Contemporary Diagnosis of Hydatidiform Mole

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The presenters have no conflicts of interest to disclose

Gestational Trophoblastic Disease

- Hydatidiform mole
- Invasive mole
- Choriocarcinoma
- Placental site trophoblastic tumor (PSTT)
- Epithelioid trophoblastic tumor (ET)
Specific Diagnosis of Gestations With Hydropic Villi Important

- Need to differentiate among
  - Complete hydatidiform mole
  - Partial hydatidiform mole
  - Hydropic abortion
- Different diseases
- Different prognosis
- Different management

What is adequate study of a POC Specimen?

- TAB
  - Probably reasonable to sign out on one slide with normal villi
- Spontaneous/missed abortion
  - Must have a good view of villous morphology
  - Suggest at least 2 slides
  - Lage found that it could require as many as 10 (!) slides to diagnose PM

Complete Mole

- Diploid
- Androgenetic – all chromosomes are paternal *
- Loss of maternally expressed imprinted transcripts, gain of paternally expressed imprinted transcripts,
- 75-85% monospermic (46, XX)
- 15-25% dispermic (46, XX or 46, XY)
- Rare diploid biparental CHM *
Complete Hydatiform Mole
Scenario 1
“Homozygous” (80-90%)

23X

46XX

46XpXp

46XX

Complete Mole
Scenario 2
“Heterozygous” (10-20%)

23X or 23Y

23X or 23Y

23X or 23Y

More likely to develop postmolar trophoblastic neoplasms

Complete Mole
Clinical Presentation

- Past: Most diagnosed 16-17 weeks
  - Abnormal bleeding, high hCG, uterine enlargement > normal for gestational age, theca-lutein cysts, preeclampsia, hyperemesis, hyperthyroidism, respiratory distress, classic sonogram
- Present: Most diagnosed < 12 weeks
  - Abnormal bleeding
  - Elevated serum hCG
  - Abnormal sonogram
Ultrasound Diagnosis of Mole

- Advanced cases produce characteristic vesicular pattern
- In early cases cannot differentiate between complete mole and degenerating villi of abortion
- Cystic villi in placenta and increased transverse diameter of gestational sac predict partial mole
- In one recent study only 34% of moles suspected on ultrasound (58% complete, 17% partial)

Complete Mole Ultrasound:
Solid collection of echoes with numerous small anechoic spaces

- [Image of ultrasound findings]

- [Image of tissue samples]
Traditional Complete Mole Histology

- No fetal tissue
- All villi abnormal
- Rounded villous contours
- Marked hydropic swelling
- Central cisterns common
- Vessels rare, no fetal RBC
- Circumferential trophoblastic proliferation (CT, IT, ST)
Occasionally, the exuberance of the proliferating trophoblast taunts the pathologist into diagnosing choriocarcinoma. No matter how atypical the trophoblast is, nor how extensive the trophoblast hyperplasia, the presence of villous tissue, by definition, precludes a diagnosis of choriocarcinoma in a first or second-trimester placenta.

Janice Lage, M.D.
“Gestational Trophoblastic Diseases”
in
Robboy, Anderson, and Russell, Pathology of the Female Genital Tract
Early Complete Mole

- Lack uniform hydropic change and circumferential trophoblastic hyperplasia.
- Large bulbous or lobulated villi.
- Basophilic, colloidal iron + villous stroma
- Hypercellular villous stroma.
- Karyorrhectic debris in villous stroma
- Immature labyrinthine stromal vessels.
- Focal trophoblastic hyperplasia.
- Atypical IT in the implantation site.

Early Complete Hydatidiform Mole
Trophoblastic Hyperplasia in Early Complete Hydatidiform Mole

Atypical IT in complete mole implantation site
Diploid Biparental Complete Mole

- Very rare
- Usually diagnosed when:
  - A patient has recurrent CHM
  - There is a familial tendency for CHM
- Autosomal recessive inheritance
- Mutations in a maternal gene involved in setting imprints in the ovum
  - 80% in NLRP7, a maternal gene on chromosome 19q
  - 5% in C6orf221
  - 15% unknown

Partial Mole and Triploidy

- 64 of 832 specimens triploid (~8%)
- 2/3 are diandric
  - Half, or 1/3 total were partial moles
  - Most of the remainder showed some but not all features of a partial mole.
- 1/3 are digynic
  - None suspicious for partial mole

Partial Mole

Clinical Findings

- Most present with vaginal bleeding
- Clinical diagnosis missed or incomplete abortion
- hCG titers rarely > 100,000mIU/ml
- Advanced complete mole type symptoms rare (preeclampsia, excessive uterine enlargement, theca-lutein cysts, hyperemesis, hyperthyroidism)

Human Pathol 29:505-511, 1998
Partial Hydatiform Mole, Scenario 1: Dismespermic – Heterozygous (Most Common – 90%)

23X or 23Y

69, XnXpXp
69, XnXpYp
69, XnYpYp

Partial Hydatiform Mole, Scenario 2: Monoospermic – Homozygous 10%

23X

69, XnXpYp

46 XY

one sperm fertilizes the egg, followed by reduplication of the paternal chromosome set due to failure of meiosis I or II
Partial Mole

Histologic Features

- Two populations of villi
  - Large hydropic villi with scalloped outlines
  - Normal to small fibrotic villi
- Cisterns less conspicuous than in CM
- Villi have inclusions
- Trophoblastic proliferation moderate
- Fetal parts or amnion may be present
- Vessels and nucleated RBC may be present
Invasive Mole

- Persistent mole in which villi are:
  - Mainly in endometrium with early invasion of myometrium (“accreta pattern”)
  - Within myometrium, especially in blood vessels
  - Penetrate to serosa
  - Spread beyond uterus (vagina, lungs)

Invasive Mole

Courtesy Michael Wells, MD

Courtesy Kyu –Rae Kim, MD
How well do pathologists diagnose hydatidiform mole?

- Conran et al (AFIP, 1993)
  - Poor agreement with histology alone
  - Gross pathology data did not markedly improve concordance
  - Good agreement when flow cytometric data available
- Crisp et al (Sheffield, 2003)
  - 40 cases, 2 revised on histology and 6 with special studies (20%)
- Fukunaga et al (5 placental pathology experts, 2005)
  - Agreement of 4 or 5 in 30/50 cases – PHM vs HA main problem
  - With ploidy data agree in 39/50 cases – still problems with some cases of CHM vs HA
- Vang et al (2012)
  - 80% accuracy for CHM with H&E, 96% with p57
  - 78% accuracy for PHM
Ancillary Studies in the Diagnosis of Hydatiform Mole

- Cytogenetics
- FISH
- Flow cytometry
- Immunohistochemistry
- Molecular genotyping

Flow cytometry: Is it triploid or not?

- Complete mole or hydropic abortion
- Partial mole
- Complete mole, or, rarely, hydropic abortion

p57\textsuperscript{kip2}

- CDKN1C Gene - Cyclin dependent kinase inhibitor
- Located on 11p15.5
- Paternally imprinted – expressed from maternal chromosome only
- PHM and hydropic abortion – maternal present
  - Positive in cytotrophoblastic and stromal cells, in villous and extravillous intermediate trophoblastic cells and decidua
  - Negative in syncytiotrophoblastic cells
- CHM – maternal absent
  - Negative in cytotrophoblastic, syncytiotrophoblastic, and stromal cells
  - Positive in villous and extravillous intermediate trophoblastic cells and decidua
Gestational Trophoblastic Neoplasia

- In most patients treated for a mole, hCG returns to baseline within 60 days
- GTN is persistent or recurrent trophoblastic disease after treatment of a mole
  - 15-20 % after complete mole
  - 0.2-4 % after partial mole
- Most likely diagnoses are persistent mole, invasive mole or choriocarcinoma
- The clinician will treat the HCG titer and clinical features, not the diagnosis; a biopsy may not even be performed
Prognostic Scoring System for GTN

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>≤40</td>
<td>&gt;40</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antecedent</strong></td>
<td>Mole</td>
<td>Abortion</td>
<td>Term</td>
<td></td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
<td>Interval</td>
<td>&lt;4</td>
<td>4-6 m</td>
<td>7-12m</td>
</tr>
<tr>
<td><strong>hCG (IU/L)</strong></td>
<td>&lt;10^2</td>
<td>10^2-10^4</td>
<td>10^4-10^6</td>
<td>&gt;10^6</td>
</tr>
<tr>
<td><strong>Largest</strong></td>
<td>&lt;3 cm</td>
<td>3-5 cm</td>
<td>&gt;5 cm</td>
<td></td>
</tr>
<tr>
<td><strong>tumor</strong></td>
<td>Spleen, kidney</td>
<td>GI tract</td>
<td>Brain, liver</td>
<td></td>
</tr>
<tr>
<td><strong>Metastatic</strong></td>
<td>1-4</td>
<td>4-8</td>
<td>&gt;8</td>
<td></td>
</tr>
<tr>
<td><strong>sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. Mets</strong></td>
<td>Single drug</td>
<td>Multidrug</td>
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≤6 = low risk; ≥7 = high risk

Staging of Gestational Trophoblastic Neoplasia

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>Confined to the uterus</td>
</tr>
<tr>
<td>II</td>
<td>Extends to other genital structures (ovary, tube, vagina, ligaments)</td>
</tr>
<tr>
<td>III</td>
<td>Lung metastasis</td>
</tr>
<tr>
<td>IV</td>
<td>All other distant metastases</td>
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Substages:
A = low risk
B = high risk

Is There a Way to Further Increase Diagnostic Accuracy?

Molecular Genotyping for Accurate Diagnosis of Hydatidiform Moles
DNA contains microsatellites or short tandem repeats (STRs)

- Short tandem repeats with repeating unit of 2-6 base pairs
- Distributed throughout the genome
- Non-coding DNA
- Heritable and stable
- Number of repeats is polymorphic (many different possible alleles at each locus)

STR genotyping has many applications

- DNA fingerprinting for forensic cases
- Paternity testing
- Specimen Identity
- Can apply this technology for the diagnosis of hydatidiform moles
Each individual inherits 2 alleles at every STR locus

- The maternal and paternal alleles can sometimes be the same

AmpFISTR Identifier Kit (Applied Biosystems)

- Single multiplex PCR for 15 STR loci and the Amelogenin locus for sex determination.

Any normal individual should have 2 alleles at each locus
How does this work for molar pregnancy?

Extract DNA from Villi (FETUS)
Extract DNA from Endometrium (MOM)

Normal or Hydropic Gestation

Monospermic Complete Mole
Dispermic Partial Mole

Digynic Triploid (Non-molar)

Benefits of molecular genotyping

- Can determine parental origin of DNA which increases diagnostic accuracy (ex. Digynic triploidy)
- Can determine monospermic versus dispermic status of moles which has been reported to show different risk of GTD
- Can identify trisomies, which accounts for ~1/3 of hydropic abortuses
- Faster turn-around time than DNA ploidy send-out (1-2 days vs. 4-5 days)
- Cost-effective
Limitations of molecular genotyping

- Cannot identify familial cases of complete mole (biparental diploid). Thought to result from mutations in the NLRP7 gene on chromosome 19. Use p57 to diagnose, confirm biparental nature with genotyping.

- Cases from Donor Eggs present a diagnostic pitfall. Know clinical history.

### Possible Hydatidiform Mole

- **p57 immunohistochemistry**
  - Morphology c/w Complete Mole? No → Molecular Genotyping
  - Morphology c/w Complete Mole? Yes → Diandric Diploid, Diandric Triploid, Digenic Triploid, Biparental Diploid +/- Trisomies

### Practice Cases

3 Cases
Answers and Discussions follow the cases.
No cheating!
Case 1
What is your diagnosis?
Endometrium

Case 2
What is your diagnosis?
Endometrium

Case 3
What is your diagnosis?
Endometrium
Quiz Cases 1-3
Answers and Explanations

Case 1
Answer and Explanation

• The correct diagnosis is a dispermic complete hydatidiform mole.

• The D8S1179, D21S11, and CSF1PO STR loci all show paternal-only alleles that are not present in the endometrium. Furthermore, the D8S1179 and CSF1PO STR loci both show 2 different paternal alleles indicating dispermy.

• The D7S820 STR locus is non-informative.

Case 2
Answer and Explanation

• The correct diagnosis is a monospermic complete hydatidiform mole.

• The D19S433, vWA, and TPOX STR loci all show paternal-only alleles that are not present in the endometrium. All 4 STR loci show a homozygous single allele indicating monospermy.

• The D18S51 STR locus is non-informative.
Case 3
Answer and Explanation

- The correct diagnosis is a monospermic partial hydatidiform mole.
- The paternal allele at each STR locus is roughly twice the height of the maternal allele, indicating a triploid genotype with 2 homozygous alleles coming from one sperm.