Pediatric Melanoma Forum:  
Making the Diagnosis of Melanoma in Children

Whitney A. High, MD, JD, MEng  
Associate Professor, Dermatology & Pathology  
Director, Dermatopathology Laboratory  
University of Colorado School of Medicine

September 26, 2015  
Grapevine, Texas

The Basics of The Skin

- The skin of an:
  - infant covers ~2500 cm²
  - adult covers ~18000 cm²
- The skin of an adult weighs 3-5 kg  
  (~20% TBW)
- The skin plays a vital role in: protection, homeostasis, and even aesthetics

Skin as Three Layered Cake

- Epidermis: outer protective layer
- Dermis: supports and nourishes the epidermis
- Subcutis: insulates and protects

One of the functions of the skin is protection…  
… from the environment.

The Sun and UV Radiation

- The sun is basis of essentially all life on earth
- Without the sun we would all perish (quickly at that)
- However, ultraviolet light causes damage to DNA

UV Radiation and Skin

UV-A penetrates more deeply into skin
The Melanocyte
Genetic Defender

- Located along the "dermoepidermal junction"
- Produce "melanin" (pigment) that absorbs the energy of ultraviolet light (protects DNA)
- All people have about the same # in the skin

Melanocytes stained brown

Embryology

Nevus

When these migrating melanocytes become "stuck" and agglomerated together…

that is called a "mole" or "nevus."

Conceptual differences exist between a:

Mole – benign aggregation of melanocytes
Melanoma – malignant aggregation of melanocytes

GROUNDS RULES

- Melanoma is rare in kids
- "Children" (<20 y/o) account for just 2% of all melanoma
- Prepubescent children account for just 0.4% of all melanoma
- That said, melanoma in kids has increased 2.8% per annum since the 1970s
So, how do we diagnose melanoma, both clinically and histologically?

Nevi vs. Melanoma
Sometimes it is obvious!

Clinical Examination
Classic Teachings
- A – asymmetry
- B – border irregularity
- C – color variegation
- D – diameter > 6 mm
- E – evolution

Clinical Features of Atypical/Dysplastic Nevi

<table>
<thead>
<tr>
<th>Common Nevi</th>
<th>Dysplastic nevi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymmetric</td>
<td>Symmetric elements</td>
</tr>
<tr>
<td>Distinct</td>
<td>Border</td>
</tr>
<tr>
<td>Uniform brown</td>
<td>Color</td>
</tr>
<tr>
<td>&gt;6 mm</td>
<td>Diameter</td>
</tr>
<tr>
<td>Usually asymptomatic</td>
<td>Evolution</td>
</tr>
</tbody>
</table>

The “Ugly Duckling”
One mole that looks nothing like the other "signature" nevi!

But in children especially…

melanoma may not be detected by the ABCDEs!
Cordoro et al. 2013

- Group A (0 to 10 years old)
  - 60% did NOT present with “classic” ABCDEs
- Group B (11 to 19 years old)
  - 40% did NOT present with “classic” ABCDEs
- Proposed different +ABCD algorithm for kids:
  A – amelanotic
  B – bleeding bump
  C – color uniformity
  D – de novo, any diameter

Grim Reaper and Atypical Nevi?

One of the strongest risk factors for melanoma is the presence of multiple atypical nevi (AMS)

### Table 1. Risk factors for MM

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2-82</td>
</tr>
<tr>
<td>Atypical nevi</td>
<td>0.127</td>
</tr>
<tr>
<td>Family history of MM</td>
<td>1.444</td>
</tr>
<tr>
<td>History of melanoma</td>
<td>0.000</td>
</tr>
<tr>
<td>&gt;200 nevi</td>
<td>0.844</td>
</tr>
<tr>
<td>Congenital nevi</td>
<td>0.118</td>
</tr>
<tr>
<td>Phakomata</td>
<td>0.39</td>
</tr>
<tr>
<td>Freckles</td>
<td>0.16</td>
</tr>
<tr>
<td>Bleeding bump</td>
<td>0.016</td>
</tr>
<tr>
<td>Red hair</td>
<td>0.9</td>
</tr>
<tr>
<td>Tendency to sunburn</td>
<td>0.5</td>
</tr>
<tr>
<td>Sensitivity to light</td>
<td>2.0</td>
</tr>
<tr>
<td>Sun exposure</td>
<td>2.8</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>2.4</td>
</tr>
<tr>
<td>History of non-MM skin cancer</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Numerous studies from different parts of the world have shown that the relative risk for developing melanoma increases as the number of dysplastic nevi increase.
Atypical/dysplastic nevi in children may have particular significance as well.

Dysplastic/atypical nevi may also be precursor lesion for melanoma...

- 20-80% of melanoma arises within a precursor nevus
- ? premalignant condition (controversial)
- Alternative explanation:
  - we are simply bad at distinguishing atypical nevi from melanoma

So what happens, after a biopsy has been performed, to make a diagnosis of MELANOMA
Atypical Nevus – Architectural

- Shoulder

Atypical Nevus - Architectural

- Bizarre nest size and arrangement

Atypical Nevus - Architecture

- Lymphocytes
- Melanophages
- Fibrosis

Atypical Nevus – Cytology

- Clear cytologic atypia
  - large cells
  - pleomorphic nuclei

Atypical Nevus Cytology

- Hyperchromasia
- Thick nuclear membrane
- Pleomorphism
- Large nucleolus

Atypical Nevus - Cytology

- Poor Vertical Maturation
Atypical Nevus - Cytology

Atypical Nevus - Architecture

Pagetoid extent

Regional Approaches to Atypical Nevi

- Grading
  - usually "mild," "moderate," "severe"
  - based on cytology or architecture or both

- No Grading ("allegedly")
  - nevus, Clark’s nevus, Clark’s nevus – excise

“Everybody’s a critic…”

How good is grading?

- Duncan et al. (1993)
  - 10 cases of nevi, dysplastic nevi (mild, mod, sev) and melanoma
  - concordance 69-80% for nevus vs dysplastic nevus vs melanoma
  - only 35-58% concordance for grading of dysplasia

- Piepkorn, et al. (1994)
  - 149 atypical nevi graded by 6 expert dermatopathologists
  - re-interpreted 6 mos later by same dermatopathologist
  - often did not “agree” with themselves ("moderate correlation")

- Farmer, et al. (1996)
  - 37 pigmented lesions shared among 8 expert dermpaths
  - only “complete” agreement on benign vs. malignant in 1/3 of cases

Dermatopathology Eight-Ball Says…

Interesting Trends

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

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

What factors complicate the histologic diagnosis of melanoma?

Representative Sampling

SIMPLE RULES TEND TO BREAK-DOWN:
“Always biopsy the thickest part of the lesion.”

4 mm Punch Biopsy by Volume
- Assume 4 mm cylinder
  Volume of punch is = 50.3 mm³
- Assume is two 3.5 um ‘silhouettes’ on slide
  “Volume” inspected is = 0.112 mm³
  The dermatopathologist is inspecting 1/450th of the overall volume of the sampling!!!!

So, what can be done to figure out difficult cases…
Immunostains in Pigmented Lesions - Bottom Line

There is NO single “Melanoma Stain.”

**Melan-A/MART-1**
- Melanocytic-associated Antigen (A103)
- Melanoma Antigen Recognized by T cells-1 (M2C107)
- Highly specific, just not as sensitive (particularly for desmoplastic MM)

Is there a pigmented lesion here?

Mart1 stains melanocytes and allows you to see that there are too many present.

**HMB-45**
- Benign melanocytic lesions:
  - junctional/superficial component stain
  - normal zonation in the deeper dermis
- Confusing in situations of dusty cells:
  - deeply pigmented nevi
  - deep penetrating nevi
  - clonal nevi

Mart1 stains melanocytes and allows you to see that there are too many present.
P16

- A tumor suppressor protein that prevents cells from dividing inappropriately
- P16 expression is good, and the pattern of expression is important:
  - loss of nuclear staining (<25% of cells)
    - 3 fold more likely to be melanoma
  - loss of nuclear and cytoplasmic staining
    - 8 fold more likely to be melanoma

Combination stains…

D2-40/MART-1 (“DuMart”)

“Therapeutic” Stain
(possibly even diagnostic)
BRAF (VE1)

Use of Stains in Action

Kimart (Ki67/Mart)
By the way…

The patient this patient is a 14 year old boy!

Comparative Genomic Hybridization

• Shows gains or losses in copy # of genes in cells
• Gains or losses are often observed in melanoma
• Benign lesions don’t often have gains or losses
• Takes weeks, costs $2000


FISH for Gains/Deletions

• Arose from CGH
• Can only look at a limited number of loci (4 or 5)
• Argued how well it works in children
• Takes days to weeks, costs $1800

Gene Expression Profiling

• qRT-PCR on FFPE that is macrodissected
• Developed and validated on N=400+ nevi/MM

• The final gene signature consists of 23 genes (Figure 3).
  - Component #1 regulates melanocyte differentiation
  - Component #2 is a group of 5 genes that have multiple functions including some immune regulation
  - Component #3 represents 8 genes involved in immune signaling
  - Housekeeper genes are necessary for normalization of gene expression
• Validation study of N=437
• Reported Sensitivity = 94%, Specificity = 90%
• 9% of lesions classified as “Indeterminate”

Spitz Nevus
• Originally termed “juvenile melanoma”
• Dr. Sophie Spitz reported these in 1948
  – patients were all young
  – the vast majority did quite well
• Posses a problem under microscope for those with lesser experience

Clinical Spitz Nevus

Classic Spitz Nevus

Tale of the “Spitz tumor”...
• 17 year-old girl seen in 2007
• Eruptive lesion on leg
  – only noticed for a “few weeks”
• No personal or FH of melanoma
• Otherwise healthy
No win situation.

- **Overcall** and a 17-year-old receives:
  - huge disfiguring scar
  - ruined insurance status
  - chronic leg edema
  - no real hope

- **Undercall** and there are:
  - medicolegal issues for doc
  - is the patient harmed?

Her Sentinel Lymph Node
Can we always reliably distinguish these so-called “atypical Spitz tumors” from melanoma?

No.

Pithy Quotes from Major Papers (2014-15)

- “Retrospective review at a large tertiary institution… demonstrated that 13% of melanocytic lesions defied diagnosis.”
- “Histopathologic findings and be ambiguous… no diagnostic ‘gold-standard’ for Spitz-like lesions has been established.”
- “Accurate classification… of some subsets of melanocytic neoplasms remains a challenge, even for those with the most expertise.”

Demarchis et al. 2014

“FISH may serve as a helpful adjunct in the classification of controversial melanocytic tumors in young patients.”

Massi et al. 2015

Classic FISH was not of real utility
FISH modified to detect homozygous loss of 9p21 was significant
Perhaps [FISH is] not appropriate for the differential diagnosis of spitzoid tumors in children.