Predictors for single-agent resistance in FIGO score 5 or 6 gestational trophoblastic neoplasia: a multicentre, retrospective, cohort study

Antonio Braga, Gabriela Paiva*, Ehsan Ghorani*, Fernanda Freitas, Luis Guillermo Coca Velarde, Baljeet Kaur, Nick Unsworth, Jingky Lozano-Kuehne, Ana Paula Vieira dos Santos Esteves, Jorge Rezende Filho, Joffre Amim Jr, Xianne Aguiar, Naveed Sarwar, Kevin M Elias, Neil S Horowitz, Ross S Berkowitz†, Michael J Seckl†*

Summary

Background Patients with gestational trophoblastic neoplasia who have an International Federation of Gynaecology and Obstetrics (FIGO) risk score of 5 or 6 usually receive non-toxic single-agent chemotherapy as a first-line treatment. Previous studies suggest that only a third of patients have complete remission, with the remaining patients requiring toxic multiagent chemotherapy to attain remission. As stratification factors are unknown, some centres offer multiagent therapy upfront, resulting in overtreatment of many patients. We aimed to identify predictive factors for resistance to single-agent therapy to inform clinicians on which patients presenting with a FIGO score of 5 or 6 are likely to benefit from upfront multiagent chemotherapy.

Methods We did a multicentre, retrospective, cohort study of patients with gestational trophoblastic neoplasia presenting with a FIGO score of 5 or 6, who received treatment at three gestational trophoblastic neoplasia reference centres in the UK, Brazil, and the USA between Jan 1, 1964, and Dec 31, 2018. All patients who had been followed up for at least 12 months after remission were included. Patients were excluded if they had received a non-standard single-agent treatment (eg, etoposide); had been given a previously established first-line multiagent chemotherapy regimen; or had incomplete data for our analyses. Patient data were retrieved from medical records. The primary outcome was the incidence of chemoresistance after first-line or second-line single-agent chemotherapy. Variables associated with chemoresistance to single-agent therapies were identified by logistic regression analysis. In patient subgroups defined by choriocarcinoma histology and metastatic disease status, we did bootstrap modelling to define thresholds of pretreatment human chorionic gonadotropin concentrations and identify groups of patients with a greater than 80% risk (ie, a positive predictive value [PPV] of 0·8) of resistance to single-agent chemotherapy.

Findings Of 5025 patients with low-risk gestational trophoblastic neoplasia, we identified 431 patients with gestational trophoblastic neoplasia presenting with a FIGO risk score of 5 or 6. All patients were followed up for a minimum of 2 years. 141 (40%) of 351 patients developed resistance to single-agent treatments and required multiagent chemotherapy to achieve remission. Univariable and multivariable logistic regression revealed metastatic disease status (multivariable logistic regression analysis, odds ratio [OR] 1·9 [95% CI 1·1–3·2], p=0·018), choriocarcinoma histology (3·7 [1·9–7·4], p=0·0002), and pretreatment human chorionic gonadotropin concentration (2·8 [1·9–4·1], p<0·0001) as significant predictors of resistance to single-agent therapies. In patients with no metastatic disease and without choriocarcinoma, a pretreatment human chorionic gonadotropin concentration of 411 000 IU/L or higher yielded a PPV of 0·8, whereas in patients with either metastases or choriocarcinoma, a pretreatment human chorionic gonadotropin concentration of 149 000 IU/L or higher yielded the same PPV for resistance to single-agent therapy.

Interpretation Approximately 60% of women with gestational trophoblastic neoplasia presenting with a FIGO risk score of 5 or 6 achieve remission with single-agent therapy; almost all remaining patients have complete remission with subsequent multiagent chemotherapy. Primary multiagent chemotherapy should only be given to patients with metastatic disease and choriocarcinoma, regardless of pretreatment human chorionic gonadotropin concentration, or to those defined by our new predictors.

Funding None.

Introduction

Gestational trophoblastic neoplasia is a rare cancer arising from the placenta that affects around 20 000 women per year globally. Early diagnosis is important, as it is associated with long-term survival rates greater than 99%.[1–5] Several forms of gestational trophoblastic neoplasia exist, including invasive mole, choriocarcinoma, and the rare placental-site trophoblastic (PSTT) and epithelioid trophoblastic tumours (ETT). Most (ie, 50–80%) patients present with the premalignant hydatidiform mole, and a

Lancet Oncol 2021

Published Online June 25, 2021 https://doi.org/10.1016/S1470-2045(21)00262-X

See Online/Comment https://doi.org/10.1016/S1470-2045(21)00327-2

For the Portuguese translation of the abstract see Online for appendix 1

*Joint first authors

†Joint senior authors

Rio de Janeiro Trophoblastic Disease Centre, Maternity School of Rio de Janeiro Federal University, Antonio Pedro University Hospital of Fluminense Federal University, Niterói, Rio de Janeiro, Brazil (A Braga MD, G Paiva MD, F Freitas MD, A P V dos Santos Esteves PhD, Prof J Rezende Filho MD, J Amim Jr MD); **Postgraduate Programme in Perinatal Health, Faculty of Medicine, Maternity School of Rio de Janeiro Federal University, Rio de Janeiro, Brazil** (A Braga, G Paiva, F Freitas, A P V S Esteves, Prof J Rezende Filho, J Amim Jr); **Postgraduate Programme in Medical Sciences, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil** (A Braga, L G C Velarde PhD); **Young Leadership Physicians Programme, National Academy of Medicine, Rio de Janeiro, Brazil** (A Braga); **Trophoblastic Tumour Screening and Treatment Centre, Charing Cross Hospital, Imperial College Healthcare NHS Trust, London, UK** (E Ghorani MD, B Kaur FRCPath, N Unsworth MSc, J Lozano-Kuehne PhD, X Aguiar BSc, N Sarwar MD, Prof M J Seckl FMedSci); **Department of Obstetrics, Gynecology and Reproductive Biology, Division of Gynecologic Oncology,**

New England Trophoblastic Disease Centre, Brigham and Women's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA (K M Elias MD, N S Horowitz MD, Prof R S Berkowitz MD)

Correspondence to: Prof Michael J Seckl, Trophoblastic Tumour Screening and Treatment Centre, Charing Cross Hospital, Imperial College Healthcare NHS Trust, London W2 1NY, UK
m.seckl@imperial.ac.uk

Research in context

Evidence before this study

We searched MEDLINE, PubMed, and Embase databases on Oct 31, 2020, using the following medical subject headings "gestational trophoblastic neoplasia" AND "low-risk" OR "choriocarcinoma" OR "metastatic disease". We searched for cohort studies or case series investigating the treatment of patients with low-risk gestational trophoblastic neoplasia (ie, those with an International Federation of Gynaecology and Obstetrics [FIGO] risk score of 0–6). Studies published in English were included in the review if they had more than ten patients; contained information on low-risk gestational trophoblastic neoplasia treatment; and had been published between Jan 1, 2000 (when scoring of disease risk according to FIGO criteria was universally adopted), and Oct 31, 2020. We found ten studies investigating treatment of patients with low-risk gestational trophoblastic neoplasia. Patients with low-risk gestational trophoblastic neoplasia should preferably be treated with single-agent chemotherapy, as almost all patients eventually have complete remission. However, only a third of those presenting with a FIGO risk score of 5 or 6 enter remission following treatment with non-toxic, first-line, single-agent treatments; the remaining patients receive first-line or second-line multiagent chemotherapy. Consequently, some investigators recommend that all women with a FIGO risk score of 5 or 6 receive first-line multiagent therapy, which is considerably more toxic than single-agent therapy.

Added value of this study

In the world's largest cohort of patients with gestational trophoblastic neoplasia who have a FIGO score of 5 or 6, we identified several risk factors as independent predictors of resistance to single-agent treatments, including the presence or absence of metastases or choriocarcinoma. Crucially, our analysis revealed specific human chorionic gonadotropin concentration cutoff values that can be used to improve the accuracy of selecting patients with a FIGO risk score of 5 or 6 for multiagent, as opposed to single-agent, first-line and second-line therapy.

Implications of all the available evidence

Based on the results of our study, multiagent chemotherapy should only be given to patients with gestational trophoblastic neoplasia who present with a FIGO score of 5 or 6 if they have no metastases or choriocarcinoma and a pretreatment human chorionic gonadotropin concentration of 411 000 IU/L or higher, metastases or histopathologically confirmed choriocarcinoma and a pretreatment human chorionic gonadotropin concentration of 149 000 IU/L or higher, or if they have metastatic choriocarcinoma. Importantly, almost 60% of the remaining patients will enter remission with one or two sequential single-agent treatments, and almost all patients who develop resistance will have complete remission. Our results could help stop some international centres from automatically treating all patients with a FIGO score of 5 or 6 with multiagent chemotherapy.

plateau or increase in the concentration of human chorionic gonadotropin pregnancy hormone after surgical evacuation[6,7] is a sensitive indicator of malignant change to gestational trophoblastic neoplasia.

Fortunately, post-molar gestational trophoblastic neoplasia and choriocarcinoma arising from any other type of pregnancy (ie, abortion, ectopic pregnancy, term, or preterm) are highly sensitive to either single-agent or more toxic multiagent chemotherapy.[8–10] To decide between single-agent and multiagent therapies, several prognostic scoring systems have been developed.[11–13] These scoring systems were eventually consolidated into the International Federation of Gynaecology and Obstetrics (FIGO) scoring system.[14] Patients with gestational trophoblastic neoplasia presenting with a FIGO risk score of 6 or less are considered to have a low-risk of developing resistance to single-agent chemotherapy, which is less toxic than multiagent chemotherapy. These individuals are usually given either methotrexate plus folinic acid or dactinomycin. Patients with chemoresistance to one single-agent regimen are often switched to the other regimen before they are offered multiagent chemotherapy.[9,15,16] By contrast, patients with a FIGO risk score of 7 or higher are considered to have a high risk of resistance to single-agent chemotherapy, and therefore receive multiagent chemotherapy from the outset.[14]

The proportion of patients with low-risk gestational trophoblastic neoplasia who attain clinical remission with single-agent chemotherapy varies considerably. For instance, the proportion of patients who have complete remission after single-agent chemotherapy is greater than 90% in individuals with a FIGO risk score of 0 or 1 compared with around only 33% of patients with a FIGO risk score of 5 or 6.[15] The proportion of patients with a FIGO risk score of 5 or 6 who have complete remission has served as a rationale for some investigators to advocate using multiagent chemotherapy in these patients from the outset.[17] However, as virtually all patients with low-risk gestational trophoblastic neoplasia are long-term survivors,[5] others have suggested that it would be better to elucidate existing or new factors that could refine the FIGO scoring system.[18–20] This refinement would enable better identification of most patients with a FIGO risk score of 5 or 6 who are unlikely to respond to methotrexate plus folinic acid, dactinomycin, or both regimens, and avoid treating the one-third of patients who are sensitive to single-agent treatment with more toxic multiagent chemotherapy.

Various strategies for choosing between single-agent and multiagent therapy in patients with a FIGO risk score of 5 or 6 have been proposed, including increased tumour vascularity, as assessed by doppler ultrasound,[21] an

absolute pretreatment human chorionic gonadotropin concentration of more than 400 000 IU/L,[22] and the presence of metastases in patients with choriocarcinoma.[23] Following initiation of single-agent chemotherapy, the rate of human chorionic gonadotropin decline during the first few weeks has also been suggested as an early marker of single-agent therapy failure.[24] These studies have been done in single centres, often with low patient numbers, and, consequently, none of the recommended approaches have been broadly adopted by the clinical community. A new, larger evaluation is needed to help identify factors that could improve categorisation of patients with a FIGO risk score of 5 or 6 into groups of single-agent versus multiagent chemotherapy responders.

In this report, we present the results of an international collaborative study of the largest global dataset of patients with gestational trophoblastic neoplasia presenting with a FIGO risk score of 5 or 6. We did an in-depth investigation to identify predictors of resistance to single-agent chemotherapies with the aim of improving the robustness of the criteria used to select patients for single-agent or multiagent chemotherapy. We suggest new parameters to select which patients with a FIGO risk score of 5 or 6 are most likely to respond to treatment with primary single-agent as opposed to multiagent chemotherapy.

Methods

Study design and participants

We did a multicentre, retrospective, cohort study of patients with gestational trophoblastic neoplasia presenting with a FIGO risk score of 5 or 6, who received treatment between Jan 1, 1964, and Dec 31, 2018, at three gestational trophoblastic neoplasia reference centres: Charing Cross Trophoblastic Disease Centre (Charing Cross Hospital, Imperial College of London, London, UK); Rio de Janeiro Trophoblastic Disease Centre (Maternity School of Rio de Janeiro Federal University, Rio de Janeiro, Brazil); and the New England Trophoblastic Disease Centre (Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA). These are three of the largest gestational trophoblastic neoplasia reference centres in the world (appendix 2 p 1).

All patients with low-risk gestational trophoblastic neoplasia, presenting with a FIGO risk score of 5 or 6, who were followed up for at least 12 months after remission were included, hence the inclusion of patients up to Dec 31, 2018. Patients were excluded if they had received a non-standard single-agent treatment, such as etoposide; had been given a previously established first-line multiagent chemotherapy regimen before etoposide and methotrexate plus folinic acid rescue and dactinomycin, alternating with cyclophosphamide and vincristine (EMA/CO); had a histopathological diagnosis of ETT or PSTT; or had incomplete data for our analyses. This study was approved by the Maternity School of Rio de Janeiro Federal University Review Board and by the Brigham and Women's Hospital Review Board. This study was also approved as a National Health Service (NHS) service evaluation and improvement exercise by Imperial College NHS Healthcare Trust, with all patient data deidentified. Considering the retrospective collection of data from anonymised patient records, the study was exempted by local ethics committees from the need to obtain written informed consent from patients (appendix 2 p 1).

Procedures

The FIGO scoring system[14] (appendix 2 p 4) was retrospectively applied to all patients given single-agent chemotherapy before 2000, to correctly identify those with a risk score of 5 or 6 for inclusion in this study. Since 2012, patients with an elevated but declining human chorionic gonadotropin concentration at 6 months after uterine evacuation were considered not to have post-molar gestational trophoblastic neoplasia in the absence of other features, as described previously.[25]

Patients were staged according to FIGO 2000 gestational trophoblastic neoplasia anatomical staging criteria. Patients included in this study underwent a centralised histopathological review when pathology samples were available, and only samples obtained before first-line treatment was started were analysed. Depending on when patients had been diagnosed and evaluated, arteriography, ultrasonography, chest radiography, chest CT, or head imaging were used for staging of the disease. During the 1960s, uterine or pelvic lesions were assessed by arteriography, and from the 1970s to currently, uterine disease was evaluated by ultrasonography. Chest radiography was the primary mode of detecting lung metastases from 1960 onwards, but chest CT imaging became routinely available in the 1970s for patients in whom the chest radiography yielded equivocal results. Only lesions greater than 1 cm in diameter[14] on chest radiography or CT scan were counted. Head imaging was introduced in the 1970s for patients with lung metastases and comprised of a contrast-enhanced CT scan in the 1970s and MRI from the 1980s to currently. Serum human chorionic gonadotropin was measured by radioimmunoassay with a rabbit polyclonal antibody or with a sandwich chemiluminescence immunoassay method, both with the reference value for normal serum of less than 5 IU/L.

See Online for appendix 2

In patients with low-risk gestational trophoblastic neoplasia, the treatment of choice was an 8-day methotrexate plus folinic acid regimen. In patients with methotrexate chemoresistance, the preferred regimen was either dactinomycin or a multiagent chemotherapy regimen. EMA/CO has been the most commonly used multiagent chemotherapy regimen over the past 30 years. Before 1990, most patients with a FIGO risk score of 5 or 6 were given a multiagent chemotherapy regimen as part of the Bagshawe middle-risk group,[11] which is why few patients received methotrexate plus folinic acid between 1964 and 1990.

After human chorionic gonadotropin normalisation, most patients received consolidation chemotherapy, which has been standardised to three cycles since 2012.[26] Post-treatment monitoring of human chorionic gonadotropin concentrations in serum, urine, or both varied from once every 2 weeks to monthly during the first 12 months and with decreasing frequency thereafter, according to local centre preferences.[3,5,27]

The following clinical, biochemical, and pathological variables were retrieved from medical records: age, gravidity, parity, pretreatment human chorionic gonadotropin concentration, histopathological diagnosis of choriocarcinoma, antecedent pregnancy, FIGO stage, sites of metastases, and FIGO risk score.

Regarding the treatment outcomes, the following variables were studied: time between the end of antecedent pregnancy and the beginning of chemotherapy, chemotherapy regimen used (single-agent or multiagent) and number of cycles needed to reach remission, occurrence of chemoresistance, need for surgery due to chemoresistance, time to remission, and occurrence of relapse or death.

Remission was defined as normalisation of human chorionic gonadotropin concentrations (to <5 IU/L), measured at least weekly for 4 weeks. Chemoresistance was considered only after 1–2 cycles of chemotherapy when at least three human chorionic gonadotropin values indicated a plateau (with each reading varying by no more than 10%) or when at least two human chorionic gonadotropin values, measured over at least 2 weeks, showed an increase. Relapse was diagnosed when, after remission, human chorionic gonadotropin concentrations increased, with or without the appearance of metastases and in the absence of a new pregnancy.

Outcomes

The primary outcome was the incidence of chemoresistance after first-line or second-line single-agent chemotherapy. Secondary outcomes were time to remission and the occurrence of relapse or death due to gestational trophoblastic neoplasia.

Statistical analysis

No sample size calculation was done for this cohort study; all eligible patients who completed follow-up were included in the analysis.

Factors associated with chemoresistance in patients with low-risk gestational trophoblastic neoplasia presenting with a FIGO risk score of 5 or 6, who were given single-agent chemotherapy were initially identified by use of univariate tests for association of categorical variables (χ^2 test or Fisher's exact test), and Student's *t* test for continuous variables. Statistical significance was set at a two-tailed p value of less than 0·05.

Univariate logistic regression models were constructed by use of the glm function of the statistics package (R version 3.4.3) to analyse the association between variables of interest and risk of resistance. We generated nested multivariable models including variables with a significant and non-significant association with single-agent resistance. These models were compared by evaluation of the Akaike information criterion, use of likelihood ratio tests, and use of cross-validation to generate an optimal predictive model. Multicollinearity was tested by evaluation of the variance inflation factor (with car package in R; appendix 2 p 3).

The association between pretreatment human chorionic gonadotropin concentration and positive predictive value (PPV) within risk subgroups, predefined according to choriocarcinoma and metastatic disease status, was assessed by bootstrap modelling. The bootstrap method enabled us to calculate the human chorionic gonadotropin concentration values at PPV thresholds of interest with 80% CIs by resampling from a single dataset. Patients were selected at random with replacement from the original dataset to create 10 000 resampled datasets of the same sample size as the original. The median pretreatment human chorionic gonadotropin concentration and 80% CIs were then calculated at PPV values of interest.

This procedure enabled us to determine human chorionic gonadotropin concentration thresholds and associated 80% CIs above which patients should be offered first-line multiagent chemotherapy, according to a prespecified PPV cutoff of 0·8. We considered human chorionic gonadotropin concentrations corresponding to a PPV for resistance of at least 0·8 (ie, of 100 patients with a value above a specific concentration, 80 would be expected to be resistant to one or two sequential single-agent treatments) as a threshold to define an unacceptably high risk of resistance to single-agent chemotherapy, and we predefined the upper bound of the 80% CI at this PPV as a cutoff, above which multiagent chemotherapy should be given. To test the utility of the proposed hCG thresholds among contemporary patients, we evaluated their performance in patients treated since the year 2000, when the updated FIGO risk classification was introduced. We additionally explored human chorionic gonadotropin concentration thresholds at a range of PPV cutoffs in a post-hoc analysis to help guide patients and clinicians in shared decision making around the optimal choice of first-line therapy depending on individual factors.

Role of the funding source

There was no funding source for this study.

Results

Between Jan 1, 1964, and Dec 31, 2018, 5025 patients with low-risk gestational trophoblastic neoplasia were diagnosed and treated, of whom 506 had a FIGO risk score of 5 or 6 (figure 1). Of these patients, 75 (15%) were subsequently excluded. As such, 431 patients with a FIGO risk score of 5 of 6 were included for analysis in this study. The clinical and therapeutic characteristics of these patients are shown in table 1. Baseline characteristics

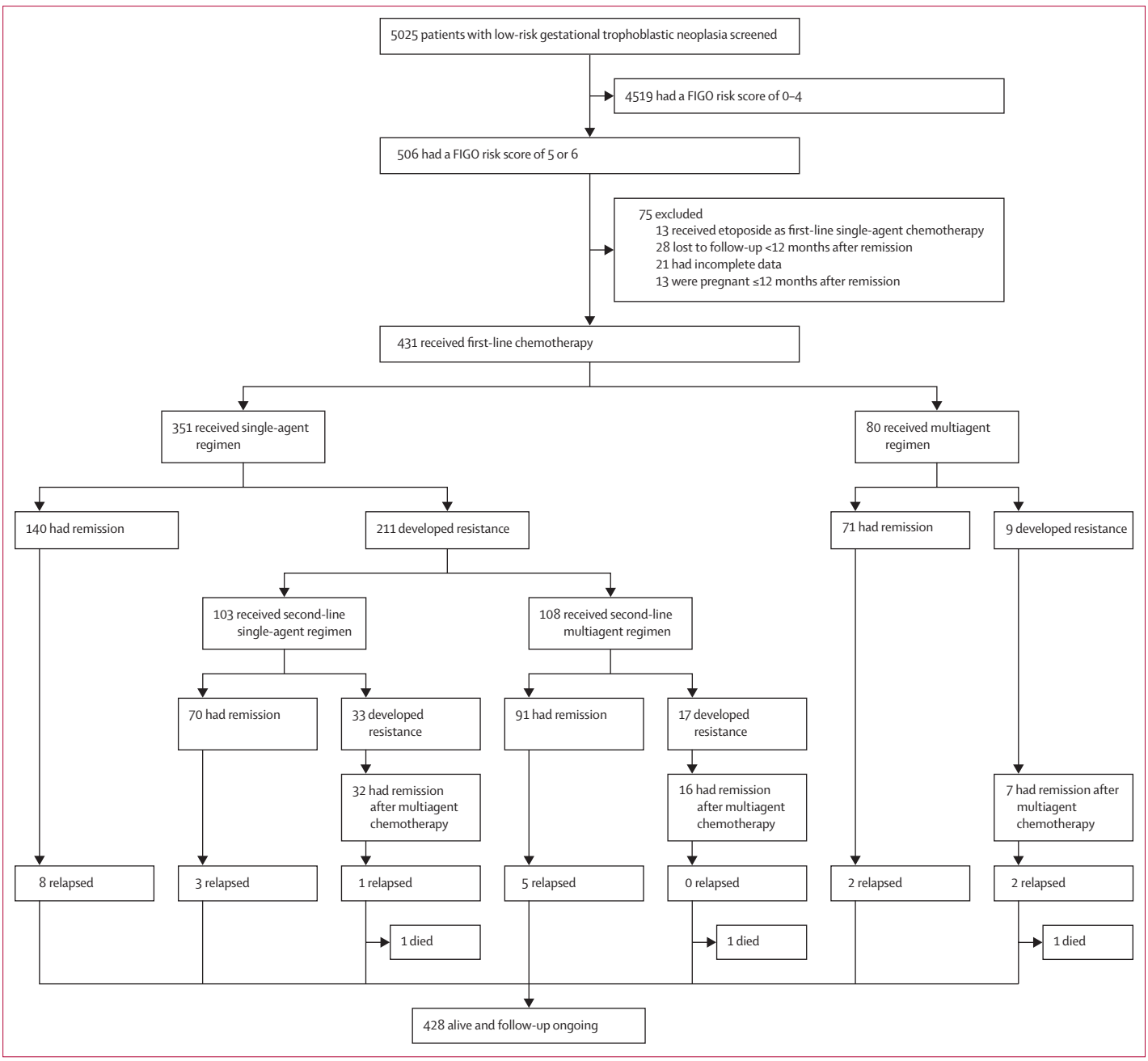

Figure 1: **Flow diagram summarising the derivation and outcomes of the study population**
FIGO=International Federation of Gynaecology and Obstetrics.

were similar across reference centres for important variables, including age, pretreatment human chorionic gonadotropin concentration, resistance to first-line chemotherapy, need for surgery after chemoresistance, and incidence of relapse or death (appendix 2 p 5). The duration of human chorionic gonadotropin follow-up after completion of treatment varied between the three centres, as previously described.[3,5,27] However, all patients included in this study had a minimum of 2 years follow-up, which is known to be when nearly all relapses will have occurred.[27] Median time to remission was 18 weeks (IQR 14–22). 21 (5%) patients had a relapse due to gestational trophoblastic neoplasia and three (1%) died because of gestational trophoblastic neoplasia.

351 (81%) patients received a first-line single-agent regimen, 103 (29%) of whom developed resistance and were then given a second single-agent regimen (figure 1). Most patients (259 [74%] of 351) were treated from the year

	Patients (n=431)
Age, years	31 (26–40)
Gravidity	2 (1–3)
Parity	1 (0–2)
Pretreatment hCG concentration, IU/L	65 035 (18 255–159 335)
<10 000	77 (18%)
10 000–99 999	169 (39%)
≥100 000	185 (43%)
Histopathology of choriocarcinoma	72 (17%)
Molar origin of gestational trophoblastic neoplasia	365 (85%)
Non-molar origin of gestational trophoblastic neoplasia	66 (15%)
Abortion	30 (7%)
Ectopic pregnancy	3 (1%)
Term or preterm	33 (7%)
Stage	
I	309 (72%)
II	15 (3%)
III	107 (25%)
Metastatic disease at presentation	122 (28%)
Choriocarcinoma with metastases	33 (7%)
Site of metastases at presentation	
Lung	107 (25%)
Vagina	15 (3%)
Time between the end of pregnancy and beginning of chemotherapy, months	1 (1–2)
FIGO risk score	
5	235 (55%)
6	196 (45%)
Single-agent first-line treatment regimen	351 (81%)
Number of cycles	6 (3–8)
Multiagent first-line treatment regimen	80 (19%)
Number of cycles	8 (5–9)
Resistance to first-line chemotherapy	220 (51%)
Surgery after chemoresistance	51 (12 %)
Total abdominal hysterectomy	34 (8%)
Lung lobectomy	9 (2%)
Other	8 (2%)
Time to remission, weeks	18 (14–22)
Relapse	21 (5%)
Death	3 (1%)

Data are median (IQR) or n (%). FIGO=International Federation of Gynaecology and Obstetrics. hCG=Human chorionic gonadotropin.

Table 1: **Clinical and therapeutic characteristics of patients with low-risk, FIGO stage 5 or 6 gestational trophoblastic neoplasia**

2000 onwards, and 329 (94%) patients, who were treated from 1990 onwards, had access to modern imaging techniques (ie, CT and MRI). The clinical features of the 351 patients who received a first-line single-agent regimen according to treatment outcome are shown in appendix 2 (p 6). 141 (40%) of 351 patients developed resistance to single-agent treatments, used once or twice in a sequential manner, and required multiagent chemotherapy to achieve remission. Consequently, 210 (60%) of 351 patients had remission with one or two sequential single-agent therapies. Compared with those who had remission, those with chemoresistance had higher pretreatment human chorionic gonadotropin concentrations (p<0·0001), more frequent histopathology of choriocarcinoma (p=0·0007), and a higher prevalence of metastatic choriocarcinoma (p=0·0022; appendix 2 p 6).

To identify variables associated with chemoresistance, we initially used univariable logistic regression analysis. Metastatic disease status (odds ratio [OR] 1·6 [95% CI 1·0–2·6], p=0·044), choriocarcinoma histology (3·0 [1·6–5·6], p=0·0006), and a pretreatment human chorionic gonadotropin concentration of more than 100 000 IU/L (3·8 [2·0–7·5], p<0·0001) were identified as significant predictors of resistance to single-agent chemotherapy; all other variables (age, time to antecedent pregnancy, antecedent pregnancy, tumour stage, and pretreatment human chorionic gonadotropin concentration of 100 000 IU/L or less) were not found to be significant predictors (figure 2A). Notably, as a continuous variable, pretreatment human chorionic gonadotropin concentration was also significantly associated with risk of resistance (2·4 [1·7–3·4], p<0·0001). Consistent with these results, nested multivariable logistic regression models also identified metastatic disease status (1·9 [1·1–3·2], p=0·018), choriocarcinoma histology (3·7 [1·9–7·4], p=0·0002), and pretreatment human chorionic gonadotropin concentration (2·8 [1·9–4·1], p<0·0001) as significant predictors of resistance to single-agent chemotherapy (figure 2B). These three factors were independently significantly associated with risk of chemoresistance (figure 2B). Multicollinearity was not present between these variables (variance inflation factor <1·1; appendix 2 p 7).

The finding that metastatic disease status and choriocarcinoma histology were independently associated with risk of resistance to single-agent chemotherapy suggests that patients can be stratified on the basis of these variables. The notion that the effect of pretreatment human chorionic gonadotropin concentration on risk of resistance varies according to choriocarcinoma histology and metastatic disease status was supported by a significant interaction between these terms and pretreatment human chorionic gonadotropin concentration (appendix 2 p 2).

To improve our ability to develop a new risk classification system, we divided patients into the following four clinical subgroups based on choriocarcinoma histology and metastatic disease status: (1) no choriocarcinoma and no metastatic disease; (2) choriocarcinoma but no metastatic disease; (3) no choriocarcinoma but with metastatic disease; and (4) choriocarcinoma with metastatic disease. Visualisation of the association between pretreatment human chorionic gonadotropin concentration and risk of resistance predicted by the multivariable model revealed substantial differences in the relationship between

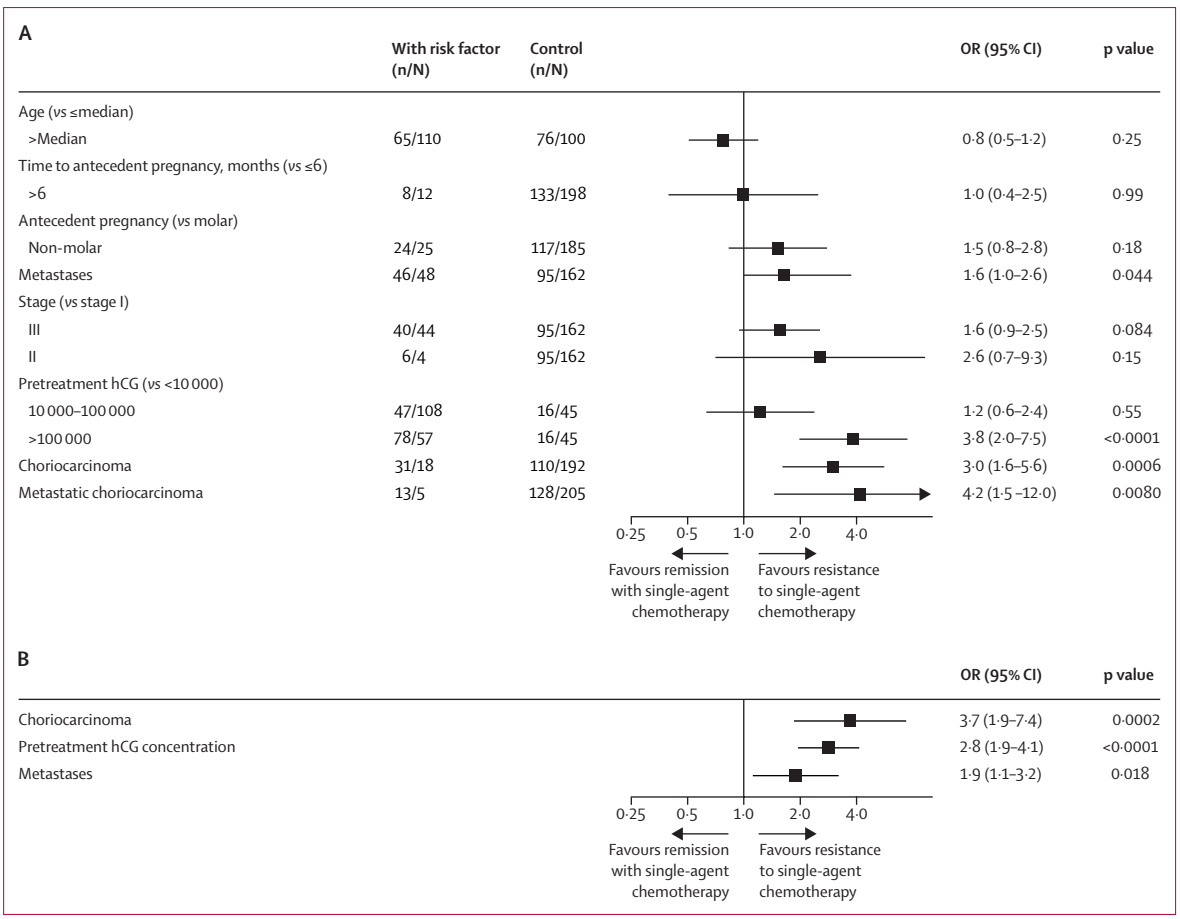

Figure 2: **Predictors of resistance to single-agent therapy**
Forest plots show univariable analysis (A) and multivariable analysis (B) of factors contributing to risk of single-agent chemotherapy failure. OR=odds ratio. hCG=human chorionic gonadotropin.

pretreatment human chorionic gonadotrophin concentration and risk of resistance among the four groups, consistent with the described interaction between this variable, metastatic disease status, and choriocarcinoma histology (appendix 2 p 8).

Based on these findings, we next modelled the association between human chorionic gonadotropin concentration and risk of resistance to single-agent chemotherapy separately across the four groups, with the aim of simplifying our new classification system.

Given that most patients (13 [72%] of 18) with metastatic choriocarcinoma who received single-agent chemotherapy did not have remission, regardless of pretreatment human chorionic gonadotropin concentration, we reasoned that this group could confidently start multiagent chemotherapy and be excluded from further modelling analyses. Additionally, there were only few patients (31 [9%] of 351) with choriocarcinoma but without metastatic disease. Consequently, we combined this group of patients with those who had metastatic disease without choriocarcinoma (n=76) to form a new composite category of patients with a single-risk factor (group 1; n=107). We then separately modelled the association between pretreatment human chorionic gonadotropin concentration and the PPV of resistance to single-agent chemotherapy in patients without choriocarcinoma or metastases (ie, those with no risk factors; group 0; n=226) and those with one risk factor (ie, either choriocarcinoma or metastases; group 1). Human chorionic gonadotropin values were similar between patients in each group, across the three centres (appendix 2 p 9).

In group 0 patients, we found that a median pretreatment human chorionic gonadotropin concentration of 264 000 IU/L (80% CI 195 000–411 000) identified high-risk patients (PPV=0·8). Taking the upper boundary of the 80% CI, nine (4%) of 226 patients in this group would be selected to proceed directly to multiagent chemotherapy (figure 3A). In group 1 patients, we found that a median pretreatment human chorionic gonadotropin concentration of 86 000 IU/L (62 600–149 000) identified high-risk patients. Taking the upper boundary of the 80% CI, 15 (14%) of 107 patients in this group were selected for primary multiagent chemotherapy (figure 3A). To verify

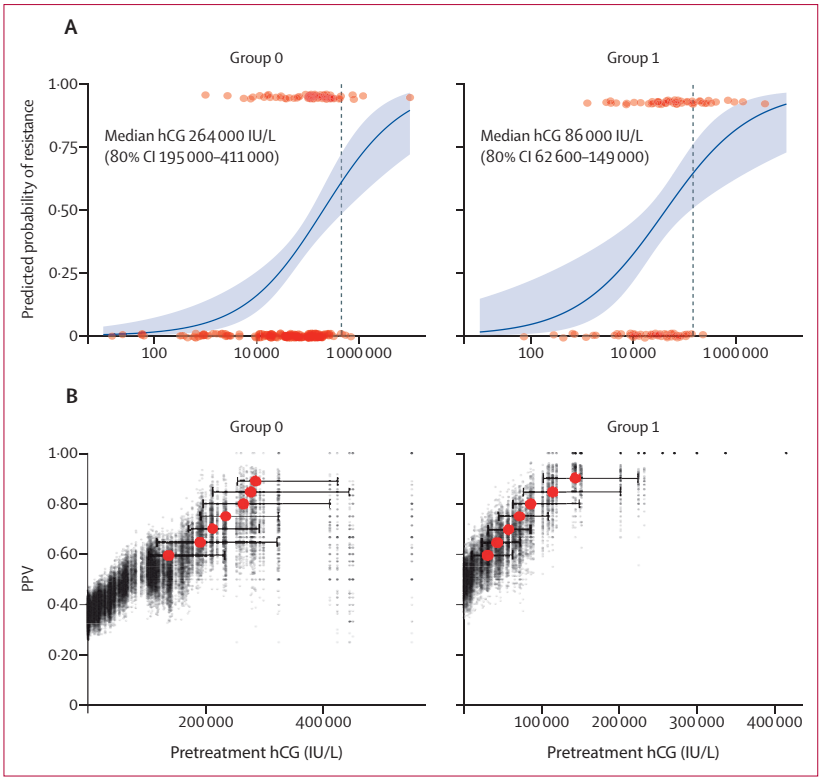

Figure 3: **Association between pretreatment hCG concentration and risk of resistance to single-agent therapy**
(A) Plots showing the predicted probability of resistance according to pretreatment hCG concentration, generated by use of separate logistic regression models for patients in group 0 (those without metastases and choriocarcinoma) and group 1 (those with either metastases or choriocarcinoma). Dashed lines represent upper boundary hCG thresholds to reach a PPV of 0·8 (411 000 IU/L in group 0 and 149 000 IU/L in group 1). The proportion of patients with a hCG concentration above these thresholds are indicated in table 2. The shaded bands represent 95% CIs around the predictions. (B) Association between PPV and pretreatment hCG concentration for group 0 and group 1 patients. At selected PPV values, the median hCG concentration (red dots) and 80% CIs (error bars) are shown. hCG=human chorionic gonadotropin. PPV=positive predictive value.

	Upper hCG boundary, IU/L	Number of patients*
PPV in group 0 patients		
0·60	234 000	28
0·65	322 000	13
0·70	292 000	15
0·75	324 000	11
0·80	411 000	9
0·85	444 000	8
0·89	424 000	8
PPV in group 1 patients		
0·60	63 000	38
0·65	73 000	31
0·70	86 000	25
0·75	109 000	22
0·80	149 000	15
0·85	202 000	11
0·90	225 000	11

The hCG value at the upper boundary of the 80% CI to reach each target PPV of 0·8 is shown. Group 0 included patients without choriocarcinoma or metastases, and group 1 included patients with choriocarcinoma or metastases. hCG=human chorionic gonadotropin. PPV=positive predictive value. *Represents the number of patients with a pretreatment hCG concentration higher than the upper boundary in each group.

Table 2: **Association between pretreatment hCG concentration and PPV in group 0 and group 1 patients.**

that these thresholds apply to contemporary patients, we tested their performance in patients who had received treatment since Jan 1, 2000. Among 182 patients in group 0, the PPV for a pretreatment human chorionic gonadotropin concentration threshold of 411 000 IU/L was 0·86 (seven patients had a pretreatment human chorionic gonadotropin concentration above this threshold, of whom six were resistant to single-agent chemotherapy). Among 68 patients in group 1, the PPV for a pretreatment human chorionic gonadotropin concentration threshold of 149 000 IU/L was 1·00 (12 patients had a pretreatment human chorionic gonadotropin above this threshold, all of whom were resistant to single-agent chemotherapy).

Finally, to further guide patients and clinicians in decisions regarding choice of therapy, we investigated the association between PPV and pretreatment human chorionic gonadotropin concentration, revealing an approximately linear response up to a concentration of 300 000 IU/L in group 0 and 150 000 IU/L in group 1 (figure 3B). Pretreatment human chorionic gonadotropin concentrations at different PPV thresholds are presented to assist with the shared decision making of clinicians and patients in the selection of single-agent versus multiagent chemotherapy (table 2). Based on our findings, we propose a treatment algorithm for patients with low-risk gestational trophoblastic neoplasia with a FIGO risk score of 5 or 6 (figure 4).

Discussion

The FIGO prognostic risk scoring system is widely used to guide the treatment of patients with gestational trophoblastic neoplasia. Patients with low-risk gestational trophoblastic neoplasia should be given single-agent chemotherapy, and those classified as having high-risk gestational trophoblastic neoplasia should receive multiagent chemotherapy regimens. Controversy remains as to whether patients with a FIGO risk score of 5 or 6 should initially receive single-agent chemotherapy or more toxic multiagent chemotherapy, as it is widely thought only a third of patients have remission with initial single-agent treatment.[9] Indeed, some clinicians argue that patients with a FIGO risk score of 5 or 6 should all simply be given multiagent chemotherapy to ensure the maximum likelihood of attaining rapid remission.[17] However, in the world's largest retrospective multicentre study, we show that 60% of patients with a FIGO risk score of 5 or 6 had remission with single-agent chemotherapy, either when used as a first-line or second-line treatment. Consequently, our data show that patients with a FIGO risk score of 5 or 6 deserve a more nuanced

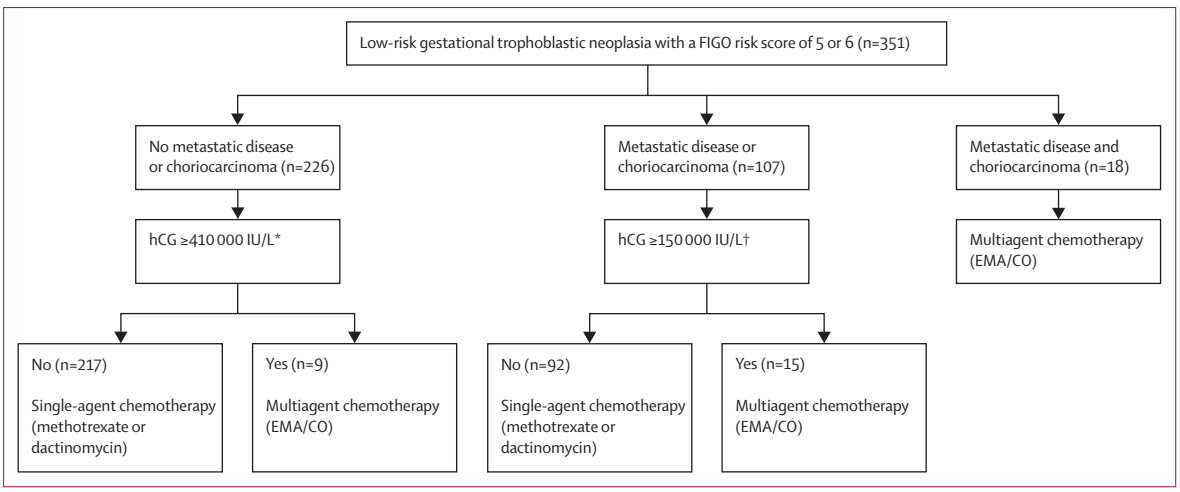

Figure 4: **Proposed algorithm for treating patients with low-risk gestational trophoblastic neoplasia presenting with a FIGO risk score of 5 or 6**
FIGO=International Federation of Gynaecology and Obstetrics. hCG=human chorionic gonadotropin. EMA/CO=etoposide and methotrexate plus folinic acid rescue and dactinomycin, alternating with cyclophosphamide and vincristine. *Rounded from 411 000 to 410 000 to facilitate memorisation and clinical use. †Rounded from 149 000 to 150 000 to facilitate memorisation and clinical use.

approach than current practices in some parts of the world.

The question arises of why the overall proportion of patients who have remission after treatment with single-agent chemotherapy is much higher in our cohort of patients with a FIGO risk score of 5 or 6 than in most previously published series and reviews.[9,15,18–20,28] Several explanations are possible, including the fact that previous studies had smaller numbers of patients with a FIGO risk score of 5 or 6, and that they only considered one rather than two sequential single-agent therapies as a means to achieve remission. Indeed, the choice of whether and when to use a second single-agent regimen rather than a multiagent regimen varies greatly between centres and reports.[17,22,23] However, such sequential single-agent chemotherapy is substantially less toxic to patients than multiagent chemotherapies, such as EMA/CO. Sequential single-agent chemotherapy is associated with occasional and mild side-effects (typically only grade 1–2 in severity), including oral mucositis, nausea, and hair thinning. By contrast, EMA/CO frequently causes grade 1–4 toxic effects, including myelosuppression with a risk of neutropenic sepsis, total alopecia, nausea and vomiting, profound lethargy, peripheral neuropathy, and long-term risks of early menopause.[9,10] Additionally, long-term multiagent chemotherapy can cause secondary cancers.[9,10] Finally, we noted that 80 (19%) of 431 patients received multiagent chemotherapy from the outset (figure 1). This choice of therapy occurred largely because of the results of previous studies suggesting that a pretreatment human chorionic gonadotropin concentration of more than 400 000 IU/L[22] or the presence of metastatic choriocarcinoma[23] indicated a high risk of resistance to single-agent chemotherapy. Indeed, our study verifies and advances these observations. Even though patients given multiagent chemotherapy were not included in our subsequent analysis, we cannot exclude the possibility that this omission has somehow artificially enhanced the observed response rates to single-agent chemotherapy in the remaining patients. Differences between human chorionic gonadotropin assays used among the three centres are unlikely to compromise our findings, since the distribution of pretreatment human chorionic gonadotropin values were similar across the centres.

A key question for us was how to establish which of the remaining 141 (40%) of 351 patients who will go on to develop resistance to single-agent chemotherapy might be suitable for first-line multiagent chemotherapy. Previous studies evaluating the pretreatment uterine artery pulsatility index and a human chorionic gonadotropin concentration of 400 000 IU/L as predictors of resistance to single-agent chemotherapy, and genetic studies of tumoral microRNA have not been validated.[3,4,6,9,22] Our data show that pretreatment serum human chorionic gonadotropin concentrations, a diagnosis of choriocarcinoma, metastatic disease, and combined choriocarcinoma and metastatic disease are all predictive of resistance to single-agent chemotherapy with methotrexate plus folinic acid or dactinomycin when used as first-line or sequential first-line and second-line therapies. These findings alone are not surprising. However, we have shown for the first time, to our knowledge, that patients with a FIGO risk score of 5 or 6 can be stratified to receive initial EMA/CO chemotherapy by clinical subgroup and pretreatment human chorionic gonadotropin concentration, and we proposed a clinical decision algorithm based on our findings. For patients with no metastatic disease and no choriocarcinoma, single-agent chemotherapy is appropriate for more than 95% of patients, and we recommend considering treatment with multiagent chemotherapy only in patients

with a pretreatment human chorionic gonadotropin concentration of 410 000 IU/L or higher. For patients with metastatic disease or histopathological evidence of choriocarcinoma, single-agent chemotherapy is still appropriate for most patients. However, a pretreatment human chorionic gonadotropin concentration of 150 000 IU/L or higher in this subgroup is associated with an approximately 80% risk of chemoresistance to single-agent treatment, either in the first-line or second-line of chemotherapy, indicating that primary treatment with a multiagent chemotherapy regimen is appropriate. Finally, all patients with metastatic disease and choriocarcinoma should be given upfront multiagent chemotherapy.

Limitations of the present study include the inherent bias introduced by its retrospective nature. Ideally, our findings should be validated in a prospective and preferably randomised trial, but given the rarity of the disease, this type of study is not practical. Additionally, over the 54-year period of study there were changes in clinical management, including in the radiological techniques used to detect metastases, and in human chorionic gonadotropin assays, and we had to re-score patients treated before 2000 according to FIGO criteria.[14] However, our analysis of patients treated after Jan 1, 2000, revealed similar results to the analysis of all patients, indicating that our proposed pretreatment human chorionic gonadotropin thresholds for choosing EMA/CO in group 0 and 1 patients are relevant for present-day practice. Most patients in our study had a uterine evacuation and a histologically diagnosed molar pregnancy, which, on subsequent monitoring, showed plateaued or increasing serum human chorionic gonadotropin concentrations indicative of malignant change to gestational trophoblastic neoplasia. A repeat biopsy to identify the type of gestational trophoblastic neoplasia at this point is contraindicated because of the risk of causing life-threatening haemorrhage. It is therefore possible that a proportion of patients classified as having molar disease, in fact had choriocarcinoma or other histologies. However, our data show that we can effectively stratify patients in a clinically meaningful way based on the real-world diagnosis of gestational trophoblastic neoplasia, including in those who have had a miscarriage or an ectopic pregnancy, without histology. In terms of possible differences in the distribution of patient human chorionic gonadotropin concentrations between centres, our study was not powered to detect between-site differences, particularly given the small size of particular patient subgroups.

We acknowledge that hysterectomy is an alternative method to avoid chemotherapy for any woman with localised disease who does not intend to have more children. Notably, deriving the study population from different health-care models and countries allows grouping of patients with a FIGO risk score of 5 or 6 who have differing demographic characteristics; therefore, the results of our study could potentially be applicable internationally.

In conclusion, identifying patients with low-risk gestational trophoblastic neoplasia presenting with a FIGO risk score of 5 or 6 who have a higher chance of chemoresistance to single-agent chemotherapy is possible by use of widely available clinical findings at diagnosis. Indeed, the prognostic factors of pretreatment human chorionic gonadotropin concentration, metastatic disease status, and choriocarcinoma histology (when tissue can be obtained safely) are readily ascertained by simple diagnostic tests. Most patients with a FIGO risk score of 5 or 6 can start therapy with single-agent methotrexate plus folinic acid, with the expectation that 60% of patients will enter remission with this regimen or after sequential use of dactinomycin.[29,30] First-line multiagent chemotherapy should be reserved only for the following patients: (1) those with no metastatic disease and no choriocarcinoma, who have pretreatment human chorionic gonadotropin concentrations of 410 000 IU/L or higher; (2) those with metastatic disease or histopathological evidence of choriocarcinoma and pretreatment human chorionic gonadotropin concentrations of 150 000 IU/L or higher; and (3) those with metastatic disease and choriocarcinoma. This risk-stratified approach might help maximise initial responses to therapy, while minimising unnecessary excessive exposure to toxic multiagent chemotherapy. Finally, this rare group of patients should be referred to a gestational trophoblastic neoplasia reference centre whenever possible to obtain expert diagnosis and ensure proper treatment.

Contributors

AB, EG, GP, KME, MJS, NS, NSH, and RSB designed the study. GP, FF, JRF, JAJ, and XA enrolled patients and participated in data collection. APVdSE was responsible for the ethical requirements during the design and execution of the study. EG, JL-K, and LGCV statistically analysed the data. AB, BK, EG, FF, GP, KME, LGCV, MJS, NS, NU, NSH, and RSB contributed to data analysis, verification, and interpretation. All authors wrote and approved the final version of the manuscript. All authors had full access to all the raw data in the study. MJS had final responsibility to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Deidentified individual participant data will be made available on request to the corresponding author (m.seckl@imperial.ac.uk), subject to ethical approval. Data will be made available with publication.

Acknowledgments

AB thanks the National Council for Scientific and Technological Development. RSB, NSH, and KME thank the Donald P Goldstein MD Trophoblastic Tumor Registry Endowment and the Dyett Family Trophoblastic Disease Research and Registry Endowment. EG is supported by a Wellcome Trust Clinical Research Fellowship and a National Institute for Health Research (NIHR) Fellowship. JL-K is supported by the Imperial Experimental Cancer Medicine Centre, funded by Cancer Research UK and the NIHR. MJS is supported by the UK Department of Health, NIHR Imperial Biomedical Research Centre, and Imperial Experimental Cancer Medicine Centre, funded by Cancer Research UK and the NIHR. We thank Matteo Quartagno for useful discussions. We thank the study participants and their families.

References

1 Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376:** 717–29.

2 Lurain JR. Gestational trophoblastic disease II: classification and management of gestational trophoblastic neoplasia. *Am J Obstet Gynecol* 2011; **204:** 11–18.

3 Elias KM, Berkowitz RS, Horowitz NS. State-of-the-art workup and initial management of newly diagnosed molar pregnancy and postmolar gestational trophoblastic neoplasia. *J Natl Compr Canc Netw* 2019; **17:** 1396–401.

4 Braga A, Mora P, Melo AC, et al. Challenges in the diagnosis and treatment of gestational trophoblastic neoplasia worldwide. *World J Clin Oncol* 2019; **10:** 28–37.

5 Freitas F, Braga A, Viggiano M, et al. Gestational trophoblastic neoplasia lethality among Brazilian women: a retrospective national cohort study. *Gynecol Oncol* 2020; **158:** 452–59.

6 Seckl MJ, Sebire NJ, Fisher RA, et al. Gestational trophoblastic disease: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24:** vi39–50.

7 Berkowitz RS, Goldstein DP. Current advances in the management of gestational trophoblastic disease. *Gynecol Oncol* 2013; **128:** 3–5.

8 Hertz R, Li MC, Spencer DB. Effect of methotrexate therapy upon choriocarcinoma and chorioadenoma. *Proc Soc Exp Biol Med* 1956; **93:** 361–66.

9 Parker VL, Pacey AA, Palmer JE, Tidy JA, Winter MC, Hancock BW. Classification systems in gestational trophoblastic neoplasia - sentiment or evidenced based? *Cancer Treat Rev* 2017; **56:** 47–57.

10 Savage P, Cooke R, O'Nions J, et al. Effects of single-agent and combination chemotherapy for gestational trophoblastic tumors on risks of second malignancy and early menopause. *J Clin Oncol* 2015; **33:** 472–78.

11 Bagshawe KD. Risk and prognostic factors in trophoblastic neoplasia. *Cancer* 1976; **38:** 1373–85.

12 Soper JT, Evans AC, Conaway MR, Clarke-Pearson DL, Berchuck A, Hammond CB. Evaluation of prognostic factors and staging in gestational trophoblastic tumor. *Obstet Gynecol* 1994; **84:** 969–73.

13 Kim SJ, Bae SN, Kim JH, et al. Effects of multiagent chemotherapy and independent risk factors in the treatment of high-risk GTT–25 years experiences of KRI-TRD. *Int J Gynaecol Obstet* 1998; **60:** S85–96.

14 Fédération Internationale de Gynécologie et d'Obstétrique Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000. *Int J Gynaecol Obstet* 2002; **77:** 285–87.

15 Sita-Lumsden A, Short D, Lindsay I, et al. Treatment outcomes for 618 women with gestational trophoblastic tumours following a molar pregnancy at the Charing Cross Hospital, 2000–2009. *Br J Cancer* 2012; **107:** 1810–14.

16 Lok C, van Trommel N, Massuger L, Golfier F, Seckl M, on behalf of the Clinical Working Party of the EOTTD. Practical clinical guidelines of the EOTTD for treatment and referral of gestational trophoblastic disease. *Eur J Cancer* 2020; **130:** 228–40.

17 Ngan HYS, Seckl MJ, Berkowitz RS, et al. Update on the diagnosis and management of gestational trophoblastic disease. *Int J Gynaecol Obstet* 2018; **143:** 79–85.

18 Osborne RJ, Filiaci V, Schink JC, et al. Phase III trial of weekly methotrexate or pulsed dactinomycin for low-risk gestational trophoblastic neoplasia: a Gynecologic Oncology Group study. *J Clin Oncol* 2011; **29:** 825–31.

19 Chapman-Davis E, Hoekstra AV, Rademaker AW, Schink JC, Lurain JR. Treatment of nonmetastatic and metastatic low-risk gestational trophoblastic neoplasia: factors associated with resistance to single-agent methotrexate chemotherapy. *Gynecol Oncol* 2012; **125:** 572–75.

20 Taylor F, Grew T, Everard J, et al. The outcome of patients with low risk gestational trophoblastic neoplasia treated with single-agent intramuscular methotrexate and oral folinic acid. *Eur J Cancer* 2013; **49:** 3184–90.

21 Sita-Lumsden A, Medani H, Fisher R, et al. Uterine artery pulsatility index improves prediction of methotrexate resistance in women with gestational trophoblastic neoplasia with FIGO score 5–6. *BJOG* 2013; **120:** 1012–15.

22 McGrath S, Short D, Harvey R, Schmid P, Savage PM, Seckl MJ. The management and outcome of women with post-hydatidiform mole 'low-risk' gestational trophoblastic neoplasia, but hCG levels in excess of 100 000 IU l(-1). *Br J Cancer* 2010; **102:** 810–14.

23 Frijstein MM, Lok C, van Trommel NE, et al. Lung metastases in low-risk gestational trophoblastic neoplasia: a retrospective cohort study. *BJOG* 2020; **127:** 389–95.

24 Dekeister K, Bolze PA, Tod M, et al. Validation of an online tool for early prediction of the failure-risk in gestational trophoblastic neoplasia patients treated with methotrexate. *Cancer Chemother Pharmacol* 2020; **86:** 15–24.

25 Agarwal R, Teoh S, Short D, Harvey R, Savage PM, Seckl MJ. Chemotherapy and human chorionic gonadotropin concentrations 6 months after uterine evacuation of molar pregnancy: a retrospective cohort study. *Lancet* 2012; **379:** 130–35.

26 Lybol C, Sweep FC, Harvey R, et al. Relapse rates alter two versus three consolidation courses of methotrexate in the treatment of low-risk gestational trophoblastic neoplasia. *Gynecol Oncol* 2012; **125:** 576–79.

27 Balachandran K, Salawu A, Ghorani E, et al. When to stop human chorionic gonadotrophin (hCG) surveillance after treatment with chemotherapy for gestational trophoblastic neoplasia (GTN): a national analysis on over 4,000 patients. *Gynecol Oncol* 2019; **155:** 8–12.

28 El-Helw LM, Coleman RE, Everard JE, et al. Impact of the revised FIGO/WHO system on the management of patients with gestational trophoblastic neoplasia. *Gynecol Oncol* 2009; **113:** 306–11.

29 Prouvot C, Golfier F, Massardier J, et al. Efficacy and safety of second-line 5-day dactinomycin in case of methotrexate failure for gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2018; **28:** 1038–44.

30 Maestá I, Nitecki R, Desmarais CCF, et al. Effectiveness and toxicity of second line actinomycin D in patients with methotrexate-resistant postmolar low-risk gestational trophoblastic neoplasia. *Gynecol Oncol* 2020; **157:** 362–68.