# Predicting factors associated with resistance to initial Methotrexate treatment in women with low-risk gestational trophoblastic neoplasia: a retrospective study

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**Abstract**

Objectives To compare clinical characteristics and factors predictive of resistance to initial treatment with Methotrexate-folinic acid (MTX-FA) in women with low-risk gestational trophoblastic neoplasia (GTN). Design A retrospective study Setting A tertiary center Populations Patients diagnosed with low-risk GTN. Methods: Demographic data, disease characteristics, treatment response, toxicity and data of the subsequent pregnancy were collected and analyzed. Main outcome measures Groups of patients who were responsive or resistance to treatment were compared. Stepwise logistic regression analysis was used to identify factors predictive of resistance to Methotrexate chemotherapy. Results Totally, 113 patients were eligible for analysis. The primary remission rate was 55.8% with first-line MTX-FA. All others patients achieved remission by subsequent treatment with Actinomycin D or multiple-agents chemotherapy. Relapse of disease was found in 4.4% and the overall survival rate was 99.1%. Univariate analysis showed that pre-treatment serum hCG, neutrophil-to-lymphocyte ratio at baseline, and serum hCG ratio of the first three consecutive cycles (C) were significantly associated with resistance to MTX-FA. Independent factors that predict failure to respond to first-line MTX-FA were pre-treatment serum hCG [?]15,000 IU/L (OR 3.95; 95%CI = 1.48-10.52; p=0.006), a less than 4.8-fold reduction of serum hCG between cycle 1 and cycle 2 (C1/C2) (OR 4.08; 95%CI = 1.60-10.39; p=0.003), and a less than 7-fold reduction of serum hCG from cycle 2 to cycle 3 (C2/C3) (OR 10.15; 95%CI = 3.10-33.30; p<0.001). Conclusions First-line MTX-FA treatment is effective in 55.8% of patients. Pre-treatment serum hCG, and hCG ratio between consecutive treatment cycles predicts initial treatment failure.

## Introduction

Gestational trophoblastic neoplasia (GTN) is a rare malignancy with approximately 18,000 women diagnosed per year worldwide. The prevalence varies widely and higher in Asian, American Indian and African American descent.[1] About 50% of GTN follows molar pregnancy, but it can be a consequence of any pregnancy events including abortion, ectopic or term pregnancy. The histopathology of GTN encompasses fours malignant forms; invasive mole, choriocarcinoma, placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor (ETT).[2,3] Most GTN patients are diagnosed clinically using the definition proposed by the collaboration of the International Society for the Study of Trophoblastic Disease (ISSTD), the International Gynecologic Cancer Society (IGCS) and the Society of Gynecologic Oncologists (SGO) in the International Federation of Gynecology and Obstetrics (FIGO) 2000 meeting, known as the "FIGO 2000

criteria".[4-6] FIGO established the GTN staging and scoring system which modified the World Health Organization (WHO) 1983/Charring Cross scoring system based on prognostic factors that were first stated by Bagshawe in 1976.[7] Patients with FIGO stage I and stage II-III with score <7 are classified as low-risk GTN and could be cure using single agent chemotherapy, either Methotrexate (MTX) or Actinomycin-D (Act-D), with a primary remission rate of 50-90%, depending on dosage, schedule, and route of administration. Patients with FIGO score [?]7 are classified as high-risk GTN and should be treated with combination chemotherapy, such as Etoposide, MTX, Act-D, Cyclophosphamide, and Vincristine (EMA-CO) regimen.[2,4,8,9]

Retrospective studies based on small sample sizes have demonstrated that FIGO stage III, high score, present of metastatic disease, large tumor size, high levels of serum hCG, and long interval from antecedent pregnancy are associated with resistance to single-agent chemotherapy in patients with low-risk GTN.[10-12]

We aimed to compare the clinical characteristics of patients with low-risk GTN between patients that responded to MTX-FA and those who were resistance, and to identify independent predictors of resistance to MTX-FA therapy.

## Methods

After approval by the Siriraj Institutional Review Board (SIRB), a retrospective review of medical records of patients diagnosed with low-risk GTN in Siriraj hospital between January 2002 and June 2018 was conducted. The exclusion criteria included placental-site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ETT), incomplete treatment or loss to follow-up during MTX-FA treatment. Data collected including age, body mass index (BMI), gravidity, contraceptive method, histopathology of antecedent pregnancy, diagnosis criteria for GTN and its pathology, FIGO stage and score, complete blood counts, serum hCG, chemotherapy regimens and number of cycles, toxicity, response of treatment, follow-up oncologic outcomes and subsequent pregnancy.

Histopathologic diagnosis of molar pregnancy or choriocarcinoma was made or reviewed by gynecologic pathologist, but not all cases had p57 confirmation. The FIGO 2000 criteria are used for GTN diagnosis, staging and scoring. This criterion was either (i) rising serum hCG ([?]10% increase over the previous weekly value) for [?]3 measurements over a period of [?]2 weeks, (ii) serum hCG plateau (<10% change over the previous weekly value) for four measurements over three consecutive weeks, (iii) persistence of elevated serum hCG for [?]6 months after pregnancy termination, or (iv) histological diagnosis of choriocarcinoma.[4-6,13] After receiving a diagnosis of GTN, patients underwent a metastatic survey by history taking, physical examination, complete blood testing, chest x-ray, and whole abdominal ultrasonography or computer tomography scan. Patients classified as low-risk GTN were treated with MTX 50 mg intramuscular on days 1, 3, 5, 7 alternating with Folinic acid 15 mg intramuscular on days 2, 4, 6, 8 and repeated every 2 weeks, which adapted from regimen used in Charring Cross hospital.[8,14] Serum hCG, complete blood counts, liver function, renal function and electrolytes were evaluated after/during every cycle of chemotherapy. Neutrophil-Lymphocyte ratio (NLR) was calculated by dividing the absolute neutrophil count by absolute lymphocyte count, before MTX-FA initiation. The first three cycles of chemotherapy were designated as "C1, C2, C3" and comparison of serum hCG between each treatment cycle; C1/C2 and C2/C3, which is defined by the serum hCG at the previous cycle divided by the serum hCG of the following cycle. Toxicity was assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4. Patients who achieved normal serum hCG (<3 IU/L) received an additional 2-3 courses of MTX-FA as consolidation treatment. Primary remission was defined as a normal serum hCG for three consecutive weeks and complete remission for [?]6 months. Patients who achieved primary remission were defined as responsive group. Patients who did not achieve complete remission by first-line MTX-FA were classified as resistance group. A diagnosis of resistance was made if the serum hCG declined less than 10% over 3 consecutive samples, increased over two consecutive samples, or a new metastatic lesion was detected.[2,8,13] In the MTX-FA resistance group, Act-D or combination regimens were used as salvage treatment according to physicians'preference. After remission was achieved, serum hCG was evaluated monthly for one year and then annually. A combined oral contraceptive pill was encouraged and pregnancy was allowed after one year of normalized serum hCG. A re-elevation of serum hCG after three

weeks of normalization was defined as a relapse.[15,16]

Statistical analyses were performed using SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). Data are presented as number and percentage, mean ± SD or median and interquartile range [IQR]. Categorical variables were compared using the chi-square test or Fisher's exact test, and continuous variables were compared using Student's t-test or Mann-Whitney U test. Stepwise logistic regression analysis was used to identify variables independently associated with the failure to respond to first-line MTX-FA. The results are presented as odds ratio (OR) and 95% confidence interval (CI). A receiver operating characteristic (ROC) curves analysis was applied to define the optimal cut-off to predict MTX-FA resistance status. All $P$ -values were two-sided and less than 0.05 was considered statistically significant.

**Results**

Between January 2002 and June 2018, 120 patients met the inclusion criteria. Seven patients were excluded because they had refused chemotherapy and loss to follow-up. Totally, 113 patients were eligible for analysis (Figure 1). The mean age was 31.5 ± 7.9 years. First-line MTX-FA treatment resulted in 63 patients achieving complete remission and 50 patients that were resistance (Table 1). The median number of cycles for all study patients was seven [IQR 5-10]. There were no significant differences in demographic data and pathology of antecedent pregnancy between responsive and resistance groups. Most patients in both groups had either a complete or partial mole as their antecedent pregnancy. Serum hCG levels before and after termination of the antecedent pregnancy were significantly associated with the failure to respond to MTX-FA treatment. No significant differences in diagnosis criteria, FIGO scoring, Hammond classification, or duration from GTN diagnosis to date of MTX-FA initiation were detected between groups. Serum hCG at the time of GTN diagnosis and pre-treatment serum hCG was significantly higher in the resistance group. Most factors in the FIGO 2000 scoring system were also not significantly different between the groups except for pre-treatment serum hCG. For easily utilized in clinical practice, we using serum cut-off 15,000 IU/L instead of median value of 17,303 IU/L, and the difference was still significance. No patient with pre-treatment serum hCG >100,000 IU/L achieved remission with first-line MTX-FA treatment. The NLR was significantly higher in the resistance group. The number of MTX-FA cycles was comparable (8.4 vs 7.4 cycles) between responsive and resistance groups, respectively ($P = 0.112$).

Primary remission following MTX-FA treatment was found in 63 patients (55.8%). In the resistance group, all patients received second-line chemotherapy and achieved complete remission. Forty-one patients received Act-D, eight patients received EMA-CO and one patient received the Methotrexate, Actinomycin, Cyclophosphamide (MAC) regimen. After remission, two patients that had responded to MTX-FA and three patients from the resistance group experienced a disease relapse. One of the latter suffered from a second relapse, then received a multi-agent regimen and ultimately died (Figure 1). The overall survival rate of study patients was 99.1%.

During treatment, a reduction of serum hCG between cycles was significantly associated with failure to respond to MTX-FA ($P< 0.001$). Specifically, serum hCG C1/C2 less than 4.8 AUC 0.734 (95% confidence interval [CI], 0.641-0.827; $P< 0.001$), and serum hCG C2/C3 less than 7 AUC 0.757 (95% CI 0.669-0.845; $P < 0.001$) are the optimal cut-points for predicting predictive failure to respond to MTX-FA. (Appendix S1). Four significant factors predicting failure to respond to MTX-FA were identified by univariate analysis; pre-treatment serum hCG [?]15,000 IU/L, reduction of serum hCG C1/C2 <4.8 times, reduction of serum hCG C2/3 <7 times and neutrophil/lymphocyte ratio [?]2). These variables were further analyzed in a multivariate model using forward stepwise logistic regression (Table 2). Pre-treatment serum hCG [?]15,000 IU/L (odd ratio [OR] 3.95, 95% CI 1.48-10.52*; $P = 0.006$*), a reduction of serum hCG between C1/C2 using a cut-point of <4.8 (OR 4.08; 95%CI, 1.60-10.39; $P = 0.003$), and a reduction of serum hCG C2/C3 using a cut-point of <7 (OR 10.15; 95%CI, 3.10-33.30; $P < 0.001$) were individually prognosticative for failure to respond to MTX-FA. Using the hCG cut-off for C1/C2 together with C2/C3, 74.3% of patients with two criteria were resistant to first-line MTX-FA therapy while only 6.5% of patients that did not meet either criteria were resistant.

MTX-FA induced gastrointestinal toxicity was found in 18 patients (28.6%) and four patients experienced grade 3-4 toxicity. Hepatotoxicity was found in nine patients (9.5%) and three were grade 3-4. All toxicities were manageable (Appendix S2).

Twelve patients subsequently became pregnant after complete remission for seven months to six years, including nine patients in first-line MTX-FA remission group and three patients in the resistance group. There were 10 term deliveries, one abortion and one still birth. Two pregnancy with unfavorable outcome were in MTX-FA remission group.

**Discussion**

Main Findings and interpretation

Gestational trophoblastic neoplasia is a malignancy that does not require histopathologic confirmation. GTN was once regarded as a lethal neoplasia due to its highly metastatic behavior. However, since the development of effective chemotherapy in 1956, it became highly curable with remission rates approaching 90-100% even in metastatic disease. Sequential monochemotherapy initiated with MTX or Act-D is the globally accepted standard of treatment for low-risk GTN.[2,3,17,18] Many retrospective studies have compared the efficacy of different chemotherapy protocols for low-risk GTN. These studies reported that the commonly used first-line regimens for treating low-risk GTN were 1) 8-day regimen of MTX-FA intramuscularly alternating with FA repeated every 14 days, 2) weekly 30-50 mg/m$^2$MTX intramuscular, 3) MTX 5-day regimen given intravenously or intramuscularly and repeated every 14 days, 4) pulse intravenous MTX every 14 days, 5) Act-D single dose repeated every 14 days, and 5) Act-D intravenous 5-day repeated every 14 days.[19] Among these, weekly 30-50 mg/m$^2$ MTX intramuscular showed inferior efficacy with only 49-74% primary remission rate while other regimens were 69-94%. A Cochrane review concluded that Act-D regimen was more likely to achieve primary remission than MTX with risk ratio (RR) 0.65 (95% CI 0.57-0.75). Take into account that most women with low-risk GTN were cured with first-line or salvage treatment regardless of the regimen used, 5-day MTX and 8-day MTX-FA regimen was more commonly used as first-line chemotherapy in low risk GTN due to its fewer toxicity.[19]

In our institute, as well as in Europe and North America, the 8-day MTX-FA alternating regimen is the standard initial treatment. The second-line therapy may be Act-D or a multiple drug regimen based on serum hCG cut-points of 300 IU/L or 1,000 IU/L or the clinician's judgement.[2,8,16,20,21] The previously reported remission rate by initial MTX and overall remission rate together with subsequent line of chemotherapy, relapse rate and subsequent pregnancy outcomes was shown in Table 3.[10-12,15,16,20-43] The 8-day MTX-FA regimen has been widely used due to its good efficacy and manageable toxicity. We observed a remission rate of 55.8% which is consistent with other published reports that have ranged from 50-72%.[16,21,23,25,28] The variation in remission rates may result from different patient characteristics and ethnicity.

Adherence to established criteria for the diagnosis of treatment failure may result in delayed detection of drug resistance.[2,8,13] Patients in our resistance group received an average of seven courses of MTX before changing to second-line therapy. This can lead to a prolonged treatment course, decreased patient satisfaction and increased toxicity. Many factors have been studied for possible association with single-agent resistance including a FIGO score of 5-6, presence of metastatic disease, tumor size >3 cm, higher pretreatment serum hCG at cut-off 30,000 or 100,000 IU/L, longer interval from antecedent pregnancy at cut-off 2 or 4 months, and pathology of choriocarcinoma.[11,12,44]Patients with a FIGO score of 5-6 are considered an "intermediate-risk group" and have only 30-35% remission rate with single agent MTX.[21] On the contrary, we did not observe these factors to be significantly associated with failure to respond to 8-day MTX-FA.

A few studies have explored FIGO score, ultrasonographic appearance, Doppler study, and serum hCG level with the likelihood of resistance to MTX. A prospective study in 147 women with low-risk GTN, found that FIGO score [?]4 was associated with increased risk of resistance to 5-day MTX (OR 6.80; 95% CI, 2.54-18.96).[45] Epstein et al, studied ultrasonographic findings in 36 patients with low-risk post-molar GTN and reported that uterine lesion size [?]4 cm was significantly associated with MTX-FA resistance (72.7% vs 27.3%,$P = 0.008$.[46] The Doppler study of uterine artery pulsatile index (UAPI) [?]1 which represent high-

vascularity was reported to be a predictor for MTX-FA resistance in intermediate-risk GTN with resistance proportion of 67% compared with 42% in patient who had UAPI >1 ($P = 0.036$).[47] A combined UAPI [?]1 and a FIGO score of 6 was reported to lead to MTA resistance in 100% of patients.[48] The high time-averaged mean velocity of uterine artery ([?]29.5 cm/sec) had higher risk of resistance to 5-day MTX intramuscular protocol with OR 5.57 (95% CI, 2.14-14.44).[45]

Interestingly, our study found that none of the eight patients with pre-treatment serum hCG >100,000 IU/L responded to first-line MTX-FA treatment. This finding was consistent with a report by McGrath et al. that examined women with low-risk postmolar GTN and serum hCG level >100,000 IU/L. They used the same protocol of MTX-FA as our study, and found that 70.3% of study patients did not respond to MTX-FA and required additional chemotherapy.[27] In order to use serum hCG to predict MTX resistance, previous study use regression in 1 log, or regression curve preceding the fourth and sixth cycles, or used mathematic equation.[49,50] Mono-exponential equation modeling of hCG kinetics (hCGres) was the predictor of resistance to 30 mg/m$^2$ MTX weekly protocol.[50]

We propose a cut-off point of serum hCG between C1/C2 and C2/C3 that could be more easily applied in clinical practice. This could result in fewer courses of chemotherapy and decreased time to remission. This hypothesis merits further study using a multicenter prospective design together with cost analysis and patient satisfaction survey.

The Neutrophil-lymphocyte ratio is a prognostic factor for survival outcome in various types of cancer such as liver, prostate, lung and melanoma. Neutrophils are involved with tumor-related chronic inflammation, suppress cytotoxic T-cell activity and promote metastasis. Lymphocytes also play a role in the cell-mediated adaptive immune response.[51] Thus, the imbalance between neutrophils and lymphocytes ratio can reflect systemic inflammation and anti-apoptotic effect.[52] A retrospective cohort of 5,363 patients diagnosed with breast, pancreas, liver, prostate, esophagus, colon, ovary or skin cancer between 1986-2014 reported that the prognostic value of NLR differed between subgroups of patients. Patients with stage IV or melanoma were associated with more accurate prognostic potential from NLR.[51] To our knowledge, there has been no previous study of GTN that included NLR as a prognostic factor. Our study found that a baseline NLR [?]2 was significantly associated with resistance to first-line MTX-FA (OR 2.90; 95%CI, 1.30-6.80*;* $P = 0.010$). This finding is consistent with previous studies in liver and lung cancers that are also share highly vascularized nature. Although there was no standard cut-off for NLR (range >1.0 to 6.0), a systematic review in patients with liver cancer using a cut-off value of >2.0 but <3.0 had poor prognostic value for overall survival with hazard ratio (HR) 1.35 (95%CI, 1.23-1.48*;* $P < 0.001$).[53] We suggest that NLR as a predicting factor for resistance to first-line MTX is useful and convenient because it can be calculated from the peripheral blood count obtained from every patient before initiating chemotherapy.

There were no serious adverse effects that required changes to the treatment regimen. Toxicity of MTX and Act-D were comparable, but Act-D tends to be associated with more grade 3-4 hematotoxicity and GI toxicity. EMA-CO regimen associated with 62.5% grade 3-4 hematotoxicity. However, toxicity was not well defined and reported due to retrospective nature.

Strengths and limitations

To our knowledge, this was the first study to demonstrate a cut-off point of reduction of serum hCG and use NLR for predicting MTX-FA resistance. All specimens were reviewed by gynecologic pathologists. The drawback of our study was retrospective nature and inadequate toxicity profile and subsequent pregnancy outcomes. No histopathology report of placenta or conceptive product after termination of subsequent pregnancy.

## Conclusion

The first-line 8-day MTX-FA regimen has a fair efficacy and tolerable toxicity. All study patients with low-risk GTN achieve remission whether from first-line or salvage therapy. Independent predictors for first-line MTX resistance were pre-treatment serum hCG [?]15,000 IU/L, reduction of serum hCG between C1/C2

using a cut-point of <4.8 and serum hCG C2/C3 using a cut-point of <7. Using the predictive factors known at earlier period of treatment may aid to early identify MTX-FA resistance and timely switch to second-line treatment.

**Disclosure of interests**

All authors declare that there are no personal or professional conflicts of interest related to the preparation and publication of this manuscript. The authors did not receive financial support from the companies that produce or distribute the tests described in this report.

**Contribution to authorship**

RP designed this study, acquired data, interpreting the results, writing the manuscript, and approved the final version. IR, SK, NJ, and SU designed this study, interpreting the results, revised the manuscript and approved the final version.

**Details of ethics approval**

This study was approved by the Siriraj Insitutional Review Board (COA no. Si 684/2018). The study was performed in accordance with the Declaration of Helsinki.

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**Supporting Information**

Appendix S1 ROC curve of reduction of serum hCG C1/C2 and C2/C3 to predict Methotrexate resistance

*Appendix S2* Treatment related toxicity among different chemotherapy regimens

**References**

1. Ghorani E., Kaur B., Fisher R.A., Short D., Joneborg U., Carlson J.W., et al. Pembrolizumab is effective for drug-resistant gestational trophoblastic neoplasia. *Lancet* 2017;390:2343-5.

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

3. Ngan H.Y.S., Seckl M.J., Berkowitz R.S., Xiang Y., Golfier F., Sekharan P.K., et al. Update on the diagnosis and management of gestational trophoblastic disease. *Int J Gynaecol Obstet*2018;143:79-85.

4. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000. *Int J Gynaecol Obstet* 2002;77:285-7.

5. Kohorn E.I. The new FIGO 2000 staging and risk factor scoring system for gestational trophoblastic disease: description and critical assessment. *Int J Gynecol Cancer* 2001;11:73-7.

6. Ngan H.Y., Bender H., Benedet J.L., Jones H., Montruccoli G.C., Pecorelli S., et al. Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003;83:175-7.

7. Bagshawe K.D. Risk and prognostic factors in trophoblastic neoplasia. *Cancer* 1976;38:1373-85.

8. Seckl M.J., Sebire N.J., Fisher R.A., Golfier F., Massuger L., Sessa C., et al. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*2013;24:39-50.

9. Lurain J.R. Gestational trophoblastic disease II: classification and management of gestational trophoblastic neoplasia. *Am J Obstet Gynecol* 2011;204:11-8.

10. Chapman-Davis E., Hoekstra A.V., Rademaker A.W., Schink J.C., Lurain J.R. Treatment of non-metastatic and metastatic low-risk gestational trophoblastic neoplasia: factors associated with resistance to single-agent methotrexate chemotherapy. *Gynecol Oncol*2012;125:572-5.

11. Lee Y.J., Park J.Y., Kim D.Y., Suh D.S., Kim J.H., Kim Y.M., et al. Comparing and evaluating the efficacy of methotrexate and actinomycin D as first-line single chemotherapy agents in low risk gestational trophoblastic disease. *J Gynecol Oncol* 2017;28,e8

12. Yarandi F., Mousavi A., Abbaslu F., Aminimoghaddam S., Nekuie S., Adabi K., et al. Five-Day Intravascular Methotrexate Versus Biweekly Actinomycin-D in the Treatment of Low-Risk Gestational Trophoblastic Neoplasia: A Clinical Randomized Trial. *Int J Gynecol Cancer*2016;**26** :971-6.

13. Mangili G., Lorusso D., Brown J., Pfisterer J., Massuger L., Vaughan M., et al. Trophoblastic disease review for diagnosis and management: a joint report from the International Society for the Study of Trophoblastic Disease, European Organisation for the Treatment of Trophoblastic Disease, and the Gynecologic Cancer InterGroup. *Int J Gynecol Cancer* 2014;24:S109-16.

14. Bagshawe K.D., Dent J., Newlands E.S., Begent R.H., Rustin G.J. The role of low-dose methotrexate and folinic acid in gestational trophoblastic tumours (GTT). *Br J Obstet Gynaecol*1989;96:795-802.

15. Maesta I., Nitecki R., Horowitz N.S., Goldstein D.P., de Freitas Segalla Moreira M., Elias K.M., 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* 2018;148:161-7.

16. Mangili G., Cioffi R., Danese S., Frigerio L., Ferrandina G., Cormio G., 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* 2018;151,449-52.

17. Goldstein D.P., Berkowitz R.S., Horowitz N.S. Optimal management of low-risk gestational trophoblastic neoplasia. *Expert Rev Anticancer Ther* 2015;15:1293-1304.

18. Hertz R., Li M.C., Spencer D.B. Effect of methotrexate therapy upon choriocarcinoma and chorioadenoma. *Proc Soc Exp Biol Med*1956;93:361-6.

19. Lawrie T.A., Alazzam M., Tidy J., Hancock B.W., Osborne R. First-line chemotherapy in low-risk gestational trophoblastic neoplasia.*Cochrane Database Syst Rev* 2016;6:CD007102.

20. Chalouhi GE, Golfier F, Soignon P, Massardier J, Guastalla JP, Trillet-Lenoir V, et al. Methotrexate for 2000 FIGO low-risk gestational trophoblastic neoplasia patients: efficacy and toxicity. *Am J Obstet Gynecol* 2009;200:643,e1-6.

21. Sita-Lumsden A., Short D., Lindsay I., Sebire N.J., Adjogatse D., Seckl M.J., et al. Treatment outcomes for 618 women with gestational trophoblastic tumours following a molar pregnancy at the Charing Cross Hospital, 2000-2009. *Br J Cancer* 2012;107:1810-4.

22. Yarandi F., Eftekhar Z., Shojaei H., Kanani S., Sharifi A., Hanjani P. Pulse methotrexate versus pulse actinomycin D in the treatment of low-risk gestational trophoblastic neoplasia. *Int J Gynaecol Obstet* 2008;103:33-7.

23. Khan F, Everard J, Ahmed S, Coleman RE, Aitken M, Hancock BW. Low-risk persistent gestational trophoblastic disease treated with low-dose methotrexate: efficacy, acute and long-term effects. *Br J Cance* r 2003;89:2197-201.

24. Taylor F, Short D, Winter MC, Tidy J, Savage PM, Sarwar N, et al. A retrospective study to evaluate single agent methotrexate treatment in low risk gestational choriocarcinoma in the United Kingdom.*Gynecol Oncol* 2015;136:258-63.

25. Baptista AM, Belfort P. Comparison of methotrexate, actinomycin D, and etoposide for treating low-risk gestational trophoblastic neoplasia.*Int J Gynaecol Obstet* 2012;119:35-8.

26. Braga A, de Souza Hartung Araujo C, Mora PAR, Paulino E, de Melo AC, Velarde GC, et al. Comparison of treatment for low-risk GTN with standard 8-day MTX/FA regimen versus modified MTX/FA regimen without chemotherapy on the weekend. *Gynecol Oncol* 2020;156: 598-605.

27. McGrath S, Short D, Harvey R, Schmid P, Savage PM, Seckl MJ. The management and outcome of women with post-hydatidiform mole 'low-risk' gestational trophoblastic neoplasia, but hCG levels in excess of 100 000 IU l(-1). *Br J Cancer* 2010;102:810-4.

28. McNeish IA, Strickland S, Holden L, Rustin GJ, Foskett M, Seckl MJ, 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* 2002;20:1838-44.

29. Kizaki S, Hashimoto K, Matsui H, Usui H, Shozu M. Comparison of 5-day MTX and 5-day ETP treatment results and early predictors of drug resistance to 5-day MTX in patients with post-molar low-risk gestational trophoblastic neoplasia. *Gynecol Oncol* 2015;139:429-32.

30. Mousavi A, Cheraghi F, Yarandi F, Gilani MM, Shojaei H. Comparison of pulsed actinomycin D versus 5-day methotrexate for the treatment of low-risk gestational trophoblastic disease. *Int J Gynaecol Obstet*2012;116:39-42.

31. Prouvot C., Golfier F., Massardier J., You B., Lotz J.P., Patrier S., et al. Efficacy and Safety of Second-Line 5-Day Dactinomycin in Case of Methotrexate Failure for Gestational Trophoblastic Neoplasia.*Int J Gynecol Cancer* 2018;28:1038-44.

32. Smith E.B., Weed J.C., Jr., Tyrey L., Hammond C.B. Treatment of nonmetastatic gestational trophoblastic disease: results of methotrexate alone versus methotrexate–folinic acid. *Am J Obstet Gynecol*1982;144:88-92.

33. Soper J.T., Clarke-Pearson D.L., Berchuck A., Rodriguez G., Hammond C.B. 5-day methotrexate for women with metastatic gestational trophoblastic disease. *Gynecol Oncol* 1994;54:76-9.

34. Uberti E.M., Fajardo M.do.C., da Cunha A.G., Frota S.S., Braga A., Ayub A.C. 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* 2015;37:258-65.

35. Gilani M.M., Yarandi F., Eftekhar Z., Hanjani P. Comparison of pulse methotrexate and pulse dactinomycin in the treatment of low-risk gestational trophoblastic neoplasia. *Aust N Z J Obstet Gynaecol*2015;45:161-4.

36. Homesley H.D., Blessing J.A., Rettenmaier M., Capizzi R.L., Major F.J., Twiggs L.B. Weekly intramuscular methotrexate for nonmetastatic gestational trophoblastic disease. *Obstet Gynecol* 1988;72:413-8.

37. Osborne R.J., Filiaci V., Schink J.C., Mannel R.S., Alvarez Secord A., Kelley J.L., et al. Phase III trial of weekly methotrexate or pulsed dactinomycin for low-risk gestational trophoblastic neoplasia: a gynecologic oncology group study. *J Clin Oncol* 2011;29:825-31.

38. Roberts J.P., Lurain J.R. Treatment of low-risk metastatic gestational trophoblastic tumors with single-agent chemotherapy.*Am J Obstet Gynecol* 1996;174:1917-23.

39. Shahbazian N., Razi T., Razi S., Yazdanpanah L. Comparison of the efficacy of methotrexate and actinomycin D in the treatment of patients with stage I low risk gestational trophoblastic neoplasia (GTN).*Med J Islam Repub Iran* 2014;28:78

40. Lertkhachonsuk A.A., Israngura N., Wilailak S., Tangtrakul S. Actinomycin d versus methotrexate-folinic acid as the treatment of stage I, low-risk gestational trophoblastic neoplasia: a randomized controlled trial. *Int J Gynecol Cancer* 2008;19:985-8.

41. Abrao R.A., de Andrade J.M., Tiezzi D.G., Marana H.R., Candido dos Reis F.J., Clagnan W.S. Treatment for low-risk gestational trophoblastic disease: comparison of single-agent methotrexate, dactinomycin

and combination regimens. *Gynecol Oncol* 2008;108:149-53.

42. Elit L., Covens A., Osborne R., Gerulath A., Murphy J., Rosen B., et al. High-dose methotrexate for gestational trophoblastic disease. *Gynecol Oncol* 1994;54:282-7.

43. Shobeiri M.J., Vejdani R., Melli M.S., Medarek E.O.S., Garebaghi P.M., Ghojazadeh M., et al. Comparison of methotrexate-folinic acid versus pulsed actinomycin-d in treatmetn of stage I, low risk gestational trophoblastic neoplasia: a randomized clinical trial. *Iranian J Obstet Gynecol Infertility* 2014;91:1-11.

44. Mousavi A.S., Zamani A., Khorasanizadeh F., Gilani M.M., Zendehdel K. Resistance to single-agent chemotherapy and its risk factors in low-risk gestational trophoblastic neoplasms. *J Obstet Gynaecol Res* 2015;41:776-83.

45. Qin J., Zhang S., Poon L., Pan Z., Luo J., Yu N., et al. Doppler-based predictive model for methotrexate resistance in low-risk gestational trophoblastic neoplasia with myometrial invasion: prospective study with 147 Patients. *Ultrasound Obstet Gynecol*2020;doi: 10.1002/uog.22069.

46. Epstein E., Joneborg U. Sonographic characteristics of post-molar gestational trophoblastic neoplasia at diagnosis and during follow-up, and its relation to methotrexate resistance. *Ultrasound Obstet Gynecol* 2020;56:759-65.

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

48. Agarwal R., Harding V., Short D., Fisher R.A., Sebire N.J., Harvey R., et al. Uterine artery pulsatility index: a predictor of methotrexate resistance in gestational trophoblastic neoplasia. *Br J Cancer*2012;106:1089-94.

49. van Trommel N.E., Massuger L.F., Schijf C.P., ten Kate-Booij M.J., Sweep F.C., Thomas C.M. Early identification of resistance to first-line single-agent methotrexate in patients with persistent trophoblastic disease. *J Clin Oncol 2006;* 24:52-8.

50. You B., Deng W., Henin E., Oza A., Osborne R. Validation of the Predictive Value of Modeled Human Chorionic Gonadotrophin Residual Production in Low-Risk Gestational Trophoblastic Neoplasia Patients Treated in NRG Oncology/Gynecologic Oncology Group-174 Phase III Trial. *Int J Gynecol Cancer 2016;2* 6:208-15.

51. Howard R., Kanetsky P.A., Egan K.M. Exploring the prognostic value of the neutrophil-to-lymphocyte ratio in cancer. *Sci Rep*2019;9:19673.

52. Yin Y., Wang J., Wang X., Gu L., Pei H., Kuai S., et al. Prognostic value of the neutrophil to lymphocyte ratio in lung cancer: A meta-analysis. *Clinics (Sao Paulo)* 2015;70:524-30.

53. Min G.T., Li Y.M., Yao N., Wang J., Wang H.P., Chen W. The pretreatment neutrophil-lymphocyte ratio may predict prognosis of patients with liver cancer: A systematic review and meta-analysis. *Clin Transplant* 2018;32:e13151.

**Table 1.** Disease characteristics of 113 study patients classified by treatment outcomes

| Variables | Remission group, n (%) n=63 | Resistant group, n (%) n=50 | p value |
|---|---|---|---|
| Age, years | 31.9 ± 10.1 | 31.0 ± 8.4 | 0.759 |
| Histology of antecedent pregnancy | | | 0.097 |
| Complete mole (n=85) | 51 (60.0) | 34 (40.0) | |
| Partial mole (n=7) | 3 (42.9) | 4 (57.1) | |

| Variables | Remission group, n (%) n=63 | Resistant group, n (%) n=50 | p value |
|---|---|---|---|
| Molar, not specified type (n=15) | 7 (46.7) | 8 (53.3) | |
| No pathology report (n=6) | 2 (33.3) | 4 (66.7) | |
| Serum hCG before molar termination, IU/L* | 273,888 [161,019-510,606] | 509,372 [200,000-1,000,000] | 0.008 |
| Serum hCG at GTN diagnosis, IU/L | 5,322 [1,089-16,993] | 17,303 [2,146-45,196] | 0.020 |
| Interval from antecedent pregnancy, weeks | 5.9 [4.4-10.7] | 5.8 [4.3-12.3] | 0.956 |
| Pathology at initiated chemotherapy | | | 0.326 |
| Postmolar GTN (n=102) | 55 (53.9) | 47 (46.1) | |
| Invasive mole (n=5) | 4 (80.0) | 1 (20.0) | |
| Choriocarcinoma (n=6) | 4 (66.7) | 2 (33.3) | |
| FIGO stage | | | 0.650 |
| I (n=90) | 49 (54.4) | 41 (45.6) | |
| II (n=1) | 1 (100) | 0 (0) | |
| III (n=22) | 13 (59.1) | 9 (40.9) | |
| FIGO score | | | 0.084 |
| 0-2 (n=66) | 40 (60.6) | 26 (39.4) | |
| 3-4 (n=32) | 18 (56.3) | 14 (43.8) | |
| 5-6 (n=15) | 5 (33.3) | 10 (66.7) | |
| Pre-treatment serum hCG, IU/L | | | 0.007 |
| <15,000 (n=70) | 46 (65.7) | 24 (34.3) | |
| [?]15,000 (n=43) | 17 (39.5) | 26 (60.5) | |
| Neutrophil/Lymphocyte ratio* | 2.0 ± 0.7 | 2.5 ± 1.3 | 0.017 |
| Serum hCG at C1, IU/L | 4,880 [776-18,775] | 16,028 [2,146-42,026] | 0.023 |
| Serum hCG at C2, IU/L | 490 [75-1,738] | 2,772 [242-12,860] | <0.001 |
| Serum hCG at C3, IU/L* | 42 [6-493] | 893 [ 136-4,066] | <0.001 |
| Reduction of serum hCG C1/C2, times | 7.97 [4.93-18.57] | 3.98 [2.21-8.35] | <0.001 |
| <4.8 (n=44) | 14 (31.8) | 30 (68.2) | |
| [?]4.8 (n=69) | 49 (71.0) | 20 (29.0) | |
| Reduction of serum hCG C2/C3, times | 7.29 [2.70-13.98] | 2.46 [1.22-4.76] | <0.001 |
| <7.0 (n=73) | 29 (39.7) | 44 (60.3) | |
| [?]7.0 (n=38) | 33 (86.8) | 5 (13.2) | |
| Number of cycles | 8.4 ± 3.6 | 7.4 ± 3.4 | 0.112 |

C, cycle; hCG, human chorionic gonadotropin; GTN, Gestational trophoblastic neoplasia; FIGO, the International Federation of Gynecology and Obstetrics.

* = data missing in some patients

| Variables | Univariate analysis | Univariate analysis | Multivariate analysis | Multivari |
|---|---|---|---|---|
| | OR [95% CI] | P | OR [95%CI] | P |
| Pre-treatment serum hCG, [?]15,000 IU/L | 2.90 [1.30-6.40] | 0.010 | 3.95 [1.48-10.52] | 0.006 |
| Reduction of serum hCG C1/C2, <4.8 times | 5.25 [2.31-11.92] | <0.001 | 4.08 [1.60-10.39] | 0.003 |
| Reduction of serum hCG C2/C3, <7 times | 10.00 [3.50-29.00] | <0.001 | 10.15 [3.10-33.30] | <0.001 |
| Neutrophil/lymphocyte ratio [?]2 | 2.90 [1.30-6.80] | 0.010 | | |

**Table 2.** Univariate and multivariate analysis of predictors for resistance to initial Methotrexate treatment

C, cycle; hCG, human chorionic gonadotropin; OR, odds ratio.

**Table 3.** Methotrexate protocol for treatment of low-risk gestational trophoblastic neoplasia

| **Protocol of MTX** | **Designs** | **No.** | **MTX remission (%)** | **Overall remission (%)** | **Relapse (%)** | **Subsequent pregnancy** |
|---|---|---|---|---|---|---|
| **MTX 50 mg/D im. D1, 3, 5, 7 FA 15 mg oral D2, 4, 6, 8** | | | | | | |
| McNeish IA. et al. 2002* | Retrospective 1992-2000 Postmolar GTN | 485 | 69.1 | 100 | 3.3 | No report |
| Khan F. et al. 2003* | Retrospective 1987-2000 Low-risk GTN | 250 | 72 | 98 | 3.2 | 128 full term 22 abortion 2 molar |
| McGrath S. et al 2010 | Retrospective 1980-2008 Postmolar GTN hCG >100,000 | 37 | 29.7 | 100 | No report | No report |
| Baptista AM. et al 2012 | RCT 2009-2010 Low-risk GTN | 20 | 50 | No report | No report | No report |
| Sita-Lumsden A. et al 2012 | Retrospective 2000-2009 Postmolar GTN | 554 | 57 | 100 | 2.9 All cured | No report |
| Taylor F. et al. 2015 | Retrospective 2000-2011 Low-risk CCA | 65 | 35 | 100 | 17 All cured | 6 full term 1 stillbirth 1 abortion |
| Mangili G. et al. 2018* | Retrospective Low-risk GTN | 99 | 71.1 | 100 | 3 | No report |

| **Protocol of MTX** | **Designs** | **No.** | **MTX remission (%)** | **Overall remission (%)** | **Relapse (%)** | **Subsequent pregnancy** |
|---|---|---|---|---|---|---|
| Current Study+ | Retrospective 2002-2018 Low-risk GTN | 113 | 55.8 | 100 | 4.4 | 10 full term 1 abortion 1 stillbirth |
| **MTX 1mg/kg/D im. D1, 3, 5, 7 FA 0.1 mg/kg oral D2, 4, 6, 8** | | | | | | |
| Chalouhi GE. et al. 2009 | Retrospective 1999-2006 Low-risk GTN | 142 | 77.5 | 99.9 | 4.9 | No report |
| Uberti EM. et al. 2015 | Retrospective 1992-2012 Low-risk GTN | 115 | 75.7 | 100 | 2.6 | No report |
| Lee YJ. et al 2017++ | Retrospective 2000-2013 Low-risk GTN | 16 | 68.8 | 100 | No report | No report |
| Maesta I. et al. 2018 | Retrospective 1974-2014 Postmolar GTN | 151 | 84.1 | 100 | 3.3 | No report |
| Mangili G. et al. 2018* | Retrospective Low-risk GTN | 77 | 77.9 | 100 | 6.5 | No report |
| Prouvot C. et al 2018 | Retrospective 1999-2017 Low-risk GTN | 877 | 88.4 | 100 | No report | No report |
| Braga A. et al 2020 | Retrospective 1990-2017 Low-risk GTN | 538 | 46.7 | 100 | 1.7 Death 0.4 | No report |
| **MTX 1mg/kg im. D1, 3, 5, 7 FA 0.1 mg/kg im. D2, 4, 6, 8** | | | | | | |
| Smith EB. et al 1982 | Retrospective 1975-1978 Nonmetastatic GTN | 29 | 72.5 | 100 | 3.4 | No report |
| Lertkhachonsuk AA. et al. 2009+ | RCT 1994-2005 Low-risk GTN | 27 | 73.6 | 96.3 1 loss during second-line | No report | No report |
| **MTX 0.4 mg/kg/D im. D1-5** | | | | | | |

| **Protocol of MTX** | **Designs** | **No.** | **MTX remission (%)** | **Overall remission (%)** | **Relapse (%)** | **Subsequent pregnancy** |
|---|---|---|---|---|---|---|
| Smith EB. et al 1982 | Retrospective 1975-1978 Nonmetastatic GTD | 39 | 92.3 | 100 | 5.1 | No report |
| Soper JT. et al. 1994 | Retrospective 1975-1990 Metastatic GTN | 52 | 60 | 100 | 0 | No report |
| Chapman-Davis E. et al. 2012 | Retrospective 1979-2009 Low-risk GTN | 358 | 81 | 100 | No report | No report |
| Mousavi A. et al 2012 | RCT 2008-2010 Low-risk GTN | 25 | 68 | 100 | No report | No report |
| Kizaki S. et al 2015§ | Retrospective 1980-2014 Postmolar GTN | 102 | 64.7 | No report | 2 | No report |
| **MTX 0.4 mg/kg/D iv. D1-5** | | | | | | |
| Roberts JP. et al 1996 | Retrospective 1962-1992 Low-risk GTN | 61 | 65.6 | 100 | No report | No report |
| Yarandi F. et al 2016 | RCT 2010-2013 Low-risk GTN | 23 | 78.1 | 100 | No report | No report |
| **MTX 20 mg/m2/D im. D1-5** | | | | | | |
| Abrao RA. et al 2008 | Retrospective 1980-2002 Low-risk GTN | 42 | 69 | No report | No report | No report |
| **MTX 30 mg/m$^2$ im. weekly** | | | | | | |
| Homesley HD. et al. 1988 | Prospective Nonmetastatic GTN | 63 | 81 | 100 | 0 | No report |
| Gilani AA. et al 2005 | RCT 2001-2003 Low-risk GTN | 28 | 50 | No report | No report | No report |
| Yarandi F. et al 2008 | RCT 2003-2006 Low-risk GTN | 81 | 48.1 | 100 | No report | No report |
| Osborne RJ. et al 2011 (GOG 174) | RCT 1999-2007 Low-risk GTN | 107 | 53.3 | 100 for who complete follow-up | 0.9 | No report |

| Protocol of MTX | Designs | No. | MTX remission (%) | Overall remission (%) | Relapse (%) | Subsequent pregnancy |
|---|---|---|---|---|---|---|
| **MTX 40 mg/m$^2$ im. weekly** | | | | | | |
| Shahbazian N. et al 2014 | RCT 2009-2011 GTN stage I | 15 | 53.3 | No report | No report | |
| **MTX 100 mg/m$^2$ iv. bolus & 200 mg/m$^2$ iv. drip FA 0.1 mg/kg oral q 12 hrs*4** | | | | | | |
| Maesta I. et al. 2018 | Retrospective 1974-2014 Postmolar GTN | 174 | 62.1 | 100 | 3.4 | No report |

D, Day; FA, Folinic acid; GOG, Gynecologic Oncology Group; GTN, Gestational trophoblastic neoplasia; im., Intramuscular; iv., Intravascular; MTX, Methotrexate; RCT, randomized controlled trial.

* = Folinic acid dose 7.5 mg

+ = Folinic acid intramuscular route

++ = not defined Folinic acid route & dose

§ = dose in range of 0.35-0.4 mg/kg

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