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The role of deoxyribonucleic acid image cytometric and interphase cytogenetic analyses in the differential diagnosis, prognosis, and clinical follow-up of hydatidiform moles

A report from the central molar registration in the Netherlands

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OBJECTIVES: To assess the value of deoxyribonucleic acid ploidy in the differential diagnosis and clinical follow-up of hydatidiform moles, the histopathologic features, deoxyribonucleic acid ploidy, and clinical follow-up were compared in 347 cases: 143 complete moles, 52 partial moles, and 152 abortions, of which 56 cases were hydropic abortions with histologic features of triploidy but lacked trophoblastic hyperplasia.

STUDY DESIGN: In all cases deoxyribonucleic acid image cytometry was performed, and in 85 of these cases interphase cytogenetics was also performed.

RESULTS: With use of deoxyribonucleic acid image cytometry and interphase cytogenetics, a bimodal polyploid deoxyribonucleic acid pattern was present in 97% of complete moles, 27% of partial moles, and 4% of abortions. All these cases of partial mole were reclassified to complete mole on the basis of this deoxyribonucleic acid pattern and the histopathologic features in spite of the presence of fetal blood cells, amnion, or yolk sac. Deoxyribonucleic acid triploidy was found in 95% of the remaining partial moles, in 77% of hydropic abortions with histologic features of triploidy, and in 14% of the remaining abortions. Reliable differentiation between deoxyribonucleic acid triploid partial moles and hydropic abortions with histologic features of triploidy was not possible on basis of the histopathologic features (trophoblastic hyperplasia) or 3.5c exceeding rates. Deoxyribonucleic acid diploidy was found in 1% of complete moles, 23% of hydropic abortions with features of triploidy, and 78% of the remaining abortions. Deoxyribonucleic acid tetraploidy was rarely found (1% of complete moles, 2% of partial moles, 1% of abortions). Persistent gestational trophoblastic disease developed in 33% of the bimodal deoxyribonucleic acid polyploid cases (all complete moles), in 1% of the diploid cases (concerning one of the two diploid complete moles), and in 1% of the triploid cases (partial moles). CONCLUSION: Deoxyribonucleic acid analysis is essential in the diagnosis of hydatidiform moles to decide on clinical follow-up. (Am J Obstet Gynecol 1997;177:1219-29.)

Key words: Hydatidiform mole, ploidy, in situ hybridization, persistent gestational trophoblastic disease

Hydatidiform mole is defined as an excessive growth of placental tissues with trophoblastic hyperplasia and cystic swelling of the chorionic villi. After evacuation the majority of cases resolve spontaneously, but some persist and show progression toward metastatic disease or choriocarcinoma. On the basis of histopathologic and cytogenetic criteria, two entities are recognized^{1, 2}: the complete mole and the partial mole. Complete moles show diffuse villous edema and trophoblastic hyperplasia in the absence of an embryo. Complete moles have a diploid karyotype that is entirely paternally derived (androgenesis, either by duplication of the haploid genome of one sperm (always XX; YY is nonviable) or by dispermy (XX or XY in a ratio of 1:2).³⁵ Partial moles show focal villous edema and trophoblastic hyperplasia and evidence of an embryo. Partial moles have a triploid karyotype with one maternal and two paternal contributions to the genome (diandry)⁶.

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Hydatidiform mole must be differentiated from hydropic abortions. They show mild villous edema without trophoblastic hyperplasia. Various cytogenetic abnormalities can be found, with diploid or triploid karyotypes. Hydropic abortions have no increased risk for persistent gestational trophoblastic disease, which implies a major difference in clinical follow-up. In spite of well-described histopathologic criteria, the differential diagnosis of complete mole, partial mole, and hydropic abortion remains difficult.⁷ Several deoxyribonucleic acid (DNA) flow cytometric^{5, 7-14} and few DNA image cytometric studies^{10, 12, 13} have established the additional value of DNA cytometry in the differential diagnosis, but they have also reported a wider spectrum of DNA ploidy than the cytogenetic studies have. A high percentage of tetraploid complete moles and few cases of diploid and tetraploid partial moles were found.^{9, 11} The value of DNA analysis to predict the risk of persistent gestational trophoblastic disease after hydatidiform moles is controversial,^{8, 9, 11, 15} and up to now no reliable predictor is known. Therefore clinical follow-up with anticonceptive counseling and toring for ≥ 1 year is recommended.

Pathology Information System, a national automated registration of pathologic diagnosis in which all pathology laboratories in the Netherlands participate. Clinical follow-up after molar evacuation included serum hCG levels measured weekly until they returned to normal (<2 ng/ml) for 3 consecutive weeks and then monthly for 1 year. Persistent gestational trophoblastic disease was diagnosed when hCG levels persisted at a plateau or were elevated for ≥ 3 consecutive weeks. All hCG measurements were performed by means of a radioimnunoassay that measures both native and free β -subunits, as described before.¹⁹ The initial treatment of women with persistent gestational trophoblastic disease was methotrexate (1 mg/kg given intramuscularly on days 1, 3, 5, and 7) with folinic acid rescue (15 mg orally on days 2, 4, 6, and 8). When it was impossible to diminish the hCG levels to normal, the methotrexate regimen was changed to the EMA-CO regimen (etoposide 100 mg/m² infused) intravenously over 30 minutes on days 1 and 2; actinomycin D 0.5 mg intravenous push on days 1 and 2; methotrexate 100 mg/m² intravenous push, 200 mg/m² infused intravenously in 1000 ml of 5% dextrose in water over 12 hours on day 1; folinic acid 15 mg intramuscularly or by mouth every 12 hours for four doses beginning 24 hours after the start of methotrexate; cyclophosphamide 60 mg/m² intravenously on day 8; vincristine 1.0 mg/m^2 intravenous push on day 8) or the Hoog Brabant regimen (etoposide 100 mg/m² infused intravenously over 1 hour on days 1 to 5; methotrexate 100 mg/m^2 intravenous push, 200 mg/m² infused intravenously over 12 hours on day 1; cyclophosphamide 600 mg/m² intravenous push on day 1; actinomycin D 0.6 mg/m² intravenously over 3 hours on day 2; folinic acid, depending the methotrexate level, on day 2; cisplatin 60 mg/m² over 4 hours on day 4). The use of these two polychemotherapy regimens depended on the different institutions. The histologic slides of all cases were reviewed according to the criteria of Szulman and Surti.^{1, 2} A diagnosis of hydatidiform mole was not made if trophoblastic hyperplasia was lacking. If scalloped villous outlines and trophoblast inclusions were present but villous edema was mild and trophoblastic proliferation not present, a diagnosis of hydropic abortion with histologic features of triploidy was preferred instead of partial mole. **DNA image cytometry.** DNA image cytometry was performed on all cases, as previously described.¹⁰ A brief description is given here. Paraffin tissue blocks were preferentially selected on the amount of trophoblastic hyperplasia. Intact nuclei were isolated from $50 \ \mu m$ thick paraffin tissue sections with use of 0.1% protease digestion for 20 minutes. Maternal decidual tissue was processed separately and served as internal control for normal diploid cells. If decidual cells were not available, maternal lymphocytes were used as an alternative. The

In the current study we analyzed 347 cases of complete mole, partial mole, hydropic abortion, and nonhydropic abortion with respect to histopathologic and DNA image cytometric results and, in a selected number of these cases (n = 85), the results of interphase cytogenetic analysis as well. Of the 347 cases, 244 cases were referred to the Central Molar Registration of the Netherlands for consultation or review. The goals of this study were as follows: (1) comparison of the submitted histologic diagnosis with the review diagnosis, (2) to gain insight into the wide variability of DNA ploidy, (3) evaluation of the additional value of DNA ploidy analysis in the differential diagnosis, and (4) evaluation of the additional value of DNA ploidy analysis in the assessment of the risk of persistent gestational trophoblastic disease and consequently in the clinical follow-up policy. Preliminary results from this study have been published previously.^{10, 16-18}

Material and methods

Patients. Between 1988 and 1993 the 347 cases were collected at the University Hospital of Nijmegen. Of these cases, 244 cases were obtained from different pathology laboratories throughout the country who referred their cases to the Central Molar Registration of the Netherlands at the University Hospital of Nijmegen, where national registration of hydatidiform moles and persistent gestational trophoblastic disease is provided. This registration includes recording of data on clinical follow-up, histologic consultation and review, and supraregional laboratory services for hCG monitoring. Data on incidences were also obtained from the Dutch National

nuclear suspension was centrifuged on a glass slide with use of a cytocentrifuge (10 minutes at 500 revolutions/ min), air dried, and fixed in a mixture of methanol, 37% formaldehyde, and acetic acid (85:10:5 by volume) for 1 hour. The nuclei were then stained with pararosaniline-Feulgen, after which the DNA content of 200 intact nuclei of villous stromal cells and of trophoblast were selectively measured with use of the CAS 100 Image Analysis System (Cellular Imaging Systems, Becton-Dickinson, Leiden, the Netherlands). At least 30 rat liver cells (DNA tetraploid) were measured as an external control

Classification	Major peak	Minor peak		
	DNA-index	DNA-index		
DNA-diploid	1.0 ± 0.10	2.0 ± 0.20		
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for DNA content, whereas ≥ 20 decidual cells were used as internal control.

The DNA histograms were classified according to the criteria shown in Fig. 1. In the diploid or polyploid DNA histograms the percentage of nuclei with a DNA content exceeding that of the first diploid G_0/G_1 peak was determined as the 2.5c exceeding rate, defined as all nuclei with a DNA content of >2.5c (DNA index = 1.25) as a fraction of the total number of nuclei measured. The nuclear fractions with a DNA content exceeding the 3c G_0/G_1 peak in DNA triploid cases, or the 4c G_0/G_1 peak (G_0/G_1 exceeding rate) in DNA tetraploid cases, were calculated as the 3.5c and 4.5c exceeding rates, respectively. A 2.5c ER of ≥40% was used as a discriminator between DNA diploidy and bimodal DNA polyploidy.¹⁰ *Statistical analysis.* The mutual differences in exceeding



ing rates between the groups were analyzed by the Student *t* test for unpaired observations. Logistic regression analysis was performed on the subgroups of partial mole with DNA triploidy and hydropic abortion with DNA triploidy to assess whether the 3.5c exceeding rate could be used as a classifier to allocate individual patients to one of these two groups.

Interphase cytogenetics. In 85 selected cases for which the imaging cytometric DNA histogram was available, interphase cytogenetic analysis was performed on 6 μ m thick paraffin-embedded tissue sections. The 85 cases consisted of 41 cases of complete mole (40 cases bimodal DNA polyploid, 1 case DNA tetraploid), 7 twins with complete mole, 21 cases of partial mole (6 cases DNA triploid, 14 cases bimodal DNA polyploid, and 1 case DNA diploid), 9 cases of hydropic abortion (6 cases DNA

triploid, 1 case DNA diploid, 2 cases DNA tetraploid), and 7 cases of abortions without hydropic degeneration (6 cases DNA diploid, 1 case bimodal DNA polyploid). The following chromosome-specific DNA probes were used^{10, 16}: the satellite III DNA probe for chromosome 1 (pUC 1.77), the alphoid DNA probe for chromosome X (pBam X5), and the satellite III DNA probe for chromosome Y (DYZ3), recognizing tandem repeats in the (peri)centromeric regions of chromosomes 1 and X and in the q arm of chromosome Y, respectively. Biotinylation of the probes was performed with use of



Fig. 1. Classification of DNA histograms.

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Table I. Comparison between submitted histopathologic diagnosis and revised diagnosis

	Submitted diagnosis							
Revised diagnosis	CM	PM	Twin with CM	NM	Total			
CM	77	34	()	4	115			
PM	12	24	()	8				
Twin with CM	l	3	3	()	7			
NM	8	27	0	18	53			
TOTAL	98	88	3	30	219			

CM, Complete mole; PM, partial mole; NM, nonmolar pregnancy.

result of tissue sectioning at 6 μ m, not all nuclei showed the maximum copy number. This percentage of nuclei with the maximum copy number decreased with increasing copy number of chromosomes.^{16, 20} In situ hybridization ploidy was based on the results of all three DNA probes. A specimen was considered to be diploid if the maximum in situ hybridization copy number did not exceed two signals in >10% of the nuclei. In the same manner, in triploid cases the maximum copy number should not exceed three signals in >10% of the nuclei, and in tetraploid cases this maximum in situ hybridization copy number should not exceed four signals in >10% of the nuclei. The occurrence of focal numeric aberrations in, for example, extravillous trophoblast was evaluated by counting 500 nuclei in these areas. In otherwise diploid cases focal polyploidy was considered to be present if in that area >10% of the nuclei displayed more than two in situ hybridization signals. Likewise, in triploid cases focal polyploidy was considered to be present if more than three signals and in tetraploid cases more than four signals were present.

Fig. 2. A, Complete mole illustrating formation of new villi, giving branching aspect with deep invaginations and progressive edematous degeneration. Trophoblastic hyperplasia is prominent. Note presence of capillary (*arrow*). (Hematoxylin-eosin stain. Original magnification $\times 50$.) **B**, Partial mole showing scalloping of villous outlines with small inclusion cyst (*arrow*) and excess of vacuolated syncytiotrophoblast. (Hematoxylin-eosin stain. Original magnification $\times 150$.)

Bio-14-deoxyadenosine triphosphate (BRL, Gaithersburg, Md.) according to the supplier's instructions. The in situ hybridization procedure in paraffinembedded tissue sections was performed as previously described,^{10,16} with minor modifications in the immunohistochemistry step: mouse antibiotin (1:100 in phosphate-buffered saline solution-Tween with 5% nonfat dry milk, Dakopatts, Glostrup, Denmark) was followed by biotin-labeled horse antimouse (1:200 in phosphate-buffered saline solution-Tween, 5% nonfat dry milk, Vector, Burlingame, Canada) and avidinbiotin complex (1:100 in phosphate-buffered saline solution-Tween, 5% nonfat dry milk, Vector). Evaluation of in situ hybridization signals. In all cases DNA probes specific for chromosomes 1, X, and Y were used to determine in situ hybridization ploidy and sex chromosome composition. The overall in situ hybridization ploidy of the whole specimen was determined in the chorionic villi by counting the number of signals in 500 nuclei of fibroblasts. Because of truncation of nuclei as a

Results

Histopathologic features. In 219 of the 244 referred cases a submitted diagnosis was provided by the pathologists who had initially seen the cases. The overall agreement between the submitted diagnoses and the revised diagnoses was only 55.7% (Table I). The percentage of agreement for the diagnosis partial mole was especially low (27.3%). Of the submitted cases of partial mole, 42.0% were revised to a diagnosis of complete mole and 30.7% to a diagnosis of nonmolar abortion. Histopathologic features of complete mole that frequently resulted in a false diagnosis of partial mole were found to be (1) presence of chorionic villi of seemingly normal size, as a result of gradual accumulation of edema in newly formed villi, giving the impression of edema to be focal (Fig. 2, A); (2) the presence of irregular branching of chorionic villi, giving the appearance of deep invaginations and large inclusion cysts on tangential cut, which are different from the scalloping

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Fig. 4. In complete mole trophoblastic hyperplasia shows prominent nuclear atypia. (Hematoxylin-eosin stain. Original magnification $\times 350$.)

presence or absence of trophoblastic hyperplasia, which was found to be a subjective criterion for a diagnosis of partial mole.

In 7 of the 347 cases a diagnosis of a twin pregnancy with complete mole was suspected on the basis of the presence of two separate villous populations. One part of the villi was normal with capillaries containing intact nucleated red blood cells, whereas the other villi were clearly abnormal with stromal edema and karyorrhexis, no capillaries or rudimentary capillaries without embryonal red blood cells, and with markedly atypical trophoblastic hyperplasia. In three cases an embryo was found. Part of these cases have been published in detail elsewhere.¹⁷

Fig. 3. In complete mole capillaries can be present, usually containing cellular debris (A) and rarely vital nucleated red blood cells (B, *arrow*). (A, Hematoxylin-eosin stain. Original magnification $\times 250$. B, Hematoxylin-eosin stain. Original magnification $\times 400$.)

villous outlines with small inclusion cysts in partial mole (Fig. 2, B); and (3) the presence of capillaries. The presence of rudimentary capillaries in the villous stroma of otherwise typical complete moles was found to be a frequent phenomenon. In 17% of the cases of complete mole these capillaries were prominent with lumina containing cellular and nuclear debris suggesting degenerated embryonal red blood cells (Fig. 3, A). In 11 cases of otherwise typically complete moles nucleated red blood cells, amnion, or yolk sac were found (Fig. 3, B), on the basis of which a diagnosis of partial mole was formally given, but with a side note that atypical trophoblastic hyperplasia was similar to that found in complete mole. In our experience the most reliable criterion for a diagnosis of complete mole was trophoblastic hyperplasia with prominent nuclear atypia, especially of the intermediate trophoblastic cells (Fig. 4). This was never found in a typical partial mole. Syncytiotrophoblast with vacuolization was not exclusive for partial mole. It was also frequently found in complete mole. The disagreement between the diagnoses of partial mole and hydropic nonmolar abortion was mostly based on the

DNA image cytometry. The results of DNA image cytometry are given in Tables II and III and Fig. 5.In complete mole a bimodal DNA polyploidy was found in 97% of cases, and one case was DNA tetraploid.The case that appeared to be DNA triploid was considered as a misclassified case of partial mole.

In the seven cases of twin pregnancy with complete mole, the normal and the molar villi had been separately processed. The normal villi showed a bimodal DNA diploid pattern and the molar villi a DNA polyploid pattern similar to that found in the cases of complete

mole.

The majority of partial moles (69%) showed the expected DNA triploidy. One case was DNA tetraploid and histopathologically very similar to the case of DNA tetraploid complete mole. Both cases were histologically borderline cases between complete and partial mole with slight to moderate nuclear atypia of the hyperplastic trophoblast. The G_0/G_4 exceeding rate (4.5c) was low, 29.5% and 22.0%, respectively. Embryonal tissues were found in the case of partial mole but not in the case of complete mole. In 11 of the 14 cases of partial mole with a bimodal DNA polyploidy, embryonal tissues (nucleated)

Table	II.	DNA	ploidy in	hydatidiform	moles	and abortion	s by in	nage cytometi	ic analysis
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	DNA ploidy pattern							
Histologic diagnosis before DNA analysis	Total	Diploid cases (No., %)	Triploid cases (No., %)	Tetraploid cases (No., %)	Polyploid cases (No., %)			
Abortion	152	88 (58)	56 (37)	2(1)	6 (1)			
Without hydropic degeneration	4.8	41 (85)	3 (6)		4(8)			
With hydropic degeneration	-18	34(71)	10(21)	2(4)	2(.1)			
With hydropic degeneration and histologic features suggestive of "triploidy"	56	13 (23)	43 (77)					
Partial mole	52	1 (2)	36(69)	1 (2)	14(27)			
Twin with complete mole	7				7 (100)			
Complete molê	136	2(1)	1 (1)	1 (1)	132 (97)			
TOTAL	3.17	91	93		159			

Table III. Two-tailed *p* values of statistical differences among complete moles, partial moles, and hydropic abortions

Diagnostic group	CM	ЪЛА	PM	PM + T	FIA + Z
CM		p < 0.0001	p = 0.02	p < 0.0001	p < 0.0001
HA		*	p < 0.0001	p = 0.87	p < 0.0001
PM				p < 0.0001	p < 0.0001
PM + T					p = 0.001
HA + T					·····

CM, Complete mole; *HA*, hydropic abortion with DNA diploidy or polyploidy; *PM*, partial mole; *T*, DNA triploidy.

Fig. 5. G_0/G_1 exceeding rates of DNA diploid and DNA polyploid cases (2.5c exceeding rate) and of DNA triploid cases (3.5c exceeding rate). The 2.5c exceeding rate of seven twins with complete mole (*CM*) and 4.5c exceeding rate of four DNA tetraploid cases are not shown. G_0/G_1 exceeding rate of DNA diploid and DNA triploid cases are in same range and did not exceed value of 40%. In DNA polyploid cases G_0/G_1 exceeding rate of 40%. In DNA polyploid cases G_0/G_1 exceeding rate of 40%. (*H*)A, Hydropic abortion; *PM*, partial mole; *HA*+*T*, hydropic abortion with histopathologic features of triploidy.

red blood cells, amnion, or yolk sac) were found, and these cases were histopathologically classified as partial mole, as described above, with a side note of atypical trophoblastic hyperplasia as usually found in complete mole. significant difference was found between the 3.5c exceeding rate of DNA triploid partial mole and the lower 3.5c exceeding rate of DNA triploid hydropic abortion (p = 0.001), there was a considerable overlap between the 3.5c exceeding rate of each subgroup. Logistic regression analysis showed that it was not possible to distinguish between these two groups.

Interphase cytogenetics. The 40 cases of complete mole, all with a bimodal polyploid DNA image cytometric pattern, showed in situ hybridization diploid chorionic villi and polyploid extravillous intermediate trophoblastic cells (Fig. 6). The mean percentage of in situ hybridization polyploid nuclei in the 6 μ m thick tissue sections was 23% (range 10% to 44%). The majority of this percentage of in situ hybridization polyploid nuclei showed three signals (14%) or four signals (7%) nuclei, but a small percentage (3%) of nuclei showed signals of up to twelve spots. These in situ hybridization signals were often larger in size and more intensely stained then the signals in the chorionic villi. In situ hybridization tetraploid villi were not found.

In the majority of cases of (hydropic) abortion DNA diploidy or DNA triploidy was found. Few cases were DNA polyploid with a maximum 2.5c exceeding rate of 54%. Implantation trophoblast was prominent in these cases but without the nuclear atypia found in complete mole. Two cases of hydropic abortion were DNA tetraploid. Histopathologically they were not different from the other cases of hydropic abortion.

Fig. 5 gives the range of G_0/G_1 exceeding rate (2.5c and 3.5c) of the DNA diploid, DNA polyploid, and DNA triploid cases. Table III gives the two-tailed *p* values of statistical differences between the G_0/G_1 exceeding rate of the subgroups, as depicted in Fig. 5. Although a

In the seven twin cases the molar villous population showed the same pattern as complete mole, with in situ hybridization diploid villi and polyploid extravillous trophoblast, whereas the normal villi with implantation trophoblast showed a diploid pattern. There were three

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cases with different sex chromosomes in the normal and molar villi, demonstrating dizygosity.

The six cases of partial mole and the six cases of hydropic abortion with both histologic features of triploidy and image cytometric results of triploidy all showed a uniform in situ hybridization triploid pattern in both villi and extravillous trophoblast. Nuclei with more then three signals were only rarely (4%) found in the latter component, mainly associated with fibrinoid deposits in which nuclei often showed degeneration and disintegration of the in situ hybridization spots. All 14 cases of partial mole with a bimodal polyploid DNA image cytometric pattern were investigated and showed the same pattern as complete mole: in situ hybridization diploid villi and polyploid extravillous trophoblast. In 11 cases with nucleated red blood cells, amnion, or yolk sac, these structures showed the same sex chromosome composition as the molar villous cells. In the eight cases of abortion, seven cases with a diploid DNA image cytometric pattern and one case with a bimodal polyploid DNA image cytometric pattern (2.5c exceeding rate 54%), in situ hybridization diploidy was found in chorionic villi and in implantation trophoblast. In the latter areas only a few in situ hybridization polyploid nuclei were found (5%).

The cases of complete mole, partial mole, and hydropic abortion in which a tetraploid DNA image cytometric pattern was found showed a uniform in situ hybridization tetraploid pattern in both villi and extravillous trophoblast. The sex chromosome composition was XXXY in complete mole, XXYY in partial mole, and XXXX and XXYY in the hydropic abortions. Polyploidization in extravillous trophoblast as seen in the DNA polyploid complete mole was not found. Clinical follow-up. Clinical follow-up for ≥ 1 year was available for all cases of hydatidiform mole, with exception of eight cases (six cases of complete mole and two cases of partial mole) in which patients were lost to clinical follow-up. In the cases of hydropic abortion with histologic and cytometric features of triploidy, inquiries for data of clinical follow-up were retrospectively made and obtained in all cases. Table IV gives the results on the occurrence of persistent gestational trophoblastic disease requiring chemotherapy. All women with persistent gestational trophoblastic disease were given methotrexate and folinic acid rescue 5 to 20 weeks (median 10 weeks) after evacuation of the mole. In 32 (58%) women the indication for treatment was a rise in the serum hCG levels; the other woman showed a plateau in the serum hCG level for ≥ 3 consecutive weeks. The median of the hCG level before treatment was 980 ng/ml (range 19 to 10,000 ng/ml). We gave 5 to 14 courses (median 7 courses). Two women had lung metastases during methotrexate treatment and were switched to polychemotherapy. One woman under-

Fig. 6. Interphase cytogenetics on tissue sections 6 μ m thick with use of biotinylated DNA probe specific for pericentromeric region of chromosome 1 (hematoxylin counterstain). In complete mole nuclei of villous trophoblast and stromal cells show disomy (*arrow*), in contrast to polysomy found in markedly enlarged and atypical nuclei of extravillous trophoblast (*double arrow*). (Original magnification $\times 400$.)

went a hysterectomy because the hCG level rose during treatment. In 7 women (13%) it was impossible to get hCG levels to the normal range, or there was an elevation, so they received polychemotherapy. Three women received the EMA-CO regimen and the other 4 the Hoog Brabant regimen. None of these women was lost to

follow-up.

We found only one women with a correctly classified (DNA triploid) partial mole in whom persistent gestational trophoblastic disease developed (see Table IV). Four weeks after the first evacuation a suction curettage was repeated because the hCG level was elevated. Eight weeks after the first evacuation the hCG level was elevated again to 250 ng/ml and we started methotrexate and folinic acid. The patient received five courses, after which she was in complete remission.

In complete mole and bimodal DNA polyploid cases initially diagnosed as partial moles, no differences were

Table IV. Frequency of persistent gestational trophoblastic disease in hydatidiform mole related to DNA ploidy

	Cases with persistent gestational trophoblastic disease							
Histologic diagnosis	Total	Diploid cases	Triploid cases	Tetraploid cases	Polyploid cases			
Abortion	0/152	0/88	0/56	0/2	0/6			
Without hydropic degeneration	0/48	0/41	0/3		0/4			
With hydropic degeneration	0/48	0/34	0/10	0/2	0/2			
With hydropic degeneration and histologic features suggestive of triploidy	0/56	0/13	0/43					
Partial mole	5/52	0/1	1/36	0/1	4/14			
Twin with complete mole	2/7		. Jania Manuella III.		2/7			
Complete mole	47/136	1/2	0/1	0/1	46/132			
TOTAL	54/347	1/91	1/93	0/4	52/159			

Table V. DNA classification of hydatidiform moles in different studies

		Complete moles							Partial moles					
Study	No.	Diploid (%)	Tetraploid (%)	Polyploid (%)	Triploid (%)	Aneuploid (%)	No.	Diploid (%)	Tetraploid (%)	Polyploid (%)	Triploid (%)	Aneuploid (%)		
Hemming et al. ⁸	49	94	0		4	2	32	31	3		63	3		
Lawler et al. ⁵	151	85	0		1	- Andrewsky and a state of the	49	2	4		86			
Lage et al. ⁹	56	50	43		2	0	49	2	2	8	82	2		
Conran et al. ⁷	21	90	10		0		29	0	0		100			
Fukunaga et al. ¹¹	129	71	19		0	9	43	5	0		95	0		
Berezowsky et al. ¹²	32	53	47	· · · · · · · · · · · · · · · · · · ·	0		18	11	0	Statistica in the second s	89			
Jeffers et al. ¹³	14	36	- Andrew Connector	50	0	14	15	13	a Tai - Thriannit	0	87	0		
Paradinas et al. ¹⁴	150	99*		· · · · · · · · · · · · · · · · · · ·	1		150	0*	***		97			
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 Current study
 143
 1
 1
 97
 1
 -- 52
 2
 27
 2
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*DNA diploid and DNA tetraploid cases were taken together in this study. +Cases were reclassified as complete moles on basis of the DNA cytometry results.

found between the cases with and without persistent gestational trophoblastic disease with respect to histologic features, DNA ploidy, 2.5c exceeding rate, percentage of in situ hybridization polyploid nuclei in the extravillous trophoblast, or the XX/XY ratio. A detailed study on this subject, in which part of the cases of the current study was used, has recently been published.¹⁸ Persistent gestational trophoblastic disease developed in 4 of the 14 bimodal DNA polyploid cases of partial mole. All 4 cases showed nucleated red blood cells, amnion, or yolk sac structure. The case of persistent gestational trophoblastic disease after DNA triploid partial mole had a 3.5c exceeding rate of 19 and showed no differences from the other cases of DNA triploid partial mole or hydropic abortion with histologic features of triploidy. Pretreatment serum hCG levels were known in 32 patients with complete mole not followed by persistent gestational trophoblastic disease and in 15 patients with complete mole followed by persistent gestational trophoblastic disease. No correlation was found between the hCG level and the 2.5c exceeding rate or the occurrence of persistent gestational trophoblastic dis-Case.

Comment

In the current study the results of DNA image cytometry in the cases of complete mole seem quite different from those of all other studies published in the literature (Table V). The image cytometric DNA histograms that were found to be characteristic of complete mole in this study, as well as the differences with data of previous studies, can be explained by the results of the DNA in situ hybridization findings. The bimodal polyploid DNA histogram of complete mole has a DNA diploid G_0/G_1 peak because of the presence of in situ hybridization diploidy in the different cell types of the chorionic villi and a second major DNA tetraploid G_0/G_1 peak with S-phase cells and a small DNA octaploid G_2/M peak because of the presence of proliferating in situ hybridization tetraploid cells in the extravillous trophoblast. In most cases some trophoblastic cells showed more than four chromosome copies in these areas, most likely the consequence of doubling of the chromosomes of the tetraploid cells, which was not followed by nuclear and cell division (endomitosis or endoreduplication). An increase in spot size also indicates the presence of doubling of the chromatides without division of the chromosome (polyteny). Both mechanisms result in polyploidization. It is

difficult to detect such high copy numbers of chromosomes in thin tissue sections because it has been shown that the probability to find all in situ hybridization spots in a truncated nucleus decreases with the increase of chromosome copies.²⁰ The polyploidy found in the extravillous trophoblast corresponded well with the prominent nuclear atypia in these areas, which, in our experience, was the most typical and constant histopathologic feature of complete mole.

Our interphase cytogenetic studies have disclosed that the bimodal polyploid DNA histogram must be discerned from the unimodal diploid DNA histogram and the unimodal tetraploid DNA histogram. The former is characterized by a major DNA diploid G_0/G_1 peak with S-phase cells and a small DNA tetraploid G₆/M peak and was seen in cases with in situ hybridization diploidy in both the chorionic villi and extravillous (implantation) trophoblast. A good criterion to distinguish the DNA diploid and the bimodal DNA polyploid histogram appeared to be a 2.5c exceeding rate >40.¹⁰ The unimodal tetraploid DNA histogram is characterized by missing the major DNA diploid G_0/G_1 peak of the bimodal DNA polyploid histogram. All our cases with a tetraploid DNA histogram exhibited in situ hybridization tetraploidy in both the chorionic villi and extravillous trophoblast. The bimodal polyploid DNA histogram was found in 97% of the histopathologically diagnosed complete moles and in all cases histopathologically diagnosed as twin pregnancies with complete mole. Unimodal DNA diploidy was found in only 1% of our cases of complete mole, which could be explained by the small amounts of extravillous trophoblast in the paraffin tissue blocks that were analyzed. Unimodal DNA tetraploidy was found to be rare (1%) in complete moles. The bimodal polyploid DNA pattern was probably not always recognized or differently interpreted in other studies (see Table V). The results of the classic karyotyping studies, showing that complete moles are diploid, can easily be explained by the fact that these studies are based on cultures of fibroblasts, which are derived from the diploid chorionic villi. When molar trophoblast is selectively cultured, a 2.4 times greater percentage of polyploid nuclei is obtained than in molar fibroblast or normal trophoblast.²¹ The results of some DNA flow cytometric studies^{5, 7-9, 14-14} showing variable frequencies of DNA diploid and DNA tetraploid complete moles can be partly explained by a variable admixture of maternal cells obscuring the DNA tetraploid G_0/G_1 peak in cases classified as DNA diploid and by incorrectly ascribing the DNA diploid peak to the maternal cells and the DNA tetraploid peak to the placental cells in cases classified as DNA tetraploid. In one of these studies¹² DNA image cytometry was also performed on nuclear suspensions and tissue sections. The studies on tissue sections confirmed the presence of tetraploid cells in the extravillous trophoblast next in

which DNA flow and image cytometry on nuclear suspensions were performed; with use of both methods DNA tetraploidy and DNA polyploidy were respectively found in 47% and 50% of the cases of complete mole. The discrepancy with our study with use of DNA image cytometry in which DNA polyploidy was found in 97% of complete moles might be explained by differences in the selection of the paraffin tissue blocks used for DNA analysis and by differences in the isolation procedure of nuclei from these paraffin tissue blocks.

It must be discouraged to interpret a bimodal polyploid DNA histogram in complete moles as DNA tetraploid because this will give a false impression of a high incidence of DNA tetraploid complete moles. Genuine tetraploid moles have been described with a triple paternal DNA contribution and one copy of maternal DNA.⁵ Some of these cases were originally histologically classified as complete moles but are in fact partial moles. Because of a relative larger contribution of paternal DNA compared with triploid partial moles, the morphologic features of these tetraploid cases might be in between those of partial and complete moles. This may also be the case for the risk of persistent gestational trophoblastic disease, but this is not yet known. Therefore correct classification and clinical follow-up is mandatory.

In 27% of the histopathologically diagnosed cases of partial mole a bimodal polyploid DNA pattern was found. In all these cases interphase cytogenetic analysis disclosed in situ hybridization diploidy in chorionic villi and polyploidy in extravillous trophoblast, which showed a corresponding prominent nuclear atypia. On basis of these DNA image cytometry and in situ hybridization findings, we concluded that these cases must be considered as misclassified cases of complete mole in spite of the presence of fetal red blood cells, amnion, or yolk sac in 11 cases. In two of these cases the androgenetic origin could be proved in a separate experiment with cytosine adenosine repeat analysis (data not shown). From experimental mouse studies²² and from our own observations and those of others,¹⁴ it must be concluded that initial embryonal development takes place in complete mole and that the presence of capillaries with nuclear debris and the occasional presence of vital nucleated red blood cells, amnion, or yolk sac does not exclude a diagnosis of

complete mole.

In the remaining cases of partial mole, virtually all cases were DNA triploid (95%). One DNA diploid case was on review considered as a misclassified case of hydropic abortion. Differentiation between partial mole and hydropic abortion is extremely difficult, as has been illustrated by previous publications⁷ and by our own findings. Partial moles are characterized by histopathologic features that are present in a variable and focal way. If trophoblastic hyperplasia is taken as an indispensable condition for the diagnosis of a hydatidiform mole, a

diagnosis of partial mole can be made in only few cases. Differentiation from implantation trophoblast in hydropic abortion is often difficult and subjective. If partial mole and hydropic abortion with histopathologic features of triploidy are taken together, then the trophoblast is often more hypoplastic than hyperplastic. With use of DNA image cytometry a significant difference was found between the 3.5c exceeding rate of these two groups (p = 0.001), but the overlap was too extensive to be of any help in the diagnosis. Reliable differentiation between partial mole and hydropic abortion with histopathologic features of triploidy is not possible on the basis of histopathologic or DNA image cytometric features. It is possible that both groups have the same cytogenetic background of diandric triploidy and must be considered as partial moles.²³ DNA analysis showed that the histopathologic criteria of triploidy cannot be relied on, even when used by pathologists with ample experience. In 10% to 20% of the cases DNA diploidy was found. These patients would have been given clinical follow-up unnecessarily if DNA analysis was not performed. With image cytometry DNA triploidy was found in 14% of the cases of abortion without histopathologic features of triploidy. It is assumed that these cases represent digynic triploids without increased risk of persistent gestational trophoblastic disease.²⁴ From Table II and the conclusions drawn above, it is evident that it can be misleading to rely solely on the histopathologic features for the diagnosis of complete mole, partial mole, and hydropic abortion. The histopathologic diagnosis is subject to interobserver variability and differences in experience between pathologists. The use of additional techniques, such as described in this study, adds an objective parameter to the diagnosis on which the histopathologic criteria can be tested and revised and that helps in the classification of unusual cases. In this study persistent gestational trophoblastic disease developed in 33% of the bimodal DNA polyploid cases (all being cases of complete mole). No correlation was found between the development of persistent gestational trophoblastic disease and the degree of DNA polyploidization, as determined from the 2.5c exceeding rate and from the results of interphase cytogenetics.¹⁸ Unlike in somatic tumors, in complete mole nuclear atypia and a corresponding increase in DNA content do not seem to be associated with tumor progression. This is in accordance with the results of most other studies^{8, 9, 11} that were based on flow cytometry. Of the DNA triploid cases, only one case progressed to persistent gestational trophoblastic disease (1%). This case was diagnosed as a partial mole. No differences were found in histopathologic features or 3.5c exceeding rate with the other cases of partial mole. Different data on the incidence of persistent gestational trophoblastic disease after partial mole are reported in the literature, varying from $0.5\%^{25}$

to 5.5%,⁹ the investigators assuming 1% of pregnancies to be triploid and 86% of triploids to be partial moles.²³ For the Netherlands, with 200,000 pregnancies each year, this means that each year a diagnosis of partial mole would be made in about 1720 patients, and that 9 to 95 patients would have persistent gestational trophoblastic disease. However, the mean number of registered cases each year is 25 (virtually all are complete moles), which renders the data for development of persistent gestational trophoblastic disease after partial mole given in the literature very unlikely. In these studies, as in our own study, a strong selection bias occurred because reference centers are involved that are likely to handle more complicated cases. When all cases of hydropic abortion with histologic and DNA cytometric features of triploidy are taken as partial moles, then the frequency of persistent gestational trophoblastic disease is very low (<0.5%). If DNA triploidy is not confirmed by DNA analysis, many cases will be missed or overdiagnosed. Therefore a rational basis for justifying a clinical follow-up policy as in complete mole (postponement of pregnancy and continuing monitoring of the serum β -hCG levels after normalization) is lacking. Differences in the risk of persistent gestational trophoblastic disease after complete or partial mole can be important for the individual patient with respect to emotional distress, duration of follow-up,

and the planning of future pregnancies.

In conclusion, we state that the following. (1) Complete moles have specific histopathologic features (e.g., atypical trophoblast hyperplasia) and a characteristic bimodal polyploid DNA pattern. Genuine tetraploid hydatidiform moles are rare. (2) Partial moles have histopathologic features that are focal and nonspecific. A DNA triploid pattern easily differentiates partial mole from complete mole but not from nonmolar hydropic abortion. If trophoblastic hyperplasia is not considered as an indispensable condition for the diagnosis of partial mole, many cases of partial mole have been missed in the past and many cases of nonmolar hydropic abortion are likely to be overdiagnosed as partial mole in the future when DNA analysis is not performed. (3) Persistent gestational trophoblastic disease must be considered as a rare phenomenon of partial mole. (4) DNA analysis is mandatory for the correct classification of hydatidiform moles and nonmolar hydropic abortions. (5) DNA analysis has no direct predictive value for the development of persistent gestational trophoblastic disease.

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