Hydatidiform Moles: Ancillary Techniques Improve Morphologic Diagnosis

Brigitte M. Ronnett, MD
Department of Pathology
The Johns Hopkins University School of Medicine
Baltimore, MD

The Question

“Does accurate diagnose of hydatidiform moles in routine practice require ancillary techniques, such as an immunostain (p57) and/or DNA/molecular analysis, or just a good H&E and some experience?”

The Modern POC* Specimen and Clinical Diagnostic Expectation

*POC: products of conception

POC specimen (usually 1st trimester)

Hydatidiform mole

Non-molar abortus

Complete hydatidiform mole (CHM)

Partial hydatidiform mole (PHM)

Classification/Differential Diagnosis

• Hydatidiform mole
  • Complete hydatidiform mole (CHM)
    • Early complete hydatidiform mole (eCHM)
  • Partial hydatidiform mole (PHM)
• Non-molar (NM) specimens capable of simulating hydatidiform moles
  • Hydropic abortus (HA)
  • Early abortus (EA) with trophoblastic hyperplasia
  • Abnormal villous morphology (AVM) related to other genetic abnormalities (e.g., trisomy)
  • Androgenetic/biparental mosaic/chimeric conceptions (MOS/CHI), some with molar component

Diagnosis of Molar and Non-Molar Specimens

• Morphologic criteria are imperfect
• Overlap in microscopic features
• Significant inter- and intra-observer variability, even for experienced GYN pathologists

Value of Refined Diagnosis of Molar Specimens

• Identify biologically distinct entities with different risks of persistent gestational trophoblastic disease (GTD):
  • ~15-20% for CHM versus <5% for PHM
    – Choriocarcinoma, placental site trophoblastic tumor, and metastatic GTD can occur with PHM
• Guide clinical management
  • Contraception and hCG levels for molar pregnancy (CHM & PHM) but not for non-molar abortus
    – Implications for patients with infertility
Diagnostic Tools for Evaluating POC Specimens

- H&E stained slide: a great tool with limitations
  - Can slides be reliably read by any board certified pathologist or only "experienced" pathologists?
  - Is it clinically acceptable to expect accuracy only from experienced pathologists?
  - What defines experience?
  - Is experience ever validated??
  - What is the performance of experienced (gynecologic) versus junior pathologists measured against diagnostic truth?

- p57 immunohistochemistry (IHC)
  - Can be routinely performed in laboratories with IHC capability
  - Can be reproducibly interpreted (k=0.9)

- Molecular genotyping
  - Establishes diagnostic truth (discerns distinct genetics of CHMs, PHMs, and NMs)
  - Requires specialized laboratory and skilled interpretation
  - Adds expense to the diagnostic evaluation

H&E stained slide: a great tool with limitations

Which cases should be tested?

Diagnostic approach

- H&E + p57
- H&E + p57 +/- Genotyping (triage by p57)
- H&E + Genotyping

Non-molar Specimen

Biparental diploidy
(1 paternal and 1 maternal chromosome complements)

Non-molar Specimen: Biparental Diploidy

Positive in nuclei of villous stromal cells, cytotrophoblast, and intermediate trophoblast

Complete Hydatidiform Mole (CHM)

Androgenetic diploidy (~85% monospermic/homozygous)
(2 paternal and 0 maternal chromosome complements)
Complete Hydatidiform Mole: Androgenetic Diploidy

- RNA → p57 protein
- p57 protein is paternally imprinted, maternally expressed
- CH_3
- CH_3
- CH_3

No maternal DNA

Paternal Chr 11 (2 copies)

Negative in villous stromal cells and cytotrophoblast (intermediate trophoblastic cells +)

Partial Hydatidiform Mole (PHM)

- CH_3
- CH_3
- CH_3

Diandric triploidy (~90% dispermic/heterozygous) (2 paternal and 1 maternal chromosome complements)

Partial Hydatidiform Mole: Diandric Triploidy

- RNA → p57 protein
- p57 protein is paternally imprinted, maternally expressed
- CH_3
- CH_3
- CH_3

Maternal Chr 11

Positive in nuclei of villous stromal cells, cytotrophoblast, and intermediate trophoblast

Diagnostic Reproducibility of Hydatidiform Moles: Performance of Gynecologic Pathologists and Fellows Measured Against Diagnostic Truth Determined with Ancillary Techniques (p57 IHC + genotyping)

- 80 genotyped cases (1 slide per case) selected from a series of 200 potentially molar POC specimens
- Case distribution (per genotyping diagnosis):
  - 27 CHMs, 27 PHMs, 26 NMs
- 3 diagnostic rounds without any training sessions:
  - 1st & 2nd = H&E, 3rd = H&E + p57
- Pathologists masked to p57 results (rounds 1 & 2) and to genotyping results (all rounds)
  - Faculty: 5 to >30 years experience
  - Fellows: completed AP/CP training

% Correct Classification (sensitivity)

Genotyping-confirmed Complete Hydatidiform Moles

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<tr>
<th>Sensitivity</th>
<th>Fac1</th>
<th>Fac2</th>
<th>Fac3</th>
<th>Round 1</th>
<th>Fel4</th>
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% Correct Classification (sensitivity)

Genotyping-confirmed Partial Hydatidiform Moles

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p = 0.13
p < 0.01
p < 0.01
% Correct Classification (sensitivity)
Genotyping-confirmed Non-molar Specimens

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<tr>
<th>Diagnosis category</th>
<th>Round 1</th>
<th>Round 2</th>
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<td>CHM (moderate)</td>
<td>0.59</td>
<td>0.46</td>
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<td>PHM (poor)</td>
<td>0.15</td>
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<td>NM (poor)</td>
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Inter-observer Agreement (kappa)

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<td>0.44 (moderate)</td>
<td>0.66 (good)</td>
</tr>
<tr>
<td>Fellow</td>
<td>0.67 (good)</td>
<td>0.63 (moderate)</td>
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Intra-observer Agreement*
(round 1 versus round 2)

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<th>Pathologist</th>
<th>Kappa (interpretation)</th>
<th>Agreement</th>
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<td>Faculty 1</td>
<td>0.44 (moderate)</td>
<td>64%</td>
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<td>Faculty 2</td>
<td>0.66 (good)</td>
<td>79%</td>
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<td>Faculty 3</td>
<td>0.67 (good)</td>
<td>79%</td>
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<td>Fellow 1</td>
<td>0.53 (moderate)</td>
<td>69%</td>
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<td>Fellow 2</td>
<td>0.63 (good)</td>
<td>75%</td>
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<td>Fellow 3</td>
<td>0.37 (fair)</td>
<td>59%</td>
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*Consistency does not guarantee achieving diagnostic truth

Case 1
34 year old;
“R/O partial mole”
Case 2

35 year old;
Estimated gestational age = 6-7 weeks;
β-HCG = 105,599 mIU/mL;
Ultrasound: “multicystic mass filling endometrium, possible small abnormal gestational sac, suspicious for hydatidiform mole—could be partial”
FacR1: NM/CHM/PHM; FelR1: CHM/CHM/CHM
FacR2: NM/CHM/NM; FelR2: CHM/CHM/CHM
FacR3: CHM/CHM/CHM, p57- (3/3); FelR3: CHM/CHM/CHM, p57- (3/3)

Molecular Genotyping: Androgenetic Diploidy = CHM

Case 3
49 year old;
Incomplete abortion
Case 4
19 year old;
Recurrent missed abortions

Molecular Genotyping: Androgenetic Diploidy + CHM
Molecular Genotyping: Diandric Triploidy = PHM

FacR1: NM/PHM/NM;   FelR1: NM/NM/NM
FacR2: NM/NM/NM;   FelR2: NM/NM/NM
FacR3: NM/PHM/NM, p57+ (3/3);   FelR3: PHM/NM/NM, p57+ (3/3)

Case 5
41 year old;
Rising quantitative β-HCG;
Ultrasound: no evidence of fetal heart tones or definite fetal pole;
R/O molar pregnancy

Conclusions
- Gynecologic pathologists, regardless of experience, correctly diagnose molar and non-molar specimens by morphology in ~50-75% of cases
  - Distinction of PHMs and NMs remains problematic
  - Failure to recognize all CHMs persists
- p57 immunostaining improves recognition of CHMs (~100%) and is highly reproducibly interpreted
  - No impact on distinction of PHMs and NMs
- Genotyping provides a definitive diagnosis for the ~25-50% of cases that are misclassified by morphology and the 20-30% that are unresolved by p57 immunostaining
Algorithmic Approach to Diagnosis of Hydatidiform Moles

Possible Hydatidiform Mole
  p57 immunohistochemistry
    p57 negative (villous stroma, cytotrophoblast)
      Androgenetic diploidy
        Complete hydatidiform mole
      Diandric triploidy
        Partial hydatidiform mole
    p57 positive (villous stroma, cytotrophoblast)
      Biparental diploidy
        Non-molar

Correct classification focused on both risk of persistent GTD and refined management (abbreviated follow-up of PHM versus CHM, correct approach for infertility patients)

CHM  PHM  NM
  H&E + p57  H&E + Genotyping

Correct classification focused on practical identification of the entity with the greatest risk of persistent GTD

CHM  PHM & NM
  H&E + p57

Diagnostic approach

H&E  H&E + p57
  H&E + Genotyping
  +/- Genotyping
    (triage by p57)

Testing threshold: Any clinical or pathologic suspicion of a mole