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Quantitative morphology

A study of the trophoblast*

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Summary. It is difficult to predict the possible development of a malignant trophoblastic tumor after the evacuation of a hydatidiform mole. In order to help resolve this difficulty, a morphometric study has been carried out. The mean nuclear area of the trophoblast in a group of hydatidiform moles, followed by a trophoblastic malignancy, was found to be statistically significantly larger than that of the trophoblast in a group of hydatidiform moles which were not followed by malignant trophoblastic disease. However, the mean trophoblast/nontrophoblast ratio in villi demonstrated no statistically significant difference between those 2 groups of hydatidiform moles. Therefore it is not advisable to grade hydatidiform moles on the basis of trophoblastic proliferation alone.

It is suggested that the trophoblastic lining of hydropic villi in the placental tissue of hydatidiform moles has malignant features already, but these are more pronounced in those hydatidiform moles which are subsequently followed by a choriocarcinoma.

Key words: Hydropic degeneration – Hydatidiform mole – Choriocarcinoma – Quantitative morphology

Introduction

In a previous article (Franke et al. 1983), we described a qualitative morphological study of placental tissue and the serum hCG disappearance time, in an attempt to predict the development of choriocarcinoma after the evacuation of hydatidiform mole. The results of this study, agreed with those of Curry et al. (1975) in showing no statistically significant difference be-

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tween various degrees of trophoblastic proliferation. In several publications measurements of nuclear features have been made to quantify the nuclear changes associated with malignancy (Goldfarb et al. 1971; Baak et al. 1981; Ooms 1981; Kwee 1982).

In this study some variables in histological material of 5 groups of patients have been investigated in order to differentiate between the various groups and to predict the development of choriocarcinoma after a hydatidiform mole.

Material and methods

The histological samples of placental tissues were taken from 5 different groups of patients, as presented in Table 1. The gestational ages of the 5 groups of patients were between 75 and 105 days, from the first day of the last menstrual period to the evacuation of the uterine contents. The histological material was fixed in 5 per cent buffered formalin. The approximately 5 μm thick sections, embedded in paraffin, were stained with haematoxylin and eosin (HE).

Histological criteria. The differentiation between hydatidiform mole and hydropic degeneration was made according to Franke et al. (1983).

Hydatidiform mole. Hydropic swelling of stromal tissue of the chorionic villi is evident. Embryonal structures are absent and therefore no erythroblasts can be found in capillaries of the villi. The trophoblast lining of the hydropic villi is characterized by irregular proliferations, variable degrees of pleomorphism and vacuolization of cytoplasm (Fox 1978; Szulman and Surti 1978; Harris et al. 1981).

Hydropic degeneration. Large swollen villi are found with a thin trophoblastic lining. Trophoblastic cells have little cytoplasm. Stromal inclusions and erythroblasts in the stromal capillaries are sometimes seen. Hydropic degeneration is associated with chromosomal abnormalities such as triploidy (69 chromosomes) and trisomy (Szulman et al. 1981). The stromal trophoblastic inclusions are the representation in histological sections of an irregular surface (Franke et al. 1983).

Two groups of hydropic degeneration were studied, depending on to the presence or absence of stromal trophoblastic inclusions.

Morphometric methods

Profile radii of villi. Black-and-white photographs were taken at 11 × magnification of villi in microscopic sections of 24 patients. The first 2 groups comprised 6 patients, the other 3 groups comprised 4 patients per group. Due to the variation in size of the villi in the different groups, variant numbers of villi in each group could be studied.

The areas of villous profiles, the two-dimensional images of the villi (= spheres), were measured on a Bit-pad digitizing tablet (Summagraphics Corp.) connected to a PDP 11/40 computer (Digital Equipment Corp.) and the profile radii of villi in normal and pathological

Table 1. Placental tissues were taken from the following 5 groups of patients and studied

Group 1. Hydatidiform mole, not followed by a malignant trophoblastic tumor
Group 2. Hydatidiform mole, subsequently followed by a histologically proven choriocarcinoma
Group 3. Hydropic degeneration without stromal trophoblastic inclusions
Group 4. Hydropic degeneration with stromal trophoblastic inclusions
Group 5. Normal placental tissue taken from induced abortions

placental tissue were calculated according to Weibel (1979). In the 5 groups of patients, the mean profile radius of villi of every patient and thereafter the mean, standard deviation and maximum profile radius in placental tissues of each group were calculated.

Trophoblast/nontrophoblast ratio in villi. Black-and-white photographs of villi were taken at 160× magnification in microscopical sections of the 5 different groups of patients. The trophoblast/nontrophoblast ratios in villi were calculated from data obtained by the Bit-Pad digitizing tablet. The villi were divided into small (radius less than 0.325 mm) and large villi (radius more than 0.975 mm). This division was made because the group of small profiles of villi is heterogeneous, since they may be the result of sectioning small villi as well as large villi.

The group of large profiles of villi is homogeneous, being sections through large villi only.

The mean trophoblast/nontrophoblast ratio in randomly selected small villi in placental tissue of every patient in Groups 1 to 5 was calculated. From these the mean, standard deviation and range of the trophoblast/nontrophoblast ratios in small villi of each group were obtained.

The mean trophoblast/nontrophoblast ratio in randomly obtained large villi in placental tissue of every patient in Groups 1 to 4 was calculated. Again, the mean, standard deviation and range of each group were obtained. The trophoblast/nontrophoblast ratios in large villi in placental tissue, taken from patients undergoing induced abortions (Group 5), could not be calculated because large villi are rarely found in normal placental tissue of a gestational age between 75 and 105 days (Jurkovic and Muzelak 1970).

Nuclear area of the trophoblast. Black-and-white photographs of nuclei of the trophoblast in microscopic sections of the 5 groups of patients were taken at 1,000× magnification. The nuclear areas were measured on the Bit-Pad digitizing tablet. We randomly selected 4 patients from each of the 5 groups of patients. In the placental tissue of each of the selected patients, areas of 80 nuclei of the trophoblast lining were randomly obtained and measured. The mean nuclear area was calculated for each individual patient. Again, the mean, standard deviation and range of the nuclear area of the trophoblast in each group were obtained.

In Groups 1 and 2 a division was made between the area of the nuclei, located in the double and 3 or more layered trophoblastic lining of small and large villi.

The statistical method applied was Student's *T*-Test.

Results

Profile radii of villi

The calculated mean, standard deviation and maximum profile radius of villi in placental tissues of the 5 different groups of patients is presented in Table 2.

Table 2. Profile radii data of the 5 groups of patients

	Mean in mm	S.D. in mm	Maximum in mm
Group 1 (No. of villi 1,104)	0.41	0.08	3.25
Group 2 (No. of villi 981)	0.38	0.10	2.39
Group 3 (No. of villi 1,794)	0.29	0.09	2.09
Group 4 (No. of villi 1,535)	0.24	0.05	1.13
Group 5 (No. of villi 4,918)	0.11	0.02	0.86

[a] S.D.: Standard Deviation

Table 3. Statistically significant differences of the mutually compared profile radii of villi of the 5 groups of patients ($p < 0.05$)

Groups	Group with statistically significantly larger profile radii of villi
1 versus 4	1
1 versus 5	1
2 versus 4	2
2 versus 5	2
3 versus 5	3
4 versus 5	4

Table 4. Trophoblast/nontrophoblast ratios in 108 randomly selected small villi in placental tissue of the 5 groups of patients

	Mean	S.D.[a]	Range
Group 1 (No. of ratios 24)	1.24	0.47	0.09–3.90
Group 2 (No. of ratios 20)	0.83	0.46	0.11–4.14
Group 3 (No. of ratios 16)	0.42	0.10	0.14–1.20
Group 4 (No. of ratios 16)	0.70	0.30	0.28–2.46
Group 5 (No. of ratios 32)	0.79	0.30	0.22–1.99

[a] S.D.: Standard Deviation

The statistically significant differences between the mutually compared profile radii of villi of the 5 groups of patients, are presented in Table 3.

From our study it appears that the mean profile radii of villi in placental tissues of hydatidiform moles (Groups 1 and 2) and of hydropically degenerated placentas (Groups 3 and 4) are significantly larger than the mean profile radius of villi in normal placental tissue (Group 5). A difference in this parameter between the pathological groups has not been observed.

Trophoblast/nontrophoblast ratio in villi

In Table 4 the mean, standard deviation and range of the trophoblast/nontrophoblast ratios in the randomly selected small villi in placental tissue of the 5 groups of patients are shown. The differences of the relative mean trophoblast/nontrophoblast ratios in small villi in placental tissues was only significant ($p < 0.05$) between the Groups 1 and 3.

In Table 5 the mean, standard deviation and range of the trophoblast/nontrophoblast ratios in the randomly selected large villi in placental tissue of 4 groups of patients are presented. In each group 12 ratios were calculated. It was not possible to show statistically significant differences between the relative trophoblast/nontrophoblast ratios of large villi in the 4 groups of patients.

Table 5. Trophoblast/nontrophoblast ratios in 48 randomly selected large villi in placental tissue of 4 groups of patients

	Mean	S.D.[a]	Range
Group 1	0.47	0.30	0.07–2.10
Group 2	0.09	0.02	0.04–0.23
Group 3	0.12	0.11	0.03–0.52
Group 4	0.15	0.07	0.05–0.44

[a] S.D.: Standard Deviation

Table 6. Nuclear area of the trophoblast of the 5 groups of patients. (no of nuclei in each group: 320)

	Mean in μm^2	S.D.[a] in μm^2	Range in μm^2
Group 1	42.6	5.6	9.1–177.5
Group 2	54.1	5.2	11.0–227.1
Group 3	30.7	8.7	3.5–141.6
Group 4	31.8	2.8	2.2–124.8
Group 5	33.4	4.8	5.4–121.3

[a] S.D.: Standard Deviation

Nuclear area of the trophoblast

In Table 6 the mean, standard deviation and range of the nuclear area of the trophoblast in the 5 groups of patients are presented. In Fig. 1 the distribution of the nuclear area of the trophoblast of the 5 various groups of patients are graphically demonstrated. The statistically significant differences between the relative mean nuclear areas of the trophoblast of the 5 groups of patients are shown in Table 7.

In order to find more differences between the Groups 1 (hydatidiform moles not followed by malignant trophoblastic disease) and 2 (hydatidiform moles subsequently followed by a histologically proven choriocarcinoma), the areas of the nuclei located in the double and 3 or more layered trophoblastic lining of small and large villi in placental tissue of those groups were compared. In Table 8 the mean, standard deviation and range of the nuclei located in the double and multi-layered trophoblastic lining of small and large villi in placental tissue of the Groups 1 and 2 are presented. In each of the 4 patients of the two Groups the mean area of 20 nuclei located in the double and multi-layered trophoblastic lining of small and large villi were calculated, as well as the standard deviation and the range of the nuclear areas. A comparison between the mean area of the nuclei of the trophoblastic lining of the small and large villi in placental tissue of Groups 1 and 2 showed that only the differences of the mean area of the nuclei located in the multi-layered trophoblastic lining of the large villi was statistically significant. Group 2 had larger nuclei than Group 1 ($p < 0.05$). Figure 2 shows a graphic demonstration of the distribution of the

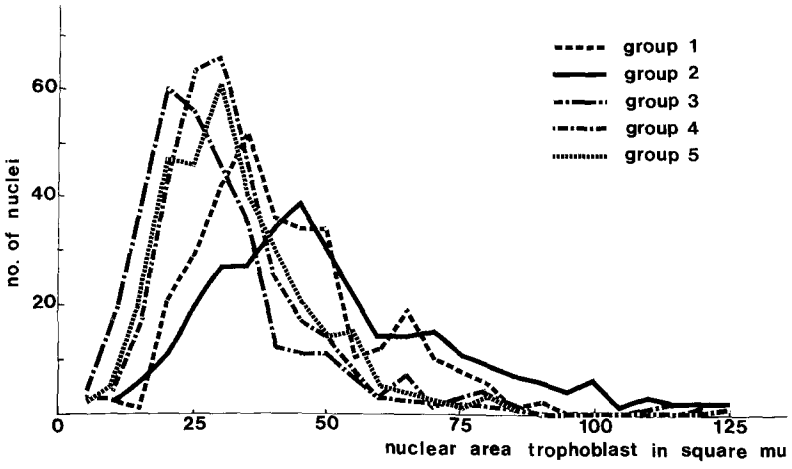

Fig. 1. Graphic demonstration of the distribution of the nuclear areas of the trophoblast of the 5 groups of patients.
Group 1: Hydatidiform mole, not followed by malignant trophoblastic disease.
Group 2: Hydatidiform mole, subsequently followed by a histologically proven choriocarcinoma.
Group 3: Hydropic degeneration without stromal trophoblastic inclusions.
Group 4: Hydropic degeneration with stromal trophoblastic inclusions.
Group 5: Normal placental tissue taken from induced abortions

Table 7. Statistically significant differences of the mutually compared mean nuclear areas of the trophoblast of the 5 groups of patients ($p < 0.05$)

Groups	Group with statistically significantly larger mean nuclear area
1 versus 2	2
1 versus 4	1
1 versus 5	1
2 versus 3	2
2 versus 4	2
2 versus 5	2

areas of the nuclei located in the multi-layered trophoblastic lining of large villi of Groups 1 and 2.

Discussion

Hydropic swelling of the connective tissue of villi is one of the typical features of a hydatidiform mole or a hydropically degenerated placenta. The profile radii of villi in placental tissues of 5 groups of patients were calculated in order to establish whether there was a statistically significant difference in size between the various groups. No statistically significant difference existed between the mean profile radii of villi in placental tissues of hydatidiform moles (Groups 1 and 2) when compared with the mean profile radius of villi in placental tissue of hydropically degenerated placen-

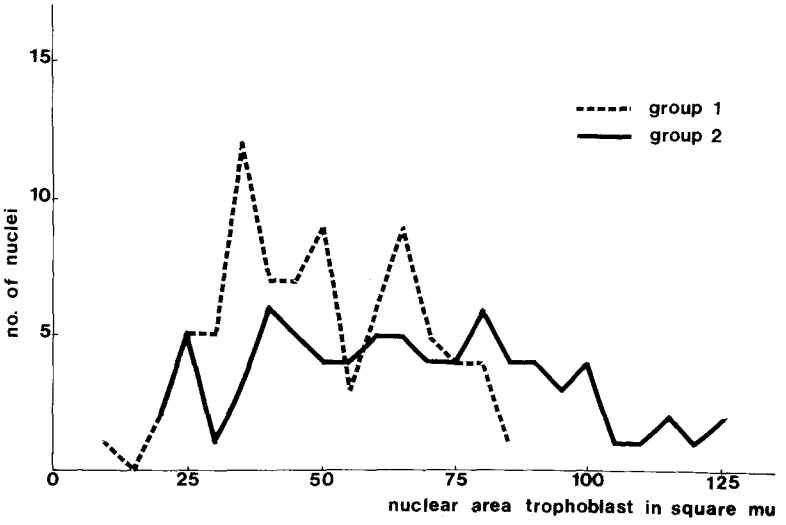

Fig. 2. Graphic demonstration of the distribution of the areas of nuclei located in the 3 or more layered trophoblastic lining of large villi of Groups 1 and 2.
Group 1: Hydatidiform mole, not followed by malignant trophoblastic disease.
Group 2: Hydatidiform mole, subsequently followed by a histologically proven choriocarcinoma

tas without stromal trophoblastic inclusions (Groups 3). Also no statistically significant difference existed between the mean profile radius of villi in hydatidiform moles not followed by a malignant trophoblastic tumor and the mean profile radius of villi in hydatidiform moles which were followed by a choriocarcinoma. The largest mean and maximum profile radius of villi were found in Group 1 (hydatidiform moles not followed by a malignant trophoblastic tumor). The maximum diameter of 6.5 mm of villi in placental tissue of Group 1 is comparable with the maximum diameters of villi of hydatidiform moles found in the literature, ranging from 1–30 mm.

As cross-sections through villi represent a heterogeneous population as to size, it was of importance to estimate the distribution of profile radii of villi.

In conclusion no prediction as to the development of choriocarcinoma after a hydatidiform mole can be given by calculating the profile radii of villi.

Increased thickness of the trophoblast is caused by hyerplasia, a characteristic feature in the histological grading of hydatidiform moles (Hertig and Sheldon 1947). Furthermore, the size of the villus is supposed to be closely related to the amount of trophoblastic epithelium (Park 1971). Therefore the trophoblast/nontrophoblast ratio was calculated. However there was no statistically significant difference between the mean trophoblast/nontrophoblast ratios in small and large villi in the 5 groups. Consequently the degree of trophoblastic proliferation cannot be considered as a parameter sufficient to discriminate between those hydatidiform moles which are followed by trophoblastic malignancies and those that are not. This is in contrast with the conclusions of Hertig and Sheldon (1947). However it

Table 8. Areas of the nuclei located in the double and 3 or more layered trophoblastic lining of small and large villi of the Groups 1 and 2

	Mean in μm^2	S.D.[a] in μm^2	Range in μm^2
Group 1 (small villi, double layered)	37.7	7.0	9.9–71.9
Group 1 (small villi, 3 or more layered)	46.3	12.5	20.1–177.5
Group 1 (large villi, double layered)	37.4	7.8	14.7–75.2
Group 1 (large villi, 3 or more layered)	49.2	11.7	9.1–85.4
Group 2 (small villi, double layered)	41.1	8.2	17.3–88.9
Group 2 (small villi, 3 or more layered)	49.0	2.2	16.7–121.4
Group 2 (large villi, double layered)	52.4	12.4	11.0–187.2
Group 2 (large villi, 3 or more layered)	73.8	14.4	19.5–227.1

[a] S.D.: Standard Deviation

is in accordance with Elston and Bagshawe (1972), who demonstrated that from a series of 70 patients with persistent trophoblastic disease, 31 developed this condition after the evacuation of a histologically clearly benign hydatidiform mole.

In previous reports various authors have correlated the mean nuclear area with the degree of malignancy. In general the nuclear area is directly related to the DNA content of the nuclei. In the course of malignant transformation the DNA content of the nucleus increases (hyperchromatism), often in combination with rapid mitotic activity. As a result the nuclear area may increase (nuclear anisokaryosis) (Atkin 1964; Robbins 1967; Levi et al. 1969). Although the nuclear area is influenced by other factors (glycogen content in the liver, water content etc), it seems to be a useful variable in distinguishing tumour-like conditions (Atkin 1964; Baak et al. 1981).

The mean nuclear area of the trophoblast in hydatidiform moles subsequently followed by a choriocarcinoma is comparable with those found in malignant ovarian tumours, metastatic mesotheliomas, grade II bladder tumours and is larger than the mean nuclear area of carcinoma of the nasal mucosa (Baak et al. 1981; Ooms 1981; Kwee 1982; Baak and Oort 1983; Helander 1984).

The mean nuclear areas of the trophoblast which line villi in hydatidiform moles (Groups 1 and 2) are significantly larger than those of the trophoblast in hydropically degenerated placentas (Groups 3 and 4) and normal placental tissue (Group 5). There is one exception, because no statistically significant difference existed between the nuclear area of the trophoblast in hydatidiform moles not followed by trophoblastic malignancies (Group 1) and hydropically degenerated placentas without stromal trophoblastic inclusions (Group 3).

These findings are in agreement with the observations of Goldfarb in 1971. They therefore are suggestive that a progressive change in nuclear area takes place in nuclei, from normal placental tissue to nuclei in hydatidiform moles, followed by a malignant trophoblastic tumor. Thus features indicative of malignancy may already be present in curettings of those hydatidiform moles that are eventually followed by choriocarcinoma. Assessment of the nuclear area allows for discrimination between a group of hydatidi-

form moles which are followed by trophoblastic malignancies and a group which are not (Table 8). The increase of the mean nuclear area of the trophoblast might be related to an increase in the thickness of the trophoblastic epithelium as a sign of general hyperplasia. This however is not the case.

Although it was possible to demonstrate a difference in nuclear size in a group of molar pregnancies developing choriocarcinoma, this study does not permit conclusions for individual patients. Further investigation is therefore required.

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