Original Article

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Serial Progesterone Kinetics after Methotrexate Treatment for Persistent Pregnancy of Unknown Location and Presumed Ectopic Pregnancy: A Retrospective Cohort Study

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ABSTRACT

Purpose: Medical treatment with methotrexate (MTX) is an established option for some patients with ectopic pregnancies or persistent pregnancy of unknown location (PUL). This study evaluated whether serial progesterone kinetics provide clinically useful information beyond beta-human chorionic gonadotropin (β-hCG) kinetics during early follow-up after MTX treatment for persistent PUL or presumed ectopic pregnancy.

Methods: In this retrospective cohort study, hemodynamically stable patients treated with systemic MTX between 1 January 2025 and 1 September 2025 were screened. Serum β-hCG and progesterone were measured on day 1, day 4, and day 7, with day 1 defined as the day of MTX administration. The primary outcome was insufficient early biochemical response, defined as a β-hCG decrease of less than 15% from day 4 to day 7. The main secondary outcome was actual second-dose MTX administration.

Results: Among 73 assessed patients, 50 (68.5%) met the inclusion criteria. Seven (14%) patients had sonographic findings compatible with tubal ectopic pregnancy, and 43 (86%) were managed as persistent PUL or presumed ectopic pregnancy. Twelve patients (24.0%) had an insufficient day 4-day 7 β-hCG response, and 9 (18.0%) received a second MTX dose. Day 7 β-hCG was higher in patients with insufficient early response, whereas day 1, day 4, and day 7 progesterone concentrations did not differ significantly between response groups. In exploratory receiver operating characteristic analysis for actual second-dose MTX administration, day 7 β-hCG and lower day 4-day 7 β-hCG decline showed greater discrimination than progesterone-derived variables. Five clinically stable patients with subthreshold β-hCG decline refused additional MTX and achieved biochemical cure with close follow-up.

Conclusion: In this predominantly PUL/presumed ectopic pregnancy cohort, early β-hCG kinetics appeared more informative than serial progesterone kinetics for identifying insufficient response and second-dose MTX administration. Serial progesterone measurement was feasible but showed limited additional discriminatory value. These preliminary findings require validation in larger prospective cohorts.

Keywords: Ectopic pregnancy, methotrexate, pregnancy of unknown location, progesterone, beta-human chorionic gonadotropin

INTRODUCTION

Medical treatment with methotrexate (MTX) is an established option for hemodynamically stable patients with selected ectopic pregnancies when close follow-up is feasible.[1] Early studies of the single-dose MTX regimen evaluated patients with unruptured ectopic pregnancy and established this approach as a conservative treatment option in appropriately selected cases.[2,3] Single-dose MTX has also been evaluated in selected patients with persistent pregnancy of unknown location (PUL).[4] Comparative evidence and larger clinical series have also supported its surgery-sparing role in appropriate patients.[5,6] Safe use requires structured biochemical surveillance.[1]

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Post-MTX follow-up relies mainly on serial serum beta-human chorionic gonadotropin (β-hCG). In single-dose protocols, β-hCG is measured on day 1, day 4, and day 7. A decrease of at least 15% from day 4 to day 7 is accepted as indicating an adequate early response, whereas a smaller decrease, plateau, or increase prompts reassessment and possible additional treatment.[2,3,6] Predictive and validation studies support this day 4-day 7 β-hCG rule.[7,8] The National Institute for Health and Care Excellence recommends β-hCG measurements on days 4 and 7 after MTX, followed by weekly monitoring until a negative result; plateauing or rising β-hCG should prompt review.[1]

Although useful, the day 4-day 7 β-hCG rule is not a direct biological measure of trophoblastic regression. An adequate decrease has high positive predictive value for treatment success, but a subthreshold decrease does not always exclude cure without further MTX.[7,8] Alternative early β-hCG patterns and variation in time to resolution after MTX also support interpreting early β-hCG kinetics within the broader clinical course.[9-11] Additional biomarkers may therefore be relevant in stable patients with an intermediate response.

Progesterone differs biologically from β-hCG and has been studied as a marker of early pregnancy viability, pregnancy failure, and MTX response.[12,13] Earlier studies suggested that pretreatment progesterone may be associated with MTX success and may fall to low levels during ectopic pregnancy resolution.[12,13] However, serial progesterone kinetics measured at the same follow-up points as β-hCG remain poorly defined.

This study evaluated serial progesterone concentrations measured concurrently with β-hCG after MTX treatment in a cohort of patients with persistent PUL and presumed ectopic pregnancy. The primary objective was to assess whether early progesterone kinetics were correlated with insufficient day 4-day 7 β-hCG response. Secondary objectives were to compare progesterone patterns by second-dose MTX administration and to describe clinically stable patients with subthreshold β-hCG decline who achieved biochemical cure after declining additional MTX.

METHODS

Study Design and Setting

This retrospective cohort study was conducted in the Department of Obstetrics and Gynecology of a tertiary referral hospital in Turkey. Patients managed with systemic MTX for persistent PUL or presumed ectopic pregnancy between 1 January 2025 and 1 September 2025 were retrospectively identified from the electronic medical records and re-reviewed.

The study was approved by the University of Health Sciences Turkey, Kayseri City Hospital Non-Interventional Clinical Research Ethics Committee (approval no: 634, date: 04.11.2025), and the requirement for informed consent was waived because anonymized retrospective data were used.

Patients were eligible if they were hemodynamically stable, were initially managed with systemic MTX for persistent PUL or presumed ectopic pregnancy, and had serum β-hCG and progesterone measurements on day 1, day 4, and day 7. Day 1 was defined as the day of MTX administration. In this manuscript, day 1 refers to the calendar day on which MTX was administered; this corresponds to day 0 in some single-dose MTX protocols. We retained the day 1 terminology because it reflected the nomenclature used in our institutional records and follow-up forms. The cohort included patients with sonographic findings compatible with tubal ectopic pregnancy and patients with persistent PUL managed as presumed ectopic pregnancy. Patients were excluded if they were hemodynamically unstable, had suspected or confirmed rupture, underwent primary surgery, had ectopic fetal cardiac activity, had a contraindication to MTX, lacked day 1, day 4, or day 7 β-hCG/progesterone data, or were lost to follow-up before cure. Cesarean scar, cervical, interstitial or cornual, ovarian, and heterotopic pregnancies were excluded.

Diagnosis and treatment decisions were based on symptoms, serial β-hCG findings, transvaginal ultrasonography and, when indicated, β-hCG response after uterine evacuation. Persistent PUL was defined as no intrauterine or extrauterine gestational sac on transvaginal ultrasonography with an abnormal β-hCG pattern. After diagnostic uterine evacuation, persistent PUL or presumed ectopic pregnancy was diagnosed when the β-hCG decrease was less than 15% at follow-up. Complete biochemical cure was defined as serum β-hCG below 10 mIU/mL.

Treatment and Follow-Up Protocol

MTX was administered intramuscularly at 50 mg/m^2. Body surface area was calculated using the Mosteller formula.[14] Serum β-hCG and progesterone were measured on day 1, day 4, and day 7. After day 7, patients were followed weekly with serum β-hCG measurement until biochemical cure. Progesterone was also measured at the same available follow-up points as β-hCG. Measurements were performed in the hospital central laboratory using electrochemiluminescence immunoassays on a Cobas 8000 modular analyzer series (Roche Diagnostics, Mannheim, Germany). β-hCG was reported as mIU/mL and progesterone as ng/mL. A previously published analytical process evaluation from the same hospital central laboratory reported two-level internal quality-control coefficients of variation of 3.68% and 3.21% for β-hCG and 3.10% and 4.47% for progesterone.[15] Patient analyte concentration values were extracted from electronic medical records.

An adequate early biochemical response was defined as at least a 15% decrease in β-hCG from day 4 to day 7. A smaller decrease, plateau, or increase was considered insufficient and prompted reassessment. A second MTX dose at the same body-surface-area-based dose was recommended based on clinical judgement in patients with insufficient early response and was also considered when an initially adequate day 4-day 7 decrease was followed by plateauing or rising β-hCG during weekly follow-up. Final management incorporated hemodynamic stability, symptoms, ultrasonographic findings when available, follow-up reliability, and patient preference. Clinically stable patients

who declined a recommended second dose were followed closely with serial clinical and biochemical assessment.

Variables and Outcomes

Baseline variables were age, gravidity, parity, abortus, previous ectopic pregnancy, previous tubal surgery, assisted reproduction, intrauterine device use, vaginal bleeding, abdominal or pelvic pain, admission hemoglobin level, MTX dose, body weight, height, body surface area, day 1 β-hCG, and day 1 progesterone.

The primary outcome was insufficient early biochemical response, defined as a β-hCG decrease of less than 15% from day 4 to day 7. The main secondary outcome was actual second-dose MTX administration. A descriptive subgroup analysis included clinically stable patients with insufficient early response who declined a recommended second MTX dose and later achieved biochemical cure with close surveillance.

Progesterone variables were day 1, day 4, and day 7 concentrations; percentage changes from day 1 to day 4 and from day 4 to day 7; and day 7 progesterone below 1.5 ng/mL. Percentage change was calculated as the later value minus the earlier value divided by the earlier value and multiplied by 100; negative values indicate a decrease. The 1.5 ng/mL threshold was selected because previous work reported this level as associated with resolution of ectopic gestation.[13]

Statistical Analysis

Continuous variables are summarized as median and interquartile range (IQR), and categorical variables as number and percentage. Due to the small sample size and non-normal biomarker distributions, Mann-Whitney U and Fisher's exact tests were used. Spearman correlation assessed associations between β-hCG and progesterone at corresponding time points and between early percentage changes.

Exploratory receiver operating characteristic (ROC) analyses were restricted to actual second-dose MTX administration. ROC analysis was not performed for the primary outcome using day 4-day 7 β-hCG decline or closely related variables, because the primary outcome was defined by this same interval change. Area under the curve (AUC) values were reported with bootstrap 95% confidence intervals using 2000 resamples. ROC findings were interpreted only as exploratory and hypothesis-generating because actual second-dose MTX administration was a management-dependent endpoint. Multivariable regression was not performed because the limited number of outcome events would make adjusted estimates unstable. Across Tables 1 and 2, 44 unadjusted group comparisons were performed. No adjustment was made for multiple comparisons; therefore, isolated statistically significant findings from unadjusted analyses should be interpreted cautiously because of the risk of false-positive results. Two-sided *p* values below 0.05 were considered statistically significant in unadjusted analyses.

RESULTS

During the study period, 73 potentially eligible patients were identified. Eight were excluded because they underwent primary surgical management without MTX, seven because day 1, day 4, or day 7 β-hCG/progesterone data were missing, and eight because they discontinued follow-up before biochemical cure. The final cohort included 50 (68.5%) patients (Figure 1).

Of these 50, seven had sonographic findings compatible with tubal ectopic pregnancy, while 43 were managed as persistent PUL or presumed ectopic pregnancy; six of these were diagnosed after an insufficient β-hCG decrease following uterine evacuation. Nine patients (18.0%) received a second MTX dose, and 41 (82.0%) achieved biochemical cure after a single dose. Twelve patients (24.0%) had an insufficient day 4-day 7 β-hCG response; seven received a second MTX dose, whereas five clinically stable patients declined additional MTX and achieved biochemical cure with close follow-up. The remaining two second-dose patients initially had an adequate day 4-day 7 β-hCG decrease but later developed plateauing or rising β-hCG during weekly follow-up. Among the final 50 patients followed until biochemical cure, no patient developed tubal rupture, hemodynamic instability, or needed emergency surgery or hospitalization for clinical deterioration during post-MTX follow-up.

Baseline and early biomarker characteristics according to the primary outcome are shown in Table 1. Patients with insufficient early β-hCG response had higher day 7 β-hCG values than those with adequate response. Day 1, day 4, and day 7 progesterone concentrations were not significantly different between groups. Both absolute and percentage progesterone changes are presented in Table 1. Day 1-day 4 progesterone percentage change differed between groups, with a greater early progesterone decrease in patients with adequate β-hCG response; however, this exploratory finding should be interpreted cautiously because multiple unadjusted comparisons were performed.

Baseline characteristics and early β-hCG/progesterone kinetics according to actual second-dose MTX administration are presented in Table 2. Median age, baseline day 1 β-hCG, and day 1 progesterone were similar between the single-dose cure and second-dose groups. Abortus count differed between groups, but this isolated finding should be interpreted cautiously because of the small second-dose group and exploratory comparisons. The second-dose group had higher day 7 β-hCG values and a markedly lower day 4-day 7 β-hCG decline. In contrast, progesterone concentrations and early progesterone percentage changes did not differ significantly between groups.

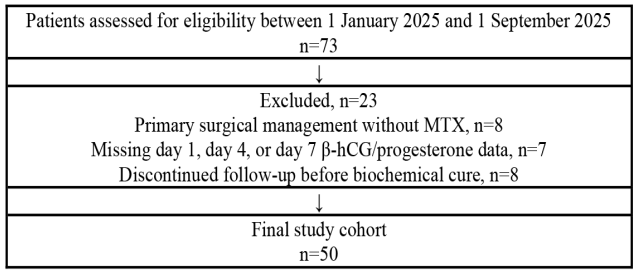

Figure 1. Flow diagram of patient selection

β-hCG: Beta-human chorionic gonadotropin, MTX: Methotrexate

Table 1. Baseline and early biomarker characteristics according to early β-hCG response

Variable	Adequate early β-hCG response (n=38)	Insufficient early β-hCG response (n=12)	*p* value
Age, years	32 (25-33)	28 (25-34)	0.802
Gravidity	3 (2-3)	2 (2-4)	0.735
Parity	1 (0-2)	1 (0-2)	0.629
Abortus	0 (0-1)	0 (0-1)	0.430
Admission hemoglobin, g/dL	12.6 (12.1-13.3)	12.6 (11.7-13.3)	0.682
MTX dose, mg	85 (81-92)	84 (77-86)	0.198
Body surface area, m^2	1.74 (1.63-1.89)	1.72 (1.57-1.85)	0.481
Day 1 β-hCG, mIU/mL	730 (112-1912)	838 (472-2094)	0.329
Day 4 β-hCG, mIU/mL	420 (97-1703)	1071 (576-2238)	0.143
Day 7 β-hCG, mIU/mL	111 (44-736)	1206 (642-2408)	0.002
Day 1 progesterone, ng/mL	3.73 (1.00-8.10)	3.91 (2.80-4.71)	0.982
Day 4 progesterone, ng/mL	2.41 (0.76-6.62)	4.22 (2.42-6.09)	0.358
Day 7 progesterone, ng/mL	1.80 (0.33-4.29)	2.97 (1.70-4.05)	0.195
Day 1-day 4 progesterone absolute change, ng/mL	-0.40 (-2.42 to 0.71)	0.03 (-0.78 to 1.19)	0.192
Day 4-day 7 progesterone absolute change, ng/mL	-0.65 (-2.37 to 0.11)	-0.92 (-1.72 to -0.47)	0.420
Day 1-day 4 progesterone change, %	-44.8 (-70.8 to 16.3)	2.0 (-17.2 to 29.9)	0.042
Day 4-day 7 progesterone change, %	-31.7 (-55.7 to 25.9)	-29.1 (-36.6 to -19.6)	0.776
Day 7 progesterone <1.5 ng/mL	17/38 (44.7)	2/12 (16.7)	0.100
Actual second-dose MTX	2/38 (5.3)	7/12 (58.3)	<0.001

Data are median (IQR) or n/N (%). *p* values were calculated with the Mann-Whitney U test or Fisher's exact test. Progesterone absolute change was calculated as the later value minus the earlier value. Progesterone percentage change was calculated as the later value minus the earlier value divided by the earlier value and multiplied by 100; negative values indicate a decrease from the earlier to the later time point
IQR: Interquartile range, MTX: Methotrexate, β-hCG: Beta-human chorionic gonadotropin

Table 3 presents three clinical subgroups: adequate early β-hCG response with single-dose cure; insufficient response with second-dose refusal and subsequent cure; and actual second-dose MTX. The observed insufficient-response subgroup had higher day 7 β-hCG than the adequate-response single-dose group but still achieved biochemical cure without further MTX. Day 7 progesterone was not uniformly low in this subgroup.

Exploratory ROC analyses for actual second-dose MTX administration are shown in Table 4. Day 7 β-hCG showed moderate discrimination, whereas a smaller or negative day 4-day 7 β-hCG decline showed good discrimination for actual second-dose MTX administration. In contrast, progesterone-derived variables showed weak or limited discrimination. Once again, these ROC findings should be interpreted cautiously because only nine (18%) patients received a second MTX dose and because second-dose administration was influenced by clinical judgment, follow-up findings, and patient preference rather than by biomarker values alone.

Spearman analysis showed moderate positive correlations between β-hCG and progesterone measured at the same time points on day 4 (rho=0.52, $p<0.001$) and day 7 (rho=0.55, $p<0.001$), whereas the day 1 correlation was weak and was not statistically significant in unadjusted analysis (rho=0.27, $p=0.055$). In contrast, day 4-day 7 β-hCG decline was not meaningfully correlated with day 4-day 7 progesterone change (rho=0.11, $p=0.459$). day 7 β-hCG was also not meaningfully correlated with day 4-day 7 progesterone change (rho=0.07, $p=0.641$).

DISCUSSION

In this retrospective cohort of 50 patients treated with MTX for persistent PUL or presumed ectopic pregnancy, early β-hCG kinetics appeared more informative than serial progesterone measurements for identifying patients with insufficient early biochemical response and those who ultimately received a second MTX dose. Although a greater day 1-day 4 progesterone decline was observed in patients with an adequate day 4-day 7 β-hCG response, progesterone concentrations at days 1, 4, and 7 and the day 4-day 7 progesterone change showed limited discriminatory performance overall. In practical terms, these findings suggest that progesterone may reflect some aspects of endocrine regression after MTX, but it does not currently provide decision-making value comparable to β-hCG during routine post-treatment surveillance.

Several physiological mechanisms may explain why progesterone and β-hCG kinetics were not closely parallel after MTX. β-hCG is produced by trophoblastic tissue and is therefore more directly linked to trophoblastic burden and post-treatment trophoblastic regression. Progesterone, in contrast, reflects corpus luteum function as well as pregnancy viability, and its secretion during early pregnancy

Table 2. Baseline characteristics and early β-hCG/progesterone kinetics according to actual second-dose MTX administration

Variable	Single-dose cure (n=41)	Second-dose MTX (n=9)	*p*
Age, years	31 (25-33)	33 (25-34)	0.310
Gravidity	3 (2-3)	3 (2-4)	0.217
Parity	1 (0-2)	1 (0-2)	0.865
Abortus	0 (0-1)	1 (0-2)	0.019
Admission hemoglobin, g/dL	12.6 (11.9-13.3)	12.8 (12.0-13.7)	0.527
MTX dose, mg	84 (81-92)	84 (74-86)	0.210
Body weight, kg	65 (61-80)	68 (60-72)	0.527
Body surface area, m^2	1.70 (1.62-1.90)	1.74 (1.65-1.81)	0.640
Day 1 β-hCG, mIU/mL	785 (133-1892)	676 (595-2169)	0.189
Day 1 progesterone, ng/mL	3.66 (1.30-8.11)	4.29 (2.99-5.23)	0.743
Previous ectopic pregnancy	6/41 (14.6)	2/9 (22.2)	0.623
Previous tubal surgery	3/41 (7.3)	0/9 (0.0)	1.000
Assisted reproduction/IVF	6/41 (14.6)	1/9 (11.1)	1.000
Intrauterine device use	4/41 (9.8)	0/9 (0.0)	1.000
Vaginal bleeding	29/41 (70.7)	9/9 (100.0)	0.092
Abdominal/pelvic pain	32/41 (78.0)	9/9 (100.0)	0.183
Day 4 β-hCG, mIU/mL	520 (110-1716)	878 (674-2239)	0.207
Day 7 β-hCG, mIU/mL	157 (44-739)	1041 (770-2409)	0.016
Day 4-day 7 β-hCG decline, %	50.8 (26.1-59.9)	-7.6 (-15.9 to 12.5)	<0.001
Insufficient day 4-day 7 β-hCG response (<15% decline)	5/41 (12.2)	7/9 (77.8)	<0.001
Day 4 progesterone, ng/mL	3.20 (0.80-7.62)	4.10 (2.12-5.90)	1.000
Day 7 progesterone, ng/mL	2.45 (0.47-5.50)	2.49 (1.64-3.85)	0.950
Day 1-day 4 progesterone change, %	-31.9 (-68.3 to 26.3)	0.7 (-35.6 to 18.9)	0.350
Day 4-day 7 progesterone change, %	-30.5 (-52.0 to 2.7)	-34.7 (-38.4 to -26.5)	0.900
Day 7 progesterone <1.5 ng/mL	17/41 (41.5)	2/9 (22.2)	0.452

Data are median (IQR) or n/N (%). *p* values were calculated with the Mann-Whitney U test or Fisher's exact test. Negative β-hCG decline values indicate an increase from day 4 to day 7. Two patients in the second-dose MTX group had an adequate day 4-day 7 β-hCG decrease but received a second dose because of plateauing or rising β-hCG during subsequent weekly follow-up
IQR: Interquartile range, MTX: Methotrexate, β-hCG: Beta-human chorionic gonadotropin, IVF: In vitro fertilization

Table 3. Clinical subgroups according to early β-hCG response and treatment course

Variable	Adequate response with single-dose cure (n=36)	Insufficient response, observed after second-dose refusal (n=5)	Actual second-dose MTX (n=9)
Day 1 β-hCG, mIU/mL	628 (103-1899)	1040 (340-1083)	676 (595-2169)
Day 4 β-hCG, mIU/mL	420 (93-1582)	1264 (283-1716)	878 (674-2239)
Day 7 β-hCG, mIU/mL	111 (44-733)	1371 (257-1479)	1041 (770-2409)
Day 4-day 7 β-hCG decline, %	52.6 (42.2-64.3)	9.2 (7.6-10.7)	-7.6 (-15.9 to 12.5)
Day 1 progesterone, ng/mL	3.73 (1.08-8.18)	3.53 (2.22-4.54)	4.29 (2.99-5.23)
Day 4 progesterone, ng/mL	2.41 (0.79-6.62)	4.46 (3.20-8.59)	4.10 (2.12-5.90)
Day 7 progesterone, ng/mL	1.80 (0.33-4.67)	4.03 (2.75-7.55)	2.49 (1.64-3.85)
Day 4-day 7 progesterone change, %	-31.7 (-57.8 to 17.8)	-14.1 (-21.4 to -12.1)	-34.7 (-38.4 to -26.5)
Day 7 progesterone <1.5 ng/mL	16/36 (44.4)	1/5 (20.0)	2/9 (22.2)

Data are median (IQR) or n/N (%). The observed insufficient-response subgroup included clinically stable patients who declined an additional MTX dose and achieved complete biochemical cure with close surveillance
IQR: Interquartile range, MTX: Methotrexate, β-hCG: Beta-human chorionic gonadotropin

Table 4. Exploratory ROC analysis for actual second-dose MTX administration

Predictor	AUC (95% CI)
Day 4 β-hCG	0.64 (0.46-0.80)
Day 7 β-hCG	0.76 (0.57-0.91)
Lower day 4-day 7 β-hCG decline	0.88 (0.67-1.00)
Day 7 progesterone	0.51 (0.33-0.67)
Higher day 1-day 4 progesterone change	0.60 (0.43-0.78)
Higher day 4-day 7 progesterone change	0.51 (0.35-0.68)

AUC values are exploratory because of the small number of second-dose MTX events. Progesterone change was calculated as the later value minus the earlier value divided by the earlier value and multiplied by 100; higher values therefore indicate less decline or an increase
MTX: Methotrexate, β-hCG: Beta-human chorionic gonadotropin, ROC: Receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval

is supported by hCG. Thus, progesterone is not biologically independent of β-hCG. Nevertheless, serum progesterone has been reported to reach low levels earlier than β-hCG during ectopic pregnancy resolution.[13] Previous studies suggesting prognostic value for progesterone provided the rationale for evaluating whether its serial kinetics could add information to routine β-hCG monitoring.[12,13] In the present study, however, serial progesterone changes showed limited additional discriminatory value, supporting β-hCG as the more informative marker for post-MTX surveillance.

The most important comparator for the present study is the prospective Human Reproduction study by Kirk et al.,[8] which remains one of the most informative post-MTX biomarker studies in this field. In that study, serum β-hCG and progesterone were measured on days 1, 3, 4, 5, and 7 in 69 women treated with single-dose MTX, and the conventional day 4-day 7 β-hCG rule correctly predicted outcome in 90.3% of cases, with sensitivity 93.0% and specificity 84.2%.[8] Importantly, Kirk et al.[8] also showed that progesterone concentrations at several follow-up time points differed between successful and unsuccessful outcomes, whereas progesterone changes between days did not. Our findings are directionally consistent with that observation. In our cohort, progesterone showed only a limited exploratory signal, mainly in the day 1-day 4 interval, but serial progesterone kinetics did not outperform β-hCG and did not clearly distinguish patients who required additional MTX. Taken together, the two studies suggest that progesterone may have a biological association with the treatment course, yet its serial changes appear less robust than β-hCG kinetics for clinical decision-making after MTX.

The comparison with Kirk et al.[8] is also informative because our cohort differs in clinically relevant ways. Their population included tubal ectopic pregnancies, interstitial ectopic pregnancies, persisting PUL, and persistent trophoblastic tissue, whereas most patients in our cohort were managed as persistent PUL or presumed ectopic pregnancy rather than sonographically confirmed tubal ectopic pregnancy. In addition, our analyses focused not only on inadequate day 4-day 7 β-hCG response but also on actual second-dose MTX administration, a pragmatic endpoint that reflects real-world management after reassessment. This distinction is important, because in routine care the decision to repeat MTX is influenced not only by biochemical behavior but also by symptoms, ultrasound findings, later β-hCG trends, follow-up reliability, and patient preference. Progesterone may therefore be too indirect a marker to function as a stand-alone determinant of second-dose treatment in this setting.

Kirk et al.[8] also highlighted a clinically relevant gray zone that parallels our own data. In their cohort, five women had successful single-dose outcomes without a documented >15% day 4-day 7 β-hCG decline; two of these women had suboptimal declines of 8% and 13%, refused a second MTX dose, and still resolved successfully.[8] Our study reproduced this clinically important gray zone in a contemporary real-world setting: five clinically stable patients with a subthreshold day 4-day 7 β-hCG decline did not accept additional MTX and nevertheless achieved biochemical cure with close follow-up. However, these patients represented a highly selected subgroup. They were clinically stable, had no evidence of rupture or hemodynamic compromise, were considered suitable for close surveillance by the treating clinicians, and were willing to comply with follow-up. Therefore, this observation should not be interpreted as evidence that a subthreshold day 4-day 7 β-hCG decline is generally benign. Rather, it supports interpreting the 15% threshold as a safety-oriented trigger for reassessment, counseling, and individualized management, not as an absolute biological boundary proving that cure without further MTX is impossible.

The broader literature also supports keeping β-hCG, rather than progesterone, at the center of post-MTX follow-up. Earlier studies suggested that pretreatment progesterone may be associated with MTX success and that progesterone may fall to a low threshold during ectopic pregnancy resolution.[12,13] However, those studies addressed a somewhat different question from the present one, being more informative about baseline prognosis or endocrine resolution than about treatment escalation during the first post-MTX week. By contrast, more recent work has focused on refining early β-hCG -based prediction. Skubisz et al.[9] and Mackenzie et al.[11] examined whether earlier post-treatment β-hCG changes could predict MTX success before day 7, whereas Davenport et al.[10] showed that time to resolution after MTX remains variable even among tubal ectopic pregnancies. Considered together with Kirk et al.,[8] these studies suggest that the field has continued to improve β-hCG -based risk stratification, but no alternative biomarker has clearly superseded the conventional day 4-day 7 framework. Our findings extend that message by showing that serial progesterone, at least when measured on days 1, 4, and 7, does not yet fill that gap.

The clinical implication of the present study is therefore not to dilute established MTX surveillance algorithms, but to refine how their results are interpreted. An insufficient day 4-day 7 β-hCG decline should still prompt reassessment and consideration of further treatment, because this remains the most widely validated early safety signal.[7,8] However, the final decision should continue to integrate the absolute β-hCG level, the direction of subsequent β-hCG change, symptoms,

ultrasonographic findings, and the patient's ability and willingness to comply with close follow-up. In this intermediate zone, progesterone remains a research marker rather than a practice-changing test. Conversely, an initially adequate day 4-day 7 β-hCG decline should not end follow-up, because some patients may later plateau or rise and still require additional MTX, as observed in our cohort.

Study Limitations

This study has several limitations. Its retrospective single-center design and modest sample size limited precision, particularly for the insufficient-response, second-dose MTX, and refusal subgroups. The low number of outcome events also limited statistical power. Therefore, the absence of significant differences in most progesterone-related parameters may reflect a type II error rather than a definite absence of association. As most patients had persistent PUL or presumed ectopic pregnancy, the findings should be interpreted within this mixed and predominantly PUL/presumed ectopic pregnancy population and may not translate directly to cohorts composed predominantly of sonographically confirmed tubal ectopic pregnancies. In addition, baseline β-hCG concentrations were relatively low, which may have contributed to the high overall treatment success rate and may limit generalizability to higher-risk patients with greater trophoblastic burden. We also could not perform robust multivariable adjustment for baseline β-hCG level, ultrasound characteristics, pre-treatment β-hCG trajectory, previous ectopic pregnancy, clinical presentation, or other potentially relevant confounders. Actual second-dose MTX administration was clinically meaningful but partly management-dependent, because it was influenced by symptoms, ultrasound findings, later β-hCG trends, follow-up reliability, clinician judgment, and patient preference. Finally, multiple unadjusted comparisons were performed without adjustment for multiple comparisons; therefore, isolated statistically significant findings from unadjusted analyses, including the day 1-day 4 progesterone change, should be considered exploratory.

Nevertheless, the study has an important strength: progesterone was measured at the same scheduled time points as routine β-hCG monitoring, allowing a direct head-to-head comparison of serial biomarker kinetics in real practice. These data support the central role of β-hCG, confirm that the <15% subgroup is clinically heterogeneous, and suggest that serial progesterone still lacks sufficient discriminatory performance to modify routine post-MTX management.

CONCLUSION

In conclusion, in this predominantly persistent PUL/presumed ectopic pregnancy cohort treated with MTX, early β-hCG kinetics appeared more informative than progesterone kinetics for identifying insufficient response and second-dose MTX administration. Serial progesterone measurement was feasible but did not show clear additional discriminatory value in this small retrospective cohort. However, because of the limited sample size, low number of outcome events, PUL-predominant population, and limited statistical power, these findings should be considered preliminary and hypothesis-generating. Larger prospective studies, particularly in cohorts with sonographically confirmed tubal ectopic pregnancy and higher baseline β-hCG levels, are needed before progesterone can be recommended as a supportive marker in post-MTX surveillance.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, Kayseri City Hospital Non-Interventional Clinical Research Ethics Committee (approval no: 634, date: 04.11.2025).

Informed Consent: Due to the retrospective design and the use of anonymized clinical data, the requirement for informed consent was waived by the ethics committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Footnotes

Authorship Contributions

Surgical and Medical Practices: C.Ü., H.A., H.G., Concept: C.Ü., H.A., Design: C.Ü., H.A., G.T.E., Data Collection or Processing: C.Ü., H.G., M.G., E.G., Analysis or Interpretation: C.Ü., H.A., G.T.E., Literature Search: C.Ü., G.T.E., Writing: C.Ü., H.A., H.G., M.G., E.G., G.T.E.

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REFERENCES

1. 2019 exceptional surveillance of ectopic pregnancy and miscarriage: diagnosis and initial management (NICE guideline NG126) [Internet]. London: National Institute for Health and Care Excellence (NICE); 2019.
2. Stovall TG, Ling FW, Gray LA. Single-dose methotrexate for treatment of ectopic pregnancy. Obstet Gynecol. 1991;77(5):754-757.
3. Stovall TG, Ling FW. Single-dose methotrexate: an expanded clinical trial. Am J Obstet Gynecol. 1993;168(6 Pt 1):1759-1765.
4. Wu J, Ludlow JP, De Vries B, Black K, Beale P. Single-dose methotrexate treatment for ectopic pregnancy and pregnancy of unknown location and progesterone as a predictor of success. Aust N Z J Obstet Gynaecol. 2014;54(5):469-474.
5. Barnhart KT, Gosman G, Ashby R, Sammel M. The medical management of ectopic pregnancy: a meta-analysis comparing single-dose and multidose regimens. Obstet Gynecol. 2003;101(4):778-784.
6. Lipscomb GH, Bran D, McCord ML, Portera JC, Ling FW. Analysis of three hundred fifteen ectopic pregnancies treated with single-dose methotrexate. Am J Obstet Gynecol. 1998;178(6):1354-1358.

7. Lipscomb GH, McCord ML, Stovall TG, Huff G, Portera SG, Ling FW. Predictors of success of methotrexate treatment in women with tubal ectopic pregnancies. N Engl J Med. 1999;341(26):1974-1978.
8. Kirk E, Condous G, Van Calster B, et al. A validation of the most commonly used protocol to predict the success of single-dose methotrexate in the treatment of ectopic pregnancy. Hum Reprod. 2007;22(3):858-863.
9. Skubisz MM, Dutton P, Duncan WC, Horne AW, Tong S. Using a decline in serum hCG between days 0-4 to predict ectopic pregnancy treatment success after single-dose methotrexate: a retrospective cohort study. BMC Pregnancy Childbirth. 2013;13:30.
10. Davenport MJ, Lindquist A, Brownfoot F, Pritchard N, Tong S, Hastie R. Time to resolution of tubal ectopic pregnancy following methotrexate treatment: a retrospective cohort study. PLoS One. 2022;17(5):e0268741.
11. Mackenzie SC, Moakes CA, Doust AM, et al. Early (days 1-4) post-treatment serum hCG level changes predict single-dose methotrexate treatment success in tubal ectopic pregnancy. Hum Reprod. 2023;38(7):1261-1267.
12. Ransom MX, Garcia AJ, Bohrer M, Corsan GH, Kemmann E. Serum progesterone as a predictor of methotrexate success in the treatment of ectopic pregnancy. Obstet Gynecol. 1994;83(6):1033-1037.
13. Saraj AJ, Wilcox JG, Najmabadi S, Stein SM, Johnson MB, Paulson RJ. Resolution of hormonal markers of ectopic gestation: a randomized trial comparing single-dose intramuscular methotrexate with salpingostomy. Obstet Gynecol. 1998;92(6):989-994.
14. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med. 1987;317(17):1098.
15. Öztürk KN, Ekici Günay N, Koçer D. Six Sigma and analytical process in biochemistry laboratory of Kayseri City Hospital. J Turk Clin Biochem. 2024;22(2):72-82.