

# **Diagnosis and Subclassification of Hydatidiform Moles Utilizing p57 Immunohistochemistry and Molecular Genotyping: Validation and Prospective Analysis in Routine and Consultation Practice Settings with Development of an Algorithmic Approach**

Thomas G. McConnell, M.D.<sup>1</sup>, Kathleen M. Murphy, Ph.D.<sup>1</sup>, Michael Hafez<sup>1</sup>, Russell Vang, M.D.<sup>1,2</sup>, Brigitte M. Ronnett, M.D.<sup>1,2</sup>

Departments of Pathology<sup>1</sup> and Gynecology & Obstetrics<sup>2</sup>, The Johns Hopkins University School of Medicine and Hospital, Baltimore, MD

## **Abstract**

Distinction of hydatidiform moles (HM) from non-molar specimens and sub-classification of HMs as complete hydatidiform mole (CHM), partial hydatidiform mole (PHM), or early CHM (eCHM) are important for clinical practice and investigational studies but diagnosis based solely on morphology suffers from poor interobserver reproducibility. Recent studies have demonstrated the utility of p57 immunostaining and molecular genotyping for improving diagnosis of HMs. After performing a validation study of both techniques on 24 archival products of conception specimens (7 CHM, 8 PHM, 9 non-molar), we prospectively analyzed 42 cases, largely obtained from a gynecologic pathology consultation practice, for which there was any consideration of a diagnosis of HM. After satisfactory experience with prospective cases, a modified approach was adopted, with p57 immunostaining used in conjunction with morphology to triage cases for molecular genotyping. Final diagnoses for the prospective cases based on combined morphology and ancillary testing were 24 CHMs (including 7 eCHMs), 7 PHMs, and 11 non-molar specimens. P57 immunostaining, performed on all 66 cases, was negative in all CHMs, with the exception of 1 case of molecularly confirmed CHM with diffuse p57 expression, and positive in all PHMs and non-molar specimens, with the exception of 3 cases of molecularly confirmed PHMs with an equivocal extent of p57 expression. Molecular genotyping of 51 cases (24 validation, 27 prospective) yielded data consistent with p57 results in the 47 cases with unequivocal p57 expression patterns and was used to establish the diagnoses for the 4 cases with aberrant or equivocal p57 results. All 17 genotyped CHMs demonstrated androgenetic diploidy, including the CHM with retained p57 expression; this case also demonstrated trisomy of chromosome 11 (retained maternal allele), accounting for the aberrant p57 expression. The remaining 14 CHMs were diagnosed by morphology and negative p57 results alone. All 15 PHMs demonstrated diandric triploidy. All genotyped non-molar specimens demonstrated biparental diploidy. This study validates p57 immunostaining as a prospectively applicable triage assay for diagnosis of CHMs based on morphology and a negative p57 result. Molecular genotyping is validated as a method to confirm a diagnosis of CHM by demonstrating androgenetic diploidy and to resolve p57-positive cases into diandric triploid PHMs, biparental diploid non-molar specimens, and the rare CHM with aberrant p57 expression.