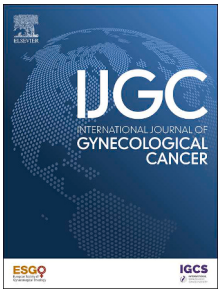
ORIGINAL RESEARCH

Impact of atypical extra-villous trophoblast foci on the natural history and management of post-molar gestational trophoblastic neoplasia



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ABSTRACT

Objective: Approximately 15% to 20% of complete hydatidiform moles progress to post-molar gestational trophoblastic neoplasia. The presence of atypical extra-villous trophoblast foci, described in complete hydatidiform moles, has been associated with an increased risk of developing post-molar gestational trophoblastic neoplasia. The primary objective of this study was to evaluate the predictive value of atypical extra-villous trophoblast foci for treatment response in post-molar gestational trophoblastic neoplasia. Secondary objectives were to assess the clinical impact of these foci on disease characteristics, the International Federation of Gynecology and Obstetrics (FIGO) score, disease stage, and human chorionic gonadotropin (hCG) kinetics.

Methods: A retrospective multi-center study was conducted by the Belgian Gestational Trophoblastic Diseases Registry (French-speaking center) between January 2017 and December 2022. All cases of complete hydatidiform mole were centrally reviewed by expert pathologists specialized in placental pathology from 3 university hospitals. Post-molar gestational trophoblastic neoplasia was diagnosed according to FIGO 2000 criteria. Clinical features were compared according to the presence or absence of atypical trophoblast foci.

Results: Among 216 patients diagnosed with complete hydatidiform mole, 56 (26%) developed post-molar gestational trophoblastic neoplasia. Atypical extra-villous trophoblast foci were identified in 38 of 56 (68%) cases. Baseline demographic characteristics, including age, were comparable between the 2 groups. Patients with atypical foci more frequently had FIGO scores ≥ 6 ($p = .044$) and pulmonary metastases (18.4% vs 5.6%). All patients requiring multi-agent chemotherapy belonged to the atypical foci group ($p = .073$). Pre-treatment hCG nadir levels were higher, and hCG slopes steeper in the atypical group ($p = .0027$ and $p = .0052$).

Conclusions: Post-molar gestational trophoblastic neoplasia arising from complete hydatidiform moles with atypical extra-villous trophoblast foci is more frequently associated with an unfavorable prognosis and the need for multi-agent chemotherapy than disease arising from moles without atypical foci.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Atypical extra-villous trophoblast foci, identified in some complete hydatidiform moles, are associated with a significantly higher risk of developing post-molar gestational trophoblastic neoplasia.

WHAT THIS STUDY ADDS

In addition to the parameters included in the International Federation of Gynecology and Obstetrics/World Health Organization (FIGO/WHO) scoring system, the presence of atypical extra-villous trophoblastic foci may serve as an additional prognostic and predictive marker for treatment response in post-molar gestational trophoblastic neoplasia.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

Gestational trophoblastic neoplasia following a complete mole with atypical extra-villous trophoblast foci appears to be associated with a higher FIGO/WHO score and a more frequent need for multi-agent chemotherapy. These findings should be confirmed in larger patient cohorts.

Keywords:
Complete Hydatidiform Mole; Atypical Extra-villous Trophoblast Foci; Post-Molar Gestational Trophoblastic Neoplasia; Predictive Histological Marker

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INTRODUCTION

Gestational trophoblastic diseases represent a group of rare and heterogeneous pregnancy-related disorders. Hydatidiform moles, whether partial or complete, are classified as pre-malignant lesions.[1] Among complete hydatidiform moles, approximately 15% to 20% progress to post-molar gestational trophoblastic neoplasia, according to the International Federation of Gynecology and Obstetrics (FIGO) 2000 criteria[2,3]: (i) a rise in serum human chorionic gonadotropin (hCG) levels exceeding 10% over a period of at least 2 consecutive weeks; (ii) a plateau in hCG levels over at least 3 consecutive weeks; or (iii) a histopathological diagnosis of choriocarcinoma.

Atypical extra-villous trophoblast foci in complete hydatidiform moles are defined as clusters of pleomorphic, biphasic trophoblastic cells located in the inter-villous space. They are characterized by a mixture of syncytiotrophoblasts and cytotrophoblasts that exhibit marked cytological atypia, analogous to that observed in choriocarcinoma. These foci are associated with an increased risk of post-molar gestational trophoblastic neoplasia.[4]

The gold standard treatment for gestational trophoblastic neoplasia is chemotherapy, with the specific regimen determined by the FIGO/WHO clinical scoring system, which assesses resistance to single-agent chemotherapy.[3,5-7] Low-risk disease (FIGO/WHO score $\leq$ 6) is treated with single-agent chemotherapy, achieving cure rates close to 100%. Intra-muscular or intravenous methotrexate is the first-line treatment, with reported success rates ranging from 67% to 91%.[8,9] In cases of resistance or toxicity, second-line therapy with actinomycin D or a multi-agent regimen is required. The efficacy of single-agent treatment decreases with increasing FIGO/WHO scores; 70% to 80% of patients with a score of 5 to 6 exhibit resistance.[10,11] In patients with low FIGO scores, a second curettage may be beneficial to avoid chemotherapy.[12] For women who have completed childbearing, hysterectomy can be considered as an alternative treatment option.[3,13]

High-risk gestational trophoblastic neoplasia (FIGO/WHO score $>$ 6) requires multi-agent chemotherapy, typically the etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine (EMA-CO) regimen, with cure rates approaching 90%.

The objective of this study, conducted in a gestational trophoblastic diseases reference center within the Belgian Gestational Trophoblastic Diseases Registry, was to evaluate the impact of atypical extra-villous trophoblast foci on the prognosis of post-molar gestational trophoblastic neoplasia and to assess their predictive value for treatment response.

METHODS

Study Design

This retrospective cohort study was conducted by the French-speaking center of the Belgian Gestational Trophoblastic Diseases Registry, which covers approximately 5 million inhabitants, between January 2017 and December 2022. All patients diagnosed with a complete hydatidiform mole were considered eligible. Case registration is based on voluntary referrals, most commonly initiated by the initial diagnosing pathologist or the managing gynecologist, and more rarely directly by the patient. Despite the voluntary nature of recruitment, comparison of registry data with epidemiological data has previously demonstrated good concordance, supporting effective and representative case ascertainment.[14] Informed consent was obtained from each participant at the time of enrollment in the national registry. The study received ethical approval from the Institutional Review Board of the University Hospital of Liège (approval number: 2023/343). Clinical and pathological data were recorded in a secure electronic database.

Pathological Analysis

Tissue samples obtained by uterine curettage were fixed in 10% neutral-buffered formalin and subsequently embedded in paraffin. Standard hematoxylin and eosin staining was employed for morphological assessment. When needed, ancillary studies (specifically immunohistochemical staining for p57) were used to confirm the diagnosis of complete hydatidiform mole. Atypical extra-villous trophoblast foci were identified in complete moles as clusters of biphasic trophoblastic cells in the inter-villous space, as described in our previous study (Fig. 1).[4] All initial diagnoses were re-evaluated by a panel of nationally recognized expert pathologists specialized in placental pathology. These specialists, affiliated with academic institutions (the University Hospitals of Liège and Brussels [Saint-Luc and Erasmus]) performed centralized slide reviews upon receipt of material from referring laboratories.[14]

Clinical Outcomes

The primary objective of this study was to evaluate whether atypical extra-villous trophoblast foci have predictive value for treatment response in post-molar gestational trophoblastic neoplasia. Specifically, we assessed their association with the number of lines of treatment administered, methotrexate resistance, and the need for multi-agent chemotherapy. Secondary objectives included examining the relationship between these foci and other clinical characteristics of the neoplasia, such as the FIGO/WHO risk score, disease stage, and hCG kinetics.

Post-molar neoplasia was diagnosed according to the criteria established by the FIGO in 2000.[2] Patients who were lost to follow-up prior to hCG normalization were excluded from the analysis.

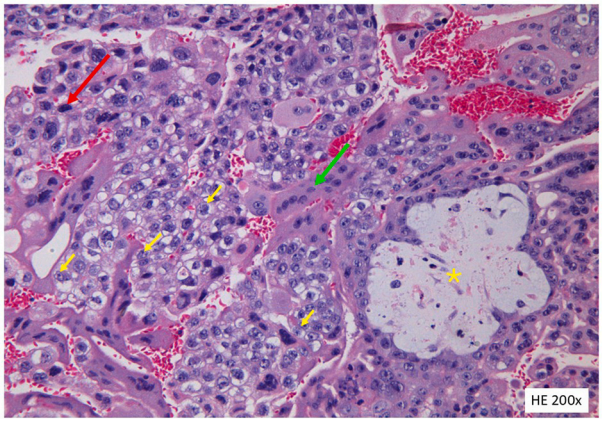

Figure 1 Hematoxylin and eosin—stained section (×200) showing atypical extra-villous trophoblastic foci, characterized by pleomorphic extra-villous trophoblast composed of mononucleated cytotrophoblast (red arrow) and multi-nucleated syncytiotrophoblast (green arrow), with marked cytologic atypia (yellow arrows), surrounded by chorionic villi (asterisk).

Low-risk disease (FIGO/WHO score $\leq$ 6) was managed with first-line intra-muscular methotrexate combined with folinic acid.[15] In the absence of metastatic disease, a second uterine evacuation, via repeat uterine curettage, hysteroscopic resection, or hysterectomy in patients not desiring future fertility, was considered. For FIGO score 5 or 6 diseases, first-line multi-agent chemotherapy might be considered in cases of metastatic disease, choriocarcinoma histology, or pre-treatment hCG $>$ 150,000 IU/L.[16] High-risk disease (FIGO/WHO score $>$ 6) received first-line multi-agent chemotherapy using the EMA-CO regimen.[15]

Statistical Analysis

Quantitative variables were expressed as the median with interquartile range, and categorical variables were summarized using absolute frequencies and percentages. The characteristics of post-molar neoplasia were compared according to the presence or absence of atypical trophoblast foci using Fisher's exact test for proportions and the Kruskal-Wallis test for medians. Odds ratios with 95% confidence intervals were also reported. Statistical significance was set at $p < .05$. Statistical analyses were performed using SAS version 9.4 and R version 4.4.1.

In accordance with the journal's guidelines, we will provide our data for independent analysis by a team selected by the Editorial Board for the purposes of additional data analysis or for testing the reproducibility of this study in other centers if such is requested.

RESULTS

A total of 709 patients were registered at the French-speaking center of the Belgian Registry for Gestational Trophoblastic Diseases. Among them, 244 (34.4%) patients were diagnosed with complete hydatidiform mole. p57 labeling was performed in 96% of cases. Twenty-eight patients were excluded due to loss to follow-up before normalization of hCG levels. Among the remaining 216 complete mole cases, 56 patients (25.9%) met the FIGO criteria for post-molar gestational trophoblastic neoplasia and were included in the analysis. Of these, 18 patients (32.1%) had no atypical extra-villous trophoblast foci, whereas 38 patients (67.9%) had atypical extra-villous trophoblast foci.

The clinical characteristics of post-molar neoplasia in the groups with and without atypical extra-villous trophoblast foci are presented in Table 1. The median age did not differ significantly between the groups (36.7 years in the group with atypical foci vs 34.4 years in the group without).

Post-molar neoplasia was diagnosed based on a sustained rise in hCG levels in 48 of 54 cases (88.9%). Less frequently, in 11.1% cases, the diagnosis was established following a plateau in hCG levels. There was a non-statistically significant trend toward more frequent hCG ascension in the atypical foci group (94.6% vs 76.5%, $p = .07$).

The median FIGO score was comparable between the 2 groups (1.5 vs 2; $p = .11$). However, a higher proportion of patients with neoplasia following a mole with atypical foci presented with a FIGO score $\geq$ 6, observed in 8 patients (21.1%) compared with none in the group without atypical foci ($p = .044$).

In this series of post-molar neoplasia, most patients presented with FIGO 2000 anatomical stage I disease at diagnosis: 94.4% in the group without atypical foci and 81.6% in the group with atypical foci ($p = .41$). Among patients with metastatic disease, all metastases were pulmonary (defining FIGO anatomical stage III disease) and occurred more frequently in the group with post-molar neoplasia and atypical foci (18.4%) than in the group without atypical foci (5.6%), although this numerical difference did not reach statistical significance ($p = .41$). Notably, all patients with lung metastases had normal brain magnetic resonance imaging findings at diagnosis.

Treatment characteristics for post-molar neoplasia are summarized in Table 2. Twenty-eight patients underwent surgical intervention, either a second uterine evacuation (53% of post-molar neoplasia cases with atypical foci and 44% of those without) or hysterectomy (47% and 56%, respectively). Surgery alone was curative in 68% of these patients ($n = 19$). Notably, among those who underwent surgery, adjuvant chemotherapy was subsequently required more often when atypical trophoblast foci were present (8 of 19 patients, 42%) compared with post-molar neoplasia without atypical foci (1 of 9 patients, 11%), but this difference was not statistically significant ($p = .19$).

Regarding chemotherapy, the 8-day methotrexate regimen was administered as first-line treatment for low-risk disease. The 8-day methotrexate protocol alone was curative in 9 of 10 post-molar neoplasia cases without atypical foci (90%) and in 13 of 23 cases with atypical foci (56.5%) ($p = .09$).

In the group without atypical foci, only 1 patient (10%) developed methotrexate resistance and required second-line single-agent actinomycin D. Among the 20 post-molar neoplasia cases with atypical foci receiving methotrexate as first-line therapy, 7 (35%) developed resistance: 3 were treated with second-line actinomycin D, 2 required the multi-agent EMA-CO regimen after failure of actinomycin D, and 2 were directly treated with EMA-CO regimen as second-line treatment.

Notably, all patients who required multi-agent EMA-CO chemotherapy belonged to the group with atypical extra-villous trophoblast foci (7 of 23 cases; 30.4%, $p = .07$). Three patients received first-line multi-agent chemotherapy due to high-risk disease: 1 had a FIGO/WHO score of 7, and 2 had a score of 8. The remaining 4 patients (57%) were initially classified as having low-risk disease but developed methotrexate resistance: 1 patient had a FIGO/WHO score of 0, 1 had a score of 2, and 2 had scores of 6.

Among the 4 patients with a FIGO/WHO score of 6, 1 was cured with a first-line 5-day methotrexate regimen. The remaining 3 patients developed resistance to methotrexate. Of these, 1 was successfully treated with second-line single-agent actinomycin D (5-day regimen), whereas the other 2 required multi-agent chemotherapy (EMA-CO). All patients ultimately achieved complete remission.

Three patients in the atypical foci group did not require treatment. They presented with low-risk disease (FIGO score 0 or 1) and hCG levels below 200 IU/L. By the time the post-molar neoplasia staging work-up was completed, their hCG kinetics had spontaneously declined, allowing follow-up until marker normalization without the need for additional therapy.

There was also a trend toward a higher number of chemotherapy courses and treatment lines in post-molar cases with atypical foci: 28.9% ($n = 11$) of patients with atypical foci required 2 or more lines of treatment, compared with 5.6% ($n = 1$) of patients without foci ($p = .08$) (Fig. 2).

Table 1 Clinical Characteristics of Post-Molar GTN According to Presence or Absence of Atypical Extra-villous Trophoblastic Foci

GTN features	Post-molar GTN				*p*-Value[a]
	No atypical foci (*N* = 18)		Atypical foci (*N* = 38)		
	No. of patients	Number (%) Median [Q1-Q3]	No. of patients	Number (%) Median [Q1-Q3]	
Age at diagnosis (y)	18	34.4 [27.4-47.3]	38	36.7 [29.1-48.0]	.37
hCG kinetic[b]	17		37		.07
Stagnation		4 (23.5)		2 (5.4)	
Ascension		13 (76.5)		35 (94.6)	
FIGO/WHO score	18	1.50 [1.00-2.00]	38	2.00 [1.00-4.00]	.11
0		2 (11.1)		4 (10.5)	
1		7 (38.9)		8 (21.1)	
2		6 (33.3)		14 (36.8)	
3		3 (16.7)		2 (5.3)	
4		0 (0.0)		2 (5.3)	
5		0 (0.0)		0 (0.0)	
6		0 (0.0)		4 (10.5)	
7		0 (0.0)		2 (5.3)	
8		0 (0.0)		2 (5.3)	
FIGO/WHO score ≥ 6	18		38		.044
No		18 (100.0)		30 (78.9)	
Yes		0 (0.0)		8 (21.1)	
Metastases	18		38		.41
No		17 (94.4)		31 (81.6)	
Yes		1 (5.6)		7 (18.4)	
Stage	18		38		.41
I		17 (94.4)		31 (81.6)	
III		1 (5.6)		7 (18.4)	

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; GTN, gestational trophoblastic neoplasia; hCG, human chorionic gonadotropin; Q1, quartile 1; Q3, quartile 3; WHO, World Health Organization.

[a] Fisher exact test for comparison of proportions or Kruskal-Wallis test for comparison of medians.

[b] The 2 missing data points are histological type gestational trophoblastic neoplasia.

The hCG levels and kinetics in both groups are summarized in Table 3. The hCG kinetic curves for both groups are shown in Fig. 3. Pre-evacuation hCG levels were similar between the 2 groups (median: 190,750 IU/L in the group without atypical foci vs 160,439 IU/L in the group with atypical foci; $p = .35$). Post-evacuation hCG levels, measured 1 week after curettage, also remained comparable (median: 5335 vs 5811 IU/L; $p = .81$). The decline slopes of hCG from post-evacuation to nadir were similar between groups (−199 vs −214 IU/L per day, $p = .46$). However, the time to reach hCG nadir before diagnosis of post-molar neoplasia was significantly shorter in post-molar cases with atypical foci (median: 12.5 vs 33 days; $p = .0042$), and the hCG levels at nadir were significantly higher in this group (median: 3653 vs 782 IU/L, $p = .0027$).

After reaching the nadir, hCG levels increased significantly faster in the atypical foci group, with a median rise slope of 640 IU/L per day compared with 45 IU/L per day in the non-atypical group ($p = .005$), up to treatment initiation.

Similarly, the decline in hCG between treatment initiation and completion of first-line therapy was significantly steeper in the atypical group (median: −260 vs −29.1 IU/L/day, $p = .011$).

The median time to hCG normalization from the initial diagnosis was shorter in post-molar cases with atypical foci (median: 101 vs 141 days, $p = .022$).

DISCUSSION

Summary of Main Results

The analysis of this series of post-molar gestational trophoblastic neoplasia suggests that the presence of atypical extra-villous trophoblast foci is associated with a more severe disease course (both in terms of natural history and clinical management) compared with post-molar gestational trophoblastic neoplasia without such foci.

These atypical foci were significantly associated with FIGO/WHO scores >6. In addition, there was a trend toward higher methotrexate resistance, a more frequent need for multi-agent

Table 2 Treatment Characteristics of Post-Molar GTN According to Presence or Absence of Atypical Extra-villous Trophoblastic Foci

GTN features	Post-molar GTN				*p*-Value[a]
	No atypical foci (N = 18)		*Atypical foci (N = 38)*		
	No. of patients	**Number (%)**	**No. of patients**	**Number (%)**	
GTN treatment	18		38		.25
None		0 (0.0)		3 (7.9)	
Surgery alone		8 (44.4)		11 (28.9)	
Chemotherapy alone		9 (50.0)		16 (42.1)	
Surgery + chemotherapy		1 (5.6)		8 (21.1)	
Type of surgery	9		19		1.00
Second uterine evacuation		4 (44.4)		10 (52.6)	
Hysterectomy		5 (55.6)		9 (47.4)	
Sequence of chemotherapy regimens	10		23[b]		.57
MTX alone		9 (90.0)		13 (56.5)	
MTX - Act D		1 (10.0)		3 (13.0)	
MTX - EMA-CO		0 (0.0)		2 (8.7)	
MTX - Act D - EMA-CO		0 (0.0)		2 (8.7)	
EMA-CO alone		0 (0.0)		3 (13.0)	
MTX resistance	10		20		.21
No		9 (90.0)		13 (65.0)	
Yes		1 (10.0)		7 (35.0)	
Multi-agent chemotherapy (EMA-CO) during treatment	10		23		.07
No		10 (100)		16 (69.6)	
Yes		0 (0.0)		7 (30.4)	
No. of chemo courses	10		23		.14
		6 (5-7)		8 (6-12)	
Lines of treatment	18		38		.08
1		17 (94.4)		27 (71.1)	
≥2		1 (5.6)		11 (28.9)	

Abbreviations: Act D, Actinomycin D; EMA-CO, etoposide, methotrexate, actinomycin D — cyclophosphamide, vincristine; GTN, gestational trophoblastic neoplasia; MTX, methotrexate

[a] Fisher exact test for comparison of proportions or Kruskal-Wallis test for comparison of medians.

[b] 1 missing data.

chemotherapy, a greater number of chemotherapy cycles, and an increased number of treatment lines required to achieve remission.

Although some of these associations did not reach statistical significance due to the small sample size, the overall findings support the hypothesis that post-molar gestational trophoblastic neoplasia following moles with atypical extra-villous trophoblast foci may represent a distinct clinico-pathological entity compared with post-molar neoplasia lacking these foci.

Results in the Context of Published Literature

Our previous research demonstrated that atypical extra-villous trophoblast foci, identified in complete hydatidiform moles, are associated with more than a 2-fold increased risk of developing post-molar neoplasia in multi-variate analysis. However, the natural history and therapeutic response of these neoplasms had not previously been characterized.

The present study contributes to the identification of patients with gestational trophoblastic neoplasia at high risk of resistance to single-agent chemotherapy, which is critical for optimizing the management of gestational trophoblastic neoplasia. Well-established predictive factors for treatment response include maternal age, type of antecedent pregnancy, the interval between the end of the index pregnancy and the initiation of treatment, pre-treatment hCG level, size of the largest tumor, site and number of metastases, and prior chemotherapy failure. These variables constitute the FIGO/WHO risk score, introduced in 2000.[6]

Despite being classified as a low-risk disease, the rate of methotrexate resistance varies between 7.3% and 34.5%, depending on the regimen used.[17-22] For FIGO/WHO scores of 5

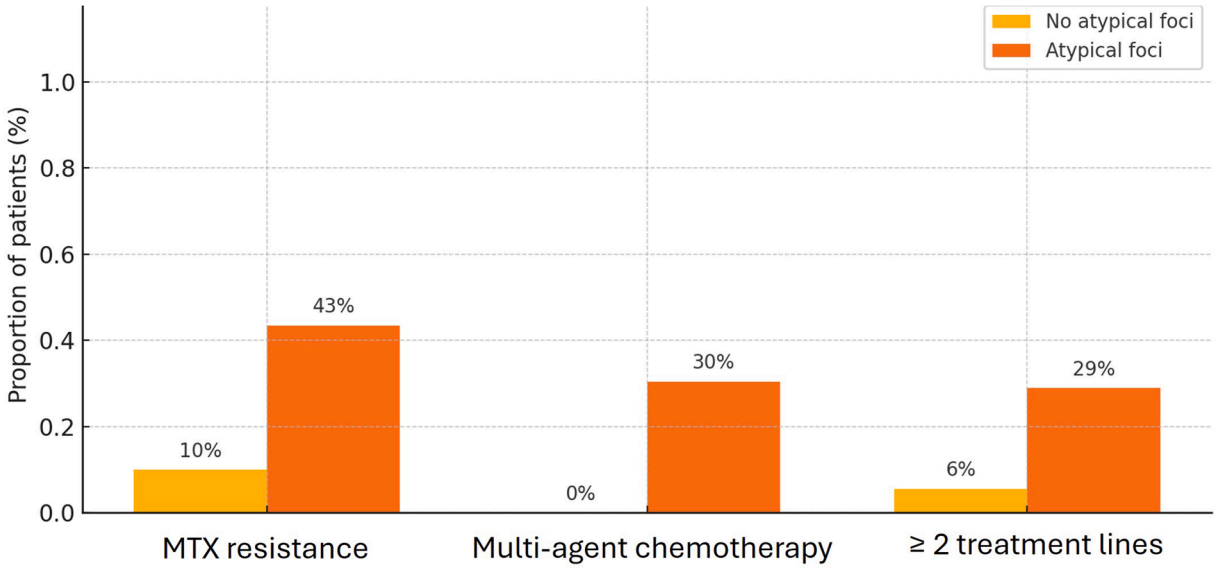

Figure 2 Comparison of MTX resistance, multi-agent chemotherapy treatment, and number of treatment lines according to the presence of atypical extra-villous trophoblast foci. MTX, methotrexate.

and 6, previously considered intermediate-risk disease, resistance to monotherapy may reach up to 70% to 75%.[10,23]

Patients with histologically confirmed post-molar gestational choriocarcinoma tend to have higher FIGO scores, higher pre-treatment hCG levels, and a greater likelihood of methotrexate resistance (hazard ratio 6.01; You and colleagues,[22] 2010; odds ratio 2.67; Strohl and Lurain,[19] 2016), with approximately 50% of patients developing resistance, regardless of the FIGO score.

Although the FIGO/WHO score incorporates only clinical, biological, and radiological parameters, additional histopathological and molecular markers could further improve risk stratification. In 1978, a correlation was first described between the morphology of the neoplasia and the therapeutic response.[24] Specifically, neoplasms exhibiting high mitotic activity, nuclear atypia, and prominent cytotrophoblastic proliferation required more intensive multi-agent chemotherapy. In gestational choriocarcinoma,

Table 3 hCG Kinetics of Post-Molar GTN According to Presence or Absence of Atypical Extra-villous Trophoblastic Foci.

hCG parameters	Post-molar GTN				*p*-Value[a]
	No atypical foci (*N* = 18)		*Atypical foci* (*N* = 38)		
	No. of patients	Median [Q1-Q3]	No. of patients	Median [Q1-Q3]	
hCG (IU/L)					
Before evacuation	14	190,750 [87,171-415,175]	30	160,439 [70,896-219,562]	.35
After evacuation	16	5335 [1895-18,218]	22	5811 [2259-13,294]	.81
Nadir hCG before treatment	17	782 [91.0-1408]	34	3653 [529-9532]	.0027
GTN treatment initiation	17	1430 [910-5524]	35	13000 [2449-34,892]	.0082
Time in relation to diagnosis (days)					
Before evacuation	14	−2.00 [−4.00 to −1.00]	30	−2.00 [−6.00 to −1.00]	.3
After evacuation	16	6.50 [5.00-8.00]	22	7.00 [5.00-11.0]	.64
Nadir hCG before treatment	17	33.0 [14.0-49.0]	34	12.5 [8.00-21.0]	.0042
GTN treatment initiation	17	63.0 [33.0-75.0]	35	33.0 [24.0-40.0]	.0066
hCG slope (IU/L/day)					
Between post-evacuation hCG and nadir	15	−199 [−327 to −51.0]	19	−214 [−5800 to −97.5]	.46
Between hCG nadir and treatment initiation	17	45.5 [1.84-134]	34	640 [101-2358]	.0052
Between treatment initiation and the end of first-line treatment	17	−29.1 [−190 to −6.93]	36	−260 [−929 to −63.3]	.011

Abbreviations: GTN, gestational trophoblastic neoplasia; hCG, human chorionic gonadotropin Q1, quartile 1; Q3, quartile 3.
[a] Kruskal-Wallis test for comparison of medians.

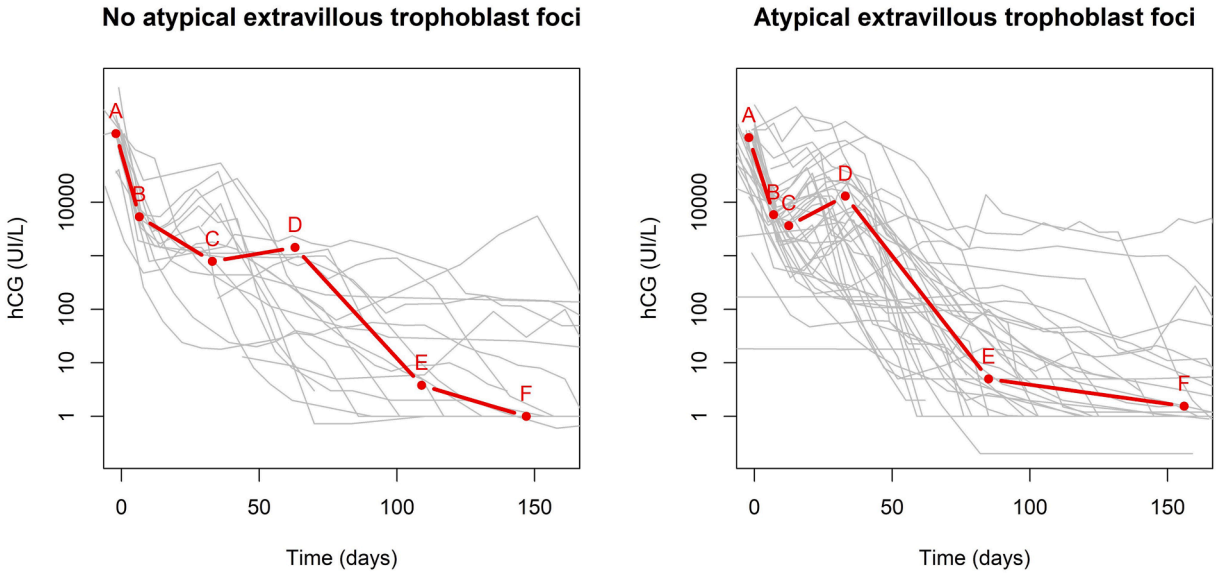

Figure 3 hCG kinetics curves of post-molar gestational trophoblastic neoplasia according to the presence of atypical extra-villous trophoblastic foci. Each gray line represents the hCG trajectory of an individual patient. The red lines connect the median hCG values at key clinical time points. The (x, y) coordinates of each red dot correspond to the median time (days) and the median hCG value (IU/L) at the following time points: A = before uterine evacuation; B = one week after evacuation; C = hCG nadir; D = initiation of post-molar neoplasia treatment; E = end of first-line therapy; F = hCG normalization. hCG, human chorionic gonadotropin.

immunohistochemical characteristics such as RASAL1 (RAS protein activator like 1) promoter hypermethylation and HLA-G (Human Leukocyte Antigen G) overexpression have been associated with chemoresistance.[25,26] However, to date, no histological marker has been identified in complete hydatidiform moles that reliably predicts the behavior of subsequent post-molar neoplasia.

The hCG kinetic profiles also provide valuable insights. Following uterine evacuation, hCG levels were similar between groups, suggesting comparable completeness of the initial treatment. However, in the group with atypical foci, the hCG nadir occurred earlier and was higher, followed by a significantly steeper rise prior to chemotherapy initiation. This pattern likely reflects the more aggressive biological behavior of post-molar disease in these patients.

These clinical findings support the hypothesis that post-molar neoplasia arising from complete moles with atypical extra-villous trophoblastic foci follows a more aggressive clinical course. Multiple lines of evidence from this study suggest the hypothesis that these foci, composed of a biphasic population of atypical syncytiotrophoblasts and cytotrophoblasts morphologically similar to choriocarcinoma, may represent an intermediate state between benign hydatidiform moles and malignant gestational choriocarcinoma. This transitional histological profile may explain the observed association with higher FIGO/WHO scores, increased methotrexate resistance, the need for more complex treatment regimens, and distinct hCG dynamics.

Implications for Practice and Future Research

The present study suggests that gestational trophoblastic neoplasia arising from complete moles with atypical extra-villous trophoblast foci follows a more aggressive clinical course. In this sub-group, clinicians should be aware of the increased risk of resistance to first-line single-agent methotrexate therapy. Given the limited sample size of the present study, our findings do not support any immediate modification of current clinical guidelines. At present, patient management should continue to follow established international recommendations for gestational trophoblastic disease, including those recently outlined by Lok and colleagues.[15]

If validated in independent and larger cohorts, our findings could nonetheless contribute to clinically meaningful refinements in patient management by improving risk stratification at 2 complementary levels.

First, the identification of atypical extra-villous trophoblastic foci could help stratify the risk of developing post-molar neoplasia. Systematic reporting of these foci by expert pathologists could be incorporated into the routine pathological assessment of complete hydatidiform moles, thereby allowing more individualized post-molar surveillance strategies, with closer follow-up in higher-risk patients and potentially reduced surveillance in lower-risk patients.

Second, in patients who develop post-molar neoplasia, the presence of atypical extra-villous trophoblastic foci could help identify those at increased risk of resistance to first-line single-agent chemotherapy, prompting closer monitoring of treatment response and earlier therapeutic adaptation when needed. Conversely, in the absence of atypical extra-villous trophoblast foci, surgery alone was successful in treating low-risk post-molar neoplasia in 44% of cases. This finding highlights a potential therapeutic option for selected patients, allowing the avoidance of chemotherapy and a shortened follow-up period.

Finally, we are continuing our work to identify additional prognostic factors. Integration of atypical extra-villous trophoblastic foci with other clinical and pathological variables into a nomogram could ultimately allow earlier identification of patients at high risk of progression, potentially supporting individualized

management strategies even before FIGO criteria for gestational trophoblastic neoplasia are met.

In the context of personalized medicine, the identification of novel predictive markers for treatment response in post-molar neoplasia represents an important step toward refining and individualizing clinical management. To date, no histological marker has been identified in complete hydatidiform moles that can predict the natural history and treatment response of subsequent neoplasia. The presence of atypical trophoblastic foci may therefore constitute a promising histopathological feature for improving risk stratification. Beyond its clinical relevance, this feature could also provide insights into the molecular mechanisms underlying neoplastic transformation, potentially representing an intermediate state between benign hydatidiform mole and malignant gestational choriocarcinoma.

Further translational research is warranted to elucidate the pathophysiological links between these entities and to determine whether atypical trophoblastic foci could serve as a reliable biomarker in gestational trophoblastic neoplasia.

Strengths and Weaknesses

A major strength of this study is its multi-center design within a national registry, combined with a systematic centralized pathological review of gestational trophoblastic diseases by nationally recognized expert pathologists.

However, we acknowledge the limitations of the retrospective design and the relatively small sample size. A larger cohort study is needed to validate these findings. When evaluating the 3 therapeutic outcomes that showed a non-significant trend between post-molar neoplasia following complete moles with or without atypical extra-villous trophoblast foci (namely, the use of multi-agent chemotherapy regimens, methotrexate resistance, and the requirement for more than 1 line of treatment) a power analysis (80% power, alpha = 0.05) estimated that cohorts of 92, 192, and 98 patients, respectively, would be required to reach statistical significance.

CONCLUSIONS

This study suggests that post-molar neoplasia following complete moles with atypical extra-villous trophoblast foci may have a more aggressive clinical course. These foci could represent a novel, cost-effective, and widely accessible histological marker for predicting disease aggressiveness and guiding therapeutic decisions.

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Author Contributions FG and SS had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: FG, KD and SS. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: SS. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: LS. Supervision: FG.

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