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ORIGINAL PAPER

THE APPLICABILITY OF METHOTREXATE INTOLERANCE SEVERITY SCORE IN JUVENILE IDIOPATHIC ARTHRITIS PATIENTS WITH MTHFR POLYMORPHISMS

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Abstract

Introduction. Many patients with juvenile idiopathic arthritis (JIA) experience gastrointestinal side effects after methotrexate (MTX) administration. Methotrexate Intolerance Severity Score (MISS) discriminates between patients with and without MTX intolerance, and helps to assess severity of adverse reactions, dose adjustment, mode of administration, or manage intolerance.
The objective of the study was to evaluate the applicability of MISS regarding methylenetetrahydrofolate reductase *(MTHFR)* polymorphisms in JIA.
Materials and methods. The study included 68 JIA patients on MTX, *MTHFR* evaluated through CFX96 Touch Real-Time polymerase chain reaction (PCR) System, Bio-Rad. The assay is a qualitative method with 100% clinical specificity. MISS consists of four domains: abdominal pain, nausea, vomiting, and behavioural symptoms. It records anticipatory and associated symptoms after MTX administration. A score of ≥6 on MISS, including at least one anticipatory, associative, or behavioural symptom, described as MTX intolerance.
Results. The samples included combined and isolated 677TT and 677CT + 1298AC patients. An average

Résumé

Applicabilité du Score de Sévérité de l'Intolérance au Méthotrexate chez les patients atteints d'arthrite juvénile idiopathique porteurs de polymorphismes du gène *MTHFR*

Introduction. De nombreux patients atteints AJI présentent des effets secondaires gastro-intestinaux après l'administration du MTX. Le MISS a été validé dans l'AJI pour discriminer les patients avec et sans intolérance au MTX. Ce score permet d'évaluer la sévérité des réactions indésirables et d'orienter l'ajustement posologique, le mode d'administration ou la prise en charge de l'intolérance.
L'objectif de l'étude était d'évaluer l'applicabilité du MISS en lien avec les polymorphismes du *MTHFR* chez les patients AJI.
Matériel et méthodes. L'étude a inclus 68 patients AJI sous MTX, avec analyse du *MTHFR* réalisée par CFX96 Touch Real-Time PCR System (Bio-Rad). Le test est une méthode qualitative avec une spécificité clinique de 100%. Le MISS comprend quatre domaines: douleurs abdominales, nausées, vomissements et symptômes comportementaux. Il enregistre les

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score of 10.22 (95% CI: 8.73–11.71) found in the study group, defining gastrointestinal intolerance to MTX. A disproportionate score 2.84 (95% CI: 2.29–3.40) resulted in the control group, not corresponding the definition of MISS intolerance. The adverse effects came in response to previous symptoms often not clinically apparent and often inadequately managed. The predominant factors driving MTX intolerance are behavioural factors.

Conclusions. JIA patients may develop anticipatory, behavioural, and associated symptoms when they think about MTX. MISS helps in determining the onset and evolution of these symptoms.

Keywords: juvenile idiopathic arthritis, *MTHFR*, MISS, intolerance, methotrexate.

List of abbreviations:
A – adenine
C – cytosine
CDC – Centers for Disease Control and Prevention
DNA – deoxyribonucleic acid
ILAR – International League of Associations for Rheumatology
JIA – juvenile idiopathic arthritis
MISS – Methotrexate Intolerance Severity Score
MTHFR – methylenetetrahydrofolate reductase
MTX – methotrexate
PCR – polymerase chain reaction
USA – The United States of America
T – thymine

symptômes anticipatoires et associés après l'administration du MTX. Un score ≥6, incluant au moins un symptôme anticipatoire, associatif ou comportemental, définit l'intolérance au MTX.

Résultats. Les échantillons incluaient des patients 677TT isolés ou combinés et 677CT + 1298AC. Un score moyen de 10.22 (IC95%: 8.73–11.71) a été observé dans le groupe étudié, indiquant une intolérance gastro-intestinale au MTX. Un score de 2.84 (IC95%: 2.29–3.40) a été retrouvé dans le groupe contrôle. Les effets indésirables étaient liés à des symptômes préexistants souvent non reconnus. Les facteurs comportementaux prédominaient.

Conclusions. Les patients AJI peuvent développer des symptômes anticipatoires, comportementaux et associés. Le MISS aide à identifier leur apparition et leur évolution.

Mots-clés: arthrite idiopathique juvénile, MTHFR, MISS, intolérance, méthotrexate.

Introduction

The biochemical structure of methylenetetrahydrofolate reductase (MTHFR) was first described in 1991, and its genetic topography on the short arm of chromosome 1 was determined in 1995[1]. The 677C>T variant of the MTHFR gene has increased the conditions associated with this genotype, such as congenital malformations, pregnancy complications, carcinogenesis, cardiovascular disease in adults, and psychiatric disorders. Although many of these associations remain unconfirmed or controversial, the goal of researchers remains to examine the geographic and ethnic distribution of associated genotypes. Accurate information about such distribution can contribute to the study of associations between individual genetic deficits and the diseases that may result (by providing reference data in the population) and population genetics (by highlighting evolutionary geographic and ethnic variations), as well as to the assessment of the impact of these associations[1,2].

The number of people who possess each of these genotypes will vary from population to population. When consuming folic acid, people with the MTHFR 677 TT genotype have an average blood folic acid concentration that is approximately 16% lower than people with the MTHFR 677 CC genotype[3].

Another variant of the gene polymorphism is the heterozygous MTHFR A1298C variant. This variant of the gene occurs at locus 1298 in the MTHFR gene, where the expected deoxyribonucleic acid (DNA) base Adenine (A) is replaced by Cytosine (C). Currently, there is insufficient evidence to demonstrate that the MTHFR A1298C variant can significantly affect how the human body processes folic acid[4].

The latest data provided by the Centers for Disease Control and Prevention (CDC) in 2009[5] show that the highest frequency of the C allele is in the non-Hispanic white population, at 31.1% (95% CI: 28.2–34.1; p<0.001), as well as the heterozygous genotype 1298 AC at 41.3% (95% CI: 38.2–44.4; p<0.001). Regarding the thymine (T) allele, the highest prevalence is among Hispanics (Mexicans) at

44.6% (95% CI: 41.7–47.5; p<0.001). The heterozygous genotype 677 CT has values of 49.4% (95% CI: 46.3–52.5; p<0.001) and the homozygous genotype 677 TT has values of 19.9% (16.7–23.5; p<0.001) for the same ethnic group. The overall resource shows a distribution of the C allele of 25.2% (95% CI: 22.2–28.5; p=0.030) and of the 1298 AC genotype of 37.8% (32.9–42.9; p=0.041) for the 12–19 age range. For the same age group, the T allele has a prevalence rate of 30.7% (95% CI: 26.5–35.3; p=0.764), while the 677 CT and 677 TT genotypes are prevalent in 39% (95% CI: 32.2–46.4; p=0.551) and 11.2% (95% CI: 8.4–14.7; p=0.551), respectively.

The prevalence of alleles and genotypes according to gender was also investigated. Thus, a statistically insignificant difference was demonstrated between males and females in the prevalence of allele C (28.6% vs. 28.2%; p=0.784) and allele T (30.4% vs. 31.1%; 0.628). In the case of genotypes, the situation remains similar for genotype 1298 AC (38.6% vs. 39%; p=0.935), for 677 CT (38.8% vs. 40.7%; p=0.731) and for 677 TT (11% vs. 10.8%; p=0.731). The overall prevalence of the C allele in relation to age 12–19 years was 27.5% (95% CI: 23.1–32.2) for non-Hispanic whites, and for the T allele for Hispanics (Mexicans) for the same age group – 48.1% (95% CI: 42.5–53.7). The 1298 AC genotype recorded a value of 39.5% (95% CI: 33.8–45.5) in non-Hispanics, while the 677 CT and 677 TT genotypes recorded values of 47% (95% CI: 37.9–56.4) and 24.6% (95% CI: 17.2–33.8)[6].

Studies have also been conducted, which found that the frequency of genotypes and alleles of the C677T polymorphism among the population of the Republic of Moldova is: CC – 40.84%, CT – 43.66%, TT – 15.49%, allele C – 62.68%, and allele T – 37.32%. In the case of the Moldovan population, an average level of C677T polymorphism was established (Ho=0.437), and the distribution of genotypes and alleles does not differ significantly from the theoretical value resulting from the Hardy-Weinberg equilibrium ($\chi 2$=2.76, p=0.09). The frequencies of genotypes observed for the A1298C polymorphism were as follows: AC – 45%; AA – 43%, CC – 12%. The distribution of genotype class frequencies falls within the limits of Hardy-Weinberg equilibrium (p=0.91)[7, 8].

Comorbidities can vary depending on the specific subtype of juvenile idiopathic arthritis (JIA) and the severity of the disease and managing them can be key to improving patients' quality of life. Uveitis is one of the most common eye complications associated with JIA and is an inflammation of the uveal tract, which can lead to vision loss if not treated properly.

Due to chronic joint inflammation in JIA, structural changes in the joints, such as bone erosions and joint space narrowing, may occur. These can lead to joint dysfunction and long-term deformities. Similarly, children with JIA may experience growth and developmental delays, especially in cases of active disease and treatment with corticosteroids or other immunosuppressive drugs. Due to chronic inflammation and corticosteroid treatment, patients with JIA are prone to bone loss and osteoporosis, which can increase the risk of fractures. There is evidence that patients with JIA may have an increased risk of cardiovascular disease, such as hypertension and endothelial dysfunction[9,10]. The chronic impact of the disease and long-term treatment can affect the mental health and emotional well-being of children with JIA, leading to anxiety, depression, and social problems.

The proper management of comorbidities is essential for improving the prognosis and quality of life of patients with JIA. It is important that they are monitored regularly by a multidisciplinary medical team, including rheumatologists, ophthalmologists, paediatricians, and other relevant specialists, to detect and manage these comorbidities correctly.

A double-blind randomized trial studied the side effects of low-dose methotrexate in patients with RA[11]. Of the 2,391 subjects randomized to MTX, 2,156 (90.20%) experienced any adverse effect and 2,080 (87.0%) experienced an organ system-specific adverse effect. The relative rates of gastrointestinal side effects (HR 1.91; 95% CI: 1.75-2.10), pulmonary (HR 1.52; 95% CI: 1.16–1.98), infectious (HR 1.15; 95% CI: 1.01–1.30), and hematologic (HR 1.15; 95% CI: 1.07–1.23) side effects were increased for low-dose MTX compared with placebo. Liver pathology was more common in the MTX group with five cases of cirrhosis (rate 0.11; 95% CI: 0.01–0.20, per 100 person-years) compared to placebo, which had no cirrhosis (p = 0.03).

The use of folic acid in the treatment of JIA is common practice, especially when patients are receiving MTX. Folic acid plays an essential role in cell division processes. Folic acid supplementation during MTX treatment can help maintain adequate folic acid levels in cells, thereby supporting the effectiveness of treatment. Despite the use of folic acid, many patients with JIA experience gastrointestinal side effects after receiving MTX. As a result, the discontinuation rate of background treatment is estimated to be as high as 35%, which increases the risk of relapse in patients already in remission, or of achieving remission in subsequent complex therapy[12]. Patients with JIA and RA also develop anticipatory and associative gastrointestinal adverse effects, such as vomiting or abdominal pain, which occur before and after MTX administration, as well as behavioural symptoms, such as restlessness and crying, when taking MTX, possibly because of the physical symptoms[13].

In this regard, for greater tolerability, increased attention is given to relaxation therapy or antiemetic medication, even though no extensive studies have been conducted for the paediatric population to date. Only for patients with RA is the balancing of adverse symptoms with folic acid supplementation considered. In children, these recommendations are currently classified as level IIIC and are valid only for minor adverse effects[14].

To quantify these side effects, in 2011, Bulatovic and colleagues published the development and validation of the Methotrexate Intolerance Severity Score (MISS)[15]. The initial MISS questionnaire was validated in patients with JIA at the University Medical Centre Utrecht by determining its ability to discriminate between patients with and without MTX intolerance, as established by the gold standard. This score can help clinicians assess the severity of adverse reactions to MTX and make decisions regarding dose adjustment, change in mode of administration, or other measures to manage intolerance to this drug.

It is important to note that the MISS score is a subjective assessment tool and that its interpretation should be performed by healthcare professionals with experience in managing MTX therapy and associated adverse reactions. Since there were no tools available designed to measure MTX intolerance, the physician's description of the presence or absence of MTX intolerance was considered the gold standard. Physicians considered the patient to be intolerant to MTX if the patient or his or her parents confirmed the presence of gastrointestinal symptoms before and after MTX administration, the persistent nature of these symptoms, and/or a negative effect of these symptoms in the days following MTX administration[15].

The same group of authors published a study conducted in the Netherlands on a sample of 291 patients who received MTX for the treatment of RA and psoriatic arthritis [13]. The patients reported at least one adverse gastrointestinal side effect, with a prevalence of 42.3%. Intolerance was more common in young people.

A multicentre study in Saudi Arabia, which included 117 patients with RA, systematized demographic and MTX-related information to study the predominant factors that can induce therapeutic intolerance. The systematization included educational level, smoking, route, and time of MTX administration. The behavioural component of MISS was involved in most cases of intolerance, which was accurately represented graphically[16].

Another example of assessing the need for early intervention in therapeutic intolerance is a 3-year prospective clinical study. Of the total of 150 Indian patients with RA, a MISS score greater than 6 points was noted in 14% of respondents. Most patients with intolerance administered MTX orally[17].

It has been noted that intolerance to MTX occurs gradually during the first months of administration and does not significantly alter the MISS score at 1 year[18]. Responders have the potential to progress positively to at least an ACR Pedi 70 index during the same period. The factors that may influence responders in JIA include disease severity, where patients with more severe forms of JIA may have a poorer response to MTX compared to those with milder forms of the disease. Other factors include the duration of treatment, genetic factors, and concomitant disease management through combination with other drugs, such as biologics or other immunosuppressive drugs.

The objective of the study was to investigate whether the MISS can be reliably applied when assessing the influence of MTHFR gene polymorphisms on treatment response in JIA.

Material and methods

To meet the study objectives, we employed an analytical, observational case-control design. The study population comprised 68 probands, aged between 1 and 18 years, all diagnosed with JIA based on International League of Associations for Rheumatology (ILAR) criteria. Genetic testing allowed classification into two groups: 23 patients in the case group and 45 patients in the control group. The patients presenting with either acute or chronic liver disease were excluded from participation.

Genetic method

Blood samples obtained from participants in both groups were preserved at –20°C and anonymized by the laboratory, following the referral procedures specified in Annex 2 of service contract no. 20112017 dated 20.11.2017. All storage units were locked, with access permitted only to authorized staff under strict monitoring. Upon completion of genetic analysis, both the extracted peripheral cell DNA and the remaining blood fractions were disposed of, in accordance with the provisions of paragraph 5.8 of the service contract. DNA amplification and detection were carried out using the CFX96 Touch Real-Time PCR Detection System, Bio-Rad, The United States of America (USA)[8], employing a qualitative method with confirmed 100% clinical specificity.

Clinical method

MTX intolerance was assessed using the MISS. This questionnaire consists of four domains: abdominal pain, nausea, vomiting, and behavioural

Table 1. MISS score in patients included in the study according to group (points)

MISS	*Study sample n=23*	*Control sample n=45*	*Total n=68*
Mean (SD)	10.22 (3.00)	2.84 (1.54)	5.32 (4.25)
Median (IQR)	9.50 (5.0)	3.0 (2.0)	4.0 (6.0)
Minimum – Maximum	7.0 – 18.0	0 – 6.0	0 – 18.0
	F; p		210.62; <0.001

symptoms, and records anticipatory symptoms after MTX administration as well as associative symptoms, i.e., symptoms that occur when the patient thinks about MTX. Symptoms are assessed at the time of questionnaire response, without a specific time interval. Behavioural symptoms include restlessness, irritability, and refusal of administration in response to gastrointestinal symptoms. Each of the 12 items on the MISS scale is scored on a psychometric scale (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms; 3 = severe symptoms) for a maximum score of 36. A score of ≥6 points on the MISS questionnaire, including at least one anticipatory, associative, or behavioural symptom, is defined as methotrexate intolerance.

Results

In this study, children with JIA treated with MTX for at least six months underwent genetic screening to detect MTHFR polymorphisms. Within the case group, the C/T heterozygous variant at locus 677 was identified in 60.9% of patients (14 subjects; 95% CI: 40–81), while 39.1% (9 subjects; 95% CI: 19–60) carried the homozygous T/T genotype for the pathological allele (χ^2 = 37.88; df = 2; p < 0.001). For locus 1298, the heterozygous A/C variant was observed in 65.2% (15 subjects; 95% CI: 44.4–85), the homozygous A/A genotype in 34.8% (8 subjects; 95% CI: 15–55.6), while no C/C homozygous variants were detected (χ^2 = 4.03; df = 2; p = 0.133). Notably, compound heterozygosity involving the polymorphic alleles T and C (C/T at locus 677 and A/C at locus 1298) was found in 60.9% of cases (14 subjects; 95% CI: 40–81).

In the control group, 71.1% (32 subjects; 95% CI: 57.5–84.4) displayed the normal C/C homozygous genotype at locus 677, while 28.9% (13 subjects; 95% CI: 15.6–42.5) carried the heterozygous C/T genotype. For locus 1298, genotype distribution included A/A homozygotes in 51.1% (23 subjects; 95% CI: 35.7–65.2), A/C heterozygotes in 42.2% (19 subjects; 95% CI: 28.3–57.1), and C/C homozygotes in 6.7% (3 subjects; 95% CI: 0–15.6).

Additionally, isolated heterozygous variants (C/T and A/C) were observed in both loci. However, these polymorphisms, when not expressed in homozygous T/T or combined heterozygous (C/T + A/C) form, did not appear to influence the clinical phenotype under investigation. The distribution of these isolated variants was similar across the control group.

The case cohort (n = 23) was further stratified according to genotype: homozygous T/T carriers (χ^2 = 20.29; df = 1; p < 0.001) and compound heterozygotes C/T and A/C (χ^2 = 34.49; df = 1; p < 0.001), in order to evaluate genotype-phenotype associations.

To determine the utility of polymorphism testing, allele frequencies at loci 677 and 1298 were calculated. The presence of homozygous T/T, as well as heterozygous C/T and A/C genotypes, was found to influence both treatment-related side effects and therapeutic response among JIA patients. Allele frequencies were estimated using the Hardy–Weinberg equilibrium model, with acceptable error margins, based on observed genotype distributions. The calculated allele frequencies in JIA subjects were: C = 0.67, T = 0.33 (locus 677), and A = 0.71, C = 0.29 (locus 1298). No deviations from Hardy–Weinberg equilibrium were detected.

MTX intolerance was measured using the MISS questionnaire. This score allowed us to assess gastrointestinal toxicity non-invasively through the questionnaire, by directly interviewing the subjects. All subjects in the general groups completed the questionnaire. After processing the results, a mean score of 10.22 (95% CI: 8.73–11.71) was found for the study group, which is a clear criterion for defining gastrointestinal intolerance to MTX. Table 1 shows the disproportionate average score in the control group, which was 2.84 (95% CI: 2.29–3.40), which does not correspond to the definition of intolerance according to MISS.

The Mann-Whitney U test in this case confirms the alternative hypothesis regarding the score obtained for assessing MTX intolerance in patients with genetic polymorphism. The distribution curves in Figure 1 differ in location and shape (p<0.001).

The anticipatory, associative, and behavioural symptoms of the MISS score were investigated and assessed, which, in relation to genetic polymorphism, show statistically significant relationships. The latter increase the diagnostic power of testing these

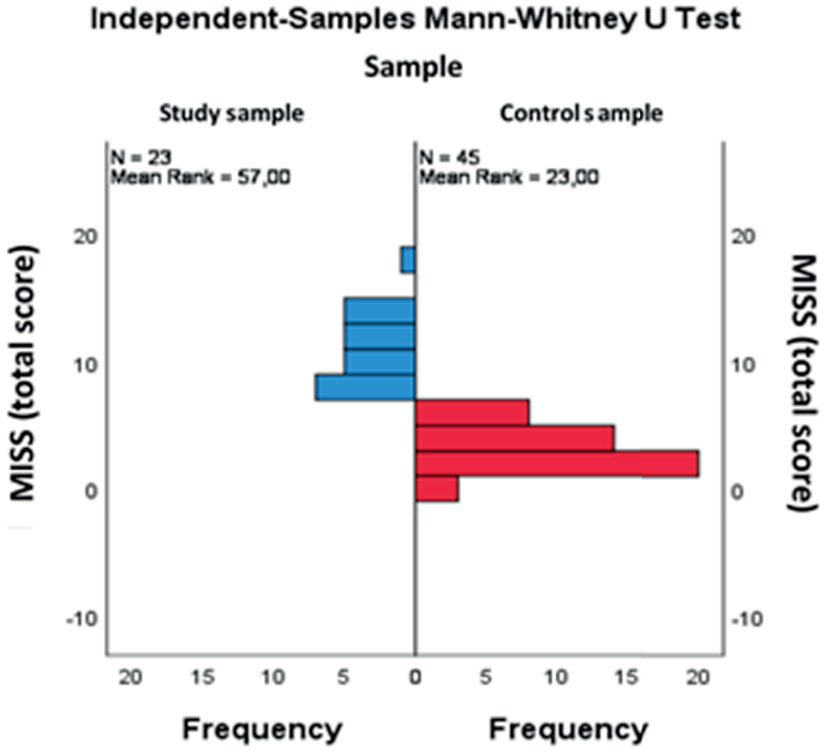


Figure 1. Distribution of MISS scores by groups

polymorphic variants, even prior to the initiation of MTX therapy.

In terms of abdominal pain after MTX administration, in the study group 11 (47.8%; 95% CI: 26.9–70.0) respondents characterized it as mild, and 12 (52.2%; 95% CI: 30.0–73.1) as moderate. In the control group, 16 (35.6%; 95% CI: 21.4–51.0) subjects experienced mild abdominal pain, and 29 (64.4%; 95% CI: 49.0–78.6) did not report abdominal pain.

Another symptom identified after administration of the preparation was nausea, which in the base group was mild in intensity in 9 (39.1%; 95% CI: 20.0–60.0) patients and moderate in intensity in 5 (21.7%; 95% CI: 5.3–41.4) patients. Nausea was absent in 9 (39.1%; 95% CI: 19.3–59.3) subjects. For the control group, there were 7 (15.6%; 95% CI: 5.3–27.5) subjects who experienced mild nausea. In total, in the control group, 38 (84.4%; 95% CI: 72.5–94.7) patients did not experience nausea after medication.

Vomiting in the subjects analysed in the case group was assessed as a mild symptom by 6 (26.1%; 95% CI: 8.7–44.8) subjects and as a moderate symptom by 4 (17.4%; 95% CI: 3.7–33.3) subjects. No vomiting was reported after MTX administration in 13 (56.5%; 95% CI: 37.0–77.8) respondents. In the control group, 1 (2.2%; 95% CI: 0 – 7.7) patient experienced mild vomiting after MTX, while the rest of the patients included in the study did not experience this post-administration symptom.

In addition, patients may develop anticipatory symptoms, which occur before MTX intake, and associative symptoms, when patients think about the drug, as well as behavioural symptoms. These adverse effects occur in response to previous symptoms experienced by patients treated with MTX and are often not clinically apparent; therefore, they are often inadequately managed. Although folic acid supplementation during MTX treatment can reduce such effects, many patients discontinue treatment, which negatively affects disease control and quality of life. The results obtained from processing the questionnaire data are shown in Table 2.

The results clearly indicate that behavioural components are the primary drivers of MTX intolerance. This trend is visually emphasized in the radar chart presented in Figure 2, where behavioural scores consistently exceed those of other symptom domains, underscoring the substantial impact of patient behaviour and medication-related distress on treatment tolerability.

Discussion

Currently, due to the cost of therapy, efficacy, and low rate of clinically manifested side effects, MTX is the treatment of choice for JIA[19–21]. Unfortunately, nearly one-third of patients do not respond adequately to MTX, due to insufficient improvement in

Table 2. The importance of MISS score symptoms for study samples

Symptoms	*Type*	*Form*	*Study sample n (%)*	*Control sample n (%)*	χ^2	*P*
Abdominal pain	Anticipatory	Absent	9 (39.1)	44 (97.8)	30,495	<0,001
		Mild	12 (52.2)	1 (2.2)		
		Moderate	2 (8.7)	-		
	Associative	Absent	12 (52.2)	41 (91.1)	13.762	0.001
		Mild	10 (43.5)	4 (8.9)		
		Moderate	1 (4.3)	-		
Nausea	Anticipatory	Absent	19 (82.6)	44 (97.8)	5.141	0.023
		Mild	4 (17.4)	1 (2.2)		
	Associative	Absent	10 (43.5)	40 (88.9)	16.901	<0.001
		Mild	11 (47.8)	5 (11.1)		
		Moderate	2 (8.7)	-		
Vomiting	Anticipatory	Absent	18 (78.3)	43 (95.6)	4.930	0.026
		Mild	5 (21.7)	2 (4,4)		
Behavioural symptoms	Restlessness	Absent	2 (8.7)	16 (35.6)	22,921	<0.001
		Mild	4 (17,4)	21 (46,7)		
		Moderate	13 (56.5)	8 (17.8)		
		Severe	4 (17.4)	-		
	Cry	Absent	5 (21.7)	33 (73.3)	16.500	<0.001
		Mild	16 (69.6)	11 (24.4)		
		Moderate	2 (8.7)	1 (2.2)		
	Irritability	Absent	3 (13.0)	30 (66.7)	21.791	<0.001
		Mild	13 (56.5)	14 (31.1)		
		Moderate	7 (30.4)	1 (2.2)		
	Refuses to take MTX	Absent	1 (4.3)	27 (60.0)	25.611	<0.001
		Mild	12 (52.2)	16 (35.6)		
		Moderate	10 (43.5)	2 (4.4)		

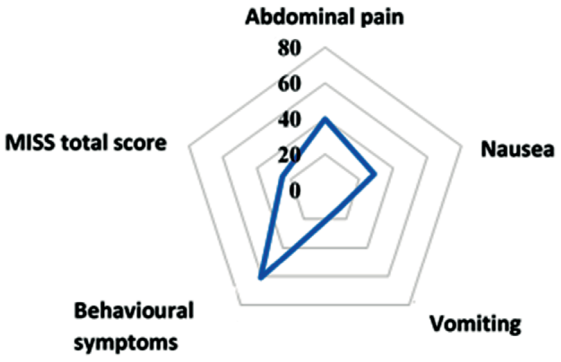


Figure 2. Radar chart of the MISS and related components.

disease symptoms or the occurrence of adverse reactions, leading to discontinuation of treatment. These patients miss the optimal window of opportunity for initiating biological therapy and experience more severe manifestations of the disease[22]. To enable prediction of response to MTX therapy, numerous pharmacogenetic studies have been conducted on the genes that encode the proteins involved in the mechanism of action of MTX.

The key aspects of personalized medicine are based on genetic analysis, namely sequencing an individual's DNA to identify genetic variations that may influence the risk of developing certain medical conditions or how they respond to treatment. Knowing an individual's genetic profile can help identify their personal risk of developing certain diseases, thus enabling more personalized preventive interventions and more effective screening programs.

The spread of MTHFR allele variants in populations is diverse, with Mexicans and Italians having the highest homozygous ratio[23]. The single nucleotide polymorphism of this gene reduces the thermostability of the MTHFR enzyme, due to its low activity at 37°C or higher. MTHFR enzyme activity in homozygous subjects is 50-60% lower at 37°C and 65% lower at 46°C [23,24].

The presence of a particular genetic variant does not automatically cause a disease or condition. The association of genetic variants of the MTHFR gene

with certain health conditions, such as neural tube defects, cardiovascular risk, and others, is still a subject of research and debate in the scientific community. In addition, genetic factors are only one of many factors that contribute to overall health and the risk of developing certain diseases.

The most well-studied polymorphism of the MTHFR gene is known as C677T. This polymorphism can influence homocysteine levels and may affect how the body processes and uses folic acid.

In this study, the homogeneity of allele distribution in relation to Hardy-Weinberg equilibrium was demonstrated in subjects with JIA. Similarly, a quantitative analysis of the polymorphic variant type was performed for both study groups, which constituted about one-third of the sample. Testing of polymorphic variants in MTHFR in patients with rheumatic disease is carried out exclusively at the local level and cannot be compared with other national studies. It is possible to associate, by way of example, a prediction model for these tests with the prediction models already created for the pathologies described above at the national level.

For adults who show clinical symptoms such as nausea or abdominal pain, the severity score for MTX intolerance is well defined, including these symptoms according to their severity. The score can also be used in paediatric patients receiving long-term MTX, as it has been validated in the context of increased MTX intolerance prevalence[15]. At the same time, some studies on these symptoms report their nature, with female adolescents receiving subcutaneous MTX for more than one year being typically affected[25].

Our results are comparable to other studies on the MISS score, with the maximum score obtained in the research group exceeding the maximum score for the control group by three times. It has been shown that attention must be paid to the patient's behavioural symptoms to understand one of the causes of poor response to treatment, resulting from non-compliance.

Given the small MTX-treated subgroup, it is possible that the study does not reflect the broader clinical and paraclinical variability of JIA. Representative score use, together with the patients' genetic data, are undoubtedly specific to each individual child and therefore prone to subjectivity in terms of the population. Physical and behavioural symptoms in stationary conditions are assessed verbally by the child or their parents, which are not always quantified and may change with the change in disease activity or depending on the degree of doctor-patient compliance. However, confidence in scores based on quantitative parameters, as well as the overall assessment of the patient's condition by the physician and patient, mitigates these limitations. The MISS score, as a test for assessing gastrointestinal toxicity, can show objective results and is routinely used at each hospitalisation for patient follow-up.

Conclusions

More than one-third of the study population carried polymorphic variants of the MTHFR gene. These individuals were distinguished by elevated rates of compound heterozygosity, with values of 0.43 and 0.44, and by a homozygosity level of 0.48 for the rs1801133 and rs1801131 polymorphisms. The application of the MISS questionnaire provided valuable insights into the tolerability of MTX among patients with MTHFR polymorphic variants. A subgroup of fourteen individuals reported abdominal pain following MTX administration. This discomfort appeared to precipitate moderate behavioural symptoms, including hesitation or outright refusal to take further doses. These findings suggest a clinically relevant intolerance to MTX in this genetically defined subset of patients.

Author Contributions:

V.I., A.C., I.M., N.R. were responsible for clinical diagnosis, paraclinical investigations, treatment decisions and follow up of the patients. I.V., A.C. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from the patients included in the study"

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