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Original Article

NeuN, a DNA-binding neuron-specific protein expressed by Merkel cell carcinoma: analysis of 15 cases

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Summary

Aim. Merkel cell carcinoma (MCC) is an aggressive cutaneous neuroendocrine carcinoma, with an increasing worldwide incidence. It presents as a painless red to purple nodule on sun-exposed skin. MCC is presumed to arise from resident cutaneous Merkel cells. The pathogenesis of MCC is likely multifactorial with immunosuppression, UV-induced skin damage, and Merkel cell polyomavirus contributing to the development. The diagnosis of MCC relies upon characteristic morphologic features and use of immunohistochemical stains. Histologically, the differential diagnosis of Merkel cell carcinoma includes the 'small round cell' tumor group, particularly metastatic small cell carcinoma and hematological malignancies. This study investigates the expression of NeuN antibody, which recognizes the protein NeuN, normally present in most neuronal cell types and neuronal tumors, in Merkel cell carcinomas.

Methods and results. Fifteen cases of Merkel cell carcinoma (7 men and 7 women; mean age 74 years) were retrieved from the institute database between the years 2011-2020. The immunohistochemical profile was investigated: CK20 (14/14), Neurofilament, (12/12), Synaptophysin (14/14); Chromogranin A (11/13), PAX5 (10/12), TDT (5/12), CK7 (1/14), TTF1 (0/14). Infection by Polyoma virus was detected in 11 of 14 patients. Most tumors showed middle/strong expression of NeuN. No cutaneous structures, or epidermal Merkel cells, showed expression of NeuN. The expression of NeuN was investigated in 17 primary small cell lung carcinomas: 2 cases were positive for Neu-N.

Conclusions. Awareness of the staining pattern of Neu-N could aid in diagnosis of Merkel cell carcinoma, avoiding misinterpretation and erroneous diagnosis with other tumors.

Key words: Merkel cell carcinoma, neuroendocrine carcinoma, NeuN, Merkel cell polyomavirus, small celllung carcinoma

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Conflict of interest
The Authors declare no conflict of interest.

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Introduction

In 1972, Toker [1] described rare primary cutaneous neuroendocrine carcinoma, with cytoplasmatic neurosecretory granules, resembling those in Merkel cell mechanoreceptor cells [2]. Merkel cell carcinoma (MCC) is an aggressive tumor: the 10-year survival rates are 71%, 48%, and 20% for localized, regional, and distant disease, respectively [3]. It has a predilection for sun-exposed skin of the head and neck (50%) and extremities (40%); primary tumors of the trunk are uncommon (10%) [4-6]. The clinical presentation usually appears as an erythematous or purplish nodule [4]. MCC accounts for less than 1% of all cutaneous malignancies, and is increasing over the last few decades [7]. White people are affected 25-fold more than other ethnic groups [8]. At diagnosis, the median age is 76 years [6]. Patients with immunosuppression and solid organ transplant show an increased risk of MCC development [4,6,9]. Large

local surgical excision, with 1-2 cm margins and sentinel lymph node biopsy is indicated. MCC may arise from two independent mechanisms: Merkel cell polyomavirus (MCPyV) or ultraviolet-induced mutagenesis [10-13]. MCC can therefore be distinguished into two molecular subclasses with diagnostic and prognostic significance [14]: virus-negative MCC (VN-MCC) and virus-positive MCC (VP-MCC). MCC is composed of monotonous, blastic-appearing cells, with a salt-and-pepper chromatin and high nuclear to cytoplasmic ratio. It is centered on the dermis and the growth pattern can be nodular, infiltrative, or a mixture. By immunohistochemistry, MCC stains with a broad-spectrum cytokeratin (CAM5.2, AE1/AE3, and cytokeratin 903) with almost 100% sensitivity. The classic marker of MCC is cytokeratin 20 (CK20), especially with dot-like reactivity. CK20 is highly sensitive (about 95% of MCC) and relatively specific [15]. Chromogranin A, synaptophysin, CD56 (neural cell adhesion molecule), and neurofilament are expressed in most tumors. Thyroid transcription factor 1 (TTF-1) is typically not expressed by MCC.

NeuN antibody (NEUronal Nuclei) specifically recognizes the DNA-binding neuron-specific protein NeuN, which is present in most central nervous system and peripheral nervous system neuronal cell types and tumors. NeuN protein distributions are apparently restricted to neuronal nuclei, perikaryal and some proximal neuronal processes in both fetal and adult brain.

In this study, we examined Neu-N immunoreactivity in 15 MCCs (14 patients) and compared NeuN staining with frequently used immunohistochemical markers utilized in the diagnosis of MCC. Nuclear staining intensity and extent were semi-quantitatively analyzed. Moreover, we explored Neu-N immunoreactivity in 17 primary neuroendocrine small cell lung carcinomas.

Materials and methods

Formalin-fixed paraffin-embedded tissue samples of 15 MCC were retrospectively selected from the cases diagnosed in Departments of Pathology, between the years 2011-2020. Clinical data including the tumor site and size were collected from the medical documents. Immunohistochemistry was performed on whole-section and semi-quantitatively graded as extent: 0 (< 5%), 1 + (5-25%), 2 + (25-50%), and 3 + (> 50). Fixation tissue is carried out in 10% neutral buffered formalin for 24 hours. Once tissue is embedded in paraffin, a 3-4 micron tissue section is cut onto charged glass slides. The detection system for immunostaining is BOND Polymer Refine Detection on staining platform LEICA BOND III for these antibodies: Cytokeratin 7 (clone OV-TL 12/30; Menarini; 1:500), 10 min at 37°C with Bond Epitope Retrieval Enzyme for antibodies; synaptophysin (clone 27G12; Leila; 1:200); chromogranin A (clone DAK-A3; Dako; 1:100); Cytokeratin 20 (clone KS20.8; Menarini; 1:500); PAX5 (polyclonal; Biocare; 1:50); TDT (E9266; Leica; 1:100); TTF1 (8G7G3/1; Menarini; 1:100); neurofilament (2F11; Menarini; 1:500); NeuN (A60; Millipore; 1:500); Merkel Cell Polyomavirus (CM2B4; Santa Cruz; 1:100) (test performed in Laboratory of ASST Brescia).

Results

At review, the diagnosis of MMC was confirmed for all cases. Clinical and histopathological data of the cases are summarized in Table I. There were 7 men and 7 women with age range from 52-95 years; mean age was 74 years. In our series, 71% of the tumors were present in the extremities, 21% in the head and neck region and 8% in the trunk. A female of 72 years old had MCC in two different sites, right knee (case 13a) and left elbow (case 13b). Largest diameter of the tumor was ≤ 2 cm in 5 cases (pT1 in AJCC 8th Ed.), and > 2 cm but ≤ 5 cm in 5 cases (pT2 in AJCC 8th Ed.); in 5 cases it was not available. Ten patients had lymph node removal, with lymph node metastasis in 7 patients. Case 6 had metastasis to both the breasts.

Detailed characteristic of the immunophenotype of our cases of MCC (Fig. 1) is given in Table II. All cases were positive for Neu-N (middle or strong nuclear expression; cytoplasmic or membranous staining were not observed), CK20 (cytoplasmic paranuclear dot staining), Neurofilament (cytoplasmic paranuclear dot staining), and Synaptophysin, and negative for thyroid transcription factor-1 (TTF1). Most cases (78.5%) showed > 25% of tumor cells positive for NeuN. NeuN showed no staining of cutaneous structures, neither normal epidermal Merkel cells. Infection by Polyoma virus was detected in 12 out of 15 MCCs (80%).

Two cases (11.7%) of 17 primary small cell lung carcinomas were positive for Neu-N in 25% to 50% of tumor cells (score 2 +), with low nuclear intensity (Fig. 2).

Discussion

MCC is an aggressive tumor arising on sun-damaged skin of the elderly, associated with immunosuppression and senescence. The course of the disease is characterized by lymph node metastases and local recurrences. MCPyV and UV-induced mutagenesis play fundamental roles in the pathogenesis of

Table I. Clinicopathological characteristics.

Case No.	Sex/Age	Location	pT AJCC 8th Ed.	Lymph node removal	Lymph node location	Lymph node metastases	Number of metastatic lymph nodes	Number of isolated lymph nodes
Case 1	F/73	left knee	pT2	Yes	left groin	Yes	3	3
Case 2	F/70	right buttock	pT2	Yes	right groin	Yes	1	1
Case 3	F/88	right ear	NA*	No	-	-	-	-
Case 4	F/61	left forearm	pT1	Yes	left axilla	Yes	1	1
Case 5	M/74	right arm	NA*	Yes	right axilla	Yes	1	4
Case 6	F/95	face	pT2	Yes	left axilla	Yes	1	1
Case 7	F/83	right forearm	pT1	Yes	right axilla	No	0	1
Case 8	M/78	right arm	NA*	Yes	right axilla	No	0	2
Case 9	M/81	left forearm	pT2	Yes	left axilla	No	0	4
Case 10	M/69	left shoulder	NA*	Yes	left axilla	Yes	6	9
Case 11	M/80	face	pT1	No	-	-	-	-
Case 12	M/52	chest	NA*	Yes	right axilla	Yes	4	14
Case 13a	F/72	right knee	pT2	No	-	-	-	-
Case 13b	F/72	left elbow	pT1	No	-	-	-	-
Case 14	M/66	right calf	pT1	No	-	-	-	-
7M 7F	5 pT1 5 pT2	10 Yes 4 No	8 Axilla 2 Groin	7 Yes 3 No				

*NA - Not avable.

Table II. Detailed characteristics of the immunophenotype. Staining extent (0: < 5%, 1+: 5%-25%, 2+: 25%-50%, and 3+: > 50%) was semi-quantitatively analyzed.

Case No.	CK7	CK20	Chromog.	Synapt.	TTF 1	Polyoma virus	PAX5	NF	TDT	Neu-N
Case 1	0	3+	3+	3+	0	3+	3+	1+	0	1+
Case 2	0	3+	3+	3+	0	3+	3+	2+	0	3+
Case 3	0	3+	3+	3+	0	3+	3+	1+	0	1+
Case 4	0	2+	2+	3+	0	3+	3+	3+	0	3+
Case 5	0	3+	3+	3+	0	3+	3+	2+	0	1+
Case 6	0	3+	1+	3+	0	3+	3+	1+	1+	3+
Case 7	0	3+	0	1+	0	3+	3+	1+	1+	1+
Case 8	0	3+	0	3+	0	3+	3+	2+	3+	3+
Case 9	0	3+	3+	3+	0	3+	3+	1+	1+	3+
Case 10	0	2+	3+	3+	0	0	0	1+	0	2+
Case 11	2+	2+	2+	3+	0	0	3+	1+	1+	3+
Case 12	0	3+	2+	3+	0	0	0	3+	0	3+
Case 13a	0	3+	-	3+	0	3+	-	-	-	2+
Case 13b	0	3+	2+	3+	0	3+	-	-	-	2+
Case 14	0	3+	3+	1+	0	3+	-	3+	-	3+
	1/14 7,1%	14/14 100%	11/13 84,6%	14/14 100%	0/14 0%	11/14 78,6%	10/12 83,3%	13/13 100%	5/12 41,6%	14/14 100%

MCPyV-positive and MCPyV-negative, respectively. MCPyV-positive MCCs constitute approximately 80% of all cases [12]. MCC is recognized by its morphologic features and by its classic immunophenotypic properties. The differential diagnosis of MCC includes cutaneous carcinomas, especially basal cell carcinoma, melanoma, lymphoma, and Ewing sarcoma. The characteristic immunohistochemical profile of MCC in-

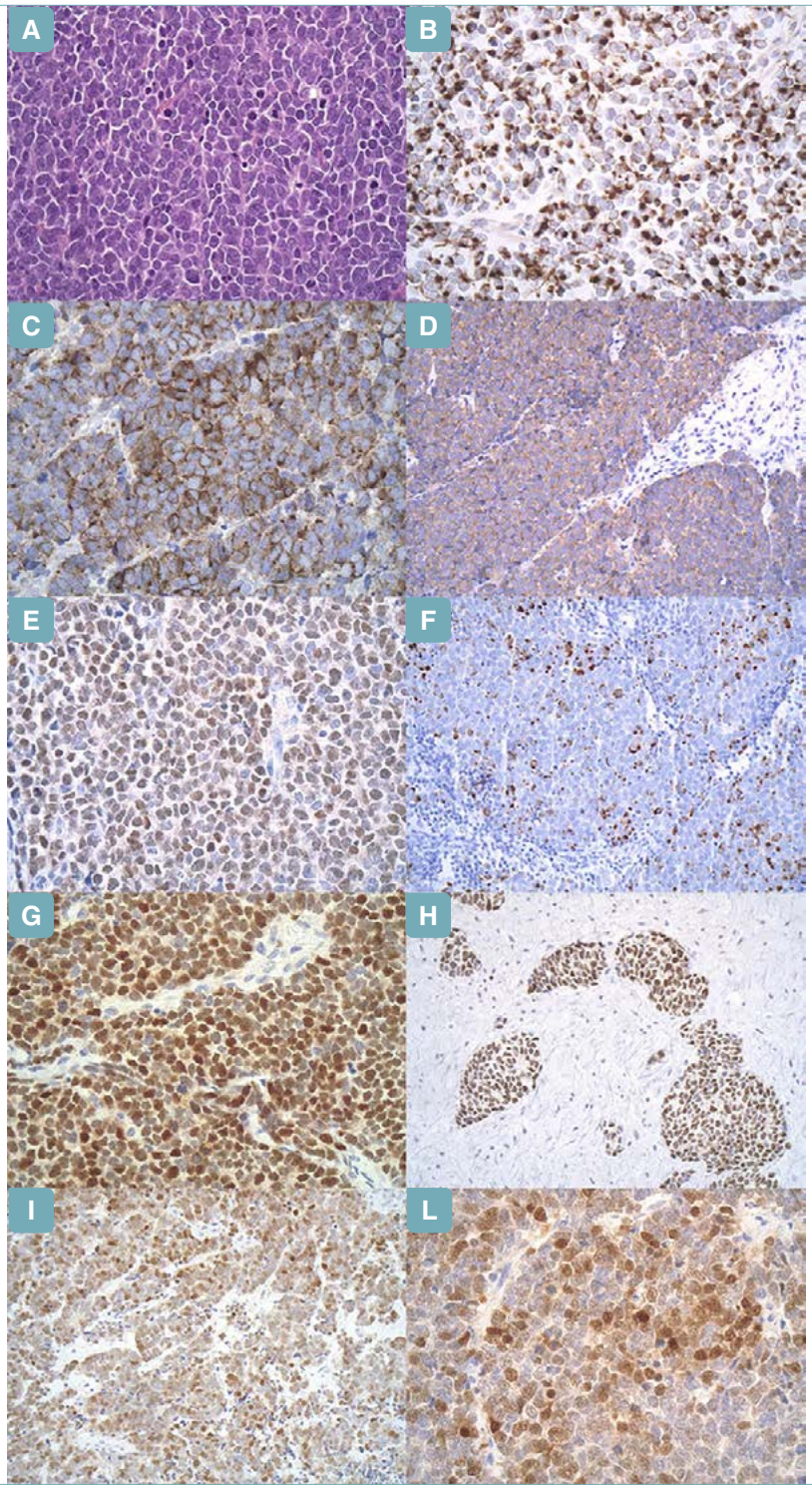

Figure 1. Merkel Cell Carcinoma. (A) (Hematoxylin & Eosin; x40) - Classic cytomorphology displaying small round blue cells with neuroendocrine chromatin, scant cytoplasm, and frequent mitoses. (B) (Immunohistochemistry; x40) - CK20, perinuclear dot pattern. (C) (Immunohistochemistry; x40) - Chromogranin A, granular cytoplasmic pattern. (D) (Immunohistochemistry; x20) - Synaptophysin, granular cytoplasmic pattern. (E) (Immunohistochemistry; x40) - PAX5, nuclear expression. (F) (Immunohistochemistry; x40) - Neurofilament, perinuclear dot pattern. (G) (Immunohistochemistry; x20) - MC Polyoma virus, nuclear expression. (H) (Immunohistochemistry; x20) - TDT, nuclear expression. (I) (Immunohistochemistry; x20) - NeuN, nuclear expression. (L) (Immunohistochemistry; x40) - NeuN, nuclear expression.

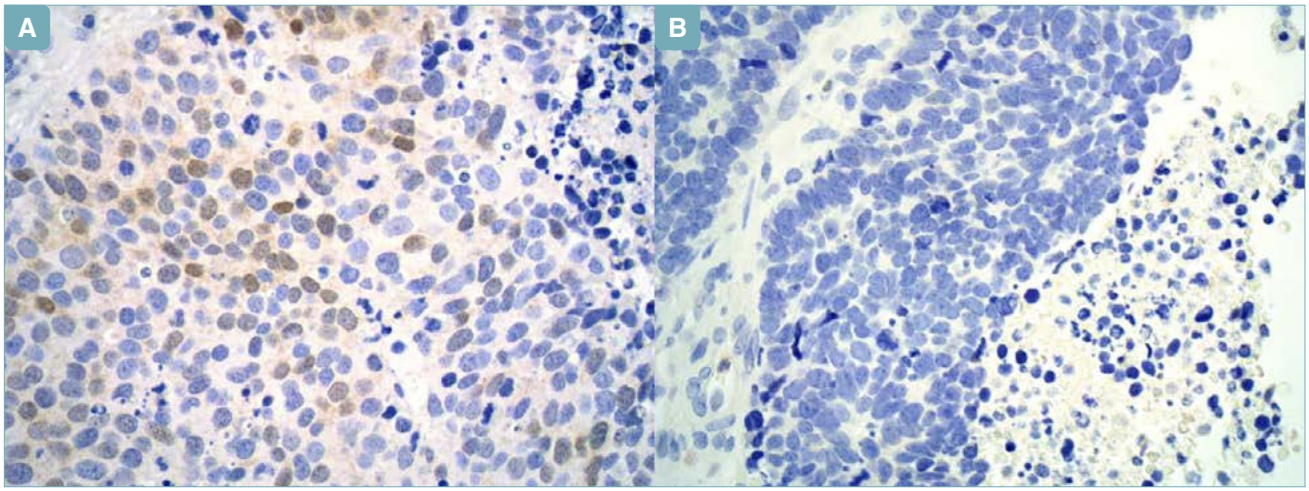

Figure 2. Small Cell Lung Carcinoma. (A) (Immunohistochemistry; x40) - NeuN, nuclear expression in > 25%, but < 50% cells. (B) (Immunohistochemistry; x40) - NeuN, negative.

cludes expression of cytokeratins and neuroendocrine markers (chromogranin A, synaptophysin). Metastatic neuroendocrine carcinomas can be indistinguishable from MCC. Pathologists use CK20 to confirm the diagnosis of MCC, as 80-90% of tumors express CK20 positivity, especially in paranuclear dot-like pattern. However, CK20 is focally expressed in 14% of small cell carcinoma of the lung, and it is frequently positive in neuroendocrine carcinomas arising in the salivary glands and genitourinary tract [15]. Moreover, CK20 may be focal or absent in a minority of MCC, especially MCPyV-negative [17]. In 2010, McCalmont et al. [17] showed that expression of neurofilament, especially in paranuclear dots, could be demonstrated in 95% of MCCs, and that this antibody can be included in the panel for evaluation of MCCs. Neurofilament has lower sensitivity in MCPyV-negative (50-75%) than MCPyV-positive (75-100%) [12]. Neurofilament is often expressed even in CK20-negative cases [18]. MCCs are typically described as negative for CK7. However, up to 30% in various studies express CK7. In our study, we detected one out of fifteen cases (7.1%). CK7 and TTF-1 help to distinguish between MCC and a cutaneous metastasis from a small cell carcinoma of the lung or other site. However, no marker is completely sensitive and specific to distinguish MCC from small cell lung carcinoma. Indeed, Pasternak et al. [14] reported that about a third of MCCPy-N express TTF-1, although only weakly and focally. Moreover, CK20 can occasionally be positive in small cell carcinoma of the lung [16].

Paired box gene 5 (PAX5) a B-cell transcription factor expressed early on cell B-cell development, was found to be reactive against various non-hematopoietic tissues, including neuroendocrine carcinomas and MCC [19]. Sur et al. [20] showed that 53% (8/15) of MCC expressed terminal deoxynucleotidyl transferase (TdT), a DNA polymerase that has greatest activity in early B cells and T cells. We detected expression of TdT in 5/12 (41.6%) MCCs. Merkel cell carcinoma expressing TdT and/or PAX5 can be a potential diagnostic pitfall, as it may result in misdiagnosis as B-lymphoblastic lymphoma, particularly since the latter is often negative for CD45.

The aim of our study was to evaluate the expression of a neuron-associated protein in primary cutaneous neuroendocrine carcinoma to determine whether it has a role in diagnostic surgical pathology. In this study, we detected NeuN as nuclear staining in all 15 cases of MCC, mostly in > 25% cells (78.5%), enabling easy and rapid decision of positivity and negativity, and only in 2 (11.7%) of 17 primary small cell lung carcinomas. In 2005, Shuangshoti et al. [21] demonstrated NeuN immunoreactivity in 56% of epithelial neuroendocrine tumors (19/34): 4 of 7 (57%) carcinoid; 4 of 5 (90%) atypical carcinoid; 11 of 22 (50%) small and large cell neuroendocrine carcinoma. The authors investigated cases of neuroendocrine epithelial tumor with pulmonary, gastric, ovarian, hepatic, colic, pancreatic, cervical, and vaginal primitiveness, but only one case of skin neuroendocrine carcinoma that was negative. The two lung carcinoids studied by the authors were negative, while the only lung neuroendocrine carcinoma was positive, although the cytoplasmic or nuclear site of expression was not specified. Shuangshoti et al. [21] used a different antibody (mouse-derived monoclonal

antibody A60, Chemicon International) and although NeuN is known to express primarily in the neuronal nuclei, cytoplasmic granular staining reminiscent of thatobserved with chromogranin was detected in 11 of NeuN positive cases. The immunoreactivity was localized in the nucleus in 8 cases. None showed nuclear/cytoplasmic co-expression.

In our study, with the exception of neurofilament and synaptophysin compared to NeuN, other immunohistochemical markers, i.e. chromogranin A, showed expression in a lesser number of patients. CK20 was expressed in all MCC, but as noted previously, there are tumors, both MCC and pulmonary neuroendocrine showing aberrant immunophenotype. Therefore, clinical correlation, radiologic studies, and novel antibodies may be required to achieve the correct diagnosis.

The expression of NeuN expands the spectrum of immunoreactivity of this rare tumor and may prove to be a useful addition to a diagnostic panel to prevent diagnostic pitfalls. However, the expression by primary small cell lung carcinomas must be considered, so it is necessary to evaluate NeuN expression in neuroendocrine carcinomas of other sites and other simulating tumors as well.

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