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Comparison of treatment for low-risk GTN with standard 8-day MTX/FA regimen versus modified MTX/FA regimen without chemotherapy on the weekend

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HIGHLIGHTS

- Low-risk GTN didn't appear to have compromised oncologic outcomes when treated with modified 8-day MTX/FA.
- Modified 8-day MTX/FA didn't appear to increase chemoresistance, number of chemotherapy cycles to achieve remission or toxicity.
- When treatment on weekends isn't an option, the modified 8-day MTX/FA appears to be an acceptable alternative.

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ABSTRACT

Objective. To compare the outcomes of patients with low-risk gestational trophoblastic neoplasia (GTN) treated with standard 8-day methotrexate/folinic acid (MTX/FA) versus modified regimen.

Methods. Retrospective cohort study of patients with low-risk GTN followed at Rio de Janeiro Federal University, from January/1990-December/2017 with standard 8-day MTX/FA or modified regimen (MTX administered on the 8th day rather than 7th) to avoid treatment on the weekend.

Results. From 937 patients with low-risk GTN, 538 were treated with standard MTX/FA and 98 patients received modified regimen. Both groups were comparable in age ($p = .749$), antecedent pregnancy ($p = .221$), time to initiate chemotherapy ($p = .926$), hCG pretreatment level ($p = .112$) and WHO/FIGO prognostic risk score ($p = .723$). Patients treated with modified MTX/FA had twice of cases of metastatic lung disease compared with the standard regimen (22.5% vs 10.6%; $p = .002$). The rate of remission ($p = .999$), number of cycles to remission in the first-line ($p = .966$), chemoresistance ($p = .500$), time to switch to second-line therapy ($p = .176$), need for multiagent chemotherapy ($p = .084$), relapse ($p = .122$) or death ($p = .475$) was the same for both MTX/FA regimen. However, although patients receiving modified MTX/FA required a higher total number of remission cycles (6 vs 5 cycles; $p = .004$) and longer time to remission (19 vs 16 weeks; $p < .001$) when compared with the standard regimen, these variables showed no significant differences after multivariate logistic regression adjusted for lung metastasis.

Conclusion. The modified 8-day MTX/FA regimen didn't compromise oncologic outcomes for women with low-risk GTN. This regimen appears to be an acceptable alternative to standard 8-day MTX/FA when treatment on weekend isn't an option.

1. Introduction

Gestational trophoblastic neoplasia (GTN) encompasses various neoplastic trophoblastic lesions, comprising invasive mole,

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choriocarcinoma (CCA), placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT). Since Li et al. [1] reported the sensitivity of gestational trophoblastic neoplasia (GTN) to methotrexate (MTX), most of these tumors became curable without the need for surgery [2].

MTX irreversibly binds to the enzyme dihydrofolate reductase, preventing the formation of tetrahydrofolate, active form of folic acid, that acts as a cofactor for thymidylate synthetase, which is fundamental in cell replication, notably the S phase of the cell cycle. MTX causes rapid disruption of DNA synthesis, promoting cell death, resulting in the reduction of the sensitive serum tumor marker, human chorionic gonadotrophin (hCG), as well as tumor disappearance and consequent clinical improvement.

More than a half century after the seminal publication of the exceptional antineoplastic results of MTX, there is no established best regimen of this drug for GTN treatment, and the numerous schemes proposed suggest that this issue is still under discussion and unresolved [3].

Monitoring the balance between the toxicity and the therapeutic effects of MTX, Bagshawe established postulates that guide the design of MTX regimens for GTN treatment: low doses MTX are effective; folinic acid may increase the tolerability of the treatment and intervals between cycles are desirable to minimize toxicity but should be as short as possible to avoid drug resistance [4,5]. Considering these principles, the 8-day regimen that alternates intramuscular MTX (50 mg fixed dose or 1 mg/kg on days 1, 3, 5 and 7) with oral folinic acid (FA) (15 mg fixed dose or 0.1 mg/kg on days 2, 4, 6 and 8) has been implemented around the world [6–8]. A recent comparison of fixed as opposed to adjusted dose MTX/FA has suggested no significant differences in remission rate and outcomes in an Italian population [9]. In Brazil we have favored the adjusted dose mostly as the first choice for low-risk GTN patients in all GTN Reference Centers (GTN-RC) [10].

However, since the 8-day MTX/FA regimen necessarily includes a day of MTX treatment on the weekend, we have observed that some Brazilian clinicians have utilized a modified MTX/FA regimen, which eliminated MTX administration during the weekend, postponing it to Monday. This is basically due to two distinct reasons, depending on where the patient receives chemotherapy. When patients are treated fully in the GTN-RC, this situation occurs due to scheduling problems in the clinical oncology unit, determined by vacations, holidays or by generally limited weekend staff. This scenario occurs more commonly when patients with GTN receive chemotherapy in private clinical oncology practices, which do not ordinarily offer treatment on the weekends.

The convenience in administering 5-day MTX (0.4 mg/kg/day, at a maximum of 25 mg/day, without FA rescue), applied from Monday to Friday, has motivated GTN-RC to adopt this treatment for low-risk GTN [11–14]. While 5-day MTX seems to have similar clinical response as the 8-day MTX/FA regimen, some studies have reported more toxicity with 5-day MTX regimens without FA rescue as compared to 8-day regimens [10,15,16].

The best chemotherapy regimen for patients with low-risk GTN is unknown [17], but 8-day MTX/FA is a common choice globally [6,7,17]. However, as far as we know, there is no study evaluating the efficacy of the modified 8-day MTX/FA regimen in which MTX is administered on day 8 rather than on day 7 thus avoiding treatment during the weekend. It is unknown whether this change in the timing of chemotherapy administration would lead to an increased number of chemotherapy cycles to achieve remission or even a higher development of chemoresistance.

This study describes the results of treatment of Brazilian patients with low-risk GTN with modified 8-day MTX/FA compared to the standard regimen. This study may be important for settings where oncology services have difficulty administering chemotherapy over the weekend, and despite that, clinicians maintain the decision to perform the 8-day MTX/FA treatment.

2. Material and methods

2.1. Study design

This is a retrospective cohort study of patients with low-risk GTN followed at the Rio de Janeiro Trophoblastic Disease Center – Maternity School of Rio de Janeiro Federal University (Rio de Janeiro – RJ, Brazil, data entered by CSHA and audited by AB), from January 1990 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 16365019.1.0000.5275 and 16365019.1.0000.5275).

2.2. Study participants

All patients with low-risk GTN treated with modified or standard 8-day MTX/FA were included. All cases of molar pregnancy that developed GTN or choriocarcinoma had their diagnosis confirmed by the Pathology Department of the Reference Center. All patients were followed for at least 12 months after remission with rigorous contraception program [18]. Patients diagnosed with high-risk GTN, PSTT and ETT, and those initially treated outside the GTN-RC or received Actinomycin-D (Act-D) as first line treatment for low-risk GTN were excluded. Additionally, patients who were pregnant <12 months after the end of chemotherapy, who were lost to follow-up with <12 months after remission or those patients who were treated with 8-day MTX 50 mg fixed dose regimen were also excluded [7].

2.3. 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 [19].

2.4. 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 [19]. Lung metastases were detected using a chest X-ray [7,19]. Magnetic resonance imaging of the brain and abdomen and chest CT scan were used for patients with visible or suspected pulmonary metastasis on chest X-ray or genital metastasis. [7,19]. Lung metastasis images were reviewed centralized in the Reference Center and counted from metastatic nodules larger than 1 cm [7].

During the entire cohort study, we used the Siemens Diagnostic Products Corporation (DPC) Immulite® assay, with the reference value for normal serum hCG results below 5 IU/L.

The standard 8-day MTX-FA regimen consisting of MTX at 1 mg/kg intramuscularly on days 1, 3, 5 and 7 alternating with FA at 0.1 mg/kg or 15 mg 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 [7,19]. Before 2013, FA was regularly administered orally at 0.1 mg/kg and patients were often instructed to break tablets into pieces to use the correct dose as closely as possible. However, after 2013, we administered FA orally at a 15 mg fixed dose, regardless of patient weight, for more convenience, following the European Society of Medical Oncology GTD Guideline [7]. In exceptional cases, where it was not possible to schedule standard 8-day MTX/FA treatment during the weekend, or in cases where patients were treated in private clinical oncology clinics, that are unable to administer chemotherapy on the weekends, MTX was not administered in the 7th

day, but postponed to the 8th day, avoiding treatment during the weekend. The patients were informed about the nature of the modified treatment and its alternatives and formalized the informed consent for the chosen treatment. To minimize the potential effect of this delay, treatment with MTX was done at the end of the day, generally at 4:00 p.m. on days 1, 3, 5, and then at 8:00 am on day 8, thus resulting in an approximate 16 h delay in administration of the last dose of MTX. Similarly, FA on day 9 was given 24 h after administration of the last MTX of the cycle.

In cases of MTX/FA resistance, second-line chemotherapy was administered with single agent (Act-D 1.25 mg/m^2, maximum 2.0 mg, IV pulse every 2 weeks or Carboplatin AUC = 6 every 21 days with maximum dose of 900 mg or Etoposide, dose of 100 mg/m^2, day 1–5, every 14 days) [20] or multiagent regimen (etoposide, MTX/FA, Act-D, cyclophosphamide, and oncovin (vincristine) – EMA/CO regimen, with or without Act-D, during shortages of Act-D) [7,10,20].

After hCG normalization, patients received 3 consolidation cycles of chemotherapy, and were monitored monthly with hCG serum levels for 12 months, when they were discharged from follow-up [7,10].

2.5. Outcomes

The primary outcome was the occurrence of remission following 8-day MTX/FA. Secondary outcomes were toxicity in the standard and modified 8-day MTX/FA regimen, number of cycles required to attain GTN remission, time to remission and occurrence of relapse and death.

2.6. Variables

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

Regarding the clinical aspects of gestational trophoblastic neoplasia, the following variables were studied: the antecedent pregnancy (molar pregnancy, term/preterm pregnancy, abortion or ectopic pregnancy), time between the end of antecedent pregnancy and the beginning of chemotherapy with 8-day MTX/FA, hCG pre-treatment level (IU/L), the GTN stage and FIGO/WHO prognostic score [19].

The following pathological variables were evaluated: the histopathological diagnosis of gestational trophoblastic disease (complete or partial hydatidiform mole, invasive mole or CCA).

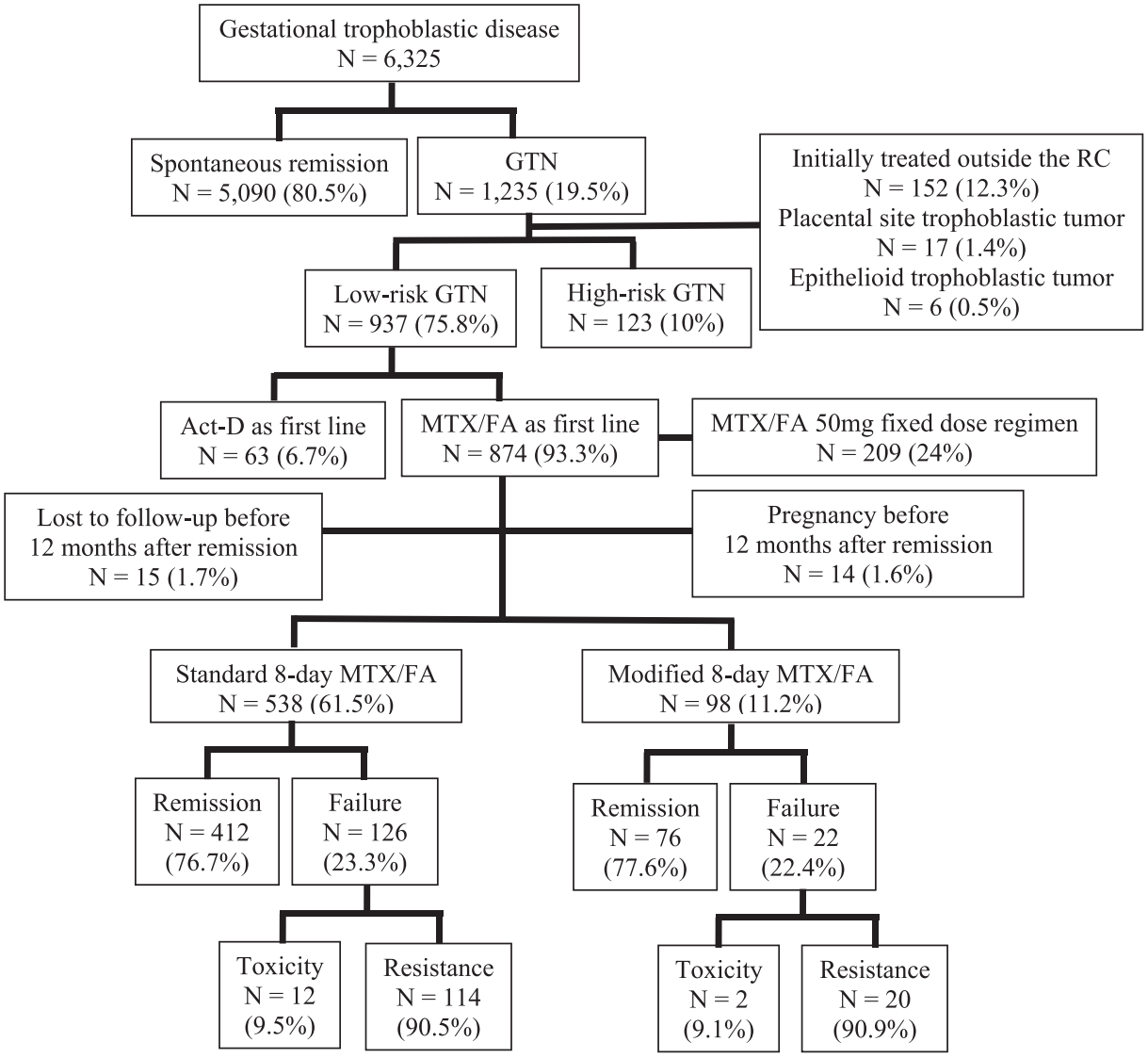

Fig. 1. Flow diagram summarizing the derivation of the study population. GTN – gestational trophoblastic neoplasia. RC – reference center. MTX/FA – methotrexate / folinic acid. Act-D – Actinomycin-D.

Considering the GTN therapeutic variables, we evaluated the occurrence of remission, the number of cycles of MTX/FA needed to attain remission or done before resistance, the type and intensity of toxicity of first-line chemotherapy, excluding episodes after remission during consolidation chemotherapy, according to Common Terminology Criteria for Adverse Events, Version 5.0, 2017 (CTCAE, 2017) [21], the time required to change to the second-line treatment (in weeks), reason to switch to second-line therapy, the level of hCG at the time of MTX/FA resistance (IU/L), occurrence of remission with the second-line regimen, number of cycles of second-line chemotherapy (without consolidation cycles), total number of cycles to remission, time to remission (weeks), occurrence of relapse or death.

Remission was defined as normalization of hCG levels - lower than 5 IU/L – which was maintained for at least 4 weeks [6]. Resistance was characterized by hCG plateau of ±10% after 2 cycles of chemotherapy or its re-elevation. Toxicity as a reason to switch to a second-line regimen was attained 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 first-line regimen. Relapse was diagnosed by the re-elevation of hCG levels after remission, in the absence of a new pregnancy.

2.7. Statistical analysis

To analyze the association between the first-line chemotherapy treatments (8-day standard MTX/FA versus modified regimen) and each of the categorical variables, Chi-square test was used.

To compare continuous variables among the first-line chemotherapy treatment regimens studied, the Kruskal-Wallis test was used.

Differences were considered statistically significant when p-values were <0.05.

For outcomes of interest, adjusted odds ratios with 95% confidence intervals (95% CI) were calculated using the Wald test for logistic regression. Variables were selected for inclusion into the multivariate model by the Akaike Information Criteria. To correct for multiple hypothesis testing, $p < .01$ was used as the threshold for significance in analyzing. To compare the time until remission, the log-rank test was used.

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 1990 to December 2017, 6325 patients were diagnosed with gestational trophoblastic disease (GTD). Among these, 5090 (80.5%) achieved spontaneous remission and 1235 (19.5%) developed GTN, among which 937 (75.8%) were categorized as low-risk GTN and 538 (61.5%) were treated with standard 8-day MTX/FA and 98 (11.2%) received modified 8-day MTX/FA.

The presenting characteristics of patients with low-risk GTN treated with standard 8-day MTX/FA were comparable to those who received modified 8-day MTX/FA regimen, including age ($p = .749$), gravidity ($p = .217$), parity ($p = .154$), antecedent pregnancy ($p = .221$), time between the end of pregnancy and the initiation of chemotherapy ($p = .926$) and hCG pretreatment ($p = .112$) (Table 1). In general these patients were young, childless, had postmolar GTN, notably after complete hydatidiform mole, whose GTN diagnosis occurred about 4 months after termination of pregnancy, and with most hCG pretreatment levels ranging from 1000 to 100,000 IU/L.

Table 2 showed that there was no increase in the prevalence of CCA ($p = .533$) and no difference in WHO/FIGO prognostic risk score ($p = .723$) among patients with low-risk GTN treated with the two chemotherapy regimens studied. However patients who were treated with modified 8- day MTX/FA had twice the number of cases of metastatic disease (22.5%) than those who received the standard 8-day MTX/FA regimen (10.6%; $p = .002$).

No difference was observed in the occurrence of remission among patients with low-risk GTN treated with standard 8-day MTX/FA versus modified 8-day MTX/AF regimen (76.7% × 77.6%; $p = .999$). Table 3 showed that treatment with modified 8-day MTX/FA did not increase the number of cycles needed to achieve remission in the first-line (5.0 versus 5.0 cycles; $p = .966$) or postpone the switch to second-line therapy (10 versus 10 weeks; $p = .176$). Likewise, no increased chemoresistance was observed among patients initially treated with modified 8-day MTX/FA when compared to standard 8-day MTX/AF (90.9% x 90.5%, $p = .500$). The two 8-day MTX/FA regimens did not differ significantly in predisposing patients to an increased need for multiagent chemotherapy ($p = .084$), occurrence of relapse ($p = .122$) or death ($p = .475$). However, in patients needing second-line chemotherapy and receiving modified 8-day MTX/FA, they required a higher total number of remission cycles (6 versus 5 cycles; $p = .004$), as well as longer time to achieve remission (19 versus 16 weeks; $p < 0.001$) compared to patients treated with standard 8-day MTX/FA.

Still, when these variables were analyzed excluding the effect of lung metastasis occurrence through multivariate logistic regression, it can be observed that the odds ratio for patients treated with modified 8-day MTX/FA receiving >5 cycles of chemotherapy to achieve remission in the treatment of second-line chemotherapy was 0.81 (CI 95%: 0.38–1.68) and according to the comparison of the curves for time to remission, the protocols presented no difference ($p = .55$) as shown in Fig. 2.

In assessing the adverse events due to MTX/FA treatment for low-risk GTN, no differences were observed when comparing standard treatment with the modified 8-day MTX/FA regimen according to Common Terminology Criteria for Adverse Events [20], as shown in Table 4. Regardless of the MTX/FA regimen administered, the most prevalent adverse events were fatigue (44.7 versus 39.7%; $p = .790$), dry eye (37.9 versus 36.7%; $p = .543$), mucositis oral (35.1 versus 33.6%; $p = .500$) and nausea (21.9 versus 19.4%; $p = .500$), not differing between the standard or modified regimens studied, respectively. It is also noteworthy that toxicity as a cause of MTX/FA regimen replacement was

Table 1
Characteristics of patients with low-risk gestational trophoblastic neoplasia treated according to different methotrexate with folinic acid rescue (MTX/FA) regimens.

Variables	Standard 8-day MTX/FA N = 538	Modified 8-day MTX/FA N = 98	p-Value
Age (years) #	26.0 (20.0–32.0)	25.0 (21.0–32.0)	0.749 K
Gravidity #	2.0 (1.0–2.0)	2.0 (1.0–2.75)	0.217 K
Parity #	0.0 (0.0–1.0)	1.0 (0.0–1.0)	0.154 K
Antecedent pregnancy			0.221 C
Hydatidiform mole	518 (96.3%)	90 (92.8%)	
Complete	*357 (69%)*	*64 (71%)*	
Partial	*161 (31%)*	*26 (29%)*	
Term/Preterm gestation	1 (0.2%)	0 (0%)	
Abortion	19 (3.5%)	7 (7.2%)	
Ectopic pregnancy	0 (0%)	0 (0%)	
Time between the end of pregnancy and the initiation of chemotherapy (months) #	4 (3–5)	4 (3–5)	0.926 K
< 4	479 (89.0%)	87 (88.8%)	
4–6	55 (10.2%)	10 (10.2%)	
7–12	3 (0.6%)	1 (1.0%)	
> 12	1 (0.2%)	0 (0%)	
hCG * (IU/L) pretreatment levels #	12,000 (900–50,000)	14,000 (4000–45,000)	0.112 K
$< 10^3$	70 (13.0%)	12 (12.2%)	
10^3–10^4	190 (35.3%)	47 (47.9%)	
10^4–10^5	232 (43.1%)	32 (32.6%)	
$> 10^5$	46 (8.6%)	7 (7.3%)	

Median and interquartile range. * hCG – human chorionic gonadotropin (IU/L – International units per liter).
K – Kruskal-Wallis test. C – Chi-squared test.

Table 2
Oncological profile of patients with low-risk gestational trophoblastic neoplasia treated according to different methotrexate with folinic acid rescue (MTX/FA) regimens.

Variables	Standard 8-day MTX/FA N = 538	Modified 8-day MTX/FA N = 98	p-Value
Stage, n (%)			0.002[C]
I	454 (84.4%)	74 (75.5%)	
II	27 (5.0%)	2 (2.0%)	
III	57 (10.6%)	22 (22.5%)	
Histology of GTN			0.533[C]
None	524 (97.4%)	96 (97.9%)	
Invasive mole	2 (0.4%)	1 (1.0%)	
Choriocarcinoma	12 (2.2%)	1 (1.1%)	
Occurrence of metastasis [¥]	84 (15.6%)	24 (24.5%)	0.002[C]
WHO/FIGO[*] Prognostic Risk Score[#]	2.0 (2.0–2.0)	2.0 (2.0–2.0)	0.723[C]
0	5 (0.9%)	0 (0%)	
1	96 (17.8%)	14 (14.3%)	
2	308 (57.2%)	60 (61.2%)	
3	61 (11.3%)	14 (14.3%)	
4	32 (5.9%)	3 (3.1%)	
5	31 (5.8%)	6 (6.2%)	
6	5 (1.1%)	1 (0.9%)	

[#]Median and interquartile range.
[¥]Metastatic GTN involving the pelvis (vagina/uterine cervix) and lung.
[*]WHO/FIGO – World Health Organization/International Federation of Gynecology and Obstetrics.
K – Kruskal-Wallis test. C – Chi-squared test.

infrequent, comparable between standard or modified regimen (9.5 versus 9.1%; p = .500) and no differences in severity of these events were observed considering the MTX/FA regimen studied.

4. Discussion

Low-risk GTN is widely cured with single-agent chemotherapy, and MTX/FA regimens were preferred as first-line treatment in the present series. No differences were observed in the primary remission rate, the number of cycles required for remission in first-line treatment or even the frequency or severity of toxicities when comparing patients treated with standard 8-day MTX/FA and those receiving modified 8-day MTX/FA regimen. Likewise, regardless of the MTX/FA regimen administered, the development of chemoresistance as an indication for second-line chemotherapy, the need for multi-agent chemotherapy in second-line treatment, or the occurrence of relapse or death did not vary.

Single-agent MTX therapy for low-risk GTN has been associated with a 65–90% remission rate, which is comparable to our 76–77% remission rate with 8-day MTX/FA, either with the standard or the modified regimen [22–25]. Although we do not recommend the modified 8-day MTX/FA regimen, the efficacy and safety established with our data suggest that this alternative scheme maybe reasonable if eliminating MTX administration during the weekend is necessary.

MTX is one of the most potent anticancer agents. Following its intramuscular administration, the peak serum concentration occurs within 30 to 60 min, with 80% bioavailability of the drug. After absorption, MTX binds to plasma proteins, notably albumin. MTX metabolization occurs intracellularly, giving rise to its active metabolites, including MTX-polyglutamate [26]. This byproduct of MTX is trapped intracellularly, has a large molecular chain that limits efflux from the cell, and exerts its metabolic action of inhibiting multiple enzymes, including dihydrofolate reductase, in a stable manner for up to 48 h after MTX administration, even after renal clearance of serum MTX [27–29]. It is precisely these chemical properties and pharmacodynamics of MTX polyglutamate that may explain comparable results in primary remission rate in low-risk GTN patients treated with different 8-day MTX/FA regimens.

Nevertheless, patients treated with modified 8-day MTX/FA required an increased total number of chemotherapy cycles to achieve remission, leading to longer time to remission than those treated with standard 8-day MTX/FA. It should be noted, however, that patients treated with modified 8-day MTX/FA had twice number of cases of metastatic lung disease as those receiving standard 8-day MTX/FA. This rather than the delay in the last day of MTX treatment may explain the need for an increased total number of chemotherapy cycles to achieve remission in this group. Since adjusting the effect of the occurrence of lung metastases through multivariate logistic regression, it was observed that there was no increased need to use >5 cycles of chemotherapy or more time to achieve remission in patients treated with second-line chemotherapy for low-risk GTN, regardless of the 8-day MTX/FA regimen previous used.

Data from patients treated with MTX at the New England Trophoblastic Disease Center showed that the presence of metastasis was also a strong independent predictor of requiring additional cycles of chemotherapy (OR = 13) [24]. However, although the occurrence of pulmonary metastases may worsen the prognosis of patients with low-risk GTN [30], this does not appear to modify the occurrence of

Table 3
Outcomes of patients with low-risk gestational trophoblastic neoplasia treated according to different methotrexate with folinic acid rescue (MTX/FA) regimens.

Variables	Standard 8-day MTX/FA N = 538	Modified 8-day MTX/FA N = 98	p-Value
Remission in first-line, n (%)	412 (76.7%) 95% CI: 73.0–80.1%	76 (77.6%) 95% CI: 69.3–85.9%	0.999[C]
Number of cycles needed to remission in first-line [W #]	5.0 (4.0–5.0)	5.0 (4.0–5.0)	0.966 [K]
Number of cycles done before resistance in first-line [#]	5.0 (4.0–5.0)	5.0 (4.75–5.0)	0.417 [K]
Time to switch to second-line therapy (weeks) [#]	10.0 (8.0–10.0)	10.0 (10.0–12.0)	0.176 [K]
hCG (IU/L)[**] at switch to second-line therapy	9812 (1120–30,001)	22,500 (13,814–36,875)	0.535 [K]
Reason to switch to second-line therapy			0.500[C]
Chemoresistance, n (%)	114 (90.5%)	20 (90.9%)	
Toxicity, n (%)	12 (9.5%)	2 (9.1%)	
Remission in second line, n (%)	91 (72.2%)	12 (54.5%)	0.055[C]
Second line treatment, n (%)			0.084[C]
Single agent	98 (77.8%)	14 (63.6%)	
Multiagent regimen	28 (22.2%)	8 (36.4%)	
Number of cycles to remission in patients who needed second-line therapy [#]	5.0 (4.0–7.0)	6.0 (5.0–7.0)	0.004 [K]
Time to remission (weeks) [#]	16.0 (12.0–20.0)	19.0 (18.0–24.0)	<0.001 [K]
Relapse, n (%)	9 (1.7%)	4 (4.1%)	0.122[C]
Death, n (%)	2 (0.4%)	1 (1.0%)	0.475[C]

[W]Without consolidation.
[#]Median and interquartile range.
[**]hCG – human chorionic gonadotropin (IU/L – International units per liter).
K – Kruskal-Wallis test. C – Chi-squared test.

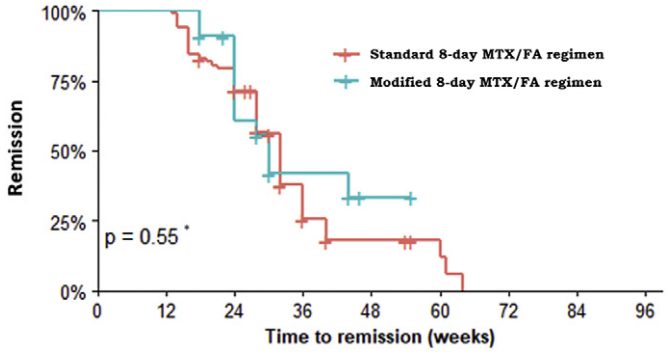

Fig. 2. Time to remission in the second-line treatment for low-risk GTN after resistance with standard or modified 8-day MTX/FA, adjusted for the occurrence of metastasis.

MTX chemoresistance [31], as shown in this paper. Frijstein et al. concluded that the presence of lung metastases among patients initially treated with MTX increases the risk of chemoresistance although it does not decrease the overall complete response. However, the authors highlighted that cases of low-risk choriocarcinoma patients with lung metastases should be initially treated with a multiagent regime due to the higher chance of chemoresistance to single-agent chemotherapy [32]. Unfortunately, the cases of choriocarcinoma in the population studied were too small to assess its impact on MTX treatment response.

Our study does have several limitations. The main limitation of this study was a retrospective non-randomized comparison of these two regimens and the small number of patients with low-risk GTN evaluated. However, considering that the remission with first-line treatment was the main response variable in this study, the results were used to estimate the probability of type II error as 3.91%, giving this result 96%

Table 4
Adverse events due to different methotrexate with folinic acid rescue (MTX/FA) regimens for treatment of low-risk gestational trophoblastic neoplasia, graded according to Common Terminology Criteria for Adverse Events, version 5.0 (2017).

Variable	Standard 8-day MTX/FA (N = 538)					Modified 8-day MTX/FA (N = 98)					p-Value for Adverse event (Chi-square test)	p-Value for CTC grade 3 + 4 (Chi-square test)
Disorders by System	Adverse event N (%)	CTC grade (N/%)				Adverse event N (%)	CTC grade (N/%)					
		1	2	3	4		1	2	3	4		
Blood												
Anemia	98 (18.2)	55 (10.2)	25 (4.6)	15 (2.8)	3 (0.6)	17 (17.3)	9 (9.2)	5 (5.1)	2 (2)	1 (1)	0.525	0.500
Febrile neutropenia	6 (1.1)	–	–	6 (1.1)	–	1 (1)	–	–	1 (1)	–	0.500	*
Cardiac												
Chest pain	6 (1.1)	–	4 (0.7)	2 (0.4)	–	1 (1)	–	1 (1)	–	–	0.500	*
Eye												
Dry eye	204 (37.9)	192 (35.7)	12 (2.2)	–	–	36 (36.7)	34 (34.7)	2 (2)	–	–	0.543	*
Gastrointestinal												
Mucositis oral	189 (35.1)	151 (28.1)	33 (6.1)	5 (0.9)	–	33 (33.6)	26 (26.5)	6 (6.1)	1 (1)	–	0.565	0.500
Nausea	118 (21.9)	90 (16.7)	22 (4.1)	6 (1.1)	–	19 (19.4)	14 (14.3)	4 (4.1)	1 (1)	–	0.666	0.500
Stomach pain	27 (5)	20 (3.7)	7 (1.3)	–	–	4 (4.1)	3 (3.1)	1 (1)	–	–	0.556	*
Vomiting	22 (4)	18 (3.3)	4 (0.7)	–	–	4 (4.1)	3 (3.1)	1 (1)	–	–	0.500	*
General												
Fatigue	241 (44.7)	201 (37.3)	40 (7.4)	–	–	39 (39.7)	33 (33.6)	6 (6.1)	–	–	0.790	*
Infections												
Upper respiratory	20 (3.7)	–	15 (2.8)	5 (0.9)	–	3 (3.1)	–	2 (2)	1 (1)	–	0.510	0.500
Urinary tract	5 (0.9)	–	5 (0.9)	–	–	1 (1)	–	1 (1)	–	–	0.500	*
Vaginal	15 (2.7)	13 (2.3)	2 (0.4)	–	–	2 (2)	2 (2)	–	–	–	0.532	*
Investigations												
Aspartate aminotransferase ↑	38 (7)	31 (5.7)	6 (1.1)	1 (0.2)	–	7 (7.1)	5 (5.1)	2 (2)	–	–	0.500	*
Lymphocyte count ↓	80 (14.8)	52 (9.6)	22 (4.1)	6 (1.1)	–	13 (13.2)	8 (8.1)	4 (4.1)	1 (1)	–	0.602	0.500
Neutrophil count ↓	35 (6.5)	20 (3.7)	9 (1.7)	6 (1.1)		6 (6.1)	4 (4.1)	1 (1)	1 (1)	–	0.500	0.500
Platelet count ↓	35 (6.5)	20 (3.7)	9 (1.7)	6 (1.1)		6 (6.1)	4 (4.1)	1 (1)	1 (1)		0.500	0.500
Reproductive												
Irregular menstruation	32 (5.9)	26 (4.8)	6 (1.1)	–	–	6 (6.1)	5 (5.1)	1 (1)	–	–	0.500	*
Menorrhagia	22 (4)	18 (3.2)	2 (0.4)	2 (0.4)		4 (4.1)	3 (3.1)	1 (1)	–	–	0.500	*
Respiratory												
Pleuritic pain	34 (6.3)	15 (2.8)	14 (2.6)	5 (0.9)	–	6 (6.1)	3 (3.1)	2 (2)	1 (1)	–	0.500	0.500
Skin												
Photosensitivity	14 (2.6)	7 (1.3)	4 (0.7)	3 (0.6)	–	3 (3.1)	3 (3.1)	–	–	–	0.500	*

* Numbers too small to compare.

power. Additionally, data was collected from a GTN-RC and may not reflect the outcomes that could be seen the Brazilian general population or patients with GTN with different nationalities or ethnicities. Although logistic regression excluding the effect of pulmonary metastasis occurring showed that the number of chemotherapy cycles to achieve second-line remission did not differ between patients treated with the different MTX regimens studied, other biases may also have influenced this scenario, such as different second-line therapies that were used between the two groups, the hCG level at the time of switching to second-line treatment or even the low numbers of cases that received second-line therapy included in this study. Due to the retrospective nature of this study, it is important to highlight that adverse events for the different 8-day MTX/FA were identified through medical record review, rather than in real time, which could introduce ascertainment bias or be incomplete.

Although there is no consensus regarding the best first-line GTN treatment [33], MTX appears to be an effective option with limited toxicity. In cases where physicians decide to administer 8-day MTX/FA, our recommendation is to adhere to the standard D 1, 3, 5, 7 administration. When treatment cannot be performed on a weekend, our data suggest the modified 8-day MTX/FA regimen is a reasonable alternative. In our practice, when we employ the modified regimen this is our standard operating procedures. First, patients sign a consent form after being informed that they are receiving a non-standard chemotherapy regimen and advised on alternatives for their treatment. Second, treatment always starts on a Monday. This allows maximum cell exposure during 3 doses of MTX (Monday, Wednesday and Friday) and minimized the delay for the 4th dose of MTX. If MTX were started on Tuesday, in addition to offering only 2 doses before the weekend (Tuesday and Thursday), the patient would not receive MTX for 3 days (delaying in Friday, Saturday and Sunday), one day longer if MTX is started on Monday. Third, in order to minimize MTX delay after the weekend, the 3 weekday MTX treatment delivery are scheduled at the end of the day as the latest possible infusion time while the MTX after the weekend is schedule as the very first appointment on Monday.

In conclusion, we have shown that modified 8-day MTX/FA had a primary remission rate similar to the standard regimen. Eliminating MTX/FA administration during the weekend, postponing it to early Monday, did not increase, in this study, the occurrence of chemoresistance, the need for more cycles of chemotherapy to achieve remission in the first-line of treatment, or the occurrence of relapse or death. The toxicity of both 8-day MTX/FA regimens was similar, not differing in the frequency of adverse effects or in its severity. In the absence of more robust evidence to define the best first-line treatment for women with low-risk GTN, it is reasonable for clinicians to adopt modified 8-day MTX/FA in settings where oncology services have difficulty working over the weekend, and alternatives to MTX are more toxic or less effective.

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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. AB, CSHA, PARM, EP, AM, JAJ and JRF treated all patients studied. CSHA collected data, audited by AB, JAJ and JRF. GCV was responsible for statistical analysis. All authors contributed to data analysis, interpretation and wrote the paper, approving the final version.

Declaration of competing interest

The authors declare no conflict of interests.

References

[1] M.C. Li, R. Hertz, D.B. Spencer, Effect of methotrexate therapy upon choriocarcinoma and chorioadenoma, Proc. Soc. Exp. Biol. Med. 93 (2) (1956) 361–366.
[2] A.T. Hertig, Human Trophoblast, Charles C Thomas, Springfield, IL, 1968.
[3] J.R. Lurain, Gestational trophoblastic disease II: classification and management of gestational trophoblastic neoplasia, Am. J. Obstet. Gynecol. 204 (1) (2011) 11–18.
[4] K.D. Bagshawe, J. Dent, E.S. Newlands, R.H. Begent, G.J. Rustin, The role of low-dose methotrexate and folinic acid in gestational trophoblastic tumours (GTT), Br. J. Obstet. Gynaecol. 96 (7) (1989) 795–802.
[5] K.D. Bagshawe, Choriocarcinoma: The Clinical Biology of the Trophoblast and its Tumours, 1969, Edward Arnold Ltd, London, 1969 259.
[6] D.P. Goldstein, R.S. Berkowitz, N.S. Horowitz, Optimal management of low-risk gestational trophoblastic neoplasia, Expert. Rev. Anticancer. Ther. 15 (11) (2015) 1293–1304.
[7] M.J. Seckl, N.J. Sebire, R.A. Fisher, F. Golfier, L. Massuger, C. Sessa, ESMO guidelines working group. Gestational trophoblastic disease: ESMO clinical practice guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 24 (Suppl. 6) (2013 Oct) vi39–50.
[8] I. Maestá, R. Nitecki, N.S. Horowitz, D.P. Goldstein, M. de Freitas Segalla Moreira, K.M. Elias, et al., Effectiveness and toxicity of first-line methotrexate chemotherapy in low-risk postmolar gestational trophoblastic neoplasia: the New England trophoblastic disease center experience, Gynecol. Oncol. 148 (1) (2018) 161–167.
[9] G. Mangili, R. Cioffi, S. Danese, L. Frigerio, G. Ferrandina, G. Cormio, et al., Does methotrexate (MTX) dosing in a 8-day MTX/FA regimen for the treatment of low-risk gestational trophoblastic neoplasia affect outcomes? The MITO-9 study, Gynecol. Oncol. 151 (3) (2018) 449–452.
[10] A. Braga, P.O. Souza, A.P.V.S. Esteves, L. Padron, E. Uberti, M. Viggiano, et al., Brazilian network for gestational trophoblastic disease study group consensus on management of gestational trophoblastic disease, J Reprod Med 63 (3) (2018) 261–270.
[11] C.B. Hammond, R. Hertz, G.T. Ross, M.B. Lipsett, W.D. Odell, Primary chemotherapy for nonmetastatic gestational trophoblastic neoplasms, Am. J. Obstet. Gynecol. 98 (1) (1967) 71–78.
[12] J.T. Soper, D.L. Clarke-Pearson, A. Berchuck, G. Rodriguez, C.B. Hammond, 5-day methotrexate for women with metastatic gestational trophoblastic disease, Gynecol. Oncol. 54 (1) (1994) 76–79.
[13] J.R. Lurain, E.P. Elfstrand, Single-agent methotrexate chemotherapy for the treatment of nonmetastatic gestational trophoblastic tumors, Am. J. Obstet. Gynecol. 172 (2Pt1) (1995) 574–579.
[14] L.C. Wong, Y.C. Choo, H.K. Ma, Methotrexate with citrovorum factor rescue in gestational trophoblastic disease, Am. J. Obstet. Gynecol. 152 (1) (1985) 59–62.
[15] E.B. Smith, J.C. Weed Jr., L. Tyrey, C.B. Hammond, Treatment of nonmetastatic gestational trophoblastic disease: results of methotrexate alone versus methotrexate-folinic acid, Am. J. Obstet. Gynecol. 144 (1) (1982) 88–92.
[16] R.S. Berkowitz, D.P. Goldstein, M.R. Bernstein, Methotrexate with citrovorum factor rescue as primary therapy for gestational trophoblastic disease, Cancer 50 (10) (1982) 2024–2027.
[17] H.Y.S. Ngan, M.J. Seckl, R.S. Berkowitz, Y. Xiang, F. Golfier, P.K. Sekharan, et al., Update on the diagnosis and management of gestational trophoblastic disease, Int. J. Gynaecol. Obstet. 143 (Suppl. 2) (2018) 79–85.
[18] P.R.S. Dantas, I. Maestá, J.R. Filho, J.A. Junior, K.M. Elias, N. Horowitz, et al., Does hormonal contraception during molar pregnancy follow-up influence the risk and clinical aggressiveness of gestational trophoblastic neoplasia after controlling for risk factors? Gynecol. Oncol. 147 (2) (2017) 364–370.19.
[19] Fédération Internationale de Gynécologie et d'Obstétrique Oncology Committee, FIGO staging for gestational trophoblastic neoplasia 2000, Int. J. Gynaecol. Obstet. 77 (3) (2002) 285–287.
[20] P.A.R. Mora, S.Y. Sun, G.C. Velarde, J.R. Filho, E.H. Uberti, A.P.V. Dos Santos Esteves, et al., Can carboplatin or etoposide replace actinomycin-d for second-line treatment of methotrexate resistant low-risk gestational trophoblastic neoplasia? Gynecol. Oncol. 153 (2) (2019) 277–285.
[21] Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, Published: November 27, 2017 (v5.0: November 27, 2017), U.S. Department of Health and Human Services National Institutes of Health National Cancer Institute. Available in: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50.
[22] I.A. McNeish, S. Strickland, L. Holden, G.J. Rustin, M. Foskett, M.J. Seckl, et al., Low-risk persistent gestational trophoblastic disease: outcome after initial treatment with low-dose methotrexate and folinic acid from 1992 to 2000, J. Clin. Oncol. 20 (7) (2002) 1838–1844.
[23] H. Matsui, K. Suzuka, K. Yamazawa, N. Tanaka, A. Mitsuhashi, K. Seki, et al., Relapse rate of patients with low-risk gestational trophoblastic tumor initially treated with single-agent chemotherapy, Gynecol. Oncol. 96 (3) (2005) 616–620.
[24] W.B. Growdon, A.J. Wolfberg, D.P. Goldstein, C.M. Feltmate, M.E. Chinchilla, E.S. Lieberman, et al., Evaluating methotrexate treatment in patients with low-risk postmolar gestational trophoblastic neoplasia, Gynecol. Oncol. 112 (2) (2009) 353–357.
[25] E.M. Uberti, M.C. Fajardo, A.G. da Cunha, S.S. Frota, A. Braga, A.C. Ayub, Treatment of low-risk gestational trophoblastic neoplasia comparing biweekly eight-day methotrexate with folinic acid versus bolus-dose Actinomycin-D, among Brazilian women, Rev Bras Ginecol Obstet 37 (6) (2015) 258–265.

[26] K. Barnhart, C. Coutifaris, M. Esposito, The pharmacology of methotrexate, Expert. Opin. Pharmacother. 2 (3) (2001) 409–417.
[27] B.A. Chabner, C.J. Allegra, G.A. Curt, N.J. Clendeninn, J. Baram, S. Koizumi, et al., Polyglutamation of methotrexate. Is methotrexate a prodrug? J. Clin. Invest. 76 (3) (1985) 907–912Sep.
[28] A. Yamauchi, T. Ichimiya, K. Inoue, Y. Taguchi, N. Matsunaga, S. Koyanagi, et al., Cell-cycle-dependent pharmacology of methotrexate in HL-60, J. Pharmacol. Sci. 99 (4) (2005) 335–341.
[29] M.I. Danila, L.B. Hughes, E.E. Brown, S.L. Morgan, J.E. Baggott, D.K. Arnett, et al., Measurement of erythrocyte methotrexate polyglutamate levels: ready for clinical use in rheumatoid arthritis? Curr. Rheumatol. Rep. 12 (5) (2010) 342–347.
[30] J. Kumar, A. Ilancheran, S.S. Ratnam, Pulmonary metastases in gestational trophoblastic disease: a review of 97 cases, Br. J. Obstet. Gynaecol. 95 (1) (1988) 70–74.
[31] M. Vree, N. van Trommel, G. Kenter, F. Sweep, M. Ten Kate-Booij, L. Massuger, et al., The influence of lung metastases on the clinical course of gestational trophoblastic neoplasia: a historical cohort study, BJOG 123 (11) (2016) 1839–1845.
[32] M.M. Frijstein, C.A.R. Lok, N.E. van Trommel, M.J. Ten Kate-Booij, L.F.A.G. Massuger, E. van Werkhoven, et al., Lung metastases in low-risk gestational trophoblastic Neoplasia: a retrospective cohort study, BJOG (2019) https://doi.org/10.1111/1471-0528.16036Dec 3. (Epub ahead of print).
[33] T.A. Lawrie, M. Alazzam, J. Tidy, B.W. Hancock, R. Osborne, First-line chemotherapy in low-risk gestational trophoblastic neoplasia, Cochrane Database Syst. Rev. 9 (6) (2016), CD007102.