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Case report

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Atypical presentation of Merkel cell carcinoma positive to polyomavirus DNA detection: Experience from a single center

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Abstract

Merkel cell carcinoma (MCC) is a rare malignant tumor of the skin with tendency to rapid local progression and frequent spread to regional lymph nodes. In this paper we retrospectively describe the atypical presentation of 5 cases of Merkel cell carcinoma observed in our surgical department in the last ten years. Four patients had buttock localization whilst one patient had primary nodal presentation. Since integration of Merkel cell polyomavirus (MCPyV) DNA into the tumor genome is frequently recorded in this type of cancer, we analyzed formalin-fixed paraffin embedded MCC tissue samples from our five patients for the presence of MCPyV DNA by means of polymerase chain reaction (PCR). MCPyV DNA was present in all five carcinomas. All patients were treated with wide surgical excision of the tumor and sentinel node biopsy. One patient had stage I disease, three patients had stage II disease, and one patient had stage III disease. Adjuvant radiotherapy was administered in all cases for local control. Chemotherapy was administered to the patient with primary nodal presentation and in stage III disease. Median time of follow-up was 84 months. None of the patients relapsed. Despite the low number of patients examined, our experience suggests that surgery is a necessary step whereas implementation of adjuvant therapy, radiotherapy and chemotherapy depends on individual risk assessment. Treatment outcome was very good, probably due to early detection of MCC.

Keywords: Merkel cell carcinoma; polyomavirus; DNA detection; chemotherapy

Introduction

Merkel cell carcinoma (MCC), first described by Toker in 1972 [1] is an aggressive neuroendocrine tumor arising in the dermo-epidermal junction of the skin. MCC incidence increases progressively with age. The tumor is rarely diagnosed in patients younger than 50 years, whereas the median age at diagnosis is about 65 years [2, 3]. MCC occurs most frequently in Europe and North America (75-90%) while only 24% of cases are reported in Australian patients; there are very limited data about the prevalence in Asian population [4-10]. These tumors are typically found on the head and neck, arms, chest, with a slight female predominance, especially in sun-exposed areas of the skin, and often presents as a painless bluish-red nodule [11]. Increased incidence of MCC has also been recorded in subjects heavily treated with methoxsalen (psoralen) and ultraviolet A (PUVA) for psoriasis, and in patients with chronic immunosuppression, as in chronic lymphocytic leukemia, human immunodeficiency virus infection, and prior solid organ transplantation [4, 12]. In 2008, a novel polyomavirus (MCPyV) was first reported in MCC tumor specimens and approximately 80% of the examined MCC are MCPyV positive [10]. Further studies confirmed the presence of this newly identified MCPyV in MCC tumor specimens and its role in influencing

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the clinical outcome. Currently, there are no evidence-based treatment modalities for MCC because of the low incidence of this entity. Indeed, only single cases or small numbers of patients have thus far been described in the literature. Here, we contribute to the knowledge of this tumor by reporting five MCPyV-DNA-positive cases with atypical presentation and earlier age onset observed at our institution.

Patients and methods

The medical records of five patients with MCC observed and treated between 2003 and 2008 at the Surgical and Oncology Department of Second University of Naples, Italy, were reviewed. Four patients had buttock presentation while one patient presented with nodal involvement with no identified primary tumor. Thus, all patients had an uncommon presentation. Diagnosis was confirmed by conventional hematoxylin & eosin staining and immunohistochemical staining using the following markers: CK20, chromogranin, synaptophysin, neurogen-specific enolase (NSE), S100, thyroid transcription factor-1 (TTF-1), and pan-cytokeratin. Standard surgical procedures were planned according to clinical presentation. The margins of excision were at least 2.5 cm around the lesion. Sentinel lymph node biopsy (SLNB) was performed in all cases following diagnosis of Merkel cell carcinoma except in the patient presenting with nodal involvement. Staging was performed with total body PET-TC scan. Adjuvant chemotherapy was administered if necessary, according to published guidelines [13]. Patients were encouraged to receive adjuvant treatment consisting in local radiotherapy (total dose: 60 Gy) and/or chemotherapy (carboplatin at AUC 4 on day 1 and etoposide at 80 mg/m^2 on days 1-3, repeated every three weeks for 4–6 cycles) according to the stage. All patients were then included in a follow-up program consisting in total body PET-TC scan and assessment of serum levels of chromogranin A and NSE every six months for the first three years and then every year for the following five years. MCPyV genome integration into tumor DNA was investigated according to standard procedures. Briefly, DNA was obtained from formalin-fixed paraffin embedded tumor samples following deparaffinization with xylene, digestion with proteinase K until complete tissue lysis, and phenol/chloroform extraction with sodium acetate/ethanol precipitation. PCR was performed with 200 ng of genomic DNA in a reaction mixture containing 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM dNTP, and 2.5 units of Taq DNA polymerase (Roche Diagnostics) in a final volume of 50 μl. The reaction was carried out in a DNA thermal cycler (Mastercycler gradient, Eppendorf, Milan, Italy). For MCPyV detection, the LT1, LT3, LT5, VP1, and M1/2 primer sets were used [6]. In addition, the LT1 and M1/2 primer sets were used for nested PCR, using 31 cycles for each primer set. β-actin PCR was performed to confirm suitability of genomic DNA for PCR analysis. PCR products were analyzed by electrophoresis on 1.8% agarose gel in TBE.

Case 1

A 41 year-old woman was referred to our institution in July 2003 because of a subcutaneous formation in the right inguinal region associated with moderate pain. She was in good clinical performance; her medical history was remarkable only for severe hypertension treated with ACE inhibitors. CT scan revealed a solid expansive oval formation of 5x6x7 cm in the right inguinal area with well-defined contours. She was subjected to radical excision of the lesion and inguinal lymph nodes. A total of five lymph nodes were excised. Histology revealed an intranodal MCC, with diffuse infiltration by tumor cells which turned imunohistochemically positive for CK20, chromogranin, synaptophysin, and NSE, and negative for S100, TTF-1 and pan-cytokeratin. The remaining lymph nodes were uninvolved. Physical examination, routine blood tests and total body PET/TC scan did not yield clues to the primary site of tumor. This atypical presentation prompted us to implement adjuvant chemotherapy with carboplatin at AUC 4 on day 1 and etoposide at 80 mg/m^2 on days 1-3, repeated every 21 days for 4 courses. Chemotherapy was well tolerated and no serious adverse events were observed. PCR analysis revealed the presence of MCPyV DNA in tumor samples. No recurrence of disease was detected after a ten-year follow-up.

Case 2

In September 2004, a 57 year-old woman was seen at our institution with an indolent, soft, bluish-red swelling (maximum diameter of 2 cm) in the superior-internal quadrant of the left buttock. She was in good clinical performance; her medical history was positive for moderate arterial hypertension of one-year duration and chronic hepatitis C. Ultrasound revealed a hypoechoic nodule, with irregular margins, measuring 20x8 mm. Surgical removal of the lesion was performed. Microscopic examination revealed the diagnosis of a Merkel cell carcinoma. Sentinel node biopsy was negative. PET-CT scan was negative, and the disease was categorized as stage II. The patient received an exclusive radiation treatment at 60Gy. FFPE tissue specimens were tested positive for MCPyV DNA by PCR. No recurrence of disease was detected after an eight-year follow-up.

Case 3

In February 2005, a 59 year-old man with an indolent, soft, nodular lesion (maximum diameter of 3 cm) in the inferior-internal quadrant of the right buttock was visited in our outpatient clinic. His medical history disclosed severe hypertension. Ultrasound examination revealed a hypoechoic nodule, with irregular margins, measuring 30x6 mm. Wide excision and SLNB were performed. Microscopic examination showed MCC. Sentinel node was negative. The patient was classified as having stage II disease. Radiotherapy was planned following surgery. FFPE tissue specimens were tested positive for MCPyV DNA by PCR. No recurrence of disease was detected after a seven-year follow-up.

Case 4

In May 2005, a 55 year-old woman was seen at our institution with an indolent, soft, nodular lesion (maximum diameter of 2 cm) in the internal region of the left buttock. Her medical history was remarkable for moderate hypertension and chronic hepatitis C. Ultrasound showed a hypoechoic nodule, with irregular margins, measuring 22x14 mm. Surgical excision of the lesion with wide margins and SLN dissection were performed. Microscopic examination revealed a MCC. SLN was negative. The patient was defined as having stage II disease. Adjuvant radiotherapy to the primary site of disease was administered following surgery. The patient was then included in the follow-up program. FFPE tissue specimens were tested positive for MCPyV DNA by PCR. No recurrence of disease was detected after a seven-year follow-up.

Case 5

In January 2008, a 68 year-old man, with mild hypertension and type 2 diabetes mellitus, noted an indolent nodular lesion on the external region of the left buttock. The maximum diameter of the lesion was 2 cm. PET-CT scan disclosed a 2.5 cm left inguinal lymphadenopathy with a max SUV of 7. Surgical removal of the lesion with wide margins and left inguinal node dissection were performed. Microscopic examination showed MCC with nodal involvement (stage III). Adjuvant radiotherapy was administered to the primary site of disease and to the left inguinal region following surgery. Concomitant chemotherapy with carboplatin at AUC 4 on day 1 and etoposide at 80 mg/m^2 on days 1-3 was given and repeated every three weeks for four cycles. The patient was then followed-up. FFPE tissue specimens were tested positive for MCPyV DNA by PCR. No recurrence of disease was detected after a five-year follow-up.

Patients and tumor characteristics are shown in Table 1 and Table 2.

Table 1 Patients' characteristics.

	Age	*Sex*	*Comorbidity*	*MCPyV DNA*	*Site of primary tumor*	*SLND*	*Stage*	*CHT*	*RT*	*Overall survival*
Case 1	41	Female	Hypertension	Present	Inguinal node	Yes	I	Yes	No	10 years
Case 2	57	Female	Hypertension HCV infection	Present	Buttock	Yes	II	No	Yes	9 years
Case 3	59	Man	Hypertension	Present	Buttock	Yes	II	No	Yes	8 years
Case 4	55	Female	Hypertension HCV infection	Present	Buttock	Yes	II	No	Yes	8 years
Case 5	68	Man	Hypertension Diabetes mellitus	Present	Buttock	Yes	III	Yes	Yes	5 years

Table 2 Tumor characteristics.

	CK20	*chromogranin*	*synaptophysin*	*NSE*	*S100*	*TTF-1*	*Pan-cytokeratin*
Case 1	+	+	+	+	-	-	-
Case 2	+	+	+	+	-	-	-
Case 3	+	+	+	+	-	-	-
Case 4	+	+	+	+	-	-	-
Case 5	+	+	+	+	-	-	-

Abbreviations: += positive; -= negative

Discussion

MCC is a rare tumor usually occurring in elderly people on sun-exposed areas such as head, neck or extremities. Areas of involvement suggest a role for the solar radiation in favoring development of disease [14]. Indeed, in a recent cohort study including 195 patients, 81% of primary MCCs occurred on ultraviolet-exposed sites; only 5 % of cases were localized on buttocks. In contrast, all MCC cases observed at our institution did not arise on sun-exposed areas; moreover, the median age of our patients was lower than that reported in literature. Specifically, median age at presentation has been reported to be 65 years, with 90% of patients being older than 50 years [15].

Although the exact origin and function of the Merkel cell remains under investigation, it is thought to have features of both epithelial and neuroendocrine derivation and to arise in cells with touch-sensitivity function (mechanoreceptors) [16-21]. This would explain the origin of the tumour in areas not exposed to the sun but to mechanical stress such as the buttock region.

MCC confined to lymph nodes without an apparent primary site has been rarely reported. To our knowledge, the largest series has been described by Eusebi et al. [22], who reported five inguinal, two axillary, and one submandibular primary lymph node cases. MCC in lymph nodes may be the result of metastatic spread from an occult or regressed primary MCC. Alternatively, lymph nodes may be the primary site of disease. Although this latter hypothesis is generally not accepted because Merkel cells have never been identified in lymph nodes, we favour this hypothesis for three reasons. First, MCC may arise de novo in lymph nodes from epithelial or stem cells of the lymph reticular system [22, 23] and Second, PET-TC failed to disclose lesions elsewhere. Third, the long disease-free survival in our patient with primary MCC nodal presentation is unusual for metastatic MCC.

Chronic immunosuppression, as seen in HIV infection, CLL, or iatrogenic (organ transplantation), appears to be an important risk factor for MCC [24]. However, in our series, none of the patients had concomitant immunological disorders. Diagnosis of MCC remains difficult and requires immunohistochemical confirmation (positivity for CK20, chromogranin, synaptophysin, and NSE; negativity for S100, TTF-1 and pan-cytokeratin). Recently, the Merkel cell polyomavirus has been implicated in the pathogenesis of MCC [5, 7-9, 25-27].

MCPyV DNA has been recently detected as a potential pathogenic factor for MCC. In the general population, its seroprevalence is 9 % in children younger than four years of age and increases to 35 % by 4–13 years of age [27]. Using immunoassays, Tolstov et al., found that 80% of healthy North American adults (blood donors) showed evidence for past MCPyV exposure. Consistent with this, MCPyV was detected in 80% of cutaneous swabs from healthy volunteers, suggesting it may be a common inhabitant of the human skin [28]. Although MCPyV is strongly associated with MCC and many studies support its role in pathogenesis, the presence of virus is not sufficient to induce MCC carcinogenesis. MCPyV encodes a large T tumor antigen (LT) and a small antigen tumor (sT), which both play a role in MCC pathogenesis by targeting several tumor suppressor genes [29].

Conclusion

All of our patient tissue specimens were tested positive for MCPyV. In particular, 4 tissue specimens were tested positive by the LT1 and M1/2 primer sets (nested PCR), whereas only 1 specimen resulted positive either by using the primer sets LT1 and M1/2 or LT3. These findings, along with the notion that no case arose on sun-exposed areas, may strengthen the role of MCPyV in the pathogenesis of this peculiar type of tumor. However, further studies are needed to address this issue.

Conflict of interest

The authors wish to express that they have no conflicts of interest.

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