GYNECOLOGY

Presence of atypical extravillous trophoblast foci is an independent predictor of the risk of progression to postmolar neoplasia

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BACKGROUND: Amongst complete hydatidiform moles, 15% to 20% will progress to postmolar neoplasia. Scientists have long searched for routinely applicable prognostic markers that can efficiently predict the risk of postmolar neoplasia.

OBJECTIVE: To assess the prognostic value of atypical extravillous trophoblast foci within complete hydatidiform moles on the development of postmolar gestational trophoblastic neoplasia.

STUDY DESIGN: Between January 2017 and December 2022, a retrospective multicenter study was conducted in the Belgian Gestational Trophoblastic Diseases Registry (French-speaking center). All complete hydatidiform moles were included after being confirmed by a systematic centralized pathological review in 3 academic units with a high exposure to placental pathology. Postmolar gestational trophoblastic neoplasia was diagnosed according to the International Federation of Gynecology and Obstetrics 2000 criteria. Atypical extravillous trophoblast foci were defined as trophoblastic clusters in the intervillous chamber with a biphasic pattern of mononucleated cytotrophoblasts and multinucleated syncytiotrophoblasts. Their prognostic value for the development of postmolar gestational trophoblastic neoplasia was assessed using univariate analysis, followed by a multivariate model with stepwise selection of variables having a *P* value below .10 in the univariate analysis. A risk score, the "R-score," was developed based on the most significant variables of the multivariate model. The intercept and coefficients were obtained by fitting a logistic regression model. To facilitate its use in clinical practice, we established a score ranging from 0 to 10.

RESULTS: Of the 216 patients with complete hydatidiform mole, 56 patients subsequently developed postmolar gestational trophoblastic neoplasia (25.9%). Atypical extravillous trophoblast foci were found in 105/216 cases (48.6%). The risk of postmolar neoplasia was significantly associated with the presence of this pathological feature in univariate analysis (odds ratio, 2.93, *P*=.0010) and in multivariate logistic regression adjusted for confounding variables (odds ratio, 2.34, *P*=.0152). The R-score is based on the presence of atypical extravillous trophoblast foci, age, and postevacuation human chorionic gonadotropin levels, with an area under the curve of 0.721. A score below 6 indicates a low risk of postmolar neoplasia (15%), while a score of 7 or higher indicates a high risk (42%).

CONCLUSION: The presence of atypical extravillous trophoblast into complete moles is associated with the risk of developing postmolar gestational trophoblastic neoplasia. These foci may represent a new inexpensive and widely available histological prognostic marker.

Key words: atypical extravillous trophoblast foci, complete hydatidiform mole, postmolar gestational trophoblastic neoplasia, prognostic histological feature

Introduction

Gestational trophoblastic diseases are a spectrum of heterogeneous and rare placental pathologies characterized by abnormal proliferation of trophoblastic tissue. Premalignant lesions include complete (CHM) and partial hydatidiform moles (PHM), while malignant conditions, termed gestational trophoblastic neoplasia (GTN), encompass invasive mole, gestational choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor.[1] The incidence of molar pregnancies ranges from 0.57 to 2/1000 pregnancies.[2] There is no marked geographical variation, although a slightly higher incidence has been reported in Asia.[2] Amongst CHMs, 15% to 20% will progress to postmolar GTN, of which 3% will be choriocarcinoma. The follow-up of hydatidiform mole is based on blood monitoring of human chorionic gonadotropic (hCG) hormone. Postmolar GTN is diagnosed biologically or histologically according to the International Federation of Gynecology and Obstetrics (FIGO) 2000 criteria[3]: a rise in hCG levels (more than 10%) over a period of at least 2 weeks; a plateau in hCG levels over a period of at least 3 weeks; or a histological diagnosis of choriocarcinoma. Low-risk GTN (FIGO score $\leq$6) are treated with single-agent chemotherapy, with cure rates close to 100%. High-risk GTN (FIGO score >6) require multiagent chemotherapy, which may be combined with surgery, with cure rates approaching 90%.[4]

The pathophysiological mechanism of the progression from mole to neoplasia is not yet well understood. Identification of prognostic factors for neoplastic transformation could allow adjustment of the follow-up of hydatidiform moles by intensifying surveillance in patients at higher risk of developing neoplasia. Scientists have been trying to identify such prognostic factors for many years. Traditional risk factors are age, history of hydatidiform mole, and the presence of theca lutein cysts.[5–7] Studies have shown that hCG levels and kinetics also predict the risk of progression to postmolar

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AJOG at a Glance

Why was this study conducted?

No routinely applicable pathological feature is available to predict a neoplastic transformation of a complete hydatidiform mole (CHM) after uterine evacuation.

Key findings

The study shows that atypical extravillous trophoblast foci, diagnosed in certain CHMs, are associated with a significant higher risk of developing postmolar gestational trophoblastic neoplasia (GTN).

What does this add to what is known?

Identifying patients at higher risk of developing postmolar GTN could contribute to early GTN diagnosis and ensure more rigorous follow-up for this subgroup of patients, particularly in developing countries where close monitoring is not always feasible for practical reasons.

GTN.[8−13] Immunohistochemical assessment of several markers (MutL homolog 1, MutS homolog 2, nonmetastatic protein 23, telomerase reverse transcriptase, tumor protein 53, endothelial growth factor receptor, erythroblastic oncogene B) showed no characteristic expression in complete moles that subsequently developed postmolar GTN.[14] Several attempts have been made to define other early biological or biomolecular prognostic markers such as microRNA expression,[15] oncogene expression,[16] or apoptotic index,[17,18] but these are not routinely investigated in clinical practice. Molecular genetic studies have failed to identify useful risk markers.[19,20]

Extravillous aggregates of proliferating trophoblasts with biphasic growth patterns mimicking choriocarcinoma have been described in the literature.[21,22] Their prognostic value for the development of postmolar GTN has never been investigated. The objective of this study, conducted in a gestational trophoblastic disease reference center within the Belgian Gestational Trophoblastic Disease Registry, was to evaluate the prognostic value of atypical extravillous trophoblast foci in complete moles.

Materials and methods

Study design

This retrospective study was conducted in the Belgian Gestational Trophoblastic Diseases Registry (French-speaking center) between January 2017 and December 2022. Cases were referred on a voluntary basis, either by the initial pathologist, the gynecologist or the patient herself. All patients with a diagnosis of CHM were included. Data were recorded in an electronic database. All patients signed an informed consent form at the time of inclusion in the Belgian Registry. The study was approved by the local Institutional Review Board of the University Hospital of Liège (reference number 2023/343).

Pathological analysis

Curettage specimens were initially fixed in 10% formalin and embedded in paraffin. Histological-morphological analyses were performed on routine hematoxylin-eosin−stained slides. Ancillary techniques were used, when necessary, p57 immunohistochemistry was performed to confirm complete moles. Each initial histological diagnosis referred to the reference center was systematically reviewed by nationally recognized expert pathologists working in an academic setting with high exposure to placental pathology.[23] Histological slides were requested from the local pathology laboratories and reviewed by referral pathologists from the University Hospitals of Liège, Saint-Luc, and Erasmus.

Young CHM are diagnosed before 12 gestational weeks and have specific histological features that differ from well-developed CHM: smaller polypoid villi, mild or focal edema, focal or absent trophoblastic hyperplasia, and prominent stromal karyorrhexis.[24]

Atypical extravillous trophoblast foci within complete moles are defined as clusters of pleomorphic biphasic trophoblastic cells (CTs) located in the intervillous chamber characterized by an admixture of syncytiotrophoblast (ST) and mononucleated CTs with striking cytological atypia analogous to choriocarcinomas (Figure 1). For each case, the presence or absence of foci was assessed by an expert pathologist, who was blinded to the clinical characteristics and outcome, without quantification. Concordance between the expert pathologists was analyzed: it was considered "concordant" when all experts agreed on the presence of atypical extravillous trophoblast foci, and "discordant" when at least 1 expert disagreed on the presence of these foci.

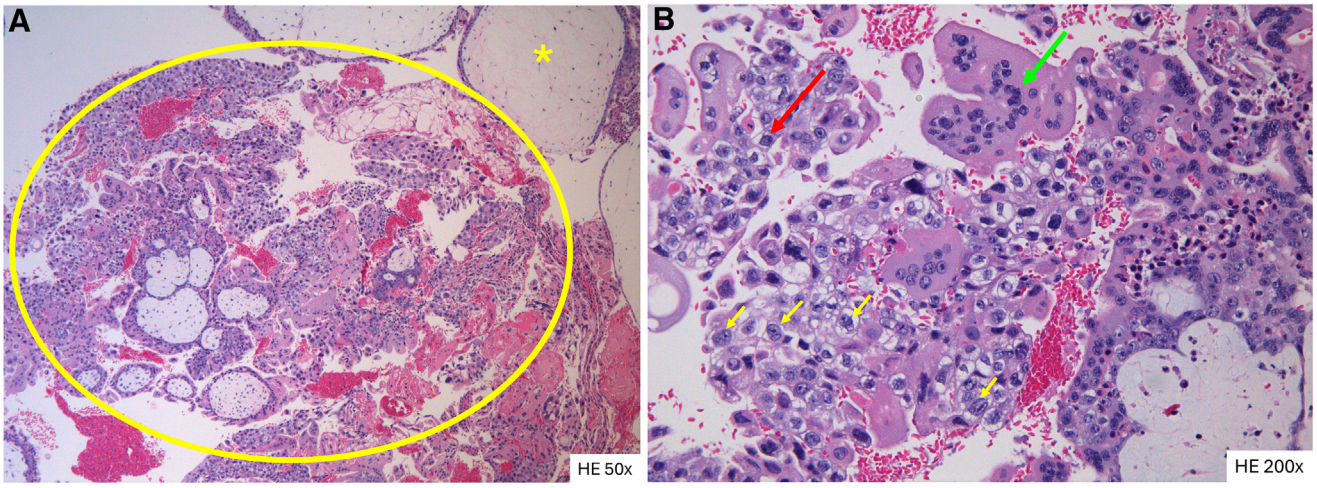
Clinical outcomes

The objective of the study was to assess the prognostic value of atypical extravillous trophoblast foci described in CHM on the risk of developing postmolar GTN. Postevacuation remission was defined as 2 consecutive normal hCG levels within the laboratory's normal range, measured at least 2 weeks apart. Postmolar GTN was diagnosed according to the criteria defined by the FIGO in 2000.[3] Cases that were lost to follow-up before the normalization of hCG levels were excluded.

Statistical analysis

Results are presented as mean±standard deviation or median and interquartile range and range for quantitative parameters and frequencies (%) for qualitative parameters. To assess the normality of the parameter distribution, the Shapiro-Wilk test was performed. hCG measurements were log-transformed to achieve a normal distribution. Cohen kappa coefficient was used to assess the agreement between pathologists regarding the presence of atypical extravillous trophoblast foci. Statistical analysis of the incidence of postmolar GTN was performed using logistic regression analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Multivariate models were run with a stepwise selection of variables based on

FIGURE 1
Histopathological features of atypical extravillous trophoblast foci in complete moles



A, (HE 50×) shows enlarged hydropic villi (*asterisk*) and atypical extravillous trophoblast foci (*yellow circle*). **B,** (HE 200×) shows a focus of pleomorphic atypical extravillous trophoblast with mononucleated cytotrophoblast (*red arrow*), multinucleated syncytiotrophoblast (*green arrow*), and striking cytologic atypia (*yellow arrows*).

HE, hematoxylin-eosin.

the parameters with a *P* value of less than .10 in the univariate analysis.

A risk score, the "R-score," was developed based on the most significant variables of the multivariate model. The intercept was obtained by fitting the logistic regression model, and the coefficients for the other variables were subsequently calculated. To facilitate its use in clinical practice, we aimed to obtain a score out of 10. Therefore, we calculated the minimum value $R(X)_{min}$ and the maximum value $R(X)_{max}$ of the score. The final score on a scale of 10 was obtained using the formula: $R(X)* = [R(X)-R(X)\ min]/[R(X)-R(X)\ min] \times 10$. The Youden method was used to determine a cutoff for R.

Results were considered significant at the 5% level of uncertainty ($P<.05$). Calculations were performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina).

Results

Study population

Between January 2017 and December 2022, a total of 709 patients were recorded by the French-speaking center of the Belgian Gestational Trophoblastic Diseases Registry. Among these, 244 were diagnosed with CHM and 191 with PHM. After excluding 28 cases lost to follow-up before hCG normalization, 216 CHM cases were eligible for analysis (Figure 2). The mean age of the patients was 35±9.1 years (range 16–56). Most CHM cases were diagnosed during the first trimester of pregnancy (n=190, 98.4%). Pathological features of young CHM were described in 44 cases (20.8%). The median preevacuation hCG level was 107,111 mIU/mL (range 19–16,733,190). The median first hCG level available after evacuation was 2017 mIU/mL (range 0–604,327), after a median of 8 days.

Atypical extravillous trophoblast foci

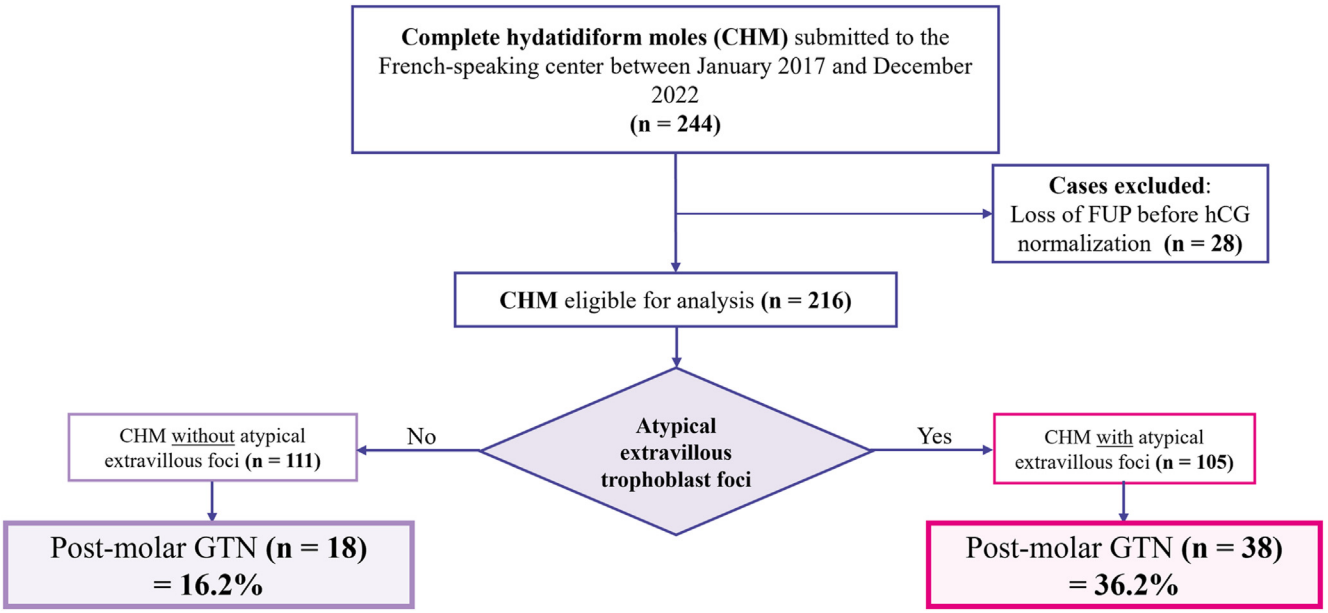
The expert pathologists diagnosed atypical extravillous trophoblast foci in 105 CHM (48.6%). Histological slides were reviewed by at least 2 expert pathologists in 88% of the cases (n=190). The interobserver correlation between the 3 institutions was statistically satisfactory with a kappa coefficient of 0.76 (95% CI, 0.65–0.86). In 5 cases (2.3%), only 1 pathologist out of the 3 diagnosed these foci. These cases were therefore included in the group without atypical extravillous trophoblast foci.

In 5 cases of PHM (5/191=2.6%), pathologists identified foci resembling atypical trophoblast foci.

Gestational trophoblastic neoplasia diagnosis

According to the FIGO criteria, 56 patients (25.9%) developed postmolar GTN, and 160 patients (74.1%) had postevacuation remission with hCG normalization (Figure 2). The diagnosis of postmolar GTN was made histologically in 2 patients and biologically in 54 patients. According to the FIGO criteria, hCG kinetics showed a plateau in 11% (n=6) and an increase in 89% (n=48) of cases. Most patients had FIGO stage I disease (n=48, 85.7%), while 8 patients had stage III disease (n=8, 14.3%). The FIGO/World Health Organization risk scores ranged from 0 to 8: 0 in 6 patients (11%), 1 in 15 patients (27%), 2 in 20 patients (36%), 3 in 5 patients (7%), 4 in 2 patients (4%), 6 in 4 patients (7%), 7 in 2 patients (2%), and 8 in 2 patients (4%).[3] Metastases were present at diagnosis in 8 cases, all of which were in the lungs. Twenty-seven patients had undergone surgery prior to a possible diagnosis of GTN: 22 patients (10.2%) had a second curettage due to vaginal bleeding or trophoblastic remnants on

FIGURE 2
Study flowchart



Flowchart describing the selection of cases for the study and GTN occurrence according to atypical trophoblast foci.
FUP, follow-up; *GTN*, gestational trophoblastic neoplasia; *hCG*, human chorionic gonadotropin.

ultrasound, and 5 patients (2.3%) who no longer wished to conceive had hysterectomy following a diagnosis of CHM. Among the 22 patients who underwent a second curettage, 14 (64%) had atypical extravillous trophoblast foci. None of the 5 patients with PHM and atypical foci developed subsequent postmolar neoplasia.

In the univariate analysis, the risk of postmolar GTN increased with age (OR=1.05 for each additional year, *P*=.0071), the presence of atypical extravillous trophoblast foci (OR=2.93, *P*=.0010), a second curettage before GTN diagnosis (OR=2.68, *P*=.032), and hCG levels before and after uterine evacuation (OR=1.62, *P*=.0029 and OR=1.38, *P*<.0001, respectively) (Table 1).

The different age groups in our population were analyzed using rounded age values close to the quartiles (Q1=28.0 and Q3=40.3 years): ≤30, 30 to 40, and >40 years. The logistic regression model based on these 3 categories showed that the risk of GTN was the same in the 30 to 40 age group as in the <30 group. Therefore, the 2 groups were merged, and the results showed that the risk of postmolar GTN was significantly higher in patients older than 40 years (OR=2.58 for patients >40 vs ≤40 years, *P*=.0044). Among patients under 20 years old (n=6), 2 developed postmolar GTN (33.33%); however, due to the small sample size, the results were not statistically different from those of the other groups under 40 years.

The variables for the multivariate model were selected using a stepwise method, based on those with a *P* value of less than .10 in the univariate analysis. Only 3 variables emerged in the multivariate model using nominal logistic regression: the presence of atypical extravillous trophoblast foci, age, and hCG level after uterine evacuation.

Multivariate analysis showed that postmolar GTN was statistically more frequent in the presence of atypical trophoblast foci (OR=2.34, *P*=.0152). The risk of postmolar GTN also increased with age at diagnosis (OR=2.24, *P*=.0258) and a high hCG level after uterine evacuation (OR=1.31, *P*=.0009) (Table 2). The presence of atypical extravillous trophoblast foci thus predicted the risk of progression to postmolar neoplasia, with an area under the curve (AUC) of 0.628 (95% CI, 0.55−0.70).

We developed a risk score, called the R-score, based on a combination of these 3 variables (X) to assess the risk of postmolar GTN (Figure 3). The first variable (X_1) was the presence or absence of atypical extravillous trophoblast foci. The second variable (X_2) was age, with a cutoff set at 40 years. The third variable (X_3) was the continuous variable hCG level after uterine evacuation, for which the Youden index was calculated at 1846 IU/L and rounded up to 1850 IU/L. Based on the logistic regression model, the risk score equation was: $R(X)=-2.4610+0.9469\times X_1+0.7665\times X_2+1.0771\times X_3$. To facilitate its use in clinical practice, we standardized the score between 0 and 10 by computing the minimum and maximum values (R[X] min=−2.461 and R[X] max=0.3295). The resulting score equation was: $R(X)=3.3933\times X_1+2.7468\times X_2+3.8599\times X_3$. Rounding the coefficients, we obtained the final R-score: $R\text{-}score=3\times X_1+3\times X_2+4\times X_3$.

An R-score below 6, a cutoff obtained using Youden' method, indicates a low

TABLE 1
Distribution of studied variables according to progression to postmolar neoplasia

Variables	No postmolar GTN (N=160)		Postmolar GTN (N=56)		OR (95% CI)	*P* value
Age at diagnosis (y), mean±SD	160	33.6±8.52	56	37.5±10.2	1.05 (1.01–1.08)	.0071
Age at diagnosis >40 y, n (%)	160	34 (21.3)	56	23 (41.1)	2.58 (1.34–4.96)	.0044
Diagnosis after the 1st trimester of pregnancy, n (%)	147 MD 13	2 (1.4)	46 MD 10	1 (2.2)	1.61 (0.14–18.2)	.70
Presence of atypical extravillous trophoblast foci, n (%)	160	67 (41.9)	56	38 (67.9)	2.93 (1.54–5.57)	.0010
Young CHM, n (%)	156 MD 4	33 (21.2)	56	11 (19.6)	0.91 (0.43–1.95)	.81
Second curettage before GTN diagnosis, n (%)	160	12 (7.5)	56	10 (17.9)	2.68 (1.09–6.61)	.032
Hysterectomy before GTN diagnosis, n (%)	160	3 (1.9)	56	2 (3.6)	1.94 (0.32–11.9)	.47
hCG level[a] before uterine evacuation, median [Q1–Q3]	129 MD 31	88,607 [29,301–156,896]	47 MD 9	191,367 [74,422–225,000]	1.62 (1.18–2.22)	.0029
hCG level[a] after uterine evacuation, median [Q1–Q3]	159 MD 1	1381 [148–4814]	55 MD 1	4833 [1865–13,294]	1.38 (1.18–1.61)	<.0001

Results of univariate logistic regression analyses. The ORs for hCG levels were calculated on the log scale.

CHM, complete hydatidiform mole; *CI*, confidence interval; *GTN*, gestational trophoblastic neoplasia; *hCG*, human chorionic gonadotropin; *MD*, missing data; *OR*, odds ratio; *SD*, standard deviation.

[a] A logarithmic transformation was applied.

risk of developing postmolar neoplasia (negative predictive value=85%). In contrast, an R-score of 6 or higher indicates an increased risk, with a sensitivity and specificity of 67%. In our cohort, we were able to calculate the R-score for 211 patients. Among them, 122 (58%) had an R-score below 6, and 89 had an R-score of 6 or higher (42%). In the low-risk group (R-score <6), 18 (15%) developed postmolar neoplasia. In the high-risk group (R-score ≥6), 37 (42%) developed postmolar neoplasia. The AUC of this score is 0.721 (95% CI, 0.65–0.80) (Figure 4). The AUC of the R-score is therefore higher than the CI of the atypical extravillous trophoblast foci alone.

Human chorionic gonadotropin kinetic

For the 216 patients, the median time to hCG normalization was 70 days (interquartile range, 52–101 days). The median time to hCG normalization was 63 days in the 111 patients without atypical trophoblast foci and 77 days in the 105 patients with atypical trophoblast foci. Among the 56 patients with postmolar GTN, the median time to hCG nadir was 18 days (interquartile range, 9–35 days). The median time to hCG nadir was 34 days in the 18 patients without atypical extravillous trophoblast foci and 14 days in the 38 patients with atypical extravillous trophoblast foci.

Discussion

Principal findings

This study showed, in the multivariate analysis, a significant correlation between the presence of atypical extravillous trophoblast foci in complete moles and the development of postmolar GTN. There was a satisfactory level of agreement between the 3 independent expert pathologists in the description of these foci.

Results in the context of what is known

To date, no routine widely accepted biomarker has been validated to predict the risk of postmolar neoplasia. Atypical extravillous trophoblast foci are a histological feature that allows pathologists to

TABLE 2
Multivariate logistic regression analyses of postmolar gestational trophoblastic neoplasia occurrence (N = 211, AUC = 0.738)

Variables	OR (95% CI)	*P* value
Age at diagnosis >40 y	2.24 (1.10–4.56)	.026
Presence of atypical extravillous trophoblast foci	2.34 (1.18–4.66)	.015
hCG level after uterine evacuation[a]	1.31 (1.12–1.54)	.0009

Three variables were selected using a stepwise method based on variables with a *P* value less than .10 in univariate analysis: atypical extravillous trophoblast foci, age, and human chorionic gonadotropin level after uterine evacuation.

AUC, area under the curve; *CI*, confidence interval; *hCG*, human chorionic gonadotropin; *OR*, odds ratio.

[a] A logarithmic transformation was applied.

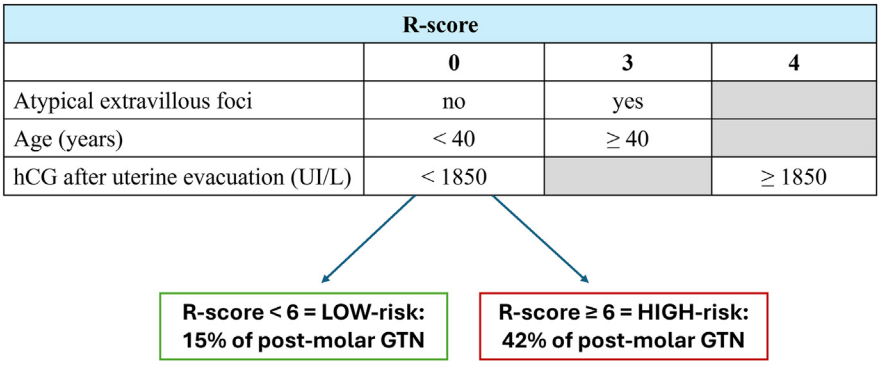
identify patients at elevated risk of developing neoplasia. Multivariate analyses have shown that the presence of these foci more than doubles the risk of neoplasia. The advantage of histological markers from uterine evacuation products is that they allow early identification of high-risk patients. Other biomarkers have been reported, including the caspase-3 apoptotic index,[17] proteomic identification, selenoprotein iodothyronine deiodinase 3, and microRNA expression.[25] However, the search for these markers is time-consuming, costly, and difficult to access in clinical practice, especially in developing countries where diagnosis and management of GTN is challenging.[26]

Regarding histological markers, several studies have historically evaluated the degree of trophoblastic villous hyperplasia as a predictor of progression to postmolar neoplasia. In 1947, Hertig and Sheldon[27] established a grading system for hydatidiform moles into 6 grades based on trophoblastic hyperplasia and anaplasia. In 1956, this classification was simplified to 3 grades: slight, moderate, or marked hyperplasia.[28] Results were controversial, with some studies observing a correlation between histological grade and postmolar neoplasia,[27–30] while others did not, concluding that the histological appearance of the original hydatidiform mole should not influence treatment and follow-up.[31,32] This histological concept focuses on trophoblastic hyperplasia in the ST border of the villi. In this study, we focused on atypical trophoblast foci present in the intervillous space with a biphasic appearance and striking atypia mimicking intraplacental choriocarcinoma. These foci have already been described in the literature as "intramolar choriocarcinoma."[21,22] The presence of trophoblastic foci with a biphasic population of atypical cells is not universally accepted as intraplacental choriocarcinoma because the diagnosis of choriocarcinoma classically requires the absence of villi. We have termed such foci "atypical extravillous trophoblast foci." The pathophysiological mechanism remains to be elucidated, but the results show that they more than double the risk of postmolar neoplasia.

Several studies have analyzed hCG kinetics as a tumor marker, focusing on the ratio of preevacuation to postevacuation levels, subunit ratios, and the linear regression slope.[8,9,11,33] Linear regression of postevacuation hCG concentrations appears to be a useful tool for identifying patients at risk of developing GTN, with an AUC of 0.906,[11] and predicting 52% of patients with postmolar GTN with a specificity of 97.5%. However, this strategy requires a delay of at least 3 weeks, from days 7 to 28 after evacuation. The present study confirms that high pre- and postevacuation hCG levels are associated with a higher risk of postmolar neoplasia (OR=1.62 and OR=1.38, respectively). In cases of postmolar GTN, the time to reach the nadir of hCG prior to neoplasia occurs earlier in the group with atypical extravillous foci, indicating that the increase in hCG levels after uterine evacuation is sooner in the presence of these foci (mean of 4 days). The time to diagnose postmolar GTN is therefore shorter in the presence of atypical extravillous trophoblast foci.

The rate of postmolar GTN in this study is 25.9% for complete moles. Other studies evaluating prognostic markers for neoplasia have reported similar rates, ranging from 24% to 36%.[9,11,13,17,25,34] This is higher than the 15% to 20% expected in the general literature and the recommendations for gestational trophoblastic diseases.[1] This discrepancy may be due to referral bias toward national tertiary reference

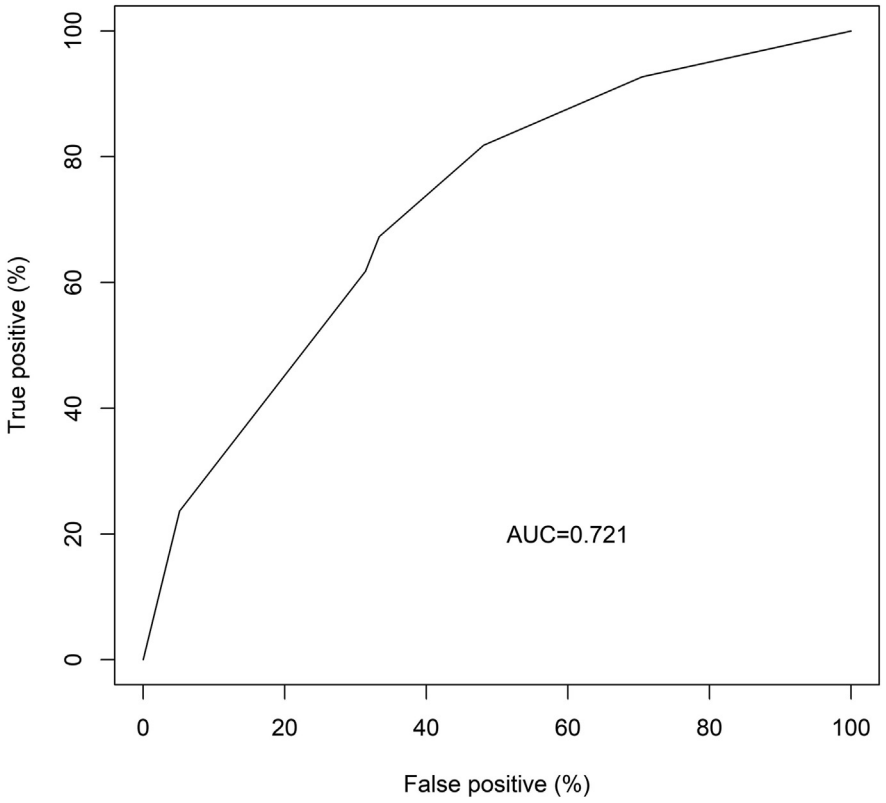
FIGURE 3
The R-Score: a GTN risk assessment tool



The R-score is a GTN risk score based on 3 factors: the presence of atypical extravillous trophoblast foci, age at diagnosis, and hCG levels after uterine evacuation. The R-score ranges from 0 to 10. Patients with an R-score lower than 6 have a 15% risk of developing postmolar GTN, while patients with an R-score of 6 or higher have a 42% risk of GTN.

GTN, gestational trophoblastic neoplasia; *hCG*, human chorionic gonadotropin.

FIGURE 4
Performance of the R-Score: receiver operating characteristic curve and AUC



Receiver operating characteristic curve and AUC value of the R-score.
AUC, area under the curve.

centers with voluntary registration, where more complex cases are centralized.

Maternal age is a well-established prognostic factor for postmolar neoplasia, with an increased risk in both younger (less than 20 years old) and older (more than 40 years old) age groups.[35–37] The results of this study confirm that patients older than 40 years have a 2.6-fold increased risk of developing postmolar neoplasia. The findings also suggest a trend toward a higher risk in patients younger than 20 years, though this was not statistically significant due to the small sample size of this group.

Studies have shown a therapeutic benefit of second uterine curettage as initial treatment for low-risk nonmetastatic GTN, with cure rates ranging from 9.4% to 45%.[38–40] A second uterine evacuation may be necessary prior to the diagnosis of GTN due to vaginal bleeding or unsatisfactory hCG evolution before meeting the FIGO criteria. Our data show that a second curettage before GTN is associated with a higher risk of postmolar neoplasia in univariate analysis, but 64% (n=13) of the patients had atypical extravillous trophoblast foci, which now appears to be independently associated with a higher risk of postmolar GTN. On the other hand, the conditions leading to the second curettage may indicate more extensive and less favorable disease. In the multivariate model, the second curettage was not a risk factor for the development of GTN.

Clinical implications

Atypical extravillous trophoblast foci in complete moles represent a histological diagnosis that does not require any specific equipment or preparation, apart from routine hematoxylin-eosin staining. This histological description can be widely used regardless of a country's healthcare resources. However, it requires a pathologist with expertise in trophoblastic/placental pathology or gynecopathology.

In placental pathology, the number of slides examined for each patient can influence the diagnostic rate.[41] For term placentas, there are recommendations for a minimum of 4 blocks to be studied, but no consensus for hydatidiform moles.[24,42] In the Belgian Gestational Trophoblastic Diseases Registry, we receive slides from external laboratories for pathological review. The number of slides varies depending on the laboratory. We therefore examine all the slides and assess the presence of atypical extravillous trophoblast foci. The key is a thorough initial macroscopic analysis to select the tissue of interest to be placed in the cassette. Unfortunately, we have not been able to determine the ideal number of slides per patient that should be examined to detect atypical foci.

After the evacuation of a complete mole, current guidelines recommend weekly hCG monitoring until hCG normalizes, followed by monthly hCG monitoring for 6 months. Studies have shown that the risk of recurrence is very low after normalization of serum hCG levels.[43,44] Identifying an early indicator capable of predicting postmolar GTN at the time of CHM diagnosis could potentially be of clinical benefit. The R-score may help to assess the risk of GTN, with a 15% risk of GTN when the score is <6 and a 42% risk when the score is ≥ 6. Further studies are needed to determine whether a shortened follow-up period could be proposed for low-risk patients, to reduce patient anxiety, healthcare costs, and the time needed to postpone another pregnancy.

Prophylactic chemotherapy after hydatidiform mole evacuation is not routinely recommended. A systematic review including 3 randomized controlled trials showed that prophylactic chemotherapy may reduce the incidence of GTN in high-risk patients, but it is associated with induced toxicity, delays in GTN diagnosis, and drug resistance.[45] However, in resource-poor countries, a single dose of dactinomycin may be effective in reducing the incidence of GTN when

hCG monitoring is difficult, in highly selected cases.[46] Identifying patients at high risk of GTN in this context may therefore be important.

In the era of personalized medicine, the discovery of a new prognostic factor for transformation into postmolar neoplasia helps guide and individualize decision-making. It allows for personalized monitoring and reduces anxiety in low-risk patients. This new marker also paves the way for a molecular understanding of neoplastic transformation. One might wonder whether atypical extravillous trophoblastic foci represent a transition between molar premalignancy and neoplasia.

Research implications

The results of this study need to be validated in a prospective study with a larger cohort of patients. Spalt-like transcription factor 4 (SALL4) is a transcription factor primarily expressed in stem cells and certain types of tumors, including germ cell tumors and gestational choriocarcinomas. It is a useful marker to distinguish gestational choriocarcinomas from other trophoblastic tumors, and it has a prognosis value.[47,48] The investigation of SALL4 expression within the atypical extravillous foci may provide valuable information for the diagnosis and understanding of these foci, which could represent a critical step before the progression to gestational choriocarcinoma.

Strengths and limitations

The strength of our study is the multicentric setting of a national registry with a systematic centralized pathological review of gestational trophoblastic diseases by recognized expert pathologists. The presence of atypical extravillous trophoblast foci is an easy-to-use marker in clinical practice, as it can be diagnosed on hematoxylin-eosin–stained slides and does not require special equipment. We acknowledge that the retrospective design and the relatively small number of cases limit the applicability of our study.

Conclusions

This study demonstrates that the presence of atypical extravillous trophoblast foci in CHMs is associated with the development of postmolar neoplasia. These foci may represent a new, inexpensive, and widely available histological prognostic marker. ■

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