Hydatidiform Moles: Ancillary Techniques Improve Morphologic Diagnosis

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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

Complete hydatidiform mole (CHM)

Partial hydatidiform mole (PHM)

Non-molar abortus
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?
Diagnostic Tools for Evaluating POC Specimens

• 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
Diagnostic approach

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

Which cases should be tested?
Non-molar Specimen

Biparental diploidy
(1 paternal and 1 maternal chromosome complements)
Non-molar Specimen: Biparental Diploidy

- p57 protein
- RNA
- p57: paternally imprinted, maternally expressed
- Maternal Chr 11
- Paternal Chr 11
- 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

Paternal Chr 11 (2 copies)

p57 protein

RNA

IHC

p57: paternally imprinted, maternally expressed

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

No maternal DNA
Partial Hydatidiform Mole (PHM)

Diandric triploidy (~90% dispermic/heterozygous)
(2 paternal and 1 maternal chromosome complements)
Partial Hydatidiform Mole: Diandric Triploidy

Maternal Chr 11

Paternal Chr 11 (2 copies)

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

p57: paternally imprinted, maternally expressed

IHC
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 NMNs
- 3 diagnostic rounds without any training sessions:
  - 1\textsuperscript{st} & 2\textsuperscript{nd} = H&E, 3\textsuperscript{rd} = 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

Sensitivity

Fac1 Fac2 Fac3 Fel4 Fel5 Fel6
Round 1

Fac1 Fac2 Fac3 Fel4 Fel5 Fel6
Round 2

Fac1 Fac2 Fac3 Fel4 Fel5 Fel6
Round 3

95% CI Sensitivity

p=0.13

p=0.67

p=0.54
% Correct Classification (sensitivity)
Genotyping-confirmed Partial Hydatidiform Moles

Fac1 Fac2 Fac3 Fac4 Fac5 Fac6
Round 1

Fac1 Fac2 Fac3 Fac4 Fac5 Fac6
Round 2

Fac1 Fac2 Fac3 Fac4 Fac5 Fac6
Round 3

95% CI Sensitivity

p=0.04
p<0.01
p<0.01

Sensitivity

0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1
% Correct Classification (sensitivity)
Genotyping-confirmed Non-molar Specimens

![Graph showing sensitivity with 95% CI and p-values](image)
% Correct Classification (sensitivity)
All Molar and Non-molar Specimens

![Graph showing the percentage of correct classification (sensitivity) for all molar and non-molar specimens. The graph includes data points for Fac1, Fac2, Fac3, Round 1, Round 2, and Round 3. The sensitivity values range from 51% to 78%, with 95% CI and p-values indicated for each condition. The p-values are 0.69, <0.01, and 0.15 for Round 1, Round 2, and Round 3, respectively.](#)
## Inter-observer Agreement (kappa)

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Round 1</th>
<th>Round 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Faculty</td>
<td>Fellow</td>
</tr>
<tr>
<td>CHM</td>
<td>0.59    (moderate)</td>
<td>0.46    (moderate)</td>
</tr>
<tr>
<td>PHM</td>
<td>0.15    (poor)</td>
<td>0.23    (fair)</td>
</tr>
<tr>
<td>NM</td>
<td>0.13    (poor)</td>
<td>0.16    (poor)</td>
</tr>
</tbody>
</table>

Round 3 p57 interpretation: 0.96/0.93 (almost perfect)
## Intra-observer Agreement*
(round 1 versus round 2)

<table>
<thead>
<tr>
<th>Pathologist</th>
<th>Kappa (interpretation)</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faculty 1</td>
<td>0.44 (moderate)</td>
<td>64%</td>
</tr>
<tr>
<td>Faculty 2</td>
<td>0.66 (good)</td>
<td>79%</td>
</tr>
<tr>
<td>Faculty 3</td>
<td>0.67 (good)</td>
<td>79%</td>
</tr>
<tr>
<td>Fellow 1</td>
<td>0.53 (moderate)</td>
<td>69%</td>
</tr>
<tr>
<td>Fellow 2</td>
<td>0.63 (good)</td>
<td>75%</td>
</tr>
<tr>
<td>Fellow 3</td>
<td>0.37 (fair)</td>
<td>59%</td>
</tr>
</tbody>
</table>

*Consistency does not guarantee achieving diagnostic truth
Case 1

34 year old;
“R/O partial mole”
FacR1: CHM/PHM/PHM;  FelR1: CHM/PHM/NM
FacR2: PHM/CHM/PHM;  FelR2: CHM/PHM/CHM
FacR3: CHM/CHM/CHM, p57- (3/3);  FelR3: CHM/CHM/CHM, p57- (3/3)

Molecular Genotyping: Androgenetic Diploidy = CHM

p57-
Case 2

35 year old;
Estimated gestational age = 6-7 weeks;
$\beta$-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
FacR1: PHM/CHM/NM; FelR1: CHM/CHM/CHM
FacR2: PHM/CHM/NM; FelR2: CHM/CHM/NM
FacR3: CHM/CHM/NM, p57- (3/3); FelR3: CHM/NM/CHM, p57-/f+/-

Molecular Genotyping: Androgenetic Diploidy = CHM
Case 4

19 year old;
Recurrent missed abortions
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
Diagnostic approach

Testing threshold: Any clinical or pathologic suspicion of a mole

H&E

H&E + p57

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

H&E + Genotyping
Correct classification focused on practical identification of the entity with the greatest risk of persistent GTD
Correct classification focused on both risk of persistent GTD and refined management (abbreviated follow-up of PHM versus CHM, correct approach for infertility patients)
Algorithmic Approach to Diagnosis of Hydatidiform Moles

Possible Hydatidiform Mole

p57 immunohistochemistry

p57 negative (villous stroma, cytotrophoblast)

Molecular genotyping

Androgenetic diploidy

Complete hydatidiform mole

Diandric triploidy

Partial hydatidiform mole

Biparental diploidy

Non-molar