

# The current state of pathology and immunohistochemistry in gestational trophoblastic disease therapeutic management: A tertiary center experience

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## ABSTRACT

Gestational trophoblastic disease (GTD) is derived from the abnormal development of trophoblastic tissue and covers a broad range of benign and malignant disorders. The histological diagnosis of GTD continues to be a challenge, even among expert pathologists. The present study evaluates the roles of pathology and immunohistochemistry in GTD diagnostic and therapeutic approaches. A retrospective study was conducted in "Filantropia" Clinical Hospital, Bucharest, Romania, involving cases of GTD between 2010 and 2020. We reported 19 clinically diagnosed and histologically confirmed cases of GTD, 15 moles (complete and partial), along with 4 cases of gestational trophoblastic neoplasia (GTN). The mean age of the patients studied was  $31.3 \pm 7.5$  years. The percentage of cases diagnosed after the sonographic evaluation was 94.7%. The rate of progression of GTD to GTN was 5.2%. Seven patients with low-risk GTD (risk score six or less) were treated with first-line chemotherapy (CT), and two cases required second-line CT. Twelve patients with high-risk GTD (risk score seven or more) were treated with first-line CT ( $n = 4$ ), first-line CT followed by second-line CT ( $n = 4$ ), first-line CT with adjuvant surgery ( $n = 3$ ), and first-line surgery (one case). No causal relationship has been established between the forms of gestational trophoblastic disease regarding hCG level, the rate of normalization of hCG, and the rate of progression to GTN. After chemotherapy for GTD, only 42.1% of patients who wanted a future pregnancy could conceive. Underutilized in the current practice (5.2%), the immunohistochemical markers for trophoblasts and DNA genotyping are particularly useful tools in the fast and accurate diagnosis and prognosis of GTD.

**Keywords:** gestational trophoblastic disease, immunohistochemistry, histology, choriocarcinoma, hydatiform mole, fertility outcome

## INTRODUCTION

Gestational trophoblastic disease (GTD) is a rare condition in tertiary centers, which continues to raise several problems, despite the attempts to standardize therapeutic behavior, monitoring, and reproductive prognosis of patients (1). GTD brings together a heterogeneous group of conditions that result in abnormal proliferation of trophoblastic tissue. The GTD component includes both forms: benign (partial and total hydatidiform mole) and ma-

lignant (invasive mole, choriocarcinoma, placental site trophoblastic tumor, epithelioid trophoblastic tumor) (2).

The lack of randomized clinical trials and a global consensus make behavioral management protocols, and the impact on reproduction vary within medical centers worldwide (3). The European Organization for the Treatment of Trophoblastic Diseases (EOTTD) is constantly trying to develop treatment schemes standardizing and disease moni-

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toring. The difficulty lies in the fact that several diseases are brought together under the same pathological entity (4).

Epidemiological studies have reported an overall incidence of GTD, which varies according to geographic distribution (1/1500 in the United States and 1/1000 in Europe). The incidence of complete hydatidiform mole (CHM) is about 1-3 per 1000 tasks, and the incidence of partial hydatidiform mole (PHM) is about 3 per 1000 tasks. The risk of occurrence is 5.6 times higher in patients aged < 15 and > 45 years, with a 5-10 times higher risk after 45 years (5).

The diagnosis of GTD is based on the clinical picture, serial levels of human chorionic gonadotropin ( $\beta$ -hCG), suggestive ultrasound images, and histopathological findings on samples after uterine curettage.

The transvaginal ultrasound findings represent an important tool for GTD and gestational trophoblastic neoplasia (GTN) diagnosis (6). Magnetic resonance imaging (MRI) can evaluate the degree of invasion in the myometrium and adjacent tissues. Computed tomography (CT) of the whole body and brain MRI is used to assess metastatic forms and PET-CT identifies metastases or chemo-resistant primary areas (6).

$\beta$ -hCG is a marker of diagnosis, evolution, response assessment, and subsequent post-treatment monitoring. Levels greater than 100,000 mIU/mL before discharge are seen in approximately half of the patients with CHM and less than 10% of patients with PHM (5).

PHM or CHM normally have a favorable prognosis after uterine evacuation, but the increase of the  $\beta$ -hCG level allows the early detection of the evolution towards GTN, which takes place in 0.5% to 5% of cases and 15% to 20%, respectively (6). Thus, the risk of GTN after normalization of  $\beta$ -hCG is very low, being 0% after PHM, 0.36% after CHM, and 9.5% after multiple pregnancies with hydatidiform mole (HM).

In the case of CHM, Braga et al. (7) showed that four weeks after uterine evacuation, the existence of a  $\beta$ -hCG level  $\geq 20,000$  mIU/mL is found in 6.1% of women and is an important risk factor for the development of GTN.

In order to capture the progression of the disease and make an early diagnosis of GTN, weekly monitoring of the  $\beta$ -hCG should be performed. Increasing  $\beta$ -hCG levels allows the establishment of chemotherapy according to the FIGO score (8). Careful monitoring of  $\beta$ -hCG progression has improved survival by using targeted and effective therapies, increasing the cure rate. Depending on the treatment, on the evolution and prognosis of GTD, patients who want a future pregnancy are selected.

The risk of permanent impairment of fertility depends on the age of the patients, the severity of GTD, and the extent of therapy (9,10).

The definitive diagnosis of GTN is histopathological, made either for primary or metastatic disease. Establishing histological criteria and implementing them as accurately as possible is an important step in developing the pathological diagnosis of GTD. Unlike the complete hydatidiform mole, whose histopathological diagnosis is clear, partial hydatidiform moles can raise diagnostic problems.

In the complete hydatidiform mole, the histological characteristics are given by the presence of the villous hydrops increased with the tendency to form cisterns and by the diffuse hyperplasia of the trophoblast. The histological appearance, sometimes nonspecific, of partial hydatidiform moles is represented by villous hydrops, two villous types, mild-to-moderate trophoblast hyperplasia and trophoblastic pseudo-inclusions. The differential diagnosis of PHM is made with non-molar hydropic abortions, chromosomal trisomies, and digynic triploidy.

Availability of new immunohistochemistry and molecular genetic techniques allows establishing an accurate diagnosis of HM and GTN. However, there is a lack of data regarding the gestational trophoblast anatomopathology and immunohistochemistry in gestational trophoblastic disease and its contribution in the therapeutic management.

## AIM

The purpose of this study was to evaluate the roles of anatomopathology and immunohistochemistry in GTD diagnostic and therapeutic approaches. The Research Medical Ethics Committee of the "Filantropia" University Hospital approved the survey protocol (No. 26/10.2020). The ethical standards of the declaration of Helsinki were followed. Before the debut of the therapeutic procedure, all patients were apprised and signed the informed consent form regarding the clinical study's intervention and enrollment agreement.

## MATERIALS AND METHODS

We performed a retrospective analysis of data on women diagnosed with a gestational trophoblastic disease on a period of ten years, admitted to our clinic 2010-2020. Patient data were taken from hospital records, following personal pathological history, risk factors, diagnostic criteria, GTD staging, pathological outcome and treatment schedule.

We performed a retrospective study on gestational trophoblastic disease for ten years (from 2010 to 2020).

The study was performed in “Filantropia” Clinical Hospital. Our clinic is a tertiary medical unit specialized in managing the gestational trophoblastic disease. We collected and analyzed data from nineteen patients diagnosed and treated according to our clinic protocol during the study period. The inclusion criteria in the study were: women of reproductive age, molar pregnancy, abortion, or term delivery in history records. The exclusion criteria were: women who were lost to follow-up or with missing data on the records.

Data analysis was performed by using the SPSS® 27.0 software (IBM®Corp., NY, USA). We described the continuous variables using the median (range), mean and standard deviation (SD) with a 95% confidence interval (CI) or count (percent, %), when appropriate. Results with  $p < 0.05$  were considered statistically significant.

## RESULTS

Between 2010 and 2020, 19 patients were clinically diagnosed with GTD and included in the study. All patients included in the study were histologically confirmed to have GTD ( $n = 7$  cases of molar pregnancy and  $n = 12$  cases of GTN). The total number of live births in “Filantropia” Hospital during the same period was over 42,000; thus, the incidence of GTD in our clinic is estimated to be 0.44 per 1,000 live births. The mean age of the patients with GTD was  $31.36 \pm 7.52$  years with 10.5% ( $n = 2$ ) of the cases over 40 years. Seven patients (36.8%) had a previous molar pregnancy.

The socio-demographic criteria of the patients included in the study are presented in table 1.

**TABLE 1.** Description of study participant group ( $n = 19$ ) structure and demographic characteristics

Demographic data	
Maternal age, mean (SD)	31.36 ± 7.52
Gestational trophoblastic disease	19
– hydatiform mole	7
– gestational trophoblastic neoplasia	12
History of previous molar pregnancy	7 (36.8%)
Risk class	
– low risk	7 (36.8%)
– high risk	12 (63.2%)
Treatment	
– first-line single-agent chemotherapy	18 (94.7%)
• intramuscular methotrexate-folinic acid	• 15 (78.9%)
• intramuscular methotrexate-folinic acid + adjuvant hysterectomy	• 3 (15.7%)
- upfront hysterectomy	1 (5.2%)
- second-line chemotherapy after first-line treatment failed attempt	9 (47.3%)
• etoposide and cyclophosphamide combination	• 4 (21.1%)
• methotrexate and actinomycin D	• 1 (5.2%)
• methotrexate only	• 2 (10.5%)
• actinomycin D	• 2 (10.5%)

Demographic data	
Birth rate	3 (15.7%)
– Cesarean section	• 2 (10.5%)
– Vaginal delivery	• 1 (5.2%)
Follow-up	
– desire for future pregnancy	5 (26.3%)
– no desire for future pregnancy	7 (36.8%)
– conceived naturally	3 (15.7%)
– underwent hysterectomy	4 (21.1%)
Area of residence	
– Urban (n)	10 (52.6%)
– Rural (n)	9 (47.3%)
Parity	
– primiparous	10 (52.6%)
– multiparous	9 (47.3%)

Five GTN cases were reported; two neoplasia cases resulted after the progression of molar cases during follow-up, and three cases initially presented as GTN. Seven cases (36.8%) were low-risk (FIGO score six or less) and twelve (63.2%) patients with high-risk GTD (risk score seven or more).

7 patients (36.8%) with low-risk GTD (risk score 6 or less) were treated with first-line chemotherapy (CT), and two patients (10.5%) required second-line CT. Twelve patients (63.2%) with high-risk GTD (risk score 7 or more) were treated with first-line CT ( $n = 4$ ; 21%), first-line CT followed by second-line CT ( $n = 4$ ; 21%), first-line CT with adjuvant surgery ( $n = 3$ ; 15.7%), first-line surgery ( $n = 1$ ; 5.2%).

18 cases received first-line single-agent chemotherapy, fifteen with a weekly regimen of intramuscular methotrexate-folinic acid, and three cases plus adjuvant hysterectomy. One case of choriocarcinoma aged 51 years was treated with upfront hysterectomy, and after that, the  $\beta$ -hCG levels were normal. 9 cases failed to respond and were shifted to second-line chemotherapy. Four cases shifted to etoposide and cyclophosphamide combination and one case to methotrexate and actinomycin D combination. Two cases shifted to methotrexate only, and the other two cases with actinomycin D.

From 15 patients with preserved fertility after treatment, three patients (20%) gave birth, two of them by C-section and one by vaginal delivery. Five patients (33.3%) had the desire for a future conception and seven (46.7%) gave up on their reproductive desire.

Of the 19 cases included in the study, only one patient with choriocarcinoma had the associated immunohistochemistry (5.2% of the study group).

The specimen obtained by hysterectomy was processed automatically and embedded in paraffin blocks, with a histological and immunohistochemical examination. The microscopical examination revealed choriocarcinoma with the uterine parenchyma invaded by the cyto- and syncytiotrophoblast mass, hemorrhagic areas, with numerous mitotic figures, and a biphasic proliferation of mononuclear cytotrophoblast (Figure 1-4).

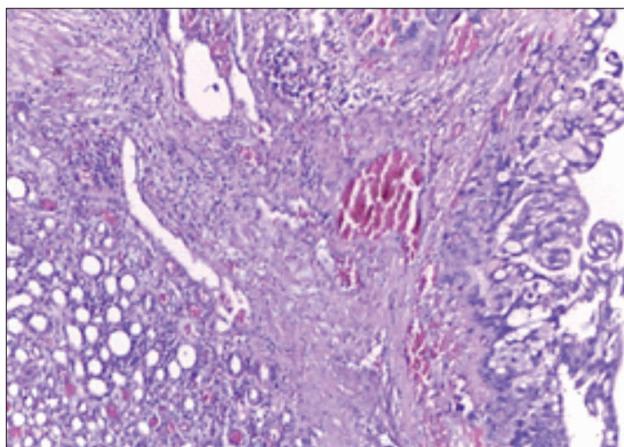
**TABLE 2.** Treatment in accordance with diagnosis and FIGO/WHO classification

Name	Age	Diagnosis	First-line treatment	Second line treatment	FIGO/WHO score
PI	30	IM	M (9 cycles)	-	I: 3
BD	24	IM*	Surgery, M (9 cycles)	D (3 cycles)	II: 8
GI	24	CHM	M (13 cycles)	E, C (2 cycles)	I: 2
DL	43	CHM	M (3 cycles)	E, C (2 cycles)	I: 10
MG	29	PHM	M (9 cycles)	-	I: 7
TG	20	CHM	M (9 cycles)	E, C (4 cycles)	I: 3
VO	32	CHM	M (5 cycles)	-	I: 8
ME	38	CHM	M (10 cycles)	E, C (5 cycles)	I: 8
GN	32	PHM	M (7 cycles)	-	I: 3
TL	31	CHM	M (9 cycles)	-	I: 8
VM	19	ETT	M (6 cycles)	D (6 cycles)	II: 10
MS	30	PHM	M (11 cycles)	M (3 cycles)	I: 12
IR	27	CHM	M (8 cycles)	-	I: 5
DG	32	CHM*	M (4 cycles), Surgery	M (12 cycles)	I: 7
DA	36	PHM	M (3 cycles), Surgery	M (9 cycles), D (11 cycles)	I: 12
CC	35	PHM	M (10 cycles)	-	I: 3
SMI	51	Cc	Surgery	-	I: 10
SE	32	PTD	M (10 cycles)	-	I: 7
SM	31	PHM	M (9 cycles)	-	I: 4

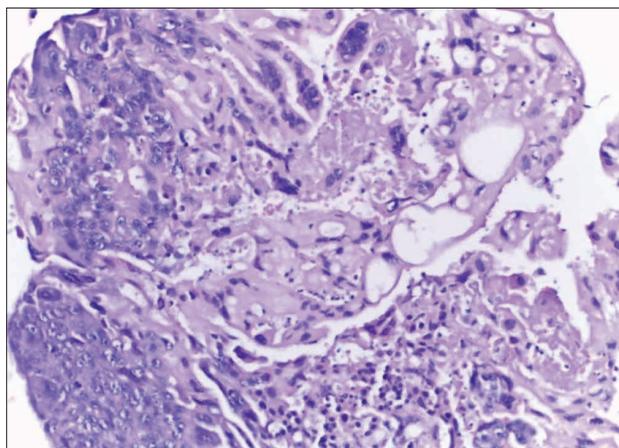
\* treatment-resistant; complete hydatiform mole (CHM); partial hydatiform mole (PHM); persistent trophoblastic disease (PTD); invasive mole (IM); epithelioid trophoblastic tumor (ETT); choriocarcinoma (Cc). A – C-section 3,350 grams after spontaneous conception; B – C-section 2,950 grams after spontaneous conception; C – two vaginal births (3550 grams and 3750 grams) after spontaneous conception. Drugs used: M- Methotrexate; D- Dactinomycin; E- Etoposide; C- Cisplatin

We found positive reaction in intravascular syncytiotrophoblastic tumoral embolus and negative reaction in the vascular wall for  $\beta$ -hCG; pancytokeratin exhibited positive cytotrophoblast reaction and negative reaction in tumor syncytiotrophoblast for MNF116.

The patient's postoperative evolution was good, with marked lowered  $\beta$ -hCG levels and no associated chemotherapy.

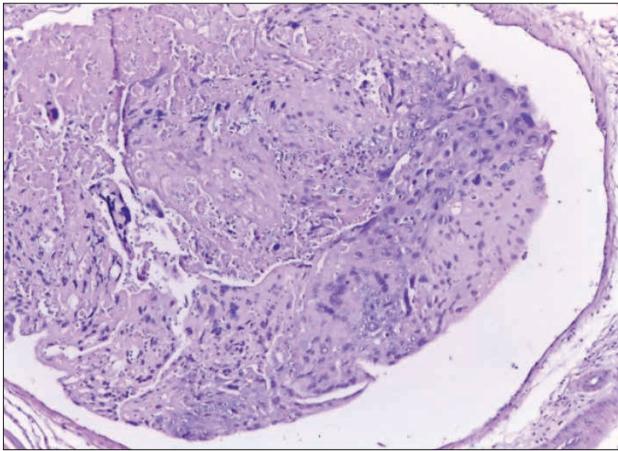


**FIGURE 1.** HE staining 40x. Uterine parenchyma invaded by the cyto- and syncytiotrophoblast mass with hemorrhagic areas

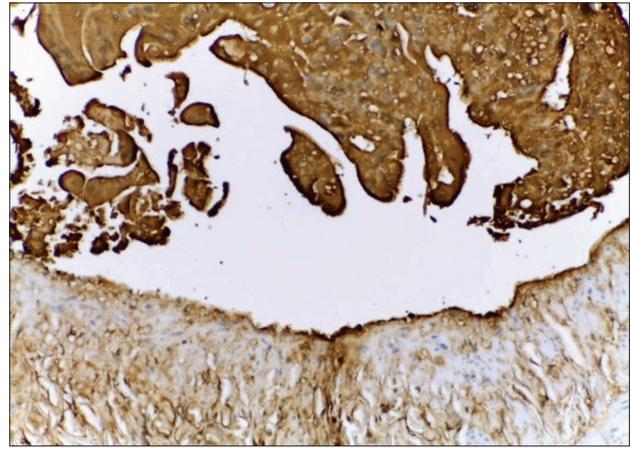


**FIGURE 2.** HE staining 100x. Detail cyto- and syncytiotrophoblast tumor (size large cell cytoplasm amfopila, voluminous nuclei, sometimes vesicular nucleoli, coarse chromatin clusters)

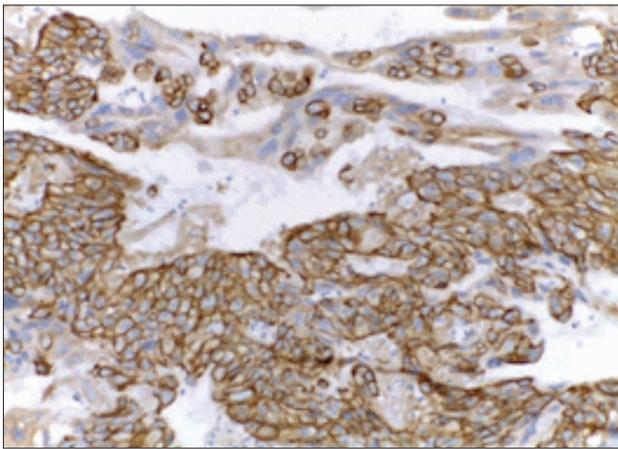
The hysterectomy specimen (Figure 7) contains areas of invasive myometrial trophoblastic proliferation with marked cytological atypia, with or without associated molar villi. In literature, the patient's average age at presentation is 30 years, the case of our patient (51 years old, menopause) being rare.



**FIGURE 3.** he staining 100x. choriocarcinoma. tumor embolus in the uterine vessel. Sincitio– and cytotrophoblastic cells clustered in the lumen of a lymphatic vessel



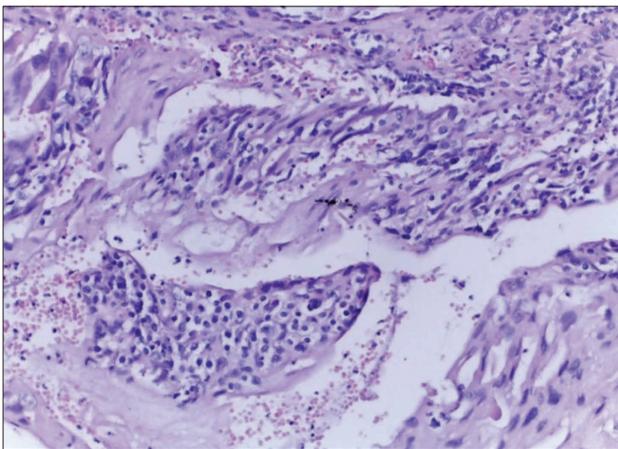
**FIGURE 6.** beta HCG 40x. Positive reaction in intra-vascular syncytiotrophoblastic tumoral embolus and negative reaction in the vascular wall



**FIGURE 4.** HE staining 100x. Choriocarcinoma - different types of cytotrophoblast



**FIGURE 7.** Sample of hysterectomy of the patient with choriocarcinoma



**FIGURE 5.** MNF116 200x. Pancytokeratin exhibits positive cytotrophoblast reaction and negative reaction in tumor syncytiotrophoblast

**DISCUSSION**

The histological diagnosis of GTD is still based on the experience and overspecialization of gynecological pathologists due to the adverse clinical consequences, the unfavorable prognosis, and sometimes reproductive repercussions.

Several lesions can be confused with hydatidiform mole or GTD, such as placental mesenchymal dysplasia, trophoblast inclusions, chorangioma with trophoblastic proliferation, or trophoblastic proliferation encountered in early pregnancy and tubal ectopic pregnancy (11). Diagnosis of moles by evaluating slides stained with hematoxylin and eosin is still difficult, having great interobserver variability, even in the case of experienced pathologists (12). Partial moles have lower histological features than complete moles, with less edema and trophoblastic proliferation. The differential diagnosis of partial moles with pregnancies with chromosomal abnormalities is done based on the absence of proliferation of trophoblasts (13).

The algorithm for the diagnosis of GTD and GTN must include the triad histology, immunohistochemistry, and DNA genotyping, in this order of analysis. In the routine practice of pathology, P57 immunohistochemistry is used to diagnose CHM, not being useful in differentiating PHM from non-molar abortions that contain maternal genetic material.

Histologically, choriocarcinoma presents as a particularly aggressive form of GTN characterized by the presence of biphasic proliferation of multinucleated tumor cells, with areas of macro- and microscopic hemorrhage, as well as areas of necrosis. In addition, increased mitotic activity is observed, tumor cells being positive for cytokeratin (CK AE1/AE3) and hCG and hPL present diffuse positivity in syncytiotrophoblastic cells (61). Choriocarcinoma can be accurately diagnosed by DNA genotyping (12).

Histologically, the trophoblastic epithelioid tumor (ETT) presents as grouped areas of mononuclear tumor cells or regions of extensive hyalinization, with a mitotic activity of one to 10 mitoses in ten high-power fields (HPF). Immunohistochemical ETT is diagnosed by p63 positivity and poorly stained with hPL. Placental site trophoblastic tumor (PSTT) is p63 negative and exhibits strong diffuse staining with hPL (14).

Although the decisive role of histopathology and immunohistochemistry in the positive and differential diagnosis of gestational trophoblastic tumors has been established, the reality is unfortunately completely different. Of the 19 cases mentioned, practically only one patient (presented in detail) had the associated immunohistochemistry, representing a percentage of 5.2%. This must be considered in the context in which immunohistochemistry is not settled by the national health insurance house, and it is paid by the patient.

Thus, the analysis of the situation leads to the conclusion that although the number of immunohistochemical analyses has increased in recent years, it is still below the real need for investigations related to the diagnosis of various forms of GTD. Therefore, the future will also integrate DNA genotyping into the mandatory algorithm for detecting various GTD subtypes.

Treatment of hydatidiform mole consists of two steps. The first one is the immediate evacuation of the mole when possible, and the second is the follow-up evaluation for persistent trophoblastic proliferation or malignancy. The treatment of choice for hydatidiform moles is vacuum aspiration. Under anesthesia, the cervix is dilated to allow the insertion of a suction cannula, through which the aspiration of the molar tissue is performed, and the sample is sent to the pathological anatomy. Subsequently, oxytocic drugs are administered for uterine retraction, and ultrasound performed to assess the emptiness of the uterine cavity (15,16,17).

In patients with non-metastatic GTD not interested in preserving fertility, hysterectomy is recommended as the initial therapy because it reduces the toxicity of chemotherapy by decreasing the duration of treatment. In addition, using single-agent

chemotherapy at the time of surgery can reduce tumor spread (18). In our study, one patient with choriocarcinoma (pT1bNxMx) had a hysterectomy as first-line treatment with no subsequent adjuvant chemotherapy.

The treatment of choice in patients who want to maintain their fertility is chemotherapy with a single agent. Treatment continues until three subsequent normal  $\beta$ -hCG levels, two rounds of chemotherapy being administered after the first normal  $\beta$ -hCG level (19).

The choice between mono- and polychemotherapy is based on the prognostic FIGO score. The FIGO classification and staging system, based on which the initial therapy is instituted in patients diagnosed with gestational trophoblastic disease in order to ensure the best prognosis (20).

In patients with GTD or low-risk GTN (WHO risk score 0-7), the protocol that includes single-agent chemotherapy (methotrexate or actinomycin D) is recommended, which coincides with the hospital-approved guideline. The overall cure rate of mono-chemotherapy was almost 100%. A 2012 study published in Cochrane, which was performed in five randomized controlled trials on 513 patients, showed the superiority of actinomycin D (Act-D) with a significantly lower drug failure rate compared to methotrexate (21).

Replacement of drugs in the chemotherapy protocol with a single agent is to be considered if serum  $\beta$ -hCG levels remain above normal during treatment or if the initial treatment's high toxicity and adverse effects are found. The switch of methotrexate with Act-D in the second-line regimen provides a good response rate between 76% and 87% in patients with relatively low  $\beta$ -hCG levels. Thus, compared to other cytotoxic drugs, actinomycin D is the preferred agent for patients with low-risk GTD (22). Nevertheless, if we have an inadequate response to the initial mono-chemotherapy, the second line of treatment is initiated with a polychemotherapy regimen. This approach can also be seen in the case of a significant increase in  $\beta$ -hCG levels or the emergence of resistance to single-agent chemotherapy.

Although several schemes and protocols for the treatment of low-risk GTD have been developed in recent years, a standard regimen has not yet been established to meet all requirements regarding the efficacy, toxicity, and quality of life of the patient. Various methotrexate and actinomycin D protocols for the treatment of low-risk GTD have been studied (22-25).

Abrão et al., in a retrospective study of patients with low-risk GTD treated with 5-day actinomycin D regimens or methotrexate or a combination of actinomycin D and methotrexate (23), showed no differences in remission rates of these regimens. In

contrast, the adverse event rate was 19% with five-day actinomycin D, 28.6% with five-day methotrexate, and 62.5% in combination therapy. In this study, the weekly intramuscular methotrexate remission rate was 47.3%, lower than the rate reported in other studies (19,20,23).

Intravenous administration of actinomycin D has been shown to be more effective than weekly intramuscular methotrexate (25). One study reported a failure rate of 20% for treatment with pulsed actinomycin D and 8% with actinomycin D (26).

In our study, the serum level of  $\beta$ -hCG before initiating first-line therapy was the main prognostic factor regarding the therapeutic response, similar to those reported by Yarandi et al. (27).

In the treatment of low-risk GTD, etoposide has been shown to be the most effective single-agent cytotoxic drug (28). In cases of persistent disease, actinomycin D or polychemotherapy is used. If the response to the initial treatment with mono CT is incomplete, it will be switched to a multiagent regimen so that there is no significant increase in  $\beta$ -hCG levels in case of progression of the high-risk disease, no metastatic disease or chemoresistance occurs (29).

In patients with a high WHO risk score ( $>7$ ) and clinicopathological diagnosis of GTN, the establishment of mono-chemotherapy is associated with an increased risk of chemoresistance to the action of a single therapeutic agent. The initial use of polychemotherapy is still debatable in low-risk patients. After normalization of  $\beta$ -hCG levels, it is recommended to administer 2-3 cycles of chemotherapy to reduce the risk of recurrence so that the complete remission rate is close to 100% (5).

Usually, mono-chemotherapy does not affect fertility and subsequent outcomes, whereas treatment regimens that use multiple agents depending on the age of the patients may induce ovarian reserve damage, infertility, or induce menopause (30). Patients at high risk due to the increased rate of hysterectomy show disastrous reproductive results (31).

Instead, the treatment of aggressive forms, high-risk GTN, uses polychemotherapy regimens, the

most used regimen being EMA-CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine). The survival rate for these patients is about 95% (28,32).

Combination chemotherapy is considered if resistance to sequential chemotherapy with a single agent occurs. In order to achieve remission between patients treated with single-agent chemotherapy, approximately 10-15% will require chemotherapy with several therapeutic agents with or without surgery. For patients with high-risk metastatic GTD the initial multiagent chemotherapy with or without adjuvant radiotherapy or surgery should be the first choice (33). The EMA-CO regimen is good for high-risk metastatic GTD. Surgical removal of foci of chemotherapy-resistant disease or controlling hemorrhage has supplemental options of the basic treatment (34).

Follow-up monitoring for GTD is at least six months, for GTN one year and up to 2 years for cases with metastases other than the lung.

## CONCLUSIONS

With recent advances in our understanding of the molecular basis of gestational trophoblastic disease and the availability of new immunohistochemistry and molecular genetic techniques, now we can precisely subclassify and diagnose hydatidiform moles and gestational trophoblastic tumors. However, traditional morphologic assessment should still remain the first step and cornerstone of the diagnosis of these entities. The immunohistochemical markers for trophoblasts and DNA genotyping are particularly useful tools in the fast and accurate diagnosis and prognosis of these tumor entities, however they are underutilised in the current practice (5.2%) in our retrospective study, costs being supported by the patient; in the future, a different approach for the patient's benefit is needed. Integration of new ancillary techniques into a diagnostic algorithm can provide high diagnostic accuracy as well as cost-efficiency.

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