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PHARMACOGENOMIC IMPACT OF METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM IN JUVENILE IDIOPATHIC ARTHRITIS

322.01 PAEDIATRICS AND NEONATOLOGY

Summary of the PhD thesis in medical sciences

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The thesis was developed in the Department of Paediatrics of the *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova

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INTRODUCTION

Children with juvenile idiopathic arthritis are treated and monitored throughout their lives [1]. This chronic disease has an unclear genesis and, despite new discoveries in disease pathogenesis, its course remains unpredictable. In the last decade, several molecular and genetic pathways have been investigated, which may provide the basis for a model to predict therapeutic success and long-term disease remission [2,3].

Changes in the osteoarticular and immune system under conditions of high disease activity cause sequelae and disability in the child's growth process [4]. JIA, with onset at any age and of any type, has consequences for the physical and psycho-emotional status of both the child and the family. At the same time, depending on the degree of disability confirmed, treatment of the disease involves additional costs for care and social integration [5].

At the onset and during the acute phase of juvenile idiopathic arthritis, locomotor and associated psychosomatic functions are compromised. With the initiation of stepped disease-modifying therapy, these functions partially recover, after which patients with difficulty accept continued administration of these medications. As a result, patients return to the rheumatology unit to associate or escalate treatment to the next step, over time, quality of life is reduced, some children remain disabled and require daily care and attention. The subsequent therapeutic step is inherently the introduction of steroidal anti-inflammatory medication, which also carries a dual character, on the one hand relieving systemic manifestations, and on the other inducing long-term side effects [6]. Recovery of musculoskeletal function depends on the degree of disease activity, the form of the disease and the patient's therapeutic compliance.

At present, significant progress has been made in identifying the mechanisms, which have an impact on the onset of disease remission and restoration of locomotor function in JIA. These are biomolecular pathways and genetic susceptibility, which are target factors involved in the acute phase of the disease.

The term "genetic susceptibility" refers to structural changes within the genome that have occurred in-vivo over the course of the child's development in response to internal and external factors. Susceptibility implies that the structure of a particular gene influences the function of the protein it encodes and phenotypically expresses the positive or negative character of a state or action. For JIA, this susceptibility favours the ability to tailor individual medication selection to follow the Treat-to-Target concept. Genetic variations would favour the explanation of some intolerance phenomena in the recovery of patients with JIA. Currently, expression of metabolic factor genes, especially mutant ones, is hypothesized as potential prognostic markers of nonresponders to disease-modifying therapy in JIA [7].

A wide spectrum of genes are associated with response to glucocorticosteroid, *methotrexate* and biologic therapy. Thus, methylenetetrahydrofolate reductase (*MTHFR*), being part of the metabolic cycle of the folates, is involved in modulating the response to initial therapy with *methotrexate* [8–10].

MTHFR is well known as a thrombophilia gene, and is implicated in causing adverse pregnancy outcomes [11–13], as well as cardiovascular risk, which has been investigated mainly in adults [14,15], and, last but not least, in neuropsychiatric pathology [16]. Furthermore, there is limited evidence that *MTHFR* is a maternal risk factor for chromosomal abnormalities in the fetus [17]. *MTHFR* is not directly affected by MTX, but displays the next step in the dysfunction of the folate cycle after MTX binding to dihydrofolate reductase. Indeed, mutations in the gene of this

enzyme increase the risk of abolished protein synthesis and elevate serum homocysteine levels [18].

To date, determining whether patients with or without mutation for the rs1801133 and rs1801131 variants of the *MTHFR* gene combined would have a worse outcome following MTX administration remains a contradictory scientific pursuit [19]. New evidence has shown that treatment response and toxicity are multifactorial and require validation as a comprehensive predictive model.

The aim of this study was to assess the role of *MTHFR* gene polymorphisms as predictors of *methotrexate* efficacy and toxicity in idiopathic juvenile arthritis in order to develop recommendations for optimizing *methotrexate* treatment.

Study objectives:

1. Determining the presence of mutations in the *MTHFR* gene in juvenile idiopathic arthritis.
2. Clinical evaluation of disease activity scores and *methotrexate* intolerance in juvenile idiopathic arthritis.
3. Evaluation of *methotrexate* liver toxicity in relation to the presence of *MTHFR* gene mutation in juvenile idiopathic arthritis.
4. Functional assessment of *methotrexate* cardiac toxicity in relation to the presence of *MTHFR* gene mutation in juvenile idiopathic arthritis.
5. Development of recommendations for genetic polymorphism testing in JIA

Study hypothesis: Children with juvenile idiopathic arthritis who carry mutations in the methylenetetrahydrofolate reductase enzyme gene have reduced therapeutic efficacy and increased hepatic and cardiac toxicity when administered *methotrexate***.**

Summary of the thesis sections: The thesis is written in Romanian as a manuscript. It is presented on 141 pages, computer written and contains: introduction, literature review, research materials and methods, results, discussion, general conclusions and practical recommendations. The bibliography includes 160 titles. The work is illustrated with 17 tables, 38 figures and 23 appendices.

The Introduction chapter highlights the importance and actuality of pharmacogenetic issues in the treatment of JIA. To this end, we reviewed relevant worldwide epidemiological data, risk factors, predictive models of ineffectiveness and toxicity in *methotrexate* treatment. We also assessed the impact of *MTHFR* gene testing on these outcomes.

Chapter 1 contains a review of the literature addressing the genetic aspects of juvenile idiopathic arthritis, mechanisms of metabolic regulation of the folate cycle, general notions of genetic susceptibility and the role of *MTHFR* gene polymorphism in the treatment of JIA. This chapter presents the achievements of the last few years, the unsolved problems to date and the future directions of research in this field of rheumatology. Standard methods of measuring predictive indices of organic impairment in JIA are discussed and reviewed, as well as the implication of behavioural symptoms in treatment compliance.

In **Chapter 2** we describe the criteria for inclusion of subjects in the study, the methodology for calculating the sample size and the research design. The chapter provides details of the clinical, paraclinical and genetic methods applied during the study. Similarly, the statistical evaluation methods for assessing the results of the study, which formed the background to the overall conclusions, are described.

Chapter 3 presents the results of the study. In this chapter the subjects of both study groups are described on the basis of demographic and social indicators, clinical and paraclinical examinations and their relationship with the *MTHFR* gene polymorphism is established. The sub-

chapter dedicated to the results of genetic investigations on rs1801133 and rs1801131 polymorphisms of the *MTHFR* gene in patients with juvenile idiopathic arthritis, analyses the groups comparatively on the basis of the detected genotypes. Thus, we obtained algorithms for individual testing of efficacy and toxicity to *methotrexate* treatment.

Chapter 4 includes discussion of all results, comparison with similar data in the literature and highlighting aspects of national novelty developed in the paper.

The thesis concludes with **General Conclusions** and **Practical Recommendations**.

General research methodology: In order to accomplish the proposed objectives, the study was conducted as an analytical case-control research. The research was approved by the Research Ethics Committee of the State University of Medicine and Pharmacy "Nicolae Testemitanu" (protocol no. 44/52 of 12.04.2018). A total of 68 patients with juvenile idiopathic arthritis receiving *methotrexate* treatment for more than 6 months were included in the study. All participants underwent a complex examination, which included: completion of a specially developed questionnaire, standard clinical examination with assessment of parameters of interest for inclusion in clinical disease assessment scores, laboratory examinations with assessment of acute phase reactants (erythrocyte sedimentation rate, C-reactive protein), traditional blood biochemical indicators for assessment of liver toxicity to *methotrexate* (ALT, ALP, total bilirubin, AST), imaging examinations (abdominal ultrasonography, echocardiography + tissue Doppler, Fibro Scan). Subjects were analysed according to the clinical form of the disease: systemic, oligoarticular, polyarticular, duration of the disease, and treatment: monotherapy, combination therapy. The control group consisted of 45 children who did not carry the genotype investigated. Based on the results, conclusions were drawn and practical recommendations were developed.

Scientific novelty of research results: a) it has been demonstrated that the combined heterozygous and homozygous mutant genotypes for *MTHFR* is significant in one third of subjects with JIA on *methotrexate*; b) the application of clinical scores to assess the efficacy and toxicity of *methotrexate* demonstrates high specificity and sensitivity for testing *MTHFR* mutations in JIA; c) one-dimensional transient elastography demonstrated the individual character of liver damage in the treatment of JIA; d) the importance of tissue Doppler in the assessment of ventricular systolic and diastolic function in patients with JIA has been demonstrated.

Theoretical importance of the research

Evaluation of *MTHFR* genetic polymorphisms in relation to clinical and paraclinical features of patients with JIA using the following tools: standard clinical examination, DAS 28 score, JADAS 71 score, ACRPedi 30% index, *Methotrexate* Intolerance Severity Score, standard 12-lead ECG parameters, standard 2D liver ultrasound parameters, FibroScan parameters, standard 2D ultrasound + cardiac tissue Doppler parameters. The study generated additional knowledge on genetic testing with a role in determining the appropriateness of MTX initiation in subjects with JIA, facilitating individualized prediction of the likelihood of intolerance.

Application value of the work

Based on the obtained results, algorithms for predicting treatment efficacy and possible toxic effects were proposed. The results of the research will be implemented as methodological support in the work of rheumatologists, cardiologists, gastroenterologists and pediatric hepatologists as well as geneticians and pharmacologists working in pediatric medical institutions involved in the process of evaluating the behavior of children with JIA.

The approval of the results obtained was carried out in accordance with the fundamental steps of the study. The main results were presented, discussed and approved at the meetings of the Department of Pediatrics of the State University of Medicine and Pharmacy "Nicolae Testemitanu"

and the Scientific Seminar, as well as at national and international scientific conferences: Conferința Națională de Pediatrie ”Progrese în Tratamentul Artritei Juvenile Idiopatice” (2020, Chișinău, Moldova); Conferința Științifică Anuală a USMF ”Nicolae Testemițanu” (2021, Chișinău, Moldova); A 5-a Conferință Națională cu Participare Internațională "Biennale Chișinău-Sibiu" -"Managementul Interdisciplinar al Copilului" (2022, Chişinău, Moldova); Conferința Științifică Anuală a USMF ”Nicolae Testemițanu” (2022, Chişinău, Moldova); Conferința Științifică Anuală a USMF ”Nicolae Testemițanu” (2023, Chişinău, Moldova); şi internaţionale: The 7th International Medical Congress for Students and Young Doctors MedEspera (2018, Chișinău, Moldova); Congresul al 25-lea al Societății Europene de Reumatologie Pediatrică (2018, Lisabona, Portugalia; Conferința Națională de Pediatrie ”Ghiduri și Protocoale în Pediatrie” (2018, București, România; A 3-a Conferință Națională cu Participare Internațională "Biennale Sibiu-Chișinău" (2019, Sibiu, România); The 8th International Medical Congress for Students and Young Doctors MedEspera (2020, Chișinău, Moldova); Conferința Națională de Pediatrie ”Ghiduri și Protocoale în Pediatrie” (2021, București, România); XXIII Конгресс Педиатров России с Международным Участием “Актуальные проблемы педиатрии” (2021, Москва, Российская Федерация; International Black Sea Coastline Countries Scientific Research Symposium – VI (2021, Giresun, Turkey); A XXXIV-a Ediție a Conferinței Naționale ”Zilele Pediatriei Ieșene N.N.Trifan” cu Participare Internațională (2022, Iași, România); Congresul al 28-lea al Societății Europene de Reumatologie Pediatrică (2022, Praga, Cehia); A XXXV-a Ediție a Conferinței Naționale ”Zilele Pediatriei Ieșene N.N.Trifan” cu Participare Internațională (2023, Iași, România); Ediția a 7-a a Conferinței Naționale de Reumatologie Pediatrică cu Participare Internațională (2023, Iași, România); Conferinţa Națională de Pediatrie cu Participare Internațională (2023, Chișinău, Moldova); Young Investigators Meeting (2023, Rotterdam, Olanda); Congresul al 29-lea al Societății Europene de Reumatologie Pediatrică (2023, Rotterdam, Olanda).

Key words: juvenile idiopathic arthritis, *MTHFR*, children, *methotrexate*, FibroScan, toxicity, efficacy.

1. GENERAL CHARACTERISTICS OF THE STUDY MATERIAL AND RESEARCH METHODS

1.1. General presentation of the performed study

The present research presents a case-control study, which was conducted during 2017-2021 in the Department of Pediatrics, State University of Medicine and Pharmacy "Nicolae Testemitanu", Republic of Moldova, at the clinical base PMSI Institute of Mother and Child, Pediatric Rheumatology Clinic. Sixty-eight children with established diagnosis of juvenile idiopathic arthritis according to ILAR classification criteria were included in the study.

At the initiation of the trial, all parents/legal guardians of the selected subjects were informed about the stages of the research and enrolled in the study only with personal consent, and were explained in detail the requirements imposed and the way of carrying out the necessary investigations, participant's rights, confidentiality of personal data, risks, through individual discussion. All procedures were carried out after signing the informed consent by the legal representative and with the assent of children aged ≥ 14 years. They were not reimbursed and incurred no financial costs related to participation. The study was approved by the Research Ethics Committee of the State University of Medicine and Pharmacy "Nicolae Testemitanu" (decision no.52 of 12.04.2018).

The research sample was calculated using the standard formula (2.1) for case-control studies:

$$\mathrm{n} = \frac{1}{1-f} x \frac{2(Z\alpha + Z\beta)^2 x\, P(1-P)}{(P0 - P1)^2} \tag{1.1}$$

where:

P0 - Patients with juvenile idiopathic arthritis with MTX and polymorphism of the *MTHFR* gene. According to literature data, the prevalence of this polymorphism is 15,49% (P0 = 0.1549)

P1 - Patients with juvenile idiopathic arthritis with MTX and without polymorphism of *MTHFR* gene. We assume, that in the research group the value will be 50% (P1 = 0,5), then:

$$P = (P0 + P1)/2 = 0{,}32 \tag{1.2}$$

Zα - tabulated value. For statistical significance of the results 95% coefficient Zα=1.96

Zβ - tabulated value. For statistical power of comparison of 90% the coefficient Zβ = 1.28

f = Proportion of subjects expected to drop out of the study for reasons other than the effect under investigation q = 1/(1-f), f = 0% (0).

Inputting the data into the formula we obtained:

$$38{,}36 = \frac{2(1{,}96 + 1{,}28)^2 x\ 0{,}32\ x\ 0{,}68}{(0{,}1549 - 0{,}5)^2}$$

Thus, two groups were created for the research:

Case group - which included at least 19 patients with established diagnosis of JIA, MTX therapy and genetic polymorphism of *MTHFR*;

Control group - which included at least 19 patients with established diagnosis of JIA, with MTX, without genetic polymorphism of *MTHFR*.

The sample was also calculated using the computer program QUANTO v1.2.4, which is a mathematical program designed for the study of associations between genetic and environmental factors and gene-ecological or intergenic interactions in the development of multifactorial diseases. The gene-candidate association with JIA in methotrexate administration was examined.

We exemplify the estimation of the number of case-control pairs required to study the association with the predicted gene - MTHFR. We assume the following:

1. The prevalence of the disease in the pediatric population (Kp) is equal to 0.001% (according to ACR data).

2. The polymorphism of the MTHFR gene results in the formation of 2 alleles at locus 677: the null allele C (e.g. marked by the letter "A") and the functionally competent allele T (marked by the letter "a"). Carriers of the homozygous TT genotype are at increased risk for developing methotrexate toxicity and ineffectiveness. The proportion of subjects in the population carrying the genotype for the Republic of Moldova is 15.49%.

3. According to the Hardy-Weinberg equilibrium law, we found the incidence of alleles in the population. Thus, the incidence of competent MTHFR alleles in the population is qA= √15.49= 0.3935. The inheritance model is the recessive model.

4. We assume that the risk of development of toxicity and inefficacy for TT genotype carriers in JIA subjects, compared to those carrying normal alleles, is at least equal to 2.0 (Rg).

5. The power of the study must be at least 80% and the established level of significance is equal to 0.05 (type I error). Entering the data into the program, we obtained the number of 204 case-control pairs.

Outcome :		Disease: JIA		
Design:		Matched case-control		
Hypothesis:		Gene only		
Desired power: 0.800000		Significance: 0.050000, 2-sided		
Gene				
Mode of inheritance:		Recessive		
Allele frequency: 0.3935				
Disease model		Summary parameters		
*P0 0.000008		kP 0.000010		
Rg: 2.0000		(*indicates calculated value)		
Parameter	N	Null	Full	Reduced
Gene	204	βG=0	βG	----

The objective of the study is to determine the presence of polymorphic variants in the MTHFR gene in JIA in patients taking MTX. Since the study of the prevalence of polymorphic variants in the population of children with JIA in the RM does not fulfill this objective, we used the standard formula for case-control studies to calculate the sample size.

Research subjects met the following inclusion criteria:

(a) children aged between 1 and 18 years;

b) diagnosis of juvenile idiopathic arthritis;

c) patients with JIA and *methotrexate* treatment for more than 6 months;

d) consent of the patient's parents or legal representative (and of the patient in case of age 14-18 years by consent form) to perform the molecular-genetic examination and participate in the study.

Exclusion criteria were:

(a) children with juvenile idiopathic arthritis not on *methotrexate* treatment;

b) children with acute and chronic liver disease;

c) children with congenital heart malformations;

d) subjects who refused to participate in the study.

All subjects were genotyped for *MTHFR* by a set of tests, which included CBC, ESR, blood biochemistry, immunological tests, electrocardiogram, echocardiography and liver ultrasonography. Fibro Scan was performed in 50 subjects. All patients were assessed by *methotrexate* intolerance severity score, DAS28, JADAS71 and ACRPedi 30 index. The study design is represented in figure 1.

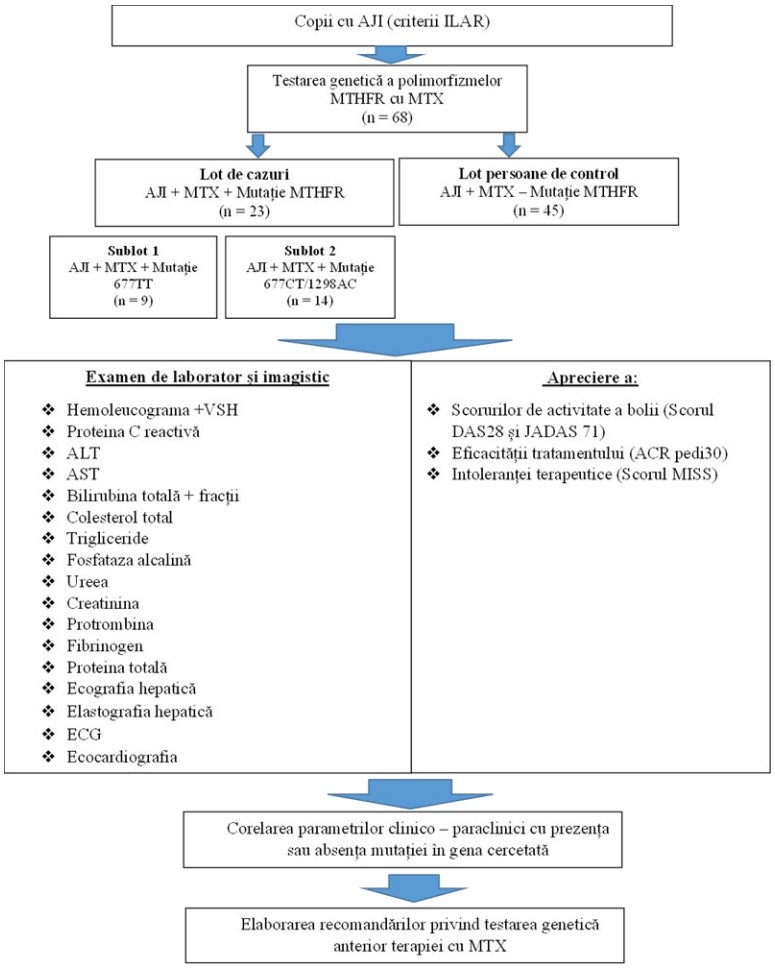

Figure 1. **Study design**

Ethical considerations. The study took into account the international rules of medical ethics with regard to the confidentiality of participants' data, as set out in the Declaration of Helsinki. The results obtained were communicated only to the participant as personal data of each subject were not used for other purposes.

1.2. Methodology applied during the research

a) Clinical method

The research protocol was used to interview both case and control groups. The questionnaire was structured for the research phase so as to allow the necessary results to be cumulated and included a number of concrete questions. This questionnaire was completed for each patient included in the study. The standard interview method allowed the collection of data on duration of symptoms and illness, global assessment of illness and symptoms such as pain, assessment of adverse reactions to treatment, and part of the information was transferred from medical documentation.

Assessment scales applied:

1) DAS28 disease activity index which consists of the assessment of 4 variables: number of painful and swollen joints, global disease assessment and erythrocyte sedimentation rate value;
2) JADAS71 disease activity index which included the following four measurements: physician global assessment of disease activity and patient or parent global assessment of disease activity, measured on the visual analogue scale of 0-10 cm where 0 = very good and 10 = very poor; erythrocyte sedimentation rate, normalised to a scale from 0 to 10 according to the formula ESRn = (ESR-20)/10;
3) ACR Pedi treatment efficacy index 30 % which is based on key variables such as physician global assessment of disease activity (0-10 cm VAS), parent/patient assessment of general well-being (0-10 cm VAS), functional capacity, number of joints with active arthritis (defined as joint effusion or limitation of movement accompanied by hyperthermia, pain or tenderness), number of joints with limited range of motion and ESR;
4) The *Methotrexate* Intolerance Severity Score (MISS) consists of four domains: abdominal pain, nausea, vomiting and behavioural symptoms and records anticipatory symptoms after *methotrexate* administration as well as associative symptoms. Each of the 12 MISS items 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 established as *methotrexate* intolerance.

b) Paraclinical method:

Paraclinical examination included the assessment of markers of inflammation - sedimentation rate of red blood cells (ESR), C-reactive protein, fibrinogen, determination of biochemical parameters - ALP, ALT, AST, total bilirubin, investigations carried out in the clinical-biochemical and immunological laboratory of the Institute of Mother and Child.

Functional exploration of the liver was performed in the Functional Diagnostic Department of the Institute of Mother and Child, according to the current requirements for the surveillance of patients with JIA (NCP - 7 Juvenile Idiopathic Arthritis, 2016).

FibroScan elastographic liver exploration was performed on 50 patients, being a painless method that does not require prior preparation and the result is released immediately. The FibroScan Touch 502 device from Echosens, Paris, France, is based on the concept of transient elastography. The reference values of median liver elasticity were taken from the European Federation of Societies for Ultrasound in Medicine and Biology.

To complete the fourth objective, according to the 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines, electrocardiography and transthoracic tissue Doppler echocardiography were performed.

c) Molecular – genetic method:

Blood samples collected from patients in both study groups were stored in a freezer at -20ºC and anonymized by the laboratory according to the referral form in Annex 2 of the contract for the provision of services no.20112017 of 20.11.2017. After submission, peripheral cell DNA and the remaining blood portions were liquidated, as per the respective amendments in paragraph 5.8 of the service contract no.20112017 of 20.11.2017.

All 68 subjects with JIA with MTX were tested by polymerase chain reaction. The analyzer used was CFX96 Touch Real-Time PCR Detection System, Bio-Rad, USA. The assay method is a qualitative method with clinical specificity of 100%.

Result interpretation

Hardy-Weinberg equilibrium is a concept in genetics that describes the situation in which the frequencies of alleles and genotypes in a population remain constant from one generation to the next in the absence of disturbing factors. This equilibrium is influenced by five conditions: random crossover, absence of natural selection, absence of migration, infinitely large population size and absence of mutations.

We used the general Hardy-Weinberg equilibrium formula which is:

$p^2 + 2pq + q^2 = 1$

where:

- p^2 is the frequency of the dominant homozygous genotype,
- 2pq is the frequency of the heterozygous genotype,
- q^2 is the frequency of the homozygous recessive genotype,
- p is the frequency of the dominant allele in the population,
- q is the frequency of the recessive allele in the population.

Correction for genotype association was done using Yates' correction, which is an adjustment utilized to improve the accuracy of genotype-disease association tests.

d) Statistical method:

The following statistical tests were applied for data analysis: for primary association investigations Pearson correlation coefficient was used, Chi-square test to assess whether there is a significant relationship between two categorical variables, ANOVA (Analysis of Variance) test to compare the mean of three or more groups to determine whether there are statistically significant differences between them, Mann-Whitney U test for independent samples in case of non-parametric data, ROC analysis in assessing the performance of diagnostic models based on different cut-off values of the diagnostic criterion.

2. RESULTS

2.1. General characteristics of the study samples

The study included 68 subjects with the diagnosis of juvenile idiopathic arthritis (JIA), who were diagnosed according to ILAR criteria, had been taking *methotrexate* (MTX) for more than 6 months, and were admitted to the Pediatric Rheumatology Department of the IMSP Institute of Mother and Child. Of these, 66.18% (45 subjects) constituted the control group and 33.82% - the study group (23 cases). The distribution of patients into the two groups was performed after genetic testing for *MTHFR* gene polymorphisms.

According to the gender distribution, 55.56% (25 cases) girls and 44.44% (20 cases) boys were registered in the control group. In the case group, boys constituted 47.83% (11 cases) and girls - 52.17% (12 cases). In both the study and control groups, the number of girls exceeded the number of boys ($\chi^2 = 0.070$; gl = 1; p = 0.791). As for the age of the subjects, both groups included in the study showed a clear age range with extremes of 24 - 215 months.

It was found that in the study group the subjects with oligoarticular form, persistent evolution were prevalent and constituted 47.82% (11 cases) of the total number of the group, followed by those with polyarticular form, seronegative which represented 39.13% (9 subjects) of the total number of the group of cases. In the control group, however, the clinical forms reflected each other with values of 48.9% (22 cases) for the polyarticular, seronegative form and 22.22% (10 cases) for the oligoarticular form with persistent evolution and the systemic form with active arthritis ($\chi^2 = 6.789$; gl = 4; p = 0.147).

The duration of *methotrexate* administration was also assessed in subjects of both groups, where patients in the study group had a mean duration of administration of 38.78 months (SD = 35.94; 95% CI: 20.9 - 56.65), while in the control group the mean duration of administration was 35.03 months (SD = 39.65; 95% CI: 20.73 - 49.33).

2.2. Analysis of the presence of mutations in the *MTHFR* gene in children with juvenile idiopathic arthritis

In the trial, subjects were initially genetically screened for polymorphism present or absent in children with JIA who had been taking *methotrexate* for at least 6 months. Thus, in the group of cases, 60.9% (14 subjects) (95% CI: 40 - 81) were noted the heterozygous C/T variant and in 39.1% (9 subjects) (95% CI: 19 - 60) - the homozygous mutation variant of the pathological T (T/T) allele at locus 677 of the *MTHFR* gene ($\chi^2 = 37.88$; gl = 2 ; $p<0.001$). For locus 1298, the heterozygous variant A/C was determined in 65.2% (15 subjects) (95% CI: 44.4 - 85), the homozygous variant after the normal allele A (A/A) - in 34.8% (8 subjects) (95% CI: 15 - 55.6), and the homozygous variant for the pathological allele C (C/C) was not determined in any subject for this group ($\chi^2 = 4.03$; gl = 2; p= 0.133).

Compared to the quantitative distribution in the analysed loci, the combined heterozygous mutation variant of the pathological alleles T and C (C/T and A/C) in loci 677 and 1298 was determined in 60.9% (14 subjects), (95% CI: 40 - 81).

It should be noted that isolated heterozygous mutations including pathological T and C (C/T and A/C) alleles in loci 677 and 1298 represent a polymorphism variant of the *MTHFR* gene that is not included in the same context of expression of the investigated clinical phenotype. Thus we observe a similar distribution in all patients without the T/T, C/T and A/C mutation variants for the control group under investigation.

The case group with an absolute number of 23 subjects was divided into two subgroups depending on the mutation variant in order to express their clinical phenotype in homozygous T/T ($\chi^2 = 20.29$; gl = 1; $p<0.001$) or heterozygous C/T and A/C ($\chi^2 = 34.49$; gl = 1; $p<0.001$) form.

Of note, the analysis of genotype frequency distribution by Hardy-Weinberg did not determine differences from this equilibrium. The allele frequency in children with JIA enrolled in the study was as follows: C = 0.67, T = 0.33 and A = 0.71, C = 0.29, respectively.

2.3. Clinical evaluation (JADAS - 71; ACR pedi index 30; DAS28 and MISS questionnaire) in relation to *MTHFR* genotypes

It was found that subjects with high disease activity values constituted the majority in the study group. Of the subjects with low JADAS-71 disease activity values, 13.33% (6 cases) were on MTX monotherapy and were homozygous for the normal allele. In MTX monotherapy, JADAS - 71 recorded high values in 8.7% (2 subjects) (95% I.I.: 0 - 22.2) with combined heterozygous polymorphism.

We determined that the JADAS71 value ≥ 11 is a threshold value that can be used to identify potential cases with *MTHFR* mutation. The sensitivity of the assay is shown to be 95.7%, the specificity is 95.3% ($\chi^2 = 53.62$; gl = 1; $p<0.001$). ROC analysis, based on the sensitivity-specificity relationship, has relevant values AUC=0.98 (IÎ 95%: 0.97 - 1.00, $p<0.001$), which confirms the correctness of the established relationship, figure 2. Therefore, it is recommended to test for genetic polymorphism of *MTHFR* gene in MTX treatment and persistence of high degree of disease activity in JIA patients.

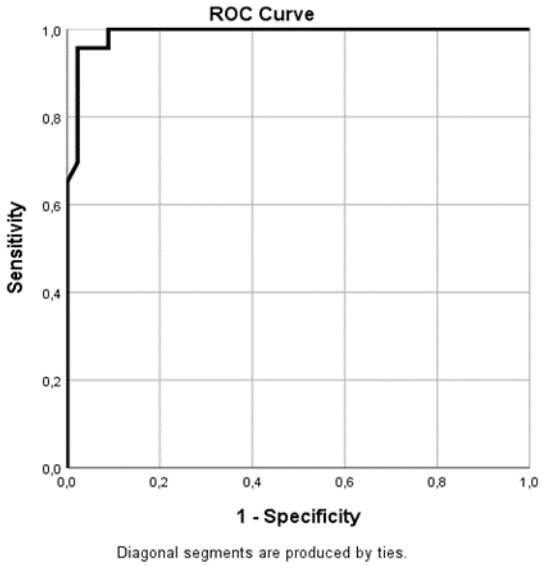

Figure 2. **ROC curve of diagnostic assessment of JADAS 71**

The clinical effect measured by the ACR Pedi 30 score was noted in 26 out of 68 (38.23%) subjects who received MTX. Among subjects in whom clinical effect was achieved, only 5 (7.35%) patients were on MTX monotherapy. Both subjects (14.3%; 95% CI: 0 - 35.7) in the study group with clinical improvement were on glucocorticosteroid combination therapy. The chance of having a score less than 30% on the ACRPedi score is 12 times higher for patients in the study group (OR = 12.0, 95% II: 2.5 - 57.3).

Simultaneously, clinical activity was assessed by using the DAS28 score. A mean score value of 3.91 (95% ÎI: 3.51 - 4.32) was found for the study group, corresponding to moderate disease activity. Also, the mean score offset for the control group was 2.15 (95% IQ: 1.83 - 2.46), corresponding to disease remission. The correlation between the degree of disease activity according to DAS28 and the duration of disease of patients on MTX treatment was also studied. This was found to be statistically insignificant ($r = 0.05$; $p < 0.687$).

The analysis determined that DAS28 ≥ 3.245 is a threshold value that can be used to identify potentially mutated cases. The sensitivity of the assay was found to be 87.0%, the specificity 93.3% ($\chi^2 = 43.83$; $p<0.001$). The ROC curve, constructed on the basis of the sensitivity-specificity relationship, has relevant AUC=0.949 (95% IÎ: 0.89 - 1.00, $p<0.001$), which confirms the correctness of the established relationship. Therefore, it is recommended to test for genetic polymorphism of *MTHFR* gene in case of continuation of MTX treatment and persistence of high degree of disease activity in patients with JIA, figure 3.

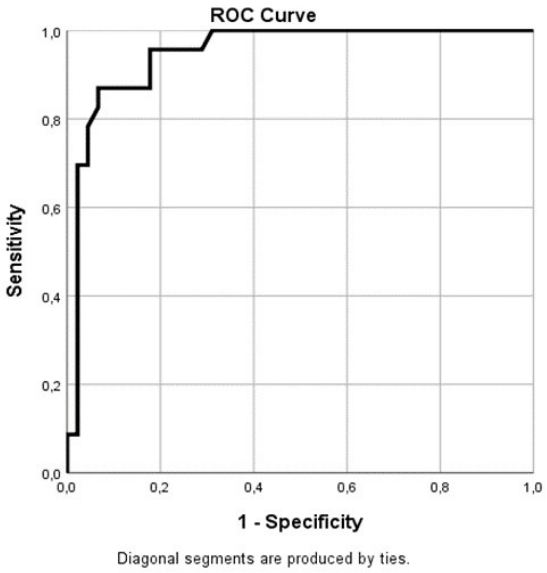

Figure 3. **ROC curve of diagnostic assessment of DAS28**

Using the Mann-Whitney U test we confirmed the alternative hypothesis regarding the MISS score obtained for the assessment of MTX intolerance for patients with genetic polymorphism. The distribution curves show different location and shape, figure 4.

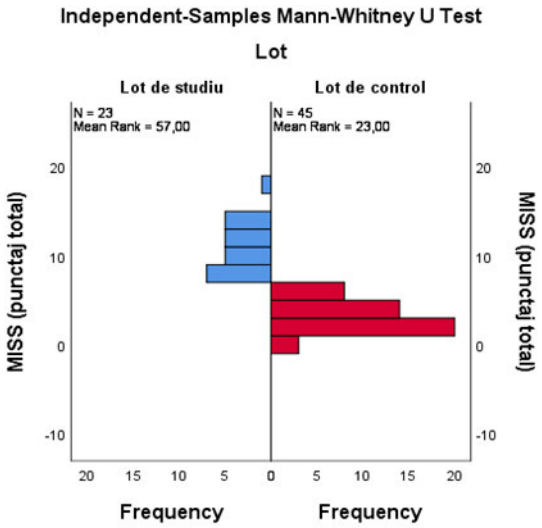

Figure 4. **Distribution of MISS Score in the samples**

When applying indices and scores to assess clinical efficacy, a mean Pearson's linear relationship was observed for the study group with combined *MTHFR* mutation, based on the numerical value of the JADAS score - 71 and DAS28 respectively. The lack of statistical evidence, in particular the strong linear relationship, is due to the small number of subjects in the subgroups, the complexity of the tests, and the variability over time of the indices and scores for each study subject.

2.4. Evaluation of MTX liver toxicity in patients with JIA in relation to mutations in the *MTHFR* gene

We aimed to assess the values of traditional markers indicating the onset of drug-induced liver injury. These include total bilirubin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase values. For liver transaminases, a substantial discrepancy of minimum and maximum values is observed which are practically below the triple value accepted by NASPHGAN which recommends interpretation of ALT based on the upper limits specific to the patient's sex (22 U/L for girls and 26 U/L for boys). The sex ratio of patients in the study group with ALT above the normal value is shown in figure 5.

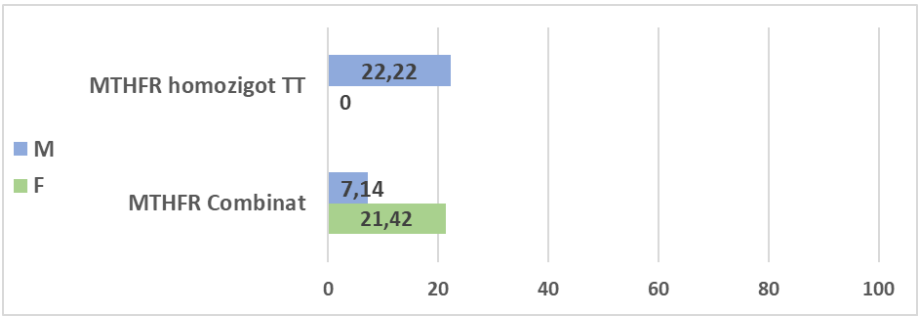

Figure 5. **Gender distribution of patients in relation to ALT (%)**

The data are also apparently similar for the control subjects except for the maximum value for ALT, which was recorded in only one patient (1.47%) in the study. Thus, JIA patients taking

MTX and presenting with polymorphism of the *MTHFR* gene do not record non-significantly different values of traditional markers compared to those without polymorphism.

Further, anticipatory, associative and behavioural symptoms of MISS score were investigated and assessed which in relation to genetic polymorphism show statistically significant relationships. The latter increase the diagnostic power of testing for these mutations prior to 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) - no abdominal pain.

Another symptom identified after administration of the preparation was nausea, which in the baseline group was mild in 9 (39.1%; 95% CI: 20.0 - 60.0) patients and moderate 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, 7 (15.6%; 95% CI: 5.3 - 27.5) subjects 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 case group was rated 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 after MTX administration - 13 (56.5%; 95% CI: 37.0 - 77.8) respondents. In the control group, there was 1 (2.2%; 95% CI: 0 - 7.7) patient with mild vomiting after MTX, i.e. the rest of the patients included in the study did not show this symptom post-administration.

In addition, patients may develop anticipatory symptoms, which occur prior to 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 evident; therefore, they are often inadequately managed. Although folic acid supplementation during MTX treatment can reduce such effects, many patients discontinue treatment, which adversely affects disease control and quality of life.
The predominant factor driving MTX intolerance has been found to be behavioural factors, as shown in the radar diagram in figure 6.

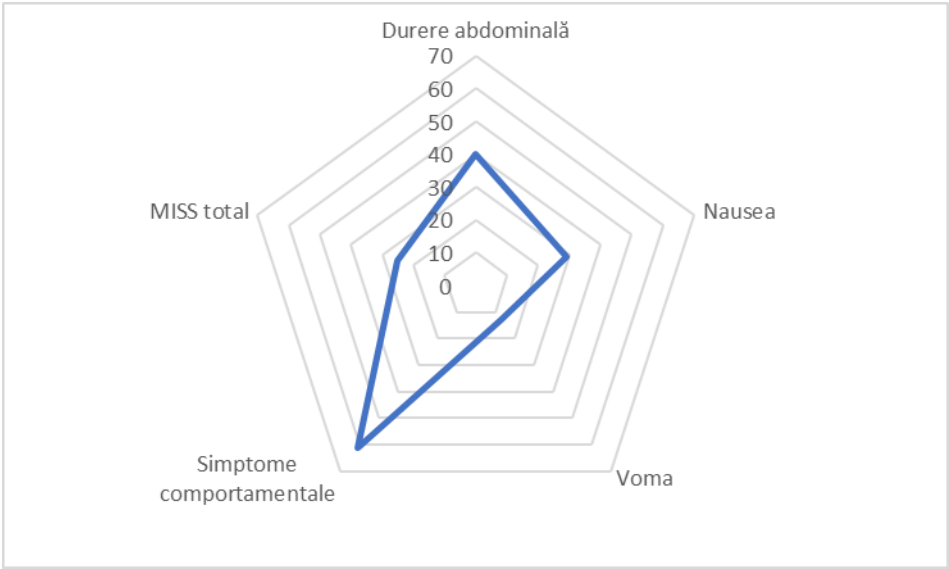

Figure 6. **Radar diagram of the MISS score and related components.**

In this work, we attempted to analyze the morphological structure of the liver following toxicity and drug intolerance events in JIA. Non-invasive imaging methods with a specificity and sensitivity suitable for the pediatric population in this aspect are standard 2D ultrasound and one-dimensional transient elastography (FibroScan). 2D ultrasound of the liver did not demonstrate statistically significant differences in the size and structural characteristics of the images obtained.

Using FibroScan we were able to assume with a high, patient-specific probability about the presence of subclinical disease based on the images and indices obtained. The elastographic picture of increased density was profiled in 37 (54.41%;) patients, predominantly in the control group (24 (53.3%; 95% CI: 39.1 - 68.3) vs. 13 (56.5%; 95% CI: 35.0 - 76.0); $\chi^2 = 0.447$; $p = 0.800$. In the study we did not obtain data on a sensitivity - specificity relationship that would help to prospectively screen patients with mutations in the *MTHFR* gene using liver elastography. ROC analysis shows no relevant values AUC=0.471 (95% CI: 0.29 - 0.64, p=0.739).

So far, a mean median liver elasticity value of 5.34 kPa (95% CI: 4.84 - 5.84) was found for the study group, which is 1.13-fold higher than the mean elasticity value for all age groups in the healthy paediatric population. Note the lack of discrepancy of the mean in the control group, which was 5.38 kPa (95% CI: 5.01 - 5.75).

The clinico-diagnostic significance of moderate and moderately-severe ultrasound lesions observed by the maximum values (7.8 kPa) of the median liver elasticity confirms the estimation of the cumulative effect of the medication and the response rate to treatment, which was individually determined in 9 (20%) patients in the control group of which 7 (15.55%) were following MTX monotherapy.

At the same time, it is important to mention the differences that were recorded for patients investigated by FibroScan who administered MTX monotherapy and those who followed combination therapy ($F = 4.16$; $p = 0.022$) (table 1).

Table 1. **Median liver elasticity in patients included in the study depending on the treatment administered (kPa)**

	N	**Media (DS)**	**IÎ 95%**	**Minimum - Maximum**
Monoterapie	19	5,85 (0,99)	5,37 – 6,33	3,6 – 7,8
Glucocorticosteroizi	28	5,11 (0,93)	4,75 – 5,48	2,8 – 6,8
Glucocorticosteroizi + Biologica	3	4,70 (0,69)	2,97 – 6,42	3,9 – 5,1
Total	50	5,37 (1,01)	5,08 – 5,65	2,8 – 7,8

2.5. Functional assessment of MTX cardiac toxicity in relation to mutations in the *MTHFR* gene

After processing the electrocardiographic data, applying the Mann-Whitney U test, we obtained a value of 525.00 ($p = 0.923$) for the frequency of cardiac contractions, a value of 665.50 ($p = 0.055$) for the PQ interval, and a value of 480.00 ($p = 0.627$), which confirms the null hypothesis regarding the importance of these indices for the prognosis of toxicity in subjects with *MTHFR* gene polymorphism. There were no differences in the values of the same indices at the subset level.

Following the analysis of the 2D echocardiography data we can report, that the sizes and volumes for the left ventricle were insignificantly different between groups. We used the Mann-

Whitney U test which confirmed the null hypothesis regarding the usefulness of these indices in assessing cardiotoxicity to MTX administration (U= 451.00; p= 0.387 for DTD VS; U= 483.00; p= 0.652 for DTS VS; U= 460.50; p= 0.460 for VTD VS; U= 500.00; p= 0.820 for VTS VS; and 463.50; p= 0.482 for EF, respectively). Both for the study group (69.33% (95% CI: 67.72 - 70.94%)) and the control group (68.72% (95% CI: 67.41 - 70.02%)) the mean values did not statistically deviate, i.e. neither met the ESC 2022 guideline condition on Cardio-oncology for a decline in LVEF ≥10% to a value of <53%.

Right ventricular systolic parameters (TAPSE and S') were also assessed. The Mann-Whitney U test for independent samples, with a p = 0.731 for TAPSE and p = 0.943 for S', respectively, demonstrates that these two parameters are not significantly different between subjects with and without mutation.

We also used tissue Doppler to assess left ventricular diastolic parameters (septal, lateral and mean E, mean E/e'). The index with high statistical significance was mean E/e´ - 8.00 (95% CI: 7.66 - 8.34).

The PSAP values for the case group were expressed as a mean of 26 mmHg (95% IÎ: 24.95 - 27.05), and for the control group the mean took values of 25.31 mmHg (95% IÎ: 24.59 - 26.03). The minimum PSAP value was 22 mmHg, and the maximum reached the threshold of 30 mmHg, with a mean of 25.5 mmHg and a median of the same value.

2.6. Algorithms for genotyping *MTHFR* in JIA

Following the analysis of the data obtained in the research, algorithms for optimizing the diagnostic work-up and therapeutic recommendation in juvenile idiopathic arthritis were developed, figure 7 and figure 8.

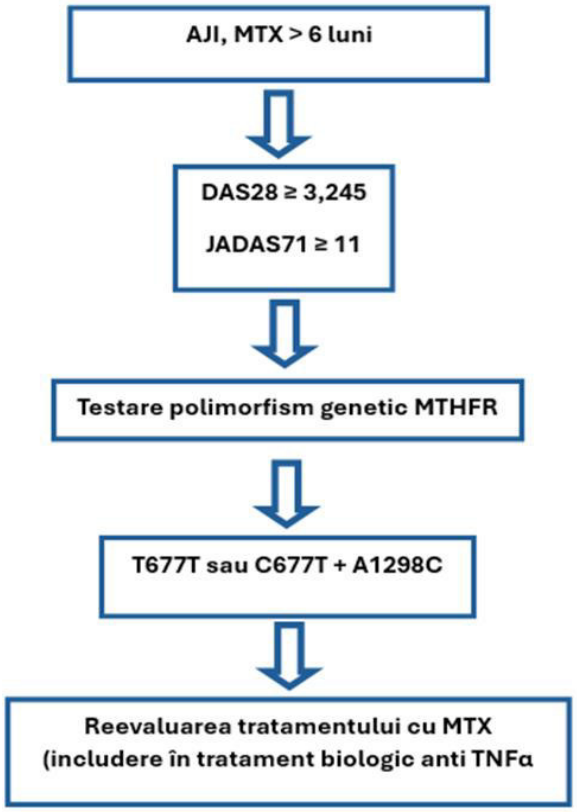

Figure 7. **Diagnostic algorithm for MTX treatment inefficiency**

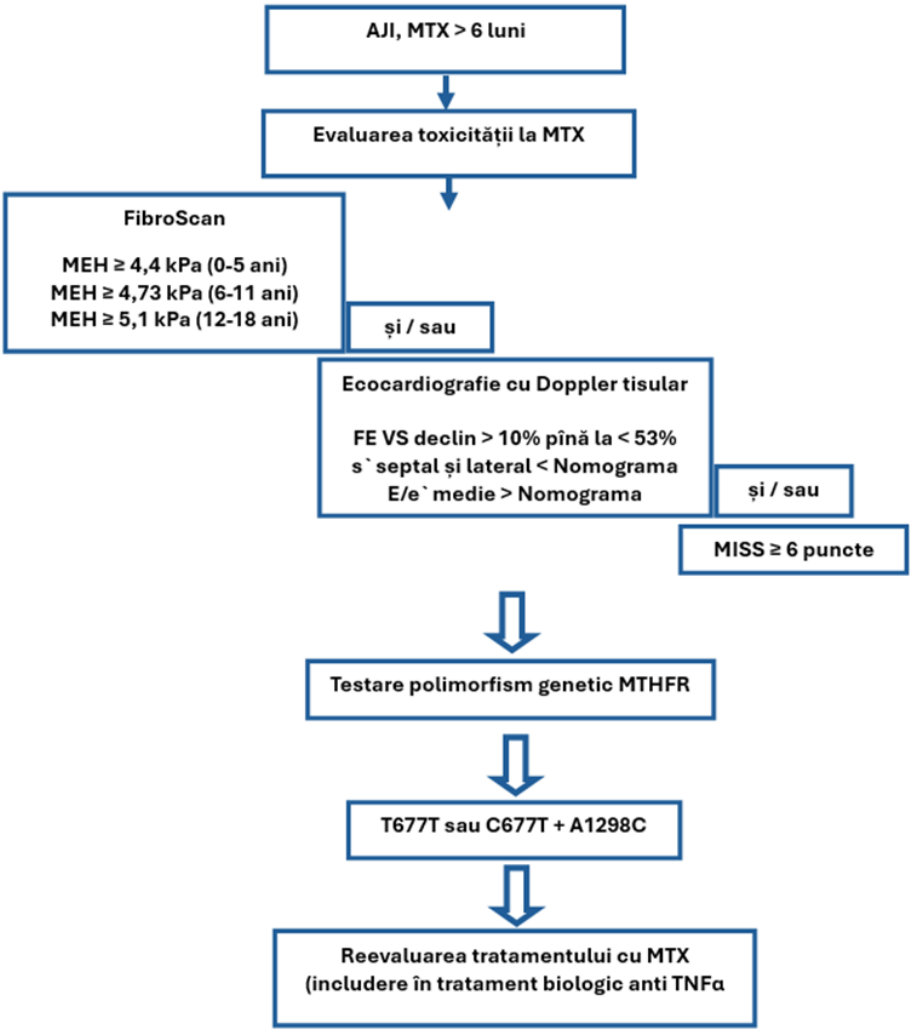

Figure 8. **Diagnostic algorithm for MTX treatment toxicity**

DISCUSSIONS

It is currently the key aspect of personalised medicine that relies on genetic analysis, i.e. 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, allowing for more personalised preventive interventions and more effective screening programmes.

The use of genetic information can help to more accurately diagnose certain medical conditions and to differentiate subtypes of diseases that may require different therapeutic approaches.

Among subjects with juvenile idiopathic arthritis, both in the experimental and control groups, a prevalence of girls over boys was observed. These data are also statistically reflected in the literature [20–22]. At the same time, there is a well-determined relationship between these values and the form of JIA manifested, in particular the oligoarticular and polyarticular seronegative form. In the seronegative form of JIA, specific laboratory tests, such as rheumatoid factor and antinuclear factor, are negative or not present in significant amounts. This makes diagnosis more difficult and requires closer clinical and imaging evaluation. Prognosis may vary depending on the severity of symptoms and response to treatment. Some children may have a complete remission of the disease over time, while others may experience persistent symptoms or associated complications.

Our results show a prevalence of comorbidities in the gastrointestinal system, which is of major importance for achieving a desired therapeutic effect, but also for generating potential drug toxicity. As for the gender distribution, we demonstrated a higher proportion of the most common comorbidities, including obesity, in male children with JIA.

The spread of allelic variants for *MTHFR* in populations is quite varied, with prevalence in the Mexican and Italian group on the ratio of homozygous [23]. The single nucleotide polymorphism of this gene reduces the thermostability of the *MTHFR* enzyme due to 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].

In our study we demonstrated the homogeneity of the allelic distribution in relation to Hardy-Weinberg equilibrium in subjects with JIA. Similarly, we performed a quantitative mutation type analysis for both study groups, which constituted about one third of the sample.

We established high predictive values of disease activity assessment scores for subjects in the case group. For the JADAS - 71 score we recorded values comparable to those in the medical literature [25]. For the DAS 28 score, subjects in the study group showed moderate disease activity while those in the control group showed minimal disease activity. We consider it very important to mention that disease activity is a dynamic parameter and is measured at each referral or hospitalization of the patient with JIA.

Our results can be comparable with other studies on the MISS score, with the maximum value of the score obtained in patients of the research group exceeding three times the maximum value for the control group. We have shown that it is necessary to draw attention to patient behavioural symptoms to understand one of the causes of poor treatment response resulting from non-compliance.

Because both the amount and duration of MTX exposure have been shown to correlate with toxicity, it has been suggested that serum MTX levels help predict certain types of toxicity, such as gastrointestinal toxicity and myelosuppression [26,27]. However, because MTX clears from serum within 24 hours of administration, serum levels are not accurate enough to predict toxicity

[28]. Therefore, steps have been established for monitoring MTX toxicity, in particular for hepatotoxicity, which do not involve measuring serum levels [27]. Furthermore, the rare potential for serious toxic harm in children and the consistency of the monitoring schedule during the transition from pediatric to adult care influenced the decision of the new ACR 2021 guideline to provide a strong recommendation for frequent monitoring [29].

Another imaging method used with minimal impact on tissue was FibroScan. Using it we were able to determine liver density values at the most vulnerable sites for the initiation of morphological change due to the toxic effect of MTX.

Studies, including national studies, which have supported the need and appropriateness of the method for JIA, have demonstrated comparable sensitivity and true specificity to liver biopsy not only in symptomatic patients [30–32].

Thus, we can explain our own results showing high median liver elasticity values for the control group compared to the baseline group, the accumulation of polyglutamylated MTX to which occurs more intensely by antagonistic effect towards folic acid at the cellular level [33]. Moreover, it is necessary to mention that the representative value is demonstrated in subjects with MTX monotherapy.

Given the need to assess cardiac function in patients with JIA, our study used traditional non-invasive functional and imaging methods. ECG and transthoracic echocardiography with tissue Doppler are included in the list of mandatory investigations according to the European Society of Cardiology guidelines, especially in patients using anti-tumor profile medication [34–37]. For example, the study by Witczak et al. demonstrates the need to apply these methods with the comparison of electrocardiographic and tissue Doppler indices in patients with different connective tissue diseases [38]. Of course, the investigations presented above are of tremendous informative value to the clinician, but there are other non-invasive imaging tests that demonstrate longitudinal and circular myocardial function, which are currently limited for the pediatric population due to the lack of age group nomograms [39].

Limitations

Due to the very small number of subjects taking MTX, they may not represent the range of clinical and paraclinical features in JIA. We used representative scores and indices that in common with patient imaging data are undoubtedly specific to each individual child, thus would be biased towards population subjectivity. Physical and behavioural symptoms in stationary conditions are verbally assessed by the child or his/her parents, which are not always quantified and may change with alterations in disease activity or depending on the degree of doctor-patient compliance. However, reliance on scores that are also based on quantitative parameters, as well as the physician's and patient's overall assessment of the patient's condition, makes these limitations alleviate. The MISS score as a test to assess gastrointestinal toxicity can show objective results by being routinely used at each hospitalization for patient follow-up.

GENERAL CONCLUSIONS

1. Genetic mutations of *MTHFR* have been identified in more than one third of enrolled subjects. These patients can be characterized by an increased level of combined heterozygosity (Ho = 0.43/0.44) and isolated homozygosity 0.48 observed for rs1801133 and rs1801131 polymorphisms.

2. The study demonstrated the importance of clinical scores (DAS 28 and JADAS 71) for estimating the risk of presence of mutations in the *MTHFR* gene, genetic confirmation of non-responders to *methotrexate* therapy. By sensitivity - specificity relationship, threshold values for *MTHFR* polymorphism testing were determined (DAS 28 ≥ 3.245; JADAS 71 ≥ 11). The ACR Pedi 30 index demonstrated a rate of up to 40% achievement of clinical effect after more than 6 months of *methotrexate* administration, of which less than 10% of subjects were on *methotrexate* monotherapy.

3. The MISS questionnaire demonstrated clinical *methotrexate* intolerance for patients with mutations in the *MTHFR* gene, through the development of behavioural symptoms of moderate intensity (refusal of *methotrexate* administration) following the development of abdominal pain (n=14).

4. Study of traditional biochemical markers for liver injury have not demonstrated statistical significance of hepatotoxicity to *methotrexate*. Examination by one-dimensional transient liver elastography revealed high median liver elasticity values in the group of children without mutation in the *MTHFR* gene, while patients with mutations in the *MTHFR* gene on *methotrexate* therapy had mean median liver elasticity values 1.13 times higher than the mean elasticity in healthy children.

5. Cardiotoxicity study in children on *methotrexate* therapy showed increased mean E/e` as determined by tissue Doppler echocardiography, indicating probable left ventricular diastolic dysfunction in drug cardiotoxicity.

6. In the study, efficacy and toxicity endpoints for *methotrexate* were determined: DAS 28, JADAS 71, MISS above threshold, presence of pathological polymorphism for rs1801133 and rs1801131 in the *MTHFR* gene, detection of low values of median liver elasticity (in *methotrexate* monotherapy) and increased values of systolic and diastolic SV indices on tissue Doppler.

PRACTICAL RECOMMENDATIONS

1. Genetic testing for *MTHFR* is recommended upon reaching/maintaining the identified threshold value for DAS 28 and/or JADAS 71 scores.

2. It is recommended to use the *methotrexate* intolerance severity score as a method to predict the effect of therapeutic compliance following initiation of *methotrexate* treatment.

3. The use of liver elastography and tissue Doppler techniques is recommended for the assessment of subclinical liver and heart target organ damage in the setting of *methotrexate* administration.

4. *MTHFR* gene mutation testing is recommended for patients in whom the clinical and/or paraclinical efficacy expected with *methotrexate* administration has not been achieved.

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LIST OF PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS

Vladimir Iacomi, Assistant Professor, Department of Paediatrics,
for his doctoral thesis in medical sciences,
with the topic **"Pharmacogenomic impact of methylenetetrahydrofolate reductase gene polymorphism in juvenile idiopathic arthritis",** 322.01 Paediatrics and neonatology,
State University of Medicine and Pharmacy "Nicolae Testemitanu" of the Republic of Moldova

✓ **Collective monograph:**

1. Revenco N., Rotaru N., Crivceanschi M., **Iacomi V.** Criterii clinice și funcționale în evaluarea copilului cu artrită. În: Revenco N., ș.a. *Reumatologie Pediatrică*. Chișinău: *Reclama*, 2018, pp. 7-39 ISBN 978-9975-58-147-9

✓ **Articles in scientific journals abroad:**

✓ **articles in ISI journals, SCOPUS and other international databases**

2. Revenco N., Cracea A., Mazur-Nicorici L., Foca S., Eremciuc R., **Iacomi V.** Treatment with golimumab in juvenile idiopathic arthritis. In: *Arch. Balk. Med. Union.* 2022, 57(2):136-143. https://doi.org/10.31688/ABMU.2022.57.2.02

3. Revenco N, Cracea A., Mazur-Nicorici L., Foca S., Eremciuc R., Gaidarji O., **Iacomi V**., Bogonovschi L. The clinical and paraclinical efficacy of tocilizumab in juvenile idiopathic arthritis. In: *Arch. Balk. Med. Union*. 2021, 56(2):185-192. https://doi.org/10.31688/ABMU.2021.56.2.07

✓ **Articles in accredited national scientific journals:**

✓ **articles in category B journals**

4. Revenco N., Cracea (Drușca) A., Foca S.-G., Eremciuc R., Gaidarji O., **Iacomi V**., Bogonovschi L. Tratamentul cu preparatul Tocilizumab la pacienții cu artrită juvenilă idiopatică – eficiența clinică și paraclinică. În: *Buletin de Perinatologie*, 2021, nr. 2(91), pp. 6-10. ISSN 1810-5289

- **Articles in scientific conference proceedings:**

✓ **national with international participation**

5. **Iacomi V**., Revenco N., Cracea A., Eremciuc R., Foca S., Bogonovschi. Determinantele genetice în aprecierea rezultatelor tratamentului în artrita juvenilă idiopatică. În: *Materialele simpozionului naţional cu participare internaţională "Registrul maladiilor reumatice la copii: experienţa Republicii Moldova".* Chișinău, 2022, pp. 53-61. ISBN 978-9975-58-285-8

✓ **national**

6. **Iacomi V**., Revenco N., Eremciuc R., Foca S., Cracea A., Gaidarji O., Monitorizarea terapiei cu *metotrexat* in artrita juvenilă idiopatică: variații farmacogenetice. În: *Materialele conferinţei naţionale "Managementul interdisciplinar al copilului", Ediţia a 5-a.* Chișinău, 2022, pp. 17-23. ISBN 978-9975-58-274-2

7. Revenco N., Cracea A., Foca S., Eremciuc R., **Iacomi V**., Eficiența clinică și paraclinică a preparatului golimumab în artrita juvenilă idiopatică. În: *Materialele conferinţei*

naţionale "Managementul interdisciplinar al copilului", Ediţia a 5-a, Chișinău, 2022, pp. 11-16. ISBN 978-9975-58-274-2

- ✓ **Patents of inventions, patents of innovation, registration certificates, materials at invention fairs**
  - ✓ **Copyright**

8. Revenco N., **Iacomi V.** *Predicţia eficacităţii tratamentului cu metotrexat în artrita juvenilă idiopatică la copii şi adolescenţi.* Nr. de înregistrare 2574, Seria OȘ Nr. adeverinței: 7776 din 23.01.2024. În: Baze de date internaționale de proprietate intelectuală http://www.db.agepi.md/opere/Details.aspx?id=734871275314514686584645538 1&nr=73487127531457468678464553 86

  - ✓ **Innovation certificates**

9. Revenco Ninel, **Iacomi Vladimir**. *Evaluarea polimorfismelor genetice T677T şi C677T/A1298C ale metilentetrahidrofolat reductazei în artrita juvenilă idiopatică.* Certificat de inovator Nr. 5981 din 07.02.2023.

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13. Revenco Ninel, **Iacomi Vladimir**. *Evaluarea toxicității hepatice a metotrexatului în artrita juvenilă idiopatică prin FibroScan.* Certificat de inovator Nr. 509 din 07.02.2023.

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  - ✓ **Implementation documents**

15. Revenco Ninel, **Iacomi Vladimir**. *Evaluarea polimorfismelor genetice T677T şi C677T/A1298C ale metilentetrahidrofolat reductazei în artrita juvenilă idiopatică.* Act de implementare Nr. 12 din 07.02.2023.

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✓ **Participation with communications in scientific forums:**

✓ **international**

21. **Iacomi V**., Revenco N., Variații genetice cu impact asupra răspunsului la *metotrexat* în AJI. *A 7-a Ediție a Conferinței Naționale de Reumatologie Pediatrică cu Participare Internațională*. Iași, România, 14-16 Septembrie 2023.

22. **Iacomi V**., Revenco N., Tratamentul Personalizat în Artrita Juvenilă Idiopatică. *Conferința Națională cu Participare Internațională "Bienala Sibiu-Chișinău", Ediția a 3-a*. Sibiu, România, 3-6 Octombrie 2019.

23. **Iacomi V**., Revenco N., Screening the C677T polymorphism of the *MTHFR* gene in assessing disease severity and response to *methotrexate* treatment in children with juvenile idiopathic arthritis. *7th International Medical Congress for Students and Young Doctors MedEspera*. Chișinău, 3-5 Mai 2018.

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Participation with posters in scientific forums:

✓ **international**

31. **Iacomi V**., Revenco N., Methylenetetrahydrofolate reductase 677 nucleotide mutation is a predictive tool in *methotrexate* non-responsive JIA patients. *The 25th PReS Congress*. Lisbon, Portugal, 5-8 September 2018.

32. **Iacomi V**., Bursacovschi D., Revenco N., Monitoring *methotrexate*-induced liver toxicity in juvenile idiopathic arthritis: new perspectives. *The 8th International Medical Congress for Students and Young Doctors MedEspera*. Chișinău, 24–26 Septembrie 2020.

33. **Iacomi V**., Revenco N., Eremciuc R., Monitorizarea tratamentului AJI prin elastografie hepatica. *Conferința Națională cu Participare Internațională "Zilele Pediatrie Ieșene N.N.Trifan", Ediția XXXIV*. Iași, România, 15-18 iunie 2022.

34. **Iacomi V**., Revenco N., Eremciuc R., Liver Stiffness in Low-Dose *Methotrexate* Use in JIA Patients. *The 28th PReS Congress.* Prague, Czech Republic, 20-23 September 2022.

35. **Iacomi V**., Revenco N., Clinical results of *methotrexate* treatment in Juvenile Idiopathic Arthritis. *Conferința Națională cu Participare Internațională "Zilele Pediatrie Ieșene N.N.Trifan", Editia XXXV*. Iași, România, 22-24 iunie 2023.

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