Maui Dermatology Meeting 2013

Dermatopathology
The Biopsy, Analysis & Report

Whitney A. High, MD, JD, MEng
Associate Professor, Dermatology & Pathology
Vice-Chairman, Clinical Affairs (Dermatology)
University of Colorado Health Sciences Center

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The Modern “Dermatopathologist”
• One of just two ABMS-recognized subspecialties in Dermatology

So, how much do you know about the procurement and processing of a biopsy?

Interesting Trends
Nine geographic areas of USA (1986-2001):
biopsy rate ↑ 2.5x (those >65 y/o)
melanoma ↑ 2.4 x

General Practice of Dermatopathology
Crudely Simplified
Biopsy Performed & Fixed in Formalin

To Lab

Accessioned at Lab

“Grossed In”

Tissue is Placed In Cassettes for Processing

“Processing”

- Water and fat removed with successive washes of ETOH & xylene
  - ETOH removes the water
  - xylene removes the ETOH
Embedding
• Voids in tissue are replaced with paraffin so tissue can be sectioned

Cutting the Block
• Cut into thin (3.5 micron) sections that allow light to pass through

Autostainer & Coverslipper
• Washes with xylene, EtOH, hematoxylin & eosin (H&E)
• Makes final H&E stained slide that is ready to "read"

Normal Skin
- Epidermis
- Stratum Corneum
- Dermis
- Hair & Seb Glands

Chain of Dependency
- Biopsy
- Courier
- Logging/Intake
- Grossing
- Embedding
- Cutting
- Labeling
- Analysis
- Typing
- Issued Report
- Distribution

Potential for error exists at each point.

Tip: Take the time to provide good insight and accurate information

You need to provide as many "clues" to the correct diagnosis as you possibly can!
Prevent An Error Before it Transpires

- “Crap in = Crap out”
  - “r/o melanoma” on everything
  - “r/o cancer” on everything
  - “rash”
  - “238.2” for everything
- Multiple specimens in the same bottle
- Curetting of a pigmented lesion
- Mismarking shaves, punches, excisions

We use bar codes and rotating ink to prevent tissue misidentification.

Ultimately, P09-41107 was a BCC but tissue placed on the slide by the tech was a benign nevus.

Not including a REALISTIC clinical impression eliminates a key method for tissue verification!

Ultimately, P09-41109 was a benign nevus but tissue placed on the slide by the tech was a BCC.

Consider taking a picture of biopsy sites!

- From 2002-2008:
  - 132 “misidentified surgical sites” reported to Colorado malpractice insurers
  - orthopedists have a 25% chance of being sued for misidentified site during a career
- Picture can be useful to dermatopathologist
- Wireless “air cards” for cameras can revolutionize this task for you!

TIP:
Secure a representative biopsy

(pssst – medicolegally, this is ALWAYS the clinician’s obligation)
The Vanishing Biopsy: The Trend Toward Smaller Specimens

- Number of shaves increased 1988-2005
- Volume of shaves decreased 1988-2005
- This impacts the accuracy of diagnosis

Basic Histology
BCC and SCC

“Rule out BCC vs. SCC”
(no other data provided)

Atypical Nevus?

How confident would you be, if you were the doctor examining this case?

Re-Excision Specimen = MIS

The Impact of Partial Biopsy on Histopathologic Diagnosis of Cutaneous Melanoma

Objective: To compare partial and excisional biopsy specimens in the accuracy of histopathologic diagnosis and staging of cutaneous melanoma

Design: Prospective, non-randomized trial

Setting: Tertiary referral center, dermatology clinic

Patients: Consecutive cases from 2000 to 2005

Inclusion: Patients with cutaneous melanoma

Rule of Thumb: No difference was found between partial and excisional biopsy specimens in the accuracy of histopathologic diagnosis and staging of cutaneous melanoma.
TIP: Choosing the right technique and biopsy site improves results

Different Biopsy Techniques
- Shave
- Deep Shave (Saucerization)
- Punch
- Incision/Excision

True story…
“Always punch the thickest part of the lesion.”

The biopsy contained:
An intradermal nevus.

The patient died of - the adjacent melanoma.

Study of Saucerization
(Pariser et al. DOJ 1999; 5:4)

Figure 1. Percentages of specimens which removed entire lesion, part of lesion with insignificant missing portion, or part of lesion with significant missing portion.

Study of Saucerization
(Pariser et al. DOJ 1999; 5:4)

Figure 2. Percentages of each specimen type resulting in a histopathologic diagnosis which was completely certain or fairly sure, or in which there was some suspicion. Shown in yellow are those cases in which there was little doubt of lesion malignancy (sum of completely certain and fairly sure).
Special Techniques

For Special Situations

Verrucous Carcinoma

• Special subtype of SCC
• Mucosa, acral skin, and genitalia
• Eponyms based upon location:
  Oral – Ackerman tumor
  Genital – Buschke-Lowenstein tumor
  Acral – epithelioma cuniculatum
• Medicolegal conundrum if insufficiently sampled (HELP!!!)

Myosis Fungoides

• Form of cutaneous T-cell lymphoma (CTCL)
• Early forms = thin patches or plaques in “double-protected” areas
• Early disease is difficult to diagnose by histology alone ("patch stage")
Mycosis Fungoides
- Dx REQUIRES clinicopathologic correlation
- No single test establishes a diagnosis MF
- Average time from onset to diagnosis is 6 years (and ≥3 biopsies)

**TIP:** shave biopsy maximizes DEJ available for inspection - may be useful in early mycosis fungoides!

Bullous Pemphigoid
- #1 immunobullous disease
- Elderly with multiple comorbidities
- Subepidermal blister
- Eosinophils present in the blister cavity

**TIP:** You can shave off the entire blister and makes examination easier
Immunofluorescence Studies

- "Direct immunofluorescence" (DIF)
  - uses patient tissue and lab antibodies
  - can only be performed on fresh tissue or tissue fixed in Michel medium
  - placement in formalin ruins the tissue

- Useful to study:
  - blistering conditions
  - connective tissue disease
  - vasculitis

Panniculitis

- Inflammation of the deep fat
- Diseases include:
  - Erythema nodosum (#1)
  - Erythema induratum
  - Pancreatic fat necrosis
  - Factitial disease (surreptitious injection)
  - Other rare diseases

Typical Clinical Image

Erythema nodosum

Markedly widened subcuticular septae

Erythema nodosum

Widened septae with granulomatous inflammation
“Rule Out Panniculitis”

What can you really say in this situation?!?!?!?

Adequate Sample

Stacked Punch Biopsy for Panniculitis

Surgical Pearl: The trophine punch for diagnosing panniculitis

Adequate Sample

“Rule out alopecia.”

Don’t even get me started.

ALOPECIA PROCESSING

Vertical Sections (like longitude)

TIP: Don’t shave the hair just trim it short! (it allows the tissue to be oriented easily)
**Lymphoid Proliferations**

- B-cell lymphoma often presents as:
  - red to purple nodules
  - middle-aged to elderly
  - single or few lesions on the head/neck

**TIP:** Lymphocytes, especially malignant ones, are sensitive to lateral pressure, and “crush” may render the biopsy useless.

**TIP:** Very nearly all tissue removed from a human being should be sent for analysis (in my humble opinion)
The utility of intraoperative microscopic analysis for histological evaluation: A retrospective study of 1335 skin tags vs. 697 moles.

5 of 1335 “skin tags” contained a malignancy
- 4 BCC, 1 SCC
- compared to 6 malignancies in 697 “moles”
- is this a reason to require submission of all tissue?

Biopsy from posterior neck of a 34 year old woman.

“Rule out tag.”

This ink can be used in clinical settings as well (and not just Mohs).
Finish Strong

Simple Things…

• 10% Neutral Buffered Formalin
  – “fixes” the specimen
  – cross-links lysine residues in proteins
  – CANNOT be used for immunofluorescence specimens

  **TIP:** volume 10% NBF should be 10x the specimen volume

Lab Boxes for After Hours Pickups

<table>
<thead>
<tr>
<th>Section 2: Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component</td>
</tr>
<tr>
<td>Formaldehyde</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
<tr>
<td>Sodium Phosphate, Dibasic</td>
</tr>
<tr>
<td>Sodium DiHydrogen Phosphate, Monohydrate</td>
</tr>
<tr>
<td>Water</td>
</tr>
</tbody>
</table>

10% NBF begins to freeze at 31.8°F and is entirely solid at 23.1°F.

**TIP:** Be careful where you position the pick-up box in cold weather!!!!

“Turn Around Time…”

• Research in pharmacy, nursing and medicine demonstrates that errors are associated with:
  – hectic work environments
  – interruptions and distractions

The effect is *independent* of the experience of the practitioner.

Big Heads and False Idols

You are here.

You are NOT here.

Even though you are likely a valued account, don’t think you are the “only thing” happening that day!
Now sit back and wait for your path report!

More tips to follow…

Initial Check

• Is this my patient?
• Does the gross information & technique match?
• Was my history and clinical impression accurate?
• Is this a plausible diagnosis?

No Path Report is Beyond Reproach

No stone tablet.
No burning bush.

No Path Report is Beyond Reproach

This is MELANOMA.
If any other diagnosis returns, it should be treated with great suspicion.

Ultimately, the diagnosis was ALMM 1.6 mm deep.

Sites of Important Information

• Diagnosis
• Comments
But also…
• Gross desc.
• Micro desc.
How much of the report is the ordering physician responsible for reading and understanding?

What are levels or step sections?
- Cutting deeper into the embedded block
- Allows for inspection of a different area of tissue (usually done in sets of 3)
- Useful when one questions the "representative" nature of the sections

Level 1
"r/o NMSC"

Level 3
"r/o NMSC"

Level 3 – Higher Magnification
"r/o NMSC"

Use of Levels Should be Documented

- If you didn’t get an expected diagnosis and levels were performed perhaps this is re-assuring
- If you didn’t get an expected diagnosis and levels weren’t performed, perhaps they should be!
Use of Immunostains or Special Stains

- Immunostains bind immunologic epitopes on tissue, are in turn bound by a chromogen
- Special stains utilize other means to mark tissue:
  - PAS for fungus
  - GMS for fungus
  - Gram stain (Brown Brenn) for bacteria
  - Fite or AFB stains for acid fast bacilli
  - Colloidal Fe stain for mucin

Case
- 79 year-old man
- "r/o NUB" on chest

Right areola – "r/o BCC"

Is this basal cell carcinoma?

Immunohistochemical Staining

BerEp4
Immunohistochemical Staining

First Stage - Mohs' (Frozen Section)

Re-Excision

Re-excision IHC Studies

Re-excision IHC Studies

Immunostains Used in Dermatology

- Melanoma/Melanocytic Processes
  - S100, Melan A, Tyrosinase, HMB45, P16
- Dermatofibroma vs DFSP
  - Factor XIIIa vs CD34
- Malignant squamous processes
  - overexpress P53
- Basal cell carcinoma
  - expresses BerEp4
- Merkel cell carcinoma
  - expresses CK20, chromogranin, EMA, etc.
Melanoma 13% of all pathology claims (44/335)
• False negative 95% (42/44) but false positive 5% (2/44)

(2006 ACS estimates: 63k melanomas, 217k breast cancers)

Immunostains for Melanocytic Lesions

- There is no “melanoma” stain
- No immunostain can “trumps” morphological inspection of the cells
- Some stains may “support” a benign or malignant diagnosis if used appropriately

IHC Techniques for Melanocytic Lesions

**Ki67** – proliferative index (<5% favorable, 5-15% borderline, >15% bad)

**HMB45** – should normally diminish with descent into the dermis

**P16** – positive expression is 3x more common in Spitz nevi than melanoma

**S100A6** – similar discriminatory power for Spitz nevus versus melanoma
P16

- Benign nevi - 100% (34/34)
- Spitz nevi - 78% (50/64)
- Melanoma - 22% (14/64)


Special Stains for Infection

- Insensitive for some infectious disease:
  - Sporotrichosis (organisms rarely seen)
  - Cellulitis (organisms rarely seen)

In most situations culture is more sensitive. Best to do both if infection is a possibility.

Where do you find stains reported in a histology report?

Special Stains for Infection

- Special stains showed NO organisms
- Culture grew a dimorphic fungus
Margins

There are important things to say about margins, and the limitations that exist in analysis.
“Breadloaf” Sections

Allow for inspection of well less than 1% of the overall margin.

Where do you find information on margin status?

- Usually present in several places upon a report (diagnosis, micro, comments)

Oriented Specimens

- Arrive at the laboratory with a suture or nick marking a specific position
- Examiner can then provide a more specific location of any residual tumor

Complete extirpation should not be “assumed” particularly when the margins are reported to be narrow.

Providing actual measurements is emerging as the preferred standard.

Synoptic Reporting

A new standard is emerging.

To make pathology report readily understandable to all.

(goal for any report)
Diagnosis: Left arm, shave
ICD-9: 172.6
DP.382B-11, MALIGNANT MELANOMA (BRESLOW DEPTH AT LEAST 0.45 MM, CLARK LEVEL AT LEAST III/IV), MARGINS POSITIVE, SEE COMMENT

Microscopic Description: The specimen is a shave biopsy of skin present as multiple H&E stained sections on one slide. The primary pathologic process is that of a malignant proliferation of atypical melanocytes along the dermoeipidermal junction and within the dermis. These atypical melanocytes manifest extreme pleomorphism, hyperchromasia, and irregular nuclear contour. There is pagetoid spread of melanocytes and confluent growth of melanocytes leading to artificial separation of the epidermis from the dermis. There is a lack of maturation within the dermal nests and cells. There is associated dermal fibrosis, inflammation, and pigmentary incontinence.

This neoplasm demonstrates the following characteristics:
- Subtype: Superficial spreading malignant melanoma, vertical growth phase
- Cell type: Epithelioid
- Ulceration: Not observed
- Regression: There is dermal fibrosis, inflammation, and pigmentary incontinence consistent with partial dermal regression
- Microsatellitosis: Not observed
- Vascular extension: Not observed
- Perineural extension: Not observed
- Mitotic activity: 1 per mm² in the dermal component
- Tumor infiltrating lymphocytes: Brisk
- Margins: The atypical melanocytes extend broadly to the deep and peripheral margins

CAP “Required” Data
- Site (with laterality)
- Procedure
- Diagnosis (with stipulations/caveats clearly identified)
- Histologic subtype
- Breslow depth (“at least, see comment.”)
- Ulceration (+/- is all CAP requires)
- Margin (measured to nearest point for excision)
- Mitotic index (<1/mm² or specify #/mm²)
- Microsatellitosis (+/-)
- Lymphovascular invasion (+/-)
- “Optional/Suggested” – Clark level, perineural invasion, TIL’s, regression (>75% or absent), growth phase

Depth
- Single most important prognostic item:
  Breslow depth (mm)
- Measured at right angle to surface from granular layer (or ulcer base) to deepest cell
- May use “at least” or “indeterminate” (with “see Comment”)

Mitotic Activity
- A “new” thing in the 7th AJCC
- Upstages from T1a to T1b (≤1.0 mm)
- Measured using “hot spot” technique
- Is it really as critical as it seems?
  - 10x ocular & 40x objective is ~0.15 mm²
  - criteria is “none or <1/mm²” versus “≥1/mm²”
  - 6 HPF x 0.15 mm² = ~ 0.9 mm²

Synoptic Reporting
- Soon to become standard for:
  - Squamous cell carcinoma
  - Merkel cell carcinoma
- Even with basal cell carcinoma it is common to identify a “growth pattern”

Thanks! Questions?