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ORIGINAL ARTICLE – MELANOMA

Merkel Cell Polyomavirus Antibody Titer Predicts Recurrence-Free Survival

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ABSTRACT

Background. Merkel cell polyomavirus (MCPyV) is associated with the development of Merkel cell carcinoma (MCC). Antibody (MCPyV-Ab) titers may have prognostic implications. This study evaluated the impact of the presence or absence of MCPyV-Ab on the 2-year overall survival (OS) and disease-free survival (DFS) of MCC patients.

Methods. This single-center, IRB-approved, retrospective cohort study evaluated 51 adult patients with MCC from 2014 to 2021 using a prospectively maintained database. Patients were compared by MCPyV-Ab status, and Kaplan-Meier analysis was used to evaluate 2-year OS and DFS.

Results. Of the 51 patients, 13 (25.4%) were seropositive, 41 (80.4%) underwent wide excision, 40 (80.0%) received radiotherapy, and 43 (84.3%) received multimodal therapy. The median follow-up period was 15.5 months (range 1–69.5 months). The median 2-year OS of the entire cohort was not reached. The median 2-year OS was not reached for either the seronegative or the seropositive patients. The difference in 2-year OS between the groups was not statistically significant (p = 0.37). Eight patients, all seronegative, were never rendered disease-free and were removed from recurrence analysis. The seropositive patients experienced no recurrences. Of the 30 seronegative patients, 9 (30.0%) experienced recurrence. The median 2-year DFS of the entire cohort was not reached. The median 2-year DFS of the seronegative group was 22.2 months. The 2-year DFS was not reached for the seropositive cohort. Seropositivity conferred a significantly better 2-year DFS than seronegativity (p = 0.04).

Conclusion. The MCPyV-Ab seropositive patients demonstrated improved 2-year DFS. The seropositive patients showed a strong trend toward improved 2-year OS, although the difference not statistically significant. This study substantiated the value of MCPyV-Ab assessment for MCC.

Merkel cell carcinoma (MCC) is an aggressive neuroendocrine cutaneous malignancy with a low overall survival and a high rate of recurrence and regional nodal metastasis.[1] From 2000 to 2013, the incidence of MCC within the United States increased from approximately 0.5 cases to 0.7 cases per 100,000, with a median age of 74–76 years at diagnosis of MCC.[2,3] This increasing incidence often is attributed to an aging population.[1] However, in addition to advanced age, Merkel cell polyomavirus (MCPyV), ultraviolet (UV) radiation, and immunosuppression are well-established risk factors for the development of MCC.[4–6]

MCPyV is a member of the non-enveloped, double-stranded DNA *Polyomaviridae* family,[7] and is detected in 60 % to 80 % of the general U.S. population.[4,8] Antibodies against MCPyV-related antigens have demonstrated promise as a useful clinical marker because their presence has been shown to correlate with improved outcomes.[9–12] However, not all studies are congruent, but this discrepancy is possibly due to the varying methods and targets used for MCPyV detection. Different detectable viral

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antigens represent different functional aspects of the virus and its contribution to tumorigenesis. Antibodies against capsid antigens represent a marker of previous infection, but are believed to play no role in tumor persistence, do not vary with disease burden,[10,13] and are found at high rates in the general population.[14] Alternatively, antibodies against MCPyV oncoproteins, found almost exclusively in MCC patients, are shown to be a more useful tumor marker because they are more specific and correlate with disease burden.[10] MCPyV oncoprotein antibodies are what is use in clinical practice and what we analyze in our study.

Studies have validated MCPyV antibodies (MCPyV-Ab) as a useful clinical marker for both prognostication and ongoing surveillance. Paulson et al.[9,10] found that MCPyV oncoprotein antibody seropositive patients had a lower risk of recurrence than seronegative patients. They found that an increase in oncoprotein antibody levels was predictive of, and sometimes preceded, disease recurrence.[12]

Further studies controlling for age, sex, disease stage, and immunosuppression demonstrated the dynamic correlation between tumor burden and antibody levels. Among patients with no recurrence, after initial treatment, antibody titers rapidly declined, and patients became seronegative by a median of 8.4 months. However, increasing oncoprotein titers had both a positive and a negative predictive value for clinically evident recurrence.[10] Paulson et. al. argued that obtaining MCPyV oncoprotein antibody titers at diagnosis for all patients allowed for the stratification of patients into high- and low-risk prognosis groups.

This single-institution, retrospective cohort study evaluated the impact that the presence or absence of MCPyV-Ab has on the 2-year overall survival (OS) and disease-free survival (DFS) of patients with MCC in order to contribute to the growing body of evidence supporting the use of MCPyV-Ab in prognostication.

METHODS

Data

This retrospective, single-institution study used a prospectively gathered database to evaluate patients with a diagnosis of MCC from June 2015 to April 2021 at the University of Tennessee Medical Center in Knoxville, Tennessee (UTMCK). An MCPyV-Ab titer was collected at the first patient visit and then at 3-month intervals if positive at the initial draw. Serology assays were performed at the University of Washington Clinical Immunology Laboratory as previously described.[14] The threshold for a negative titer was set at less than 75. Patients with titers at 75 or higher were defined as antibody-positive.[10] After exclusion of 14 patients with unknown antibody titers, 51 patients were included in the final analysis.

Analyses included multiple patient-level variables including demographics (age, gender), immunocompromised status, prior malignancies, location of MCC (head and neck, trunk, upper extremity, lower extremity, unknown primary), and pathologic stage (American Joint Committee on Cancer, 8th edition). Race/ethnicity was not included because all the patients were white. The perioperative variables included type of operation (wide local excision, sentinel lymph node biopsy, complete node dissection), margin status (positive or negative), and sentinel lymph node status (positive or negative). The reasons for not performing a sentinel lymph node biopsy (SLNB) included clinically positive nodes, failed lymph node mapping with lymphoscintigraphy, and poor surgical candidates who could not tolerate general anesthesia. The patients undergoing complete lymph node dissection (CLND) were divided into two groups: the patients with clinically occult nodal disease but positive sentinel nodes and the patients with clinically positive nodes. The indications for not performing a CLND included patients with a negative SLNB and patients who were poor surgical candidates. All resections were performed by one of three fellowship-trained surgical oncologists. Adjuvant treatment included immunotherapy (avelumab, nivolumab) and radiation.

Patient OS was defined as the time from index surgery until the date of death. Patients alive at the last recorded follow-up were considered censored for OS. Recurrence was determined by biopsy or imaging findings. Patient DFS was defined as the time from index surgery until the date of recurrence found on biopsy or imaging. Patients alive and without a documented recurrence at the time of the last follow-up visit were considered censored for DFS. Eight patients were removed from the DFS analysis because they were never rendered disease-free. All eight patients were antibody-negative. The study protocol was reviewed and approved by the Institutional Review Board (IRB) at the UTMCK Graduate School of Medicine (IRB 4337).

Statistical Analysis

The cohort was divided into two groups based on the presence or absence of MCPyV antibody. Univariate analysis using Pearson chi-square tests for categorical variables and Kruskal-Wallis tests for continuous variables was performed to determine whether patient characteristics differed across the antibody groups. In the analysis for OS and DFS, the Kaplan-Meier method was used to estimate survival curves, and the log-rank test was used to test for differences among curves. All statistical analyses were performed using STATA version 16.1 (StataCorp LLP,

College Station, TX, USA). Statistical significance for all analyses was defined as a p value lower than 0.05.

RESULTS

Patient Characteristics

The 51 patients with known antibody titers were stratified into two cohorts: 38 MCPyV antibody-negative (AbNeg) patients (74.5 %) and 13 MCPyV antibody-positive (AbPos) patients (25.4 %) (Table 1). The median age at diagnosis was 78 years for the AbNeg cohort and 71 years for the AbPos cohort. The majority of the patients were male (n = 32, 62.8 %), and all the patients were of Caucasian ethnicity. Ten (26.3 %) of the AbNeg patients were immunocompromised compared with 2 (15.4 %) of the AbPos patients, but this was not statistically significant. The majority of the AbNeg primary tumors (n = 19, 50.7 %) were found on the head and neck, whereas most of the AbPos primary tumors (n = 10, 76.9 %) were located on the upper extremity (p = 0.01). Of the AbNeg patients, 28 (73.7 %) underwent wide local excision, compared with all of the AbPos patients. Positive margins were found in four of the AbNeg patients (10.5 %), and negative margins were achieved in all of the AbPos patients.

In the AbNeg cohort, 17 patients (44.7 %) underwent SLNB, 2 patients (5.3 %) had clinically positive nodes and underwent CLND, 18 patients (47.4 %) had no nodal evaluation due to poor functional status, and 1 patient (2.6 %) did not map with lymphoscintigraphy. A CLND was not performed for 12 of the AbNeg patients (31.6 %) because their sentinel lymph node was negative. One patient (2.6 %) underwent CLND after exhibiting a positive sentinel node. Eight (66.7 %) of the AbPos patients underwent SLNB, whereas two (16.7 %) of the patients had clinically positive nodes and underwent CLND, and two (16.7%) of the patients had a poor functional status precluding nodal evaluation. Five AbPos patients (38.5 %) did not undergo CLND because their sentinel lymph node was negative. One AbPos patient (7.7%) underwent CLND after exhibiting a positive sentinel lymph node.

The majority of the patients had stage 1 disease (17 AbNeg patients and 6 AbPos patients), and there was no significant difference between the cohorts. The majority of the AbNeg and AbPos patients received radiation treatment. In the AbNeg cohort, 22 patients (59.5 %) received adjuvant radiation, and 13 patients (35.1 %) received radiation as primary treatment. Of the AbPos cohort, 11 patients (84.6 %) received adjuvant radiation, and 3 patients (23.1 %) received radiation as primary treatment.

DFS and OS For the entire cohort, the median follow-up time was 15.5 months (range, 1–70 months). Eight patients (15.7 %) were never rendered disease-free, and all of them were AbNeg. Of the remaining 43 patients who were disease-free, 9 (20.9 %) experienced recurrence, and all were AbNeg. Of those who had recurrence, 5 (55.6 %) had a locoregional recurrence, 3 (33.3 %) had a distant recurrence, and 1 (11.1 %) had both locoregional and distant recurrences. The median time to recurrence was 6.7 months (range, 1.2–22.2 months).

2-year DFS stratified by AbNeg and AbPos is shown in Fig. 1. The median 2-year DFS of the AbNeg patients was 22.2 months. 2-year DFS was not reached for the AbPos cohort. The AbPos cohort had a significantly better 2-year DFS than the AbNeg cohort (p = 0.04).

2-year OS stratified by AbNeg and AbPos is shown in Fig. 2. The median 2-year OS of the 51-patient cohort, including the patients who never were disease-free, was not reached. The median 2-year OS of the AbNeg patients was not reached, and the median 2-year OS of the antibody-positive group was not reached. The difference in 2-year OS between the AbNeg and AbPos patients was not statistically significant (p = 0.37).

DISCUSSION

Merkel cell carcinoma is an aggressive malignancy characterized by a propensity for disease progression and recurrence. Recently, significant advances in prognostication and surveillance of MCC have occurred, namely, with regard to our evolving understanding and usage of MCPyV-Ab titers. The current National Comprehensive Cancer Network (NCCN) guidelines recommend considering MCPyV serology for all patients as part of the initial MCC workup, and as a tool for ongoing surveillance among seropositive patients.[15] When obtained at diagnosis, MCPyV-Ab allows the stratification of patients into high-risk seronegative and low-risk seropositive cohorts.[9]

Seronegativity at diagnosis across multiple studies has been consistently associated with a higher risk of recurrence as well as worse DFS and OS.[9–12] Additionally, among seropositive patients, the utility of MCPyV-Ab titers for surveillance continues through the course of the disease because it has been shown to correlate with tumor activity, thus allowing for evaluation of treatment response and recurrence.[9,10]

Multiple studies have demonstrated that MCPyV oncoproteins promote cell cycle progression and inhibition of tumor suppression.[16–18] Titer levels typically decline after resection of the primary tumor, and increasing levels can be a harbinger of disease recurrence.[10,13] Paulson et al.[9] demonstrated a rapid drop in titers after resection among all AbPos patients in their study who did not experience a

TABLE 1 Characteristics of patient with Merkel cell carcinoma stratified by Merkel cell polyomavirus (MCPyV) antibody status

	Antibody-negative (n = 38) (%)	Antibody-positive (n = 13) (%)	p Value
Median age at diagnosis (years)	78	71	0.01
Sex			0.58
Male	60.5	69.2	
Female	39.5	30.8	
Immunocompromised	26.3	15.4	0.42
Prior cutaneous malignancy	65.8	53.8	0.44
Location			0.01
Head and neck	50.0	7.7	
Trunk	7.9	7.7	
Upper extremity	23.7	76.9	
Lower extremity	15.8	7.7	
Unknown primary	2.6	0.0	
Wide local excision	73.7	100.0	0.04
Positive margins	10.5	0.0	0.22
Sentinel lymph node biopsy			0.19
No, clinically positive	5.3	16.7	
Yes	44.7	66.7	
No, poor surgical candidate	47.4	16.7	
No, failed mapping	2.6	0.0	
Sentinel nodes			0.58
Negative	28.9	38.5	
Positive	15.8	23.1	
Not done	55.3	38.5	
Complete nodal dissection			0.14
No, pN0	31.6	38.5	
Yes, completion dissection	2.6	7.7	
No, poor surgical candidate	60.5	30.8	
Yes, cN1	5.3	23.1	
Stage			0.59
1	50.0	46.2	
2a	17.6	15.4	
2b	0.0	0.0	
3a	11.8	23.1	
3b	11.8	15.4	
4	8.8	0.0	
Immunotherapy	22.9	8.3	0.27
Avelumab	75.0	100.0	
Nivolumab	0.0	100.0	
Other	0.0	0.0	
Radiation	78.4	84.6	0.63
None	21.6	7.7	
Adjuvant	59.5	84.6	
Primary treatment	35.1	23.1	
Salvage	0.0	0.0	
Recurrence			0.01
None	55.3	100.0	
Recurred	23.7	0.0	

Table 1 (continued)

	Antibody-negative (n = 38) (%)	Antibody-positive (n = 13) (%)	p Value
Never disease-free	21.1	0.0	
Recurrence location			
Locoregional	13.2	0.0	0.17
Distant	10.5	0.0	0.22
Status			0.08
No evidence of disease	44.7	92.3	
Alive with disease	23.7	0.0	
Died of disease	10.5	0.0	
Died of other cause	7.9	7.7	
Died of unknown cause	7.9	0.0	
Lost to follow up	5.3	0.0	
Disease-free interval	328.5	733.2	
Overall survival	508.9	733.7	

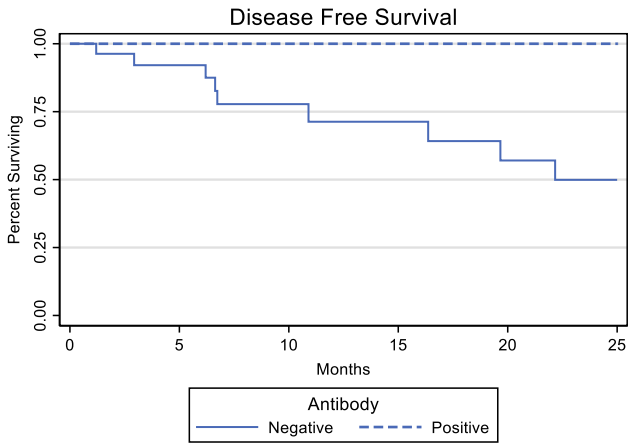

FIG. 1 2-year disease-free survival (DFS) of Merkel cell carcinoma patients stratified by Merkel cell polyomavirus (MCPyV) antibody status (p = 0.04, log-rank)

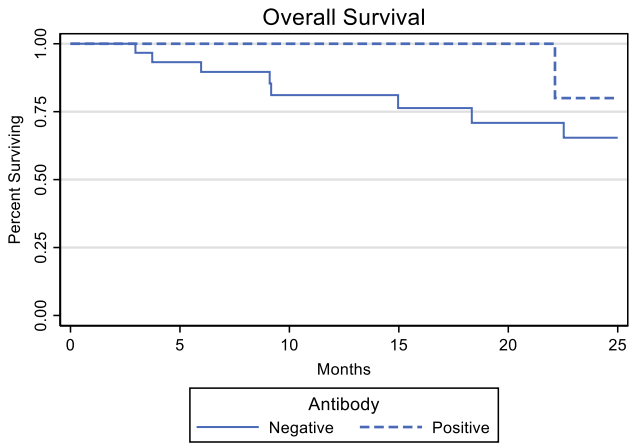

FIG. 2 2-year overall survival (OS) of Merkel cell carcinoma patients stratified by Merkel cell polyomavirus (MCPyV) antibody status (p = 0.3, log-rank)

recurrence. They also discovered rising titers in all six patients who experienced disease recurrence. Additionally, three of these six patients demonstrated elevated titers months before clinically detected recurrence. Because none of our seropositive patients experienced a recurrence, we were unable to determine whether increasing titers correlated with risk of recurrence in our population.

Antibody seronegativity at diagnosis stratifies patients into a high-risk cohort. In addition to representing an increased risk of recurrence and worse outcomes, seronegativity poses a diagnostic conundrum because serial antibody titers serve no use in ongoing surveillance of these patients. Seronegative patients require more vigilant clinical follow-up surveillance including more frequent imaging. The NCCN currently recommends the intensification of radiologic imaging surveillance for seronegative patients.[15] Although our conclusions are limited by our univariate analysis, because factors affecting survival are potentially confounded by many variables including age, locations of lesions, and radiation, the results of our study demonstrating a statistically significant worse 2-year disease-free survival among the MCPyV-Ab negative patients, together with those previously published, support these recommendations.[9–12]

Ultraviolet radiation has been identified as the likely cause of MCPyV-negative MCC. Findings show that MCPyV-negative MCC cells contain loss-of-function mutations in tumor suppressor and DNA repair genes due to UV-associated DNA damage.[5] These MCPyV-Ab negative tumors may behave more aggressively due to a higher mutational burden, neoantigen expression, and inactivation of the RB1 and p53 tumor suppressor genes.[19]

Studies have associated MCPyV-Ab seronegativity with prognostically important disease characteristics including a higher likelihood of nodal metastases at diagnosis as well as characteristics that might have an impact on the ability to treat aggressively, namely, the tendency for MCPyV-Ab-negative tumors to be located on the head and neck.[11,20] Seronegativity in our study was significantly associated with tumors located in the head and neck. Interestingly, and likely consequential to seronegative tumors being more commonly located in the head and neck, seronegativity also was significantly associated with patients who did not undergo a wide local excision.

Increasing age also has been correlated with a worse OS and DFS.[6] Additionally, age has been correlated with a higher likelihood of MCPyV-Ab seronegativity.[21] In our study, age was one variable significantly associated with antibody status. Older individuals had a higher likelihood of being seronegative. A functional immune system is required to mount a response against foreign antigens partly through antibody production. The relative immunosuppression associated with aging may account for the association between older age, MCPyV oncoprotein antibody seronegativity, and worse prognoses.[22]

Radiotherapy and wide excision are the currently recommended treatment methods for MCC.[15] Immune checkpoint inhibitors are currently under investigation and have shown promise in the treatment of both seropositive and seronegative MCC regardless of programmed death ligand 1 (PD-L1) expression.[23,24] Although it is counterintuitive that antibody-negative patients would demonstrate a reliable response to immune-based therapies, this is explained by the observation that antibody-negative tumors have a high mutational burden, a variable associated with immunotherapy responsivity.[25] Although MCPyV-Ab-positive patients might be expected to benefit less from immunotherapy because seropositive tumors typically demonstrate a low mutational burden, it is believed that the response to immunotherapy in these patients is due to their expression of targetable viral proteins.[26]

The NCCN currently recommends immunotherapy for disseminated disease, and for recurrent locally advanced and regional disease if radiation and curative surgery are not feasible.[15] Given the worsened prognosis with MCPyV-Ab seronegativity, it may be prudent to consider immunotherapy earlier in these cases, but further study is required.

Like any retrospective study, this study had limitations. First, because the study included only patients at UTMCK, an academic, tertiary referral center, the study results may not be generalizable to all medical centers that treat MCC. Due to the rarity of MCC, the overall number of patients was low, limiting the extent of the conclusions. Due to the low number of patients, we were unable to perform multivariate analysis to control for receipt of radiation, which is known to decrease the risk of local recurrence. Finally, the patient data were gathered from chart review, and any inconsistencies or errors in charting would have been carried over into our study.

To our knowledge, this is the largest single-institution study to evaluate the impact of MCPyV oncoprotein antibodies on survival outcomes. Our study demonstrating improved DFS associated with MCPyV-Ab seropositivity contributes to the growing body of evidence supporting the use of MCPyV-Ab titers. Although MCPyV was discovered years ago, knowledge of antibody titers and their use is not widespread.

Clinicians treating MCC might incorporate this information into their practice by obtaining a baseline antibody level at diagnosis to guide patient counseling and surveillance because it has been repeatedly shown to have an impact on prognosis at diagnosis and throughout the course of the disease. Antibody titers also are easily obtained with little morbidity and provide clinically important and multifaceted information. Although challenging due to the rarity of MCC, a multicenter, prospective study following outcomes of seronegative and seropositive individuals is required to address the role of MCPyV oncoprotein antibody definitively in treatment and surveillance.

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DISCLOSURES There are no conflicts of interest.

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