

## RESEARCH ARTICLE

# Human Chorionic Gonadotropin (hCG) Regression Curve for Predicting Response to EMA/CO (Etoposide, Methotrexate, Actinomycin D, Cyclophosphamide and Vincristine) Regimen in Gestational Trophoblastic Neoplasia

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## Abstract

**Background:** An hCG regression curve has been used to predict the natural history and response to chemotherapy in gestational trophoblastic disease. We constructed hCG regression curves in high-risk gestational trophoblastic neoplasia (GTN) treated with EMA/CO and identified an optimal hCG level to detect EMA/CO resistance in GTN. **Materials and Methods:** Eighty-one women with GTN treated with EMA/CO were classified as primary high-risk GTN (n = 65) and single agent-resistance GTN (n = 16). The hCG levels prior to each course of chemotherapy were plotted in the 10th, 50th, and 90th percentiles to construct the hCG regression curves. Diagnostic performance was evaluated for an optimal cut-off value. **Results:** The median hCG levels were 264,482 mIU/mL mIU/mL and 495.5 mIU/mL mIU/mL for primary high-risk GTN and single agent-resistance GTN, respectively. The 50th percentile of the hCG level in primary high-risk GTN and single agent-resistance turned to normal before the 4th and the 2nd course of chemotherapy, respectively. The 90th percentile of the hCG level in primary high-risk GTN and single agent-resistance turned to normal before the 9th and the 2nd course of chemotherapy, respectively. The hCG level of  $\geq 118.6$  mIU/mL mIU/mL at the 5th course of EMA/CO predicted the EMA/CO resistance in primary high-risk GTN patients with a sensitivity of 85.7% and a specificity of 100%. **Conclusion:** EMA/CO resistance in primary high-risk GTN can be predicted by using an hCG regression curve in combination with the cut-off value of 118.6 mIU/mL at the 5th course of chemotherapy.

**Keywords:** EMA/CO - gestational trophoblastic neoplasia - human chorionic gonadotropin - drug resistance

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## Introduction

Gestational trophoblastic neoplasia (GTN) is a group of malignant lesions that arise from abnormal proliferation of placental trophoblast cells leading to invasive mole, choriocarcinoma, placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor (ETT). GTN is highly sensitive to chemotherapy with cure rate higher than 90% (Lurain, 2011).

The patients with GTN are classified as having low-risk or high-risk disease using the modified World Health Organization prognostic scoring system as adapted by the International Federation of Gynecology and Obstetrics (Ngan et al., 2003). High-risk is defined by FIGO stage IV or any FIGO stage with a score  $\geq 7$  (Ngan et al., 2003), which indicates multi-agent chemotherapies for treatment. EMA/CO is an effective, less toxic regimen and the most frequently used primary therapy for high-risk GTN (Ngan et al., 2012). The remission rates of EMA/CO range from 71% to 86% (Bolis et al., 1988; Newlands et al., 1991; Bower et al., 1997; Escobar et al., 2003). The patients

who were chemo-refractory to EMA/CO had a worse outcome compared to patients with relapsed disease (5-year survival 43%) (Powles et al., 2007). Nowadays, there is no consensus guideline to define the criteria for resistance to chemotherapy even though some clinicians determine this condition by a plateau or a rising in serum human chorionic gonadotropin (hCG) and/or development of new metastases (van Trommel et al., 2006).

To avoid the risk of ineffective cycles of chemotherapy, many studies attempted to develop new methods to enable early identification of chemoresistance in low-risk GTN such as an hCG regression nomogram (van Trommel et al., 2006) and serum hCG concentration ratios (van Trommel et al., 2008). Unfortunately, there is a lack of studies in patients with high-risk GTN.

In 2012, Lybol introduced the hCG regression nomogram in high-risk GTN to predict EMA/CO resistance. However, due to the limitations of diagnostic performance evidence, this nomogram could not be applied in clinical practice. Therefore, we wanted to study the pattern of hCG regression in high-risk GTN patients

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and evaluate the diagnostic accuracy in predicting the risk of chemoresistance and assign a cut-off value for clinical application.

## Materials and Methods

Following approval by the Research Ethics Committee, the medical records of patients with high-risk GTN who received EMA/CO chemotherapy regimen between 1 January 2002 and 31 December 2013 were reviewed. The patients who received EMA/CO for recurrent disease were excluded. The patients who were diagnosed as placental site trophoblastic tumor (PSTT) and epithelial trophoblastic tumor (ETT) were kept out. In addition, the patients whose serum hCG levels during EMA/CO treatment were unavailable were rejected from the study. A total of 94 patients treated with EMA/CO were included. Patient characteristics were reviewed for age, antecedent pregnancy, interval months from index pregnancy, size and site of metastasis, pretreatment serum hCG level, and serum hCG level prior to each course of chemotherapy. FIGO stage and FIGO risk prognostic scores were also recalculated to ensure precise data.

Based on the indication for treatment with EMA/CO, we classified the patients into either the primary high-risk GTN group (n=77) or single agent-resistant group (n=17).

Only the patients who reached complete remission after EMA/CO treatment in each group (n=65 in the primary high-risk GTN, n=16 in the single agent resistance) were used separately to create the hCG regression curve. To approximate the normal distributions, the regression was based on 10 log transformed serum hCG level for each chemotherapy course. The percentiles of 10% (P10), 50% (P50), and 90% (P90) were calculated and plotted for each course in an hCG regression curve using the program R, version 2.14.2. The serum hCG level in each chemotherapy course of the patients who were resistant to EMA/CO were used to make receiver operating characteristic (ROC) curves and to calculate the area under the curves (AUCs). Chemoresistance was defined as at least 4 values of persistently elevated hCG plateau or sequential rise of hCG in 3 values in 2 weeks (van Trommel et al., 2006). The statistical analyses were performed using the R program, version 2.14.2. Differences in the numerical data between the two groups were tested nonparametrically (Mann-Whitney U test) and parametrically (Student's T test). All tests were considered significantly different at  $P < 0.05$ .

### Immunoassays

All hCG concentrations were measured using electrochemiluminescence immunoassay (ECLIA) with

**Table 1. Patient Characteristics**

	Primary high-risk n=65	Single agent resistance n=16	p value
Age (years)	39	39.5	0.79
Median (Min-Max)	(17-57)	(21-51)	
Parity (Median) (Min-Max)	2	2	0.1
Antecedent pregnancy	(0-10)	(0-6)	0.02
Hydatidiform mole (%)	27 (41.5%)	13 (81.2%)	
Non-molar abortion (%)	16 (24.6%)	1 (6.2%)	
Term pregnancy (%)	22 (33.8%)	2 (12.5%)	
Interval from index pregnancy (months)	11	9.5	0.74
< 4	(1-312)	(2-168)	
4-6	14 (21.5%)	3 (18.8%)	
7-12	11 (16.9%)	3 (18.8%)	
> 12	9 (13.8%)	4 (25.0%)	
> 12	31 (47.7%)	6 (37.5%)	
Pretreatment hCG (mIU/mL) (Min-Max)	264482	495.5	<0.001
Median (Min-Max)	(3173-4000000)	(70-34400)	
Tumor size (cm.)	6	2	<0.001
Median (Min-Max)	(0-16)	(0-7)	
Metastasis disease	45 (69.2%)	3 (18.8%)	<0.001
Vagina	14 (21.5%)	0	
Lung	37 (56.9%)	2 (12.5%)	
Spleen, Kidney	1 (1.5%)	0	
GI	2 (3.1%)	0	
Liver, Brain	11 (16.9%)	1 (6.2%)	
FIGO stage			0.006
I	21 (32.3%)	13 (81.2%)	
II	5 (7.7%)	0	
III	29 (44.6%)	2 (12.5%)	
IV	10 (15.4%)	1 (6.2%)	
FIGO score	12	8	<0.001
Median (Min-Max)	(7-21)	(1-11)	
No. of chemotherapy	6 (3-11)	3 (2.5)	<0.001
Median (Min-Max)	(3-11)	(2-5)	

the Elecsys 1010/2010, Modular Analytics E170, cobas e601/411 (Roche diagnostics). This assay determined intact hCG plus the hCG B-subunit in human serum and plasma. This method was standardized against the 4<sup>th</sup> International Standard for Chorionic Gonadotropin from the National Institute for Biological Standards and Control (NIBSC) code 75/589. Serum hCG concentrations were considered to be normal if less than 7 mIU/mL. The corresponding 95% confidence interval extends to an upper limit of 8.3 mIU/mL. In the author's institute, hCG levels less than 10 mIU/mL were considered normal.

## Results

The characteristics of patients with primary high-risk GTN (n=65) and patients with single agent-resistance (n=16) are shown in Table 1. Expectedly, the median pretreatment serum hCG levels of primary high-risk GTN patients were significantly elevated compared to those with single agent-resistance (264,482 vs. 495.5 mIU/mL,  $P<0.001$ ). The median tumor size also was significantly larger in the primary high-risk GTN group compared to the single agent-resistant patients (6 vs. 2cm,  $P<0.001$ ). More metastatic diseases was observed in the primary high-risk GTN group (69.2% vs. 18.8%,  $P<0.001$ ). The

lungs were the most common site of metastasis in both groups. No differences in age, parity, and the number of interval months from index pregnancy were observed between the 2 groups.

Not surprisingly, the FIGO stage for the primary high-risk GTN group was mostly stage III while the FIGO stage for the single agent-resistant patients was mostly stage I. The median FIGO prognostic score of primary high-risk GTN patients was also significantly higher than the patients with single agent-resistance (12 vs. 8,  $P<0.001$ ). There was a greater number of EMA/CO courses administered to the primary high-risk GTN patients (median 6 courses, range 3-11) compared with the single agent-resistant patients (median 3 courses, range 2-5,  $P<0.001$ ).

The hCG regression curve for the patients who were successfully treated with EMA/CO for primary high-risk GTN is shown in Figure 1A. The 90<sup>th</sup> percentile of the pretreatment hCG levels was 1,186,653 mIU/mL and turned to a normal level before the 9<sup>th</sup> course of chemotherapy. The median pretreatment hCG level of this group was 264,482 mIU/mL. Half of the patients with primary high-risk GTN had a normal hCG level before the start of the 4<sup>th</sup> course of chemotherapy. The 10<sup>th</sup> percentile of pretreatment hCG level of this group was 20,912.6 mIU/mL and turned to a normal level before the 2<sup>nd</sup> course of chemotherapy.

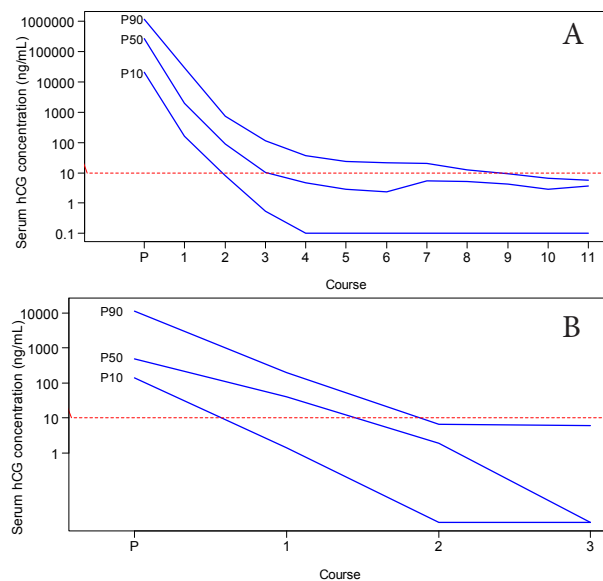
The hCG regression curve for the patients with single agent-resistance who were successfully treated with EMA/CO is shown in Figure 1. B. The 90<sup>th</sup> percentile of the pretreatment hCG level was 11,419.5 mIU/mL. Ninety percent of the patients with single agent-resistance had normal hCG levels before the 2<sup>nd</sup> course of chemotherapy. The median pretreatment hCG level of this group was 495.5 mIU/mL and half of the patients had a normal hCG level before the 2<sup>nd</sup> course of chemotherapy. The 10<sup>th</sup> percentile of pretreatment hCG level was 136.5 mIU/mL.

The hCG levels before the start of each chemotherapy of the patients with primary high-risk GTN who were resistant to EMA/CO (n=12) were plotted in an hCG regression curve as shown in Figure 2. The diagnostic accuracy to predict the patients who may be resistant to EMA/CO treatment in each course was performed by calculation of the AUC of an ROC curve in the 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> courses of chemotherapy (Figures 3A-C). The corresponding AUCs for these ROCs are listed in Table 2. The AUC of the hCG level before the start of

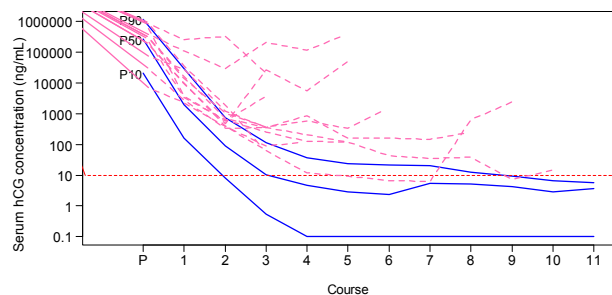
**Table 2. Diagnostic Performance of Each Course of Chemotherapy**

Course	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
Study, No of patients	12	8	7
Control, No of patients	77	70	61
hCG cut-off (mIU/mL)	≥55	≥118.3	≥118.6
Sensitivity	100%	87.50%	85.70%
Specificity	78.40%	95.10%	100%
AUC	0.892	0.913	0.928

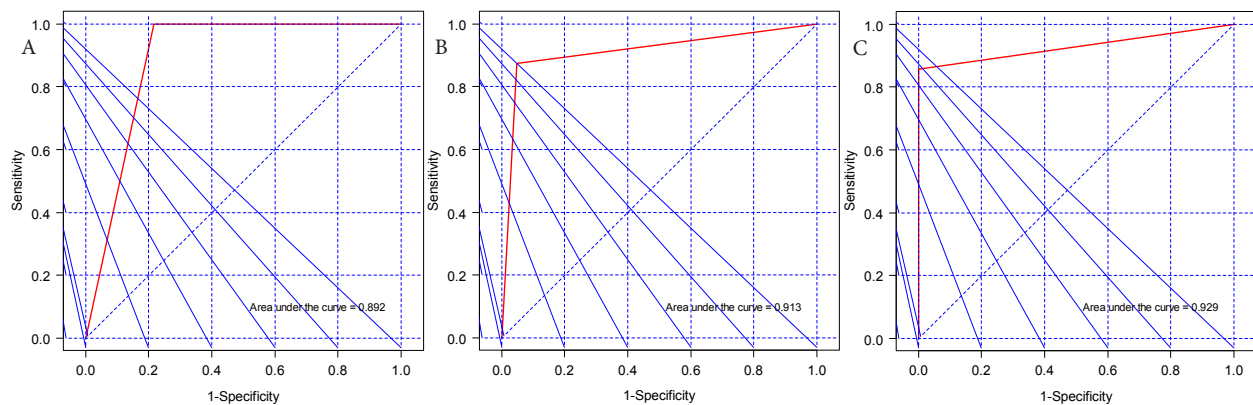
\*Abbreviation: AUC, area under the curve



**Figure 1. A) Normal hCG Regression Curve in the Patients who Successfully Treated with EMA/CO for Primary High Risk GTN (n=65); B) Normal hCG Regression Curve in the Patients who Successfully Treated with EMA/CO Single Agent-Resistance (n=16)**



**Figure 2. Serum hCG Regression of the Primary High Risk GTN with EMA/CO Resistance (n=12) were Plotted in the hCG Regression Curve**



**Figure 3. A) ROC Analysis at the Third Course of EMA/CO for the Patient with EMA/CO Resistance; B) ROC Analysis at the Forth Course of EMA/CO for the Patient with EMA/CO resistance; C) ROC Analysis at the Fifth Course of EMA/CO for the Patient with EMA/CO Resistance**

the 3<sup>rd</sup> EMA/CO treatment was 0.892. The AUC of the hCG level before the start the 4<sup>th</sup> EMA/CO treatment was 0.913, which increased to 0.928 preceding the 5<sup>th</sup> EMA/CO treatment. In accordance with the AUC, an hCG level  $\geq 118.6$  mIU/mL measured prior to the 5<sup>th</sup> course of chemotherapy provided the best diagnostic accuracy in predicting EMA/CO regimen resistance (Sensitivity 85.7%, specificity 100%).

## Discussion

The objectives of the study were to construct the hCG regression curves for the patients with high-risk GTN who were successfully treated with EMA/CO in different indications and use these curves to calculate the diagnostic performance and predict EMA/CO resistance. The median pretreatment hCG level in the primary high-risk GTN group was significantly higher than in the single agent-resistant group due to the larger tumor volumes. The 90<sup>th</sup> percentile was chosen as the upper line of the hCG regression curve because of the concern for early prediction of EMA/CO resistance as was found in a previous study (Lybol et al., 2012). We found that half of the patients in primary high-risk GTN and single agent-resistance had normal hCG levels before the start of the 4<sup>th</sup> and the 2<sup>nd</sup> course of chemotherapy, respectively. Likewise, Lybol et al. (2012) reported half of the patients with primary high-risk GTN and MTX-resistance had normal hCG levels before the 6<sup>th</sup> and the 3<sup>rd</sup> course of chemotherapy, respectively. For the 90<sup>th</sup> percentile in our study, the patients with primary high-risk GTN had normal hCG levels before the 9<sup>th</sup> course while the single agent-resistance patients had normal hCG levels before the 2<sup>nd</sup> course of chemotherapy. Comparing our results with the Lybol et al. (2012) study, they reported that the 90<sup>th</sup> percentile of the hCG level turned to normal before the 8<sup>th</sup> and the 4<sup>th</sup> course in primary high-risk GTN and MTX resistant patients, respectively.

There is a particular concern for the exposure of ineffective courses of chemotherapy. Many studies tried to identify early and accurate methods in detection of chemoresistance in low-risk GTN patients (van Trommel et al., 2006). They found a 97.5% specificity of the hCG level in the first few courses of MTX that identified

50% of the patients who were likely to need alternative chemotherapy to cure their disease. Additionally, Lybol et al. (2012) constructed an hCG regression nomogram for the high-risk GTN patients treated with EMA/CO. They did not explore the diagnostic accuracy of serum hCG level to detect EMA/CO resistance in high-risk GTN patients. To the best of our knowledge, this is the first study that explores the diagnostic performance of an hCG regression curve in patients with high-risk GTN treated with EMA/CO to identify the EMA/CO resistance.

We selected the 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> course of chemotherapy to calculate the diagnostic performance of EMA/CO resistance. There were 2 reasons. First, this selection leads to a more precise early detection of EMA/CO resistance because most of the patients with EMA/CO resistance had hCG levels higher than the 90<sup>th</sup> percentile of the hCG regression curve after the 3<sup>rd</sup> course of chemotherapy. If we had selected the 1<sup>st</sup> or 2<sup>nd</sup> course, it may have led to an over diagnosis. The second is to reduce the side effects from an ineffective course of chemotherapy. Consequently, we chose the 3<sup>rd</sup> to 5<sup>th</sup> courses to calculate the diagnostic accuracy. Of interest, we found the highest specificity (100%) and a good sensitivity (85.7%) to predict EMA/CO resistance in primary high-risk GTN patients by using the hCG cut-off level of  $\geq 118.6$  mIU/mL at the 5<sup>th</sup> course of EMA/CO chemotherapy. The use of this value would avoid an excessive treatment, from the absence of false positive result. Nevertheless, because of a lack of consensus diagnostic criteria for chemotherapy resistance and the application of rising or plateau criteria in some institutes, we suggest that if the serum hCG meets the criteria of chemoresistance before the 5<sup>th</sup> course of EMA/CO, an alternative criteria of rising or plateau hCG level would be helpful.

In patients who show resistance to the EMA/CO regimen, second line chemotherapy maybe considered. Several effective regimens of salvage chemotherapy were proposed such as EP/EMA (Bower et al., 1997; Newlands et al., 2000), TP/TE (Wang et al., 2008), and Capecitabine (Bianconi et al., 2007). Some regimens were less effective in a salvage setting such as 5-fluoracil plus Actinomycin D (Manopunya and Suprasert, 2012), and VAC (Oranratanaphan and Lertkhachonsuk, 2014). In some circumstances, hysterectomy may be required

*HCG Regression Curve for Predicting Response to EMA/CO Regimen in High-risk Gestational Trophoblastic Neoplasia* when a chemoresistant lesion was confined to the uterus (Pongsaranantakul and Kietpeerakool, 2009). In this study, five of twelve patients with primary high-risk GTN who showed resistance to EMA/CO regimen had disease remission by salvage chemotherapy with EP/EMA regimen (3), hysterectomy (1), and hysterectomy followed by EP/EMA (1). Two patients were lost to follow-up during second line chemotherapy treatment. Regrettably, another five patients failed to achieve remission; 3 patients died with disease progression and 2 patients died with febrile neutropenia during chemotherapy treatment. A patient in the MTX resistant group who failed EMA/CO treatment had disease remission with hysterectomy followed with the TP/TE regimen.

The limitation of this study is that the number of patients with single agent-resistance was too small to evaluate the diagnostic accuracy. Further prospective trials may consider the use of the hCG regression curve compared with rising or plateau criteria to study the survival and adverse effects of chemotherapy.

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