

p57 in Hydatidiform Moles: Evaluation of Antibodies and Expression in Various Cell Types

Helle Lund, MD,*† Søren Nielsen, HT,* Anni Grove, MD,* Mogens Vyberg, MD,*† and Lone Sunde, MD‡§

Abstract: The protein p57 is encoded by *CDKN1C*. This gene is known to be paternally imprinted and maternally expressed in cytotrophoblasts and villous stromal cells. We present a method for evaluating p57 antibodies (Abs) in hydatidiform mole (HM) and demonstrate the results for 4 p57 Abs in various cell types. Five cases of complete HM, diploid with 2 paternal genome sets (CHM;PP), 5 cases of partial HM, triploid with 2 paternal and 1 maternal genome sets (PHM;PPM), and 5 cases of non-HM, with diploid biparental genomes (non-HM;PM) were stained with p57 Abs: 57P06, EP183, KP10, and KP39. Assessment of the fraction of nuclei stained, and the intensity of staining of the nuclei and cytoplasm was performed. For evaluation of the Abs, the observations in cytotrophoblasts, villous stromal cells, maternal decidual cells, and intermediate trophoblasts were scored. The fraction of stained nuclei in cytotrophoblasts and villous stromal cells and the staining of cytoplasm showed to be important parameters in the evaluation of the Abs. 57P06 was evaluated as optimal. KP10 showed moderate cytoplasmic staining in maternal decidual cells and intermediate trophoblasts, and was evaluated as good. EP183 was evaluated as poor, primarily due to nuclear staining in $\geq 10\%$ of the villous stromal cells in CHM;PP. KP39 was evaluated as poor, primarily due to strong cytoplasmic staining in some cytotrophoblasts and villous stromal cells. A structured testing of p57 for diagnosing HM is recommended. No nuclear staining was observed in syncytiotrophoblasts of CHM;PP, indicating that in syncytiotrophoblasts also, *CDKN1C* is paternally imprinted.

Key Words: hydatidiform mole, antibody, immunohistochemistry, imprinting, syncytiotrophoblast

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Hydatidiform mole (HM) is an abnormal human pregnancy that imposes a significant risk of gestational trophoblastic neoplasia (GTN). On the basis of morphologic criteria, HM can be classified as complete (CHM) or partial (PHM).^{1–3} The majority of CHM cases are diploid with 2 paternal (P) genome sets (CHM;PP), whereas the majority of PHM cases are triploid with 2 paternal genome sets and 1 maternal (M) genome set (PHM;PPM).^{4–7} Subclassification of HM is important, as the risk of GTN is significantly higher in CHM;PP than in PHM;PPM.^{6,8} As regards prognosis, genetic classification is superior to morphologic classification.^{6,7} However, analysis of ploidy and parental origin is costly and ideally performed on unfixed tissue, which is often not available. In contrast, immunohistochemical (IHC) analysis of nuclear p57 expression is cheap and can be performed on formalin-fixed tissue.^{9–13} p57 is the product of the gene *CDKN1C*, located on chromosome 11p15.5. This gene is paternally imprinted and maternally expressed in cytotrophoblasts and villous stromal cells.¹⁴ Thus, p57 staining of nuclei of the cytotrophoblasts and villous stromal cells should be negative in CHM;PP, and positive in PHM;PPM, and in nonmolar pregnancy products, which possess 1 paternal and 1 maternal genome set (non-HM;PM).

The use of ancillary techniques, such as IHC of p57 to refine the diagnosis of HM, is generally recommended.^{10,12,15–20} The quality of the p57 staining is important, as both false-negative and false-positive results may lead to misclassification of HM. In the Nordic Immunohistochemical Quality Control (NordiQC) external quality assessment (EQA) of p57, run 41, data from 121 laboratories were used to evaluate p57 Abs for diagnosing HM. An insufficient result was achieved by 21% of the laboratories, mostly due to a weak staining reaction or a poor signal to noise ratio (www.nordiqc.org/downloads/assessments/41_61.pdf). The NordiQC challenge primarily compares the analytical performance of p57 staining in participating laboratories across various antibodies (Abs) used with many different protocols and various stainer platforms.²¹ To evaluate the performance of the Abs more accurately, a standardized comparison in a reference laboratory is necessary.

In this study, we present a method to assess p57 Abs for diagnosing HM in a standardized way and illustrate its use on the 3 best performing p57 Abs from the NordiQC

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From the *Department of Pathology, Aalborg University Hospital;

†Department of Clinical Medicine, Aalborg University, Aalborg;

‡Department of Biomedicine, Aarhus University; and §Department

of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark.

The authors declare no conflict of interest.

Reprints: Helle Lund, MD, Department of Pathology, Aalborg University Hospital, Ladegaardsgade 3, Aalborg 9000, Denmark (e-mail: helu@rn.dk).

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challenge, run 41, and a newly launched p57 Ab. Further, we explored the expression of p57 in syncytiotrophoblasts.

MATERIAL AND METHODS

Tissue Samples

Three tissue microarrays (TMAs) were constructed from archival paraffin blocks: one with tissue from 5 pregnancy products that morphologically were classified as CHM and genetically showed 2 paternal genome sets (CHM;PP), 1 with tissue from 5 pregnancy products that morphologically were PHM and genetically showed triploidy with 2 paternal genome sets and 1 maternal genome set (PHM;PPM), and 1 with tissue from 5 morphologically nonmolar pregnancy products with diploid biparental genomes (non-HM;PM). The morphologic diagnoses were made using standard histopathologic criteria.³ The genetic analyses were performed on unfixed tissue (Supplementary Material, Part A, Supplemental Digital Content 1, <http://links.lww.com/AIMM/A244>). From each pregnancy product, 3 cores containing cytotrophoblasts, syncytiotrophoblasts, and villous stromal cells, and 1 or 2 cores containing maternal decidual cells and/or intermediate trophoblasts, respectively, were selected (Supplementary Material, Part B, Supplemental Digital Content 1, <http://links.lww.com/AIMM/A244>).

Analysis of Staining

Four Abs were assessed: mouse monoclonal (mm) Ab 57P06, rabbit monoclonal (rm) Ab EP183, mmAb KP10, and

mmAb KP39, using protocols optimized in a reference laboratory (Supplementary Material, Part C, Supplemental Digital Content 1, <http://links.lww.com/AIMM/A244>). The process for evaluating the performance of the Abs is illustrated in Figure 1. Using a digital monitor, images of the individual TMA cores stained with the 4 Abs were viewed simultaneously and assessed by 3 authors (A.G. and H.L.; pathologists with special interest in molar diseases; S.N., histology technician and specialist in immunohistochemistry). For the cytotrophoblasts and syncytiotrophoblasts, 100 nuclei were assessed. For the villous stromal cells, maternal decidual cells, and intermediate trophoblasts, a minimum of 20 nuclei were assessed. For 1/5 cases of PHM;PPM, maternal decidual cells were not available. For 2/5 cases of PHM;PPM and 3/5 cases of non-HM;PM, intermediate trophoblasts were not available. For all pregnancy products, either maternal decidual cells or intermediate trophoblasts or both were available. The proportion of stained nuclei, the intensity of the nuclear staining, and the intensity of the cytoplasmic staining were categorized using the criteria shown in Table 1. Categorization was performed by consensus between the 3 assessors.

Evaluation of the Performance of the Antibodies

For each of the 15 pregnancy products, and each of the 4 cell types, cytotrophoblasts, villous stromal cells, maternal decidual cells, and intermediate trophoblasts, the results of the assessments were quantified by assigning an individual score, as described in Table 2. A total score was calculated by summing the individual scores for each of these cell types in each of the 15 pregnancy products.

Assessment

For each of the three to four cell types* in five samples from three types of pregnancy products**:

Assessment of three variables: Proportion of nuclei stained, intensity of nuclear staining, and intensity of cytoplasmic staining. For categorization, see Table 1.



Individual scores

For each of the three to four cell types* in five samples from three types of pregnancy products**:

Generation of individual scores. For algorithm, see Table 2.

For results, see Supplementary material, Part E.



Total scores

Generation of total scores by summing individual scores for three to four cell types** in five samples from three types of pregnancy products**. For results, see Table 4.



Final evaluation

Based on both the total score relative to the maximum total score, and on individual scores.

For algorithm, see Table 3.

FIGURE 1. Flow chart for evaluating the performance of antibodies against p57. *Cytotrophoblasts, villous stromal cells, maternal decidual cells, and/or intermediate trophoblasts. **CHM;PP, PHM;PPM, non-HM; PM. CHM indicates complete hydatidiform mole; PHM: partial hydatidiform mole; Non-HM, non-hydatidiform mole; PP: diploid with 2 paternal genome sets; PPM: triploid with 2 paternal genome sets and 1 maternal genome set; PM: diploid with biparental genome.

TABLE 1. Categories for Assessment of the Staining With p57 for Diagnosing Hydatidiform Mole

Proportion of nuclei stained	< 1%; 1%-9%; 10%-49%; ≥ 50%
Intensity of nuclear staining	None; weak; moderate; strong
Intensity of cytoplasmic staining	None; weak; moderate; strong

In the final evaluation of the Abs, both individual and total scores were used (Table 3). The p57 staining in the syncytiotrophoblasts was evaluated separately (Supplementary Material, Part D, Supplemental Digital Content 1, <http://links.lww.com/AIMM/A244>).

RESULTS

The total scores for the 4 Abs are summarized in Table 4. Supplementary Material, Part E (Supplemental Digital Content 1, <http://links.lww.com/AIMM/A244>) lists the individual scores. With 57P06, a weak staining of the cytoplasm was observed in a number of cases, resulting in the individual score “2.” However, the cytoplasmic staining in no case interfered with the interpretation of the nuclear staining. The final evaluation for 57P06 was optimal, as the total score was ≥ 2/3 of the maximum score, and no individual score was < 2 (Figs. 2A, B).

A maximum individual score was observed for EP183 in the cytotrophoblasts of the 5 cases of CHM;PP (Fig. 2C), as there was no nuclear or cytoplasmic staining. In the cytotrophoblasts and villous stromal cells for both cases of PHM;PPM (Fig. 2D) and non-HM;PM, the individual score was ≥ 2, as the nuclear staining was moderate to strong in

TABLE 2. Individual Scores Based on the Assessment of the Staining Reaction of the p57 Antibodies in Various Types of Pregnancy Products and Various Cell Types

Intensity of Nuclear Staining*	Intensity of Cytoplasmic Staining	Proportion of Nuclei Stained			
		< 1%	1%-9%	10%-49%	≥ 50%
CHM;PP (cytotrophoblasts and villous stromal cells)					
	None	3	2	0	0
	Weak	2	2	0	0
	Moderate	1	1	0	0
	Strong	0	0	0	0
CHM;PP (maternal decidual cells and intermediate trophoblasts), PHM; PPM, and non-HM;PM (all cell types)					
None/weak	None	0	0	0	0
	Weak	0	0	0	0
	Moderate	0	0	0	0
	Strong	0	0	0	0
Moderate/strong	None	0	0	2	3
	Weak	0	0	2	2
	Moderate	0	0	1	1
	Strong	0	0	0	0

*The intensity of the nuclear staining in the cytotrophoblasts and villous stromal cells in CHM;PP are not included in the assessment.

CHM indicates complete hydatidiform mole; Non-HM, non-hydatidiform mole; PHM, partial hydatidiform mole; PM, diploid with biparental genome; PP, diploid with 2 paternal genome sets; PPM, triploid with 2 paternal genome sets and 1 maternal genome set.

TABLE 3. Final Evaluation of the p57 Antibodies

Criteria	Result
Total score ≥ 2/3 of the maximum total score, and no individual score < 2 (all cell types*)	Optimal
Total score ≥ 2/3 of the maximum total score, and ≥ 1 individual score = 1 (maternal decidua and/or intermediate trophoblasts) and no individual score < 2 (cytotrophoblasts and villous stromal cells)	Good
Total score ≥ 1/3, < 2/3 of the maximum total score, and no individual score = 0 (all cell types*)	Borderline
Total score < 1/3 of the maximum total score, and/or ≥ 1 individual score = 0 (all cell types*)	Poor

*Cytotrophoblasts, villous stromal cells, maternal decidual cells, and intermediate trophoblasts.

≥ 50% of the cells. However, in 4/5 cases of CHM;PP, 10% to 49% of the nuclei in villous stromal cells showed staining with a moderate intensity (Fig. 2C), and in 1 CHM;PP, ≥ 50% of the nuclei in villous stromal cells showed moderate staining. Accordingly, the final evaluation for EP183 was poor.

For KP10, the total score was ≥ 2/3 of the maximum score, and no individual score was < 2 for cytotrophoblasts and villous stromal cells (Figs. 2E, F). However, 4/24 individual scores for the maternal decidual cells and intermediate trophoblasts were “1” primarily due to a moderate staining in the cytoplasm (inset, Fig. 2E). The final evaluation was good.

KP39 showed moderate cytoplasmic staining in villous stromal cells in 2 cases of CHM;PP (Figs. 2G, H) and strong cytoplasmic staining in the cytotrophoblasts in 2 cases of non-HM;PM, interfering with the evaluation of the staining in the nuclei. The final evaluation was poor.

In all cases of CHM;PP, the frequency of stained nuclei in cytotrophoblasts was < 1% with all 4 Abs, whereas even with the optimal/good performing Abs 57P06 and KP10, 1% to 9% of the nuclei in villous stromal cells were stained in several pregnancy products.

Supplementary Material, Part D (Supplemental Digital Content 1, <http://links.lww.com/AIMM/A244>) lists the findings in the syncytiotrophoblasts. With KP39, all 15 pregnancy products showed a strong staining of the cytoplasm, making it difficult to evaluate the nuclear staining (Figs. 2G, H). With 57P06, EP183, and KP10, all cases of CHM;PP uniformly showed < 1% stained nuclei. In contrast, most nuclei in PHM;PPM and non-HM;PM showed weak-moderate staining, with a tendency toward a higher proportion of stained nuclei and a more extensive staining in the latter.

DISCUSSION

We present a method for evaluation of the performance of Abs against p57 for diagnosing HMs and used this to evaluate 4 p57 Abs. We also explored the expression of p57 in various cell types, including the syncytiotrophoblasts.

The strengths of our study are the conduction of p57 staining in a reference laboratory. Optimizing protocols for the Abs and comparing slides on a monitor, allowing

TABLE 4. Total Scores for the Staining Reaction With the 4 p57 Antibodies

Type of Pregnancy	Type of Cells	Maximum Score	Scores for Individual Abs			
			57P06	EP183	KP10	KP39
CHM;PP	Cytotrophoblasts	15	12	15	11	10
	Villous stromal cells	15	11	0	11	6
	Maternal decidual cells	15	10	8	9	10
	Intermediate trophoblasts	15	11	10	10	11
	Sum	60	44	33	41	37
PHM;PPM	Cytotrophoblasts	15	12	14	11	15
	Villous stromal cells	15	15	14	15	15
	Maternal decidual cells*	12	8	7	8	8
	Intermediate trophoblasts*	9	6	6	6	7
	Sum	51	41	41	40	45
Non-HM;PM	Cytotrophoblasts	15	10	11	11	4
	Villous stromal cells	15	14	12	13	10
	Maternal decidual cells	15	10	7	9	11
	Intermediate trophoblasts*	6	4	4	3	3
	Sum	51	38	34	36	28
Total score		162	123	108	117	110
Final evaluation			Optimal	Poor	Good	Poor

The total score was obtained by summing up the individual scores for the various cell types for CHM;PP, PHM;PPM, and non-HM;PM.

*For PHM;PPM and non-HM;PM: <5 cores represented the maternal decidual cells and/or intermediate trophoblasts.

CHM indicates complete hydatidiform mole; Non-HM, non-hydatidiform mole; PHM, partial hydatidiform mole; PM, diploid with biparental genome; PP, diploid with 2 paternal genome sets; PPM, triploid with 2 paternal genome sets and 1 maternal genome set.

the assessors to observe the same area stained with the different Abs, contributed to a fair comparison of the Abs. Testing the antibodies on molar and nonmolar pregnancy products, well characterized both morphologically and genetically, further supports the validity of the results. The use of TMAs consisting of several cores compared with conventional slides containing more tissue, and the fact that only one staining platform was used, may have introduced limitations.

In the algorithm for using IHC of p57 in the diagnostics of HM and pregnancy products suspected of being HM, it is recommended to analyze cytotrophoblasts, villous stromal cells, maternal decidual cells, and intermediate trophoblasts.^{22,23} Our method for evaluating the performance of Abs used for p57 staining is based on the assessment of the staining of these 4 cell types, in 3 types of pregnancy products. In our method, 3 variables are assessed: the proportion of nuclei stained, the intensity of the nuclear staining, and the intensity of the cytoplasmic staining.

To convert IHC results into quantitative data, the H-score is widely used.^{24–26} The H-score is calculated by summing up the products of the percentage of stained nuclei and the staining intensity. However, as the proportion of stained nuclei is a main criteria in the algorithm rather than the staining intensity,²² the H-score is not ideal for assessing p57 Abs. In our method, the staining in the nuclei and the cytoplasm in the various cell types are quantitated in individual scores, influenced by the fraction of nuclei stained and the intensity of the staining separately. For the final evaluation, both the individual and the total scores are used.

The validity of the nuclear staining is important in every cell type used in the diagnosis of HM. However, this is most important for cytotrophoblasts and villous stromal

cells, as a staining of $\geq 10\%$ of the nuclei in these cells will argue against the diagnosis CHM;PP. This is critical, as the risk of GTN is high for CHM;PP. In this study, the staining result for EP183 was poor, mainly because the proportion of stained nuclei in the villous stromal cells was $\geq 10\%$ in all cases of CHM;PP. It is recommended that a conceptus showing negative nuclear staining in the cytotrophoblasts and a simultaneous positive nuclear staining in the villous stromal cells is investigated further by genotyping.²² However, genotyping is costly, and not all laboratories have access to this technique. Thus, when validating p57 Abs, the staining in the various cell types should be assessed separately.

Most authors apparently do not assess staining in the cytoplasm in their evaluation of HM.^{10,16,18,20,27} However, excessive nonspecific cytoplasmic staining has been suggested to influence the nuclear staining, which could lead to erroneous interpretation.²³ For cells serving as positive controls, we allowed a moderate cytoplasmic staining for the final evaluation as good. However, to avoid misjudgment of the nuclear staining, an Ab showing moderate staining of the cytoplasm in cytotrophoblasts or villous stromal cells could at the most achieve a borderline result in the final evaluation. The cytoplasmic staining was of significance for the final evaluation of 2 of the 4 antibodies. For KP10, a moderate staining in the cytoplasm in maternal decidual cells and/or intermediate trophoblasts that could interfere with the interpretation of the nuclear staining, was the main reason for the final evaluation resulting good, but not optimal. For KP39, a moderate to strong cytoplasmic staining in cytotrophoblasts and villous stromal cells interfering with the evaluation of the nuclear staining was the main cause that the final evaluation ended up as poor. These findings illustrate that assessment of the staining in the cytoplasm is important.

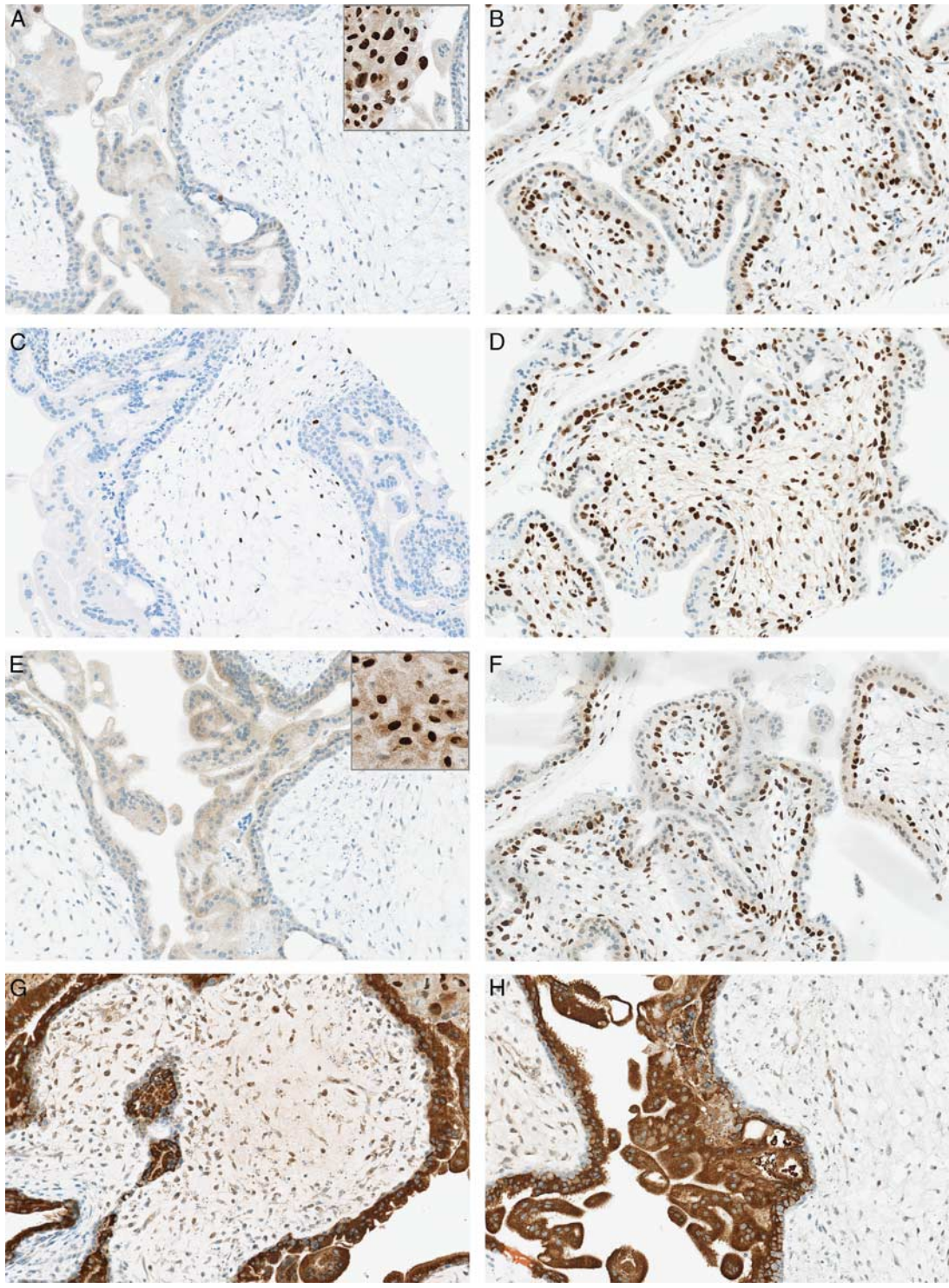


FIGURE 2. P57 staining with the Abs 57P06 (A, B), EP183 (C, D), KP10 (E, F), and KP39 (G, H). A, Immunostaining with the Ab 57P06 in a CHM;PP (case 1). There is no staining in the nuclei of the cytotrophoblasts (score 3), and 1% to 9% stained nuclei with a weak staining intensity in the villous stromal cells (score 2). As a positive internal control, the intermediate trophoblasts showed a strong staining in $\geq 50\%$ of the nuclei and a weak cytoplasmic staining (inset, score 2). In the syncytiotrophoblasts, there is no nuclear staining, but a weak cytoplasmic staining. B, Immunostaining with the Ab 57P06 in a PHM;PPM (case 6). There is a strong nuclear staining in $\geq 50\%$ of the nuclei and a weak cytoplasmic staining in the cytotrophoblasts (score 2), and $\geq 50\%$ stained nuclei with strong staining intensity in the villous stromal cells (score 3). In the syncytiotrophoblasts, there is a weak nuclear staining in 1% to 9% of the nuclei and a weak cytoplasmic staining. C, Immunostaining with the Ab EP183 in a CHM;PP (case 1). There is a moderate nuclear staining in 10% to 49% of the villous stromal cells (score 0). Both in the cytotrophoblasts and the syncytiotrophoblasts, neither nuclei nor cytoplasm are stained (score 3). D, Immunostaining with the Ab EP183 in a PHM;PPM (case 6). There is a strong staining in $\geq 50\%$ of the nuclei in the cytotrophoblasts and the villous stromal cells (score 3). The cytoplasm is clear in these cells. In the syncytiotrophoblasts, there is a weak nuclear staining in 10% to 49% of the nuclei, and no cytoplasmic staining. E, Immunostaining with the Ab KP10 in a CHM;PP (case 1). There is no nuclear staining, but a weak cytoplasmic staining in the cytotrophoblasts (score 2). In the villous stromal cells, there is a weak staining in 1% to 9% of the nuclei (score 2). In the decidual cells, $\geq 50\%$ of the nuclei were strongly stained; however, the cytoplasm showed moderate staining (inset, score 1), which was a main cause of the final evaluation resulting good. There is no nuclear staining, but a weak cytoplasmic staining in the syncytiotrophoblasts. F, Immunostaining with the Ab KP10 in a PHM;PPM (case 6). The cytotrophoblasts showed a strong staining in $\geq 50\%$ of the nuclei and a weak cytoplasmic staining (score 2). Likewise, the nuclear staining is strong in $\geq 50\%$ of the villous stromal cells, and the cytoplasm is clear in these cells (score 3). In the syncytiotrophoblasts, there is a weak staining in 1% to 9% of the nuclei, and a weak cytoplasmic staining. G and H, Immunostaining with the Ab KP39 in 2 cases of CHM;PP (G: case 2, H: case 1). There is a moderate cytoplasmic staining in the villous stromal cells interfering with the evaluation of the nuclear staining in these cells (in both cases: score 0). There is no nuclear staining, but a weak cytoplasmic staining in the cytotrophoblasts (in both cases: score 2). Furthermore, there is a very strong cytoplasmic staining in the syncytiotrophoblasts complicating the evaluation of the nuclear staining in these cells.

In the NordiQC challenge, the proportion of laboratories with sufficient results (optimal or good) was 100% (14/14) for KP10, 83% (5/6) for KP39, and 82% (32/39) for 57P06 (www.nordiqc.org/downloads/assessments/41_61.pdf). In the present study, we evaluated the staining result for KP10 as good, for KP39 as poor, and for 57P06 as optimal. In the NordiQC challenge, run 41, serial sections of TMAs of “standardly processed” tissues were circulated to a number of laboratories to be stained with the p57 Ab routinely used in the laboratory, and they were thereafter assessed by a group of experienced pathologists and biomedical scientists. Thus, the results from the NordiQC challenge were based on the various protocols and IHC staining systems used by the participating laboratories. In our study, we performed a standardized comparison of p57 Abs using in-house optimized versions of the vendor’s protocols. We obtained the best staining result with 57P06 in contrast to only 82% of the laboratories obtaining a sufficient result in the NordiQC challenge. Our observations suggest that this Ab can be recommended for diagnosing HMs, if the protocol is optimized.

In the NordiQC challenge, KP10 showed the best performance. With this Ab, we found a moderate staining in the cytoplasm in the maternal decidual cells and the intermediate trophoblasts. Accordingly, our final evaluation for KP10 was good, but not optimal. Probably, in the NordiQC challenge, there was less focus on the cytoplasmic staining, compared with our assessment. Being aware of the potential staining in the cytoplasm in the internal controls, KP10 is acceptable for diagnosing HMs.

In the NordiQC challenge, 5/6 laboratories obtained a sufficient result with KP39. In our study, KP39 was evaluated as poor, mainly because of a strong cytoplasmic staining in the cytotrophoblasts and villous stromal cells in some samples. In the NordiQC challenge, no comments on strong

cytoplasmic staining with this Ab were made. As the NordiQC challenge was performed in 2014, other lots may have been used, giving different staining reactions. To search for the explanation of the discrepancy, we purchased KP39 with another lot number from the same vendor. However, again, we observed an unacceptable strong cytoplasmic staining (data not shown). This does not exclude that some lots of this Ab may perform well. However, as at least some lots seem to cause a cytoplasmic staining interfering with the interpretation of the nuclear staining in cells that should be negative in CHM;PP, this antibody cannot be recommended. Furthermore, with this antibody, the cytoplasm of the syncytiotrophoblasts showed strong staining in all cases of CHM;PP, PHM;PPM, and non-HM;PM. For an unexperienced pathologist, the strong staining in the syncytiotrophoblasts may interfere with the interpretation of the staining in neighboring cytotrophoblasts.

Neither can the new Ab EP183 be recommended for diagnosing HMs. The positive staining in the nuclei in the villous stromal cells in CHM;PP is problematic, as these should be negative. Therefore, a CHM;PP could be overlooked. Diagnostics of mosaics, characterized by both p57-positive and p57-negative cells,^{28,29} also require reliable p57 staining in the various cell types.

As NordiQC is an EQA system, it can identify performance problems in the individual laboratories. However, both internal and external quality control for diagnostic IHC are important.^{30–32}

The method presented here complements the NordiQC challenge, because using optimized protocols and rigorous standardized assessments can reveal the reasons for suboptimal staining results. Further, our method can be used by individual laboratories to assess which p57 Ab performs best in their set-up.

The interpretation of the p57 staining is described as straightforward in the majority of cases, as the nuclei in the cell types in which p57 is differentially expressed, should be stained almost uniformly negative or positive.²² However, a limited extent of nuclear p57 staining has previously been reported in the cytotrophoblasts and villous stromal cells in CHMs, and therefore <10% stained nuclei are considered compatible with a diagnosis of CHM.^{10,15} In all cases of CHM;PP, and with all Abs, we observed staining of <1% of the nuclei of cytotrophoblasts, whereas, in some cases, 1% to 9% of the nuclei in the villous stroma were stained. It is possible that the paternal imprinting of *CDKN1C* is more “constant” in the cytotrophoblasts than in the villous stromal cells; however, we cannot exclude that, in some cases, a “foreign cell” present in the villus was mistaken as a villous stromal cell. Although our observations suggest that the cut-off for cytotrophoblasts could be lowered to 1%, we suggest that the limit 10% is maintained in order to minimize the risk of misclassifying a CHM;PP.

With antibodies against p57, the nuclei of the syncytiotrophoblasts in HMs and early non-HM pregnancy products have been reported to be uniformly negative^{10,11,15} or occasionally immunoreactive.^{14,27} With 57P06, KP10, and EP183, we observed staining of <1% of the nuclei in the syncytiotrophoblasts in all cases of CHM;PP, while some nuclear staining was observed in most cases of PHM;PPM and non-HM;PM, with a tendency for more frequent staining of the latter. The absence of staining in CHM;PP indicates that *CDKN1C* is paternally imprinted and maternally expressed in the syncytiotrophoblasts, whereas the apparent difference between the staining in non-HM;PM and HM;PPM is at present unexplained.

A small percentage of diploid HMs show mosaicism between a diploid cell line with 2 paternal genome sets and a normal biparental cell line (PP/PM). Accordingly, the phenotype of these HMs may not be that of a “classic” CHM. However, as these moles impose a risk of GTN similar to the risk after a CHM;PP,³³ it is important to identify the mosaic HMs. In mosaic HMs, discordant immunostaining of p57, for example, absence of staining in the villous stromal cells and presence of staining in the cytotrophoblasts, or vice versa, has been described.^{22,28,29} Thus, it could be tempting to include the staining pattern of the nuclei in the syncytiotrophoblasts in the diagnostics. However, in that case, one should pay attention to the fact that, in the presence of a maternally inherited allele of *CDKN1C*, the staining of nuclei of the syncytiotrophoblasts generally appear both weaker and less consistent than the staining of the nuclei of the cytotrophoblasts and the villous stromal cells.

CONCLUSIONS

We present a method for estimating the quality of p57 Abs for diagnosing HMs, wherein both the staining of nuclei and of cytoplasm are assessed, and wherein both the staining of nuclei expected to be positive and that of nuclei expected to be negative are assessed. Among 4 Abs tested, we found the Ab 57P06 to be best. However, for optimal

results, each laboratory should repeat the testing to validate the performance with the IHC methods used in the local laboratory. *CDKN1C* seems paternally imprinted and maternally expressed in the syncytiotrophoblasts. However, for clinical use, nuclear staining of p57 in the syncytiotrophoblasts should be interpreted with caution, as the nuclear staining of syncytiotrophoblasts with a maternal allele of *CDKN1C* can be weak.

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