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Can carboplatin or etoposide replace actinomycin-D for second-line treatment of methotrexate resistant low-risk gestational trophoblastic neoplasia?

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HIGHLIGHTS

- Actinomycin-D and etoposide achieved higher remission rates than carboplatin as a second-line regimen for low-risk GTN.
- Carboplatin caused more hematologic toxicity and treatment delays than actinomycin-D or etoposide.
- Carboplatin required greater utilization of G-CSF for neutropenia than actinomycin-D.

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ABSTRACT

Objective. To evaluate the impact of periodic shortage of actinomycin-D (Act-D) in the treatment of Brazilian patients with low-risk gestational trophoblastic neoplasia (GTN) after methotrexate and folinic acid rescue (MTX/FA) resistance, treated alternately with carboplatin or etoposide as a second-line regimen.

Methods. Retrospective cohort that included patients with failure of first-line MTX/FA regimen for low-risk GTN treated at Rio de Janeiro Federal University, Universidade Federal de São Paulo and Irmandade da Santa Casa de Misericórdia de Porto Alegre, from January/2010- December/2017.

Results. From 356 patients with low-risk GTN treated with MTX/FA, 75 (21.1%) developed resistance, of which 40 (53.3%) received Act-D, 23 (30.7%) carboplatin and 7 (9.3%) etoposide. Although patients treated with single-agent chemotherapy as a second-line regimen had comparable clinical and primary treatment characteristics, those treated with Act-D (80%, $p = 0.033$) or etoposide (71.4%, $p = 0.025$) had higher remission rates when compared with carboplatin (47.8%). Only 29% of patients treated with carboplatin received the chemotherapy cycles without delay compared to Act-D (98%, $p < 0.001$) or etoposide (85%, $p = 0.009$). Patients treated with carboplatin had significantly more hematological toxicity, notably anemia (30.4%, $p = 0.008$), lymphopenia (47.7%, $p < 0.001$) and thrombocytopenia (43.4%, $p < 0.001$), as well as a higher occurrence of febrile neutropenia (14.4%, $p = 0.044$) and vomiting (60%, $p < 0.001$) than those receiving Act-D (5%, none, 2.5%, none, 10%, respectively).

Conclusion. Carboplatin did not have a satisfactory clinical response rate, likely due to severe hematological toxicity, which postponed chemotherapy. Our results reinforce the preference for Act-D as a second-line agent in patients with low-risk GTN after MTX/FA resistance.

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1. Introduction

Gestational trophoblastic neoplasia (GTN) includes a group of tumors derived from the human trophoblast, comprising invasive mole, choriocarcinoma (CCA), placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT). Generally, GTN is highly responsive to chemotherapy and has a sensitive tumor marker, human chorionic gonadotrophin (hCG), that allows monitoring response to treatment and assuring disease remission [1].

Because GTN is uncommon, patients with this disease may benefit from treatment in Reference Centers, where survival rates approach 100%, especially when the GTN is diagnosed early [2]. Choice of chemotherapy for GTN is based on the International Federation of Gynecology and Obstetrics (FIGO) staging and World Health Organization (WHO) risk score system that evaluates prognostic factors for resistance to single-agent therapy [1]. Patients with stage I, II or III disease and a FIGO/WHO risk score ≤ 6, are considered to have low-risk GTN and are treated with single-agent chemotherapy, usually methotrexate and folinic acid rescue (MTX/FA) or actinomycin-D (Act-D), with primary remission rates ranging from 70 to 90% [1]. The risk for resistance to a single-agent regimen is increased among patients with low-risk GTN with FIGO/WHO risk scores of 5–6 and clinicopathologic diagnosis of choriocarcinoma. Even in these cases, salvage chemotherapy can cure >90% of these patients [1].

In Brazil, the Brazilian Association of Gestational Trophoblastic Disease (BAGTD) has established treatment protocols for patients with low-risk GTN [3]. While there is no clear global consensus on the preferred single-agent treatment [4], BAGTD recommends MTX/FA as a first-line chemotherapy for these patients [3]. In cases of MTX/FA chemoresistance, Act-D is proposed as a second-line regimen, reserving the etoposide, methotrexate, actinomycin-D, cyclophosphamide, oncovin (vincristine) (EMA/CO) regimen as salvage chemotherapy [3].

However, since 2013, Brazil has experienced periodic shortages of Act-D, compromising the treatment of GTN in patients with MTX/FA resistance. In this period, despite institutional efforts made by the BAGTD, Brazilian Federation of Gynecology and Obstetrics Associations and the Brazilian Society of Clinical Oncology, to facilitate market availability of Act-D, Brazilian physicians treating GTN have necessarily sought therapeutic alternatives. Without access to Act-D, two alternative single-agents were used to treat women with MTX/FA chemoresistance: carboplatin or etoposide.

Carboplatin is an alkylating organoplatinum compound that as part of multiagent regimens has been shown to be safe and effective in the treatment of women with GTN [5–10]. Winter et al. [11], showed for the first time that carboplatin as a single-agent induced remission in 81% of women with MTX/FA chemoresistant low-risk GTN. The authors further reported that this regimen was well tolerated, with myelosuppression as the most significant toxicity (one-third of patients had grade III/IV neutropenia/thrombocytopenia). Etoposide is a semi-synthetic derivative of podophyllotoxin, used since the 1990s, as a single-agent [12–16] or in multiagent regimens [17,18] for treatment of patients with GTN. In general, etoposide has more side effects than other single-agent chemotherapies and carries a slight increase in lifetime absolute risk of leukemia; despite this it has excellent response rates in the treatment of GTN and remains an important agent [17–19].

The best chemotherapy regimen for patients with low-risk GTN after failure of MTX/FA is unknown [1], but Act-D, which is widely used all over the world, tends to be the favored choice [19]. To date there is limited data exploring alternative options. There is only one paper reporting the experience with carboplatin [11], and limited experience with the use of etoposide [12–16]. The shortage of Act-D in the Brazilian market necessitated the treatment of our patients with MTX/FA-resistant GTN with these less common single-agent chemotherapy regimens. There are no prior Brazilian cohort studies evaluating carboplatin or etoposide in the treatment of women with MTX/FA chemoresistant low-risk GTN.

This study describes the results of treatment of Brazilian patients with MTX/FA-resistant low-risk GTN with actinomycin-D, carboplatin or etoposide, and compares our experience with the literature. Importantly, in an era when global markets have frequent shortages of chemotherapies, it is critical to evaluate the efficacy of alternative treatments [20,21].

2. Material and methods

2.1. Study design

This is a collaborative retrospective cohort study of patients with GTN followed at Maternity School of Rio de Janeiro Federal University (Rio de Janeiro – RJ, Brazil, data entered by PARM and audited by AB), São Paulo Hospital Trophoblastic Disease Center of Universidade Federal de São Paulo (São Paulo – SP, Brazil, data entered by PARM and audited by SYS) and Mario Totta Maternity Ward at Irmandade da Santa Casa de Misericórdia Hospital (Porto Alegre – RS, Brazil, data entered by PARM and audited by EHU), from January 2010 to December 2017.

This study was approved by the local Institutional Review Board associated with the Brazilian Research Ethics Committee of the Maternity School of the Rio de Janeiro Federal University (CAAE 95188418.2.0000.5275), São Paulo Hospital of Universidade Federal de São Paulo (CAAE 95188418.2.3003.5505) and Mario Totta Maternity Ward at Irmandade da Santa Casa de Misericórdia de Porto Alegre (CAAE 95188418.2.3001.5335).

2.2. Study participants

Patients diagnosed with low-risk GTN according to the FIGO 2000 criteria [22], that had a diagnosis of MTX/FA-resistance and were subsequently treated with Act-D, carboplatin or etoposide were included in this study. All patients were followed up for at least 12 months after remission. All cases of molar pregnancy that developed GTN or choriocarcinoma had their diagnosis confirmed by the Pathology Department of the Reference Centers. Patients diagnosed with high-risk GTN, PSTT and ETT, and patients treated with multiagent regimens as their second-line therapy following the diagnosis of MTX/FA-resistance were excluded. Patients with MTX/FA treatment failure due to toxicity were also excluded from this study, to make the results more comparable to those of Winter et al. [11], that only included patients who developed MTX/FA resistance.

2.3. Sample size

In the recent report by Winter, et al. [11], the complete response rate to single-agent carboplatin was 17/21 (81%; 95% confidence interval 59%–93%) and to actinomycin D was 53/59 (90%; 95% confidence interval 79%–96%). This suggests that the absolute difference between the two regimens could be as great as 37% within an accepted range of uncertainty. In the prior report, however, patients received carboplatin or actinomycin D according to a risk-adapted algorithm. Patients with hCG < 150 IU/L in the first time period of the study and hCG < 300 IU/L in the second time period of the study received single-agent actinomycin D while patients with hCG > 300 IU/L were eligible to receive second-line treatment with carboplatin. This leaves open the question as to whether carboplatin is non-inferior to actinomycin D among unselected patients requiring second-line chemotherapy.

As reported complete response rates to actinomycin D as second-line chemotherapy for methotrexate resistant low-risk gestational trophoblastic neoplasia range from 60% to 90% [11,23–26], we performed our power calculation assuming an average response rate to either second-line regimen of 80%. Based on the Winter, et al. [11] paper, we conservatively posited the null hypothesis of the regimens being similarly efficacious within an absolute range of 30%. For calculating the statistical power of the study, this meant that if there is truly no difference

between actinomycin D and carboplatin, then 44 patients (at least 22 in each group) would be required to have 80% power to ensure that the upper limit of a one-sided 95% confidence interval (or equivalently a 90% two-sided confidence interval) would exclude an absolute difference in cure rates in favor of actinomycin D of >30% [27,28].

2.4. Diagnosis of GTN

According to FIGO 2000 criteria, GTN was diagnosed when there was a histological diagnosis of choriocarcinoma or when quantitative hCG serum monitoring exhibited four hCG plateaued values over a period of at least 3 weeks, an increased hCG level in three consecutive measurements or more for at least 2 weeks, or when hCG levels remain elevated, even if they are falling, 6 months or more from evacuation of a molar pregnancy [22].

2.5. Staging, risk factors and treatment of GTN

Patients were staged according to FIGO 2000 GTN anatomical staging and assigned a prognostic score for resistance to single-agent chemotherapy following the FIGO/WHO Prognostic Scoring System [24]. Lung metastases were detected using a chest X-ray [1,3,22]. Magnetic resonance imaging of the brain and abdomen were used for patients with visible pulmonary metastasis on chest X-ray or genital metastasis [1,3,22].

Measurement of hCG in all Reference Centers employed the Siemens Diagnostic Products Corporation (DPC) Immulite® assay. The reference value for normal results was an hCG value below 5 IU/L. All patients included in this study received contraceptive counseling and received hormonal contraception [3].

The 8-day MTX/FA regimen with methotrexate 1 mg/kg intramuscular on days 1, 3, 5, and 7 alternating with folinic acid 0.1 mg/kg orally on days 2, 4, 6, and 8 was used as first-line treatment in cases of low-risk GTN if there was no contraindication [1,3,22]. In cases of MTX/FA resistance and when it was available, second-line chemotherapy was administered with Act-D 1.25 mg/m^2 (maximum 2.0 mg) IV pulse every 2 weeks [1,3,22].

In periods of Act-D shortage, patients were advised on the available therapeutic options. After counseling, the treatment choice was dictated by patient/physician preference on an individual basis with no formal randomization. Patients were treated with one of the following chemotherapy regimens:

a. Carboplatin AUC = 6 every 21 days with maximum dose of 900 mg. Dose was calculated using the Calvert formula and Cockcroft-Gault estimation of GFR, as described by Winter, et al. [11]
b. Etoposide, dose of 100 mg/m^2, day 1–5, every 14 days [13,14].

Beginning in 2013, there was a shortage and unavailability of Act-D to treat MTX-resistant low-risk GTN in Brazil, and we used etoposide as our second-line therapy in 2013–2014. When we became aware of pre-published favorable results of second-line treatment with carboplatin from the Sheffield Centre for Trophoblastic Disease, we switched to carboplatin in 2015–2017.

Treatment was delayed if the patient presented with neutropenia (<1000/mm^3) or thrombocytopenia (<75,000/mm^3) until recovery, and the carboplatin dose was reduced to an estimated AUC of 5 mg/mL/min for subsequent doses [11]. If hematological toxicity caused at least one delay in the chemotherapy cycles, granulocyte colony stimulating factor was routinely used in the subsequent cycles until remission of the disease.

In the case of resistance to second-line therapy, severe toxicity (grade III or IV in two consecutive cycles) to second-line treatment or by the patient's desire after medical advice following the first episode of grade III/IV toxicity in the second-line treatment, a third-line regimen of etoposide, MTX/FA, cyclophosphamide, and oncovin (vincristine) (EM/CO) was administered, i.e. EMA/CO without Act-D, and the paclitaxel, cisplatin and etoposide regimen (TP/TE) was used as a fourth-line regimen.

After hCG normalization, patients received 3 consolidation cycles of chemotherapy, which was interrupted in case of toxicity, and were monitored monthly with hCG levels for 12 months, when they were discharged from follow-up [3]. If patients did not attend the scheduled visits, a social worker and hospital psychologist actively tried to contact them by phone, electronic message and telegram to identify what was hindering compliance and to motivate them to return to follow-up.

2.6. Outcomes

The primary outcome was the occurrence of remission following second-line chemotherapy (Act-D, carboplatin or etoposide). Secondary outcomes were toxicity in the various second-line chemotherapy regimens, number of cycles required to attain GTN remission, time to remission, duration of follow-up, and occurrence of relapse and death.

2.7. Variables

The following population variables were studied: age (in years), number of gestations and parity of the patient.

The following clinical variables of gestational trophoblastic disease were evaluated: gestational age at diagnosis (in weeks), occurrence of medical complications at the time of diagnosis: anemia (hemoglobin <9 g/dL), vaginal bleeding, enlarged uterus for gestational age (when the uterine size is >4 cm above the expected for fundal height for gestational age), theca lutein cysts (presence of ovarian cyst exceeding 6 cm), preeclampsia (systolic blood pressure levels above 140 mm Hg or diastolic above 90 mm Hg with proteinuria higher than 300 mg/24 h), hyperemesis (>5 episodes of vomiting per day, with or without metabolic changes), hyperthyroidism (levels of thyroid stimulating hormone below 0.01 IU/L and free thyroxine above 91.5 ng/dL) and respiratory failure.

The following pathological variables were evaluated: the histopathological diagnosis of gestational trophoblastic disease (complete or partial hydatidiform mole or CCA) and the level of hCG at diagnosis of gestational trophoblastic disease (IU/L).

Regarding the clinical aspects of gestational trophoblastic neoplasia, the following variables were studied: the GTN stage and FIGO/WHO prognostic score, antecedent pregnancy (molar pregnancy, term/preterm pregnancy, abortion or ectopic pregnancy), hCG pre-treatment level (IU/L) and time between the end of antecedent pregnancy and the beginning of chemotherapy with MTX/FA.

Considering the GTN therapeutic variables, we evaluated the number of cycles of MTX/FA administered, the time required to change to the second-line treatment (in weeks), the level of hCG at the time of MTX/FA resistance (IU/L), number of cycles of second-line chemotherapy (without consolidation cycles), the type and intensity of toxicity of second-line chemotherapy, excluding episodes after remission during consolidation chemotherapy, according to Common Terminology Criteria for Adverse Events, Version 5.0, 2017 (CTCAE, 2017) [29], occurrence of delay and number of days of delay to start a new cycle of chemotherapy due to toxicity, occurrence of remission with the second-line regimen (defined as normalization of hCG levels - lower than 5 IU/L – which was maintained for at least 3 weeks), resistance to the second-line regimen (characterized by hCG plateau of ±10% after 2 cycles of chemotherapy or its re-elevation), toxicity as a reason to switch to a third-line regimen (characterized by the occurrence of grade III/IV toxicity in two consecutive cycles, or by the patient's desire after medical advice, after the first episode of grade III/IV toxicity in the second-line regimen), relapse (characterized by the re-elevation of hCG levels after remission, in the absence of a new pregnancy) and death.

2.8. Statistical analysis

To analyze the association between the second-line chemotherapy treatments (Act-D, carboplatin or etoposide) and each of the categorical variables, Chi-square test or Fisher's exact test was used when appropriate.

To compare continuous variables among the second-line chemotherapy treatments, the Kruskal-Wallis test was used. A Bonferroni-corrected Mann-Whitney *U* test was used for pairwise comparisons between two groups in cases of significance by the Kruskal-Wallis test.

Differences were considered statistically significant when *p*-values were <0.05. Statistical analysis was made using R software statistical package version 3.3.2, available at www.r-project.org.

3. Results

Fig. 1 represents a flow diagram describing the study population. From January 2010 to December 2017, 2158 patients were diagnosed with gestational trophoblastic disease (GTD). Among these, 1737 (80.5%) achieved spontaneous remission and 421 (19.5%) developed GTN, among which 377 (89.5%) were categorized as low-risk GTN and 356 (94.4%) were treated with single-agent MTX/FA. We observed 75 (21.1%) patients with MTX/FA resistance, of which 40 (53.3%) were treated with Act-D, 23 (30.7%) with carboplatin and 7 (9.3%) with etoposide.

The presenting characteristics of patients with low-risk GTN with MTX/FA resistance were similar among the three regimens studied (Table 1). The different groups of patients treated were comparable, with no statistical differences in age ($p = 0.982$), gravidity ($p = 0.902$), parity ($p = 0.601$), gestational age at the diagnosis ($p = 0.872$), occurrence of medical complications, histology of GTD ($p = 0.768$) or hCG level pre-evacuation ($p = 0.126$).

Patients with MTX/FA resistance treated with Act-D, carboplatin or etoposide similarly did not differ in clinical and therapeutic aspects of low-risk GTN management (Table 2). They were mainly patients with

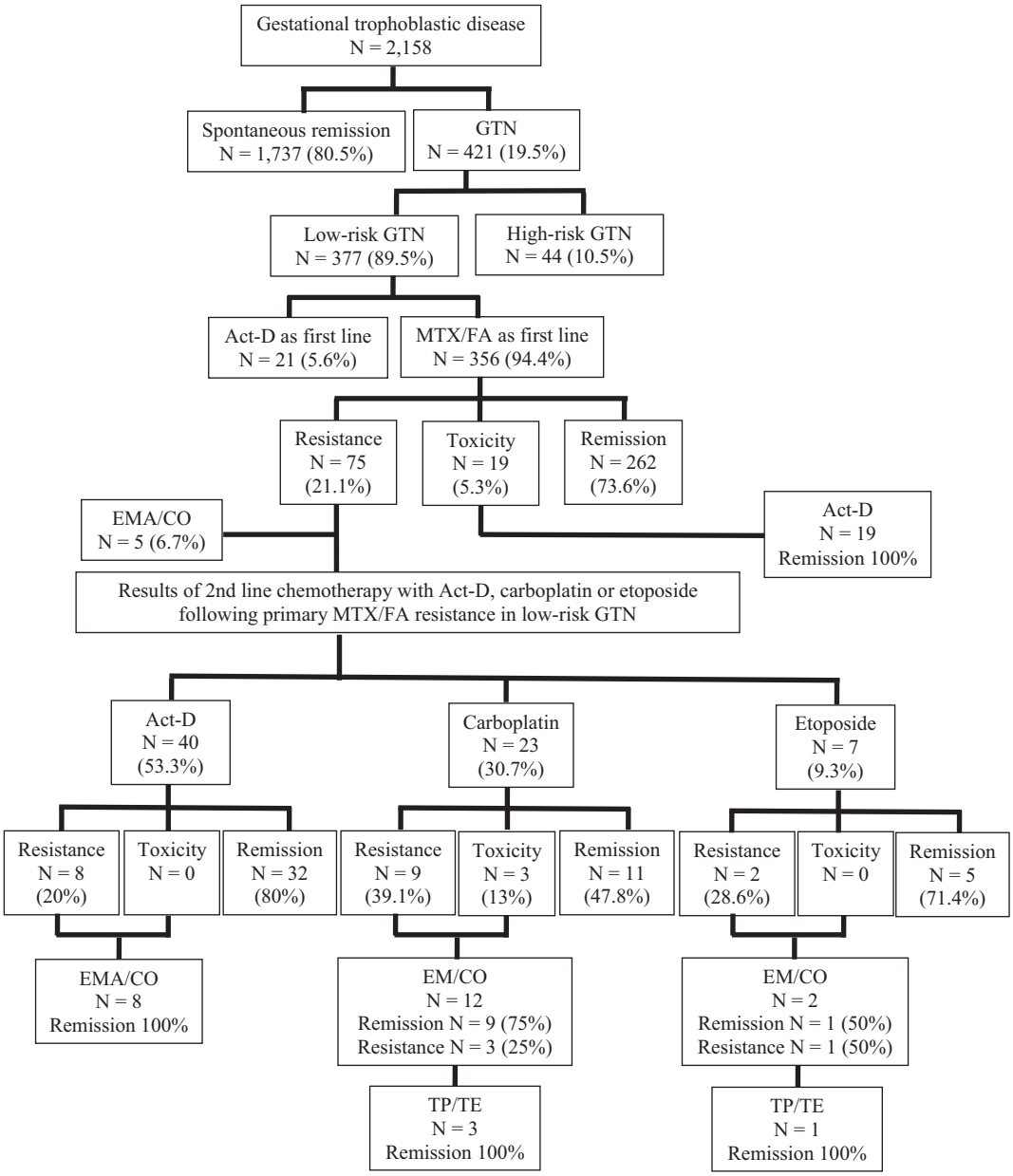

Fig. 1. Flow diagram summarizing the derivation of the study population. GTN – gestational trophoblastic neoplasia. MTX/FA – methotrexate/folinic acid. Act-D – Actinomycin-d. EMA/CO – etoposide, methotrexate, actinomycin D/cyclophosphamide, oncovin (vincristine). EM/CO - etoposide, methotrexate/cyclophosphamide, oncovin (vincristine). TP/TE – paclitaxel, cisplatin/paclitaxel, etoposide.

Table 1
Characteristics of patients with low-risk gestational trophoblastic neoplasia after MTX/FA resistance according to the second-line regimen.

Variables	Actinomycin-D $N = 40$	Carboplatin $N = 23$	Etoposide $N = 7$	*p*-Value
Age (years)[a]	25 (21–36)	25 (21–34)	29 (21−31)	0.982 [K]
Gravidity[a]	1 (1–2)	1 (1–2)	1 (1–2)	0.902 [K]
Parity[a]	0 (0–1)	0 (0–0.5)	0 (0–1)	0.601 [K]
Gestational age at diagnosis (weeks)[a]	10 (9–11)	10 (9–11)	9 (9–11)	0.872 [K]
Medical complication at presentation, n (%)				
Vaginal bleeding	23 (57.5%)	14 (60.9%)	3 (42.8%)	0.774 [C]
Anemia	5 (12.5%)	5 (21.7%)	2 (28.6%)	0.370 [C]
Enlarged uterus for gestational age	23 (57.5%)	14 (60.9%)	3 (42.8%)	0.774 [C]
Theca lutein cysts	5 (12.5%)	5 (21.7%)	2 (28.6%)	0.370 [C]
Hyperemesis	9 (22.5%)	4 (17.4%)	2 (28.6%)	0.687 [C]
Hyperthyroidism	2 (5%)	0	0	0.619 [C]
Respiratory distress syndrome	2 (5%)	0	0	0.619 [C]
Histology of GTD[b], n (%)				0.768 [C]
Complete hydatidiform mole	30 (75%)	19 (82.6%)	5 (71.4%)	
Partial hydatidiform mole	5 (12.5%)	3 (13%)	1 (14.3%)	
Choriocarcinoma	5 (12.5%)	1 (4.4%)	1 (14.3%)	
hCG[c] (IU/L) at molar evacuation[a]	175,000 (150000–212,500)	150,000 (115000–200,000)	150,000 (130000–265,000)	0.126 [K]

C – Chi-square test.
K – Kruskal-Wallis test.
[a] Median and interquartile range.
[b] GTD – gestational trophoblastic disease.
[c] hCG – human chorionic gonadotropin (IU/L – International units per liter).

non-metastatic GTN ($p = 0.952$) following molar pregnancies ($p = 0.456$) with low WHO/FIGO Prognostic Risk Scores ($p = 0.818$) and moderately elevated hCG values upon initiating primary therapy ($p = 0.989$). Most patients started chemotherapy within 4 months of pregnancy termination ($p = 0.729$) and experienced resistance after about 5 cycles ($p = 0.785$). hCG levels were similar among the three groups at the time of switching to a second-line regimen: 7890, 1385 and 4250 IU/L ($p = 0.188$), respectively.

Comparing the remission rates among the three second-line regimens, Act-D (80%) and etoposide (71.4%, $p = 0.659$) were similar, but both were higher than carboplatin (47.8%, $p = 0.033$ and $p = 0.025$, respectively) (Table 3). The number of cycles needed to achieve remission was significantly different among the studied groups ($p = 0.002$): with more cycles of Act-D needed compared to carboplatin ($p = 0.016$) or etoposide ($p = 0.027$), while there was no difference between the number of cycles of carboplatin and etoposide required to induce remission ($p = 0.479$). The total number of cycles administered of Act-D, carboplatin and etoposide were 203, 56 and 23, respectively. There was a significant increase in the occurrence of chemoresistance/toxicity among patients treated with carboplatin (52.2%) than in patients receiving Act-D (20%, $p = 0.008$) or etoposide (28.5%, $p = 0.022$). It was also observed that only 29% of the patients treated with carboplatin received the chemotherapy cycles without delay, which was significantly different when compared to the women treated with Act-D (98%, $p <$

Table 2
Clinical and therapeutic profile of patients with MTX/FA failure for treatment of low-risk gestational trophoblastic neoplasia according to the second-line regimen.

Variables	Actinomycin-D $N = 40$	Carboplatin $N = 23$	Etoposide $N = 7$	*p*-Value
Stage, n (%)				0.952 [C]
I	35 (87.5%)	19 (82.6%)	6 (85.7)	
II	1 (2.5%)	1 (4.3%)	0	
III	4 (10%)	3 (13.1%)	1 (14.3%)	
IV	0	0	0	
WHO/FIGO[b] Prognostic Risk Score, n (%)				0.995 [C]
0	1 (2.5%)	0	0	
1	11 (27.5%)	6 (26.1%)	1 (14.3%)	
2	8 (20.0%)	5 (21.7%)	2 (28.5%)	
3	6 (15.0%)	5 (21.7%)	1 (14.3%)	
4	5 (12.5%)	2 (8.7%)	1 (14.3%)	
5	4 (10%)	2 (8.7%)	1 (14.3%)	
6	5 (12.5%)	3 (13.1%)	1 (14.3%)	
WHO/FIGO[b] Prognostic Risk Score[a]	2 (1–4)	3 (1–4)	3 (2–4)	0.818 [K]
Antecedent of pregnancy, n (%)				0.456 [C]
Hydatidiform mole	35 (87.5%)	22 (95.6%)	6 (85.7%)	
Term/preterm	4 (10%)	0 (0%)	1 (14.3%)	
Abortion	1 (2.5%)	1 (4.4%)	0 (0%)	
hCG[c] (IU/L) at initial MTX/FA[d] treatment[a]	14,000 (883.5–89,250)	13,000 (4800–34,000)	12,000 (1550–30,900)	0.989 [K]
Time between molar evacuation and initial MTX/FA[d] treatment[a] (months)	4 (3–5)	4 (3–5)	4 (3–4)	0.729 [K]
Number of cycles of MTX/FA[d] treatment[a]	5 (4–7)	5 (4–5)	5 (4–6)	0.785 [K]
Time to switch to second-line therapy (weeks)[a]	10 (8–14)	10 (8–11)	10 (8–12)	0.785 [K]
hCG[c] (IU/L) at switch to second-line therapy[a]	7890 (2568–9870)	1385 (443–6824)	4250 (2580–5920)	0.188 [K]

C – Chi-square test.
K – Kruskal-Wallis test.
[a] Median and interquartile range.
[b] WHO/FIGO – World Health Organization/International Federation of Gynecology and Obstetrics.
[c] hCG – human chorionic gonadotropin (IU/L – International units per liter).
[d] MTX/FA – Methotrexate with folinic acid rescue.

Table 3
Outcomes of second-line regimen for treatment of patients with low-risk gestational trophoblastic neoplasia after MTX/FA failure.

Variables	Actinomycin-D $N = 40$	Carboplatin $N = 23$	Etoposide $N = 7$	*p*-Value			
				Among all three regimens	Pairwise comparisons		
					Actinomycin-D versus Carboplatin	Actinomycin-D versus Etoposide	Carboplatin versus Etoposide
Remission in second line, n (%)	32 (80%) 95% CI[c]: 65%–90%	11 (47.8%) 95% CI[c]: 29%–67%	5 (71.4%) 95% CI[c]: 35%–92%	0.047 C	0.033 F	0.659 F	0.025 F
Number of cycles needed to remission in second-line[a,b]	3 (3–6)	3 (2–3)	2 (1–3)	0.002 K	0.016 M	0.027 M	0.479 M
Number of cycles to remission[b]	6 (6–7)	5 (5–5.5)	4 (4–5)	0.003 K	0.003 M	0.014 M	0.748 M
Time to switch to third-line therapy (weeks)[b]	12 (10–14)	11 (9–13)	8 (7–10)	0.231 K			
hCG (IU/L)[d] at switch to third-line therapy	1035 (262.5–21,695)	900 (220–3640)	899 (88.5–4200)	0.507 K			
Reason to switch to third-line therapy Chemoresistance/toxicity, n (%)	8 (20%)	12 (52.2%)	2 (28.6)	0.022 C	0,008 F	0,609 F	0,022 F
Chemotherapy cycles on time, n (%)	39 (98%)	7 (29%)	6 (85%)	<0.001 C	<0.001 F	0.154 F	0.009 F
Number of days overdue[b]	4 (1–5)	8 (5–18)	5 (2–6)	0.001 K	0.001 M	0.051 M	0.892 M
Number of patients that needed G-CSF[e], n (%)	0	9 (39%)	2 (29%)	<0.001 K	<0.001 F	0.019 F	0.204 F
Time to remission (weeks)[b]	20 (15–26)	20 (15–23)	12 (9–18)	0.099 K			
Duration of follow up (months)[b]	16 (15–19)	17 (15–18)	15 (13–16)	0.081 K			
Relapse, n (%)	0	0	0				
Death, n (%)	0	0	0				

C – Chi-square test.
F – Fisher's exact test.
K – Kruskal-Wallis test.
M – Bonferroni-corrected Mann-Whitney *U* test.
[a] Without consolidation.
[b] Median and interquartile range.
[c] CI – confidence interval.
[d] hCG – human chorionic gonadotropin (IU/L – International units per liter).
[e] G-CSF – granulocyte-colony stimulating factor.

0.001) or etoposide (85%, $p = 0.009$). Among patients treated with Act-D, 5 (2.4%) cycles required delay, whereas among patients treated with carboplatin and etoposide this occurred in 39 (69.6%) and 4 (17.4%) cycles, respectively. The length of delay was significantly higher in patients treated with carboplatin (median of 8 days, $p = 0.001$) or etoposide (median of 5 days, $p = 0.051$) when compared to those treated with Act-D (median of 4 days).

The significant delays between the carboplatin cycles occurred due to treatment toxicity. Table 4 shows that only patients treated with carboplatin had grade III/IV toxicity. Patients treated with carboplatin had significantly more hematological toxicity, notably anemia (30.4%, $p = 0.008$), lymphopenia (47.7%, $p < 0.001$) and thrombocytopenia (43.4%, $p < 0.001$), as well as a higher occurrence of febrile neutropenia (14.4%, $p = 0.044$) and vomiting (60.9%, $p < 0.001$) than those treated with Act-D (5%, none, 2.5%, none, 10%, respectively). Alopecia was significantly reported among patients treated with etoposide (100%, $p < 0.001$), being uncommon among patients treated with Act-D (5%) or carboplatin (4.3%). Due to toxicity, 27 (48.2%) cycles of carboplatin were administered at a dose reduction of AUC5.

Compared to patients treated with Act-D, the number of women treated with carboplatin (39%, $p < 0.001$) or etoposide (29%, $p = 0.019$) who required G-CSF treatment was significantly higher (Table 3). All patients who did not respond to the second-line regimen were successfully treated with multi-agent chemotherapy, as shown in Fig. 1, and there were no cases of relapse or death among patients studied.

4. Discussion

While most patients with low-risk GTN achieve remission with single-agent chemotherapy [19], our study showed that 21% of patients needed a second-line regimen, similar to prior studies [19,24–26,30]. Although there is no consensus regarding the best second-line GTN treatment [1], the expectation is that most women can still be cured with a second-line single-agent chemotherapy, thereby avoiding multiple-agent treatment and its attendant early and late toxicities [1,25,30]. In this study, we have shown that carboplatin had a lower remission rate than Act-D and etoposide as a second-line regimen for low-risk GTN after MTX/FA resistance. Carboplatin had a higher occurrence of chemotherapy failure and a higher rate of grade III/IV toxicities, notably with hematological impairment. Although patients receiving carboplatin required more support with G-CSF than those treated with Act-D, >2/3 of patients on carboplatin experienced delayed cycles of chemotherapy, postponing the continuity of treatment when compared to patients treated with Act-D.

The only prior study evaluating the response to second-line treatment of patients with low-risk GTN after MTX/FA resistance found similar remission rates in women treated with Act-D and carboplatin (53/59–89.8% versus 17/21–80.9%, respectively) [11], higher rates than we observed in our study. Population characteristics such as race and ethnicity have been cited to explain different responses and toxicity to chemotherapy treatments with carboplatin and other agents [31,32]. It is possible that differences in patient population may partly explain differences in outcomes between the experience with Winter et al. [11] and our data. It should also be noted that in the study by Winter et al. [11], hCG levels at the time of the switch to the second-line therapy had lower median and interquartile levels between patients who were treated with Act-D than among women who were treated with carboplatin (28, interquartile: 18–69 IU/L versus 2126, interquartile: 1149–7948 IU/L, respectively). In our study, patients with low-risk GTN after MTX/FA resistance, who were treated with different second-line regimens, were comparable in variables commonly associated with response to chemotherapy, such as age, hCG level at the time of failure of first-line chemotherapy, histology of GTD, antecedent pregnancy, the interval between the end of previous pregnancy and the beginning of MTX/FA chemotherapy, presence of metastatic disease and FIGO/WHO score [25,33,34].

Table 4
Adverse events due to second-line regimen for treatment of low-risk gestational trophoblastic neoplasia after MTX/FA resistance, graded according to Common Terminology Criteria for Adverse Events, version 5.0 (2017).

Variable	Actinomycin-D ($N = 40$)			Carboplatin ($N = 23$)					Etoposide ($N = 7$)			Among all three regimens *p*-value (Chi-square test)	Pairwise comparisons *p*-Value (Fisher's exact test)		
Disorders by system	Adverse event N (%)	CTC grade (N/%)		Adverse event N (%)	CTC grade (N/%)				Adverse event N (%)	CTC grade (N/%)					
		1	2		1	2	3	4		1	2		Actinomycin-D versus Carboplatin	Actinomycin-D versus Etoposide	Carboplatin versus Etoposide
Blood															
Anemia	2 (5)	1 (2.5)	1 (2.5)	7 (30.4)	2 (8.7)	4 (19.4)	1 (4.3)	–	1 (14.3)	1 (14.3)	–	0.018	0.008	0.390	0.637
Febrile neutropenia	–	–	–	4 (14.4)	–	–	3 (13)	1 (4.3)	1 (14.3)	1 (14.3)	–	0.031	0.044	0.148	1
Gastrointestinal															
Diarrhea	10 (25)	6 (15)	4 (10)	5 (21.7)	5 (21.7)	–	–	–	1 (14.3)	1 (14.3)	–	1			
Mucositis oral	14 (35)	10 (25)	4 (10)	7 (30.4)	3 (13)	4 (17.4)	–	–	1 (14.3)	1 (14.3)	–	0.650			
Nausea	18 (45)	18 (45)	–	20 (87)	12 (52.2)	8 (34.8)	–	–	2 (28.6)	2 (28.6)	–	<0.001	0.001	0.681	0.006
Stomach pain	5 (12.5)	4 (10)	1 (2.5)	7 (30.4)	4 (17.4)	3 (13)	–	–	1 (14.3)	1 (14.3)	–	0.1702			
Vomiting	4 (10)	4 (10)	–	14 (60.9)	8 (34.8)	6 (26.1)	–	–	2 (28.6)	2 (28.6)	–	<0.001	<0.001	0.213	0.204
Infections															
Upper respiratory	3 (7.5)	3 (7.5)	–	3 (13)	3 (13)	–	–	–	1 (14.3)	1 (14.3)	–	0.5911			
Urinary tract	2 (5)	2 (5)	–	5 (21.7)	4 (17.4)	1 (4.3)	–	–	–	–	–	0.0864			
Vaginal	4 (10)	4 (10)	–	5 (21.7)	4 (17.4)	1 (4.3)	–	–	1 (14.3)	1 (14.3)	–	0.4076			
Investigations															
ALT ↑	1 (2.5)	1 (2.5)	–	11 (47.7)	3 (13)	5 (21.7)	3 (13)	–	1 (14.3)	1 (14.3)	–	<0.001	<0.001	0.278	0.193
AP ↑	1 (2.5)	1 (2.5)	–	9 (39.1)	2 (8.7)	4 (17.4)	3 (13)	–	1 (14.3)	1 (14.3)	–	<0.001	<0.001	0.278	0.371
AST ↑	1 (2.5)	1 (2.5)	–	9 (39.1)	2 (8.7)	4 (17.4)	3 (13)	–	1 (14.3)	1 (14.3)	–	<0.001	<0.001	0.278	0.371
Lymphocyte count ↓	–	–	–	11 (47.7)	4 (17.4)	4 (17.4)	3 (13)	1 (4.3)	1 (14.3)	1 (14.3)	–	<0.001	<0.001	0.148	0.030
Neutrophil count ↓	1 (2.5)	–	1 (2.5)	13 (56.5)	5 (21.7)	5 (21.7)	3 (13)	1 (4.3)	2 (28.6)	1 (14.3)	1 (14.3)	<0.001	<0.001	0.002	0.181
Platelet count ↓	1 (2.5)	–	1 (2.5)	10 (43.4)	3 (13)	3 (13)	3 (13)	1 (4.3)	1 (14.3)	1 (14.3)	–	<0.001	<0.001	0.148	0.024
Reproductive															
Irregular menstruation	1 (2.5)	1 (2.5)	–	3 (13)	3 (13)	–	–	–	–	–	–	0.300			
Menorrhagia	2 (5)	2 (5)	–	4 (17.4)	4 (17.4)	–	–	–	1 (14.3)	1 (14.3)	–	0.210			
Skin															
Alopecia	2 (5)	2 (5)	–	1 (4.3)	1 (4.3)	–	–	–	7 (100)	5 (71.4)	2 (28.6)	<0.001	0.528	<0.001	<0.001
Vascular															
Phlebitis	2 (5)	2 (5)	–	1 (4.3)	1 (4.3)	–	–	–	1 (14.3)	1 (14.3)	–	0.5369			

ALT – Alanine aminotransferases. AP – Alkaline phosphatase. AST – Aspartate aminotransferases.
G-CSF – Granulocyte-colony stimulating factor.

There was a higher occurrence of chemoresistance/toxicity among women receiving carboplatin in our series. Winter et al. [11] reported a grade III/IV toxicity of neutropenia (38.1%) and thrombocytopenia (28.6%), similar to our results, which identified bone marrow suppression as the main toxicity among patients treated with carboplatin. This led to a delay in 71.4% of patients receiving carboplatin, identical to our results. However, although Winter et al. [11] did not report the time needed to restart chemotherapy, this was significantly higher among our patients treated with carboplatin (median of 8 days). The delay between carboplatin cycles due to toxicity may have contributed to the increased resistance rates observed among these patients [35].

We agree with Winter et al. [11], that carboplatin doses may be overestimated by 30% due to possible over-estimation of renal clearance using the Cockcroft-Gault formula based on serum creatinine, and this could be related to the toxic effects observed. Likewise, it may be necessary to consider the use of prophylactic G-CSF in patients requiring carboplatin or to initiate its use early in order to avoid the delay of cycles with this regimen and possible chemoresistance.

Although etoposide attained excellent remission rates in patients with low-risk GTN after MTX/FA resistance, similar to those treated with Act-D, and with fewer cycles of chemotherapy, it has been shown to be more toxic, presenting more alopecia and neutropenia. The use of etoposide is also associated with risk of secondary malignancies, including leukemia, breast and colon cancer and melanoma [36].

Our study does have several limitations. The main limitation of this study is the small number of patients having MTX/FA resistance as first-line therapy in low-risk GTN. Only 21% of patients with low-risk GTN developed resistance to MTX/FA. Subdividing such a small cohort into different groups leads to wide confidence intervals in estimates of response rates. This was particularly true for evaluating etoposide. However, while we considered that differences between this study and the report from Sheffield could fall within the range of statistical variation, nonetheless the absolute difference in response rates between carboplatin and Act-D exceeded our very conservative criteria for assuming equivalent efficacy, suggesting that sample size alone is unlikely to be the source of variation. Data was also collected from different hospital databases and may not reflect the Brazilian general population. As referral centers, these data may overestimate the true prevalence of MTX/FA resistance, attracting patients with a worse prognosis to respond to treatment with single-agent chemotherapy. Due to the retrospective nature of this study, it is important to highlight that adverse events for the different regimens were identified through medical record review, rather than in real time, which could introduce ascertainment bias or be incomplete. Although well-established criteria were followed when discontinuing a chemotherapeutic regimen due to toxicity, clinician practice patterns and patients also can influence this decision and therefore may also explain the differences between Winter et al. [11] and our outcomes. Despite these limitations, as far as we know, this is the largest series of patients treated with carboplatin as a second-line in patients with low-risk GTN after MTX/FA resistance. In assessing the response rate of different second-line regimens in populations with a comparable profile, this study presents an important contribution to the literature on the potential role of carboplatin in the treatment of Brazilian women after failure of first-line MTX/FA for treatment of low-risk GTN.

Our results reinforce the preference for Act-D as a second-line agent in patients with low-risk GTN after MTX/FA resistance [1,25], not only because Act-D achieved an excellent clinical response rate, but also demonstrated good tolerability with few adverse effects. Although it is critical to evaluate therapeutic alternatives in a global market that has frequently suffered from shortages of oncology drugs, we must be aware that the alternatives proposed can be not only more expensive, but also more toxic and not necessarily as effective [20,21]. This work also highlights that regimens that are effective in one population may not have similar results when studied in a different country with differential racial/ethnic make-up [31,32]. Although it is not the objective of this study, we should highlight that the shortage of Act-D compromises not only the treatment of women with failure of first-line MTX/FA treatment for low-risk GTN, but will directly affect the treatment of patients who do not respond to the second-line treatment and need multiagent regimen, such as EMA/CO [1,19].

In conclusion, carboplatin as a chemotherapeutic drug used to treat Brazilian women with low-risk GTN after MTX/FA resistance during the shortage of Act-D, did not have a satisfactory clinical response rate, likely due to severe hematological adverse effects, which postponed chemotherapy cycles. Patients treated with Act-D had an excellent clinical response compared to those treated with carboplatin, without the side effects observed with carboplatin and etoposide. In the absence of more robust evidence to define the best treatment for women with low-risk GTN after MTX/FA resistance, it is reasonable for clinicians to rely on Act-D. It is imperative for both governmental and professional medical organizations to use their full influence to reduce the occurrence of global shortages of life-saving chemotherapy agents.

Author contribution

AB, KME, NSH and RSB conceived the study. AB, GCV, APVSE, KME, NSH and RSB designed the study. APVSE was responsible for the ethical requirements during the design and execution of the study. PARM, AB, JRF, SYS and EHU treated all patients studied. PARM collected data, audited by AB and JRF (data from Rio de Janeiro Federal University), SYS (data from Universidade Federal de São Paulo) and EHU (data from Irmandade da Santa Casa da Misericórdia Hospital). GCV and KME were responsible for the sample size calculation and statistical analysis. All authors contributed to data analysis, interpretation and wrote the paper, approving the final version.

Conflict of interests

The authors declare no conflict of interests.

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