Accuracy of p57^{KIP2} compared with genotyping to diagnose complete hydatidiform mole: a systematic review and meta-analysis

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Background Distinguishing hydatidiform moles (HMs) from nonmolar specimens and the subclassification of HM are important because complete hydatidiform mole (CHM) is associated with an increased risk of development of gestational trophoblastic neoplasia. However, diagnosis based solely on morphology has poor inter-observer reproducibility. Recent studies have demonstrated that the use of p57^{KIP2} immunostaining improves diagnostic accuracy for CHM.

Objectives To evaluate the accuracy of p57^{KIP2} immunostaining compared with molecular genotyping for the diagnosis of CHM.

Search strategy Major databases were searched from inception to March 2017 using the terms 'hydatidiform mole', 'p57', and 'genotyping', with their variations, and the search limit for the relevant study design.

Selection criteria Any cross-sectional study, case series, case–control study, cohort study, or clinical trial that evaluated the accuracy of p57^{KIP2} immunostaining for the diagnosis of CHM compared with genotyping was included. Case reports, narrative reviews, expert opinions, and animal testing were excluded.

Data collection and analysis Extracted accuracy data were tabulated and pooled using a hierarchical bivariate random effects model.

Main results Bivariate meta-analysis produced a summary sensitivity of 0.984 (95% CI: 0.916–1.000) and specificity of 0.625 (95% CI: 0.503–0.736) with significant heterogeneity for specificity ($I^2 = 71.8$, chi-square $P = 0.029$). The pooled summary diagnostic odds ratio was 56.54 (95% CI: 11.03–289.74) with no heterogeneity ($I^2 = 0.00\%$, chi-square $P = 0.67$). The diagnostic performance of the test was high with an area under the curve of (AUC) 0.980.

Conclusions p57^{KIP2} immunostaining is accurate when diagnosing CHM. It can be used as an adjunct test in a combination algorithmic approach.

Keywords Complete hydatidiform mole, meta-analysis, molecular genotyping, p57 immunohistochemistry, systematic review.

Tweetable abstract A meta-analysis to evaluate the accuracy of p57^{KIP2} compared with genotyping to diagnose CHM.

Linked article This article is commented on by B Kaur and NJ Sebire, p. 1234 in this issue. To view this mini commentary visit https://doi.org/10.1111/1471-0528.15330

Introduction

Hydatidiform mole (HM) is an abnormal gestational condition characterised by significant hydropic enlargement and variable trophoblastic proliferation involving part or all of the chorionic villi.[1] The incidence is approximately

1–3 in 1000 pregnancies for complete hydatidiform mole (CHM) and 3 in 1000 pregnancies for partial hydatidiform mole (PHM) in North America and Europe; both conditions appear to be diagnosed more often in Asia and Latin America.[2,3] Some studies show that cases are ten times more likely in some Asian or African countries.[4–6] In Brazil, there is no official gestational trophoblastic disease registry, and this disease, despite its important morbidity and psychosocial impact, may be underestimated at 1/200–800 gestations, depending on the geographical region.[7,8]

Histopathological examination remains the basis for the diagnosis of HM; however, the diagnosis and classification of HM have become increasingly difficult because HMs are now commonly evacuated at an earlier stage and do not satisfy the well-established classic morphological features.[1,9] Previous studies have demonstrated that a diagnosis of HM based on morphology alone is subject to inter-observer variability and therefore suboptimal diagnostic reproducibility.[10,11]

Differentiating a molar pregnancy from nonmolar specimens (NMS) and the classification of HM as CHM (including early CHM), PHM, or hydropic miscarriage are important for both clinical practice and investigational studies because of the risk of development of gestational trophoblastic neoplasia (GTN), including choriocarcinoma, which is significantly higher after a pregnancy affected by CHM (15–27%) or PHM (0.5–5%) then with any other pregnancy.[10–13]

The p57[KIP2] gene on chromosome 11p15.5 encodes a strong inhibitor of several G1 cyclin/Cdk complexes and is a negative regulator of cell proliferation. This gene is paternally imprinted and maternally expressed, and the presence of its protein product serves as a surrogate marker for the nuclear maternal genome. CHM is the only type of conceptus lacking a maternal contribution and p57[KIP2] is accordingly absent, whereas it is present in CHM mimics.[12]

In developing countries, where genetic study is not widely available, the use of immunohistochemistry with p57[KIP2] evaluation may be a more affordable, less expensive, and viable alternative. The aim of this article was to assess the diagnostic accuracy of the p57[KIP2] gene in CHM diagnosis, which may be of particular value to clinicians in regions with limited resources, where molar pregnancies are more prevalent and constitute a public health problem.

Methods

Data sources and study selection
The detailed protocol of this systematic review and meta-analysis has been published previously.[14] Briefly, we have searched databases, such as EMBASE, LILACS, MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science, up to January 2017, using the following search terms: 'hydatidiform mole', 'p57', 'immunohistochemistry', and 'genotyping'. The detailed search strategy is available in Supporting Information Appendix S1. We also screened the reference lists of relevant studies and reviews for additional articles and searched the grey literature at The Grey Literature Report, OpenGrey, and the Open Archives Initiative (OAIster).

Two independent researchers evaluated the titles and abstracts arising from the combined search and independently extracted all data from the retrieved articles, such as study population and test characteristics. A third author adjudicated any discrepancies. In the case of duplicate publications or more than one publication from a preliminary study, we attempted to maximise the use of the information by simultaneously evaluating all of the available data, but we did not include the same group of patients in the analysis more than once. The data were extracted in the form of a data sheet specifically developed for this analysis.

We have included articles from cross-sectional or case series studies that evaluated the accuracy of p57[KIP2] immunostaining for the diagnosis of CHM compared with genotyping.

No restrictions were imposed regarding publication date or language. We contacted the corresponding authors of the eligible studies to request any missing or insufficient data. A sensitivity analysis was conducted to assess the impact of including studies with 20% or more nonreported data.

Methodological quality assessment
The study protocol followed the PRISMA guidelines for the reporting of systematic reviews.[15] We assessed the quality of the studies using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool.[16] Grading of Recommendations Assessment, Development, and Evaluation (GRADE) was used to rate the quality of the body of evidence retrieved in the search.[17]

Statistical analysis
To construct two-by-two tables, we extracted true-positive, false-positive, true-negative, and false-negative results or recalculated the numbers from available parameters (sensitivity, specificity, positive predictive value, and negative predictive value). The primary outcome was the diagnostic accuracy of p57[KIP2] immunostaining for the diagnosis of CHM, which was described based on sensitivity and specificity, and positive and negative likelihood ratios wherever possible. All analyses were performed using the software META-DISC version 1.4 (Unit of Clinical Biostatistics team of the Ramón y Cajal Hospital, Madrid, Spain).[18]

The diagnostic performance of p57[KIP2] was analysed to calculate the odds ratio (OR) of the likelihood of a positive result, with 95% CI, using a random bivariate model or the

HSROC model from Rutter and Gatsonis,[19] according to the presence of heterogeneity. Summary receiving operating characteristics (SROC) with confidence interval area were generated to show the joint overall sensitivity and specificity of the diagnostic test.

Ethical aspects

This study was submitted to Plataforma Brasil (an online system run by the Brazilian government) and approved on 28 August 2015 by the Institutional Review Board of Maternity School of Rio de Janeiro Federal University, as set forth in CAAE number 47952515.7.0000.5275.

Funding

Results

Of the 178 articles identified through electronic databases and an additional record identified through the reference check, the full text of 21 potentially relevant papers was evaluated for inclusion in the systematic review (Figure 1). Seven publications met the eligibility criteria and were included in the systematic review.[1,8,12,13,20–22] The basic characteristics of the included studies were study sample size ranging from 16 to 80 women with ages ranging from 13 to 55 years old (Supporting Information Appendix S3). All studies but one,[1] were performed in the USA. A majority of the studies used a prospective design performing immunohistochemistry and genotyping and were published in 2006 2014. A detailed list of excluded studies with reasons for their exclusion can be found in Supporting Information Appendix S2. The sensitivity, specificity, and negative and positive likelihood ratios are described in Table 1.

All three included studies,[1,10,21] used the same mouse monoclonal antibody (Lab Vision/Neomarkers, Fremont, CA, USA), and all but one[8] performed the same genotyping systems.

Methodological quality of included studies

Figure 2 summarises the results of the quality assessment. Four of seven studies had a high risk of bias for the sample selection due to a lack of information about the selection process; two of these were included in the meta-analysis. The majority of studies were assessed as low risk of bias for the implementation of the reference standard and all studies for the index test. The bias for flow and timing was unclear due to lack of data in six of seven studies.

The GRADE approach was used to assess the quality and strength of a recommendation regarding the use of p57[KIP2] in the diagnosis of CHM. The evidence quality had to be downgraded by at least one level to moderate the quality of evidence, primarily due to risk of bias and statistical imprecision, because of the number of patients in the included studies, the wide confidence intervals, and the statistical heterogeneity.

Systematic review

From the seven included studies, Landolsi et al.[1] state that p57[KIP2] immunostaining can be used as successfully as genotyping. The Popiolek et al.[10] study found that p57[KIP2] immunostaining accurately identified all the investigated cases of CHM and concluded that the test is a time- and cost-effective means of distinguishing CHM from its mimics in challenging cases. The McConnell et al.[13] study validated p57[KIP2] immunostaining as a triage assay for the diagnosis of CHM and the genotyping as a confirmatory assay. The Vang et al.[21] study states that p57[KIP2] immunostaining improves the sensitivity of a diagnosis of CHM in 96% from morphological diagnosis. Lewis et al.[20] refer to the importance of recognising the distinctive p57[KIP2] expression patterns and genotyping results, as this approach can prevent misclassification as typical CHMs, PHMs, or NMS. For Gupta et al.,[22] the p57[KIP2] immunostaining significantly improved recognition of CHMs and had high reproducibility; additionally, the genotyping provides a definitive diagnosis for the ∼25 to 50% of cases that are misclassified by morphology, especially those that are also unresolved by p57[KIP2] immunostaining. Finally, Banet et al.[12] found that p57[KIP2] expression is highly correlated with genotyping, serving as a reliable marker for the CHM diagnosis, and identifying androgenetic cell lines in mosaic conceptions.

Meta-analysis

From the seven selected studies for this systematic review, only three had quantitative data included in the meta-analysis (Figure 1). Three of the studies refer to the same population.[12,21] and only two of these studies[13,21] provided quantitative data, we used only the most recent data published[21] to avoid duplication. Eligible studies for the quantitative analysis included 126 pregnant women. The pooled positive likelihood ratio was 2.45 (95% CI 1.37–4.36), and the negative one was 0.05 (95% CI 0.01–0.21) with no heterogeneity (positive likelihood ratio $I^2 = 23$ with a chi-square $P = 0.27$ and negative likelihood ratio $I^2 = 0.0\%$ with a chi-square $P = 0.94$).

Bivariate meta-analysis produced a summary sensitivity of 0.984 (95% CI 0.916–1.000) and specificity of 0.625 (95% CI 0.503–0.736) with significant heterogeneity for specificity ($I^2 = 71.8$ chi-square $P = 0.029$), and a random model was used. The pooled summary diagnostic odds ratio (SDOR) was 56.54 (95% CI 11.03–289.74) with no heterogeneity ($I^2 = 0.00\%$, Cochran chi-square $P = 0.67$).
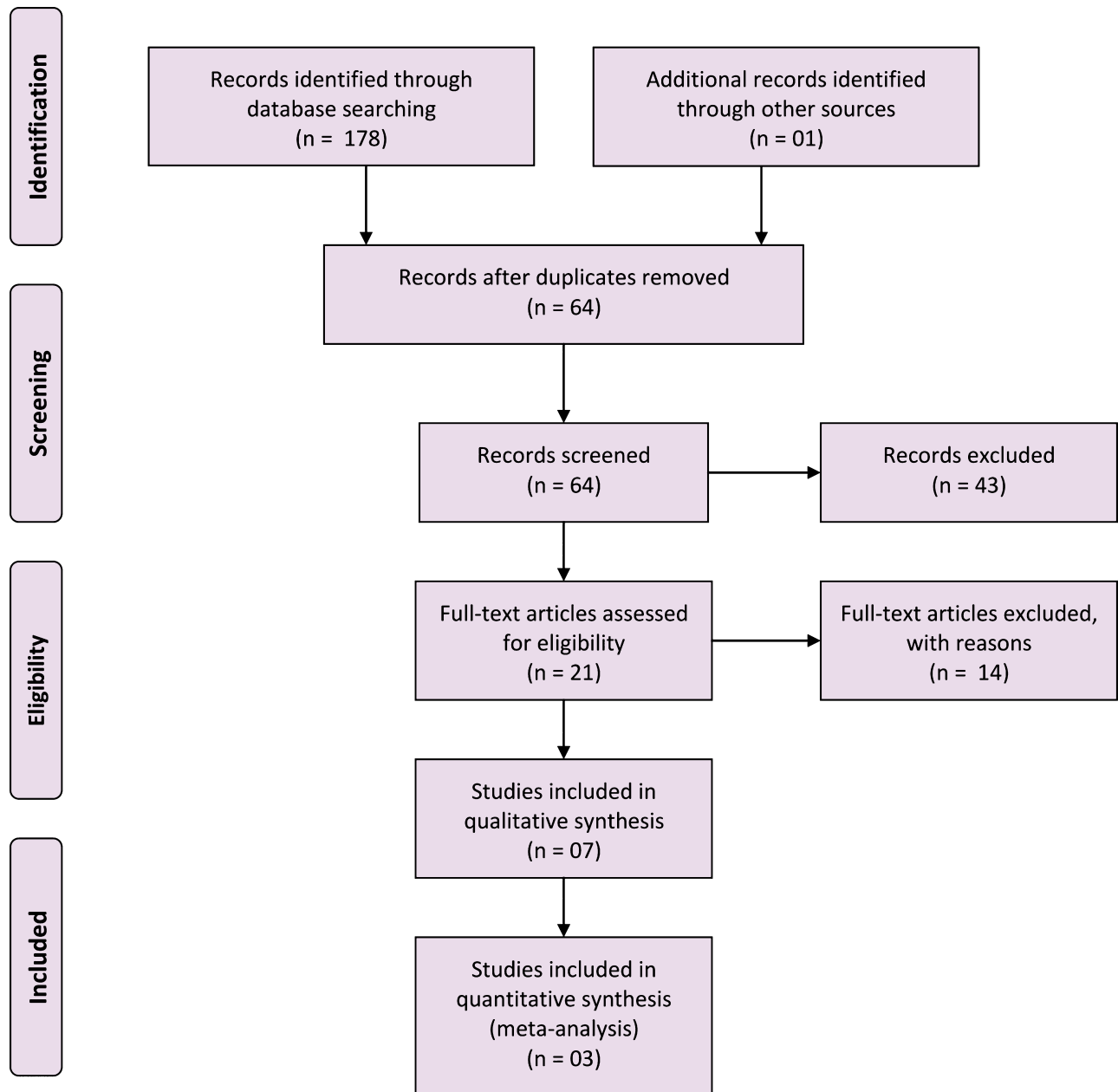
Figure 1. PRISMA flow diagram of the study selection process.

The diagnostic performance of the test was high with an AUC of 0.980 (Figure 3).

We performed a sensitivity analysis by excluding, one by one, the studies that presented zero cells, and the exclusion did not substantially change the results. The exclusion of Popiolek et al.[10] led to a pooled SDOR of 41.17 (95% CI 6.89–245.86) and by excluding Landolsi et al.,[1] we obtained an SDOR of 56.42 (95% CI 9.01–353.20). The sensitivity analysis did not improve the extremely high confidence intervals retrieved.

Discussion

Main findings

Only a small number of articles are available on this topic and include a limited number of patients. After the eligibility analysis of the retrieved studies, few studies fulfilled the criteria and even less had quantitative data to be meta-analysed. However, the selected studies consider the p57^{KIP2} an accurate and promising test for the diagnosis of CHM. The SROC showed a large AUC with

Table 1. Diagnostic properties of the included studies

First author	*n*	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Vang (2012)	80	96.0 (81–99.9)	58.7 (45.6–71.0)	2.33 (1.72–3.16)	0.06 (0.01–0.47)
Landolsi (2011)	30	100 (87.7–100)	50.0 (1.3–98.7)	1.97 (0.63–6.10)	0.03 (0.00–0.67)
Popiolek (2006)	16	100 (66.4–100)	100 (59–100)	15.2 (1.03–233.38)	0.05 (0.00–0.80)
Total	126	98 (91.6–100)	62.5 (50.3–73.6)	2.45 (1.373–4.36)	0.05 (0.01–0.21)

CI, confidence interval.
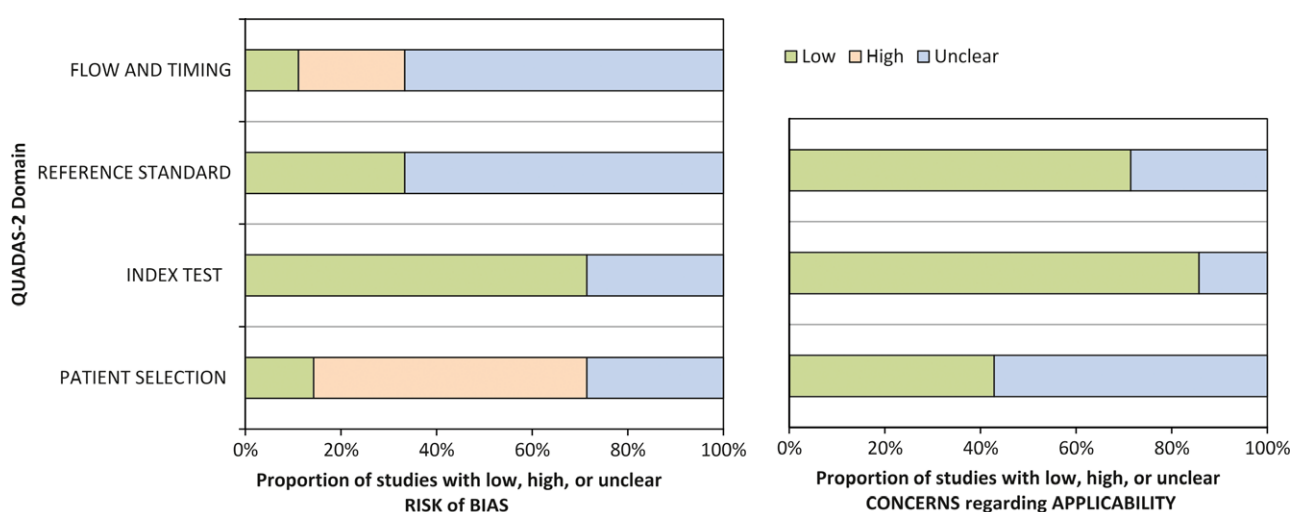
Figure 2. Methodological evaluation according to QUADAS-2 of the included studies. QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies.
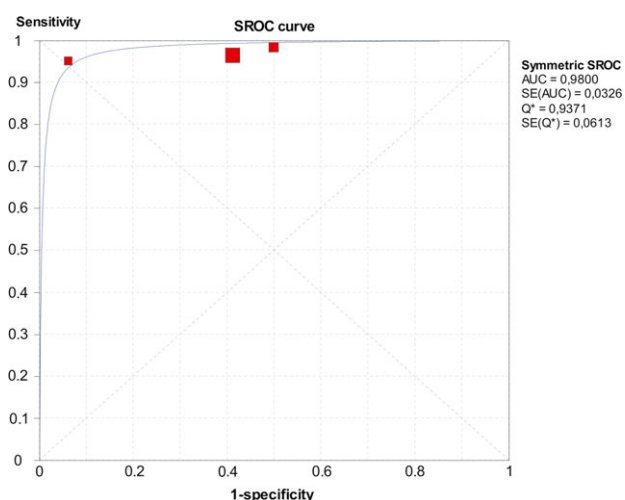
Figure 3. The summary receiver operator characteristic curve. AUC, area under the curve; SE, standard error; SROC, summary receiver operating characteristic curve.

high values of sensitivity and positive likelihood ratio. Thereby, our review considers that p57^{KIP2} is accurate to screen CHM; thus, it can be a useful tool when applied within a combined diagnostic approach in a scenario of difficult clinical cases.

Strengths and limitations

The small number of studies did not allow us to better explore and evaluate the robustness of the conclusion or the impact of article quality on the results. However, due to the large confidence intervals calculated, the interpretation of the summarised SDOR should be performed with caution. Furthermore, most of the included studies had an observational design and were performed by the same research group. As a result, evidence quality had to be downgraded using GRADE methodology, primarily due to imprecision, limiting our clinical practice recommendations.

Interpretation

Distinction of HM from NMS and the subclassification of HM as CHM versus PHM are important for both clinical practice and investigational studies. Accurate classification is critical to ascertain the actual risk of development of GTN associated with the various subtypes of HM and to

determine the appropriate nature and duration of clinical follow up.[23] As the risk of GTN after hCG normalisation in patients with PHM is negligible, it seems to be reasonable to discontinue postmolar follow up after three consecutive weekly results of hCG below 5 IU/l. Current recommendations for surveillance after hCG normalisation should be revisited, minimising lost time off from work and saving public health resources.[24]

Some individual studies[1,12] have confirmed that p57[KIP2] is a practical and accurate adjunct for the diagnosis of CHM and its mimics because this technique is a relatively simple, reliable, cost-efficient, and rapid procedure. Therefore, in some cases, the ideal method for correctly classifying HMs and NMS is a combined approach that includes the correlation of morphological features, p57[KIP2], and molecular genotyping.[12] This combined approach is particularly important when evaluating difficult and challenging cases with discordant positive p57[KIP2], when molecular techniques are still necessary and are the gold standard.[1]

Usui et al. [25] combined p57[KIP2] results with histological findings, showing a diagnostic accuracy estimated to be up to 95%. The authors conclude that p57[KIP2] can be easily applied in the routine, when necessary, thus improving the diagnostic accuracy. The combined approach is also good because the p57[KIP2] test can be performed in routine pathologic examinations,[26] and molecular diagnostic methods are technically difficult to perform, relatively costly, and unavailable in most pathology laboratories.[1] A diagnosis of HM based upon histopathology and immunohistochemistry alone, without access to selective molecular genotyping, will lead to subsequent clinical and laboratory costs in a significant proportion of patients.[27]

There is considerable overlap in histological features between molar and nonmolar pregnancies and between CHMs and PHMs, which results in significant inter-observer variability in the diagnosis of HM.[9,11,22] Therefore, correct diagnosis of these difficult cases may require molecular techniques that examine the differences in DNA content between CHM and PHM, including flow or image cytometric DNA analysis, chromosome in situ hybridisation, polymerase chain reaction-based genotyping, or human leucocyte antigen typing. However, these molecular diagnostic methods are technically difficult to perform, relatively costly, and unavailable in most pathology laboratories.[1] Banet et al. established that immunohistochemical analysis of p57[KIP2] expression is highly correlated with genotyping results and demonstrated that CHM is almost always p57-negative, with only rare examples (0.5%) displaying aberrant (positive) p57[KIP2] expression, which is attributable to retention of the maternal copy of chromosome 11.[12]

The findings of Banet et al. demonstrated that p57[KIP2] is extremely reliable for the diagnosis of CHM. Therefore, the algorithmic approach for the diagnosis of HM proposed in the study advocates that p57[KIP2] results be used to triage cases for genotyping because this technique provides a highly reliable method for accurately diagnosing CHM in routine practice using a single immunohistochemical stain, with very little risk of misclassification of CHM. Consequently, genotyping for CHM is not necessary in routine practice and can be reserved for problematic cases, such as when p57[KIP2] is suboptimal or unsatisfactory or when there is a discrepancy between morphology and p57[KIP2] results.[12]

The Banet et al. study also confirmed that p57[KIP2] analysis is useful for identifying androgenetic/biparental mosaic/chimeric conceptions, which include uniformly androgenetic/biparental mosaic specimens without molar features (probably early forms of placental mesenchymal dysplasia, which is characterised by androgenetic/biparental mosaicism and a lack of trophoblastic hyperplasia), androgenetic/biparental mosaic specimens with a molar component (typically CHMs), and twin gestations composed of CHM and nonmolar specimen components. Recognition of the discordant and divergent staining patterns in these specimens is the key to correctly interpreting these complex specimens and is necessary for specific microdissection of the different components to assure accurate molecular genotyping.[12]

Hui et al. stated that the use of an algorithmic approach that combines p57[KIP2] and molecular genotyping for improving the diagnosis of HM is recommended. The publication also shows an approach that uses universal assessment of p57[KIP2] expression for all potentially molar specimens with triage to genotyping based on the p57[KIP2] result. Another approach of the publication uses universal genotyping based on morphologic assessment, which suggests any kind of HM, with selective application of p57[KIP2] to address any discordance between morphology and genotyping. This approach can be modified to incorporate some triage to p57[KIP2] versus genotyping, based on morphologic assessment, as favouring CHM versus PHM, respectively. When the morphology suggests a CHM and the p57[KIP2] is negative (with satisfactory internal positive control), then a diagnosis of a CHM is established.[23]

Conclusion

In agreement with previous studies, our findings suggest that p57[KIP2] expression appears to be a practical and accurate adjunct in the diagnosis of HM and can be an optimal technique to help in distinguishing CHM from its mimics in a subset of challenging cases, when used as a combined approach with genotyping.

Our results so far show that p57[KIP2] immunostaining has high values of sensitivity and positive likelihood ratio and

can be used as an adjunct test in cases of equivocal or difficult results of CHM. Although we found high performance of SDOR, the results are inconsistent due to the small number of studies and patients, and our results should be evaluated with extreme caution.

This meta-analysis adds to the current body of evidence on the accuracy of the p57$^{\text{KIP2}}$ immunostaining, which may be used as an adjunct test for CHM after the routine morphologic assessment. Additionally, again, in a scenario of intriguing cases, the cost-effectiveness of the test could limit its universal. Thereby, molecular techniques are still required for the evaluation of some cases with discordant positive p57$^{\text{KIP2}}$ staining. Therefore, our study reinforces that the use of an algorithmic approach, which combines p57$^{\text{KIP2}}$ and molecular genotyping for improving the diagnosis of CHM, is recommended. Additionally, we suggest future research evaluating the reduced follow up in GTN.

Disclosure of interests
None declared. Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship
JMM, AB, MPP, IEL, and EMW contributed to the conception of the study and search strategy, which was refined by MPP and EMW. MPP, IEL, and EMW designed the statistical analysis plan. The manuscript was drafted by JMM, MPP, IEL, and EMW and was critically revised by AB. JMM registered the protocol with the PROSPERO database. All authors have approved the publication of the manuscript.

Details of ethics approval
This study was submitted to Plataforma Brasil (an online system run by the Brazilian government) and approved on 28 August 2015, by the Institutional Review Board of Maternity School of Rio de Janeiro Federal University, as set forth in CAAE number 47952515.7.0000.5275.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Search strategies

Appendix S2. List of studies included or excluded (with their respective justification)

Appendix S3. Description of the studies ■

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