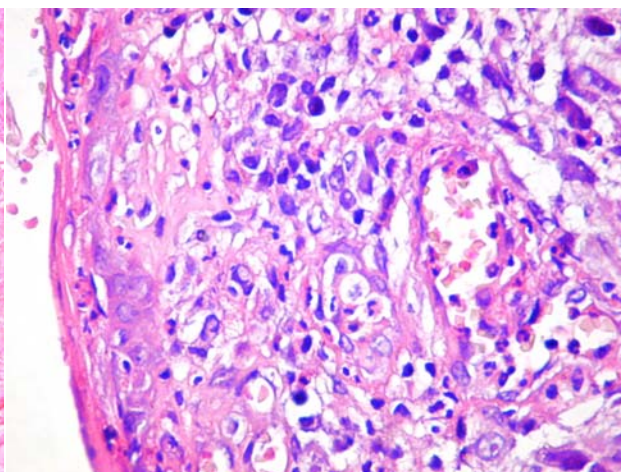


Figure 2 – The ulceration of the overlying epithelium on large areas (HE, ob. $\times 40$)

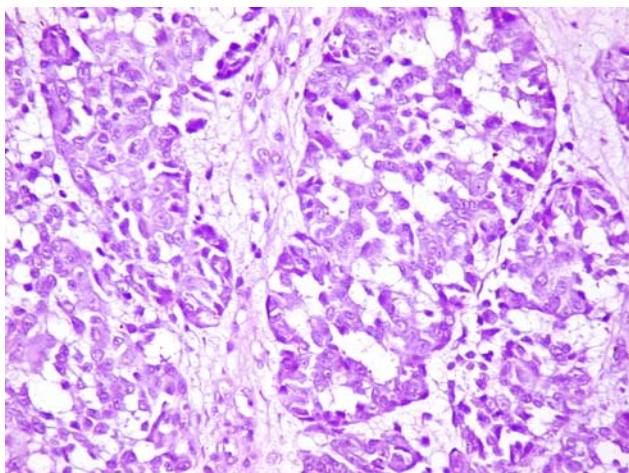


Figure 3 – Histopathological section showing an infiltrating dispersed cell population with alveolar pattern of growth (HE, ob. $\times 20$)

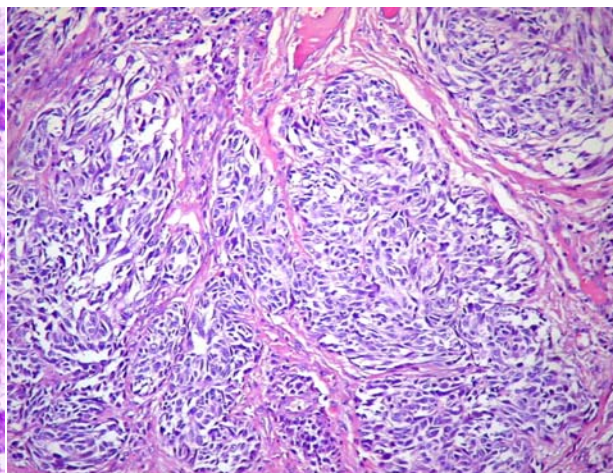


Figure 4 – Spindle tumor cells that infiltrate the connective tissue (HE, ob. $\times 10$)

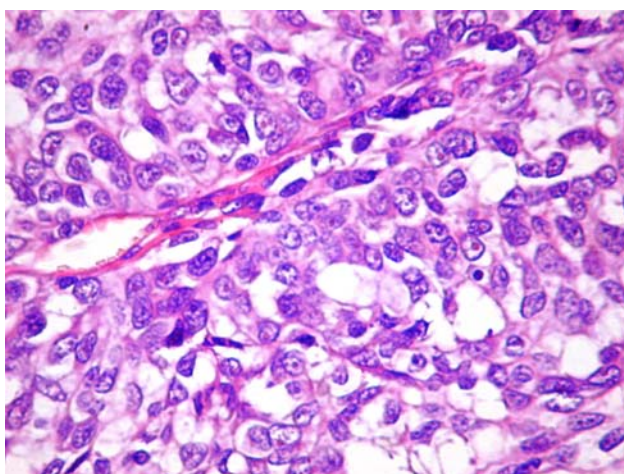


Figure 5 – Loose aggregates of pleomorphic tumor cell, with relatively abundant lacy cytoplasm and granular hyperchromatic large nuclei, with occasional prominent nucleoli (HE, ob. $\times 40$)

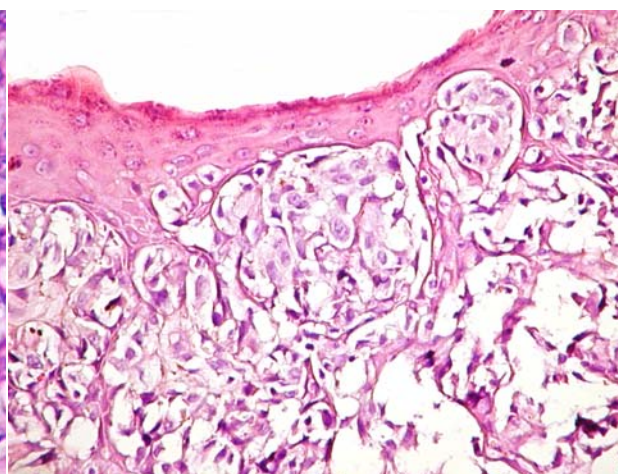


Figure 6 – Clusters of atypical melanocytes at the epithelial-connective junction (HE, ob. $\times 40$)

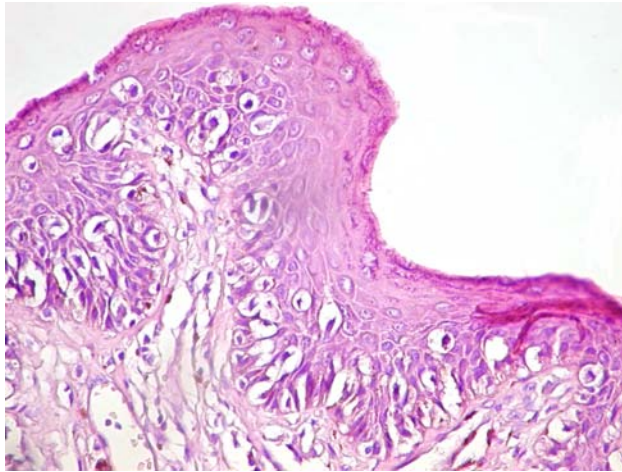


Figure 7 – The overlying epithelium revealed area of superficial spreading melanoma, with atypical melanocytes, isolated or in small clusters, distributed in all epithelial layers (HE, ob. $\times 20$)

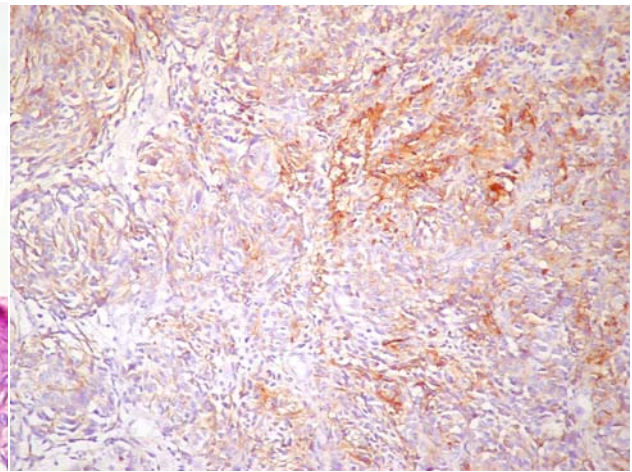


Figure 8 – Immunohistochemical staining with HMB-45 in the vulvar tumor tissue. Note the heterogeneous reaction of tumor cells (ob. $\times 10$)

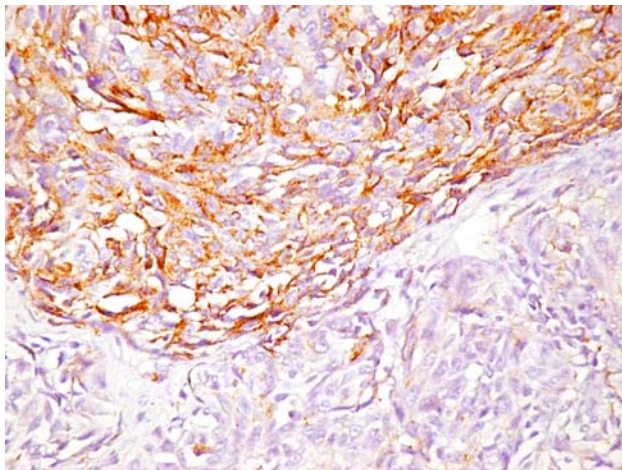


Figure 9 – Intense and diffuse HMB-45 expression of tumor cells. Note diminishment in both intensity of staining and size of the melanocytes at the lower edge of the tumor (ob. $\times 20$)

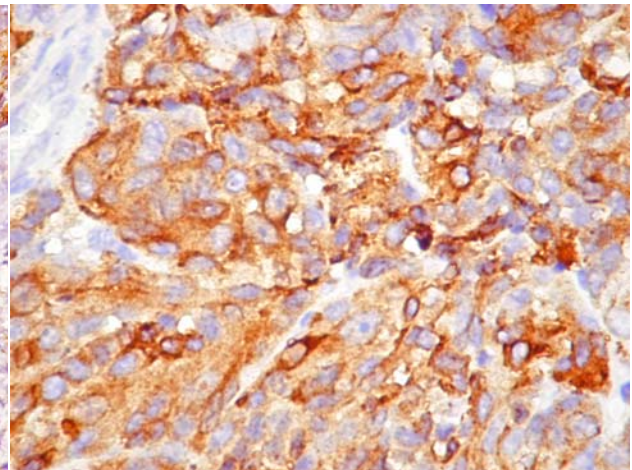


Figure 10 – Intense, diffuse and heterogeneous expression of Melan-A in stromal atypical melanocytes (ob. $\times 40$)

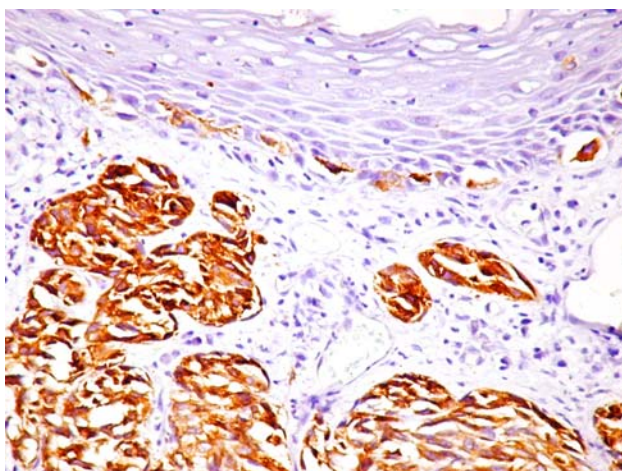


Figure 11 – Scattered pagetoid upward migration of nests and solitary melanocytes in conjunction with confluent, enlarged nested melanocytes (Melan-A immunolabelling, ob. $\times 20$)

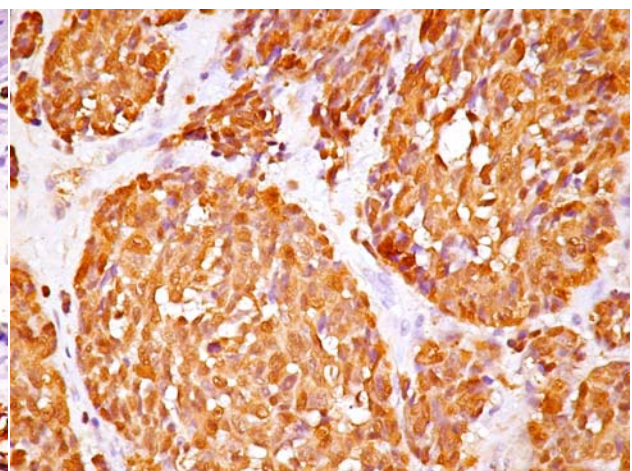


Figure 12 – Immunohistochemical stain for S-100 protein showing diffuse cytoplasmic and nuclear positivity in tumor cells (ob. $\times 20$)

Typical presentations include an asymptomatic pigmented lesion or an identified mass that may be painful or bleeding due to ulceration. Less frequently, patients presented with bleeding, pruritus, swelling, local discomfort and a cystic lesion [6, 7, 9].

Vulvar melanomas are usually pigmented; in a recent review, less than 10% were found to lack pigmentation [4]. In our case, the patient presented with a not pigmented vulvar mass, ulcerated and bleeding, that miss orientated the diagnosis to a vulvar carcinoma.

The tumor may arise from a preexisting pigmented lesion or from a normal-appearing skin. The preexisting nevi can be considered a precursor of superficial spreading melanoma but only in the vulvar hairy skin. In contrast, nodular melanomas of the glabrous skin emerged apparently *de novo* [5, 7]. Our patient had no family history of melanoma or dysplastic nevi and apparently, the tumor derived from normal vulvar mucosa devoid of preexisting lesions.

The melanoma tumor cells always derive from melanocytes, which are located in the basal layer of the epithelium and arise from the neural crest [7].

Although location of the lesion varies, some investigators believe that the location of the lesion correlates with prognosis, for example, a more central location confers a poorer prognosis [7].

Other factors such as parity, genetic, or hormonal influence seemed unrelated to either the occurrence or extent of vulvar melanoma. A correlation between exposure to ultraviolet radiation and the development of malignant melanoma has been known for sometime, but knowing this correlation does not help the understanding of the etiology of vulvar melanoma. It has been suggested that UV light may be indirectly involved, causing a cell-mediated systemic alteration of the immune response which creates a more favorable environment for the development of this vulvar malignancy [7].

There are three histopathologic subtypes of vulvar melanoma: superficial spreading melanoma, nodular melanoma and acral lentiginous melanoma. The reported frequency of these different subtypes varies widely. Some studies highlighted that the majority of the vulvar melanomas were mucosal lentiginous melanomas [5], Benda JA *et al.* [12], whereas another study [13] on 14 patients found nodular melanoma to be the most frequent subtype. Instead, other authors found that the most common histologic subtype was superficial spreading melanoma, 45% [11]. These comparisons are not given much weight because of the small number of patients studied and because of the lack of universal agreement on the classification of lesions. In our case, the amelanotic melanoma was a nodular subtype and involved the squamous epithelium and submucosa, similar with other studies [6].

The cell types in any melanoma may consist of epithelioid, dendritic (neuroid), or spindle cell types, either solely or in combination. Similar to published data of the literature, in the present case, we observed a mixture of spindle and epithelioid cells, or poorly differentiated cells, severely atypical cells, often anaplastic and/or multinucleated.

The amount of melanin can vary from none to a large amount, which may obscure examination of the cellular detail. Similar to our finding, macroscopic amelanosis of the tumor is common in vulvar melanoma of glabrous skin, but it was rare in hairy vulvar tissue.

Lymphoid cell infiltration between single tumor cells or small nests of tumor cells were recorded separately from those clustered adjacent to but outside tumor masses.

Staging

The development of a system for describing the extent of lesions from vulvar melanoma has been an important forward step in the understanding and management of this disease. Staging is now recognized as the primary prognostic indicator for melanoma.

In 1969, Clark WH Jr *et al.* [14] delineated five levels of tumor invasion in cutaneous melanoma based on the penetration of dermal connective tissue planes and their correlation with prognosis.

The following year, Breslow A *et al.* [15] published another assessment of prognostic indicators for cutaneous melanoma, agreeing that depth of invasion is important but adding tumor size, using tumor thickness as the most significant measurement of size. Tumor thickness as determined with an ocular micrometer was divided into the following categories: less than 0.76 mm, 0.76 to 1.50 mm, 1.51 to 2.25, 2.26 to 3.0 mm, greater than 3.0 mm.

Soon after these observations were reported, the *International Federation of Gynecologists and Obstetricians* adopted the system of FIGO surgical staging for vulvar melanoma. FIGO staging can be summarized as follows: (I) tumor less than 2 cm with clinically negative lymph nodes; (II) tumor greater than 2 cm with clinically negative lymph nodes; (III) tumor extending to urethra, vagina, perineum, anus, and/or clinically positive nodes; and (IV) tumor involving bladder or rectum mucosa, bone, or distant sites of metastases. Although mostly used for squamous carcinomas, most investigators have found FIGO classification to be of minimal prognostic value with vulvar melanomas.

Many reports found the Clark and Breslow microstaging systems accurate and reflective of the natural history of the disease process.

We could not apply the Clark microstaging system for vulvar melanoma arising on glabrous skin of clitoris and labia, because the subepithelial tissues of these sites differ in morphology from the rest of the skin of the body.

For melanoma of the vulva, Chung AF *et al.* [16] devised a new staging system, taking into account the lack of a papillary dermis in mucous membranes of the labia, as following: Chung level I – melanoma confined to the surface epithelium; Chung level II – invasiveness <1 mm, Chung level III – invasiveness between 1 and 2 mm, Chung level IV – invasiveness > 2 mm, and Chung level V – tumor extending into underlying adipose tissue (measurements from the granular layer of vulvar skin or the outermost epithelial layer of the squamous mucosa).

Since 1978, it is known that staging by depth of tumor invasion and tumor thickness could be used to estimate the risk of regional and distant metastases. In this belief, few patients with Chung Level II lesions had nodal metastases or died of their disease. Patients with Chung Levels I and II had 5-year survival rates of 100 percent, with survival rates decreasing to 28 percent for level V patients. These same investigators found a similar inverse correlation with survival using Breslow's stages of tumor thickness [2, 7].

Vulvar cancers have three modes of spread: (1) direct extension into adjacent organs such as the vagina, urethra, and anus; (2) embolization to regional lymph nodes; and (3) hematogenous dissemination to distant organs such as the liver, lungs, and bone.

The overall incidence of lymph node metastases in vulvar carcinoma is about 30% to 45%. The incidence of lymph node metastasis increases with the size of the primary tumor and its depth of stromal invasion. Any spread beyond the inguinal lymph nodes is considered distant metastasis. Distant metastases are uncommon at initial presentation, but are often seen in women with recurrent disease. The most common site of distant hematogenous metastasis is the lungs [2].

Prognostic factors

Data on prognostic factors for non-squamous cell vulvar malignancies are much less extensive than those of squamous carcinoma. For patients with vulvar melanoma, both the thickness and depth of invasion have been shown to correlate with the pattern of spread and prognosis [2, 17]. The size of the primary lesion did not adversely affect prognosis in Ariel's study [18] but this finding may hold true only for superficial spreading melanoma.

Ulceration was determined to be other important prognostic parameter of survival. Some authors considered ulceration the single most important factor. Ulceration in melanomas presumably reflects a very aggressive tumor growth that infiltrates and destroys the mucosal membrane.

In some studies, amelanosis was another prognostic factor. In contrast, the absence of melanin on microscopic slides had no significant impact on survival [19].

Other factors, which adversely affect prognosis, are the presence of satellite lesions, the extension of melanoma to urethra or vagina, metastases to regional lymph nodes and increased age at diagnosis [7, 18].

Vulvar melanomas carry a poor prognosis and show a tendency to recur locally as well as to develop distant metastases. However, this aggressive behavior is primarily due to the advanced stage of disease in most cases rather than especially aggressive behavior of the vulvar disease [1, 5, 7, 8].

The 5-year survival rates vary with depth of invasion and metastases. The range is between 8 and 55 percent with a mean of 36%. Patients with superficial lesions (Chung levels I and II) have an excellent chance for cure following surgical resection (5-year cumulative recurrence-free survival rates 78 and 74% respectively) [10, 20].

Bradgate MG *et al.* [21] suggest that the lower rate of survival of vulvar melanoma compared with skin melanoma may be related to the advanced stage of disease at presentation and the higher patient age, predominantly over 65 years [2]. Some authors suggested that patients who received surgery and adjuvant chemotherapy had an improved recurrence-free survival and overall survival too. Compared with chemotherapy, biochemotherapy did not improve prognosis significantly.

The vulvar melanoma has a local recurrence rate between 30 and 51 percent. The preferred site of recurrence is the groin, followed by perineum, rectum, vagina, urethra, and cervix. Distant metastases appear most often in the lung, liver, and brain, but also in the myocardium, kidneys, adrenals, stomach, and retroperitoneal lymph nodes. In patients who develop a recurrence, the disease-free interval ranges from one month to 14 years with an average of one year [22].

Treatment

Although radical surgery has been recommended in the past, gynecologists now generally recommend wide local resection as primary treatment for vulvar melanoma. Some authors found no difference in recurrence or survival between women treated with excision or hemivulvectomy compared with those who underwent radical vulvectomy and lymphadenectomy [23]. Controversies still exist over the value of regional lymphadenectomy. It is probably not indicated in women with early lesions. However, with bigger lesions the risk of nodal involvement increases and lymphadenectomy may be palliative and occasionally curative. Radiation therapy and chemotherapy have generally not proven to offer any survival benefit [1, 3, 6, 8]. Adjuvant interferon has been demonstrated to be of benefit in selected subgroups of patients with cutaneous melanomas [22].

Differential diagnosis

Only few previous reports [24] of amelanotic vulvar melanomas have been published in the recent literature.

The diagnosis of malignant melanoma is readily made if melanin pigment is present [4] but, macroscopically amelanotic tumors were observed in 27% of patients, predominantly in glabrous skin (46%); the clitoral area and labia majora were the most common primary sites [5].

Amelanotic melanoma, which is a unique variant of malignant melanoma, can be misdiagnosed as a carcinoma or sarcoma because of the lack of pigmentation. The pathologist must distinguish a melanoma from other vulvar pathologic lesions including Paget's disease, vulvar intraepithelial neoplasia and dysplastic nevi.

It has been recently reported that immunohistochemical staining with HMB-45 is useful for the cytological and histological diagnosis of amelanotic melanoma [4]. Immunohistochemistry can be helpful in distinguishing between Paget's disease and melanoma, because Paget cells show immunoreactivity for carcinoembryonic antigen (CEA), whereas melanoma

cells do not. These cells do usually show positivity for S-100 protein or HMB-45. Immunohistochemistry may be also useful for confirming diagnosis and ascertaining surgical margins. Special stains to detect melanin are not useful because amelanotic melanoma may lack melanin but Paget cells may contain it. Some poorly differentiated vulvar lesions may not be classifiable by light microscopy alone, and may require the use of a panel of immunohistochemical stains to provide identification of cellular origin. This panel should include epithelial, hematopoietic, histiocytic, neural, neuroendocrine, and melanoma markers [7, 20].

Other differential diagnosis includes a hemato-lymphoid neoplasm because most hematological malignancies primarily or secondarily may involve the genital tract: high-grade non-Hodgkin lymphoma and anaplastic myeloma could have been possible differential due to the nucleolar prominence and large cell size [25]. There is necessary to do a differential diagnosis between different types of melanocytic lesions. The histologic features that suggested a malignant melanoma are (1) their wide lateral extent, (2) lack of uniformity in sizes and shapes of melanocytes within the epidermis, (3) confluence of some junctional melanocytes, and (4) presence of melanocytes, both singly and in nests within adnexal structures.

Superficial spreading melanoma can be differentiated from nodular melanoma by sampling and microscopically evaluating the adjacent epithelium. If the radial growth of melanoma or atypical melanocytes involves four or more adjacent rete ridges, the lesion is classified as a superficial spreading melanoma. In contrast, nodular melanoma has no adjacent radial growth phase. Acral lentiginous melanoma mostly occurs in the vestibule with both vertical and radial growth phases. The spindle cells in the junctional zone show extension into the adjacent dermis in a diffuse pattern and may show a desmoplastic response.

Melanocytes overlying scars and lichen sclerosis exhibit an activated phenotype denoted by HMB-45 expression and increased proliferation. Considering this shared keratinocytic and melanocytic response to stromal fibrosis or sclerosis, the similarities of lichen sclerosis melanocytic nevi to persistent melanocytic nevi are not unexpected. Although persistent malignant melanoma also showed many of the same histologic and immunophenotypic features, the presence or absence of crucial histologic features and the higher variability and greater degree of immunophenotypic changes can distinguish malignant melanoma from both lichen sclerosis melanocytic nevi and persistent melanocytic nevi. Histologically, sharp demarcation of junctional melanocytic hyperplasia that is limited to the area above the dermal fibrosis-sclerosis and the presence of residual melanocytic nevi were not features of persistent malignant melanoma. Variable and deep dermal HMB-45 expression and significantly greater growth fraction in the form of mitotic figures and Ki-67 antigen expression can separate persistent malignant melanoma from lichen sclerosis melanocytic nevi and persistent

melanocytic nevi. Mucosal lentiginous melanoma is histologically similar to the acral lentiginous melanoma of glabrous volar, palmar, and subungual skin. The radial growth phase of mucosal lentiginous melanoma is characterized by single or small-clustered melanoma cells aligned linearly at the epithelial-subepithelial junction and along the rete ridges. At high magnification, melanoma cells dendrites are often apparent, as are tumor cells invading the papillary dermis. Occasionally, single tumor cells invade upper epithelial layers, especially in cases of ulceration, but then lack the typical "pagetoid" cell morphology and growth of superficial spreading melanoma [5].

☒ Conclusions

In conclusion, we present a rare case of amelanotic melanoma of the vulva, originally suspected to be a vulvar carcinoma, but subsequently correctly diagnosed by Melan-A, HMB-45 and S-100 protein immunohistochemistry.

We reconfirmed the usefulness of these antibodies for the diagnosis of amelanotic melanoma. In the future, to be more successful in malignant melanoma management, there is the need of more clinical studies about its biologic nature.

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Corresponding author

Flavia Baderca, Assistant Professor, MD, PhD, Department of Histology, “Victor Babeş” University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300 041 Timișoara, Romania; Phone +40745–106 101, E-mail: flaviabaderca@yahoo.com

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