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TdT expression in Merkel cell carcinoma: potential diagnostic pitfall with blastic hematological malignancies and expanded immunohistochemical analysis

Monalisa Sur, Hosam AlArdati, Cathy Ross and Salem Alowami

Division of Anatomical Pathology, Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Merkel cell carcinoma is an uncommon aggressive primary cutaneous neuroendocrine carcinoma. Histologically, the differential diagnosis includes the 'small round cell' tumor group, particularly metastatic small cell carcinoma and blastic hematological malignancies involving skin/soft tissues. Terminal deoxynucleotidyl transferase (TdT) is a DNA polymerase, which is a sensitive and specific antibody for acute lymphoblastic lymphoma with a small proportion of acute myeloid leukemia showing positivity. This study investigates the expression of TdT in 20 cases with initial diagnosis of Merkel cell carcinoma. Archival blocks and slides were retrieved and reviewed and clinical information obtained from patient charts. Immunohistochemistry was performed and graded as: 0, no staining; 1 +, less than 50% staining in the cells; 2 +, 50% or more staining in the cells. After review, 15 cases were confirmed as Merkel cell carcinoma. Immunohistochemical positivity was as follows: 8/15 cases were positive for TdT with strong nuclear staining, morphologically resembling 'blasts', AE1AE3, CAM5.2 (15/15) (both membrane and paranuclear dot positivity), CD56 and BCL-2 (15/15), Synaptophysin (13/15), Chromogranin A (11/15), NSE (15/15), CK20 (14/15), CK7 (3/15), both CK7 and CK20 (3/15), CD117 (8/15), CD99 (2/15), CD10 (1/15). One case was negative for CK7/CK20. All 15 cases were negative for thyroid transcription factor-1, LCA, CD20, CD3 and CD34. Expanded immunohistochemical panel with positive staining for epithelial/neuroendocrine markers, CK20, negative staining for hematolymphoid markers and awareness of TdT expression and other markers that show overlap with blastic hematological malignancies avoids misinterpretation in the diagnosis of Merkel cell carcinoma. This aids in further diagnosis of Merkel cell carcinoma, avoiding the potential diagnostic pitfall with other small round cell tumors and hematological malignancies primary or metastatic to the skin.
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Merkel cell carcinoma is a rare, primary neuroendocrine carcinoma of the skin occurring on sun-exposed areas in elderly patients.[1] It accounts for less than 1% of all cutaneous malignancies,[2] with approximately 53% occurring in the head and neck region and 35% present in the extremities.[3] Merkel cell carcinoma may display aggressive biological behavior and can grow rapidly, metastasizing to the regional lymph nodes and other organs such as liver, bones, lungs and brain. Histologically, the differential diagnosis of Merkel cell carcinoma includes the 'small round cell' tumor group, particularly metastatic small cell carcinoma from other organs and hematological malignancies involving skin/soft tissues, which may include acute lymphoblastic lymphoma, acute myeloid leukemia and cutaneous natural killer (NK)/T-cell lymphoma and hematodermic CD56 + /CD4 + neoplasm. Terminal deoxynucleotidyl transferase (TdT) is an intranuclear enzyme, a template independent DNA polymerase expressed in approximately 90% of acute lymphoblastic lymphoma, in a small subset of acute myeloid leukemia and hematodermic CD56 + /CD4 + neoplasm and a significant number of pediatric small round cell tumors.[4,5] This study investigates the

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expression of TdT in Merkel cell carcinoma by immunohistochemistry along with an expanded immunohistochemical profile which aids in further diagnosis of Merkel cell carcinoma, avoiding potential diagnostic pitfall with other small round cell tumors presenting in cutaneous/soft tissue locations.

Methods

Twenty cases with initial diagnosis of Merkel cell carcinoma were retrieved from the institute database and archival blocks and slides were retrieved spanning 1999–2006. The pathology material was reviewed by two hematopathologists and a dermatopathologist. Immunohistochemistry was performed by using Standard Labeled Strep Avidin Biotin technique and graded as: 0, no staining; $1+$, less than 50% staining in the cells; $2+$, 50% or more staining in the cells. Detailed immunohistochemical panel performed is listed in Table 1. This panel was used to diagnose and differentiate Merkel cell carcinoma from other small cell neuroendocrine carcinomas metastasizing to the skin. This panel was also used to distinguish a subset of Merkel cell carcinomas which can morphologically resemble 'blastic' hematological malignancies and can be confused with hematological malignancies such as acute lymphoblastic lymphoma, acute myeloid leukemia, NK//T-cell lymphoma, hematodermic $CD56+/CD4+$ neoplasm that can present in the skin and soft tissue locations. Clinical information

was obtained from patient charts and is listed in Table 2.

Results

After review, 15 cases were confirmed as Merkel cell carcinoma. Five cases that excluded from the study were diagnosed on review as follows: basaloid squamous cell carcinoma; B-cell non-Hodgkin's lymphoma; metastatic small cell carcinoma (three cases). There were 5 men and 10 women with age range from 54–86 years; mean age 70 years. In our series, 60% of the tumors were present in the extremities, 26% in the head and neck region and 13% in the trunk. Two cases also showed metastasis to left inguinal lymph node and liver respectively, at the time of cutaneous presentation. At the time of this study, eight patients (four men and 4 women) were dead and seven patients were still alive (one men and six women).

Microscopy

Nine cases demonstrated morphology similar to conventional small cell carcinoma (Figure 1a and b). Six cases showed morphology resembling high-grade hematological malignancies. The cells were large to intermediate in size with vesicular nuclei, open chromatin pattern and 1–3 nucleoli. The background exhibited a starry sky appearance with brisk mitotic activity (Figure 2a and b).

Table 1 Immunohistochemical panel

Antibody	Manufacturer	Dilution	Antigen retrieval	Clone
AE1/AE3	Dakocytomation	1/100	Protease	AE1 AE3
Cam 5.2	Beckton Dickenson	1/100	Protease	Cam 5.2
CK7	Dakocytomation	1/50	Protease	OV-TL 12/30
CK20	Dakocytomation	1/100	Protease	Ks 20.8
Chromogranin A (CGA)	Dakocytomation	1/100	HIER Citrate-6 buffer	DAK-A3
Synaptophysin (SYN)	Dakocytomation	1/100	HIER Citrate-6 buffer	Polyclonal
NSE	Dakocytomation	1/2000	HIER Citrate-6 buffer	BBS/NC/V1-H14
CD56	Novocastra	1/50	HIER DAKO buffer	1B6
CD45 (LCA)	Dakocytomation	1/200	None	2B11/PD7126
CD10	Novocastra	1/25	HIER Citrate-6 buffer	56C6
CD34	Novocastra	1/50	None	QBEnd/10
TTF-1 (thyroid transcription factor-1)	Dakocytomation	1/200	HIER EDTA-8 buffer	8G7G 3/1
TdT	Novocastra	1/25	HIER DAKO buffer	SEN 28
CD99	Dakocytomation	1/100	HIER EDTA-8 buffer	12E7
BCL-2	Dakocytomation	1/25	HIER Citrate-6 buffer	124
CD117	Dakocytomation	1/400	HIER Citrate-6 buffer	Polyclonal
CD 3	Cell margue	1/1500	HIER Citrate-6 buffer	Polyclonal
CD20	Dakocytomation	1/1000	None	L26

Table 2 Patient characteristics

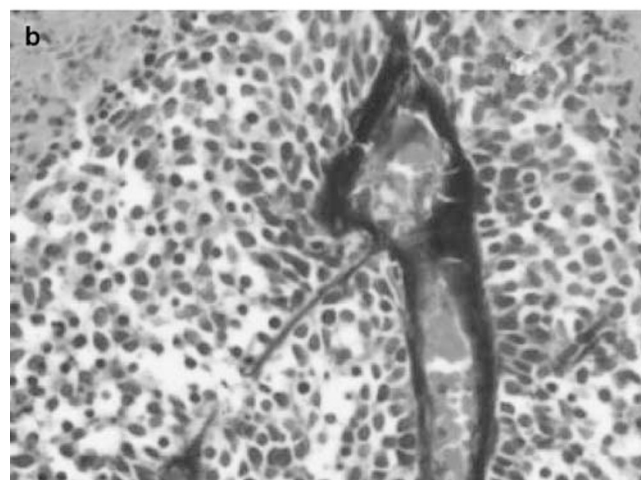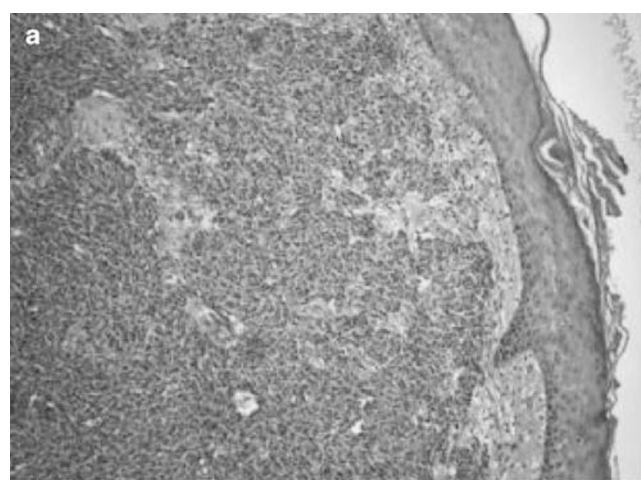| Case	Age	Sex	Site	Survival
1	72	F	Right elbow	Alive
2	85	M	Nose	Dead
3	74	M	Neck	Dead
4	79	F	Left abdominal wall with left groin lymph node metastasis	Dead
5	54	F	Right arm	Alive
6	67	F	Left thigh	Alive
7	79	F	Right thigh	Alive
8	73	M	Left thigh	Dead
9	86	F	Left calf	Dead
10	82	F	Left thigh	Alive
11	78	M	Left chest wall with liver metastasis	Dead
12	80	F	Right calf	Dead
13	62	F	Left arm	Alive
14	74	M	Right ear	Alive
15	83	F	Right eyelid	Dead

Figure 1 (**a**) Conventional Merkel cell carcinoma showing features of a small cell carcinoma (H&E) (× 100). (**b**) Merkel cell carcinoma showing small cells with high nuclear/cytoplasmic ratio, hyperchromatic nuclei and a blood vessel showing Azzopardi effect (H&E) (× 400).
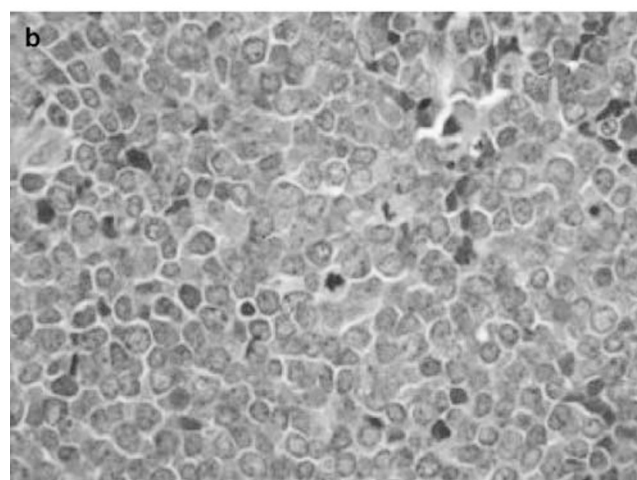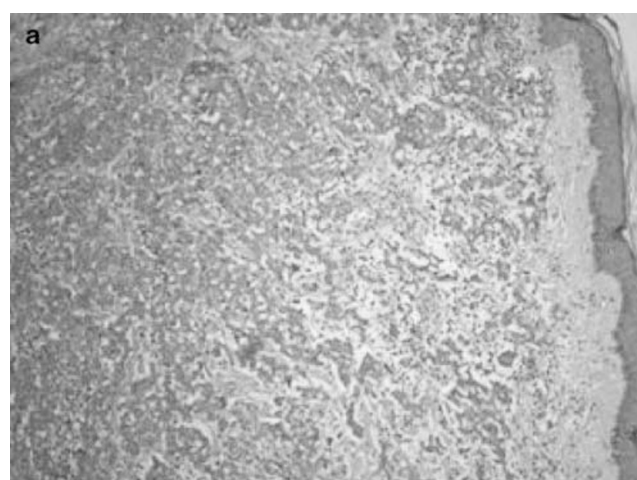

Figure 2 (**a**) Merkel cell carcinoma resembling 'blastic' hematological malignancy (H&E) (× 100). (**b**) High-power view showing large to intermediate cells with vesicular nuclei, 1–3 nucleoli and brisk mitosis (H&E) (× 400).

Immunohistochemistry

The immunohistochemical staining pattern in the neoplastic cells is shown in Table 3. Eight out of 15 cases were positive for TdT with 6 cases showing 2+ (Figure 3a and b), and 2 cases showing 1+ nuclear staining. Six of the eight cases expressing

Table 3 Immunohistochemical profile

Immunohistochemical stains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
TdT	–	–	–	–	–	+	++	++	–	++	++	–	++	++	+
CD117	–	–	–	++	–	–	++	–	++	++	++	++	++	++	–
CD99	–	–	–	–	–	–	–	+	++	–	–	–	–	–	–
TTF-1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CD56	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
CD10	–	–	–	–	++	–	–	–	–	–	–	–	–	–	–
CD34	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BCL-2	++	++	++	++	+	++	++	++	++	++	++	++	++	++	++
AE1AE3	++	+	++	++	++	++	++	++	++	++	++	++	++	+	++
Cam5.2	++	+	++	++	++	++	++	+	++	++	++	+	++	++	++
CK 7	–	–	–	++		–	+	–	–	++	+	–	–	–	–
CK 20	++	++	+	++	+	++	++	–	++	++	+	++	++	+	++
Synaptophysin (SYN)	++	–	++	++	++	++	++	–	++	++	++	++	++	++	++
Chromogranin (CGA)	++	++	++	–	++	++	–	++	–	++	++	–	++	++	++
NSE	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
CD45 (LCA)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CD20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CD3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

+, less than 50% cells staining; ++, 50% or more cells staining.

Figure 3 (**a**, **b**) 2 + nuclear staining for TdT (**a**) (× 100), (**b**) (× 400). (**c**, **d**) 2 + membrane and paranuclear dot staining for CAM5.2 (**c**), CK20 (**d**) (× 400).

TdT morphologically resembled blastic hematological malignancies. All the cases showed positive staining for CAM5.2 (Figure 3c) and AE1AE3.

Fourteen cases were positive for CK20 (Figure 3d) with three cases demonstrating positivity for both CK7 (Figure 4a) and CK20. One case was negative
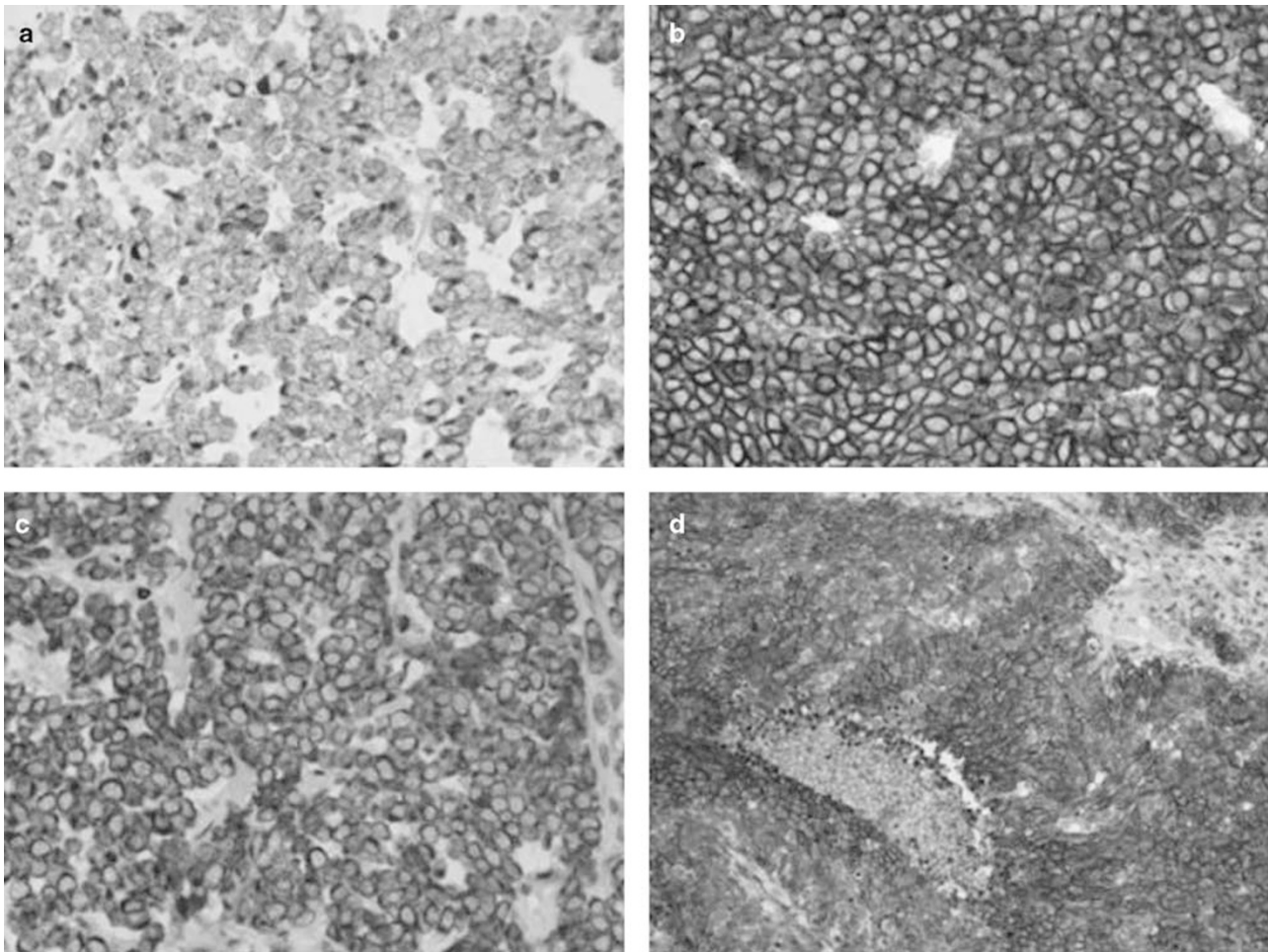
Figure 4 2+ staining for CK7 (**a**), CD56 (**b**), Bcl-2 (**c**), CD117 (**d**) ($\times 400$).

for both CK20 and CK7. The staining pattern for all epithelial markers were both membranous and paranuclear dot type. All our cases showed 2+ positivity for two or more neuroendocrine markers (NSE, CGA and Synaptophysin). CD56 and BCL2 demonstrated 2+ membrane positivity in all the cases (Figure 4b and c). Eight cases showed 2+ membrane staining for CD117 (Figure 4d), two cases demonstrated 1+ membrane staining for CD99 and one case showed 2+ membrane staining for CD10. Ki-67 showed a proliferative index of 70–85% in all the cases. All 15 cases were negative for thyroid transcription factor-1 (TTF-1), LCA, CD20, CD3 and CD34.

Discussion

Merkel cell carcinoma is a primary cutaneous neuroendocrine carcinoma found in sun-exposed areas of elderly individuals (mean age in our study being 70 years).[1] Only 5% of all reported cases were below the age of 50 years, 54 years being the youngest in our series. There is a slight male preponderance in one series,[6] with a predilection

for female preponderance in another series.[7] Our series also showed a female preponderance. In our study, patients who died were more males than females; this finding is similar to previous studies showing male gender to be an adverse prognostic factor.[8,9] Although, head and neck is the most common location,[3] the extremities (60%), followed by head and neck (26.6%), and trunk (13.3%) were the preferred sites in our study.

Merkel cell carcinoma shares many histological and immunohistochemical features with small cell neuroendocrine carcinoma from other locations.[1] Although, CK20 has been reported to be occasionally positive in small cell carcinoma of the lung,[10] it is considered to be a fairly specific and sensitive marker for Merkel cell carcinoma showing membrane and paranuclear dot positivity in the neoplastic cells,[11,12] a finding further corroborated in this study. In addition, three cases (20%) were also positive for CK7 and CK20, the pattern of staining for CK7 being similar to CK20. Although, one case was negative for both CK7 and CK20, all the cases demonstrated positivity for epithelial markers CAM5.2 and AE1AE3 and two or more neuroendocrine markers. The case that was negative for both

CK7 and CK20 did not clinically have any other lesion elsewhere. TTF-1 is a nuclear transcription protein, which is expressed in pulmonary and extrapulmonary small cell carcinomas, but not in Merkel cell carcinoma.[13] All our cases were consistently negative for TTF-1, making this an important marker to differentiate Merkel cell carcinoma from cutaneous metastasis of small cell carcinoma from other sites, particularly in the setting of CK7 positivity or in the absence of staining for CK20 and/or CK7.

TdT is an intranuclear enzyme, a template-independent DNA polymerase that catalyzes the repetitive addition of deoxyribonucleotides to the 3′-OH-terminated segment of DNA without the need for a template strand.[4] The gene responsible for expression of TdT has been found to span at least 65 kb and is located to chromosome 10 q23-q25.[14] TdT is expressed in most cases of acute lymphoblastic lymphoma, a small subset of acute myeloid leukemia, occasional cases of CD56 + /CD4 + hematodermic neoplasm and some pediatric non-hematopoietic small round cell solid tumors.[4,5] In this study, 53% (8/15) of the cases expressed strong diffuse nuclear staining for TdT. Six of these cases morphologically resembled blastic hematological malignancies with large- to intermediate-sized cells showing vesicular nuclei and one or two prominent nucleoli. This morphology could lead to an erroneous diagnosis of a blastic hematological malignancy in the skin, given the TdT positivity. To the best of our knowledge, this is the first series to identify TdT expression in Merkel cell carcinoma by immunohistochemistry, which was published as an abstract in the Modern Pathology issue of March 2007, following presentation at the 96th USCAP meeting in San Diego.[15] Since then, a recent correspondence has been published where the authors have found similar nuclear immunoreactivity in 10 out of their 28 cases of Merkel cell carcinoma (36%).[16]

The embryological origin of merkel cells remains controversial. Merkel cells are generally believed to originate from neural crest-derived cells of the amine precursor uptake and decarboxylation system, and a residual epidermal stem cell. Some investigators have suggested that neoplastic cells in Merkel cell carcinoma arise from stem cells that develop neuroendocrine properties similar to the wild-type merkel cells.[17] Recent evidence has suggested that merkel cells are derived from epidermal keratinocytes.[18] TdT is normally expressed in thymocytes and precursors of lymphocytes in the bone marrow, but not in mature lymphocytes. Most investigators support the concept that both medullary and cortical epithelia arise from a common precursor and, in the early stage of thymus ontogeny, the epithelia coexpress markers that later segregate into cortical (cytokeratin 8 positive) and medullary (cytokeratin 5 positive) epithelial compartments. This differentiation is thought to

occur under the influence of the mesenchymal cells that migrate from the neural crest to the thymic components.[19,20] There is a great deal of similarity and parallelism between the ontogenic development of the thymic cells and the merkel cells of the skin. It is possible that this positive immunoreactivity in our series is an aberrant expression of TdT by the mutant merkel cells. The functional significance of this rare biological event is not very clear. TdT is known to catalyze and promote the addition of deoxynucleotides to the 3′-OH-terminal (amino terminal end) of DNA and functions without the need for a DNA template. It is possible that this enzyme may help DNA repair in damaged neoplastic cells or apoptotic cells which are found in significant numbers in Merkel cell carcinoma, and in this manner may prolong the survival of the tumor cells, augmenting their malignant behavior. It would be interesting to further study the nature and mechanism of this expression of TdT in Merkel cell carcinoma by actual chemical extraction, isolation and purification of the coding DNA, its amplification by polymerase chain reaction and its characterization by DNA sequencing. Cytogenetic anomalies occur in 30–47 % of Merkel cell carcinomas. The most frequent change is the loss of heterozygosity due to deletions or translocations on chromosome 1. Molecular studies on neoplastic cells in Merkel cell carcinoma have indicated the involvement of two tumor suppressor genes—p73, a member of the TP53 family of tumor suppressors localized on chromosome 1, and succinate-ubiquinone oxidoreductase subunit D, localized on chromosome 11.[21,22] The multiplicity of chromosomal changes in Merkel cell carcinoma may be a reflection of the highly malignant nature of the neoplasm and possibly ongoing somatic mutations during the progressive evolution of its aggressive malignant nature. The significance of the cytogenetic and molecular changes in the induction of TdT expression by this tumor remains conjectural. The presence of multiple chromosomal changes, which include chromosome 10, may have some relevance to the activation of the gene that codes for TdT protein in Merkel cell carcinoma.[23]

CD56 (neural cell adhesion molecule) is positive in neoplastic plasma cells, leukemias with myelomonocytic differentiation, CD56 + /CD4 + hematodermic neoplasm, NK/T-cell lymphomas, myogenic tumors and tumors showing neuroendocrine differentiation.[24–26] In our series, CD56 was positive in all the cases, indicating that CD56 is a sensitive but not a specific marker for Merkel cell carcinoma and needs to be interpreted in conjunction with other markers and not in isolation in the diagnosis of Merkel cell carcinoma. This is of importance because CD56 is positive in Merkel cell carcinomas and hematological malignancies such as CD56 + / CD4 + hematodermic neoplasm, NK/T-cell lymphoma and myeloid leukemia with myelomonocytic differentiation, which can morphologically resem-

ble Merkel cell carcinoma showing blast-like histology in the cutaneous and soft tissue location.

CD99 showed 1+ positivity in two cases. CD99, a MIC2 gene product and a p30/32 cell surface glycoprotein demonstrates positive staining in a wide variety of small round blue cell tumors, which include Ewing's sarcoma/PNETs family, acute lymphoblastic lymphoma, acute myeloid leukemia, alveolar rhabdomyosarcoma, mesenchymal chondrosarcoma.[27] Epithelial tumors expressing CD99 include neuroendocrine tumors such as islet cell tumors, carcinoid tumors and pulmonary small cell carcinomas.[28,29] The immunoreactivity for CD99 in Merkel cell carcinoma should be taken into consideration when differential diagnosis with other small round blue cell tumors is considered.

CD117(C-KIT) showed 2+ membrane staining in eight cases (53%). CD117 is also a marker of acute myeloid leukemia, which can morphologically mimic Merkel cell carcinoma showing blast-like cells in the cutaneous location. A number of papers have been published on CD117 positivity in Merkel cell carcinoma.[30,31] The presence of CD117 positivity in Merkel cell carcinoma possibly indicates a c-kit mutation with a constitutive activation of this receptor tyrosine kinase. However, no correlation could be established with survival in these patients and its clinical significance is still unknown. Further, prospective studies are required to assess whether this finding could have an impact on the treatment protocol.

One of our cases was positive for CD10. The exact significance of CD10 expression in Merkel cell carcinoma is not known and may be an aberrant expression. All our cases showed strong diffuse 2+ membrane positivity for BCL-2. Expression of BCL-2 protein is frequent in Merkel cell carcinoma, but positive immunoreactivity and recurrences and survival are unrelated.[32,33]

In general, Merkel cell carcinoma resembles a typical small cell neuroendocrine carcinoma, with cohesive sheets of small cells with scant cytoplasm, powdery dispersed chromatin, irregular nested groups of infiltrating cells and azzopardi effect. In our series, interestingly, 6 of the 15 cases also exhibited a more diffuse discohesive pattern of infiltration with a starry sky appearance at low power. The neoplastic cells were medium- to large-sized with brisk mitotic activity, with no appreciable cytoplasm and vesicular nuclei with one to three nucleoli resembling blastic hematological malignancies. TdT was expressed in 53% of our cases, which is an unusual finding. In addition, Merkel cell carcinoma expressing TdT can show similar morphologic features as blastic hematological malignancies, which can be a potential diagnostic pitfall with other hematological malignancies expressing TdT. In our series, Merkel cell carcinoma was also positive for CD117 (53%), CD56 and BCL2 (100%), CD99 (13%) and CD10 (6.6%); markers that are also expressed in hematological malignancies described

above. Therefore, we recommend that when faced with a cutaneous or soft tissue lesion showing blastic morphology, the immunohistochemical panel should include epithelial markers (AE1AE3, CAM5.2, CK7 and CK20), neuroendocrine markers (NSE, Synaptophysin and CGA) and CD56, TTF-1 and first line hematolymphoid markers (LCA, CD34, TdT, CD10, CD3, CD20, myeloperoxidase and CD117). CD34 is a hematopoietic progenitor marker, which is positive in most cases of acute lymphoblastic lymphoma along with TdT and CD10 and some cases of acute myeloid leukemia but negative in Merkel cell carcinoma. CD99 positivity in Merkel cell carcinoma should be interpreted in conjunction with other markers for small round cell tumors. CD56 and BCL2 are sensitive but not specific markers and can be used in conjunction with epithelial markers, CK20 and neuroendocrine markers in the diagnosis of Merkel cell carcinoma. Although CK7 was positive in 20% of our cases, TTF-1 was negative in all our cases, making this an important marker to differentiate Merkel cell carcinoma from cutaneous metastasis of small cell carcinoma from other sites.

In conclusion, awareness of the positive staining pattern for the less commonly used markers in the diagnosis of Merkel cell carcinoma and markers which can show overlap with hematological malignancies avoids misinterpretation and erroneous diagnosis with other tumors, which may morphologically mimic Merkel cell carcinoma.

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