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CASE REPORT

# Merkel cell carcinoma - particularities and morphological aspect of a unique and rare entity

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**Introduction**: Merkel cell carcinoma is a very rare malignant neoplasm which presents high aggressivity, high recurrence rate and has metastatic potential. Our purpose is to present the histological and immunohistochemical particularities of Merkel Cell Carcinoma while reviewing potential differential diagnoses and challenges that we can encounter in daily practice.
**Case presentation**: We present the case of an 86-year-old female patient who presented with a nodular tumour located in the left forearm, raising suspicion of a soft tissue tumour. The histological appearance of this unique type of cancer is highlighted on the Haematoxylin-eosin stain as a solid tumour composed of nests and chords of monomorphic cells. The nuclei of these tumoral cells appear characteristically as enlarged with dispersed chromatin. The immunohistochemical reactions have been performed and it was observed that the tumoral cells exhibited positivity for synaptophysin, CD56, NSE, EMA, as well as a "dot-like" expression for CK20. These histopathological and immunohistochemical features were consistent with a diagnosis of MCC, stage pT3, based on the assessment of tumour size.
**Conclusions**: Sometimes, differentiating this tumour from other primary malignant neoplasms of the skin or even cutaneous metastases can be difficult. Immunohistochemistry remains the most important tool of diagnosis, especially for differentiating this neoplasm from metastatic neuroendocrine tumours that can affect the skin.

**Keywords**: Merkel Cell Carcinoma, immunohistochemistry, rare neoplasm, malignant

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## Introduction

Merkel Cell Carcinoma (MCC) is a very rare entity, a neuroendocrine carcinoma that is thought to arise in the skin from the Merkel-Ranvier cells. These cells account for less than 5% of the total number of cells located in the keratinised squamous epithelium and they are scattered in the basal layer. Larger numbers are found in mucosal tissues, while in the skin, they are seen mostly in palms, fingers, or areas of the genital organs (clitoris, prepuce). Their role is sensory discernment [1-2].

The precise incidence of Merkel cell carcinoma in Europe is not definitively established; however, estimations suggest a frequency ranging from approximately 0.13 to 0.4 cases per 100,000 individuals annually. MCC constitutes less than 1% of all cutaneous malignancies [3]. Unfortunately, according to the Global Cancer Observatory, cases of Merkel cell carcinoma in Romania remain unquantified.

The causes behind the tumour formation comprise several known factors, such as the Merkel Cell Polyoma virus, UV radiation or immune suppression. The virus is associated with around 80% of the MCC cases [4].

The tumour usually appears on the sun-exposed skin of the elderly individuals as a rapidly progressive nodule (most affected are the head, neck, superior and inferior extremities). The nodule presents with a blue, red or grey coloration, can grow twice or thrice in just couple of months and even produce the ulceration of the surface epithelium of the skin. It is usually asymptomatic. The staging of the tumour, as established and described by the American Joint Committee on Cancer (AJCC) guidelines, considers the regional lymph nodes (sentinel lymph nodes), imagistic investigations, and of course, biopsy with pathological examination [5].

The surgical resection of the tumour is the most important and the initial step in the treatment of this malignancy. However, obtaining clear resection margins can be a difficult task. The recurrence rate, appearance in areas that were previously unaffected and distant metastases have also been reported, as the metastatic potential of MCC is known to be high even in early stages [6].

Our purpose is to present the histological and immunohistochemical particularities of Merkel Cell Carcinoma while reviewing potential differential diagnoses and challenges that we can encounter in daily practice.

## Case presentations

We present the case of an 86-year-old female patient who presented with a nodular tumour located in the left forearm, raising suspicion of a soft tissue tumour. The patient described that the tumour appeared a few months ago and started to increase significantly in size. Surgical excision was performed, and the specimen was sent for analysis to our Pathology Department of the Clinical County Hospital Mureș. The patient has consented to the submission of the case report to the journal. The specimen was sampled and processed according to our department's protocols, using the routine histopathological technique. The tissue samples were histological processed, and there were stained

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using Hematoxylin-eosin staining by a routine protocol. Samples were fixed in formalin and embedded in paraffin prior to undergoing Hematoxylin-eosin staining. Immunohistochemistry reactions were performed on 4 μm-thick sections and were processed using an automated immunostainer (using Benchmark GX, Ventana Medical Systems, Inc., Tucson,AZ, USA). All reagents and incubation times were chosen based on the directions given on the antibody package inserts. Slides were developed using the Diaminobenzidine (DAB) detection kit (Ventana Medical Systems, Inc.) and were counterstained with Hematoxylin. The immunohistochemistry markers used in our case are described in Table I.

Grossing examination of the specimen revealed a cutaneous nodular tumoral mass, sized 7 x 5 mm, with a mixed blue-red coloration.

The microscopy on the routine histological stain (Hematoxiline and Eosine), revealed a solid tumour proliferation, consisting of various architecture types, arranged in chords, plaques and nests of tumoral cells, with a monomorphic aspect.

These tumoral cells had small or medium dimensions, reduced cytoplasm and hyperchromic, enlarged nuclei. Nucleoli were not absent. A significant number of nuclei presented granular, dispersed chromatin. Mitotic count was 12/HPF. Invasions (lymphatic, vascular or neural) were observed. Tumour infiltrating lymphocytes (TIL) were observed, brisk-type. The resection margins were not infiltrated by the tumour.

The immunohistochemical reactions have been performed and it was observed that the tumoral cells exhibited positivity for synaptophysin, CD56, NSE, EMA, as well as a "dot-like" expression for CK20. These histopathological and immunohistochemical features were consistent with a diagnosis of MCC, stage pT3, based on the assessment of tumour size (Tabel I, Figure 1).

**Table I. Markers used for immunohistochemical (IHC) reactions [7] [8].**

| Clone | Staining type |
|---|---|
| Anti-CD56 - (123C3) - Mouse Monoclonal Primary Antibody, VENTANA | Membranous expression of tumoral cells |
| Anti-Cytokeratin 20 - (SP33) - Rabbit Monoclonal Primary Antibody, VENTANA | "Dot-like" expression of tumoral cells |
| Anti-NSE - (MRQ-55) - Mouse Monoclonal Primary Antibody, VENTANA | Membranous expression of tumoral cells |
| Anti-EMA - (E29) - Mouse Monoclonal Primary Antibody, VENTANA | Membranous expression of tumoral cells |

## Discussions

According to College of American Pathologists (CAP), Merkel cell carcinoma has a variety of parameters that are important for the prognosis of the patient. For staging, the dimension of the tumour is the most important. Tumours sized below 2 cm, are considered to be stage pT1; tumours between 2 cm and 5 cm are included in stage pT2; tumours above 5 cm are included in stage pT3 and, pT4 stage is reserved for tumours larger than 5 cm that presented bone, muscle, fascia or cartilage invasion. In our case, the tumour had a maximum size of 7 mm, revealing no signs of invasion in any of the above-mentioned structures [9-10] (Table II).

Another important parameter is the number of mitoses. It is considered that more than 10 mitoses/1 $mm^2$ is associated with a poor prognosis and high rate of metastases and recurrence.

The inflammatory cells of the tumour microenvironment are also important and should be reported in the same way as for melanoma. There is conflicted data regarding the TILs (tumour-infiltrating lymphocyte) in MCC (Merkel Cell Carcinoma), as some authors consider that an increased number of inflammatory cells is corelated with a poor prognosis of the patient. Other data contradicts the statement and regards the parameter as being unclear so far. TIL is reported as brisk type and non-brisk type. In the no-brisk type, the inflammatory cells are present only focally in the tumour. For the brisk type, the inflammatory cells are dispersed between the tumoral cells or located entirely at the base of the tumour, surrounding it. In our case, the tumour infiltrating lymphocytes showed a brisk pattern and were distributed between the tumoral cells [11-15].

The histological aspect of MCC can differ from case to case. Mostly, the intradermal MCC of the skin appears as a tumour proliferation with tumour cells arranged in chords or nests, sometimes with cribriform pattern. The cells are large or medium in dimension and can present with a round or polygonal shape. Characteristically, the nuclei show a dispersed chromatin, with an aspect known as "salt and pepper". Sometimes, the differential diagnosis can be challenging [16-17].

There are several known entities that are similar in appearance to MCC, as it follows: metastatic neuroendocrine carcinoma, nodular basal cell carcinoma, lymphoma, leukaemia, small cell melanoma and Ewing sarcoma.

**Table II. American Joint Committee on Cancer consensus (AJCC) staging system of Merkel Cell Carcinoma 2018**

| Stage | Primary tumour | Lymph node | Distant metastasis |
|---|---|---|---|
| 0 | In situ (within epidermis only) | No regional lymph node metastasis | No distant metastasis |
| I | ≤ 2 cm maximum tumour dimension | Nodes negative by clinical exam | No distant metastasis |
| II | ≥2 cm tumour dimension | Nodes negative by clinical exam | No distant metastasis |
| II | 2 -5 cm tumour dimension | Nodes negative by clinical exam pathological | No distant metastasis |
| III | >5 cm | Nodes positive by clinical exam | No distant metastasis |
| IV | Primary tumour invades fascia, muscle, bone or cartilage | Nodes positive by clinical exam | Metastasis beyond regional lymph nodes. |

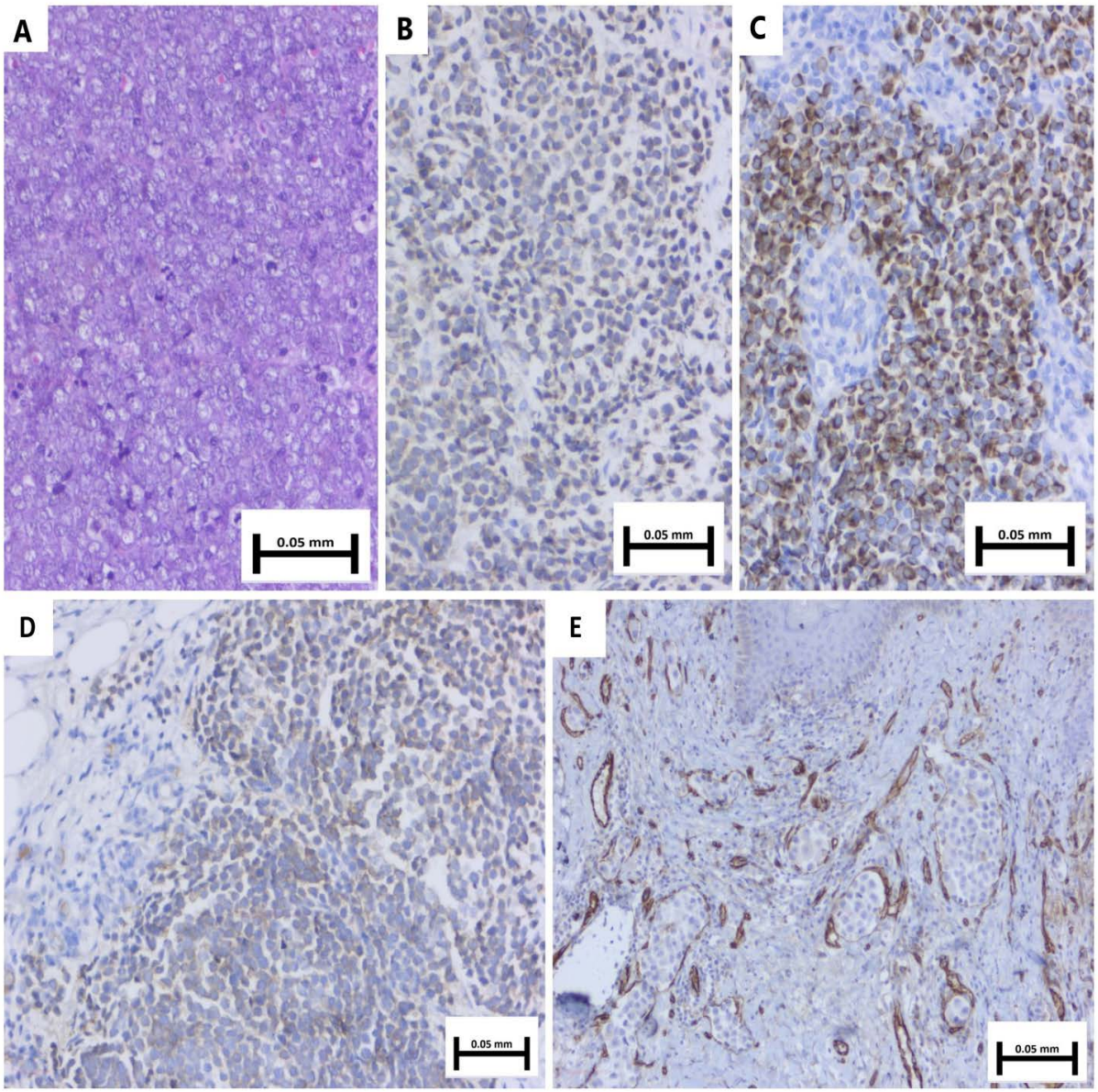


Fig. 1. Microscopic description: A. Merkel cell carcinoma (ob. 20x, H&E) - solid tumour proliferation consisting of round cells with reduced cytoplasm. Nuclei are enlarged and present the "salt and pepper" appearance; B. Synaptophysine antibody (MRQ-40, Rabbit Monoclonal Antibody, CELL MARUE, VENTANA, ob. 20x) – cytoplasmic expression; C. CK20 antibody (SP33, Rabbit Monoclonal Antibody, CELL MARUE, VENTANA, ob. 20x) – "dot-like" expression; D. CD56 antibody (123C3, Mouse Monoclonal Primary Antibody, VENTANA, ob. 20x) – membranous expression, ob. 20x); E. CD34 antibody (QBEnd/10, Primary Antibody, VENTANA, ob. 20x) - strong membranous expression.

For metastatic neuroendocrine carcinoma, the morphological aspect is indistinguishable from primary cutaneous MCC, therefore the diagnosis is established with the help of immunohistochemical reactions. MCC of the skin is positive for CK AE1/AE3, Cytokeratin 20 (CK20) with "dot-like" aspect and neuroendocrine markers, while metastatic neuroendocrine carcinoma lacks expression for CK20 and can be positive for TTF1 (Thyroid transcription factor 1), cytokeratin7 (CK7), CDX2, PAX8 (Paired-box gene 8) [18-19].

In order to differentiate MCC from nodular basal cell carcinoma, distinct morphological aspects need to be observed. Basal cell carcinoma (BCC) presents as nodular tumour proliferation which presents features such as palisading of nuclei. The stroma is frequently myxoid and stromal retraction can be observed around the tumoral nodules. In addition to the nodular aspect of BCC, other morphological architectures and aspects can be observed. Frequently, adenoid changes and cystic degeneration can be encountered in this subtype. Along with the nodular type, superficial BCC can be identified as tumour plaques located in the papillary dermis, which maintain a connection with the superficial epithelium. Not rarely, micronodular, infiltrative patterns or keratotic differentiation can also be associated. All these changes are absent in MCC. In the rare cases in which the histological appearance of the tumour is not relevant, immunohistochemistry reactions can be performed. Immunohistochemistry shows that BCC is positive for BerEP4 and negative for CK20 [20-22].

Lymphomas and leukemias can mimic the morphological aspect of MCC, however, the immunohistochemistry reveals negativity for cytokeratins and neuroendocrine

markers. These type of tumours express various CD (Cluster of differentiation) markers (CD3, CD20, CD45) depending on the subtype. Most frequent lymphomas in the skin are T cell lymphomas, such as mycosis fungoides and its subtypes. B cell lymphomas are rare in comparison, yet if encountered, the most common one is primary cutaneous follicle center lymphoma [23].

Melanoma can present melanin pigment and prominent nucleoli, features that are absent in MCC. The small cell variant of the tumour might be morphologically indistinguishable; therefore, immunohistochemistry must be performed. Small cell melanoma can be considered a particular variant of nevoid melanoma, a unique and rare tumour. Sometimes, the appearance of this neoplasm consists of melanoma in situ associated with nests of monomorphic small cells located in the dermis. In some cases, the in situ component is not observed, raising difficulties for the diagnosis. Immunohistochemistry in cases of melanoma, shows positivity for markers like SOX10 (Sry-related HMg-Box gene 10), S100, MART1/MelanA and HMB45 are constantly negative for keratins. Out of all the markers, currently, SOX10 is considered to be the most specific [24-25].

In the rare cases of Ewing sarcoma, we observed a tumor composed of round cells with basophilic reduced cytoplasm that shows negativity for CK20. This particular tumour arises in the bone and in the soft tissue. A challenge in the diagnosis occurs in the absence of marker CK20, as both MCC and Ewing sarcomas can express CD99 or NSE. CD99 is expressed in MCC mostly in cases associated with Merkel Cell Polyoma virus [26-27].

The tumoral cells exhibited positivity for neuroendocrine markers and epithelial markers which may show a characteristic perinuclear-dot pattern. These immunohistochemical features are routinely used for diagnosing MCC. A few studies reported that the lack of a characteristic stain for CK20 does not exclude the diagnosis of MCC (Tabel III) [28] .

## Conclusions

Merkel Cell Carcinoma is a very rare entity which comprises unique features. The diagnosis is challenging due to the resemblance with other tumours which can be found in the cutaneous layer, primary or metastatic. The suspicion of Merkel cell carcinoma should be elevated upon encountering non-pigmented cutaneous nodules that exhibit rapid enlargement, particularly in elderly patients. This consideration holds significance, albeit not exclusively, when such nodules manifest on sun-exposed areas of the skin. Immunohistochemistry remains the most important tool of diagnosis, especially for differentiating this neoplasm from metastatic neuroendocrine tumours that can affect the skin.

## Authors' contribution

ACT: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be published
ABL: revising the article, acquisition of data, analysis of data, final approval of the version to be published
OSC: drafting the article, interpretation of data, final approval of the version to be published, conception and design, acquisition of data, drafting the article, final approval of the version to be published

## Conflict of interest

None to declare.

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Table 3. Immunohistochemical staining markers of small, blue cells in the skin

| Cancer type | CK20 | CEA | EMA | MELAN A | HMB45 | S-100 | CD56 |
|---|---|---|---|---|---|---|---|
| Merkel Cell Carcinoma | +/- | - | + | - | - | - | + |
| Melanoma | - | - | - | + | + | + | -/+ |
| Lymphoma | - | - | - | - | - | - | - |
| Squamous carcinoma | - | + | + | - | - | - | - |

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