Risk Factors for Methotrexate-Induced Abnormal Laboratory Monitoring Results in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine risk factors for methotrexate (MTX)-induced hepatic and hematologic laboratory abnormalities in patients with rheumatoid arthritis (RA).

Methods. Measurements of aspartate aminotransferase (AST), white blood cell counts, and platelet counts were collected in a database of patients with RA receiving MTX from 1991 through 2002. Potential risk factors for toxicity were recorded on each patient.

Results. Four hundred and eighty-one patients were followed for 2,323 person-years of MTX exposure. MTX was discontinued permanently because of abnormal laboratory test results in 22 patients (4.6%), the majority of whom (17/22, 77%) had elevated AST values. The body mass index (BMI) was significantly higher in those patients where MTX was permanently discontinued than in those in whom it was not ($p < 0.03$). Independent predictors of a significantly higher percentage of abnormal AST values were lack of folate supplementation ($p < 0.001$) and untreated hyperlipidemia ($p < 0.02$). Of the 17 patients in whom MTX was discontinued permanently because of an elevated AST value, 11/17 (65%) had either lack of folate supplementation or untreated hyperlipidemia. Hypoalbuminemia correlated independently with an increased percentage of abnormal platelet counts ($p < 0.03$).

Conclusion. Lack of folate supplementation, untreated hyperlipidemia, and elevated BMI identified patients receiving MTX at risk for transaminase elevation, and low serum albumin was a risk factor for thrombocytopenia. Nonalcoholic fatty liver disease could be the underlying risk factor for transaminase elevation in patients with hyperlipidemia and obesity. (J Rheumatol 2004;31:1727–31)

Key Indexing Terms:
METHOTREXATE RHEUMATOID ARTHRITIS DRUG MONITORING RISK FACTORS

Methotrexate (MTX) is a widely used and effective disease modifying antirheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA). The American College of Rheumatology (ACR) has published guidelines for monitoring RA patients for MTX-induced hepatic toxicity[1]. When monitoring patients every 4 to 6 weeks, the number of abnormal serum aspartate aminotransferase (AST) values correlates positively with histologic progression in Roenigk grade[2]. The goal of following transaminase levels is to identify patients at risk for developing cirrhosis while receiving MTX.

The literature suggests that risk factors for hepatic fibrosis in patients with RA taking MTX may include alcohol consumption[2], lack of folate supplementation[3], obesity[2,4], total MTX dose and duration of therapy[5], presence of diabetes mellitus[4], and late age at first use of MTX[5]. Risk factors reported for hematologic MTX toxicity include renal impairment, hypoalbuminemia, low serum folic acid levels, drug interactions (trimethoprim-sulfamethoxazole), events causing increased myelopoietic cell turnover (infection, hemorrhage), presence of extra-articular disease, MTX dose, age, history of alcoholism, and elevated mean corpuscular volume[6,7].

Ideally, clinicians could identify patients receiving MTX at risk for significantly abnormal laboratory monitoring measurements by the presence or absence of a number of patient-specific variables. In this way, patients at risk for MTX toxicity could be monitored more closely.

To further define the risk factors for abnormal laboratory monitoring tests while receiving MTX and to determine whether these influence the need to maintain, decrease, or discontinue MTX, we reviewed our regional cohort of patients with RA from 1991-2002.

MATERIALS AND METHODS

Study design. This retrospective cohort study was approved by the Mayo Foundation Institutional Review Board. All patients included had given permission for their charts to be used for the purpose of medical research. Sex, date of birth, date of RA diagnosis, date of first MTX use, total number of months monitored, number of monitoring measurements, mean MTX dose, use of folate, number of abnormal AST values (defined as higher than

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the upper limit of normal, usually 31 U/l), number of abnormal leukocyte counts (defined as less than 3.5×10^9/l), and number of abnormal platelet counts (defined as less than 150×10^9/l) were abstracted from the chart. Smoking status, presence of rheumatoid factor, history of chronic viral hepatitis, concomitant DMARD or nonsteroidal antiinflammatory drug therapy, presence of hyperlipidemia (defined as low density lipoprotein cholesterol level higher than 160 mg/dl or triglycerides higher than 200 mg/dl), use of lipid-lowering drugs, presence of diabetes mellitus, use of insulin, use of alcohol (before and during MTX therapy), mean serum albumin, mean serum creatinine, and body mass index (BMI) were recorded. Patients taking a multivitamin were considered to have folate supplementation, although the usual folate supplementation in this practice is 1 mg/day. Results of liver ultrasonography and liver biopsy were noted. The rheumatologists in our practice did not have a uniform standard for deciding when to maintain, stop, or reduce the dose of MTX based on abnormal laboratory data. Such decisions were made by individual clinicians attempting to weigh the risks and benefits for each individual patient, but clearly there was heterogeneity in practice style.

Statistics. JMP version 4.0.4 (SAS Institute, Inc.) statistical software was used. The Wilcoxon rank sum test was used to assess categorical risk factors with nonparametric continuous outcomes. Continuous risk factors with continuous outcomes were assessed by linear regression analysis with application of the method of least squares. Multivariate linear regression analysis was performed using the method of least squares, after using a stepwise model selection with a list of potential predictors. To test the difference between means with unequal variances, the Welch analysis of variance F test was used. The Pearson chi-square test was used to fit categorical responses to categorical factors. A p value of less than 0.05 was used for significance.

RESULTS

Four hundred and eighty-one patients were identified for this study and their characteristics are displayed in Table 1. These patients represent 2323 person-years of MTX monitoring data and 17,849 monitoring measurements (each including a complete blood count and an AST) performed.

Of the patients, 177 (37%) had no AST abnormalities and 304 (63%) has at least 1 above the normal range. Of the patients with AST abnormalities, 251 patients had values 1-2 × normal (32-62 U/l), 37 had values 2-3 × normal (63-94 U/l), 9 had values 3-4 × normal (95-126 U/l), 4 had values 4-5 × normal (127-155 U/l), 1 had values 5-6 × normal, 2 had values 6-7 × normal, and 0 had values > 7 × normal. Leukopenia was present at least once in 51 (11%) patients, while significant thrombocytopenia (< 100×10^9/l) occurred in only 9 (1.8%) patients.

Table 1. Patient characteristics.

Female, n (%)	334 (69)
Mean age (SD) at RA diagnosis, yrs	47 (±14)
Mean age (SD) at MTX start, yrs	55 (±13)
Rheumatoid factor, n MTX	
Positive	312
Negative	92
Unknown	77
Mean (SD) monitoring tests per patient, n	37 (24)
Mean (SD) mo monitored per patient, n	58 (38)
Mean (SD) measurements per year	8.4 (3.0)
Mean (SD) MTX dose, mg/wk	12.6 (4.3)

Of the 5 patients who had liver biopsies, 3 were obese (BMI > 30 kg/m^2). One of the obese patients was also diabetic, none had hyperlipidemia, and one had lack of folate supplementation. Four of 5 patients had liver ultrasound, all of which suggested fatty infiltration of the liver. All biopsy specimens showed steatosis, 3 showed mild steatohepatitis, and one showed minimal fibrosis (this patient had a history of alcoholism and was the only patient of the 5 in whom MTX treatment was stopped because of biopsy results).

In 22 patients (4.6%), MTX was permanently discontinued because of abnormal laboratory test results, a cumulative incidence of 95 patients per 10,000 person-years of observation (Table 2). In the majority of these cases (17/22, 77%), MTX was stopped for elevated AST values (mean highest of 86 U/l). The mean BMI ± SD was significantly higher (32.1 ± 6.9 kg/m^2) in patients who required MTX to be permanently stopped compared with those who did not (28.5 ± 6.0 kg/m^2, $p < 0.03$), and this group was significantly less likely to be receiving folate supplementation ($p < 0.001$).

Sixty-seven patients had MTX stopped temporarily or the dose reduced at least once for abnormal laboratory test results. Of these, 11 eventually had MTX discontinued permanently, becoming part of the group described above. Elevated AST values were the reason for holding MTX or reducing the dose in 57 (85%) of 67 patients, and depressed leukocyte count was the cause in the other 10 (15%) patients. Serum albumin was followed routinely as a marker of liver synthetic function. Hypoalbuminemia was more prevalent in this group of 67 patients compared to the remainder ($p < 0.04$).

Because so few liver biopsies were obtained and only one showed any degree of fibrosis, the percentage of abnormal AST results (number of abnormal measurements/total

Table 2. Laboratory monitoring abnormalities prompting physician intervention.

	Patients, n	Incidence (Cases per 10,000 Person-years)	Associated Risk Factor
Permanent MTX discontinuation	22	95	Lack of folate ($p < 0.001$) Increased BMI ($p < 0.03$)
Reason			
Elevated AST	17	73	
Leukopenia	3	13	
Thrombocytopenia	2	9	
MTX dose temporarily held or decreased	67	288	Hypoalbuminemia ($p < 0.04$)
Elevated AST	57	245	
Leukopenia	10	43	
Thrombocytopenia	0	0	

AST: aspartate aminotransferase; BMI: body mass index.

number of measurements × 100) was used as a surrogate marker for potential liver damage caused by MTX. The percentage of abnormal AST values has previously been shown to be significantly associated with worsening hepatic histologic grade according to the Roenigk system from Grade I to Grade IIIA[8], and thus may be a useful surrogate for potential liver damage from MTX.

Predictors of a significantly higher percentage of abnormal AST values using a univariate analysis are shown in Table 3 and include lack of folate supplementation ($p < 0.001$), increased creatinine ($p < 0.03$), presence of untreated hyperlipidemia ($p < 0.02$), and male sex ($p < 0.04$). Male patients were less likely to be taking folate than female patients ($p < 0.004$) and were more likely to consume alcohol ($p < 0.02$). In a multivariate analysis, lack of folate and untreated hyperlipidemia were independent risk factors for a high percentage of abnormal AST values (Table 4). This model only accounts for about 7% of the variance of the percentage of abnormal AST values, so clearly other factors are involved. Of the 17 patients in whom MTX was stopped permanently for elevated AST values, 11/17 (65%) had either lack of folate supplementation, untreated hyperlipidemia, or both. Folate use has become much more common in recent years at our institution. For example, of the 28 patients who started MTX treatment in 1990, 10 (36%) were not given folate, whereas of the 30 patients started in 2000, only 1 (3%) was not given folate.

Not surprisingly, patients with higher percentages of abnormal AST values were followed more frequently ($p < 0.0001$), and because of a tendency for these patients to have the drug discontinued, they were monitored for fewer months ($p < 0.04$). None of the analyzed risk factors correlated with a higher percentage or absolute number of abnormal leukocyte determinations. Patients with an increased percentage of abnormal leukocyte counts were followed more frequently ($p < 0.01$). Hypoalbuminemia ($p < 0.03$) and male sex ($p < 0.02$) were risk factors for a higher percentage of abnormal platelet counts, and there was a trend with increasing creatinine ($p = 0.066$). In a multivariate analysis, only hypoalbuminemia remained significant.

Table 3. Univariate analysis of risk factors for increased percentage of abnormal AST results.

Factor	Increased Abnormal AST Results
Lack of folate supplementation	$p < 0.001$
Untreated hyperlipidemia	$p < 0.02$
Increased creatinine	$p < 0.03$
Male sex	$p < 0.04$
Current alcohol use	$p = 0.07$
Age at RA diagnosis	NS
Age MTX started	NS
Mean MTX dose	NS
Cumulative MTX dose	NS
Duration of MTX	NS
Positive rheumatoid factor	NS
Hyperlipidemia plus lipid lowering agent	NS
Fasting glucose	NS
Diabetes	NS
Low albumin	NS
Smoking, current or past	NS
Past alcohol use	NS
Concurrent DMARD or NSAID use	NS
BMI	NS

NS: nonsignificant.

Table 4. Stepwise model selection for percentage of abnormal AST results.

Factor	Prob > F	Parameter Estimate in Multivariate Model ± 95% CI
Lack of folate supplementation	0.0034	3.9 ± 2.1
Hyperlipidemia	0.0010	3.4 ± 1.9
Sex	0.0522	1.9 ± 1.8
Serum creatinine	0.4930	NS
Current alcohol use	0.2010	NS
Age at RA diagnosis	0.4700	NS
Age MTX started	0.6840	NS
Diabetes	0.2234	NS
Serum albumin	0.9985	NS
BMI	0.3646	NS

DISCUSSION

These data illustrate that the incidence of adverse events in the form of laboratory abnormalities requiring physician action is very low for patients with RA on MTX. Lack of folate supplementation was a risk factor for an increased percentage of abnormal AST results. Use of MTX results in decreased hepatic folate stores[3]. A recent randomized trial also found more frequent liver enzyme elevations resulting in discontinuation of therapy in patients given MTX without folic or folinic acid[9].

Hyperlipidemia was also an independent risk factor for an increased percentage of abnormal AST results. Patients with hyperlipidemia had a significantly higher mean BMI (30.9 vs 28.0 kg/m^2, $p < 0.001$) and were more likely to have diabetes ($p < 0.0001$). Hyperlipidemia, obesity, and diabetes are risk factors for nonalcoholic fatty liver disease (NAFLD). Patients with NAFLD frequently have mildly elevated transaminase levels[10]. The presence of NAFLD is already known to be a significant risk factor for liver injury in psoriatic patients receiving MTX[11]. Although studies of NAFLD treatment are few, 1 small prospective trial showed significant reductions in transaminase values in patients treated with gemfibrozil[12]. Our data support the notion that lipid-lowering therapy protects against transaminase elevation in hyperlipidemic patients on MTX, because patients on lipid-lowering therapy did not show an increased percentage

of AST abnormalities compared to those without hyperlipidemia. Treatments suggested for NAFLD, such as losing weight and controlling hyperglycemia and hyperlipidemia, may decrease the risk of MTX-induced hepatic toxicity in patients. Since the prevalence of obesity and diabetes continues to increase in the United States, rheumatologists will increasingly be faced with using MTX in patients with these comorbid conditions[13].

It is curious that alcohol consumption was not found to be a risk factor for abnormal laboratory results in this study, although there was a trend ($p = 0.071$) with the percentage of abnormal AST values. This may reflect selection by rheumatologists of patients who consume little or no alcohol and/or under-reporting of alcohol use by patients (all patients at our institution are periodically asked to fill out a form that includes questions about alcohol consumption).

Other than the association of male sex and low serum albumin with a higher percentage of low platelet counts, no other risks for hematologic toxicity from MTX were found. The presence of extraarticular features such as Felty syndrome was not assessed, and this is a limitation of our study with regard to hematologic risk factors. Also, only 51 patients had an episode of leukopenia, which may underpower this study to evaluate hematologic risk factors. Lower serum albumin may be associated with thrombocytopenia by several mechanisms. Because MTX is partially bound to albumin, lower serum albumin results in higher free MTX levels and a greater potential for hematologic toxicity[6]. Also, hypoalbuminemia may reflect occult chronic liver disease with portal hypertension and congestive splenomegaly with platelet sequestration. Men consumed alcohol more often than women ($p < 0.02$) and were less likely to be receiving folate ($p < 0.004$), which may also account in part for the higher percentage of abnormal platelet counts in men.

Patients with laboratory abnormalities were monitored more frequently ($p < 0.0001$ for the percentage of abnormal AST values). The cumulative dose of MTX had an inverse trend with the percentage of abnormal AST values ($p = 0.069$), suggesting that physicians discontinue or lower the MTX dose in the presence of transaminase abnormalities. This reflects the practice of physicians to follow tests more frequently once an abnormal result is discovered. This phenomenon potentially influences the percentage of abnormal results.

A significant limitation of our study is confounding by indication. Because obesity, hyperlipidemia, and other variables may be risk factors for AST elevations in the absence of MTX, evaluating the role MTX plays in causing AST elevations in patients with these conditions can be problematic. However, the current ACR guidelines also do not address this significant problem.

Another limitation was the lack of criteria used by physicians deciding when to stop, hold, or decrease the dose of MTX in this retrospective cohort. While this reflects real life practice, it introduces bias and variability to the study. While no definite criteria were used by clinicians, it is clear that the subset of patients who had MTX permanently stopped represent a group of patients who had higher values for AST. The mean highest AST in the group who had MTX permanently stopped was 86 U/l, while it was 78 U/l in patients who had MTX held or decreased, and 45 U/l in patients who had at least one abnormal value, but did not have MTX held, stopped, or decreased.

Further caution when interpreting our results arises from the retrospective cohort design as well as the potential for type I error, because multiple statistical comparisons were performed. Data for each risk factor were not available in all patients, which may also be a source of bias. Mayo Clinic is a tertiary-care center, where the patient population can reflect referral bias. However, the patients with RA on MTX whom we monitor typically live in Rochester and the surrounding rural communities and usually do not reflect an increased severity of disease.

In conclusion, lack of folate supplementation, presence of untreated hyperlipidemia, and a low serum albumin value may be risk factors for more frequent laboratory abnormalities in patients receiving MTX. High BMI was a risk factor requiring permanent discontinuation of MTX because of transaminase elevation. Hyperlipidemia, obesity, and lack of folate are modifiable factors. Further investigation may lead to better systems for monitoring patients that take into account risk factors for laboratory abnormalities.

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