CHAPTER 7

TIME TO TREATMENT IS AN IMPORTANT FACTOR FOR THE RESPONSE TO METHOTREXATE IN JUVENILE IDIOPATHIC ARTHRITIS

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ABSTRACT

Objective

Methotrexate (MTX) is the most commonly used disease modifying antirheumatic drug in Juvenile Idiopathic Arthritis (JIA). At present no reliable prediction of individual response to MTX can be made. Identification of clinical and genetic factors that influence the response to MTX could be helpful in realizing the optimal treatment for individual patients.

Materials & methods

A cohort of 128 JIA patients treated with MTX was studied retrospectively. Eleven clinical parameters and genotypes of 6 single nucleotide polymorphisms in 5 genes related to the mechanism of action of MTX were compared between MTX responders and non-responders using a multivariate regression analysis.

Results

The time from diagnosis to start MTX treatment, the physician's global assessment at baseline and the starting dosage were significantly associated with the response to MTX at 6 months after initiation. Patients with a shorter time from diagnosis to start MTX and a higher disease activity according to the physician, but with a lower MTX dosage showed increased response. The effect of the starting dosage on MTX response seemed to be mainly due to the influence of the systemic JIA subtype. The time from diagnosis to start MTX treatment and physicians' global assessment at baseline were highly correlated. Therefore, the precise effect size of each independent variable could not be determined.

Conclusion

In children with JIA, the time from diagnosis to start MTX appears to be an important factor for the MTX response. Our results suggest that earlier start of MTX treatment will lead to an increased response.

INTRODUCTION

Juvenile Idiopathic Arthritis (JIA) is the most common chronic rheumatic inflammatory disorder in childhood, with an incidence of around 10 per 100.000 children.[1] JIA is defined as arthritis of unknown etiology that persists for more than 6 weeks and with an onset before the age of sixteen years. Seven different subtypes have been defined according to the criteria of the International League of Associations for Rheumatology (ILAR).[2]

Methotrexate (MTX) is the most commonly used disease modifying antirheumatic drug (DMARD) in JIA, especially in the treatment of polyarticular JIA.[3] The efficacy of MTX has been shown in randomized placebo controlled trials and in subsequent clinical use.[4;5] The response rate of MTX, prescribed in a weekly standard dose of 8.5-12.5 mg per body surface area (m^2), is about 65% at 4-6 months after initiation of therapy.[4;6-8]

The precise mechanism of action of MTX remains unclear, although it is thought that MTX inhibits the de novo synthesis of purine and pyrimidine, essential components of DNA and RNA.[9;10] Thereby it inhibits the proliferation of cells, amongst which T-lymphocytes. Additionally it has been shown that the anti-inflammatory effects of MTX are mediated by an increased adenosine-release. More recently research groups have reported that genetically based differences contribute to MTX efficacy since polymorphisms in genes involved in the purine and pyrimidine synthesis have been associated with response to MTX in JIA and RA.[11-13]

However in JIA, reliable predictors for the response to MTX are yet unknown. Factors identifying JIA patients with a high likelihood to respond to MTX therapy would be very helpful for achieving the optimal treatment for individual patients in an early stage of the disease and thereby preventing damage to the joints on the long term. Therefore, the aim of this study is to identify clinical and genetic factors that are associated with the response to MTX in patients with JIA.

METHODS & MATERIALS

Patient population

The patients in this analysis are a retrospectively observed cohort of children diagnosed with JIA that were recruited from 4 pediatric rheumatology referral centers in The Netherlands, Belgium and Germany. Clinical data were collected from 347 patients of which also DNA was available. Forty-four percent of these patients were treated with MTX (n=152) and 128 patients fulfilled the inclusion criteria for this study. Twenty-four

patients were excluded because: at start of MTX> 18 years (n=5), start MTX < 6 months ago (n=5), use of MTX because of JIA associated uveitis (n=3) and missing data about follow-up (n=11). No statistical significant differences were found with regard to subtype, age at onset and gender between the 128 genotyped patients and the total group of patients receiving MTX (p>0.05). Patients with undifferentiated JIA (n=5), JIA with enthesitis (n=4) and psoriatic arthritis (n=1) were grouped together in the subgroup "other JIA". 97 Per cent of the patients were of European white ethnicity based on self-report. Written informed consent was obtained from all patients and/or parents together with approval of each institution's medical ethics board.

Clinical data

Demographic and clinical data, together with detailed information about the use of MTX and co-medication were collected from the patients' chart. At the time point of start and at 6 months after initiation of MTX, the following parameters were scored: the physician's global assessment of disease activity, the amount of joints with arthritis (defined by swelling, not due to bony enlargement, or if no swelling is present, limitation of motion accompanied either by pain on motion and/or tenderness) (32 joint count) and the erythrocyte sedimentation rate (ESR). The physician' global assessment was scored at a five point scale (1 no, 2 mild, 3 moderate, 4 severe and 5 very severe activity). In addition, the joint score was divided into categories with 0 no arthritis, 1 monoarthritis (1 joint), 2 oligoarthritis (2-4 joints), 3 polyarthritis (5-10 joints), 4 severe polyarthritis (>11 joints) and in systemic JIA patients an additional category (5) was used when systemic features were present.

Definition of response

The response to MTX was defined as follows: improvement in physician's global assessment of 1 or more categories together with an equal or improved joint score measured from baseline to 6 months after the start of MTX. The ESR was not incorporated in the definition of response because of the large number of missing data. Patients were considered non-responders to MTX if they did not fulfill the criteria of response.

Pharmacogenetics

Six single nucleotide polymorphisms (SNPs) in 5 candidate genes, related to the mechanism of action of MTX, were selected taking the following criteria into consideration: validated SNP, SNP -preferably- causing non-synonymous amino acid change, indications for clinical relevance from previous publications[11-13] and a preferred minimal genotype frequency of approximately 10%. These SNPs were located in the genes adenosine monophosphate deaminase (AMPD1) (34C>T; rs17602729), amino-

imidazole carboxamide ribonucleotide transformylase (ATIC) (347C>G; rs2372536), inosine triphosphate pyrophosphatase (ITPA) (94C>A; rs41320251) methylenetetrahydrofolate reductase (MTHFR) (677C>T; rs1801133 and 1298A>C; rs1801131) and in methylenetetrahydrofolate dehydrogenase (MTHFD1) (1958G>A; rs2236225). Genotyping was performed using real-time polymerase chain reaction (PCR) with Taqman technique according to protocols provided by the manufacturer (Taqman, Applied Biosystems, Foster City, CA, USA). 5-10% of samples were genotyped in duplicate. The mean for overall success rate was 96%. All 6 SNP genotype frequencies showed Hardy-Weinberg equilibrium.

Statistical analysis

Clinical variables considered relevant for the response to MTX at 6 months after initiation were: subtype of JIA, age at start MTX, time from diagnosis to start MTX (time-to-start MTX in months), disease activity at start MTX (physician's global score, joint score), ESR, starting dosage MTX (milligrams per body surface area per week), use of intra-articular steroids and/or sulfasalazine (SSZ) and/or other DMARDs before MTX (yes/no), use of systemic steroids before MTX (yes/no), use of systemic steroids during MTX treatment (yes/no) and use of SSZ during MTX treatment (yes/no). These variables were compared between responders and non-responders by the Student's t-test, Mann-Whitney U test or Chi-square test depending on the tested variable. Differences in genotype distribution between responders and non-responders were tested in a two-by-two cross tabulation by carrier analysis with a two-sided Chi-square test. MTHFR 677C>T and MTHFR 1298A>C were only tested as number of copies of the MTHFR1298A-677C haplotype. With the sample size of 55 non-responders and 73 responders, an increase in frequency of 2 haplotype copies (MTHFR1298A-677C) from 12% to 34% could be detected with 80% power and 95% confidence. Variables with a p-value of < 0.1 between responders and non-responders were considered relevant for influencing the response to MTX either by a true effect of by confounding. Therefore, variables with p-value <0.1 were included in the multiple binary logistic regression analysis with response as dependent. Additionally the univariate odds ratios (with 95% confidence interval) of these variables were calculated to illustrate the confounding effect of the different variables. ESR was not included in the multivariate analysis because of the large number of missing data. All statistical analyses were performed using SPSS 14.0. Variables with p-value <0.05 in the multivariate regression analysis were considered statistical significant.

RESULTS

Description of the patient population

The clinical and demographic characteristics for responders and non-responders are presented in Table 1. In our cohort (n=128) the response rate at 6 months after initiation of MTX was 44% in persistent oligoarthritis, 69% in extended oligoarthritis, 61% in RF negative polyarthritis, 82% in RF positive polyarthritis and 32% in systemic JIA, whereas the response rate in the overall JIA population with these subtypes combined was 57% (Table 1). In addition the comparison of the clinical and demographic characteristics of the MTX responders and MTX non-responders is listed in Table 1.

Table 1.
Clinical and demographic characteristics of 128 JIA patients, according to their response to MTX[#]

	MTX non-responder % (n)	MTX responder % (n)	p-value[*]
Total group of JIA patients	43 (55)	57 (73)	
Diagnosis:[1]			0.050 [P]
Persistent oligoarthritis	56 (10)	44 (8)	
Extended oligoarthritis	31 (8)	69 (18)	
RF negative polyarthritis	39 (17)	61 (27)	
RF positive polyarthritis	18 (2)	82 (9)	
Systemic JIA	68 (13)	32 (6)	
Other JIA•	50 (5)	50 (5)	
Female:male	74:26 (41:14)	79:21 (58:15)	0.51 [P]
Age at start MTX (years), mean (range; sd[2])	7.9 (1.9- 15.9; 3.7)	7.7 (1.3- 17.4; 4.3)	0.82 [t]
Time-to-start-MTX in months, median (range)	16.3 (0.0- 150)	9.5 (0.0- 94.8)	0.074 [MW]
Medication:			
Starting dosage MTX (mg/m^2) mean (range;sd)	10.4 (4.5- 23.7; 4.7)	8.6 (1.8- 16.5; 3.3)	0.018 [t]
SSZ,IAS or other DMARDs used before start MTX (yes/no)	58 (32)	56 (41)	0.82 [P]
Systemic steroids used before start MTX (yes/no)	31 (17)	16 (12)	0.053 [P]
SSZ used during MTX (yes/no)	33 (18)	32 (23)	0.88 [P]
Steroids used during MTX (yes/no)	35 (19)	52 (38)	0.048 [P]
Disease activity at start MTX:			
ESR[2] (mm/h), median (range) (n=67)	30 (7-137)	25 (2-107)	0.040 [MW]
Physician's global assessment			0.000 [lin]
1 Inactive	7 (4)	0 (0)	
2 Mild	16 (9)	4 (3)	
3 Moderate	55 (30)	56 (41)	
4 Severe	22 (12)	48 (28)	

5 Very severe	0 (0)	1 (1)	
Joint score			0.40 [lin]
0 None	7 (4)	0 (0)	
1 Monoarthritis	2 (1)	4 (3)	
2 Oligoarthritis	35 (19)	31 (23)	
3 Polyarthritis	45 (25)	51 (37)	
4 Severe polyarthritis	0 (0)	11 (8)	
5 Systemic features	11 (6)	3 (2)	

sd: standard deviation, SSZ: sulfasalazine, IAS: intra-articular steroid, DMARD: disease modifying anti rheumatic drug, ESR: erythrocyte sedimentation rate
#) all variables are presented as percentage (number of patients) unless indicated otherwise
•) other JIA consists of undifferentiated JIA (n=5), JIA with enthesitis (n=4) and psoriatic arthritis (n=1)
*) p-value of different statistical test comparing these clinical variables between the non-responders and responders.
[1] Diagnosis JIA according to the revised ILAR criteria(2)
[2] ESR not included in further analysis because of the large number of missing data
[P] p-value of Pearson Chi-square
[MW] p-value of Mann-Whitney U Test
[t] p-value of Student's t-test
[lin] p-value of linear-by- linear association

Genetic analysis

The genotype frequencies and the MTHFR haplotype frequencies in this population are presented in Table 2. Comparing MTX responders with non-responders in a haplotype-carrier analysis, a statistically significant difference in number of MTHFR1298A-677C haplotype copies was found. Non-responders showed more frequently none or 1 copy of the MTHFR1298A-677C haplotype when compared to responders (p-value= 0.039). All other pharmacogenetic association analyses showed no significant differences.

Table 2.
Genotype and (MTHFR 1298A-677C) haplotype frequencies in MTX non-responders and MTX responder %(n).

	AMPD1 34 C-T			**ATIC 347 C-G**			**ITPA 94 C-A**			**MTHFD1 1958 G-A**			**MTHFR 1298A-677C***	
	C/C	**C/T**	**T/T**	**C/C**	**C/G**	**G/G**	**C/C**	**C/A**	**A/A**	**G/G**	**G/A**	**A/A**	**0+1**	**2**
MTX non-responder	77 (40)	23 (12)	0	42 (23)	49 (27)	9 (5)	91 (50)	9 (5)	0	24 (13)	53 (29)	24 (13)	89 (47)	11 (6)
MTX responder	66 (44)	32 (21)	2 (1)	49 (35)	38 (27)	13 (9)	85 (58)	13 (9)	2 (1)	30 (21)	49 (35)	21 (15)	74 (50)	26 (18)
p°	0.183			0.748			0.279			0.503			**0.039**	

*) The number of MTHFR1298A-677C haplotype copies
°) p-value of linear-by-linear association

Univariate and multivariate analysis of variables in relation to MTX response

Variables with a p-value of <0.1 (Table 1 and 2) were considered of influence of the response to MTX and were analyzed univariately and thereafter included in a multivariate regression analysis to correct for confounding effects (Table 3).

In the multivariate regression analysis, the time-to-start MTX, the baseline physician's global assessment and the starting dosage of MTX were significantly associated with the response to MTX at 6 months after initiation. No confounding effect of the included variables on the effect of time-to-start MTX on response was observed. Briefly, responders started earlier with MTX and had a higher disease activity at baseline based on the physician's global assessment. What is more remarkable is that responders received a lower starting dosage. However, the starting dose MTX was highly influenced by the subtype of JIA (ANOVA p<0.001), especially by the systemic JIA patients who receive a higher starting dosage and have a decreased response. Repeating the multivariate analysis without the systemic JIA subtype resulted in a significant association of the time-to-start MTX and baseline physician's global assessment with MTX response (data not shown) and no effect of the starting dose on the MTX response was observed (OR 0.89, 95%CI 0.76- 1,05; p= 0.166).

Table 3 Univariate and multivariate regression analysis of clinical and genetic factors with MTX response in JIA patients (n=118) as dependent variable.

	Univariate analysis		**Multivariate analysis[1]**	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
JIA subtype*		0.069		0.441
Persistent oligoarthritis	1.00 (reference)			
Extended oligoarthritis	2.81 (0.81- 9.80)	0.104	3.41 (0.62- 18.7)	0.159
RF negative polyarthritis	1.99 (0.65- 6.03)	0.226	0.76 (0.18- 3.15)	0.700
RF positive polyarthritis	5.63 (0.94- 33.8)	0.059	1.41 (0.16- 12.4)	0.757
Systemic JIA	0.58 (0.15- 2.21)	0.422	0.68 (0.09- 5.12)	0.712
Other JIA	1.25 (0.27- 5.89)	0.778	0.92 (0.14- 6.2)	0.933
Time-to-start-MTX (months)	0.98 (0.97- 0.996)	0.013	0.97 (0.95- 0.996)	**0.021**
Starting dosage MTX (mg/m^2)	0.89 (0.81- 0.98)	0.023	0.84 (0.73- 0.97)	**0.021**
Steroid use during MTX (yes/no)	2.06 (1.00- 4.23)	0.050	2.34 (0.86- 6.34)	0.095
Steroid use before MTX (yes/no)	0.44 (0.19- 1.02)	0.056	0.43 (0.12-1.6)	0.205
Physician's global assessment at start	2.6 (1.5- 4.7)	0.001	2.4 (1.1- 5.1)	**0.026**
MTHFR-haplotype^o	2.8 (1.03- 7.7)	0.043	2.3 (0.68- 7.7)	0.178

* JIA subtype as category with persistent oligoarthritis as reference subtype.

^{o} 0 or 1 haplotype (MTHFR1298A-677C) copies versus 2 haplotype copies.

1 total R^2 (Cox & Snell): 0.27

Regarding the time-to-start MTX (Table 3), it is evident that the use of intra-articular steroids, SSZ and/or other DMARDs prior to MTX influences time-to-start MTX. Although the use of prior treatment was not related to MTX response (Table 1), we analyzed whether the association of time-to-start MTX was independent of the use of prior treatment and a true effect for MTX response. Therefore the effect of time-to-start MTX on response was assessed including only patients with prior treatments. This repeated multivariate analysis showed that the time-to-start MTX was still significantly associated to the response to MTX (OR 0.96, 95%CI 0.94-0.99; p= 0.017).

Finally, the time-to-start-MTX is strongly correlated to the physicians' global assessment at start MTX, meaning that patients with an increased disease activity are treated earlier. Interestingly, our data showed that the physicians' global assessment at baseline also reflects the disease activity as measured from diagnosis to start MTX (data not shown). Although the precise effects size of time-to-start MTX and the physicians' global assessment at baseline cannot be individually determined, from a clinical point of view it is important to observe that earlier treatment is related to increased response rates.

DISCUSSION

In this retrospectively observed cohort of JIA patients, the time-to-start-MTX, the physician's global assessment at baseline and the starting dosage are significantly associated with the response to MTX at 6 months. Patients with an earlier start of MTX and an increased disease activity show an increased response. Our finding that a lower starting dosage MTX is associated with increased response is mainly due to the systemic JIA patients, who receive a higher starting dose and show decreased response.

Although treatment with intra-articular steroids, sulfasalazine and/or other DMARDs prior to MTX is an important determinant for the delay in starting MTX, only including patients with prior treatments to MTX therapy still showed that an early start of treatment with MTX was significantly associated with an increased response. This indicates that our data were not biased by population selection.

Our data show that patients with an increased disease activity receive MTX earlier after diagnosis, which may partially reflect confounding by indication. Therefore, the independent effects of a higher physicians' global assessment at baseline and the decreased time-to-start MTX on response cannot be determined in this analysis. The best evidence for these associations remains the replication in controlled trials with JIA patients.

Because of the different treatment strategies and small numbers of patients in the different JIA subtypes, only associations with MTX response in the general JIA population were observed and no conclusion about the influences of response in individual subtypes can be drawn.

With regard to the genetic parameters, only the MTHFR 1298A-677C haplotype showed a significant decrease in lower copy number in non-responders. This finding is consistent with recent-onset rheumatoid arthritis where a higher number of haplotype copies was related to increased response to MTX.[13] Remarkably, no other significant genetic associations with the response to MTX were detected. This may be due the small sample size resulting in an increased probability to obtain false negative findings (type 2 error).

Our data analysis used a composite measure for response including the physician's global assessment and the joint score. No frequent parent/ patient global assessment of overall well-being and information about limitation of joints could be retrospectively obtained from the patients' chart. Although the definition of improvement as developed by Giannini et al.[14] includes 6 core set variables, the two variables that were included in our definition of improvement are sensitive instruments for measuring change, with the subjective assessment of disease activity by the physician as the most responsive instrument.[15] The independent factors are able to measure an improvement of 53-60%.[16] Combined in our definition of improvement it generated a 57% response. Since improvement of the physician's global assessment is part of our response definition and a higher physician's global assessment at baseline is associated with response, the response rates in our cohort might be partly due to regression to the mean and an effect of unequal distance between the categories and will not fully reflect the true effect size of MTX.

According to the distribution of subtypes, this retrospectively observed cohort is comparable to the clinical practice described by Brunner et al, indicating that no non-random exclusion of subtypes has occurred.[3] In the different subtypes the response rates in this study are comparable to previous reported efficacy of MTX, except for persistent oligoarthritis.[4-8] In the persistent oligoarthritis cohort of Brik et al an response rate of 90% has been reported in patients receiving early treatment (maximum of 4.3 months), whereas our patients with persistent oligoarthritis had a response rate of 44% and were treated after a mean of 22.2 months (range 0-104 months; sd 27.4).[17] This difference in response rate underlines that also in persistent oligoarthritis early treatment with MTX may be an important factor for efficacy.

In summary, in this JIA population, the time-to-start MTX is an important and independent factor for the response to treatment. Already in RA it has been shown that early treatment in the "widow of opportunity" is associated with an improved

clinical outcome and less radiographic damage.[18] Although it is thought that JIA patients may show similar favorable effects of early treatment, our study is the first to illustrate that JIA patients have increased responses to MTX when treated earlier. Patients with a good clinical response to other DMARDs than MTX were not included in this study. Therefore, no conclusion about the effectiveness of MTX compared to other DMARDs or the association between time-to-start-MTX and other DMARDs in JIA can be drawn.

In RA it has been shown that the onset of disease and immunologic events predate the symptoms by many years. The activation of RA is believed to be a multifactorial process that is followed by an ongoing progression of inflammation leading to bone damage already in the first year after diagnosis.[19] Chronis arthritis in JIA patients probably has as similar early onset preceding the symptoms and a subsequent progression of inflammation. The increased response to early treatment with MTX, that is described in RA and shown in JIA in this study, might reflect the fact that MTX can suppress early stages of inflammation, but that the mechanism of action is less sufficient to control well-established chronic inflammation.

It is clinically highly relevant that reducing the time-to-start MTX may lead to an increased response. Future prospective studies are needed to replicate these findings and reveal the exact window of opportunity for JIA. More importantly future studies are needed to determine if an increased early response leads to less joint damage on the long-term.

In conclusion, our study shows the time-to-start-MTX appears to be an important factor for the MTX response. Our results suggest that earlier initiation of MTX treatment will lead to an increased response.

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