Merkel Cell Carcinoma of the Head and Neck: Potential Histopathologic Predictors

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Objectives/Hypothesis: To identify or confirm any new or suggested independent histopathological predictors in Merkel cell carcinoma (MCC) of the head and neck (HN) correlated with outcome.

Study Design: Retrospective chart and pathology review.

Methods: Between 1990 and 2010, 58 patients with Merkel cell carcinoma of the head and neck HNMCC were identified for study. Pathologic specimens were reviewed and evaluated for independent prognostic factors and correlated with locoregional recurrence and disease-specific survival.

Results: The 2- and 5-year disease-specific survival (DSS) rates were 72.7% and 63.6%, respectively. The local and regional recurrence rates were 12.0% and 24.1%, respectively. A total of 25.9% of the patients developed distant metastases during follow-up. Tumor size (< 1 cm vs. > 1 cm) and the presence of a positive deep resection margin were independently found to be significantly associated with regional recurrence \((P = 0.01\) and \(P = 0.04\), respectively). No other prognostic factors could be identified.

Conclusion: Adjuvant radiotherapy cannot remediate a positive resection margin. Given these results, consideration for revision surgery should be considered for a positive deep margin. Frozen section analysis may help to define the margins in this invasive and aggressive disease.

Key Words: Merkel cell carcinoma, head and neck, histopathological predictors.

Level of Evidence: 2b.

INTRODUCTION

Over the past decades there has been a rising incidence of Merkel cell carcinoma (MCC). Usually MCC presents in the elderly patient, with the head and neck (HN) being the most common site of involvement. MCC has a propensity to spread to regional lymphatic sites, with many patients presenting with nodal metastases at the time of diagnosis.\(^1\) Ultraviolet B (UVB) radiation exposure and immunosuppression have been reported to be the primary etiologic factors.\(^2,3\) Recently Feng et al. identified a virus, termed by the authors as Merkel cell polyomavirus (MCV), in a significant proportion of patients, which may represent another potential etiology for the development of MCC\(^4,5\)

The latest edition of the American Joint Committee on Cancer (AJCC) staging system\(^6\) uses a new protocol for the examination of specimens from patients with MCC of the skin developed by the College of American Pathologists. This provides clinically useful and relevant information for the pathologist when reporting results of surgical specimen examinations.\(^7\) The protocol relies on the assessment of histological parameters that have been found to be of prognostic significance.

Since there is still some controversy regarding the optimal treatment strategy, independent histopathologic factors may elucidate the best treatment management for patients with HNMCC, especially in early stage disease. Furthermore, prognostic factors may help to identify the patient who benefits the most from different, mostly aggressive, treatment strategies in an elderly population. The aim of this study was to identify or confirm any new or suggested independent histopathological predictors correlated with outcome.

MATERIALS AND METHODS

A retrospective review of 58 patients treated with a biopsy-proven MCC between the years 1990 and 2010 at the Princess Margaret Hospital (PMH), Toronto, ON, Canada was performed. Patients were identified through the Princess Margaret Hospital Cancer Registry, and were included if they had MCC arising within the skin of the head and neck. Institutional research ethics board approval was obtained for the study.

Sociodemographic, clinicopathologic, and outcome data was obtained from the electronic medical record. The disease
was staged based on the AJCC-staging system. All histological specimens were reviewed by two dermatopathologists. Cases were evaluated according to the proposed guidelines by the College of American Pathologists (CAP), which identified the following parameters as important in histopathological description of an MCC specimen: tumor size, tumor thickness (Fig. 1A), margins (peripheral and deep margin; Fig. 1B), lymph-vascular invasion (LVI) (Fig. 1C), perineural invasion (PNI), mitotic index, tumor-infiltrating lymphocytes (TILs) (Fig. 1D), and the tumor growth pattern (nodular vs. infiltrative). In addition to these parameters, the use of immunohistochemistry (i.e., cytokeratin 20, chromogranin-A, synaptophysin) for confirmation of diagnosis was noted.

A surgical resection margin was considered involved if tumor cells were identified at the margin of the specimen.

**Statistical Analysis**

Demographic and pathologic data were summarized using descriptive statistics. Descriptive statistics are provided with median and range for continuous factors such as diagnosis age, and frequencies and proportions for categorical factors such as tumor stage and treatment. The local and regional recurrence rates and disease-free survival rates were calculated from the date of diagnosis to the date of event of interest or the last follow-up. Survival analysis was performed using the Kaplan-Meier method. Differences between survival curves were analyzed by log-rank test. Cox proportional hazard regression model was applied for multivariate analysis. Hazard ratio (HR) and 95% confidence interval was estimated based on the Cox regression model. All the statistical tests were two-sided, and a P value of < 0.05 was considered significant. Statistical analyses were performed using Version 9.2 of the SAS system and User’s Guide (SAS Institute, Cary, NC).

**RESULTS**

A total of 58 patients were eligible for inclusion. There were 32 male and 26 female patients. The median age at presentation was 78.5 years (range, 38–95). The most common primary site was the cheek (33%, n = 19/58), followed in frequency by the skin of the neck (15%, n = 9/58), lip (7), ear (5), nose (4), forehead (4), scalp (4),
eyelid (3), and the temple (3). Fourteen patients (24%) were immunosuppressed, with five patients having a history of organ transplantation and nine patients having a history of leukemia or other immunocompromising diseases. Most patients (66%, n = 38) presented with early local disease (T1/T2), while 20 patients (34%) presented with nodal metastases on clinical and/or radiographic examination. All patient and tumor characteristics can be found in Table I.

Treatment
Surgery was the primary modality of treatment in 47 (81%) patients, of which 30 (64%) received adjuvant radiotherapy. All patients underwent excision of the primary tumor, and 42.5% (20/47) underwent a neck dissection. Of the patients who did not undergo a neck dissection, 13 patients received adjuvant radiotherapy for local-regional control, while 14 patients did not have any further management of their neck. All of the 14 patients had no evidence for nodal involvement at the time of diagnosis and most of the primaries were pT1 lesions (9/14). The remaining primary lesions (5) included one pT2 lesion, one pT4 lesion, and three cases where the retrospective pathologic analysis of the primary lesion could not be performed due to the lack of specimen. Eleven patients underwent primary radiotherapy only. Both the primary and the neck were treated with RT, and four patients had their primary site only irradiated. The median dose was 50 Gy (range, 14–66). One patient receiving only 14 Gy died during the course of treatment. Chemotherapy was administered in two patients presenting with distant metastases.

Histopathological Parameters
The median tumor size was 1.2 cm (range, 0.4–10 cm) and the median tumor thickness was 6.5 mm (range, 1.5–40 mm). In the 20 patients who underwent a neck dissection, 14 (70%) showed pathologic evidence of nodal metastases. The median and mean numbers of positive lymph nodes identified in the neck dissection specimen were 1 (range, 1–36) and 4.5 respectively (std 7.6).

On margin assessment of the 47 surgically treated patients, original pathology material was available for review in 39 cases (the remaining 8 cases were seen only in pathology consultation without retained material on-site for reassessment). Nineteen of these (49%) had negative margins at the primary site (peripheral and deep margin). The remaining 20 patients had one or more reported positive margins. Of these, 12 had a positive peripheral margin and 19 had a positive deep margin, with an overlap of 11 cases showing both positive peripheral and deep margins. This translated to a negative peripheral margin achieved in 69% (27/39) and a negative deep margin achieved in 51% (20/39). The mean distance to the peripheral margin was 2.1 mm (±3.4 mm), whereas the mean distance to the deep margin was 1.3 mm (±3.9 mm).

A re-resection was immediately performed in 12 cases, whereas adjuvant radiotherapy was used as further treatment only in eight cases revealing a positive resection margin.

The cheek was the dominant site in the case of a positive margin, accounting for 45% (9/20), followed by the ear (2/20; 10%), the nose (2/20; 10%), the neck (2/20; 10%), and the lip (2/20; 10%). Further sites with positive margins were the forehead (1), eyelid (1), and scalp (1). Five out of the 20 (25%) patients with positive resection margins were immunosuppressed.

Lymphatic and vascular invasion (LVI) was present in 68% (26/38 cases) of patients and perineural invasion (PNI) in 24% (8/33 cases) of patients. The median mitotic index was 57/mm² (range, 9–141). Tumor-infiltrating lymphocytes (TILs) were found in 9/32 cases (28%). The
growth pattern showed mostly a nodular pattern (67%; 24/36 cases). Cytokeratin (CK) 20 staining was positive in 91% (31/34 cases) and synaptophysin was positive in 74% (26/35 cases). Chromogranin A was found to be positive in 45% (14/31 cases). Spindle cells were present in 15% (6/40 cases).

Outcome Analysis

The overall median and mean follow-up time was 19 months (range, 1–168) and 29.6 months (±30.9), respectively. The median and mean follow-up time for the patients alive at last follow-up was 38 months (range, 1–168) and 44.5 months (±36.5), respectively. At last follow-up 26 patients were alive without disease. Twenty-eight (48.3%) patients died during follow-up, 15 died from disease, and 13 died from other causes.

Recurrence Outcomes

The overall recurrence rate in this series was 31.0% (18/58). The median time to recurrence was 6.6 months (range, 1–90). The local and regional recurrence rates were 12.0% (7/58) and 24.1% (14/58), respectively. During follow-up 25.9% (15/58) of the patients developed distant metastases. Of the patients with pathologic material available for review, there was no association between local recurrence and tumor size (<1 cm vs. ≥1 cm, \(P = 0.11\)), tumor thickness \((P = 0.15)\), high mitotic index rate \((P = 0.40)\), TILs \((P = 0.16)\), LVI \((P = 0.65)\), PNI \((P = 0.20)\), growth pattern \((P = 0.12)\), peripheral margin status \((P = 0.70)\), deep margin status \((P = 0.24)\), or presence of spindle cells \((P = 0.43)\). Furthermore, there was no association between the presence of positive staining for CK20, chromogranin A, and synaptophysin and local recurrence \((P = 0.41, 0.44, \text{and } 0.17)\), respectively.

There was a significant correlation between tumor size \((<1 \text{ cm vs. } ≥1 \text{ cm})\) and regional recurrence \((P = 0.01\); Fig. 2). In addition, the presence of a positive deep resection margin was significantly associated with regional recurrence \((P = 0.04\); Fig. 3). However, there was no significant association between regional recurrence and tumor thickness \((P = 0.29)\), high mitotic index rate \((P = 0.13)\), TILs \((P = 0.38)\), LVI \((P = 1.0)\), PNI \((P = 0.20)\), growth pattern \((P = 0.76)\), peripheral margin \((P = 0.29)\), or presence of spindle cells \((P = 0.57)\). There was also no association between the presence of positive immunohistochemical staining for CK20, chromogranin A, and synaptophysin and nodal recurrence \((P = 0.31, 0.38, \text{and } 0.23)\), respectively.

Survival Outcomes

Survival outcomes are presented for the entire cohort of patients. The 2- and 5-year overall survival (OS) rate was 57.8% and 47%, respectively. The 2- and 5-year disease-specific survival (DSS) rates were 72.7% and 63.6%, respectively. The Kaplan-Meier curves for
OS and DSS are shown in Figures 4 and 5, respectively. On univariate analysis, tumor size ($P = 0.76$), tumor thickness ($P = 1.0$), high mitotic index rate ($P = 0.26$), TILs ($P = 0.17$), LVI ($P = 0.82$), PNI ($P = 0.44$), growth pattern ($P = 0.90$), peripheral margin ($P = 0.70$), deep margin ($P = 0.79$), or presence of spindle cells ($P = 0.56$) were not found to be significantly associated with DSS. Furthermore, there was no significant association between positive immunohistochemical staining for CK20 ($P = 1.0$), chromogranin A ($P = 0.82$), synaptophysin ($P = 0.37$), and DSS.

**DISCUSSION**

It is known that regional and distant metastases at time of diagnosis can be present in MCC in 10% to 30% and up to 6%, respectively. A combined treatment approach (surgery plus radiotherapy) is often preferred and leads to better outcome results. However, this aggressive treatment approach may not be applicable to all patients with MCC since many patients are aged or immunosuppressed. In addition, some patients present with early stage disease where combined therapy may not be required. Thus, there is a need to identify additional prognostic factors in order to select out high- and low-risk patients for recurrence or death, where the former patients may potentially be treated with a less aggressive therapy regimen.

In the current study we failed to find any histopathologic variables that were statistically significant predictors of local recurrence or survival, while increased tumor size and a positive deep margin status were noted to be associated with a higher rate of regional recurrence. Tumor size has inconsistently been found in other studies to be associated with survival. Andea et al. found in their series of patients with MCC, mostly other than head and neck, a significant correlation between tumor size and DSS on univariate analysis ($P = 0.0002$) but not on multivariable analysis. Other authors found tumor size was associated with poor survival outcome. Llombart et al. only found a significant correlation between tumors larger than 3 cm and poor prognosis. In their study all tumors with distant metastases ($n = 4$) were larger than 3 cm. In the current series there was no relationship between tumor size and distant metastases. With regard to regional recurrence, we were able to demonstrate that tumor size $>1$ cm was associated with a higher rate of regional recurrence. In contrast, Sandel et al. reported no prognostic significance of tumor size with respect to regional recurrence.

As in melanoma, tumor size may be less important than tumor thickness. However, many studies, including the current series, have failed to demonstrate a relationship between tumor thickness and recurrence or survival. Andea et al. found in their analysis that tumor thickness was associated with poor prognosis on univariate analysis, but not on multivariable analysis. Promoting the idea of tumor thickness as a prognostic indicator may work in some body subsites but does not show statistical significance for HNMCC. Given the histological anatomy of skin in the head and neck, this may be due to relative access to lymphatics and large vessels in subcutaneous tissue and skeletal muscle despite small tumor thickness measurements. Thus, tumor thickness could be interpreted in the context of other features and not be considered as a reliable independent prognostic factor for HNMCC when making decisions on a patient's prognosis.

Many authors have published on the importance of negative resection margins, although the extent of margin required in MCC is controversial. A combined treatment approach may not be applicable to all patients with MCC since many patients are aged or immunosuppressed. In addition, some patients present with early stage disease where combined therapy may not be required. Thus, there is a need to identify additional prognostic factors in order to select out high- and low-risk patients for recurrence or death, where the former patients may potentially be treated with a less aggressive therapy regimen.

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section from the most suspicious area of deep margin involvement with the provided clinical history of MCC would be a reasonable approach to intraoperative frozen-section assessment for this entity.

Another explanation for the rate of positive deep margins is the location of the tumors. When looking for which site was found to reveal positive deep margins, the cheek was found to be the most affected subsite. As with many tumors in this area, the goal is the preservation of the facial nerve knowing that the resection margin may be positive and patient may receive postoperative adjuvant radiation, especially in case of MCC being highly radiosensitive. Nevertheless, a frozen section or a re-resection is warranted in order to obtain negative margins.

Further histopathologic parameters including the mitotic index, or the presence of TILs, previously reported to be associated with poorer prognosis, were investigated. A high mitotic index was found (median 57/mm²), but this was not found to be associated with outcome. Whether this is due to the limited study size or whether its importance is less than in melanoma remains unclear. TILs were only present in 28% of the primaries in our series; therefore, sample size plays a role in the data analysis showing no correlation with poorer outcome. The addition of further analysis of histopathologic factors such as LVI, PNI, the growth pattern (nodular versus infiltrative), the presence of spindle cell component, and several immunohistochemical parameters failed to demonstrate any significant prognostic value related to outcome analysis.

The reason for not being able to demonstrate any significant association of a number of histopathologic variables with prognosis is a limitation of this study. As in many of the published studies with Merkel cell carcinoma, the sample size of our institutional review is of a substantial size but still limited in numbers. Although we have only included MCC arising from the skin of the head and neck, the heterogeneity of the different HN subsites plays an important factor. Further, the nonuniform treatment approach and the inclusion of immunosuppressed patients influence the data substantially.

**CONCLUSION**

With a single institutional series, we are able to identify prognostic factors for MCC, such as tumor size and deep resection margin involvement. Large, multicenter, and prospective studies are warranted to identify and verify more predictive histopathologic factors affecting further treatment. The deep margin of the primary, even small, is of utmost importance in this disease that shows aggressive vertical growth phase. Adjuvant radiotherapy cannot replace a positive resection margin, despite the highly radiosensitive nature of MCC. Thus, in cases with a positive deep margin on final pathologic assessment, a re-resection should be performed. Intraoperative frozen section analysis may help to define the margins in this invasive and aggressive disease.

**BIBLIOGRAPHY**