Case Report

Merkel Cell Carcinoma: A Comparison of Different Immunohistochemical Markers Useful in Diagnosis

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Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine carcinoma associated with a high mortality rate. A polyomavirus integrated into the genome of most tumors is thought to be pathogenic. MCCs may be difficult to distinguish from other small cell carcinomas, melanoma, lymphoma, and others. Immunohistochemical marker studies are essential for accurate and efficient diagnosis. Although the paranuclear dot-like pattern seen with staining for cytokeratin 20 is considered characteristic, antibodies directed at other cytokeratins such as cytokeratin 8 (CAM 5.2), pancytokeratins (AE1/AE3), and other clones that recognize low molecular weight cytokeratins are also useful for diagnosis. We set out to evaluate which stains are helpful in diagnosing the cases of MCC encountered in our patient population. A retrospective study of 59 cases of MCC from our files was performed. Each MCC was stained with a panel of IHC markers and evaluated for the staining pattern and intensity in an effort to identify the most efficient IHC stains useful in establishing a diagnosis of MCC.

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INTRODUCTION

Merkel cell carcinoma (MCC) is an aggressive tumor that often develops on sun exposed skin as a firm flesh colored nodule.[1] Tumors are often non-descript and clinicians often consider other more common skin tumors in the differential diagnosis. MCC typically arises on the sun damaged skin, typically head and neck, of older immunosuppressed adults. Fair complexion, history of extensive sun exposure, immunosuppression, and age above 65 are all associated with a higher incidence.[1] Histologic examination reveals a tumor composed of small, hyperchromatic, undifferentiated cells (**Figures 1A and 1B**). This appearance may mimic lymphoma, melanoma, and metastatic small cell lung carcinoma, as well as other entities.

Cytokeratin 20 (CK20) has been long recognized as a useful stain that can help differentiate MCC from other tumors.[7,8] A paranuclear dot-like pattern is characteristic. CK20 is found in a variety of different epithelia and is helpful in identifying colorectal, and transitional cell carcinomas, as well as other adenocarcinomas. CK20 is found in MCCs but is absent in lung carcinoma and prostate carcinoma. CAM 5.2 (low molecular weight cytokeratin) is an IgG2 antibody directed at purified human keratin 8. MCC is a type of cutaneous neuroendocrine carcinoma, which has a neuroendocrine phenotype (CD56+, synaptophysin+, chromogranin+). The significance of CK20 is that in almost all other neuroendocrine tumors, CK20 is negative (except some cases from the lower GI tract). On the contrary, AE1/AE3, CK7 and Cam5.2 are positive in the vast majority of neuroendrocrine tumors. Various other immunohistochemical (IHC) stains have been used to characterize MCCs but further information is needed to compare staining intensity and patterns commonly found in clinical practice. This study was undertaken because CAM5.2 staining has been shown to be associated with more prominent paranuclear staining on several index cases. Although CAM5.2 may stain other neuroendocrine tumors, precocious metastatic neuroendocrine carcinoma is exceptionally rare, and in clinical practice the differential is between MCC and lymphoma, and melanoma. Merkel cell polyomavirus (MCPyV) has been found in approximately 60-80% of cases of MCC and is thought to play a role in tumor development.[9] We set out to discover the prevalence of MCPyV in our patient population.

METHODS

Cases with a clinical and pathological diagnosis of MCC were retrieved from our files. A total of 59 MCCs were identified in which the tissue block was of sufficient size to complete our selected panel of markers. Ethics approval and/or informed consent were not required because all slides for this study were

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de-identified. All 59 MCCs were stained with seven different IHC markers **(Table 1)** and one negative control set. All slides were blinded to reviewers. Each slide was assessed for IHC staining pattern, which consisted of either a paranuclear dot-like pattern, a cytoplasmic pattern or a nuclear pattern. Some of the stains had one predominant staining pattern while others had multiple. After the pattern was assessed, the intensity of staining was quantified using an H-score. H-score is a previously documented formula to weigh the relative intensity of staining in IHC markers.[5] For example, to determine an H-score for membrane staining intensity, the intensity of membrane staining (weighted as 0, 1+, 2+, or 3+) is determined for cells in various fields. Each intensity is given a percentage and an H-score is assigned using the following formula: [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)]. The final score, ranging from 0 to 300, gives more relative weight to higher intensity staining in a given tumor sample. For each staining pattern, the average H-score and standard deviation were calculated.

RESULTS

A total of 59 tumors were evaluated. CK20 staining revealed a predominantly paranuclear dot-like pattern associated with some cytoplasmic staining (**Figures 2A and 2D**). 90% of MCCs stained with CK20 in both a cytoplasmic and paranuclear dot-like pattern with H-score intensity reported in **Figure 3**, along with the H-score intensity for each of the different stains. CD56 highlighted 100% of the tumors in a cytoplasmic membrane pattern (**Figure 2G**). TTF-1 was negative in all (**Figure 2F**). AE1/AE3 highlighted 98% of tumors in both a paranuclear dot-like and cytoplasmic pattern. MCPyV was positive in 54% of tumors in a nuclear pattern (**Figures 2B and 2C),** CK8 decorated neoplastic cells with a weak paranuclear dot-like) pattern in 66% of tumors and in a weak cytoplasmic pattern in 32% of tumors. CAM 5.2 was strongly positive in a paranuclear dot-like pattern in 95% of tumors and a cytoplasmic pattern in 98% of tumors, while also staining 100% of the tumors (**Figure 2E**). Many slides revealed varying intensity of staining throughout the tissue as seen in **Figure 2H**.

DISCUSSION

We demonstrated strong staining of our MCCs with CK20, CD56, AE1/AE3, MCPyV and CAM5.2. Although CK20 is an often used IHC stain for MCC, our results indicate that CAM5.2 (among others) may be more useful in the diagnosis of MCC. MCC is a very rare tumor and having a large number of 59 cases to evaluate gave us a better picture of the patterns that can be encountered than seen in smaller studies. The fact that only 89% of MCCs were positive for CK20 also leaves a bit to be desired as other stains including CD56, and CAM5.2 highlighted over 95% of MCCs. Though a relatively small difference, this can be crucial when dealing with such a rare entity.

The initial report of using CK20 for staining MCC by Moll et al[7] was comprised of a total 15 tumors that all stained positive in 1992. Another study by Scott and Helm in 1999[8] of 10 MCCs found that 9/10 stained positive for CK 20. However, Paik et al reported in 2011 that CK20 may not be as sensitive as once thought.[9] Their study reviewed 104 Australian cases of MCC and found 5% of the specimens lacked expression of CK20, similar to our findings. This point warrants that MCCs may need a wider panel of IHC stains to adequately arrive at the diagnosis if it is unclear.

CK20, and CK8/CAM5.2 had both a paranuclear dot-like and cytoplasmic pattern which was somewhat more difficult to interpret in comparison to stains with one pattern. One of the limitations in evaluation was that cytoplasmic staining was often clouded by clumps of chromagen (DAB) which complicated the evaluation of concomitant paranuclear dot-like staining. TTF-1 was negative as expected in our series of primary cutaneous MCCs. Pertinent negative staining for antigens such as TTF-1 is helpful in excluding metastatic small cell lung carcinoma that can mimic Merkel cell carcinoma on routine histologic evaluation.

Immunohistochemical staining can also help identify important prognostic markers. When large numbers of CD8 positive lymphocytes infiltrate tumor, increased survival can be expected because the brisk CD8 positive infiltrate serves as a marker of an effective immune response to tumor.[11] Unfortunately, strong tumor expression of programmed death ligand 1 (PD-L1) can mitigate the immune response.[12] However, in MCC, PD-L1 expression within the tumor microenvironment has been positively correlated with the number of infiltrating CD8 positive lymphocytes, presence of MCPyV DNA, and improved patient survival. This is an interesting finding since PD-L1 expression is believed to create a negative feedback loop protecting tumor cells from immune destruction.[13]

Our study identified that 54% of tumors had detectable staining for Merkel cell polyomavirus. This percentage is similar to the 60-80% detection of virus previously reported in MCCs.[15] All 6 of the CK20 negative tumors in our study were also MCPyV negative. MCCs not expressing MCPyV contain different mutations from their virus positive counterparts, including RB1 inactivating mutations.[16] CK20 negative and MCPyV negative tumors have been increasingly reported as of late.[14,17-20] The fact that these tumors arise independently of polyoma virus while also harboring UV signature mutations points to a different etiological source. This difference may play a role in directing targeted treatments in the future.

Although there are new digital image analysis methods to quantify IHC staining intensity,[10] our analysis was performed by manually viewing each slide. This can be a tedious method, but allows for appreciation of the IHC staining pattern and the ability to discern any non-significant background or inflammatory cell staining. Comparison of a manual review of our results to a digital image analysis would be an interesting future project.

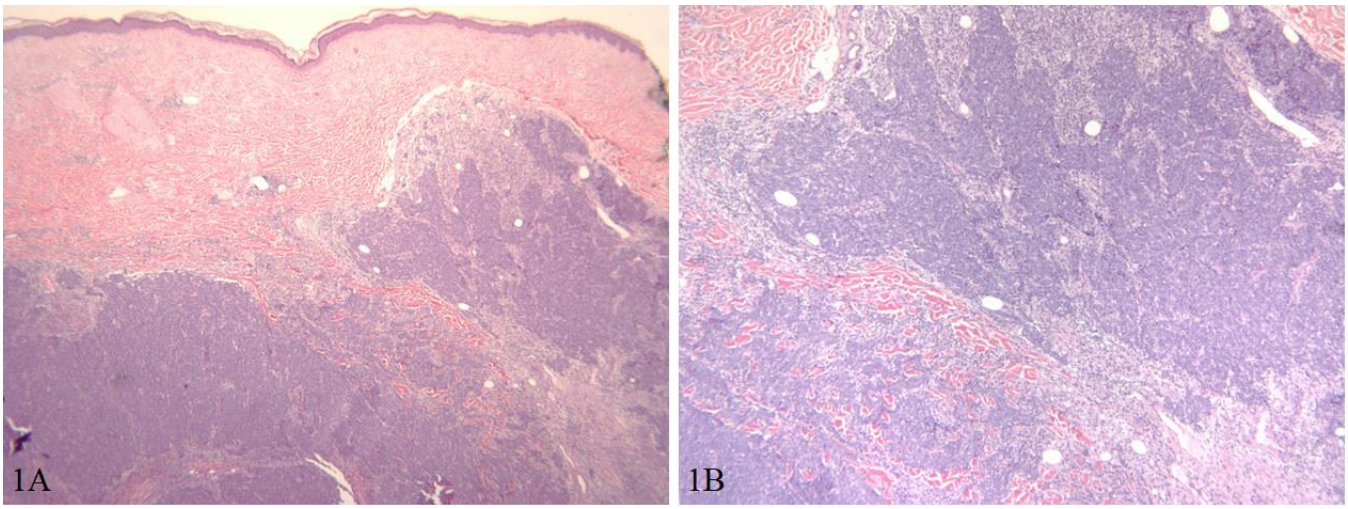

Figure 1. Merkel cell carcinoma, **1A**: H&E Stain low power. **1B**: H&E Stain low power higher power.

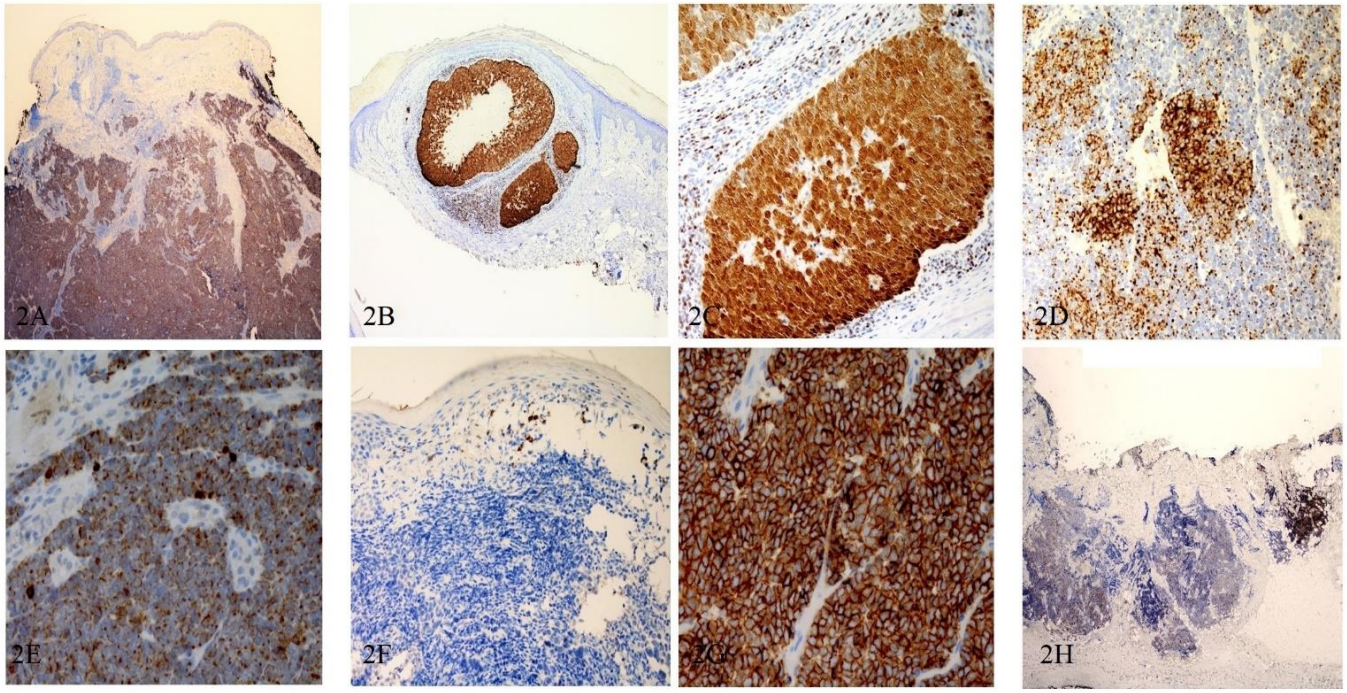

Figure 2. Merkel cell carcinoma, Immunohistochemical Stains. **2A**: Strong MCC tumor staining from low power (20x) (CK20). **2B**: MCPyY Strong MCC tumor staining from low power (40x) (MCPyV). **2C**: Strong nuclear staining at 200x with surrounding negative lymphocytes (MCPyV). **2D:** Paranuclear dot-like and cytoplasmic staining at 200x (CK20). **2E:** CAM 5.2 staining in a paranuclear dot-like pattern (CAM 5.2). **2F:** Negative tumor staining at 200x (TTF-1) with melanophages in the papillary dermis. **2G**: Strong cytoplasmic staining at 400x (CD56). **2H:** Various staining intensities in a paranucleardot-like pattern (CK20).

Table 1. Seven different IHC markers.

CAM 5.2	Cell Marque	1:50	Mouse monoclonal antibody developed using human colorectal carcinoma line HT24 directed at select keratins useful in identifying many epithelial tumors. **Low Molecular weight cytokeratins**
CD56	Dako	Pre-diluted	Mouse monoclonal antibody clone 123C3 which detects Leu-19, neural cell adhesion molecule. **Expressed in natural killer cells, small cell lung carcinoma, neural derived tumors and others.**
CK8	Cell Marque	1:100	Mouse monoclonal antibody (35betaH11) directed at cytokeratin 8. **Stains most non-squamous epithelial tumors**
CK20	Dako	Pre-diluted	Mouse monoclonal antibody K20.8 directed at protein IT antigen. This intermediate filament protein is important in cellular cytoskeleton formation.
CK AE1/AE3	Dako	Pre-diluted	Cocktail of two mouse monoclonal antibodies directed at human epidermal callus. Identifies the majority of human cytokeratins.
MCPyV large T-antigen	Biotechnology	1:50	The MCPyV large T-antigen (CM2B4) is highly specific for MCPyV large T-antigen and 57kT isoforms because it was raised against a peptide in exon 2 of the T antigen locus. It will not detect MCPyV small T-antigen.
Anti Thyroid Transcription Factor (TTF-1)	Dako	Pre-diluted	This mouse monoclonal antibody clone 8G7G3/1 identifies transcription factors expressed in thyroid, lung, and diencephalon. **Used to rule out small cell lung cancer**

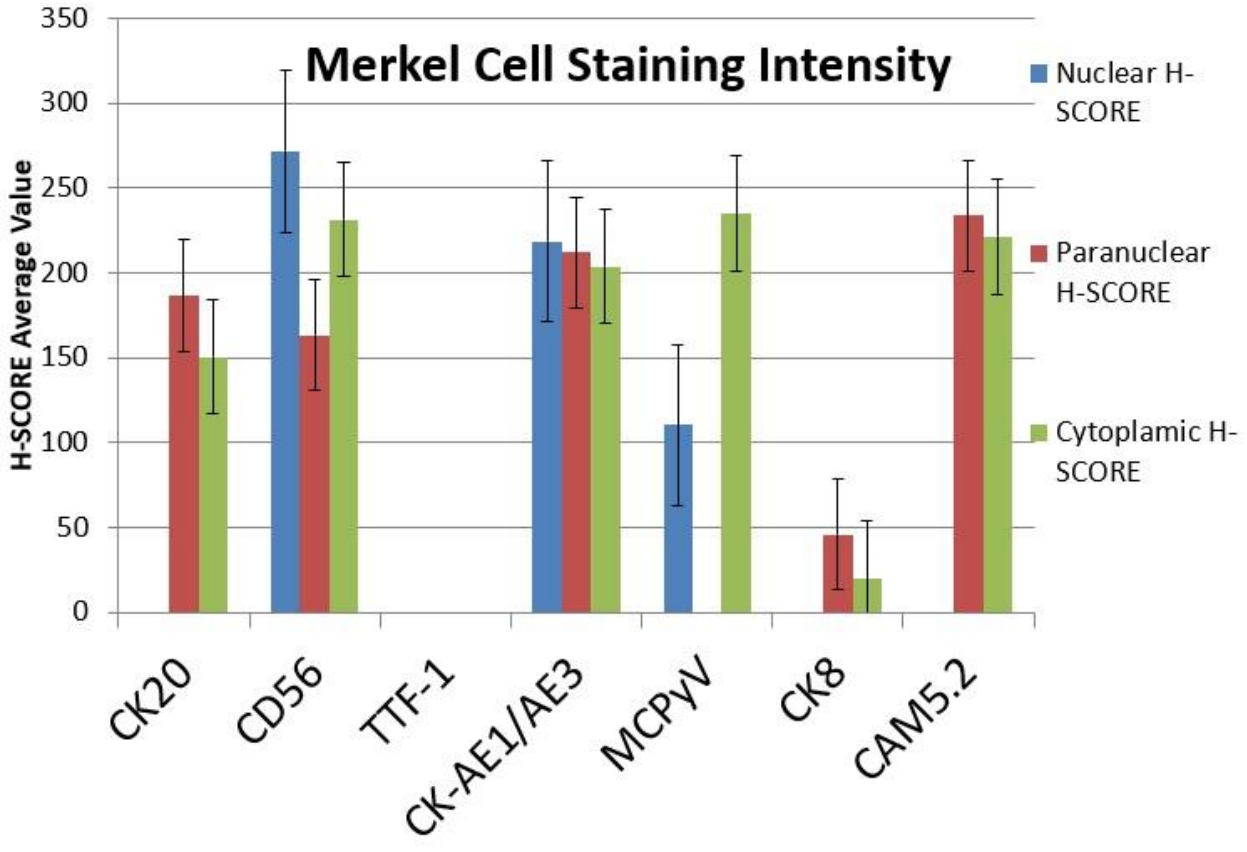

Figure 3. H-score intensity.

CONCLUSIONS

We found that CK20 can be negative in 10% of MCCs. Stains for CK20, CD56, AE1/AE3 and CAM5.2 are all associated with a high sensitivity, but loss in specificity. CK20 negative tumors have also been found more likely to be MCPyV negative (6/6 in our study). MCCs may need a wider panel of IHC stains to adequately arrive at the diagnosis if it is unclear.

CONFLICT OF INTEREST
None.

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