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METASTASES OF MERKEL CELL CARCINOMA IN TERMS OF DIAGNOSING CARCINOMAS WITH UNKNOWN PRIMARY LOCALIZATION

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Ключові слова: *карцинома з клітин Меркеля, рак без первинної локалізації, CK20, ImageJ*

Abstract. Metastases of Merkel cell carcinoma in terms of diagnosing carcinomas with unknown primary localization. Poslavska O.V. *Merkel cell carcinoma (MCC) is a rare malignant primary skin neoplasia with epithelial and neuroectodermal differentiation. The problem of MCC, which determines the relevance of its research, is often a high frequency of local recurrence, regional lymph node metastasis and further hematogenous and / or distant lymphogenous dissemination. Materials and methods. A retrospective analysis of the biopsy material of 6 patients with isolated MCC metastasis aged from 29 to 82 years (mean 63.11±16.24; median 62.5) was performed. For the control group, 9 cases of postoperative material of primary skin tumors with MCC of patients aged from 27 to 76 years (average 52.17±12.72; median 53.5) were selected to determine the differential diagnostic criteria. The results of the study. Carcinomas without primary localization can be interpreted as metastases from MCC, if they demonstrate the phenotype Cytokeratin, Ran AE1 / AE3 (paranuclear +) / CK20 (paranuclear +) / Vimentin (-) / CD45 (-) / S100 (-) / Chromogranin A (+) / Synaptophysin (+), as well as possess nuclei, the area and perimeter of which are 2 times higher than that of normal lymphocytes (all p>0.05). Conclusions. Metastatic MCC without primary localization, in comparison with locally spreading, often demonstrate low-differentiated small-cell and transitional forms, which require additional immunohistochemical studies to exclude metastases of carcinoma of other origin.*

Реферат. Метастазы карциномы из клеток Меркеля с точки зрения диагностики опухолей неизвестной первичной локализации. Пославская А.В. *Карцинома из клеток Меркеля (ККМ) – редкая злокачественная первичная неоплазия кожи с эпителиальной и нейроэктодермальной дифференцировкой. Проблемой ККМ, которая обуславливает актуальность её исследования, часто является высокая частота локального рецидива, регионального метастазирования в лимфатические узлы и дальнейшая гематогенная и/или дистанционная лимфогенная диссеминация. Материалы и методы. В работе проведен ретроспективный анализ биопсийного материала 6 пациентов с изолированным метастазом ККМ возрастом от 29 до 82 лет (среднее 63,11±16,24; медиана 62,5). Для контрольной группы были отобраны 9 случаев послеоперационного материала первичных опухолей кожи с ККМ пациентов возрастом от 27 до 76 лет (среднее 52,17±12,72; медиана 53,5) с целью определения дифференциально-диагностических критериев. Результаты исследования. Карциномы без первичной локализации могут быть интерпретированы как метастазы ККМ, если они демонстрируют фенотип Cytokeratin, Pan AE1/AE3 (парануклеарно +) / CK20 (парануклеарно +) / Vimentin (-) / CD45 (-) / S100 (-) / Chromogranin A (+) / Synaptophysin (+), а также обладают ядрами, площадь и периметр которых превышают в 2 раза показатели обычных лимфоцитов (все p>0,05). Выводы. Метастатические ККМ без первичной локализации, в сравнении с местнораспространяющимися, чаще демонстрируют низкодифференцированные мелкоклеточные и переходные формы, которые требуют дополнительного проведения иммуногистохимического исследования с целью исключения метастазов карцином другого происхождения.*

Merkel cell carcinoma (MCC) is a rare malignant skin neoplasm with epithelial and neuroectodermal differentiation. Tumor cells of MCC share morphologic, immunochistochemical and ultrastruc-

ture peculiarities with differentiated Merlel cells. But a direct histogenetic association is not proved and some researches consider them to be neoplasms from pluripotent progenitor cells. They belong to the soft tissue neoplasias of the skin. For the first time they were described by Cyril Toker as trabecular carcinoma in 1972. Other synonyms include neuroendocrine skin carcinoma, primary small-cell carcinoma of the skin and skin APUDome. MCC-associated poliomavirus is considered to be the cause of development.

According to WHO data for 2017 about 1.500 of new types of MCCs were registered in the United States, this is about 460 times as much as in 2011 (more than 3 times for 6 years). More often it occurs in men than in women, i.e. 2:1. MCC is very aggressive, more than 1/3 of the patients with this diagnosis die, this is 2 times exceeds the number of deaths from melanomas. It should be noted that up to 37% of cases are nodular form, and 6-12% present distant metastases. These tumors typically develop on the skin areas of the elderly (median age 69 years) open to the sun exposure. Anatomical and geographic distribution of carcinoma from Merkel cells depends on solar radiation as the main factor. A relatively high prevalence of these neoplasms is in in patients with chronical immunosuppression [5, 7].

The head is involved in 50% of cases and the limbs – in 40%. The trunk and genitalia are involved in less than 10% of cases, involvement of the mucosa also were described. The majority of neoplasms present solid painless enlarged nodes or thickened plaques of a red, violet or body color, sometimes with ulcer. The majority of MCC is less than 2 cm in diameter, this sometimes complicates revealing the source in dissemination of the process. The complexity of diagnostics causes the need for immunohistochemical research, and the diagnostic search includes basal cell carcinoma, melanoma, lymphoma, ecrine carcinoma, low-differentiated squamous cell carcinoma, metastatic neuroblastoma, primary primitive neuroectodermal tumor and metastases of neuroendocrine carcinoma [6, 8].

The problem of MCC which determines the relevance of its study is the high frequency of local relapse, regional metastasis to the lymph nodes and subsequent hematogenous and/or distant lymphadenic proliferation. MCC metastases without a detected primary focus belong to 20% of favorable phenotypes with better prognosis, compared with other 80% of cancer phenotypes without primary localization [3]. But clinical diagnosis after such a histological diagnosis necessarily involves a complete examination, excluding other possible primary localizations and the presence of multiple metastases.

The aim is to investigate the complex of morphological, morphometric and immunohistochemical characteristics of cases of isolated carcinoid metastases from Merkel cells, in comparison with primary tumors of the skin of this origin, for the improvement of diagnostic algorithms.

MATERIALS AND METHODS OF RESEARCH

In this work a retrospective analysis of the histological, morphometric and immunohistological characteristics of the biopsy material was performed on 6 patients (4 men and 2 women) with isolated metastatic carcinoma from Merkel cells aged 29 to 82 years (mean 63.11±16.24, median 62.5) on the basis of the morphological department of the medical and diagnostic center JSC “Pharmacies of Medical Academy” (Dnipro) for the period from 2015 to 2017. For the control group for the comparison of histological and morphometric characteristics, 9 cases of postoperative material of primary skin tumors from Merkel cells carcinoma (5 woman and 4 men) aged 27 to 76 years (mean 52.17±12.72; median 53.5). Localization of the primary material was distributed as follows: 4 areas of cheeks, 3 - in the hip area, 1 - the skin of the leg and 1 - forearm.

For the morphometric method, the Zeiss Primo Star - Axiocam ERC 5s microscope camera with the licensed ZEN 2 blue edition software was used, informative fields of view were recorded in .jpg format and processed in the ImageJ program with the definition of the perimeter, area and roundness of the nuclei (Fig. 1) according to the method described in other publications [1, 2]. The immunohistochemistry study was conducted according to the protocols of TermoScientific (TS), (USA). In sections with a thickness of more than 4 μm, the Lab Vision Quanto (TS, USA) visualization system was used to detect the protein chain using DAB Quanto Chromogen (TS, USA). Characteristics of monoclonal antibodies are listed in Table 1.

Statistical analysis of the parameters of areas, perimeters and circle coefficient of cells was carried out in the programs ImageJ and Microsoft Excel with the calculation of minimum, maximum, median, arithmetic mean and standard deviation.

RESULTS AND DISCUSSION

The primary carcinomas from Merkel cells were represented by fine-cell "blue-cell" neoplasms consisting of the same cells with a round or oval nucleus and a poor cytoplasm. Clear nuclear membrane separates disperse chromatin and nucleoli. The attention was drawn to numerous mitotic figures and nucleic fragments (pycnosis, rexis), as well as individual differentiated elongated

cells were present. The tumors were located in the center of the dermis and in 4 out of 9 cases (44.44%) spread into subcutaneous tissue. Only in 1 of the cases epidermis was involved (pagetoid type of lesion), but in other observations there was ulceration of epidermis.

Table 1

Primary monoclonal antibodies

Primary antibodies	Clon (dilution)	Reaction localization
Cytokeratin, Pan	**AE1/AE3(1:50)**	**Cytoplasma**
Vimentin	**Ab-2/sp20 (1:200)**	**Cytoplasma**
Chromogranin A	**sp12 (1:400)**	**Cytoplasma**
Synaptophysin	**sp11 (1:200)**	**Cytoplasma** **Cytoplasma** **Cytoplasma**
Cytokeratin 7 (CK7)	**RCK105 (1:100)**	**Cytoplasms**
Cytokeratin 20 (CK20)	**Ks 20.8 (1:100)**	**Cytoplasma**

In most observations, the neoplasms formed diffuse nets and solid nests in the dermis. On the periphery there were trabecular patterns, in the form of "nets" and "garlands", in 2 out of 9 (22,22%) pseudorosettes were formed. The dermis around tumor in 6 of 9 (66.67%) demonstrated a desmoplastic reaction and zonal tumor necroses with angiolymphatic involvement. According to the histological forms, primary MCCs were divided into three groups: trabecular MCC - 4 out of 9 (44,44%), transient - 3 out of 9 (33,33%), and small-cellular - 2 out of 9 (22,22%). The indicators of the morphometric study are listed in Table 2.

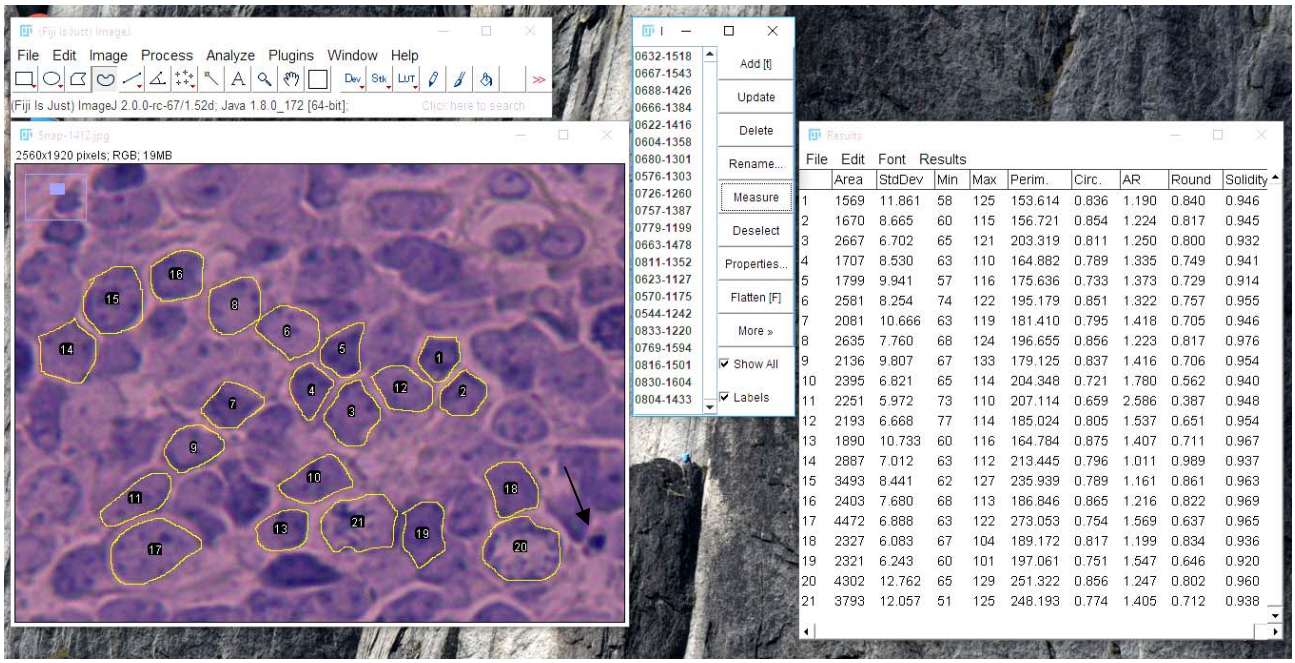

Fig. 1. Features of cell structure of primary Merkel cell carcinoma: study of morphometric parameters (area, perimeter and index of «roundness» of nuclei) by the ImageJ program: nuclei of angulated form, with clear nuclear membrane, which separates dispersion chromatin and nuclei. Arrow indicates nuclear fragments (pycnosis, rexis)

Table 2

Indicators of morphometric studies of metastases and primary carcinomas from Merkel cells in the ImageJ program

Types of primary ovarian tumors	Area (мсм^2) χ±SD	Perimeter (мсм) χ±SD	Ratio of «roundness» (ImageJ parameter) χ±SD
Primary carcinoma from Merkel cells	37,263±5,689	23,067±1,523	0,795±0,045
Metastasis of carcinomas from Merkel cells	34,862±5,933	22,454±2,093	0,802±0,064
p	p>0,05	p>0,05	p>0,05

N o t e : χ±SD – mean ± standard deviation, statistically significant difference was considered in p<0.05.

All carcinomas from Merkel cells have demonstrated epithelial and neuroendocrine differentiation. Paranuclear punctated immunohistochemical staining of CK20 cytokeratins was specific, which in half of the observations had a cap-like perinuclear staining. Positive reaction was also found on the markers synaptophysin and chromogranin, CD117. And it was completely negative for the panleukocytic antigen CD45, Vimentin, S100.

All metastatic lesions were analyzed according to the patterns of the histological structure and corresponded to the epithelioid morphology and the phenotype of the Cytokeratin, Ran AE1 / AE3 (paranuclear +) / SK20 (paranuclear +) / Vimentin (-) / CD45 (-) / S100 (-) after the primary IgG antibody panels (Fig. 2)

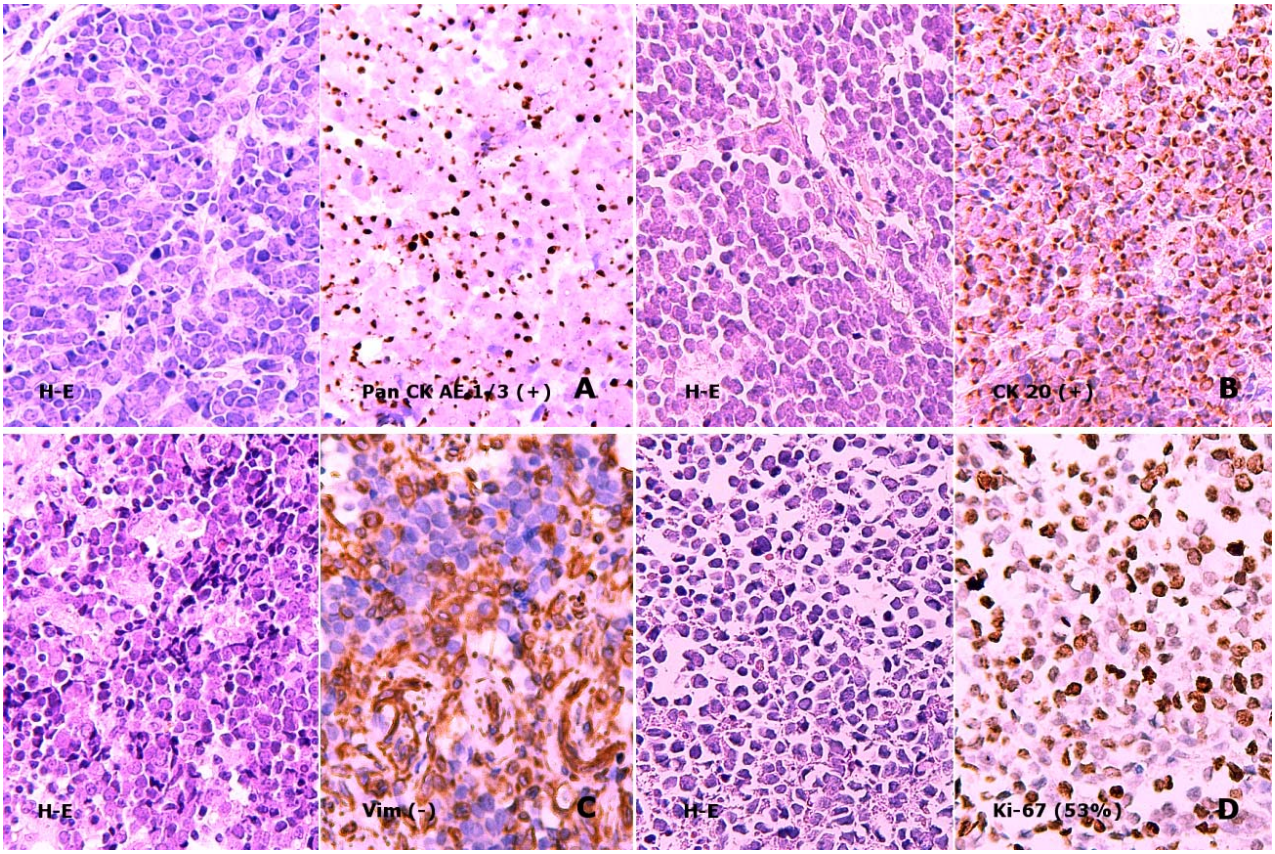

Fig. 2. Examples of metastatic lesions of lymph nodes with Merkel cell carcinoma without primary localization. A. G-E / paranuclear punctated immunohistochemical staining with Cytokeratin, Pan AE1 / AE3, ICH, additional staining with Mayer's hematoxylin (× 400). B. G -E/cap-like perinuclear staining CK20, IHC, additional staining with Mayer's hematoxylin (× 400). C. G-E / negative reaction of Vimentin in tumor cells and positive in lymphoid cells of the environment, IHC, additional staining with Meyer's hematoxylin (× 400). D. G-E / Intranuclear reaction with KI-67, proliferative activity up to 53%, IHC, additional coloring with Mayer's hematoxylin (× 400)

Morphological types of metastatic carcinoma from Merkel cells included 2 transitional forms and 4 small-cell carcinomas, which were much less commonly exhibited trabecular patterns or pseudorosettes, whereas they had solid fields of monomorphic cells with intravascular invasion and stromal response. The difference in the distribution of histological forms of primary and metastatic MCC was reflected in the decrease in the size of cells of metastatic MCC, but statistically significant differences between the study groups revealed by the indicators of their morphometric study with ImageJ program was not found (all p>0.05) (Table 2) .

For the purpose of differential diagnosis of MCC with other carcinomas, morphometric indices of primary and metastatic carcinomas from Merkel cells were compared with Vimentin-positive lymphoid cells of the environment (Table 3). A twofold difference was found in the area and perimeter of MCC tumor cells of both groups and normal lymphocytes (p (1) <0.05, p (2) <0.05).

Table 3

Indicators of morphometric studies of metastases and primary carcinomas of Merkel cells in the ImageJ program

Types of primary ovarian tumors	Area (мсм^2) χ±SD	Perimeter (мсм) χ±SD	Ratio of «roundness» (ImageJ parameter) χ±SD
Primary carcinoma from Merkel cells (1)	**37,263±5,689**	**23,067±1,523**	**0,795±0,045**
Metastasis of carcinomas from Merkel cells (2)	**34,862±5,933**	**22,454±2,093**	**0,802±0,064**
Lymphocytes of lymphatic node	**16,738±2,693**	**15,993±1,541**	**0,746±0,098**
p	**p(1)<0,05, p(2)<0,05**	**p(1)<0,05, p(2)<0,05**	**p(1)>0,05, p(2)>0,05,**

N o t e : χ±SD – mean ± standard deviation, statistically significant difference was considered in p<0.05.

By the data of previous studies, MCC often has deletion on the short arm of chromosome 1 (lp36) and is common to other neuroendocrine tumors such as melanoma and neuroblastoma. Numerous other chromosomal lesions are described, for example, trisomy by 6 chromosome, approximately to 50% of tumors. Thus, some researchers believe that this tumor originates from primitive epidermal stem cells that can become epidermis or neuroendocrine cells.

According to a combination of risk factors, progression of carcinomas from Merkel cells includes: more advanced age, localization on the head and neck, the size of more than 2 cm, immunosuppression, and progression of the disease. Histopathological and immunohistochemical risk factors show more than 10 mitotic figures with large enlargement, small-cell histological form, angiolymphatic invasion and CD45 immunoreactivity.

CONCLUSIONS

1. Carcinomas without primary localization can be interpreted as metastasis of carcinoma from Merkel cells if they exhibit the phenotype of Cytokeratin, Phen AE1 / AE3 (paranuclear +) / CK20 (paranuclear +) / Vimentin (-) / CD45 (-) / S100 (-) / Chromogranin A (+) / Synaptophysin (+), and also have nuclei whoich area and perimeter exceeds 2 times the corresponding indices of normal lymphocytes (all p> 0.05).

2. Metastatic carcinomas from Merkel cells, compared with primary ones, often show low-differentiated small-cell and transitional forms requiring additional immunohistochemical studies to exclude metastases of carcinomas of other origin.

The research was carried out within the framework of the research work of the Department of Pathological Anatomy and Forensic Medicine of the SE "Dnipropetrovsk Medical Academy of Health Ministry of Ukraine" "Development of diagnostic and prognostic criteria for neoplasms of different localizations, taking into account the biological parameters of the activity of the tumor process" (state registration number 0116U002827, execution period 2016-2018).

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