Diagnostic Quality Control Exam Review of Answers

Question 1

A 65-year-old woman received Mohs surgery treatment of a sebaceous carcinoma on the left lateral upper eyelid. Typical sebaceous carcinoma was seen on the 1st stage Mohs section (Slide 1: H and E stain and Slide 2: oil red O stain; slides submitted for your information). Please review the 2nd stage Mohs section (Slide 3: H and E stain and Slide 4: oil red O stain) and select the best answer from the following statements:

Please review the 2nd stage Mohs section (Slide 3: H and E stain and oil red O stain) and select the best answer from the following statements:

A. Focal, residual sebaceous carcinoma is present in both H and E and oil red O-stained slides; take an additional stage.

B. Focal, residual sebaceous carcinoma is present in the oil red O-stained slide but not in the H and E stained-slide; take an additional stage.

C. Focal, residual sebaceous carcinoma is present in the H and E stained-slide but not in the oil red O-stained slide; take an additional stage.

D. Margin clear of sebaceous carcinoma; no additional stage needed.

Discussion

Question 1

Correct Answer:

A. Focal, residual sebaceous carcinoma is present in both H and E and oil red O-stained slides; take an additional stage.

Main Histopathologic Features:

- Sebaceous carcinoma is typically composed of infiltrating lobules of basaloid and squamoid cells mixed with cells exhibiting sebocytic differentiation. Well-differentiated sebaceous carcinomas show well-formed sebocytes, while poorly differentiated tumors may be confused with squamous cell or basal cell carcinoma. Tumor cells are often hyperchromatic with nuclear polymorphism and frequent mitoses. Atypical keratinizing cells may be present.
- Sebaceous carcinoma may present in multicentric fashion, and exhibit central areas of comedo-type necrosis and pagetoid intraepidermal spread (40–80%).
- The oil red O stain performed on frozen sectioned material confirms the presence of lipid in cytoplasmic vacuoles. Like any special stains, oil red O has to be carefully interpreted and should only be interpreted using H and E slide as a reference.
- When necessary, submit Mohs tissue for permanent sections. Sudan IV stains, epithelial membrane antigen, Leu-M1, antiperilipin, and antiadipophilin immunostains on permanent sectioned slides may be helpful in aiding the diagnosis.

Differential Diagnosis:

- Sebaceoma
- Basal cell carcinoma with sebaceous differentiation
- Squamous cell carcinoma with sebaceous differentiation

Clinical Concerns:

- Sebaceous neoplasms are frequently associated with Muir-Torre syndrome (MTS) (5-45% incidence). MTS is a small subset of the autosomal dominant familial cancer syndrome known as Lynch syndrome/ hereditary nonpolyposis colorectal cancer (HNPCC).
- MTS is caused by germline mutations in the DNA mismatch repair genes, most commonly in MLH1or MSH2, and less commonly in MSH6, PMS1, and PMS2. Mismatch repair defects result in the loss of DNA repair protein expression (detected by immunohistochemistry staining) and microsatellite instability (manifested as variable numbers of repeats of mononucleotide, dinucleotide, trinucleotide, and tetranucleotide sequences of DNA). These two features are commonly used as screen tests for MTS.
- Confirmation of germline mutations in one of those mismatch genes by sequencing the patients' DNA from blood leukocytes is a more definitive test but only used in selected cases.
- We recommend all patients with sebaceous neoplasms be screened for Muir-Torre Syndrome (MTS) by exploring his/her personal or family history of visceral malignancies predominantly in the GI and GU systems. Family history is the most cost effective screening tool with the highest positive and negative predictive values.

References:

- Eisen DB, Michael DJ. Sebaceous lesions and their associated syndromes: part II. *J Am Acad Dermatol* 2009 Oct;61(4):563-578; quiz 579-580.

- Noriyuki M et al. Sebaceoma and related neoplasms with sebaceous differentiation-a clinicopathologic study of 30 cases. *The Am J of Dermatopathology* 24(4): 294–304, 2002
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- Goyal S, Honavar SG, Naik M, Vemuganti GK. Fine Needle Aspiration Cytology in Diagnosis of Metastatic Sebaceous Gland Carcinoma of the Eyelid to the Lymph Nodes with Clinicopathological Correlation. Acta Cytologica 2011;55:408–412

Question 2

This Mohs section is from the first stage of basal cell carcinoma on the left forehead of a 61-year-old man.

Which of the following is the best answer?

A. There is residual nodular BCC; take additional layer.

B. Highly suspicious for nodular BCC; may order a recut slide for further evaluation.

C. Margin clear with no suspicious or convincing BCC.

D. Both A and B.

Discussion

Question 2

Correct Answer:

D. Both A and B.

Main Histologic Features:

- In common nodular BCC, nodular masses of basaloid cells extend from the epidermis into the dermis with surrounding connective tissue stroma. Peripheral palisading of cells and stroma retraction artifact aid in diagnosis.
- Histological diagnosis of BCC is straightforward. Nevertheless, frequently in Mohs sections, differentiating BCC from benign hair follicles is not as simple. Sometimes ordering a recut slide may help secure the diagnosis of BCC.
- There are some clues we can use to differentiate between hair follicles and BCC when recuts are not helpful: 1) BCC shows subtle stroma retraction artifact even in frozen sections; 2) Typical peripheral palisading may not present but BCC cells tend to be more basophilic and crowded with haphazard arrangement of center cells; 3) As opposed to BCC, morphology of a hair follicle (for example, hair shaft keratin, inner root sheath, and out root sheath) may become more evident when one tracks multiple subsections; and 4) Some BCC may have follicular features; reviewing original biopsy slide can be helpful.

Differential Diagnosis:

- Trichoblastoma
- Trichoepithelioma
- Basaloid follicular hamartoma (BFH)

Clinical Concerns:

- The discrimination of BCC from benign follicles on en-face frozen sections can be occasionally challenging.
- Failure to differentiate benign follicles from basal cell carcinomas could result in unnecessary surgical excision.

References:

- Alsaad KO, Obaidat NA, and Ghazarian D, Skin adnexal neoplasms—part 1: An approach to tumours of the pilosebaceous unit. J Clin Pathol 2007;60:129–144.
- Lever's Histopathology of the skin. 11th Ed. Edited by Elder DE, 2014; Wolters Kluwer.
- Saxena A, Shapiro M, Kasper D, Fitzpatrick J, Mellette JR. Basaloid follicular hamartoma: A cautionary tale and review of the literature. *Dermatol Surg*, 2007;33:1130-1135.

Question 3

A 63-year-old Caucasian male was referred for treatment of a tumor on right supraclavicular area. A representative frozen section of the tumor is shown.

What is most likely diagnosis?

A. Desmoplastic melanoma

B. Benign fibrous histiocytoma

C. Dermatofibrosarcoma protuberans

D. Leiomyosarcoma

Discussion

Question 3

Correct Answer:

C. Dermatofibrosarcoma protuberans

Main Histologic and Immunohistochemical Features of Dermatofibrosarcoma Protuberans

- Dermal tumor, typically shows diffuse irregular infiltration of the subcutaneous fat in a typical lace-like pattern or bundles of cells which spread parallel to the epidermis
- Composed primarily of uniform spindled cells with elongated nuclei showing little or no pleomorphism and pale cytoplasm
- The cells are characteristically arranged in a storiform pattern characterized by numerous whorls of cells
- Little mitotic activity, rarely abnormal in appearance
    - Does not exceed five mitoses per 10 high-power fields
- Peripheral collections of chronic inflammatory cells are occasionally present, as are foci of myxoid degeneration, but necrosis is rarely a feature
- Immunohistochemically, tumor cells are usually diffusely positive for CD34 and negative for factor XIIIa, S-100 protein, desmin, actin and CD117 (c-kit)

Histologic Differential Diagnosis:

- Desmoplastic melanoma - Characterized by a diffusely infiltrative, occasionally paucicellular, malignant spindle cell tumor with significant interstitial fibrosis and collagenization. The subepidermal papillary dermis is unaffected, but the infiltrate often extends into the subcutaneous fat or beyond. Involvement of skeletal muscle or underlying bone frequently occurs. The cells are typically elongated and have eosinophilic or more commonly basophilic cytoplasm. Nuclei may be tapered and hyperchromatic or cigar-shaped and vesicular with prominent eosinophilic nucleoli. Mitoses are rare. Most commonly, the tumor has a distinctly fascicular arrangement, however, focal storiform areas are sometimes noted. The tumor may have a feathery appearance due to myxoid change. Lymphocytic infiltrates, which present as nodular aggregates, are a characteristic feature. Overlying epidermis frequently demonstrates atypical melanocytic hyperplasia, most often of the lentigo maligna pattern. However, in many cases, no in-situ component may be seen. Perineural involvement is also common. Immunohistochemically, desmoplastic melanoma expresses S-100 protein (94–100%), neuron-specific enolase and vimentin.

- Benign fibrous histiocytoma - Poorly demarcated dermal tumor. The overlying epidermis can demonstrate acanthosis or pseudoepitheliomatous hyperplasia and hyperpigmentation of the basal cell layer. A grenz zone is present in about 70% of cases. Tumor is composed of interlacing fascicles of spindled cells, sometimes in a focal storiform arrangement, set within a loose collagenous or myxoid stroma. In between the spindled cells are foamy histiocytes, multinucleated giant cells and thin-walled blood vessels. Foci of chronic inflammatory cells, including lymphocytes and plasma cells, and hemosiderin deposition are often noted. A common feature is the presence of individual hyaline collagen bundles surrounded by tumor cells in the periphery of the lesions. CD34 and factor XIIIa staining has been used differentiate dermatofibroma from DFSP. However, some overlap does occur.
- Leiomyosarcomas - May be 1) superficial or dermal leiomyosarcomas (at least 90% of the tumor confined to the dermis) → arising from arrector pili or genital smooth muscle, or 2) subcutaneous leiomyosarcomas → arising from vascular smooth muscle. Histopathologic features span a morphologic spectrum. Well-differentiated

leiomyosarcoma features overlap with leiomyomas, while poorly differentiated lesions closely resemble atypical fibroxanthoma, and moderately differentiated leiomyosarcomas cytologically resemble normal smooth muscle cells. Dermal leiomyosarcoma are composed of poorly circumscribed fascicles of spindled cells with blunt-ended nuclei and eosinophilic cytoplasm that infiltrate between the collagen. Leiomyosarcomas have a high nucleocytoplasmic ratio, and contain mitotic figures. Subcutaneous leiomyosarcomas may be better circumscribed and surrounded by a pseudocapsule of compressed tissue. They have a higher degree of pleomorphism and nuclear atypia, higher mitotic rate and can demonstrate focal necrosis. Immunohistochemical studies are important in supporting the cell lineage. Leiomyosarcoma will usually be actin-positive and desmin-positive, but negative for keratin, S100 protein and CD68.

Clinical Concerns:

- Locally aggressive sarcoma of intermediate malignancy with predilection for young to middle-aged adults.
- DFSP occurs on the trunk in 50–60% of patients, the proximal extremities in 20–30%, and the head and neck in 10–15%.
- Complete surgical excision (WLE or Mohs) is the standard of care.
- Because the translocation (17;22) places the platelet-derived growth factor (PDGF) β-chain gene under the control of the collagen 1A1 promoter, imatinib mesylate, which targets the PDGF receptor, is currently FDA-approved for adults with unresectable, recurrent and/or metastatic DFSP.

References:

1. Bolognia, JL, Jorizzo, JL, Schaffer, JV. (2012) Dermatology. China: Elsevier.
2. Calonje E, Brenn T, Lazar A., McKee, PH. (2012) McKee's Pathology of the Skin. (4th Edition). China: Elsevier.
3. Goldblum JR1, Tuthill RJ. CD34 and factor-XIIIa immunoreactivity in dermatofibrosarcoma protuberans and dermatofibroma. Am J Dermatopathol. 1997;19(2):147-53.
4. Weedon, D. (2010) Weedon's Skin Pathology. (3rd Edition). China: Elsevier.

Question 4

A 34-year-old Caucasian male was referred for treatment of a recurrent tumor on vertex scalp. A representative frozen section of the tumor is shown.

What is most likely diagnosis?

A. Neurofibroma

B. Dermatofibrosarcoma protuberans

C. Leiomyosarcoma

D. Palisaded encapsulated neuroma

Discussion

Question 4

Correct Answer:

B. Dermatofibrosarcoma protuberans

Main Histologic and Immunohistochemical Features of Dermatofibrosarcoma Protuberans Treated with Imatinib

- Dermal tumor composed of atypical spindle cells
- Paucicellular histological appearance with abundant collagen deposition in the stroma
- Storiform pattern is difficult to recognize
- Atypical spindle cells stain with CD34
- Procollagen I stain is strongly positive, confirming the presence of abundant collagen in the dermis

Histologic Differential Diagnosis:

- Neurofibroma - An unencapsulated mass of ill-defined cells with eosinophilic cytoplasm and wavy or comma-shaped nuclei. Mast cells are frequently seen. Collagen is present at varying amounts and sometimes there is a marked myxoid stroma. S-100 protein is generally positive throughout the tumor. CD34 is sometimes focally positive.
- Leiomyosarcoma - May be 1) superficial or dermal leiomyosarcomas (at least 90% of the tumor confined to the dermis) → arising from arrector pili or genital smooth muscle, or 2) subcutaneous leiomyosarcomas → arising from vascular smooth muscle. Histopathologic features span a morphologic spectrum. Well-differentiated leiomyosarcoma features overlap with leiomyomas, while poorly differentiated lesions closely resemble atypical fibroxanthoma, and moderately differentiated leiomyosarcomas cytologically resemble normal smooth muscle cells. Dermal leiomyosarcoma are composed of poorly circumscribed fascicles of spindled cells with blunt-ended nuclei and eosinophilic cytoplasm that infiltrate between the collagen. Leiomyosarcomas have a high nucleocytoplasmic ratio, and contain mitotic figures. Subcutaneous leiomyosarcomas may be better circumscribed and surrounded by a pseudocapsule of compressed tissue. They have a higher degree of pleomorphism and nuclear atypia, higher mitotic rate and can demonstrate focal necrosis. Immunohistochemical studies are important in supporting the cell lineage. Leiomyosarcoma will usually be actin-positive and desmin-positive, but negative for keratin, S100 protein and CD68.
- Palisaded encapsulated neuroma - Well-circumscribed dermal nodule. Encapsulation is incomplete and the superficial portion of the tumor merges with the surrounding dermis. Tumor cells are arranged in short fascicles separated by artifactual clefting. Cells have wavy hyperchromatic nuclei and cytoplasm is ill-defined and eosinophilic. Palisading of nuclei is not frequent. The epidermis is typically normal, but mild to prominent hyperplasia is sometimes seen. Frequently, a normal nerve is identified near the base of the lesion, often entering the lesional capsule. Vascularity is occasionally present. Majority of the cells are S-100 positive. Many axons can be identified with neurofilament protein and the cells in the capsule stain for EMA.

References:

1. Calonje E, Brenn T, Lazar A. (2012) McKee's Pathology of the Skin. (4th Edition). China: Elsevier.
2. Thomison J, McCarter M, McClain D, Golitz LE, Goldenberg G. Hyalinized collagen in a dermatofibrosarcoma protuberans after treatment with imatinib mesylate. J Cutan Pathol. 2008 Nov;35(11):1003-6.

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Question 5

A 68-year-old female with a basal cell carcinoma (BCC) arising on the left temple is referred for Mohs Micrographic Surgery (MMS).

A standard Mohs layer is removed and subsequently examined.

Select the most likely diagnosis and best course of action:

A. BCC is present in the deep dermis; Take another layer.

B. Invasive squamous cell carcinoma is visualized; Take another layer.

C. No residual tumor is identified; Perform closure.

D. Specimen is inadequate; Request recuts from the histotechnician.

Discussion

Question 5

Correct Answer:

D. Specimen is inadequate; Request recuts from the histotechnician.

Main Histologic Features:

- Quality Assurance:
  - Deep tissue is well-visualized without holes; subcutaneous architecture is well-preserved.
  - Staining is adequate.
  - Epidermis is present around entire section.
  - Epidermis is flipped/folded.
- Oncologic Analysis:
  - Residual focus of superficial BCC is obscured by region of flipped epidermis, thus representing a "false negative."

Differential Diagnosis:

- Adequate histologic slide without tumor present.

Clinical/Histologic Concerns:

- Mohs slides must be analyzed both for quality and for the presence/absence of tumor.
- In this case, recuts were requested due to folded epidermis, revealing a focus of superficial BCC that was subsequently extirpated.
- Studies demonstrate that a likely cause of recurrence after MMS may be identified on slide review in approximately 80% of cases.
- In these cases, slides are falsely interpreted as "negative," or free of tumor, when residual tumor is actually present.
- The root causes of "false negatives" can be stratified into errors of tissue processing and slide quality versus errors of slide interpretation and surgical execution.
- Aspects of poor slide quality leading to persistent/recurrent tumors include deep tissue drop-out (29%), missing epidermis (27%), and poor staining (3.7%).
- Errors in slide interpretation (tumor present on slide but missed by surgeon) have been associated with 20% of recurrences after MMS, while errors in surgical execution (inadequate size or location of subsequent stage leading to false negative slide) have been associated with 6% of recurrences.
- Mohs surgeons must be just as vigilant in assessing for slide quality as they are evaluating for neoplasia.

References:

- Hruza GJ. Mohs micrographic surgery local recurrences. J Dermatol Surg Oncol. 1994;20(9):573-7.
- Zabielinski M, Leithauser L, Godsey T, Gloster HM. Laboratory Errors Leading to Nonmelanoma Skin Cancer Recurrence After Mohs Micrographic Surgery. Dermatol Surg 2015;41:913-6.
- Campbell T, Armstrong AW, Schupp CW, Barr K, et al. Surgeon error and slide quality during Mohs micrographic surgery: is there a relationship with tumor recurrence? J Am Acad Dermatol 2013;69:105-11.
- Lee KC, Higgins HW II, Dufresne RG Jr. Tumor recurrence after Mohs micrographic surgery. J Am Acad Dermatol 2014;70:385-6.

Question 6

A 74-year-old male with a 2 centimeter BCC arising on the upper forehead is referred for MMS.

A standard Mohs layer is taken, bisected, with one of two blocks examined here. For the purposes of this question, assume the other block is free of tumor on histologic examination. **The first cut into this tissue block is denoted by a black dot.**

Select the most likely diagnosis and best course of action:

A. BCC is visualized and is transected at the deep margin; Take another layer.

B. BCC is visualized but is clear at the deep (true surgical) margin; Commence reconstruction.

C. SCCIS is present; Take another layer.

D. Specimen is inadequate; Request recuts from the histotechnician.

Discussion

Question 6

Correct Answer:

A. BCC is visualized and is transected at the deep margin; Take another layer.

Main Histologic Features:

- Quality Assurance:
  - Epidermis is present around entire section.
  - Staining is adequate.
  - Deep tissue is well-preserved, with few significant holes.
  - The true deep surgical margin is not present on the first cut into the block (as illustrated by a lack of surgical ink at the deep margin of this cut), but is visualized by later cuts (where ink extends along the entire deep margin).
- Oncologic Analysis:
  - Residual infiltrative BCC is present, but is not visualized on the first cut due to tangential sectioning.
  - The first cut thus represents a "false negative."

Differential Diagnosis:

- BCC is visualized but is clear at the deep (true surgical) margin.

Clinical/Histologic Concerns:

- Mohs slides must be analyzed both for quality and presence/absence of tumor.
- In this case, the tumor appears to be clear at the true surgical margin, as represented by the first cut into the tissue block. However, this cut represents a "false negative" that could lead to recurrence.
- The clue here is the lack of surgical ink outlining the entire deep margin of the first cut, which should inform the surgeon that the true deep margin is not visualized.
- Later cuts into the block demonstrate surgical ink along the entire deep margin, where infiltrative BCC is easily identified. Another deep layer should be taken to clear this patient.

Study	Recurrences (#)	Cause Identified on Slide Review (%)	Slide Quality Unsatisfactory (%)	Surgeon Error (%)
Hruza	30	77	40	30
Campbell	19	N/A	N/A	26
Lee	11	N/A	27	18
Zabielinski	22	82	23	27
Total	**82**	**79.1**	**32**	**28**

References:

- Hruza GJ. Mohs micrographic surgery local recurrences. J Dermatol Surg Oncol. 1994;20(9):573-7.
- Zabielinski M, Leithauser L, Godsey T, Gloster HM. Laboratory Errors Leading to Nonmelanoma Skin Cancer Recurrence After Mohs Micrographic Surgery. Dermatol Surg 2015;41:913-6.
- Campbell T, Armstrong AW, Schupp CW, Barr K, et al. Surgeon error and slide quality during Mohs micrographic surgery: is there a relationship with tumor recurrence? J Am Acad Dermatol 2013;69:105-11.

- Lee KC, Higgins HW II, Dufresne RG Jr. Tumor recurrence after Mohs micrographic surgery. J Am Acad Dermatol 2014;70:385-6.

Question 7

A 78-year-old woman presents for treatment of a biopsy positive basal cell carcinoma of the right nasofacial sulcus. This slide is from the second Mohs stage.

Based on the findings on this slide which of the following is correct?

A. There is no residual BCC.

B. There is residual BCC at the blue pole.

C. There is residual BCC at the red pole.

D. There is residual BCC at the deep margin.

Discussion

Question 7

Correct Answer:

A. There is no residual BCC (folliculocentric basaloid proliferation).

Main Histologic Features:

- Superficial folliculocentric nests of small epithelioid cells without peripheral palisading
- Thickened basement membrane surrounding the nests
- Normal surrounding stroma without mucinous degradation or myxoid changes
- No significant pleomorphism, apoptosis, dyskeratosis, or mitoses

Differential Diagnosis:

- Basal cell carcinoma with follicular differentiation/infundibulocystic BCC
- Basaloid follicular harmartoma
- Trichoblastoma
- Trichoepithelioma

Clinical Concerns:

- Folliculocentric basaloid proliferation (FBP) can mimic BCC on H&E.
- Often the axial orientation and connections to central follicles can be lost on horizontal Mohs sections compared to vertical sections making differentiation from BCC, particularly the infundibulocystic variant, difficult.
- Failure to differentiate FBP from BCC can result in excessive Mohs stages being taken enlarging defects for a benign adnexal proliferation.

References:

- Patel NS, Johnston RB, Messina JL, Cherpelis BS. A unique basaloid proliferation encountered during Mohs surgey: potential pitfall for overdiagnosis of basal cell carcinoma. *Dermatol Surg*. 2011;37(8):1180-8.
- Leshin B, White W. Folliculocentric basaloid proliferation: the bulge (der Wulst) revisited. *Arch Dermatol*. 1990;126(7):900-6.
- Krunic AL, Garrod DR, Viehman, et al. The use of antidesmoglein stains in Mohs micrographic surgery. *Dermtol Surg*. 1997;23(6):463-8.
- Saxena A, Shapiro M, Kasper DA, et al. Basaloid follicular hamartoma: a cautionary tale and review of the literature. *Dermatol Surg*. 2007;33(9):1130-5.

Question 8

A 55 year-old gentleman presents for treatment of a biopsy positive 2cm infiltrative basal cell carcinoma of the R preauricular cheek that has been present for several years. This slide is from the first Mohs stage.

This slide demonstrates which of the following:

A. Deeply invasive BCC masked by inflammatory peritumoral camouflage.

B. BCC with reactive lymph node formation.

C. BCC with concomitant CLL leukemic infiltrate.

D. BCC with lymphoepithelioma-like carcinoma.

Discussion

Question 8

Correct Answer:

A. BCC with reactive lymph node formation.

Main Histologic Features:

- Infiltrative metatypical BCC surrounded by dense lymphocytic aggregates
- Some aggregates demonstrate a follicular pattern with a central germinal center zone composed of larger cells surrounded by a mantle of smaller lymphocytes
- The lymphocytes do not show significant cytologic atypia

Differential Diagnosis:

- Tumor camouflage of invasive or perineural carcinoma with dense peritumoral inflammation
- BCC with associated leukemic infiltrate of chronic lymphocytic leukemia (CLL)
- BCC with follicular lymphoma

Clinical Concerns:

- Reactive lymphadenoid proliferation can be seen in the cutaneous and subcutaneous tissue surrounding longstanding skin cancers.
- It is important to differentiate these reactive inflammatory infiltrates from invasive carcinoma with T-cell predominant peritumoral inflammation.
- B-cell predominant leukemic infiltrates can be seen in over 1/3 of non-melanoma skin cancers treated in patients with chronic lymphocytic leukemia (CLL).
- In difficult cases immunoperoxidase stains can be helpful in differentiating this benign reactive process from undiagnosed CLL leukemic infiltrates.

References:

- Mehrany K, Byrd DR, Roenigk RK, et al. Lymphocytic infiltrates and subclinical epithelial tumor extention in patients with chronic leukemia and solid-organ transplantation. *Dermatol Surg*. 2003;29(2):129-134.
- Padgett HK, Parlette HL, English JC. A diagnosis of chronic lymphocytic leukemia prompted by cutaneous lymphocytic infiltrates present in Mohs micrographic surgery frozen sections. *Dermatol Surg*. 2003;29(7):769-771.
- Wilson ML, Elston DM, Tyler WB, et al. Dense lymphocytic infiltrates associated with non-melanoma skin cancer in patients with chronic lymphocytic leukemia. *Dermatol Online J*. 2010;16(3):4.

Question 9

A 71-year-old man presents for treatment of a recurrent melanoma in situ on the left neck. Both H&E and MART-1 immunostains are performed during Mohs surgery. The slide is from the first layer.

What is your next step?

A. Stop. The margin is clear. Only normal skin is appreciated.

B. Stop. The margin is clear but a solar lentigo is also present in the background.

C. The peripheral margin is positive due to melanoma in situ. Take an additional layer.

D. The peripheral margin is positive due to melanoma in situ. Invasive melanoma is also present. Take an additional layer.

Discussion

Question 9

Correct Answer:

C. The peripheral margin is positive due to melanoma in situ. Take an additional layer.

Main Histologic Features of Melanoma in Situ and Immunohistochemical Markers

- Melanoma in situ is characterized by an asymmetrical proliferation of atypical melanocytes arranged as single units and nests at the dermoepidermal junction (DEJ). Single melanocytes are also often irregularly spaced and can be present above the DEJ (pagetoid spread).
- The epidermis may be effaced or atrophic and there may be prominent solar elastosis in the dermis, particularly in the case of lentigo maligna. The presence of scattered inflammatory cells and melanophages in the dermis may be associated with occult lentigo maligna melanoma.[1]
- Melanoma antigen recognized by autologous cytotoxic T-cells (MART-1), a cytoplastic stain, is one of the most widely used antibodies during Mohs surgery for melanoma.[2,3]
- Previously published criteria for positive margins when using the MART-1 immunostain include "(1) nests of at least 3 atypical melanocytes, (2) melanocytes above the dermoepidermal junction, (3) confluence of more than 9 adjacent melanocytes, (4) "vertical stacking" of melanocytes, (5) melanocytic hyperplasia significantly different in one area as compared with the rest of the margin, and (6) presence of nests of atypical cells in the dermis."[2]
- The use of microphthalmia transcription factor (MITF), a nuclear stain for melanocytes, has also been studied in frozen sections.[4]
- Sry-related HMG-BOX gene 10 (SOX-10), a nuclear transcription factor responsible for the differentiation of neural crest cells into Schwann cells and melanocytes, is a newer melanocytic marker.[5] This nuclear stain is particularly useful in highlighting melanocytes in desmoplastic melanoma[5] which usually stains poorly with MART-1 and MITF.

Differential Diagnosis

- Solar lentigo: Histologic features include an increase in epidermal melanin and hyperplasia of the epidermis overlying solar elastosis. The pigmented rete ridges display a "dirty feet" appearance.
- Incidental melanocytic nevus: Melanocytic nevi can be divided into junctional, intradermal, and compound types. Distinguishing between an incidental junctional nevus and the edge of a melanoma in situ may be challenging during Mohs surgery as diagnostic criteria overlap. In contrast, incidental dermal and compound nevi can often be differentiated from melanoma. The dermal component may display nests and single melanocytes that shrink in size toward the deeper part of the dermis. They can be found during Mohs surgery for non-melanoma skin cancer as well. The decision to take another stage depends on multiple factors. For example, it is conceivable to remove the incidental nevus in order to prevent the development of a recurrent nevus which may mimic a melanoma (pseudo-melanoma) on histopathology.
- Chronically sun-damaged skin (melanocytic hyperplasia): It can be challenging to differentiate between changes seen in chronically sun-damaged skin and melanoma in situ as both can display melanocytic hyperplasia. In order to better characterize the extent of melanocytic hyperplasia in sun-damaged skin, Hendi et al[6] stained sun-exposed skin prepared from dog-ears with the MART-1 immunostain. The authors found

an average of 15.6 melanocytes per high power field (x400 magnification, equivalent to 0.5 mm of skin). Mild (2 adjacent melanocytes) to moderate (3 to 6 adjacent melanocytes) confluence was common in 54.0% and 34.0%, respectively, of cases. The upper limit of confluent melanocytes was 9. The authors noted that while these data are helpful as a guide, they shouldn't be used alone to differentiate between melanoma and sun-damaged skin. Other criteria for melanoma in situ, such as nesting, pagetoid spread, and degree of confluence, are also important.

Clinical Concerns

- According to the American Academy of Dermatology guidelines, surgical excision with clear histologic margins is the primary treatment modality for cutaneous melanoma.[7] The goal is to minimize local recurrence due to persistent disease.
- Two large studies have shown that Mohs micrographic surgery with the MART-1 stain achieves local recurrence rates of less than 0.5%.[2,3]

References

1. Calonje E, Brenn T, Lazar A. (2012) McKee's Pathology of the Skin. (4th Edition). China: Elsevier.

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Question 10

A 77-year-old white male presents with a 10mm squamous cell carcinoma on his left preauricular skin. The following slide represents a tissue block from Mohs stage I.

The correct diagnosis is:

A. Squamous metaplasia of the sebaceous gland

B. Squamous cell carcinoma with sebaceous differentiation

C. Squamous cell carcinoma, moderately differentiated

D. Sebaceous carcinoma

Discussion

Question 10

Correct Answer:

A. Squamous metaplasia of the sebaceous gland

Main Histologic Features:

- Reactive metaplasia of the sebaceous glandular epithelium to squamous epithelial cells, similar to those of the stratum spinosum
- Associated inflammatory infiltrate consisting of lymphocytes, neutrophils, and foreign body granuloma
- Considered a non-specific response to chronic injury, and has been described in the context of pressure ischemia and squamous cell carcinoma in situ

Differential Diagnosis:

- Squamous cell carcinoma with sebaceous differentiation
- Sebaceous carcinoma with squamous differentiation
- Squamous cell carcinoma

Clinical Concerns:

- Mimics squamous cell carcinoma, leading to false positive margin status during Mohs surgery

References:

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Question 11

These slides represent stage I and II of a Mohs-assisted excision for a poorly-differentiated squamous cell carcinoma.

On slide 2, squamous cell carcinoma involves which of the following?

A. Perichondrium

B. Cartilage

C. Dermis

D. Lymphatics/intravascular space

E. A, C and D

Discussion

Question 11

Correct Answer:

E. A, C and D

Main Histologic Features:

- This squamous cell carcinoma is characterized by invasive aggregates of malignant keratinocytes demonstrating little to no keratinization.
- Cartilage is composed of chondrocytes with round nuclei and ample cytoplasm in a hyalinized (blue) stroma. Squamous cell carcinoma does not invade this fibroconnective tissue.
- The perichondrium, clinically, is a distinct connective tissue layer providing vascular support to the cartilage. Histologically, it appears as loose collagen fibers contiguous with both the basophilic cartilage and the brightly-eosinophilic reticular dermis. Sections show a large aggregates of tumor adjacent to the cartilage involving this loose, perichondrial tissue.
- Superficial to the perichondrium, squamous cell carcinoma can be identified invading the eosinophilic collagen of the reticular dermis.
- At the junction of the reticular and papillary dermis, there are interfollicular aggregates of tumor within endothelial-lined lymph-vascular spaces occurring at a consistent depth (the lower third of the pilosebaceous units).
- Determining whether tumor is contained within artefactual clefts or within true, vascular lumina rests upon identifying endothelial cells. Endothelial cells appear as flattened, spindled cells that define a lumen. These cells can be identified in the superficial papillary plexus (the capillary plexus at the junction of the papillary and reticular dermis) where they line lymphatics, capillaries, and blood vessels. Lymphatics tend to appear most superficially at the junction of the papillary and reticular dermis as delicate, endothelial-lined structures with neither a cuticle nor vascular smooth muscle. Capillaries and blood vessels, by comparison, have variable amounts of smooth muscle in the walls and, on frozen sections, may have an apparent cuticle (an eosinophilic band that occasionally forms a ring outside of the endothelial cells).
- The consistent depth of the tumor aggregates is a clue that the tumor cells are contained within lymph-vascular lumina.
- Lymph-vascular spaces may appear dilated, making specific distinction between lymphatic or vascular origin difficult. Immunostains, including D2-40, may be used to highlight endothelial cells in cases where intravascular invasion is suspected.

Clinical Concerns:

- Intravascular invasion (LVI, lymph-vascular invasion), is a defined histologically as the presence of tumor within endothelial-lined, vascular spaces. LVI has been identified in up to 40% of patients with lymph node metastases from primary cutaneous squamous cell carcinomas of the head and neck ($P < .00001$). Though one study identified LVI as an independent prognostic factor for nodal metastasis [Moore], the correlation between LVI and metastasis failed to meet statistical significance on multivariate analysis. LVI appears to be significant in the development of in-transit metastases, however.

- The American Joint Committee on Cancer (AJCC) includes LVI as a high-risk feature in the assessment of cutaneous squamous cell carcinomas of the head and neck in both the 7th and 8th edition staging manuals. Other high risk features include patient immunosuppression, large tumor size, tumor thickness, high-grade, anatomic level,

location on the ear or non-glamorous lip, history of prior radiation, involvement of deep tissues and perineural invasion. In general, high-risk squamous cell carcinomas carry a 10-20% of metastasis as compared to all squamous cell carcinomas that carry a 5% of overall metastasis.

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