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Clinical Research Article

Effects of Low-dose Methotrexate With Methimazole in Patients With Graves' Disease: Results of a Randomized Clinical Trial

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Abstract

Context: Supplemental methotrexate (MTX) may affect the clinical course of Graves' disease (GD).

Objective: To evaluate the efficacy of add-on MTX on medical treatment in GD.

Design: Prospective, open-label, randomized supplementation controlled trial.

Setting: Academic endocrine outpatient clinic.

Patients: One hundred fifty-three untreated hyperthyroid patients with GD.

Intervention: Patients received MTX 10 mg/w with methimazole (MMI) or MMI only. MTX and MMI were discontinued at months 12 to 18 in euthyroid patients.

Main Outcome Measures: Discontinuation rate at month 18 in each group.

Results: In the MTX with MMI group, the discontinuation rate was higher than the MMI group at months 15 to 18 [50.0 vs 33.3%, $P = .043$, 95% confidence interval (CI) 1.020-3.922; and 55.6 vs 38.9%, $P = .045$, 95% CI 1.011-3.815, respectively). The decrease in thyrotropin-related antibodies (TRAb) levels in the MTX with MMI group was significant from baseline to month 6 compared to the MMI alone group [MTX + MMI 67.22% (43.12-80.32), MMI 54.85% (33.18-73.76), $P = .039$] and became more significant from month 9 [MTX + MMI 77.79% (62.27-88.18), MMI 69.55% (50.50-83.22), $P = .035$] to month 18 ($P < .01$ in 15-18 months). A statistically significant difference was seen between the levels of TRAb in the MTX with MMI group and the MMI group at 9 to 18 months. There were no significant differences in the levels of free T3, free T4, and TSH between the 2 groups. No serious drug-related adverse events were observed in either group ($P = .771$).

Conclusion: Supplemental MTX with MMI resulted in a higher discontinuation rate and improvement in decreased TRAb levels to homeostatic levels faster than methimazole treatment alone at months 12 to 18.

Key Words: methotrexate, MTX, Graves' disease, TRAb, clinical trial, autoimmunity

Graves' disease (GD) is the most frequent cause of hyperthyroidism in iodine-replete geographical areas. The incidence peaks between 30 and 50 years of age. The lifetime risk is 3% for women and 0.5% for men (1). GD is an autoimmune disorder characterized by the presence of thyrotropin-related antibodies (TRAb) stimulating the thyroidal cells and causing an overproduction of thyroid hormones (2). Although the etiology of GD remains unknown, evidence indicates a strong genetic component combined with random potential environmental insults in an immunologically susceptible individual. There is no doubt that immunologic factors are important in the pathogenesis of GD.

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Current management of GD is largely imperfect, in the absence of therapies targeting the pathogenetic mechanisms of the disease (3, 4). Major treatments for GD are antithyroid drugs (ATD), radioactive ^{131}I, and thyroidectomy. ATD therapy is the first-line treatment worldwide under most circumstances (4, 5). Standard treatment for GD includes use of ATD for a total duration of 12 to 18 months to treat overproduction of thyroid hormones, which is then discontinued if the TSH and TRAb levels are normal at that time (6). TRAb with normal levels indicating greater chance for remission. However, many patients remain positive TRAb after completing a course of methimazole (MMI), consideration should be given to treatment with radioactive ^{131}I or thyroidectomy or prolonged low-dose MMI treatment. The former 2 treatments can lead to lifelong thyroid dysfunction, while the latter may result in potential drug-related side effects such as hepatic injury and agranulocytosis (6). Therefore, a pressing clinical issue is to enable more patients to discontinue medication when TRAb levels turn negative after 12 to 18 months of MMI treatment. Because of the autoimmune nature of GD, the additional use of immunosuppressive drugs in combination with ATD may be considered to positively impact the clinical outcomes. There is great interest in looking for regimens that target key immunopathogenic mechanisms of GD with fewer side effects and a lower price. Emerging treatment choices are biologics with new targets of the immune pathway, like rituximab (7, 8) and K1-70 (9), which are not likely to become the first choice for new;y diagnosed GD patients because of prohibitive costs.

Methotrexate (MTX) is an effective immunosuppressant that has been widely used in rheumatoid arthritis (RA) (10) and multiple sclerosis (11). The advantages of MTX include cost effectiveness, weekly dosing, and moderate side effects. Adverse effects of MTX include cytopenia, infections, liver damage, mucocutaneous toxicity, and hypersensitivity pneumonitis. Serious adverse effects are uncommon in low-dose regimens (5-25 mg/week) (12). MTX is suggested to work through several mechanisms. As a potent inhibitor of dihydrofolate reductase, MTX decreases the de novo production of purines and pyrimidines and interferes with DNA synthesis. It can nonspecifically prevent T and B cells from proliferating and induce cell apoptosis (13). T cells are highly sensitive to MTX-induced apoptosis (14). MTX also confers anti-inflammatory properties through the release of adenosine and the inhibition of inflammation mediators (15). Based on these pharmacological properties, some studies have used MTX for the treatment of thyroid-associated ophthalmopathy. Low-dose MTX has shown significant efficacy in the treatment of inflammatory symptoms, with reduced glucocorticoid dosage and fewer side effects (16).

Because of its pharmacologic properties and the known benefits of MTX in the management of Graves' orbitopathy (GO) as demonstrated in previous studies, we considered MTX as a potential adjunctive therapy for GD. Hence, we report the results of a prospective, open-label, randomized controlled trial designed to assess the efficacy of low-dose MTX with MMI in untreated GD.

Materials and Methods

Subjects

All participants were recruited consecutively in the Endocrinology Department of Ruijin Hospital between January 2018 and November 2020. The inclusion criteria were (1) a new diagnosis of GD, based on hyperthyroidism associated with detectable circulating TRAb; (2) between 18 and 70 years of age; (3) informed consent; (4) absence of exclusion criteria. Patients were excluded in the following situations: (1) previous or concomitant treatments for hyperthyroidism by any means; (2) pregnancy and lactation; (3) known autoimmune disease; (4) liver or hematologic disorders, kidney failure, malabsorption/malnutrition, severe cardiac or lung diseases, active neoplasia; and (5) contraindications to MMI treatment or lack of informed consent.

Study Design

We performed a prospective, open-label, randomized controlled trial of MTX in 2 groups of newly diagnosed GD patients, treated either with MTX and MMI or with MMI only. The trial was approved by the Ethical Committee of Ruijin Hospital, Shanghai Jiao Tong Medical University, and registered at the Chinese Clinical Trial Registry in 2018 (ChiCTR1800020153). Written informed consents were signed by all participants. This study followed the Consolidated Standards of Reporting Trials reporting guideline for randomized clinical trials.

Randomization and Blinding

Starting with 001, a computer algorithm generated a 6-block-size randomization list. Eligible patients were randomly assigned to treatment with MMI monotherapy or MMI combined with MTX, according to the randomization list. The trial participants and the healthcare providers recruiting them and dispensing the study drug were not blinded to the intervention.

Treatment

Patients in both groups were treated with MMI at doses based on free T4 (FT4) levels as follows: (1) for FT4 levels ≥19.04 to <28.56 pmol/L: 5~10 mg/day for 4 weeks, followed by dose adjustment based on FT4 and TSH levels; (2) for FT4 levels ≥28.56 to <38.08 pmol/L: 10~20 mg/day for 4 weeks, followed by dose adjustment as noted; (3) for FT4 levels ≥38.08 pmol/L: 30~40 mg/day for 4 weeks, followed by dose adjustment as noted. During the study, after free T3 (FT3) and FT4 levels returned to normal, patients were followed up every 6 weeks, each time by reducing the MMI dose by half of the original dose, and then every 12 weeks after reducing to the maintenance dose. It is advised to discontinue the drug when the patient has reached 12 to 18 months of treatment and TSH and TRAb levels are normal.

In the MTX with MMI group, the patients were prescribed with MTX at 7.5 mg/week initially for 2 weeks after enrolling, then increased to 10 mg/week and maintained to the end of the study. The dose selection of MTX was based on the guideline-recommended dose for RA in China (17). In the meantime, 5 mg folate was also given for every week on the day after taking MTX. MTX and folate prescriptions were filled at Ruijin Hospital Pharmacy.

Follow-up

Clinic visits were scheduled monthly for dose adjustment and adverse event reporting. Adverse events were assessed by standard adverse event reporting. Outpatient follow-up was

every 4 weeks until thyroid function returned to normal, every 6 weeks after thyroid function returned to normal, and every 12 weeks after entering the maintenance phase of medication. Thyroid transverse area (WTAR; width multiplied by thickness of the area) (18) and the number of nodules were assessed by ultrasound at baseline and every 6 months during follow-up. The number and frequency of additional visits will be increased if the patient has a drug-related adverse event.

The following laboratory tests were monitored weekly for the first 2 weeks and monthly thereafter: complete blood counts, kidney function, and liver function. Chest X-ray was performed every 6 months. Patients were monitored for pneumonitis, infections and severe adverse events including death and hospitalization from any cause. Discontinue medication immediately upon occurrence. During follow-up monitoring, the following are defined as criteria for discontinuation: leukopenia was defined by a white blood cell count $< 3 \times 10^9$/L and thrombocytopaenia as platelet count $<100 \times 10^9$/L. Granulocytopenia or agranulocytosis was defined as a neutrophil count of 1.5×10^9/L or lower for granulocytopenia and 0.5×10^9/L or lower for agranulocytosis. Aspartate aminotransferase or alanine aminotransferase (ALT) was more than 3 times above the upper limit of normal. Persistent rash was caused by ineffective antihistamine treatment. Patients who discontinued the medication due to adverse events and withdrew from the study were included in the primary analysis.

Primary and Secondary Outcomes

Patients were evaluated at baseline and 3, 6, 9, 12, 15, and 18 months after starting treatment. Clinical examination was performed by an investigator blind to treatment. The primary outcome was the discontinuation rate at month 18. Medication discontinuation was defined as biochemical euthyroidism with thyroid-related hormones FT3, FT4, and TSH within the normal range and negative TRAb. Secondary outcomes included changes in biochemical markers (between-groups difference in the variation of FT4 and FT3 levels every 3 months over the course of 12 months and the variations of TRAb levels every month), decline of TRAb from baseline levels every 3 months, the median MMI daily dose in each month, and adverse events in each group.

Sample Size

The study used PASS software version 15.0.5 and was based on earlier research that found about 20% to 30% of patients were reported to have a lasting remission after 12 to 18 months of medication (19). With an alpha error of .05 and power of 80% and considering a 20% drop-out rate and a discontinuation rate of 30%, the minimum required sample size for each group was determined to be 53.

Serology

Serum TSH, FT3, and FT4 were measured by automated chemiluminescent immunoassays (Architect i2000SR; Abbott Laboratories, Chicago, IL). The laboratory reference ranges provided by the manufacturer were used in this study: TSH 0.35-4.94 μIU/mL, FT4 9.01-19.04 pmol/L, FT3 2.43-6.01 pmol/L. Serum levels of TRAb were measured by electro-chemiluminescence immunoassays (Cobas 601 analyzer, Roche Diagnostics) with a suggested cutoff value of 1.75 IU/L.

Quantification of MTXPGs in Peripheral Blood Mononuclear Cells

To determine the relative abundance of MTX polyglutamates (MTXPGs), blood samples were obtained at baseline, 6, 12, and 18 months after treatment start. Peripheral blood mononuclear cells (PBMCs) were isolated and stored at −80 °C until used. MTXPG1-2 were measured separately with the ultra-high-performance liquid chromatography-tandem mass spectrometry, which performs semiquantitative analysis [adapted from Den Boer et al (20)]; 5×10^6 PBMC were resuspended in 100 μL of methanol and 20 μL of 20 ng/mL internal standard. The mixture was vortexed for 2 minutes and then ultrasonic treatment for 20 minutes. After centrifugation (4 °C, 14 000 g, 15 minutes), 10 μL of the supernatant was used for ultra-high-performance liquid chromatography-tandem mass spectrometry analysis. MTXPGs in PBMCs were separated on a Kinetex® 2.6 μm C18 100 Å column (3 × 100 mm; Phenomenex, CA, USA) and detected on an AB Sciex QTrap 6500+ (AB Sciex Instruments). The relative abundance of total MTXPGs was obtained by adding the 2 individually measured MTXPGs.

Statistical Methods

The primary analysis was based on the intention-to-treat allocation. Baseline characteristics were presented in frequency (percent) values, whereas numerical data were presented using the mean (SD) if normally distributed or median (interquartile range) if not normally distributed. To assess differences between groups, categorical variables were analyzed with a Chi-square test or Fisher's exact test, while continuous variables were evaluated with a 2-sample *t*-test or Mann–Whitney U test as applicable. A pseudo-median difference calculated with the use of the Hodges–Lehmann estimate based on the Mann–Whitney U test. Subgroups analysis of discontinuation rates used a logistic regression model to investigate potential interactions between the intervention and various factors, including sex, age (above or below 40 years), FT4 levels (1.5 and 2 times the upper limit of normal), TRAb concentration (above or below median 15.35 μIU/mL), goiter (yes or no), and GO (yes or no). Subgroups were analyzed separately for the effect of intervention which is presented as odds ratio with 95% confidence intervals (CIs). All reported *P*-values were 2-sided. The significance level was set at .05. Statistical analysis was performed with the IBM SPSS Statistics Version 26.0 (IBM, Armonk, NY, USA), and graphs were drawn with GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA). A forest plot was created using R version 4.2.2.

Results

One hundred fifty-three patients were clinically diagnosed with GD between January 2018 and November 2020 in our center (Fig. 1). Nine patients did not meet the inclusion criteria for the following reasons: (1) 1 patient was ineligible for age; (2) 6 patients chose to undergo thyroidectomy or radioactive iodine; (3) 1 patient had ALT > 3 times above the upper limit of normal; (4) 1 patient became pregnant. The 144 eligible

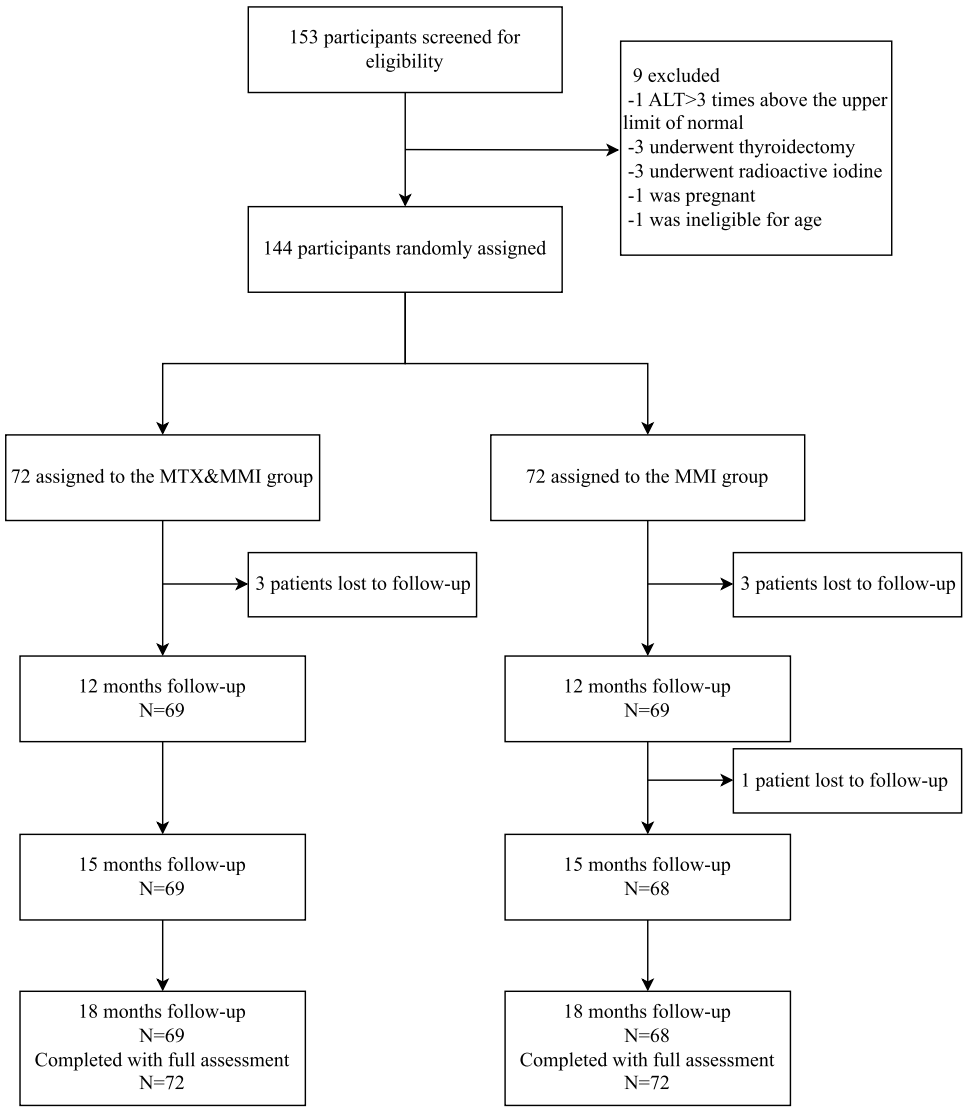

Figure 1. Flow chart depicting identification, treatment allocation, and follow-up of participants.

patients were randomized to the MTX with MMI group or to the MMI group in a 1:1 ratio. Three (4%) patients in each of the MTX with MMI group and MMI only group dropped out before the 12-month visit; 2 patients chose to seek medical care locally and 4 were lost to follow-up due to the COVID-19 pandemic. One (1%) patient in the MMI group was lost to follow-up between the 12-month and 18-month visit and was kept in the analysis according to the last observation carried forward. This patient was contacted by telephone for safety analysis up to 18 months. There were no other patients who discontinued the medications or needed dose reductions. Overall, all randomized patients received at least 1 dose of the allocated treatment and were included in the intention-to-treat analysis.

Baseline Characteristics

Demographic, clinical, and biochemical features of the 2 groups at baseline are illustrated in Table 1. There were no relevant or significant differences between the study groups pertaining to sex, age, smoking rate, goiter degrade, family history, prevalence of GO, MMI dose, or serum levels of thyroid-related hormones and TRAb.

Outcomes and Measures

Primary outcome

A discontinuation to medical treatment and biochemical euthyroidism with negative TRAb was registered in 23 of 72 patients (31.9%) and in 18 of 72 (25.0%) at month 12 in the MTX with MMI group and MMI alone groups, respectively, with no significant difference observed (95% CI, 1.020-3.922, $P = .356$). At month 15, their discontinuation rates were 50.0% (36/72) and 33.3% (24/72), respectively, with a significantly higher discontinuation rate in the MTX combined with MMI group (95% CI, 1.020-3.922, $P = .043$). At month 18, there was a statistically significant difference in efficacy, as the discontinuation rate was 55.6% (40/72) in the MTX combined with MMI group compared to 38.9% (28/72) in the MMI alone group (95% CI, 1.011-3.815, $P = .045$) (Fig. 2).

Secondary outcomes

As shown in Table 2 and Fig. 3A, compared to the MMI alone group, the MTX with MMI group exhibited a greater magnitude of TRAb level reduction in patients. Starting from the month 6 of treatment, the TRAb reduction from baseline levels in the MTX with MMI group was 67.22%

Table 1. Baseline characteristics of participants

Variables	MMI group	MTX + MMI group	*P*-value
n	72	72	
Sex (% female)	76.39	72.22	.567
Age	41.17 ± 12.80	40.08 ± 11.04	.587
At study entry			
Graves' orbitopathy, no. (%)	11 (15.3)	12 (16.7)	.849
Goiter, no (%)	48 (66.7)	43 (59.7)	.388
Current smoking, no. (%)	9 (12.5)	13 (18.1)	.354
Family history of thyroid disease, no. (%)	19 (26.4)	16 (22.2)	.560
FT3 (pmol/L)	28.75 (16.56-46.08)	26.48 (18.24-46.08)	.928
FT4(pmol/L)	36.89 (30.84-47.09)	39.44 (34.29-47.23)	.237
TSH(μIU/mL)	0.0001 (0.0001-0.0008)	0.0002 (0.0001-0.0009)	.322
TRAb (IU/L)	13.80 (9.44-22.65)	14.16 (9.09-19.42)	.646
WBC (10^9/L)	5.59 (4.80-6.80)	6.12 (5.13-6.99)	.176
Lymphocytes (10^9/L)	1.99 (1.53-2.50)	2.07 (1.70-2.58)	.454
Neutrophils (10^9/L)	2.95 (2.13-3.85)	3.13 (2.50-3.73)	.178
Monocytes (10^9/L)	0.58 (0.50-0.69)	0.60 (0.50-0.73)	.321
Thyroid nodes, no (%)	21 (29.2)	26 (36.1)	.374
MMI dose (mg)	20 (15-30)	30 (15-30)	.311

Data are n (%), mean (SD), or median (interquartile range). Baseline characteristics as measured at trial inclusion.
Abbreviations: CI, confidence interval; FT3, free T3; FT4, free T4; MMI, methimazole; MTX, methotrexate; TRAb, thyrotropin-related antibodies; WBC, white blood cell count.

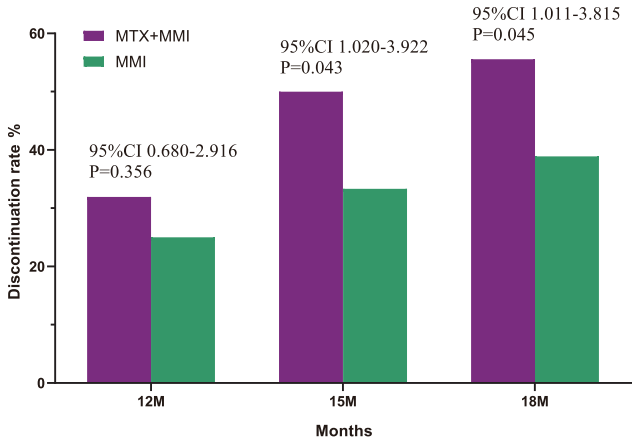

Figure 2. Comparison of the discontinuation rate with MTX with MMI or MMI alone in whole patients with GD in terms of normalizing serum FT3, FT4, and TSH levels and negative TRAb. *$P < .05$.

Abbreviations: GD, Graves' disease; FT3, free T3; FT4, free T4; MMI, methimazole; MTX, methotrexate; TRAb, thyrotropin-related antibodies.

(43.12-80.23%), while in the MMI alone group, it was 54.85% (33.18-73.76%), with the TRAb reduction in the MTX with MMI group being more significant (95% CI, −18.103 to −0.447, $P = .039$). At month 9, the TRAb reduction from baseline levels in the MMI combined with MTX group and the MMI group was 77.79% (62.27-88.18%) and 69.55% (50.50-83.22%), respectively (95% CI, −13.392 to −0.566, $P = .029$). At month 12, the TRAb reduction in the 2 groups was 85.61% (71.05-92.97%) and 77.49% (63.05-87.90%), respectively (95% CI, −10.074 to −0.396, $P = .035$). Furthermore, from month 15(95% CI, −10.522 to −1.364) to month 18(95% CI, −10.181 to −1.326), the difference in TRAb levels reduction between the 2 groups became even more pronounced ($P < .01$ in 15-18 months).

We found that after 9 months of therapy, a statistically significant difference between the levels of TRAb in the MTX with MMI group and the MMI group at 9 to 18 months (Fig. 3A). However, there were no significant differences in the levels of FT3 and FT4 between the 2 groups throughout the follow-up period (Fig. 3B and 3C). Besides, a trend similar to that of TRAb was also observed in lymphocytes. Lymphocyte counts significantly decreased in the MTX + MMI group compared to the MMI group from month 9 ($P = .034$) to month 18 ($P < .01$) after the treatment (Table 3). Other leucocytes including white blood cell count, neutrophil count, and monocyte count showed no significant differences between the 2 groups throughout the follow-up period (Supplementary Table S1) (21).

Furthermore, there was no significant difference in the numbers of patients achieving normal thyroid function (regardless of TRAb levels) between the 2 groups at 12 to 18 months (Supplementary Table S2) (21). At months 6 to 18, the median MMI dose did not differ between the 2 groups (Supplementary Table S3) (21). The median time to discontinuation was 12.0 (12.0-18.0) months for the MMI group, the same as the MTX with MMI group (data not shown).

Only the MTX with MMI group showed a reduction in the mean WTAR of the left thyroid lobe at months 12 compared to baseline ($P = .03$). There were no differences between the 2 groups in other ultrasound parameters (Supplementary Table S4) and the number of thyroid nodules (Supplementary Table S5) (21).

To investigate whether the discontinuation rate was significant in specific subgroups and the potential interactions

Table 2. The change in the decline of TRAb from baseline levels in the 2 groups of treatment at different temporal points

	3M	6M	9M	12M	15M	18M
MTX + MMI group (%)	34.77 (11.48-53.46)	67.22 (43.12-80.32)	77.79 (62.27-88.18)	85.61 (71.05-91.37)	85.96 (78.85-92.97)	86.32 (79.45-93.21)
MMI group (%)	28.02 (−1.47-51.50)	54.85 (33.18-73.76)	69.55 (50.50-83.22)	77.49 (63.05-87.90)	80.51 (63.98-89.82)	81.01 (63.89-89.78)
95% CI	−18.428, 5.332	−18.103, −0.447	−13.392, −0.566	−10.074, −0.396	−10.522, −1.364	−10.181, −1.326
P-value	.292	.039*	.029*	.035*	.008**	.009**

Data are presented as median (interquartile range).
Abbreviations: CI, confidence interval; M, months; MMI, methimazole; MTX, methotrexate.
*$P < .05$. **$P < .01$.

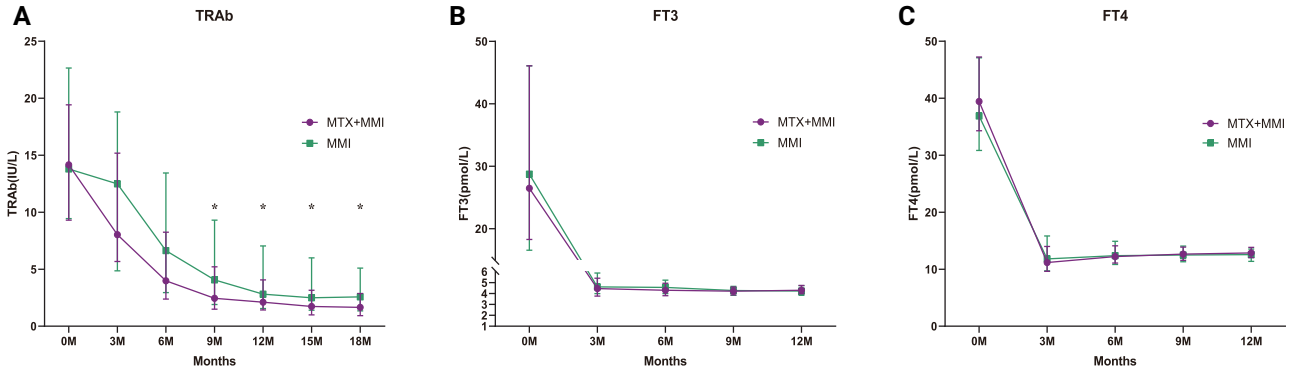

Figure 3. Effect of 18 months of the MTX with the MMI vs the MMI alone on TRAb (A), FT3 (B), and FT4 (C). Group median and quartiles. *$P < .05$, **$P < .01$.

Abbreviations: FT3, free T3; FT4, free T4; MMI, methimazole; MTX, methotrexate; TRAb, thyrotropin-related antibodies.

Table 3. The count of lymphocytes in the 2 groups of treatment at different temporal points

	MMI group	MTX + MMI group	*P*-value
0M	1.99 (1.53-2.50)	2.07 (1.70-2.58)	.454
3M	2.16 (1.62-2.50)	2.00 (1.58-2.37)	.157
6M	2.07 (1.71-2.40)	1.85 (1.55-2.30)	.055
9M	2.13 (1.72-2.54)	1.84 (1.55-2.13)	.034*
12M	2.15 (1.75-2.47)	1.72 (1.54-2.25)	.001**
15M	2.04 (1.71-2.48)	1.84 (1.55-2.27)	.039*
18M	2.13 (1.79-2.55)	1.81 (1.56-2.27)	.005**

Data are presented as median (interquartile range), units in 10^9/L.
Abbreviations: M, months; MMI, methimazole; MTX, methotrexate.
*$P < .05$. **$P < .01$.

between the intervention and various factors, we did a comprehensive analysis for subgroups based on the baseline characteristics (Fig. 4). We found that the proportional effect of supplemental MTX on discontinuation was consistent across all prespecified subgroups.

Adverse Effects

Table 4 contains a list of all the adverse events from our research. There were no MTX-related serious adverse events. The incidence of adverse events was 8.3% (6/72) and 9.7% (7/72) in the MTX with MMI group and MMI only group, respectively($P = .771$). The most frequent drug-related side effect in both groups was rash; the rate of patients with rash was 2.8% (2/72) in the MTX with MMI group and 4.2% (3/72) in the MTX only group ($P = 1.000$). There was also no difference in the occurrence of ALT elevation, which occurred in 1 and 2 patients in the MTX with MMI group and MTX only group, respectively ($P = 1.000$). The rate of patients with nausea and vomiting was 1.4% (1/72) in the MTX with MMI group and MTX only group ($P = 1.000$). The incidence of leukopenia was 1.4% (1/72) in the MTX with MMI group and MTX only group. One patient, 1.4% (1/72) in the MTX with MMI group, appeared to have an ear infection. During the study period, no adverse events leading to discontinuation were reported.

MTX Polyglutamates

We measured a total of 288 samples, including 72 samples per group at baseline and 144 samples after the last treatment during 18 months of follow-up. After the last treatment, MTXPG1-2 could be detected in patients from the MTX + MMI group, whereas they were undetectable in the MMI group, confirming the intake of MTX. According to previous studies on low-dose oral MTX in RA patients (22), the relative abundance of these MTXPGs is within the expected range (Supplementary Fig. S1) (21).

Discussion

This is the first randomized trial to test the effect of supplemental MTX on discontinuation rates from GD. We clearly showed that the addition of MTX resulted in significantly higher

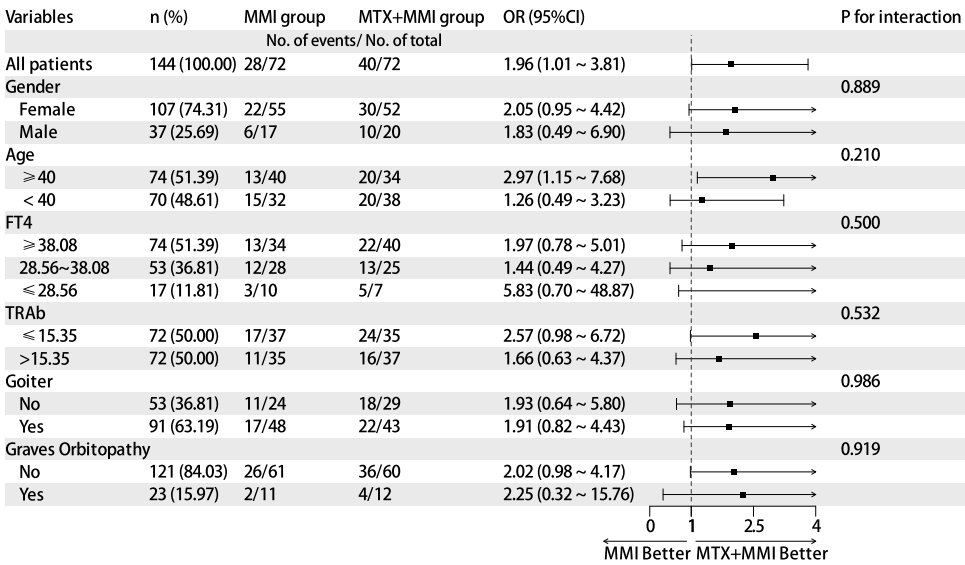

Figure 4. Forest plot of discontinuation among subgroups. The odds ratio was calculated using logistic regression model, with 95% confidence intervals presented.

Table 4. Adverse effects of the medications used in the study

Adverse effect	MMI group (n = 72)	MTX + MMI group (n = 72)	*P*-value
Any adverse events	7 (9.7)	6 (8.3)	.771
Serious adverse events	0 (0.0)	0 (0.0)	1.000
ALT elevation	2 (2.8)	1 (1.4)	
Leukopenia	1 (1.4)	1 (1.4)	
Rash	3 (4.2)	2 (2.8)	
Nausea and vomiting	1 (1.4)	1 (1.4)	
Ear infection	0 (0.0)	1 (1.4)	

Data are presented as n (%).
Abbreviations: ALT, alanine aminotransferase; MMI, methimazole; MTX, methotrexate.

discontinuation rates compared to MMI alone. In addition, patients adding MTX as an adjuvant to ATD treatment experienced a much greater decrease in TRAb levels than controls.

The TRAb level is a useful predictor for remission and relapse of patients at the end of the course of ATD therapy. In 1994, a meta-analysis including 5 prospective and 5 retrospective studies reported a decline in serum TRAb levels during ATD treatment (23). In a review by Krause et al (24), the authors demonstrated that TRAb was an important modulator of GD and concluded that blocking TRAb can be a promising combined therapeutic approach with ATDs to achieve remission in GD. The 2016 ATA guidelines suggest measurement of TRAb prior to stopping ATD at 12 to 18 months of therapy, because it helps to predict which patients can be weaned from the ATD treatment (6). Meanwhile, serum TRAb titer has been considered as a predictor of relapse at diagnosis at 12 to 18 months of ATD treatment (25). The study conducted by Cappelli et al (26) found that the rate at which TRAb levels decrease was the most accurate predictor of remission in GD, with high sensitivity and specificity. Also, our previous studies showed that TRAb level was a prognostic marker of attaining remission in GD (27). Our study found that GD patients treated with MTX in combination with MMI had a significantly higher TRAb-negative rate than controls at 12 to 18 months of the course, compared with those who received MMI only (87.5%, and 33.4%, respectively). In addition, the decrease in TRAb level was significantly faster in the MTX with MMI group compared with the MMI alone group (86.32% and 81.01%, respectively). This finding suggests that the use of MTX may contribute to higher discontinuation rates, as TRAb is considered to be the most important predictor of remission in GD.

The mechanism by which MTX reduces the key pathogenic antibody TRAb in GD is ambiguous. Both T cells and B cells are necessary for the development of GD. T cell activation and proliferation produce inflammatory factors that activate B cells to produce the pathogenic antibody TRAb (1). Additionally, the imbalance of Th17/Treg cells in peripheral blood (28) and the activation of the NF-κB signaling pathway contribute to the pathogenesis of GD (29). Studies have suggested that the effects of MTX on T cells include increased susceptibility to apoptosis, inhibition of the transcription factor NF-κB, and promotion of immunosuppressive adenosine secretion from Tregs, causing inhibition of T cell activity (30). A recent finding indicated that MTX may reduce antibody production following polysaccharide-protein antigen attacks in RA by blocking plasmablasts activation and switching memory B cells (31). Interestingly, we observed that the declining trend of TRAb is similar to the declining trend of lymphocyte count. So we hypothesize that MTX, as an immunosuppressant, may play a significant role in GD treatment by regulating T and B cell proliferation and activity, as well as reducing inflammation, potentially lowering TRAb levels.

The mechanism of MTX on thyroid function remains unclear. A study in Sweden showed that young RA patients with autoimmune thyroid diseases (AITD) had a lower initial response rate to MTX, but this effect was not found in older patients (28). Tokumaru reported a RA patient with thyroid lymphoid hyperplasia. The symptoms were alleviated after discontinuation of MTX (32). The cross-sectional study conducted by Chen et al (33) of 281 subjects showed that RA

patients treated with MTX were more likely to have lower TT4 levels than those not treated with MTX, while there was no significant difference in FT3 and FT4 levels. Our findings were consistent with no significant differences in the concentrations of FT3, FT4, and TSH between the MTX with MMI group and MMI alone group.

We established the MTX dosage based on the Chinese guidelines for the treatment of RA (17). The efficacy of MTX at a weekly dose of 10 mg has been validated in RA patients. With a tightly controlled treatment approach, MTX 10 mg/week appears to be a favorable choice for the majority of RA patients, often representing the optimal dosage (34). Our research indicates that a weekly dose of 10 mg MTX is therapeutically effective for subjects with GD, with relatively fewer side effects. In our study, only 2.8% of participants reported rash, 1.4% of participants reported leukopenia, 1.4% reported gastrointestinal reactions (nausea and vomiting), and 1.4% of participants had abnormal liver function in the MTX group. None of these adverse reactions led to treatment discontinuation. We may attribute the safe administration of MTX to low dosage.

While numerous studies and reviews in the existing literature have demonstrated the role of MTX in the management of GO, our study stands out as the first study to explore the effect of MTX on the discontinuation rate of GD. Noteworthy strengths of our investigation include a substantial sample size and clinical homogeneity, ensuring uniform patient characteristics. This homogeneity not only enhances the internal validity of the study but also diminishes the likelihood of confounding factors. Furthermore, the study's robust design incorporates the randomization of participants, a pivotal element in clinical trials to mitigate bias and ensure comparability among treatment groups. However, a notable limitation of the study is that the follow-up period was only 18 months, which may have been relatively short to evaluate long-term remission status. Additionally, the absence of blinding for both participants and researchers introduces the possibility of unintentional influences on study outcomes.

Overall, treatment of patients with GD with a novel combination therapy consisting of MTX and MMI resulted in a higher discontinuation rate and an improvement in decreased TRAb levels to homeostatic levels faster than MMI treatment alone, potentially resulting in early and long-lasting discontinuation rates in patients with GD. Furthermore, no severe side effects were observed in patients administrated with MTX. Our study suggests that MTX could be used as an efficacious adjuvant drug for patients with GD. The effects of MTX need to be further researched.

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Disclosures

The authors have nothing to disclose.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Clinical Trial Registration: Clinicaltrials.gov registration no. ChiCTR1800020153 (registered December 17, 2018).

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