Human chorionic gonadotropin (hCG) regression normograms for patients with high-risk gestational trophoblastic neoplasia treated with EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine) chemotherapy

C. Lybol1,2*, K. Westerdijk1, F. C. G. J. Sweep2,†, P. B. Ottevanger3,†, L. F. A. G. Massuger1,† & C. M. G. Thomas2,†
Departments of 1Obstetrics and Gynaecology; 2Laboratory Medicine; 3Medical Oncology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Received 5 March 2012; revised 2 May 2012; accepted 9 May 2012

Background: We present normograms for human chorionic gonadotropin (hCG) regression in patients with high-risk gestational trophoblastic neoplasia (GTN) successfully treated with multiagent chemotherapy in order to predict treatment resistance.

Patients and methods: We collected data for 46 patients with high-risk GTN treated with EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine) who had hCG values available. Patients were classified as having methotrexate (MTX)-resistant disease (n = 22) or primary high-risk disease (n = 24). The 10th, 50th and 90th percentiles of the hCG before every chemotherapy course were calculated and plotted in normograms.

Results: Half of the patients treated for MTX-resistant disease and primary high-risk disease had normal hCG levels before the third and sixth course of chemotherapy, respectively. In patients with MTX-resistant disease, the 90th percentile line fell below normal before the start of the fourth course, whereas in patients with primary high-risk disease this was not the case until the eighth course of chemotherapy.

Conclusion: Resistance to EMA/CO treatment for high-risk GTN, as illustrated by examples, could be predicted using normograms for hCG resistance. Normograms differed depending on the indication for multiagent chemotherapy due to much higher initial hCG values in patients with primary high-risk disease compared with those treated for MTX-resistant disease.

Key words: EMA/CO, gestational trophoblastic neoplasia, normogram, treatment resistance

introduction

Gestational trophoblastic neoplasia (GTN) represents a unique group of tumours which includes invasive mole, choriocarcinoma, placental-site trophoblastic tumour and epithelioid trophoblastic tumour. GTN may arise in association with any pregnancy event, although ~50% are diagnosed after a hydatidiform mole. Patients with GTN are classified as having low-risk or high-risk disease using the modified World Health Organisation prognostic scoring system as adapted by International Federation of Gynaecology and Obstetrics [1]. Patients scoring ≥7 are considered to be at high risk of developing resistance to single-agent methotrexate (MTX), and are therefore treated with multiagent chemotherapy. Worldwide, the most frequently used chemotherapy is EMA/CO (etoposide, MTX and actinomycin D, alternated weekly with cyclophosphamide and vincristine). The remission rate of EMA/CO ranges from 71% to 86% [2–5]. Patients who progress during the EMA/CO therapy have a poor outcome with a reported 5-year survival of only 43% (95% confidence interval (CI) 12%–73%) [6].

Currently an international definition of resistance to chemotherapy is lacking. Previously, van Trommel et al. developed a normogram for the regression of human chorionic gonadotropin (hCG) concentrations during the treatment of low-risk GTN with MTX to identify resistance to MTX at an early stage [7]. This normogram allows the identification of 50% of patients needing alternative therapy with a specificity of 97.5% before the fourth course of MTX. The early
identification of patients resistant to multiagent chemotherapy is even more important, since these patients are at greater risk of a fatal outcome. For these patients, treatment options are limited. Salvage chemotherapy with etoposide and platinum drug regimens often combined with surgical excision of persistent tumour will result in remission in 70%–88% of patients resistant to EMA/CO [2, 8–11]. We aimed at constructing normograms for hCG regression in patients with high-risk GTN successfully treated with EMA/CO chemotherapy in order to predict patients developing resistance to EMA/CO in an early phase of their treatment.

patients and methods

patients

In the Netherlands, patients with gestational trophoblastic disease are registered voluntarily at the Dutch Central Registry for Hydatidiform Moles (DCRHM), which also provides a national hCG assay service to gynaecologists. A total of 4190 patients were registered at the DCRHM from 1977 until 2012. Furthermore, patients with high-risk GTN are presented at the Dutch Working Party on Trophoblastic Disease. Since 1983 in the Netherlands, a clinical classification system proposed by the Dutch Working Party has been used which defines high-risk disease as the presence of one or more of the following features: insufficient response to single-agent chemotherapy; metastasis in more than one organ; metastasis in liver, spleen, kidneys, gastrointestinal tract, bones or brain; antecedent term pregnancy and an interval of >12 months between the end of the antecedent pregnancy and the start of treatment [12].

Data for all patients with high-risk GTN according to Dutch guidelines and treated with EMA/CO chemotherapy were collected from the databases of the DCRHM and the Dutch Working Party and included in the study. Patients receiving EMA/CO for recurrent disease were excluded. In addition, patients for whom the serum hCG values during EMA/CO treatment were unavailable or measured on an assay other than the in-house developed radioimmunoassay (RIA) of the assay service of the DCRHM were excluded from further analysis. Eventually, a total number of 46 patients treated with EMA/CO and with available hCG values were included in this study.

Based on the indication for treatment with multiagent chemotherapy, patients were classified as having single-agent MTX-resistant disease (n = 22) or as having a high prognostic score at the time of diagnosis of GTN (n = 24). MTX-resistant disease was defined as a rise or plateau in the hCG concentration during treatment with MTX for low-risk disease. Three patients with primary high-risk disease were treated with a hysterectomy before EMA/CO treatment, and one patient had a hysterectomy during EMA/CO treatment since she no longer wished to conceive. Separate normograms were constructed for patients receiving multiagent chemotherapy due to MTX resistance and patients treated for primary high-risk disease at diagnosis of GTN.

hCG measurements

All hCG concentrations were measured using an in-house developed RIA based on polyclonal antibody raised in rabbits [13]. This assay detects intact hCG and free β-subunit: hCG + hCGβ. A highly purified hCG β-subunit preparation labelled with iodine-125 was employed as a tracer for this assay. The RIA was calibrated with the third International Standard Preparations for intact hCG and hCG β-subunit. A value of 2 ng/ml is considered normal. hCG concentrations that were measured in serum obtained before the start of a chemotherapy course were used in the construction of the normogram. However, hCG concentrations that were measured in serum obtained during a chemotherapy course were excluded.

statistics

All hCG measurements of the patients reaching complete remission after multiagent chemotherapy without recurrent disease were used to construct the normograms for MTX-resistant disease and for primary high-risk GTN. Log transformation was carried out on all hCG values to obtain normal distribution of the data. The hCG values were evaluated cross-sectionally for each chemotherapy course. The normograms were based on a minimum of 10 log-transformed hCG values for each chemotherapy course. Serum hCG concentrations were subsequently sorted for each chemotherapy course. The 10th, 50th and 90th percentiles for every chemotherapy course were calculated and plotted for each course in a normogram using Excel (Microsoft). Resistance to EMA/CO according to the normogram was defined as the hCG concentration exceeding the 90th percentile of the normogram. Statistical analysis was carried out using SPSS 18 software. Serum hCG concentrations before the first course of EMA/CO and the number of chemotherapy courses were compared using the Mann–Whitney U test.

results

The characteristics of patients with MTX-resistant disease and patients with primary high-risk disease are shown in Table 1. The median follow-up was 26.1 months for patients with MTX-resistant disease and 26.0 months for patients with primary high-risk disease. No patients who achieved normal hCG concentrations with EMA/CO chemotherapy relapsed during follow-up.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>MTX-resistant disease (n = 22)</th>
<th>Primary high-risk disease (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range)</td>
<td>31 (23–43)</td>
<td>29 (22–54)</td>
</tr>
<tr>
<td>Antecedent pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydatidiform mole</td>
<td>22 (100%)</td>
<td>3 (12.5%)</td>
</tr>
<tr>
<td>Term</td>
<td>0 (0%)</td>
<td>20 (83.3%)</td>
</tr>
<tr>
<td>Non-molar abortion</td>
<td>0 (0%)</td>
<td>1 (4.2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site of metastases¹</th>
<th>MTX-resistant disease (n = 22)</th>
<th>Primary high-risk disease (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>18 (81.8%)</td>
<td>8 (33.3%)</td>
</tr>
<tr>
<td>Lungs</td>
<td>3 (13.6%)</td>
<td>15 (62.5%)</td>
</tr>
<tr>
<td>Vagina</td>
<td>0 (0.0%)</td>
<td>4 (16.7%)</td>
</tr>
<tr>
<td>Liver</td>
<td>1b (4.6%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Brain</td>
<td>0 (0.0%)</td>
<td>2 (8.3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interval from index pregnancy to start EMA/CO treatment (months)</th>
<th>MTX-resistant disease (n = 22)</th>
<th>Primary high-risk disease (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4</td>
<td>8 (36.4%)</td>
<td>16 (66.7%)</td>
</tr>
<tr>
<td>4–6</td>
<td>10 (45.4%)</td>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>7–12</td>
<td>4 (18.2%)</td>
<td>1 (4.2%)</td>
</tr>
<tr>
<td>&gt;12</td>
<td>0 (0.0%)</td>
<td>5 (20.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum hCG before the start of EMA/CO treatment (ng/ml)</th>
<th>MTX-resistant disease (n = 22)</th>
<th>Primary high-risk disease (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range)</td>
<td>21 (3–1300)</td>
<td>7059 (range 300–60 000)</td>
</tr>
</tbody>
</table>

¹Some patients had metastases at several sites.

bDubious lesion on computed tomography scan.

EMA/CO, etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine; hCG, human chorionic gonadotropin; MTX, methotrexate.
The normogram for patients treated with EMA/CO for MTX-resistant disease \((n = 22)\) is shown in Figure 1(a). The median hCG concentration before the onset of the first chemotherapy course was 21 ng/ml (range 3–1300 ng/ml), and before the third EMA/CO course half of the patients had normal hCG levels. The 90th percentile was at 148 ng/ml before the start of treatment. Ninety percent of the patients with MTX-resistant disease had normal hCG values before the start of the fourth EMA/CO course. The 10th percentile is just above normal (3 ng/ml) before the start of the first course but decreases to normal levels before the second course.

The normogram for patients treated with EMA/CO for primary high-risk disease is shown in Figure 1(b). The 90th percentile before the start of treatment was 32 781 ng/ml. In 90% of patients, hCG concentrations regressed to normal before commencing the eighth course of EMA/CO. The median hCG level was 7059 ng/ml (range 300–60 000 ng/ml) at the start of the first course, and after five EMA/CO courses 50% of patients had a normal hCG concentration. The 10th percentile line started at an hCG concentration of 1520 ng/ml, and ten percent of patients showed disease remission before the fourth course.

The median serum hCG concentration before the first course of EMA/CO was significantly less elevated (21 ng/ml) in the group of patients with MTX-resistant disease \((n = 19)\) compared with the group of patients with primary high-risk disease \((7059 \text{ ng/ml, } n = 14)\) \((P < 0.001)\). Significantly fewer courses of EMA/CO were administered to the group of patients.

**Figure 1.** Normogram for patients treated with (etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine) EMA/CO chemotherapy for methotrexate (MTX)-resistant disease (a) and for primary high-risk disease (b). The lower, middle and upper lines indicate the 10th, 50th and 90th percentiles. The number of human chorionic gonadotropin (hCG) values the percentiles were calculated from is shown above each course. Patients not cured on EMA/CO are plotted.
with MTX-resistant disease \( (n = 22) \) (median 5 courses; range 2–10) compared with the primary high-risk patients \( (n = 22) \) (median 8 courses; range 5–12; \( P < 0.001 \)).

Two primary high-risk patients failed to achieve normal hCG concentrations with EMA/CO. In one patient, there was a plateau of serum hCG after five courses of EMA/CO. Subsequent chemotherapy consisted of three courses of EMA/EP, one course of VIP (etoposide, ifosfamide and cisplatin) and salvage surgery consisting of a hysterectomy and surgical excision of pulmonary metastases. Her hCG level subsequently regressed to normal. The hCG concentration at the onset of EMA/CO course was between the 50th and 90th percentile line, but the level exceeded the 90th percentile before the second course. The other patient also received five courses of EMA/CO, after which a plateau of serum hCG developed. Treatment was continued with four courses of VIP and a hysterectomy, after which the hCG level was normal. The serum hCG level in this patient exceeded the 90th percentile already before the start of treatment.

**discussion**

In the present study, we constructed two normograms for the prediction of resistance to EMA/CO chemotherapy. As shown, normograms for hCG regression during multiagent treatment differed depending on the indication for multiagent chemotherapy. The initial hCG concentrations in the group of patients with primary high-risk disease are much higher due to the higher tumour load compared with those in the MTX-resistant group of patients, who already had tumour regression with MTX and subsequent decrease of the hCG concentration. It is therefore important that hCG concentrations of patients with MTX-resistant disease and patients with primary high-risk disease are not combined into one normogram.

Previous normograms for the prediction of post-molar GTN and for the prediction of resistance to MTX chemotherapy used upper percentiles of p95, p97.5 and p99, respectively \([7, 14, 15]\). Early prediction of patients who will not be cured by multiagent chemotherapy is of utmost importance to avoid fatal outcomes by an early change to a platinum-containing regimen (usually etoposide, MTX and actinomycin D, alternated with etoposide and cisplatin [EMA-EP]). Therefore, the 90th percentile was chosen as the upper line of the normogram. We feel that early prediction of a larger portion of patients not showing disease remission on EMA/CO outweighs the fact that more people will unnecessarily be treated with more toxic platinum-containing chemotherapy regimens. Patients with an hCG level >90th percentile of the normogram before the start of EMA/CO might be considered to start a platinum-containing chemotherapy regimen.

We presented for the first time a normogram for hCG regression in patients with high-risk GTN successfully treated with EMA/CO chemotherapy in order to predict treatment resistance. In addition, we demonstrated examples of two patients who could have been predicted early as developing resistance to treatment. We showed that normograms for hCG regression differed depending on the indication for multiagent chemotherapy due to higher hCG values in the group of patients with primary high-risk disease compared with those with MTX-resistant disease. Our findings need to be validated in a larger cohort of patients.

**disclosures**

The authors have declared no conflicts of interest.

**references**