

ORIGINAL ARTICLE

Expression and prognostic significance of programmed death-ligand 1 (PD-L1) in Merkel cell carcinoma

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Summary

Purpose: To detect the expression of programmed death-ligand 1 (PD-L1) in Merkel cell carcinoma (MCC) and to determine the prognostic influence of the PD-L1 expression.

Methods: A total of 13 patients with MCC were retrospectively evaluated (12 patients with primary skin lesion, one patient was diagnosed as unknown primary MCC). All patients underwent surgical resection. PD-L1 was determined by immunohistochemistry. Immunostaining results were evaluated semiquantitatively. The prognostic influence of the expression of PD-L1 on overall survival (OS) was calculated with the Kaplan-Meier method and log-rank test.

Results: PD-L1 positivity was detected in 8 patients (61.5%), in all cases the result was 1+. PD-L1 negativity was shown in the remaining 5 patients. In patients with PD-L1 positivity median OS was 42.1 months (95% CI 9.3-42.1) compared with median OS of 9.4 months (95% CI 2.1-80.9) in the group of patients without PD-L1 positivity ($p=0.417$).

Conclusions: PD-L1 positivity was found in 61.5% of patients with MCC. No prognostic significance of PD-L1 expression for OS was demonstrated.

Key words: Merkel cell carcinoma, prognostic factors, overall survival, PD-L1, immunotherapy

Introduction

Merkel cell carcinoma (MCC) is a rare but aggressive malignant skin tumour. Lately, a significant rise in the incidence of this disease was observed [1]. The most frequent place of occurrence is the head and neck (50%), followed by the thorax (30%) and extremities (10%). Apart from the skin, MCC can also originate in the mucous membranes [2,3]. Approximately 80% of MCC cases are associated with infection of Merkel cell polyomavirus (MCPyV); other cases are associated with UV radiation induced mutations [4,5]. MCC is positive for the presence of CK20, CD56, synaptophysin and chromogranin [6,7].

MCC is classified as an immunogenic malignant disease. Work in the past has already proved lymphocyte infiltration in MCC tumours [8,9]. It was later proven that patients with immunodeficiency have a worse prognosis than patients without any immune system disorder [10]. On the other hand, MCPyV positive tumours have a longer OS rate than patients with MCPyV negative tumours [11]. Patients with MCC show presence of B-lymphocytes, CD4+ and CD8+ T lymphocytes, and similarly also antibodies against MCC antigens [12-14]. These trials showed a significant role of the immune system in MCC patients. It appears that also

MCPyV-negative MCC tumours display a certain immunogenicity, most probably connected with mutation changes originating from the effects of UV radiation [15].

In recent years, immunotherapy has developed significantly and represents the current treatment standards in treating malignant melanoma, non-small cell lung cancer, kidney cancer, and other types of malignant tumours [16-18]. Immune checkpoint inhibitors are among the most significant elements of current immunotherapy. The PD-1 (programmed cell death - 1) receptor and its ligand (PD-L1) have the greatest effect. The result of the PD-1/PD-L1 reaction is a suppression of the immune reaction mediated by T-cells, which can lead to progression of tumour [19]. PD-1 (CD279) is localised on the surface of cells and belongs to the CD28/CTLA-4 family of immunoglobulin molecules. PD-1 is expressed mainly on activated T-lymphocytes [20,21]. PD-L1 (B7-H1, CD274) is a glycoprotein localised on the cell surface and belongs to the B7 family [22]. PD-L1 is expressed on the surface of tumour cells and on the antigen presenting cells in various types of malignancy [23-26]. PD-L1 is very rarely expressed in normal tissue cells [27]. The PD-1/PD-L1 interaction plays a key role in the negative regulation of the immune reaction with the importance of preventing immune-mediated tissue damage. In the case of a chronic virus infection or in the presence of cancer, this reaction is accentuated [28,29]. The prognostic and predictive significance of expression of PD-1 or PD-L1 in tumors is not clearly defined [30,31].

The purpose of our retrospective study was to detect the expression of PD-L1 in MCC cells and to determine its prognostic significance.

Methods

Patient characteristics

A total of 13 patients with MCC were diagnosed in the period between 1st January 2000 and 31st December 2018 in our hospital. All 13 patients (4 men and 9 women) were included in the evaluation. The average patient age at the time of diagnosis was 76 years \pm 12.73, and the median age was 80 years (range 42-90). The median ECOG performance status was 2. In 12 patients, MCC was primarily localized on the skin, most on the of the head area (7 patients). No skin manifestation of MCC was found in one patient, where the disease presented as a lymph node metastasis in the right inguina. According to TNM classification, 3 patients were in stage I, 5 in stage II, and 3 patients were in stage III. In 2 patients, the disease was diagnosed in metastatic stage with hepatic and massive skin involvement. The study did not require informed consent.

Therapy

All patients with primary skin localization underwent surgical resection of the primary tumour. One patient with neck lymphadenopathy has undergone dissection of the neck lymph nodes. The patient with primarily inguinal lymphadenopathy underwent dissection of this area. Adjuvant radiotherapy of the regional lymphatic area was indicated for 5 patients. Irradiation was performed by linear accelerator (Elekta Precise). We applied photon beam radiotherapy with energy 6 and 15 MeV. We used the step and shoot technique of intensity modulated radiotherapy (IMRT; Figure 1). The prescribed dose was in the range of 50-60 Gy (median 56) in normofractionation regimen (2 Gy per day). Adjuvant chemotherapy or any other type of systemic cancer treatment was not indicated in any patient.

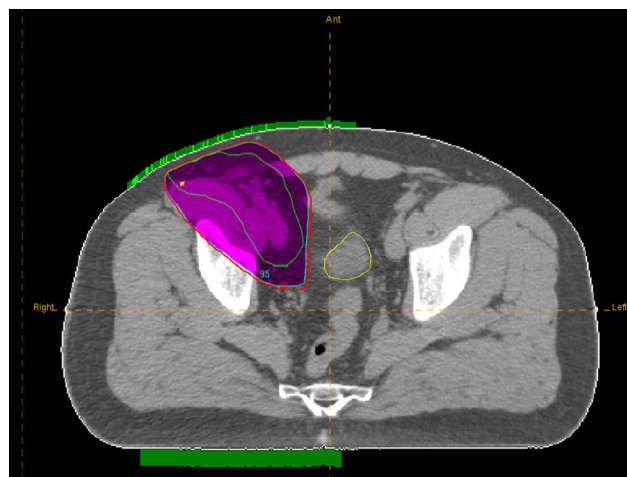


Figure 1. Isodose plan of adjuvant radiotherapy in a patient with inguinal lymphadenopathy.

Immunohistochemical determination of PD-L1 expression

The diagnosis of MCC was performed by histopathological analysis of the resected tissues in all 13 patients. All resected specimens were fixed in buffered formalin and embedded in paraffin. Five-micrometer thin sections were stained with haematoxylin and eosin. For immunohistochemical purposes the sections were placed on poly-D-lysine-coated glass slides. Rabbit monoclonal antibody recognizing PD-L1, clone 28-8, Abcam, Cambridge, UK, was diluted 1:400. Standard immunohistochemical procedure was applied to all specimens using Ventana BenchMark XT autostainer following antibody data-sheet recommended procedure. Pre-treatment with CC1 solution and OptiView detection system with 3,3-diaminobenzidine was used to visualize immunohistochemical reactions. Immunostaining results were evaluated by an experienced histopathologist (TJ) semi-quantitatively: 0- no positive staining; 1- up to 1% cells positive; 2- 1-10% cells positive; 3- 10-50% cells positive. Consensus was achieved viewing slides with multihead microscope (Olympus, Prague, Czech Republic) in a few controversial cases.

Statistics

The statistical analyses were performed by an experienced biostatistician using the NCSS statistical program (version 11.0.7). We defined the overall survival (OS) = the time from the performance of the initial surgical procedure to the date of death or the date of the last check up for the surviving patients (censored data). The OS curves were generated using the Kaplan-Meier method. The prognostic influence of the expression of

PD-L1 on OS was evaluated with log-rank test. Statistical tests were performed at a level of significance of $\alpha=0.05$.

Results

Recurrent disease was found in 6 patients in a group of 11 patients without primary metastatic involvement. The site of metastatic spread was as follows: liver, lung, peritoneum, bones and distant lymphadenopathy. In 5 patients no recurrent disease was detected after primary surgical resection. Primary metastatic stage was diagnosed in 2 patients. Currently, 4 patients are alive and 9 have died. Median OS was 18.7 months (95% CI 12.1-80.9). Two-year OS was 46.1% (95% CI 16.6-75.7 %; Figure 2).

PD-L1 positivity was detected in 8 patients (61.5 %) and in all cases the result was 1+ (Figure 3). PD-L1 negativity was found in the remaining 5 patients (Figure 4).

In patients with PD-L1 positivity the median OS was 42.1 months (95% CI 9.3-42.1) compared with the median OS of 9.4 months (95% CI 2.1-80.9) in the group of patients without PD-L1 positivity (Figure 5). No statistical significance was shown using the log-rank test between the two groups of patients ($p=0.417$).

Discussion

All patients with primary skin localization underwent surgical excision of the skin lesion. Adjuvant dissection of lymph nodes or postoperative radiotherapy on the regional lymphatic area was not routinely indicated in all patients. The reason for this was the poor performance status of the predominantly older population of patients (me-

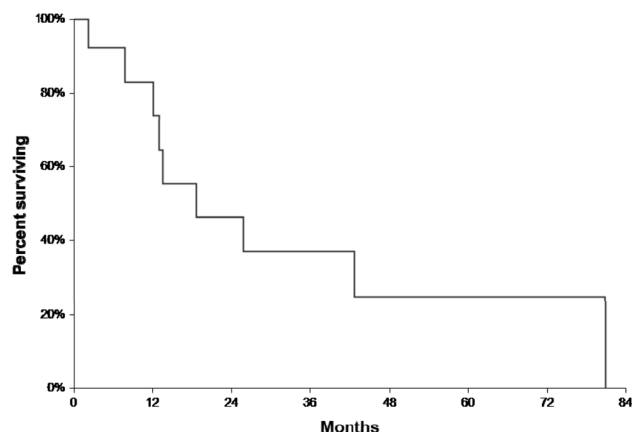


Figure 2. Kaplan-Meier overall survival.

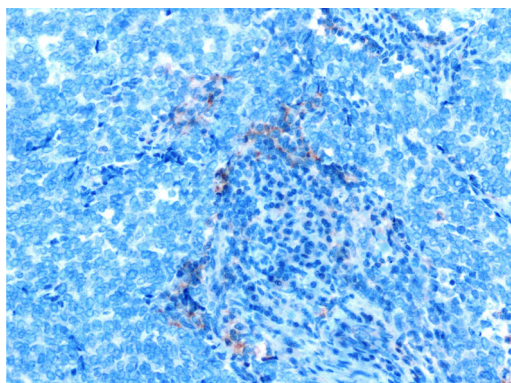


Figure 3. Expression PD-L1 1+ in MCC (magnification 200x).

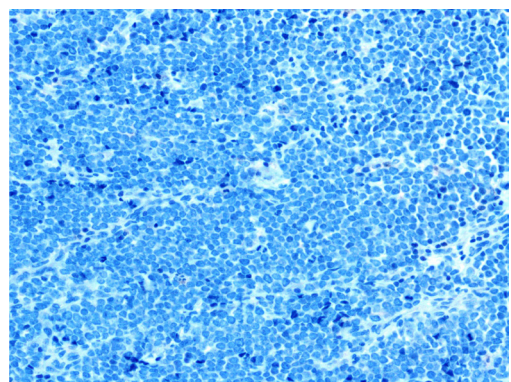


Figure 4. Negative expression PD-L1 in MCC (magnification 200x).

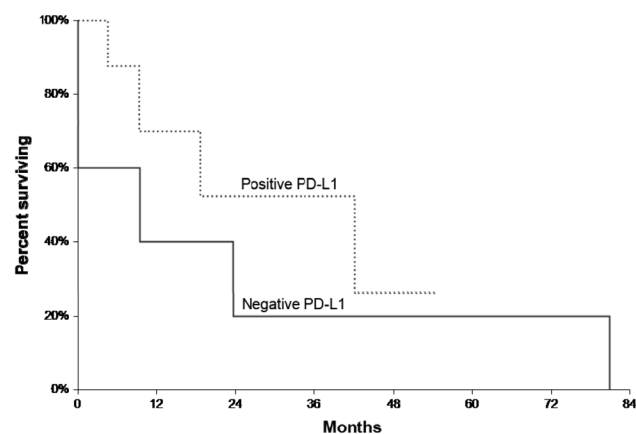


Figure 5. Kaplan-Meier overall survival. Intermittent curve: patients with PD-L1 positive; uninterrupted curve: patients without PD-L1 expression ($p=0.417$).

dian age:80). The absence of clearly defined treatment recommendations could have been another reason, especially in the period of 17 years in which we diagnosed this rare disease in our hospital. We performed adjuvant radiotherapy to stage III patients with involvement of regional lymph nodes. With respect to the various locations of the disease and the condition of the patients, the dosage of adjuvant radiotherapy was in the range of 50-60 Gy. We did not perform systemic chemotherapy for patients with metastatic disease. Again, the reason was the poor performance status, so symptomatic treatment was recommended.

One patient presented with primary inguinal lymphadenopathy. No primary skin lesion was identified. Similarly, dissemination of the disease was excluded. This was the youngest patient of the entire group (42 years old). This patient subsequently underwent dissection of the lymph node, and adjuvant radiotherapy in a dose of 2/50 Gy to the inguinal area (Figure 1).

Cases presented as lymph node metastasis without primary skin lesions are relatively rare (12-14%) [32,33]. The disease is defined as unknown primary MCC (UPMCC). Currently, it is not clear if it is primary MCC of the lymph nodes or metastasis of MCC of the skin with spontaneous regression of skin lesion [34]. It appears that patients with UPMCC have a slightly better prognosis than patients with primary MCC of the skin. Median OS in patients with UPMCC ranges from 18 to 104 months [33,35,36]. Also, our patient with UPMCC is currently without any signs of disease recurrence. On the other hand, a strong association between UPMCC and other malignancy has been described [37]. In our patient with UPMCC we demonstrated the MCPyV infection. The significance of the MCPyV infection in patients with UPMCC is interpreted differently in various publications. Pan et al describe this infection in 4 from 13 patients, while Biase et al proved this fact in all 5 evaluated patients [37,39].

In our group, we did not demonstrate statistically significant difference of the prognostic influence of the expression of PD-L1 on the OS of patients with MCC. The median OS was more than 4-fold longer in the patients with positive expression of PD-L1 than in the patients with PD-L1 negativity (42.1 versus 9.4 months, Figure 5). Despite a difference of 32.7 months, log-rank test showed the difference was not significant. A possible explanation could be related to the small number of patients in the studied group. In our documentation we recorded a total of 13 patients in 18 years. Therefore, it can be assumed that statistical significance could be achieved with a larger group of patients.

Several clinical trials tried to evaluate the prognostic influence of the expression of PD-L1 in MCC patients. Probably the largest study was a group of 49 patients where the expression of PD-L1 was proved in almost half the cases (49%) [40]. Further, a higher expression of PD-L1 in MCPyV-positive MCC was described in MCPyV-negative MCC [5,40]. As already mentioned, the PD-1/PD-L1 interaction negatively influences the immune response. For this reason, therapeutic blockade of the PD-1/PD-L1 immune checkpoint can strengthen the immune reaction with therapeutic results. A clinical trial evaluated pembrolizumab (anti-PD1) in patients with advanced MCC in first-line treatment. The treatment with pembrolizumab demonstrated 56% therapeutic responses [41]. Another study published later evaluated the predictive significance of biomarkers for the treatment with pembrolizumab. An association with the treatment results in a group of patients with PD-1 or PD-L1 positivity was revealed [42]. Avelumab (anti PD-L1) was another antibody that was evaluated in the therapy of MCC. Its efficacy was proven in clinical trials in patients with metastatic MCC in first-line treatment (therapeutic response 66%), as well as in second-line treatment in patients resistant to chemotherapy (therapeutic response 32%) [43,44]. In a I/II phase clinical trial, the antibody nivolumab (anti-PD1) demonstrated a total therapeutic response of 73% in patients without previous treatment, and in 50% response in previously treated patients [45]. All trials have proved the efficacy of pembrolizumab, avelumab and nivolumab in MCPyV-positive as well as MCPyV-negative MCC.

On the basis of the presented results, the strategy of metastatic MCC treatment is gradually changing. Further identification of possible prognostic but also predictive factors for this disease will be a challenge for current research. The issue is also becoming far more relevant due to constantly increasing incidence.

In conclusion we found PD-L1 positivity in 61.5% of patients with MCC and we have not demonstrated prognostic significance of expression PD-L1 for OS.

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Conflict of interests

The authors declare no conflict of interests.

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