Potential role of pharmacogenetics for optimalization of drug therapy in rheumatoid arthritis

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Chapter 5:

Cross-validation of a clinical pharmacogenetic model to predict the efficacy of MTX monotherapy in established RA

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Abstract

Objective

To cross-validate a clinical pharmacogenetic model to predict the efficacy of methotrexate (MTX) monotherapy in patients with established rheumatoid arthritis and previous failure of disease modifying antirheumatic drugs (DMARDs).

Methods

A clinical pharmacogenetic model developed in DMARD naive recent-onset RA patients, was applied in MTX naive RA patients and failure of other DMARDs. Patients from an RA inception cohort (N=593) were eligible if they fulfilled the ACR classification criteria for RA, received MTX monotherapy for at least 6 months and failed on at least one DMARD before MTX. Concomitant oral prednisone was allowed if the dose did not exceed 10 mg. Hundred-and-eighteen patients fulfilled these inclusion criteria, DNA and clinical data were available for 75 of those patients, and 71 patients were genotyped successfully. Risk scores for non-response as defined by DAS>2.4 at 6 months, were calculated using the pharmacogenetic model utilising eight baseline factors: gender, rheumatoid factor, smoking status, DAS, and four polymorphisms in the genes MTHFD1, AMPD1, ITPA and ATIC.

Results

The mean (SD) DAS at baseline was 3.55 (1.11). The time-averaged dose of MTX was 13.9 mg/week (range 5-25 mg/week); 48% of the patients used folic acid suppletion and 16% used prednisone. At 6 months, the DAS decreased with a mean (SD) of -0.72 (1.13) points ($p<0.0001$), resulting in 33% responders (n=25) and 67% non-responders (n=50). At baseline, risk scores ranged from 0 to 10.5. Seventy-five percent (56/75) of the patients could be categorized into predicted responders (risk score ≤ 3.5) and predicted non-responders (risk score ≥ 6) using the eight baseline variables. Comparison of the observed and predicted response at 6 months showed that the true positive response ratio was 47% (14/30) and the true negative response ratio was 81% (21/26).

Conclusion

The pharmacogenetic model predicts the efficacy of MTX monotherapy better in DMARD naive recent-onset RA patients than in patients with preceding DMARD failure. Updating the model is needed to improve its usefulness in patients with established RA and DMARD failure.

Introduction

Early treatment response in newly diagnosed rheumatoid arthritis (RA) patients is effective in decreasing functional disability and bone destruction (1,2). In addition, combination strategies of Disease Modifying Anti–Rheumatic Drugs (DMARDs), with or without anti-tumor necrosis factor (anti-TNF) agents, show higher response rates compared to monotherapy strategies in treating RA and considerably improve the prognosis (3,4).
Although combination strategies are more efficacious, such intensive approach is probably not necessary for all newly diagnosed RA patients since ~30 to 40% of the patients will experience a sustained good clinical response to methotrexate (MTX) monotherapy (5,6). To avoid unnecessary combination treatment, it is important to determine whether patients have a high probability of response to MTX monotherapy.
Recently a clinical pharmacogenetic model to predict response to MTX monotherapy has been developed in DMARD-naive patients with early onset RA (Table 1) (7). This model includes disease activity, gender, smoking and rheumatoid factor status next to four polymorphisms in genes related to the MTX mechanism of action and correctly predicts the response to MTX in early RA (7).
Next to MTX, other DMARDs such as sulphasalazine (SSZ) are still frequently used as a first DMARD for treating RA in daily clinical practice. Of note, the number of previous DMARDs and disease duration have been associated with reduced response to MTX (8,9). It is unclear whether the clinical pharmacogenetic model is still informative if MTX is intended after failure of another DMARD. In order to tailor treatment choices in RA, there is a need for prediction of response to MTX therapy in patients with established RA patients with previous DMARD failure.
The objective of this study was to cross-validate the clinical pharmacogenetic model with clinical and genetic factors to predict the efficacy of MTX monotherapy in established RA patients with previous DMARD failure.

Methods

Patients
Eligible patients were selected from the database of the inception cohort of early RA patients at the Department of Rheumatology of the Radboud University Nijmegen (10). Inclusion criteria of the cohort are: fulfillment of the ACR criteria for RA (ACR 1978), no use of DMARDs, a symptom duration < 1 year. Additional inclusion criteria for the present study were: use of MTX mono-therapy for at least 6 months, no use of oral prednisone > 10mg/day during the first 6 months of the MTX course, treatment with at least one other DMARD previously, availability of clinical data at start of MTX and after 6 months comprising the Disease Activity Score (DAS), and availability of a DNA sample.

Variable	Score
Female gender	
Premenopausal	1
Postmenopausal	1
Male gender	0
DAS at baseline	
≤3.8	0
>3.8 and ≤5.1	3
>5.1	3.5
RF-negative nonsmoker	0
RF-negative smoker	1
RF-positive nonsmoker	1
RF-positive smoker	2
MTHFD1 1958 AA genotype	1
AMPD1 34 CC genotype	1
ITPA 94 A-allele carrier	2
ATIC 347G-allele carrier	1
Other genotype	0

Table 1. The original clinical pharmacogenetic model to predict response to MTX monotherapy

Abbreviation(s): RF = Rheumatoid Factor; DAS = Disease Activity Score; *AMPD1*= adenosine monophosphate deaminase; *ATIC*= aminoimidazole, carboxamide ribonucleotide transformylase; *ITPA*= inosine triphosphate pyrophosphatase; *MTHFD1*= methylenetetrahydrofolate dehydrogenase

RA cohort

Patients in the Nijmegen RA inception cohort were regularly assessed regarding disease activity and medication use (10). Medication and clinical information was registered in a computerized database. DNA had been collected in an unselected subsample of patients. At the time of patient selection for the present study in 2007, there were N=593 patients included in the database. N=352 patients had used MTX at any time point; MTX was the first DMARD used in n=36 patients, and a total of n=151 patients had switched to MTX monotherapy after one or more previous DMARDs, most often SSZ. Out of those 151 patients on MTX monotherapy, 118 had used MTX for at least 6 months. For 76 of them, DNA and DAS values were available. One patient used oral prednisone > 10mg/day and was excluded. Consequently, genotyping was performed in 75 patients, and was complete in 71 patients. The dates of start of MTX of the included patients ranged uniformly from 1988 to 2006. There were no significant differences between patients that were included (n=75) and patients that were not included (n=76) regarding age, gender, rheumatoid factor, smoking, DAS at diagnosis and MTX dose.

Variables
Age and disease duration at the time of MTX initiation (baseline) were calculated. As menopausal status was unavailable, age categories of ≤55 and 55< were used as proxy. For Rheumatoid Factor (RF) positivity, the most recent value before baseline was used. Smoking status was defined as current smoker and nonsmoker at the time of diagnosis. Joint counts were assessed by trained research nurses, Visual Analogue Scales (VAS, 0-100 mm.) for general health and pain were filled in by the patient. ESR was determined using the Westergren method. The DAS was calculated using the Ritchie Articular Index (RAI), the 44 swollen joint count (SJC), ESR and general health, according to the original formula (11).

Treatment
All treatment decisions were to the discretion of the treating rheumatologist and the patient. Concomitant use of methylprednisolone and Non-steroidal Anti-inflammatory Drugs (NSAIDs) was allowed. Patients receiving oral prednisone > 10mg/day during the first 6 months of the MTX course were not included.

MTX response evaluation
For the purpose of the present study, the same response definition as in the previous study form Wessels et al. was used (7). Responders were defined as patients who were treated with MTX for 6 months and had a DAS≤2.4 at 6 months, nonresponders were defined as patients who were treated with MTX for 6 months but had a DAS>2.4 at 6 months (12).

Prediction rule
The variables of the prediction rule for the probability of non-response (DAS>2.4) after 6 months of MTX use were: gender, menopausal status, RF status, smoking status, DAS at baseline, and 4 polymorphisms in AMPD1, ATIC, ITPA, MTHFD1 (7). To arrive at a clinical useful prediction rule, weighted scores had been assigned by rounding the regression coefficients in the final prediction model. In the final model, menopausal status did not contribute anymore to the clinical prediction rule (7).

Genotyping
Genomic DNA was extracted from peripheral venous blood (10 ml/sample) according to standard protocols. All included patients were genotyped for SNPs in genes coding for adenosine monophosphate deaminase (*AMPD1*; rs17602729), aminoimidazole carboxamide ribonucleotide transformylase (*ATIC*; rs2372536), inosine triphosphate pyrophosphatase (*ITPA*; rs1127354) methylenetetrahydrofolate dehydrogenase (*MTHFD1*; rs17850560). Genotype frequencies of *AMPD1* (p=0.068), *ATIC* (p=0.22), *ITPA* (p=0.23), and *MTHFD1* (p=0.065) were not significantly different to the distributions as expected based on the HapMap-CEU sample (http://www.ncbi.nlm.nih.gov/sites/entrez). Genotype distributions were as follows: for *AMPD1* 34C>T, 68% CC, 31% CT, 1% TT; for *ATIC* 347C>G, 49% CC, 41% CG, 9% GG; for *ITPA* 94C>A, 80% CC, 20% CA; and for *MTHFD* 1958G>A, 23% GG, 59% GA, 18% AA. Hardy-Weinberg equilibrium was not calculated because of the small sample. Genotyping was performed using real-time polymerase chain reaction with TaqMan®, according to the protocol provided by the manufacturer (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands).

Statistical analysis

Responders and non-responders were compared at baseline regarding the clinical and genetic variables in the prediction model and regarding former medication use, using Student's t-test, Wilcoxons' test, Chi-square test or Fisher-Exact test, as appropriate.

No variable selection procedure was attempted for model building. The variables of the existing prediction formula were applied in a logistic regression model with MTX response (yes/no) as dependent variable, and the clinical variables (nongenetic model) and the clinical and genetic variables combined (pharmacogenetic model) as independent variables. The exponential of the regression coefficient, e^{β} is an estimate of the adjusted Odds Ratio. In congruence with the original publication (7), the probability of a non-response was modeled. Of both the nongenetic model and the pharmacogenetic model, discrimination was evaluated using the area-under-the-receiver-operating-characteristic-curve (c-statistic) and the variance explained (Nagelkerke R^2). The clinical prediction rule and the cut-points for nonresponse, intermediate, and response were applied as delineated in the original publication (7). The resulting clinical prediction scores were compared between responders and nonresponders using Student's t-test. Using observed and predicted responses the true positive and true negative response ratios were calculated. It was attempted to update the nongenetic and pharmacogenetic models by addition of the predefined variables: number of previous DMARDs and disease duration (8), and SSZ as previous DMARD.

All analyses were performed using SAS 8.02 (SAS Institute Inc, Cary, NC, USA).

Results

Description of the cohort

The starting dose of MTX of the included patients was in median 15 mg/week, ranging from 2.5-22.5 mg/week. After 6 months the median MTX dose was also 15 mg/week, with a range of 5-25 mg/week. The median time-averaged dose of MTX was 13.9 mg/week (range 5-25 mg/week). Of the included patients 36/75 (48%) used folic acid suppletion and 12/75 (16%) used concomitant oral prednisone in the allowed dose of ≤10 mg/day. The mean (SD) DAS at baseline was 3.55 (1.11). After 6 months, the DAS had significantly decreased with mean (SD) -0.72 (1.13) points ($p<0.0001$), resulting in 33% responders (n=25) with a DAS≤2.4 and 67% non-responders (n=50) with a DAS>2.4.

In table 2, baseline values of responders and nonresponders are compared. The prespecified variables for the pharmacogenetic model were: gender, menopausal status, Rheumatoid Factor positivity, smoking status, DAS at baseline, and 4 polymorphisms in AMPD1, ATIC, ITPA, MTHFD1 genes. Of these variables, only baseline DAS was significant at a level of $p<0.05$. Notably, none of the 4 genetic factors were statistically significant at that p-level. Moreover, of all variables tested, only RF positivity, the baseline DAS, the tender joint counts, pain, ESR and the number of previous DMARDs would satisfy a liberal selection criterion of $p<0.20$ for model building.

Baseline variable	Responders (N=25)	Non-responders (n=50)	P-value
Age	55 (13)	58 (14)	0.33
Age > 55	12 (48%)	30 (60%)	0.32
Female gender	15 (60%)	35 (70%)	0.39
RF positivity	41 (82%)	16 (64%)	0.09
Disease duration (months)	19 (7-49)	19 (5-43)	0.99
Current smoker	8 (32%)	15 (30%)	0.86
DAS baseline	3.1 (1.3)	3.8 (0.9)	0.01
SJC44	12 (5-18)	14 (10-18)	0.31
TJC53	4 (2-9)	11 (7-19)	0.002
RAI	5 (2-7)	8 (5-12)	0.0005
Pain VAS	37 (25)	48 (22)	0.07
General Health VAS	46 (25)	50 (24)	0.47
ESR	14 (7-36)	24 (13-44)	0.14
Folic acid use	10 (40%)	26 (52%)	0.33
Oral prednisone use	3 (12%)	9 (18%)	0.74
Number of previous DMARDs	1 (1-3)*	1 (1-4)*	0.15
Previous DMARDs>1	6 (24%)	20 (40%)	0.17
Previous DMARD was SSZ	21 (84%)	36 (72%)	0.25
MTHFD1 1985 AA genotype	4 (17%)	9 (18%)	0.99
AMPD1 34 CC genotype	14 (58%)	35 (73%)	0.21
ITPA 94 A-allele carrier	4 (16%)	11 (22%)	0.54
ATIC 347 G-allele carrier	13 (52%)	25 (50%)	0.87

Table 2. Baseline variables of responders and nonresponders

Abbreviation(s): RF = Rheumatoid Factor; DAS = Disease Activity Score; SJC44 = 44 swollen joint count; TJC53 = 53 tender joint count; RAI = Ritchie Articular Index, SJC44 and TJC53 both are based on the joints assessed in the RAI; VAS = visual analogue scale; SSZ=Sulphasalazine; *AMPD1*= adenosine monophosphate deaminase; *ATIC*= aminoimidazole, carboxamide ribonucleotide transformylase; *ITPA*= inosine triphosphate pyrophosphatase; *MTHFD1*= methylenetetrahydrofolate dehydrogenase.

Cross-validation of the clinical pharmacogenetic model

The variables entered in the logistic regression model to predict non-response to MTX at 6 months according to a DAS≤2.4 were prespecified by the existing prediction model (Table 3).

First, the nongenetic variables were simultaneously entered in a logistic regression model; next the genetic variables were added. The sample was too small for including interaction terms. According to the odds ratio's it is seen that the contribution of gender was small (OR near 1) and that smoking was inversely associated with non-response (OR below 1). Age, RF positivity and DAS at baseline were all predictive for non-response.

The genetic factors were not statistically significant, three of the genetic factors had a sign ('-') reversed to the expectation, the sign for AMPD1 was in agreement with the expectation according to the publication of Wessels et al. (7).

When the original clinical prediction rule was applied on the baseline variables, the resulting risk scores ranged from 0 to 10.5. The mean (SD) risk score was 5.1 (2.3) and 3.8 (2.7) for non-responders and responders, respectively. The difference between these scores was significant (p=0.03). Accordingly, 75% (56/75) of the patients could be correctly categorized into predicted responders (risk score ≤ 3.5) and predicted non-responders (risk score ≥ 6) using the eight baseline

variables of the pharmacogenetic model (Table 4). Comparison of the observed and predicted response at 6 months showed that the true positive response ratio was 47% (14/30) and the true negative response ratio was 81% (21/26).

	Nongenetic model		Pharmacogenetic model	
Variable	**β**	**OR (95% CI)**	**β**	**OR (95% CI)**
Female gender	0.028	1.1 (0.3-3.3)	-0.12	0.8 (0.2-2.7)
Age ≥55	0.78	2.2 (0.7-6.7)	0.89	2.4 (0.7-8.0)
DAS at baseline	0.70	2.0 (1.2-3.5)	0.77	2.2 (1.2-4.0)
RF-positive	1.13	3.1 (0.8-11.5)	0.98	2.7 (0.7-11.0)
Smoking	-0.47	(0.6 (0.2-2.0)	-0.47	0.6 (0.2-2.2)
MTHFD1 1958 AA genotype	--	--	-0.31	0.7 (0.2-3.2)
AMPD1 34 CC genotype	--	--	0.34	1.4 (0.4-4.9)
ITPA 94 A-allele carrier	--	--	-0.21	0.8 (0.2-3.6)
ATIC 347G-allele carrier	--	--	-0.23	0.8 (0.3-2.4)

Table 3. Regression coefficients and odds ratio's of the logistic regression models to predict non-response to MTX

Abbreviation(s): RF = Rheumatoid Factor; DAS = Disease Activity Score; *AMPD1*= adenosine monophosphate deaminase; *ATIC*= aminoimidazole, carboxamide ribonucleotide transformylase; *ITPA*= inosine triphosphate pyrophosphatase; *MTHFD1*= methylenetetrahydrofolate dehydrogenase

	Predicted response		
Observed response	*Nonresponder*	*Intermediate*	*Responder*
Pharmacogenetic model			
Nonresponder	21	11	16
Responder	5	4	14
Nongenetic model			
Nonresponder	4	16	28
Responder	1	3	19

Table 4. Observed and predicted response after 6 months of MTX, using the original pharmacogenetic and nongenetic models.

Discriminatory performance of the nongenetic model and the pharmacogenetic model was studied using the area-under-the-ROC curve (Figure 1). The area-under-the-curve was 0.73 for the nongenetic model and 0.77 for the pharmacogenetic model. The variance explained (R^2) was 0.22 and 0.28 for the nongenetic and pharmacogenetics models, respectively. To assess the relative contribution of both models in predicting (DAS-) response in comparison to a very reduced model, a model including only baseline DAS was used. It showed an area-under-the-curve of 0.70 and an R^2 of 0.14.

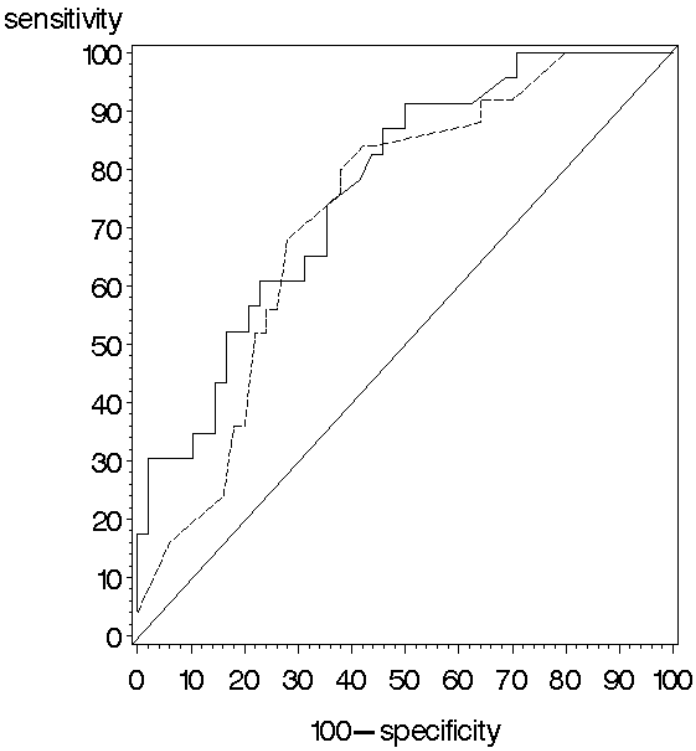

Figure 1. ROC curves for predicting the response to MTX using the original pharmacogenetic (solid line) and nongenetic (broken line) models.

Updating

Updating the clinical and pharmacogenetics models by removing the interactions of age and gender, and smoking and RF positivity, and adding the variables 'number of previous DMARDs' and 'SSZ as previous DMARD' did not improve the discriminatory performance (not shown). Disease duration was no candidate for uptake in an updated model, according to the results of the univariate analysis (Table 2).

Discussion

This study shows that the previously published clinical pharmacogenetic model to predict MTX mono-therapy efficacy in patients with established RA does not perform as good as in DMARD naïve patients with recent-onset RA. Previously it was found that the clinical pharmacogenetic model had a true positive and negative response rate of 95% and 86% respectively, whereas in the current cross-validation positive and negative response rates of only 47% and 81% were found. There are several possible explanations for these findings.

Clinical prediction rules developed in relatively small patient samples, which is not uncommon in medicine, generally perform less well in replication studies using independent samples. Therefore, cross-validation is necessary to assess the performance of clinical prediction rules in future patients (13). The original clinical pharmacogenetic model was developed in early RA patients who were DMARD naïve (7). In that same study, the model was validated in a small sample of early-onset DMARD-naïve RA patients from another hospital. In the current study, the model was applied in RA patients with established disease and a history of DMARD use, notably SSZ. By taking a different population, it was tested whether the model would be applicable for predicting MTX non-response in patients for whom MTX is not the first DMARD.

The MTX response in the present study was less in comparison with the MTX response in the study in which the clinical pharmacogenetic model was derived (7). Only one-third of the patients reached a response as defined by a DAS>2.4 at 6 months after start of MTX mono-therapy. These patients therefore appear to be more therapy-resistant, but also MTX dosages were lower than in the original study. Since MTX dosage was increased according to the discretion of the treating rheumatologist, the median MTX dosage of 15 mg/week at 6 months after initiation was less in comparison with recent tight control strategies in treating RA (2,14). Previously, it has been shown that tight disease control with increased MTX dosages, up to 30 mg/week, will lead to increased response rates even after re-employment after previous MTX failure (15-17). The clinical pharmacogenetic model was also based on a tight control strategy with increased dosages MTX, including folic acid suppletion (7). Therefore, it might be that MTX non-responders in our inception cohort would have been responders if treated with tight-control strategies instead of daily practice.

Yet, it also has been shown that RA patients failing previous DMARD monotherapy have an increased likelihood to fail on the next DMARD after switching (18,19). In this context, RA patients with longer disease duration do not respond as well to treatment compared with patients with early disease (9). Further, patients with DMARD failure are a subset of all patients. As a consequence, predictive factors for response may differ as well. In contrast with the original study, in the present study gender appeared to have no predictive value, and smoking appeared to be associated with response instead of non-response. The latter may have been caused by the fact that in the current study, smoking was only assessed at diagnosis, not at start of MTX which was in median 19 months later. Also, menopausal status was not assessed, but menopausal state is not of influence in calculating the risk score (7).

Interestingly, our results suggest that the clinical prediction model is capable to predict non-response to MTX also in patients with previous DMARD failure. This is despite the fact that the variables 'disease duration', 'number of previous DMARDs', and 'previous SSZ use' did not improve the clinical (nongenetic) model. The pharmacogenetic model showed no substantial improvement over the clinical model in this study. Failure on previous DMARDs, notably SSZ, could select patients into a subsample for which the proposed genetic factors are not discriminative. However, in our study there appeared to be no selection in direction of the less favorable genotypes, as the gene frequencies in this study were similar to the frequencies as expected based on the HapMap-CEU sample and similar to the frequencies as found by Wessels et al. earlier (7,20). Therefore, it remains

yet unclear whether the four selected genetic variants are true markers for MTX response (21,22). Fine mapping of these and other genes related to MTX treatment outcome is necessary in order to probably detect better pharmacogenetic factors for MTX response. Large collaborations between groups and continued collection of patients and data on MTX pharmacogenetics in RA are still needed in order to validate the pharmacogenetic model, in DMARD naïve early-onset RA treated with MTX according to a tight control strategy. In addition, the robustness of the pharmacogenetic model could also be tested by cross-validation in a larger population of patients again with established RA and previous DMARD failure, treated according to a tight control strategy.

In conclusion, the pharmacogenetic model predicts the efficacy of MTX monotherapy better in DMARD naive recent-onset RA patients than in patients with preceding DMARD failure. Updating the model is needed to improve its usefulness in patients with established RA and failure of previous DMARDs.

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