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Comparison of p57, c-erbB-2, CD117, and Bcl-2 expression in the differential diagnosis of hydatidiform mole and hydropic abortion

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Summary

Purpose: To explore the utility of p57, c-erbB-2, CD117, and Bcl-2 immunostaining in the differential diagnosis of complete hydatidiform mole (CHM), partial hydatidiform mole (PHM), and hydropic abortion (HA). *Materials and Methods:* Immunohistochemical expression of the p57, c-erbB-2, CD117, and Bcl-2 proteins were investigated semi-quantitatively using paraffin-embedded tissue sections from histologically unequivocal cases of CHM (n = 20), PHM (n = 23), and HA (n = 17). *Results:* All cases of CHM exhibited a striking absence of p57 expression. The percentage of positive p57 staining was similar between PHMs (73.9%) and HAs (76.5%) ($p > 0.05$). The comparison of c-erbB-2 expression revealed a significantly higher percentage of positive c-erbB-2 staining in CHMs (45%) compared with that in PHMs (8.7%) and HAs (5.9%) ($p = 0.006$ and 0.01, respectively). The CD117 expression pattern (immunoreactivity score, percentage of positive cells, and staining intensity) was significantly lower in HAs compared with that in PHMs and CHMs ($p < 0.05$ for all). A significantly increased Bcl-2 expression pattern was observed in HAs compared with that in PHMs and CHMs ($p < 0.05$ for all). *Conclusion:* Immunohistochemical examination of p57, c-erbB-2, CD117, and Bcl-2 expression represents a relatively simple, reliable, and cost-efficient procedure to definitively distinguish among CHM, PHM, and HA.

Key words: Bcl-2; CD117; c-erbB-2; Hydatidiform mole; p57.

Introduction

Hydatidiform moles (HMs) are the most common form of gestational trophoblastic disease (GTD) resulting from abnormal fertilisation and are characterised by hydropic swelling of placental villi and trophoblastic hyperplasia. They are categorised into two distinct entities of partial hydatidiform mole (PHM) and complete hydatidiform mole (CHM), based on morphological, genetic, and clinical features. In addition, hydropic abortions (HAs) can mimic HMs morphologically. Accurately distinguishing HAs from HMs and PHMs from CHMs is important for appropriate clinical management, as the risk of persistent GTD is higher in patients with CHM (10–30%) than with PHM (0.5–5%), whereas HA is completely benign and not associated with the risk of persistent GTD [1]. A diagnosis of HM can often be made based on a morphological assessment alone. However, there are significant overlaps in the histological features between HMs and HAs, as well as between CHMs and PHMs, causing considerable inter-observer and intra-observer variability in the diagnosis [2].

Genomic imprinting (gene expression based on gametes of origin) is important for the regulation of implantation and embryonic development. CHMs are derived exclusively from the paternal genome (androgenetic diploidy), whereas PHMs contain one maternally derived and two paternally derived haploid genomes (diandric triploidy), suggesting that both CHM and PHM are the result of abnormal expression of imprinted genes. The p57 gene is paternally imprinted and expressed predominantly from the maternal allele in most tissues. p57 is a potent cell cycle inhibitor and tumour suppressor, and lack of p57 expression in trophoblastic disease plays a relevant role in its abnormal proliferation and differentiation [3].

The c-erbB-2 (also known as Her-2/neu) protein is a transmembrane tyrosine kinase receptor in the epidermal growth factor receptor family. This proto-oncogene is involved in activating pathways leading to cell growth and differentiation. The c-erbB-2 protein is expressed frequently at low levels in a variety of adult epithelial cells; however, aberrant activation of c-erbB-2 due to amplification and/or overexpression can contribute to unrestrained proliferation and tumour development [4].

CD117, also known as c-kit, is a tyrosine kinase receptor that regulates cell proliferation, apoptosis, adhesion and chemotaxis. CD117 may be involved in proliferation and normal differentiation of the placenta during pregnancy

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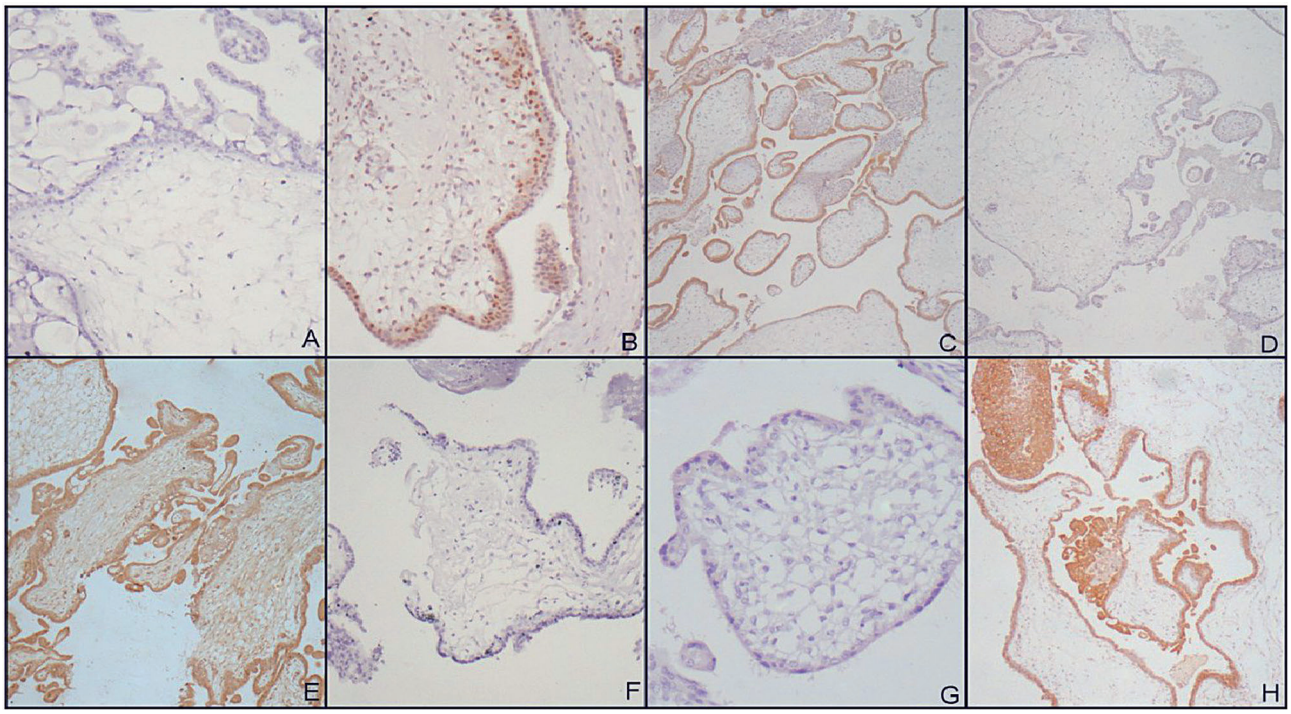

Figure 1. — A) Villi from a complete hydatidiform mole (CHM) demonstrating complete loss of p57 immunostaining of both the villous cytotrophoblast and stromal cells (magnification, ×100). B) Villi from a partial hydatidiform mole (PHM) demonstrating positive p57 immunostaining of both the villous cytotrophoblast and stromal cells (magnification, ×100). C) Bcl-2 protein cytoplasmic staining (strong intensity) is seen in the synctiotrophoblastic and cytrophoblastic cells of PHM (magnification, ×40). D) Lack of Bcl-2 staining is seen in the CHM synctiotrophoblastic and cytotrophoblastic cells (magnification, ×40). E) CD117 cytoplasmic staining (strong intensity) is seen in the CHM synctiotrophoblastic and cytotrophoblastic cells (magnification, ×100). F) Lack of CD117 staining is seen in PHM synctiotrophoblastic and cytotrophoblastic cells (magnification, ×40). G) Negative c-erbB-2 immunostaining in hydropic abortion (magnification, ×200). H) Positive c-erbB-2 immunostaining of CHM synctiotrophoblastic and cytotrophoblastic cells (magnification, ×40).

[5]. Overexpression of CD117 has been implicated in the pathogenesis of numerous tumours including choriocarcinoma [6].

Apoptosis plays an important role in normal placental morphogenesis and in the pathogenesis of GTD [7]. Bcl-2, an anti-apoptotic molecule residing in the mitochondria, plays a decisive role regulating cell death during embryogenesis and normal placental growth, and its dysregulation has been implicated in several pregnancy disorders [8].

The objectives of this study were to ascertain the expression patterns of p57, c-erbB-2, CD117, and Bcl-2 in CHMs, PHMs, and HAs, and to assess the value of these markers in the differential diagnosis of the three entities.

Materials and Methods

Case selection

Sixty-seven formalin-fixed paraffin-embedded gestational specimens with hydropic swelling of chorionic villi were retrieved from the files of the Department of Pathology, Antalya Training and Research Hospital between January 2010 and December 2012 after institutional review board approval. The diagnosis of each case (CHM, PHM or HA) was obtained from the original pathology report. Hematoxylin and eosin-stained sections of the specimens were reviewed independently by two pathologists with no knowledge of the specimens' clinical information and classified as CHM, PHM or HA according to the main morphological findings [9]. CHM is characterized by hydropic swelling of villi with central cisterns, circumferential trophoblastic hyperplasia with diffuse and marked atypia, and trophoblastic inclusions. Morphologic features of PHM include focal trophoblastic hyperplasia, a dimorphic villous population with an admixture of hydropic and normal villi, scalloping and prominent stromal trophoblastic inclusions, and mild trophoblastic atypia. HA is characterized by villous edema without trophoblastic hyperplasia. Sixty cases were histologically unequivocal for CHM (n =20), PHM (n = 23), and HA (n = 17) and constituted the study group. The remaining seven equivocal cases were difficult to classify as HA, PHM or CHM because of mixed histological features and were excluded from the final statistical analysis. These equivocal cases were subjected to molecular genotyping. Patient demographic data were obtained through a chart review. Serum beta-human chorionic gonadotropin (β-hCG) levels were measured by a two-site chemiluminescence immunoassay based on the direct sandwich technique. The inter- and intra-assay coefficients of variation were 4.1% and 1.3%, respectively.

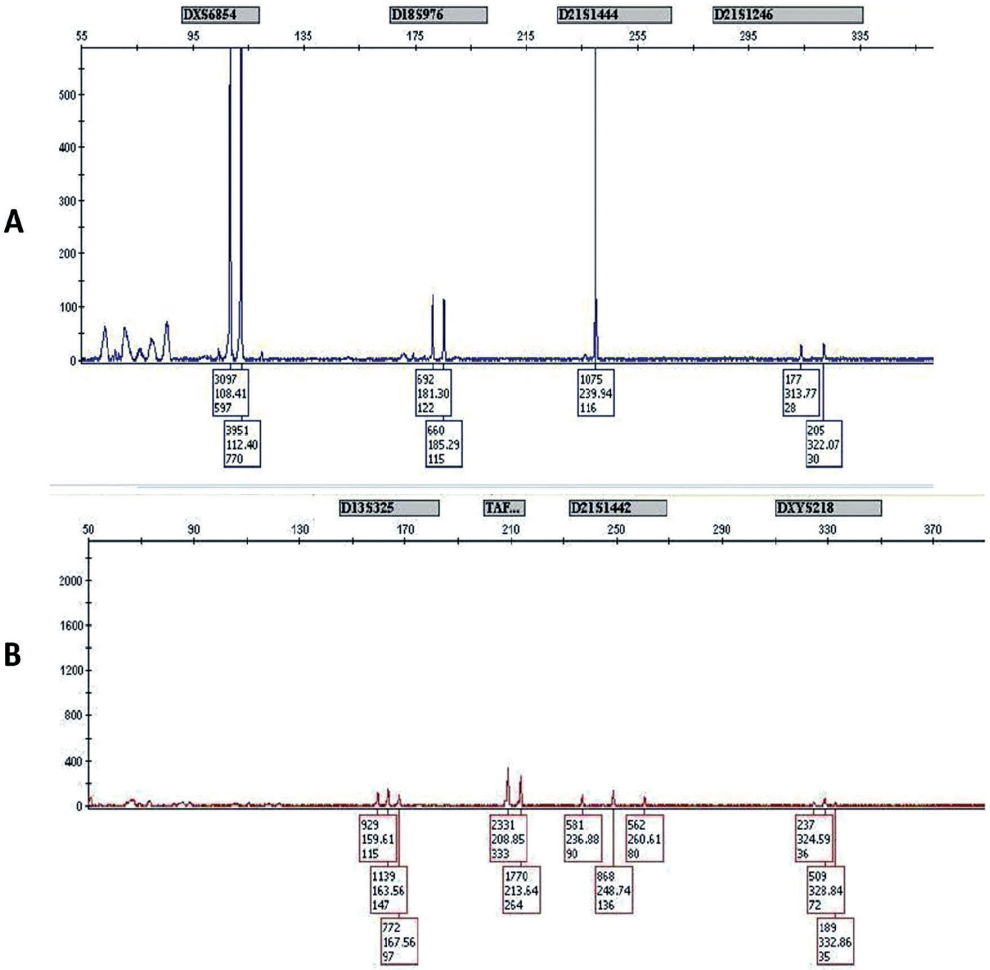

Figure 2. — Representative examples of diploid and triploid histograms produced by short-tandem repeat amplification. A) Three loci (DXS6854, D18S976, and D21S1246) each demonstrate two alleles, consistent with diploidy (two peaks with approximate 1:1 ratios). B) Three loci (D13S325, D21S1442, and DXYS218) each demonstrate three alleles, consistent with triploidy.

Tissue preparation and evaluation of immunohistochemical staining

Briefly, four-µm thick, representative sections from formalin-fixed, paraffin-embedded tissue blocks were obtained in each case, incubated for 120 minutes at 60°C and then overnight at 37°C. The tissue sections were deparaffinzed in xylene and alcohol, rehydrated, washed in a solution buffered with 10% sodium citrate in a microwave oven (800 W). The slides were left to cool at room temperature for 20 minutes. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide, and the slides were washed in phosphate-buffered saline (PBS; ten mM, pH 7.4). As the primary antibody, mouse monoclonal antibodies against p57 (clone 25B2; 1:50 dilution), c-erbB-2 (clone 10A7; 1:40 dilution), CD117 (clone 57A5D8, ready to use), and Bcl-2 (clone 3.1; 1:80 dilution) were incubated with the slides for 60 minutes at room temperature, according to the manufacturer's protocol. The slides were washed in PBS, and the sections were incubated for 20 minutes with biotinylated secondary antibody. The chromogenic reaction was performed using 3,3'diaminobenzidine. Then, the sections were washed in distilled water, counterstained with hematoxylin and mounted with Entellan. Appropriate positive and negative controls were run for each case. The evaluation of protein expression was performed independently by two pathologists (DS and BT). The stained cell types were identified as either a villous cytotrophoblast, villous intermediate trophoblast, villous syncytiotrophoblast, villous stromal cells or decidual cells. All samples were scored semi-quantitatively. Immunohistochemical results were recorded independently of the original clinicopathological diagnosis. Representative case examples are illustrated in Figure 1.

On the basis of the staining pattern reported in the literature, the specimens were interpreted as "positive" for p57 staining when distinct nuclear staining (> 50%) of villous stromal cells and cytotrophoblasts was observed. The p57 stain was interpreted as "negative" when there was no distinct staining or limited nuclear staining (< 10%) of villous stromal cells and cytotrophoblasts but intermediate trophoblasts and/or maternal decidua exhibited nuclear expression of p57 (which served as the positive internal control for all cases). Nuclear expression in villous stromal cells and cytotrophoblasts in the focally positive range (10–50%) was considered an equivocal result [10]. Syncytiotrophoblastic cells were used as negative controls.

c-erbB-2 expression was analysed by evaluation of the staining intensity and the proportion of stained villous trophoblastic cells as follows: (0): no staining or staining observed in < 10% of the villous trophoblast cells, (+): weak staining observed in > 10% of the villous trophoblast cells in part of the cell membrane, (++): weak to moderate complete membrane staining observed in > 10% of the villous trophoblast cells, and (+++): strong complete membrane staining observed in > 10% of the villous trophoblast

Table 1. — *Immunohistochemical analysis of p57, c-erbB-2, CD117, and Bcl-2 in hydropic abortions, partial, and complete hydatidiform moles.*

Marker expression	HA (n=17)	PHM (n=23)	CHM (n=20)	*p*-value	p^1-value	p^2-value	p^3-value
p57 [a]				< 0.001	< 0.001	0.999	< 0.001
Negative	4 (23.5)	6 (26.1)	20 (100)				
Positive	13 (76.5)	17 (73.9)	-				
c-erbB-2 [a]				0.003	0.006	0.999	0.01
Negative	16 (94.1)	21 (91.3)	11 (55.0)				
Positive	1 (5.9)	2 (8.7)	9 (45.0)				
CD117 [b]							
Staining intensity	1.06±0.24	1.78±0.60	2±0.72	< 0.001	0.307	< 0.001	< 0.001
Percentage of positive cells	1.88±0.70	2.65±0.89	2.75±0.91	0.002	0.857	0.005	0.005
Immunoreactivity scores	2.06±1.20	5.09±2.81	6.05±3.87	< 0.001	0.651	0.001	< 0.001
Bcl-2 [b]							
Staining intensity	2.29±0.77	1.61±0.84	0.75±0.55	< 0.001	0.001	0.014	< 0.001
Percentage of positive cells	1.35±0.1	0.72±0.21	0.70±0.47	0.005	0.015	0.009	0.005
Immunoreactivity scores	2.29±0.77	1.61±0.84	0.75±0.55	< 0.001	0.001	0.014	< 0.001

Values are given as [a] number (%) or [b] mean ± SD (standard deviation). If the Kruskal–Wallis test was positive ($p < 0.05$) then post-hoc analysis was applied.
p, between three groups; p^1, between partial and complete hydatidiform mole; p^2, between partial hydatidiform mole and hydropic abortion;
p^3, between complete hydatidiform mole and hydropic abortion. Adjusted significance level for p^1, p^2, and $p^3 = 0.017$.
HA: hydropic abortion, PHM: partial hydatidiform mole, and CHM: complete hydatidiform mole.

cells [11]. c-erbB-2 expression was categorised as positive or negative, and only the score (+++) was considered a positive reaction. Sections of two breast carcinomas known to express c-erbB-2 served as positive controls.

CD117 and Bcl-2 expression in villous cells was evaluated using an immunoreactivity score (IRS) as described elsewhere [12]. The IRS was calculated by multiplying the percentage of positive cells (PP) by the staining intensity (SI). PP was estimated by counting ~ 100 cells per slide (×400 magnification) and scored as follows: 0 = < 5% staining, 1 = 5–25% staining, 2 = 25–50%, 3 = 50–75% staining, and 4 = > 75% staining. The SI was scored as follows: 0: negative, 1: weakly positive, 2: moderately positive and 3: strongly positive. Cytoplasmic staining was the criterion for positive CD117 and Bcl-2 reactions. Sections of a lymph node with follicular hyperplasia were used as a positive control for Bcl-2, and sections of a gastrointestinal stromal tumour were used as a positive control for CD117.

DNA analysis by short tandem repeat (STR) genotyping

DNA extraction was performed on formalin-fixed, paraffin embedded tissue following a standard procedure using an automated system. Quantitative fluorescent polymerase chain reaction (QF-PCR) methodology was used to determine the diploidy status of the extracted DNA. Short-tandem repeat loci were evaluated in each sample using the ChromoQuant QF-PCR kit, which allows for DNA amplification and fluorescence analysis of 22 loci from different chromosomes and the amelogenin locus simultaneously. The amplified microsatellite fragment size data were analysed using ChromoQuant Visualizer STaR ver. 4.03 analysis software. QF-PCR amplification and capillary electrophoresis were performed according to the manufacturer's instructions. Capillary electrophoresis data from villous tissues were analysed to identify alleles at each locus. For each locus from which two alleles were identified, the allelic ratio was calculated by dividing the peak height of the longer allele by the peak height of the shorter allele. Allelic ratios of 0.8–1.4 were considered consistent with diploidy. Allelic ratios < 0.65 or > 1.8 were considered to be consistent with triploidy. Allelic ratios that fell between the normal and abnormal ranges were classed as inconclusive. In addition, loci with three and two alleles identified were consistent with triploidy and diploidy, respectively (Figure 2). At least two informative loci were required for the final interpretation.

Statistical analysis

The comparison of antibodies among histologically unequivocal cases was assessed using SPSS 18.0 software. The numeric variable comparison among the three groups was performed using the Kruskal–Wallis test, and post-hoc comparisons were assessed using the Mann–Whitney *U*-test with Bonferroni's correction. Categorical data were compared using Pearson's chi-square test. All tests were two-sided at a significance level of $p < 0.05$.

Results

The age of the 67 patients ranged from 17 to 45 years (median, 27) with gestational ages of 6–15 weeks (median, ten). Patients with PHM (median, 26 years) were younger than those with CHM (median, 29 years), and HA (median, 31 years). The median gestational age at the time of diagnosis was nine weeks for the HA cases, 11 weeks for PHM cases, and ten weeks for CHM cases. All patients with HMs were followed by clinical examination and serum β-hCG measurements. After therapeutic evacuation, one patient with CHM evolved into persistent trophoblastic disease requiring methotrexate therapy. However, the β-hCG level was negative after six months of therapy. The average follow-up period in cases of HMs was 12.8 months (range 12–14).

The comparison of p57, c-erbB-2, CD117, and Bcl-2 expression in the histologically unequivocal cases is summarised in Table 1. All 20 cases that had been morpho-

Table 2. — *Molecular genotyping results in the histologically undetermined cases.*

Case	Clinical impression	Morphologic impression	p57 staining	Genotyping results	Final diagnosis
1	Missed abortion	HA *vs.* PHM	Positive	Biparental diploidy	HA
2	Missed abortion	CHM *vs.* PHM	Negative	Androgenetic diploidy	CHM
3	Incomplete abortion	CHM *vs.* PHM	Negative	Androgenetic diploidy	CHM
4	Incomplete abortion	CHM *vs.* PHM	Equivocal	Diandric triploidy	PHM
5	Hydatidiform mole	CHM *vs.* PHM	Equivocal	Diandric triploidy	PHM
6	Hydatidiform mole	CHM *vs.* PHM	Negative	Androgenetic diploidy	CHM
7	Hydatidiform mole	CHM *vs.* PHM	Negative	Androgenetic diploidy	CHM

HA, hydropic abortion; PHM, partial hydatidiform mole; CHM, complete hydatidiform mole.

logically diagnosed as CHM exhibited a striking lack of p57 positive staining in villous cytotrophoblasts and stromal cells. Although the percentage of positive p57 staining tended to be higher in HAs (76.5%) than in PHMs (73.9%), the difference was not significant ($p = 0.999$). Maternal decidua and intermediate trophoblasts showed strong p57 expression in contrast to the syncytiotrophoblast, which showed complete negativity in all cases regardless of the diagnosis.

c-erbB-2 expression was observed in all types of villous trophoblasts. The villous stromal cells and decidual cells showed negative immunostaining. A significant between-group difference was observed in the percentage of positive c-erbB-2 staining ($p = 0.003$). Pair-wise comparisons between the groups revealed a significantly higher percentage of positive c-erbB-2 staining in CMs (45%), compared with PHMs (8.7%) and HAs (5.9%) ($p = 0.006$ and 0.01, respectively). However, no significant difference was observed between PHMs and HAs ($p = 0.999$).

Cells positively expressing CD117 were restricted mostly to villous trophoblasts, whereas decidual and villous stromal cells showed weak immunostaining. The expression pattern (SI, PP, and IRS) was significantly lower in HAs compared with PHMs and CMs, as shown in Table 1. Although the expression pattern tended to be higher in CMs than in PHMs, the difference was not significant (adjusted $p > 0.017$).

Bcl-2 expression was observed in all types of villous trophoblasts. Villous stromal cells and decidual cells showed negative immunostaining. A significance between-group difference was observed in the Bcl-2 expression pattern ($p < 0.05$). A pair-wise comparison between the groups revealed significantly increased expression in HAs compared with PHMs and CHMs, and also in PHMs compared with CHMs (adjusted $p < 0.017$ for all)

Seven histologically undetermined cases included one case with a differential diagnosis between HA and PHM, whereas the other six cases had a differential diagnosis between PHM and CM (Table 2). DNA analysis by STR genotyping was performed to refine the histological subtypes of these cases. Case with a differential diagnosis between HA and PHM showed positive p57 expression and cytogenetic diploidy, consistent with a HA. Among the six cases with a differential diagnosis between PHM and CM, two cases showed equivocal results for p57 immunostaining and cytogenetic triploidy, consistent with PHM, and the remaining four cases showed negative p57 immunostaining and cytogenetic diploidy, consistent with CM.

Discussion

Accurate clinical diagnosis is important for distinguishing among CHM, PHM, and HA. A careful microscopic evaluation of the morphological features observed on haematoxylin and eosin-stained slides remains the cornerstone of diagnosis for these three entities. However, classifying HMs based solely on histological appearance can be extremely difficult, even for an experienced pathologist. In addition, HAs may exhibit atypical trophoblast proliferation leading to an erroneous diagnosis of HM. Several ancillary techniques have been applied to resolve these diagnostic problems, including immunohistochemistry, conventional cytogenetics (karyotyping), flow cytometry, digital image analysis, fluorescence *in situ* hybridisation (FISH), and molecular genotyping [13].

The value of an immunohistochemical analysis of the paternally imprinted, maternally expressed p57 gene for improving the diagnosis of HMs has been well established. p57 is a highly specific and sensitive marker for CHM due to an absence of nuclear staining in villous stromal cells and cytotrophoblasts [14-16]. The lack of p57 activity in CHM cases can lead to loss of cell cycle control, resulting in abnormal proliferation and differentiation of trophoblasts, correlated with histological features such as trophoblastic hyperplasia. In contrast, both PHMs and HAs contain a maternal chromosomal complement and express p57. This differential p57 staining pattern is helpful for distinguishing CHMs from PHMs and HAs. Rare examples of CHMs displaying aberrant (positive) p57 expression attributable to retention of the maternal copy of chromosome 11 have been reported [17]. Conversely, PHMs with loss of maternal chromosome 11 may show negative p57 expression [18]. Several studies have evaluated p57 immunohis-

tochemical staining and have revealed high concordance of the results with morphology, ploidy, and molecular genotyping studies [19, 20]. p57 immunostaining can also be helpful when refining the diagnosis of some morphologically challenging cases and for detecting androgenetic cell lines in mosaic/chimeric conception cases [21]. The present authors identified loss of p57 expression in all CHM cases. In the present cases, nearly all p57 staining results were readily interpretable as negative or positive, with the exception of two equivocal results encountered in cases proven to be PHMs by molecular genotyping. The authors confirmed that p57 is of no value when trying to distinguish PHM from HA, although it is helpful to distinguish CHM from PHM and HA, as both conditions show similar p57 expression patterns.

CD117 is a surface marker for embryonic, hematopoietic, and mesenchymal stem cells; it allows cells to remain in their undifferentiated state [22]. The interaction between CD117 with its ligand stem cell factor (SCF) promotes phosphorylation and activation of intracytoplasmic signal cascades essential for embryogenesis, hematopoiesis, proliferation, and migration of germ cells. In addition, binding of SCF to CD117 promotes tumour growth by promoting proliferation and/or by protecting tumour cells from death. Few studies have investigated CD117 expression in HMs. Ahmed *et al.* found that the CHM trophoblast cells express CD117 with variable intensity and localisation, but no comparison was made with HAs or with PHMs [23]. A recent study found that CD117 expression pattern does not differ among CHMs, PHMs and normal pregnancy [24]. The authors found a significantly increased CD117 expression pattern in CHMs and PHMs compared with that in HAs. This finding suggests that over-expression of CD117 may play a critical role in the aggressive behavior of CHMs.

Bcl-2 is a type of proliferation or maturation-related marker of trophoblasts that shows decreased expression along with terminal differentiation and maturation [25]. Previous studies have reported contradictory results in relation to Bcl-2 expression for discriminating molar and non-molar pregnancies. A study by Fulop *et al.* demonstrated significantly stronger Bcl-2 protein expression in CHMs and choriocarcinoma compared with that in both normal placentas and PHMs [26]. Al-Bozom showed that Bcl-2 staining pattern does not differ among CHMs, PHMs, and HAs [27]. Hussein reported strong Bcl-2 expression in chorionic villi from first trimester pregnancy terminations compared with CHMs and PHMs. Author suggested that the relatively moderate Bcl-2 expression in partial and CHMs may prevent apoptotic cell death of these atypical trophoblastic cells, allowing them to acquire a more malignant potential [12]. The present results demonstrated a significant decrease in Bcl-2 expression in CHMs and PHMs compared with HAs, inferring an increased apoptotic profile in molar pregnancy. Some of these discrepancies can be attributed to differences in the immunohistochemical staining method and the evaluation of Bcl-2 expression. The present authors propose that the variations in Bcl-2 expression among CHMs, PHMs, and HAs may be used as a potential adjunctive diagnostic tool to discriminate the three entities.

Immunohistochemical analysis of c-erbB-2 overexpression in HMs has been proposed as a predictor of persistent trophoblastic disease in several studies. Some authors found increased c-erbB-2 expression in CHM that progressed to a gestational trophoblastic tumour compared with those with spontaneous remission [28, 29]; however, this finding has not been corroborated [11]. Fulop *et al.* found that c-erbB-2 staining was significantly stronger in cases of CHM and choriocarcinoma compared with normal placenta and PHM [26]. Bauer *et al.* reported that high c-erbB-2 expression in combination with DNA hyperploidy is associated with more aggressive behavior of the GTD [30]. The present study is the first attempt to assess the value of c-erbB-2 expression to distinguish HMs from HAs. The present results demonstrate a higher percentage of c-erbB-2 expression in CHMs compared with PHMs, which supports the more aggressive characteristics of CHMs. In addition, immunohistochemical staining for c-erbB-2 provided useful diagnostic information to distinguish among CHMs, PHMs, and HAs.

Previous studies have shown that histological evaluations in combination with techniques that determine DNA content (ploidy) improve the accuracy of diagnosing HMs. These ancillary molecular techniques include conventional cytogenetics (karyotyping), flow cytometry, image analysis, and FISH [31-33]. DNA ploidy analysis can readily distinguish triploid PHM from diploid conceptions but cannot distinguish between CHM and HA because both are diploid. The most recent ancillary technique, molecular genotyping using PCR amplification of STR loci, allows for determination of both ploidy and the maternal/paternal contributions of chromosome complements. Unlike karyotyping, molecular genotyping does not require fresh tissue, as it can be performed on routine formalin-fixed paraffin-embedded material, making it particularly suitable for clinical practice. Other ploidy techniques, including FISH, can be performed on fresh paraffin-embedded tissue but occasionally produce results that are difficult to interpret because of maternal tissue contamination. Recent data suggest that molecular genotyping may have advantages over other molecular methods used to distinguish androgenetic diploidy, diandric triploidy, and biparental diploidy, which are characteristic of CMs, PHMs and HAs, respectively [34,35]. One study proposed a diagnostic working algorithm in combination with molecular genotyping and p57 immunohistochemistry to refine the diagnosis of HMs [36]. However, molecular genotyping methods are technically difficult, relatively expensive, time consuming, and not universally

available. Another limitation of genotyping is that maternal decidual tissue free of fetal tissue must be present for comparison of villous and paternal alleles. In the present study, STR genotyping was performed on the equivocal cases reported herein due to mixed histological features that did not conform to the expected findings for typical CHMs, PHMs, and HAs, and its use was crucial to determine the correct diagnosis.

Conclusion

Immunohistochemical examination of p57, c-erbB-2, CD117, and Bcl-2 expression is a relatively simple, reliable, and cost-efficient procedure to definitively distinguish among CHM, PHM, and HA. However, molecular techniques are still required for evaluating some challenging cases.

References

[1] Soper J.T.: "Gestational trophoblastic disease". *Obstet. Gynecol*., 2006, *108*, 176.

[2] Fukunaga M., Katabuchi H., Nagasaka T., Mikami Y., Minamiguchi S., Lage J.M.: "Interobserver and intraobserver variability in the diagnosis of hydatidiform mole". *Am. J. Surg. Pathol*., 2005, *29*, 942.

[3] Castrillon D.H., Sun D., Weremowicz S., Fisher R.A., Crum C.P., Genest D.R.: "Discrimination of complete hydatidiform mole from its mimics by immunohistochemistry of the paternally imprinted gene product p57KIP2". *Am. J. Surg. Pathol*., 2001, *25*, 1225.

[4] Klapper L.N, Kirschbaum M.H., Sela M., Yarden Y.:"Biochemical and clinical implications of the ErbB/HER signalling network of growth factor receptors". *Adv. Cancer. Res*., 2000, *77*, 25.

[5] Sharkey A.M., Jokhi P.P., King A., Loke Y.W., Brown K.D., Smith S.K.: "Expression of c-kit and kit ligand at the human maternofetal interface". *Cytokine*, 1994, *6,* 195.

[6] Roskoski R. Jr.: "Structure and regulation of Kit protein-tyrosine kinase-the stem cell factor receptor". *Biochem. Biophys. Res. Comm*., 2005, *338*, 1307.

[7] Mochizuki M., Maruo T., Matsuo H., Samoto T., Ishihara N.: "Biology of human trophoblast". *Int. J. Gynaecol. Obstet*., 1998, *60,* S21.

[8] Sgarbosa F., Barbisan L.F., Brasil M.A., Costa E., Calderon I.M., Gonçalves C.R, *et al.*: "Changes in apoptosis and Bcl-2 expression in human hyperglycemic, term placental trophoblast". *Diabetes Res. Clin. Pract*., 2006, *73*, 143.

[9] Wells M.: "The pathology of gestational trophoblastic disease: recent advances". *Pathology,* 2007, *39,* 88.

[10] Murphy K.M., McConnell T.G., Hafez M.J., Vang R., Ronnett B.M.: "Molecular genotyping of hydatidiform moles: analytic validation of a multiplex short tandem repeat assay". *J. Mol. Diagn*., 2009, *11*, 598.

[11] Menczer J.,Schreiber L.,Berger E.,Golan A., Levy T.: "Assessment of Her-2/neu expression in hydatidiform moles for prediction of subsequent gestational trophoblastic neoplasia". *Gynecol. Oncol*., 2007, *104*, 675.

[12] Hussein M.R.: "Analysis of p53, BCL-2 and epidermal growth factor receptor protein expression in the partial and complete hydatidiform moles". *Exp. Mol. Pathol*., 2009, *87*, 63.

[13] Uzunlar A.K., Yilmaz F., Bayhan G., Akkuş Z.: Expressions of p53, proliferating cell nuclear antigen, and Ki-67 in gestational trophoblastic diseases. *Eur. J. Gynaecol. Oncol.,* 2002, *23,* 79.

[14] Jun S.Y., Ro J.Y., Kim K.R.: "P57kip2 is useful in the classification and differential diagnosis of complete and partial hydatidiform moles". *Histopathology*, 2003, *43*, 17.

[15] Merchant S.H., Amin M.B., Viswanatha D.S., Malhotra R.K., Moehlenkamp C., Joste N.E.: "P57KIP2 immunohistochemistry in early molar pregnancies: emphasis on its complementary role in the differential diagnosis of hydropic abortuses". *Hum. Pathol.,* 2005, *36*, 180.

[16] Sarmadi S., Izadi-Mood N., Abbasi A., Sanii S.: "p57KIP2 immunohistochemical expression: a useful diagnostic tool in discrimination between complete hydatidiform mole and its mimics". *Arch. Gynecol. Obstet*., 2011, *283*, 743.

[17] McConnell T.G., Norris-Kirby A., Hagenkord J.M., Ronnett B.M., Murphy K.M.: "Complete hydatidiform mole with retained maternal chromosomes 6 and 11". *Am. J. Surg. Pathol.*, 2009, *33*, 1409.

[18] DeScipio C., Haley L., Beierl K., Pandit A.P., Murphy K.M., Ronnett B.M.: "Diandric triploid hydatidiform mole with loss of maternal chromosome 11". *Am. J. Surg. Pathol,.* 2011, *35,* 1586.

[19] Landolsi H., Missaoui N., Brahem S., Hmissa S., Gribaa M., Yacoubi M.T.: "The usefulness of p57(KIP2) immunohistochemical staining and genotyping test in the diagnosis of the hydatidiform mole". *Pathol. Res. Pract*., 2011, *207*, 498.

[20] Kipp B.R., Ketterling R.P., Oberg T.N., Cousin M.A., Plagge A.M., Wiktor A.E., *et al.*: "Comparison of fluorescence in situ hybridization, p57 immunostaining, flow cytometry, and digital image analysis for diagnosing molar and nonmolar products of conception". *Am. J. Clin. Pathol*., 2010, *133,* 196.

[21] Hoffner L., Dunn J., Esposito N., Macpherson T., Surti U.: "p57KIP2 immunostaining and molecular cytogenetics: combined approach aids in diagnosis of morphologically challenging cases with molar phenotype and in detecting androgenetic cell lines in mosaic/chimeric conceptions". *Hum. Pathol*., 2008, *39*, 63.

[22] Hassan H.T.: "c-kit expression in human normal and malignant stem cells prognostic and therapeutic implications". *Leuk. Res*., 2009, *33*, 5.

[23] Ahmed A., Lacson A., Gilbert-Barness E.: "Immunohistochemical expression of endothelial nitric oxide synthase and C-kit in the placenta of complete hydatidiform mole". *Fetal Pediatr. Pathol*., 2005, *24,* 141.

[24] Byramji A.T., Yee Khong T.: "Expression of c-kit in hydatidiform mole". *Pathology,* 2009, *41*, 193.

[25] Kim C.J., Choe Y.L., Yoon B.H., Kim C.W., Chi J.G.: "Patterns of bcl-2 expression in placenta". *Path. Res. Prac*., 1995, *191*, 1239.

[26] Fulop V., Mok S.C., Genest D.R., Szigetvari I., Cseh I., Berkowitz R.S.: "c-myc, c-erbB-2, c-fms and bcl-2 oncoproteins". Expression in normal placenta, partial and complete mole, and choriocarcinoma. *J. Reprod. Med*., 1998, *43*, 101.

[27] Al-Bozom I.A.: "p53 and Bcl-2 oncoprotein expression in placentas with hydropic changes and partial and complete moles". *APMIS*, 2000, *108*, 756.

[28] Yazaki-Sun S., Daher S., de Souza Ishigai M.M., Alves M.T., Mantovani T.M., Mattar R.: "Correlation of c-erbB-2 oncogene and p53 tumor suppressor gene with malignant transformation of hydatidiform mole". *J. Obstet. Gynaecol. Res*., 2006, *32*, 265.

[29] Yang X., Zhang Z., Jia C., Li J., Yin L., Jiang S.: "The relationship between expression of c-ras, c-erbB-2, nm23, and p53 gene products and development of trophoblastic tumor and their predictive significance for the malignant transformation of complete hydatidiform mole". *Gynecol. Oncol*., 2002, *85*, 438.

[30] Bauer M., Horn L.C., Kowalzik J., Mair W., Czerwenka K.: "C-erbB2 amplification and expression in gestational trophoblastic disease correlates with DNA content and karyotype". *Gen. Diagn. Pathol*., 1997, *143*, 185.

[31] Maggiori M.S., Peres L.C.: "Morphological, immunohistochemical and chromosome in situ hybridization in the differential diagnosis of hydatidiform mole and hydropic abortion". *Eur. J. Obstet. Gynecol. Reprod. Biol*., 2007, *135*, 170.

[32] Osterheld M.C., Caron L., Chaubert P., Meagher-Villemure K.: "Combination of immunohistochemistry and ploidy analysis to assist histopathological diagnosis of molar diseases". *Clin. Med. Pathol*., 2008, *1*, 61.

[33] Le Gallo R.D., Stelow E.B., Ramirez N.C., Atkins K.A.: "Diagnosis of hydatidiform moles using p57 immunohistochemistry and HER2

fluorescent in situ hybridization". *Am. J. Clin. Pathol*., 2008, *129*, 749.
[34] Lai C.Y., Chan K.Y., Khoo U.S., Ngan H.Y., Xue W.C., Chiu P.M., *et al.*: "Analysis of gestational trophoblastic disease by genotyping and chromosome in situ hybridization". *Mod. Pathol*., 2004, *17*, 40.
[35] Bifulco C. Johnson C., Hao L., Kermalli H., Bell S., Hui P.: "Genotypic analysis of hydatidiform mole: an accurate and practical method of diagnosis". *Am. J. Surg. Pathol.*, 2008, *32*, 445.
[36] Banet N., DeScipio C., Murphy K.M., Beierl K., Adams E., Vang R., *et al.*: "Characteristics of hydatidiform moles: analysis of a prospective series with p57 immunohistochemistry and molecular genotyping". *Mod. Pathol.,* 2014, *27,* 238.

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