LITHUANIAN UNIVERSITY OF HEALTH SCIENCES
MEDICAL ACADEMY

Aušra Šnipaitienė

JUVENILE IDIOPATHIC ARTHRITIS: BLOOD, URINE AND ULTRASOUND MARKERS OF CLINICAL REMISSION

Doctoral Dissertation
Medical and Health Sciences,
Medicine (M 001)

Kaunas, 2026

Dissertation is defended extramurally.

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Dissertation will be defended at the open session of the Medical Research Council of the Lithuanian University of Health Sciences on the 26th of February 2026 at 1 p.m. in the auditorium 4004 of the Department of Paediatrics of the Lithuanian University of Health Sciences.
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LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS
MEDICINOS AKADEMIJA

Aušra Šnipaitienė

JUVENILINIS IDIOPATINIS ARTRITAS: KRAUJO, ŠLAPIMO BEI ULTRAGARSINIAI KLINIKINĖS REMISIJOS POŽYMIAI

Daktaro disertacija
Medicinos ir sveikatos mokslai,
medicina (M 001)

Kaunas, 2026

Disertacija ginama eksternu.

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Disertacija bus ginama viešajame Medicinos mokslo krypties tarybos posėdyje 2026 m. vasario 26 d. 13 val. Lietuvos sveikatos mokslų universiteto Vaikų ligų klinikos 4004 auditorijoje.
Disertacijos gynimo vietos adresas: Eivenių g. 2, LT-50161 Kaunas, Lietuva.

CONTENTS

ABBREVIATIONS

ACR	–	American College of Rheumatology
ACT	–	active juvenile idiopathic arthritis
ADA2	–	adenosine deaminase 2
AICD	–	activation-induced cell death
AJC	–	active joint count
ANA	–	antinuclear antibodies
anti-CCP	–	anti-cyclic citrullinated peptides
anti-MCV	–	antibodies to mutated citrullinated vimentin
anti-TNF	–	tumour necrosis factor inhibitor
AUC	–	area under the ROC curve
bDMARDs	–	biological disease-modifying antirheumatic drugs
BIC	–	B-cell integration cluster
CBC	–	complete blood count
C/EBP	–	CCAAT-enhancer-binding proteins
CI	–	confidence intervals
CID	–	clinically inactive disease
cJADAS	–	clinical JADAS
ClinSUM	–	total sum of the clinical features
CR	–	conventional radiology
CRP	–	C-reactive protein
CT	–	computed tomography
DA	–	disease activity
DMARDs	–	disease-modifying antirheumatic drugs
DNA	–	deoxyribonucleic acid
DOCK2	–	dedicator of cytokinesis 2
ERA	–	enthesitis related arthritis
ESR	–	erythrocyte sedimentation rate
EULAR	–	European Alliance of Associations for Rheumatology
FDA	–	the U.S. Food and Drug Administration
FLS	–	fibroblast-like synoviocytes
FoxP3	–	forkhead box P3
GCC	–	glucocorticoids
HC	–	healthy control
Hgb	–	haemoglobin
HLA B27	–	human leucocyte antigen B27
IBD	–	inflammatory bowel diseases
ICD-10	–	International Classification of Diseases and Related Health Problems
ICOS	–	inducible T cell Co-stimulator
IFN-gamma	–	interferon gamma
IL	–	interleukin
IL-13Rα1	–	interleukin 13 receptor α1
ILAR	–	International League of Associations for Rheumatology
IQR	–	interquartile range
IRAK1/2	–	interleukin-1 receptor-associated kinase 1 or 2
JADAS	–	juvenile arthritis disease activity scale
JAMAR	–	Juvenile Arthritis Multidimensional Assessment Report

JIA	–	juvenile idiopathic arthritis
IFN gamma	–	interferon gamma
KPC1	–	KIP1 ubiquitination-promoting complex
LOM	–	limited range of motion
LPS	–	lipopolysaccharide
LRG	–	leucine-rich α2 glycoprotein
LSMU	–	Lithuanian University of Health Sciences
M0	–	baseline visit
M3	–	3 months from inclusion into the study
M6	–	6 months from inclusion into the study
M9	–	nine-month visit from inclusion into the study
M12	–	twelve-month visit from inclusion into the study
MAPK	–	mitogen-activated protein kinase
MCPs	–	metacarpophalangeal joints
miRNA	–	microRNA
MMP	–	matrix metalloproteinase
MPV	–	mean platelet volume
MRI	–	magnetic resonance imaging
mRNA	–	messenger RNA
MSUS	–	musculoskeletal ultrasound
MTPs	–	metatarsophalangeal joints
MTX	–	methotrexate
n (or N)	–	number
NA	–	non applicable
NF-κB	–	nuclear factor-kappa B
NIH	–	the National Institutes of Health
NSAIDs	–	nonsteroidal anti-inflammatory drugs
OR	–	odds ratio
PaGA	–	patient/parent global assessment of disease activity
PBS	–	phosphate-buffered saline
PBMCs	–	peripheral blood mononuclear cells
PCT	–	plateletcrit
PD	–	power doppler
PDC4	–	programmed cell death 4
PD-L1	–	programmed cell death ligand 1
PDW	–	platelet distribution width
PhGA	–	physician global assessment of disease activity
PIPs	–	proximal interphalangeal joints
PReS	–	Paediatric Rheumatology European Society
PRINTO	–	Paediatric Rheumatology International Trials Organisation
RA	–	rheumatoid arthritis
Rac1	–	Ras-related C3 botulinum toxin substrate 1
REM	–	remission
RF	–	rheumatoid factor
RISC	–	miRNA-protein complex
RNA	–	ribonucleic acid
ROC	–	receiver operating characteristic curve
RORγt	–	retinoid-related orphan receptor γ-t
RT-PCR	–	real-time polymerase chain reaction

SD	–	standard deviation
sDMARDs	–	synthetic disease-modifying antirheumatic drugs
SHIP-1	–	Src homology 2-containing inositol phosphatase-1
SHJ	–	small hand joints
SMRT	–	silencing mediator for retinoid and thyroid hormone receptor
SOCS1	–	Suppressor of Cytokine Signalling 1
Stat1	–	signal transducer and activator of transcription 1
TCR	–	T-cell receptor
TLR	–	Toll-like receptor
TMJ	–	temporomandibularr joint
TNF	–	tumour necrosis factor
TRAF6	–	factor associated with the TNF receptor 6
US	–	ultrasound
V0	–	baseline visit
V1	–	follow-up visit
VAS	–	visual analogue scale
WBC	–	white blood cells
yrs	–	years

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a persistent inflammatory condition affecting the joints of children under 16 years old, and it includes a wide range of clinical presentations [1, 2]. Recent research has highlighted differences in the pathogenesis, genetic factors, and epidemiology across the various subtypes of JIA, including oligoarthritis, polyarthritis, and enthesitis-related arthritis, with regional variations observed [3–6]. Extensive studies have identified JIA as a complex disease with multiple pathogenetic pathways, which has led to the creation of targeted treatments like tumor necrosis factor (TNF) inhibitors, interleukin (IL)-1 and IL-6 inhibitors, among others [7]. The prognosis for JIA has markedly improved in recent years due to the biological therapies [8–10]. Nevertheless, diagnosing JIA still depends on clinical criteria, and a significant number of patients do not achieve long-term clinical remission [11–13]. Many patients experience relapses in previously treated joints, the progressive involvement of new joints, and/or severe complications, underscoring the necessity for accurate disease monitoring and enhanced management strategies [13, 14].

Evaluating disease activity is crucial for making informed treatment choices in JIA. Presently, validated clinical assessment tools are extensively used to track disease activity in children with JIA [15, 16]. The most frequently employed tool is the Juvenile Arthritis Disease Activity Score (JADAS) [17]. However, these tools have certain drawbacks, such as subtype-specific adjustments with varying reference values for disease activity [16, 18–20]. Additional challenges include factors like young age, which limits patient cooperation, age-related joint hypermobility, and the inability to self-report joint pain, as well as transient childhood joint disorders like transient synovitis. Moreover, research has shown poor-to-moderate interrater reliability in clinical arthritis assessments, along with various factors affecting physicians' evaluations of overall JIA disease activity [21, 22]. A multicenter study by Shoop-Worrall et al. highlighted significant inconsistencies between different clinical scoring systems when applied to the same patient groups [11]. These findings underscore the necessity for more precise and standardized methods and biomarkers to evaluate disease activity, alleviate the disease burden, and enhance the quality of life for children with JIA.

In recent years, multiple JIA disease activity monitoring approaches have emerged in paediatric rheumatology research. One area focuses on the non-invasive techniques, such as musculoskeletal ultrasound (MSUS), while another explores advancements in next-generation biomarker research, including

microRNAs (miRNAs). MSUS is a non-invasive, radiation-free, cost-effective, and patient-friendly imaging modality that enables real-time assessment of joint involvement in JIA [23–26]. Several studies have emphasized the critical role of using MSUS to detect subclinical synovitis for the classification of JIA subtypes and the assessment of active joint counts [23, 25, 27]. Recent initiatives have focused on creating uniform scanning protocols and methods for assessing synovitis across various joints in both JIA patients and healthy children, as conducted by several research groups [24, 28–30]. Nonetheless, there is still a scarcity of longitudinal data on MSUS characteristics in JIA patients over the course of the disease.

Another rapidly evolving field in paediatric rheumatology is the search for circulating biomarkers. Numerous molecules, including genetic factors, antibodies, and inflammatory proteins, have been explored in recent years [31]. However, no JIA-specific biomarker has been identified, shifting research focus toward epigenetic regulation. MiRNAs – small, non-coding ribonucleic acids (RNAs) – play a crucial role in post-transcriptional regulation and are involved in biological functions such as angiogenesis, cell proliferation, differentiation, inflammation, and immune responses. These processes are integral to the development of various chronic paediatric conditions, including JIA [32–34]. MiRNAs are considered potential biomarkers due to their exceptional stability across different bodily fluids, resistance to RNase degradation, ability to endure changes in pH, and resilience through multiple freeze-thaw cycles [35, 36]. Research on miRNAs in the context of JIA has predominantly examined their presence in plasma, serum, or synovial fluid [37–39]. However, the majority of these investigations have been conducted at the disease onset or during treatment response evaluations, while data on longitudinal miRNA expression changes throughout the disease course remain scarce.

For younger children who need frequent follow-ups, a testing method that is non-invasive and convenient is preferable to repeated blood draws. Urine serves as an appealing alternative biofluid for clinical analysis and biomarker research because it is collected with minimal invasiveness, provides a high sample yield, and is suitable for repeated measurements. Urinary miRNAs have already been demonstrated as reliable biomarkers in several paediatric diseases across different age groups, from infancy to adolescence [40–42]. However, there have been no investigations into the use of urinary miRNAs as potential biomarkers for JIA.

Novelty and relevance of the study

Early diagnosis and longitudinal monitoring in JIA remain a clinical challenge. Discrepancies among several clinical disease activity evaluation scales emphasize the need for additional biomarkers or imaging modalities to optimize patient management in JIA [11]. The inherent characteristics of miRNAs and their recognized role in various biological functions position them as promising tools for assessing chronic inflammation [32, 34]. Moreover, an increasing body of evidence supports the integration of ultrasound into routine clinical practice as a patient-friendly imaging modality that provides valuable insight into disease activity [23, 43–46].

This study is significant in the international scientific field as, as far as we know, this is the first research to examine changes in miRNA levels over a 12-month period in conjunction with MSUS markers of inflammation.

Our research highlights the potential of serum miRNAs as diagnostic tools in JIA and marks the initial exploration of urine as a new biofluid for miRNA detection in this group of patients. The study highlights the potential role of miRNAs in the pathogenesis of JIA and their utility in the longitudinal monitoring of disease activity.

Our study further indicates that even after achieving clinical remission for a period of six months or longer, a considerable number of JIA patients continue to display subclinical inflammation detectable by MSUS. Currently, the criteria for determining remission or an inactive state in JIA do not take imaging results into account [16, 47]. However, as the body of evidence supporting the relevance of MSUS grows, it becomes increasingly clear that ultrasound findings should be integrated with clinical evaluations to provide a more comprehensive assessment of disease activity. This integration may eventually lead to the inclusion of ultrasound as an additional criterion for defining JIA remission in the future.

STUDY AIM AND OBJECTIVES

Study aims

To evaluate the persistence of subclinical synovitis detected by MSUS in patients with JIA over a 12-month follow-up period, and to explore the diagnostic potential of serum and urine miRNAs in JIA, including their associations with MSUS findings and clinical disease activity measures during both active disease and remission states.

Study objectives

1. To analyse correlation of clinical disease activity indicators and signs of inflammation in MSUS in patients diagnosed with JIA.
2. To evaluate serum miRNAs, particularly miR-16, miR-146a and miR-155, potential in JIA diagnostics and follow-up.
3. To assess the presence and variation of miRNAs' levels in urine samples of JIA patients according to their disease activity, aiming to determine their potential as non-invasive biomarkers.
4. To correlate miRNAs levels with conventional inflammatory markers (complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR)) and musculoskeletal ultrasound findings across different JIA disease activity states.
5. To monitor longitudinal changes in miRNA levels over a 12-month period, capturing their fluctuations in response to disease progression.

1. REVIEW OF THE LITERATURE

1.1. Juvenile Idiopathic Arthritis (JIA)

JIA is a heterogenous group of disorders that manifests as early-onset arthritis, with varied long-term outcomes [12, 48]. All subtypes start before the age of 16, with arthritis symptoms lasting for six weeks or longer, and there is no other identifiable cause [49].

1.1.1. JIA epidemiology

Data about JIA epidemiology vary significantly among reported studies, in part because JIA is identified clinically without specific diagnostic tests. No particular country or continent exhibits clear predominance; however, most epidemiological studies have been conducted in Europe and North America [3, 4]. According to a systematic review analysing 43 studies, incidence rates range from 1.6 to 23 per 100 000, and prevalence from 3.8 to 400 per 100 000 [3]. Girls are more frequently affected than boys, with an overall female-to-male ratio of 3:2, although this ratio varies significantly between JIA subtypes [1, 3, 4, 12]. The most common subtype – oligoarticular JIA – primarily affects females, whereas enthesitis-related arthritis (ERA) shows a clear male predominance [50].

Efforts to collect global epidemiological data have been led by international research groups, such as in the EPOCA study [4]. This study highlighted significant regional variation in JIA subtype incidences. In Europe, especially in southern regions, oligoarthritis is more prevalent and presents at a younger median age than in other regions. In contrast, North America reports a higher frequency of rheumatoid factor (RF)-negative polyarthritis [4]. Systemic JIA is more common in Asia, with countries like India and Japan reporting higher frequencies compared to Europe and North America [51, 52]. These findings support earlier data from Canada suggesting that the risk of JIA is influenced by ethnicity [53]. Children of European descent in North America were found to have an increased risk of developing JIA, particularly extended oligoarticular and psoriatic subtypes, compared to other ethnic groups [53]. Similarly, Beesley et al. reported the highest JIA incidence (6.2 per 100 000 population) among White ethnic groups in England [5]. These disparities reflect the influence of genetic, environmental, and access to healthcare-related factors on the epidemiology of JIA. Reported discrepancies may also result from the fact that most studies are clinic-based rather than community-based, and access to paediatric rheumatologist,

diagnostic approaches, and different study designs for collecting and reporting data vary globally.

The true incidence of JIA in Lithuania is not known, as no unified national registry exists. According to the Lithuanian Health Statistics database (Higienos institutas: *https://stat.hi.lt/default.aspx?report_id=168*), where diagnoses are registered using International Classification of Diseases and Related Health Problems **(**ICD-10) codes, there are 1.36 JIA cases per 1 000 inhabitants. However, the figure may not reflect the actual incidence, as it is unclear who made the diagnosis (e.g., general practitioner, paediatric rheumatologist, orthopaedic surgeon, etc.), or whether it was a confirmed case or merely a suspicion.

1.1.2. Subtypes of JIA

The most widely used classification system in both clinical practice and research is the ILAR (International League of Associations for Rheumatology) classification [49]. According to it, JIA is divided into seven categories, based on the number of active joints (e.g., five or more for polyarticular JIA), laboratory markers (e.g., rheumatoid factor – RF), and extraarticular clinical features (e.g., psoriasis) (Table 1.1.2.1). While some JIA features resemble adult forms of arthritis, such as RF-positive JIA and psoriatic JIA, other subtypes, like oligoarthritis and ANA-positive RF-negative polyarthritis, show patterns unique to paediatric populations, including a predisposition to asymptomatic uveitis, which is rarely seen in adults [54, 55].

Recent advances in genetics, proteomics, and longitudinal studies have highlighted distinct clinical course and treatment responses even within the same ILAR-defined subtypes. Up to 44% of patients change JIA subtype over the course of their disease [12, 54, 56, 57]. Moreover, it has been recognized that criteria such as joint count or presence of psoriasis do not necessarily correlate with homogeneous immune pathogenesis [58]. Therefore, ILAR classification has faced increasing criticism, prompting efforts to develop a new taxonomy based on pathogenetic mechanisms and biomarker profiles [55, 59, 60]. Although debate continues over how best to revise the criteria, a consensus conference led by the Paediatric Rheumatology International Trials Organization (PRINTO) recently proposed a new classification system, which is currently undergoing validation (Table 1.1.2.1) [60].

Table 1.1.2.1. *ILAR and PRINTO classifications of JIA and possible pathogenetic pathways (adapted according [7, 49, 60].*

JIA group	ILAR criteria	PRINTO criteria	Type of disease	Immune system involved in patho-genesis	Autoanti-bodies	Key moment in patho-genesis	Main pro-inflam-matory cytokines	Main treatment targets
1	2	3	4	5	6	7	8	9
Systemic JIA	Arthritis in one or more joints with or preceded by fever of at least 2 weeks' duration that is documented to be daily ("quotidian") for at least 3 days, and accompanied by one or more of the following: 1. Evanescent (nonfixed) erythematous rash 2. Generalized lymph node enlargement 3. Hepatomegaly and/or splenomegaly 4. Serositis. Exclusions: a, b, c, d (see below the table).	Fever of unknown origin which is documented to occur daily for at least 3 consecutive days and reoccurring over a duration of at least 2 weeks and accompanied by 2 major criteria OR 1 major criterion and 2 minor criteria. Major criteria are (1) evanescent erythematous rash; and (2) arthritis. Minor criteria are (1) generalized lymph node enlargement and/or hepato-megaly and/or splenomegaly; (2) serositis; (3) arthralgia lasting 2 weeks or longer (in the absence of arthritis); and (4) leucocytosis ($\geq$ 15 000/mm^3) with neutrophilia.	Auto-inflam-matory Auto-immune	Innate	None	Abnormal activation of phagocytes leads to hypersecre-tion of pro-inflammatory cytokines	IL-1, IL-6, IL-18, IL-37, LRG and ADA2	Block of IL-1 and IL-6 signalling pathway

Table 1.1.2.1. *Continued*

1	2	3	4	5	6	7	8	9
RF-positive polyarticular JIA	Arthritis affecting 5 or more joints during the first 6 months of disease; 2 or more tests for RF at least 3 months apart during the first 6 months of disease are positive. Exclusions: a, b, c, e (see below the table).	Arthritis for ≥ 6 weeks, and association with 2 positive tests for RF at least 3 months apart or at least 1 positive test for antibodies to cyclic citrullinated peptide.	Auto-immune	Adaptive	ANA, RF, anti-CCP, anti-MCV	Imbalance between inflammatory Th1/Th17 and Treg cells	TNF-alfa, IL-17, IL-23, IFN-gamma	Inhibition of T-cell proliferation, block of TNF-alfa
RF negative polyarticular JIA	Arthritis affecting 5 or more joints during the first 6 months of disease; a test for RF is negative. Exclusions: a, b, c, d, e (see below the table)	**Early-onset ANA-positive JIA:** Arthritis for ≥6 weeks, and early-onset (≤6 years), and presence of 2 positive ANA tests with a titter ≥1/160 (tested by immunofluorescence) at least 3 months apart. Exclusions are systemic JIA, RF-positive arthritis, and enthesitis/ spondylitis-related JIA.	Auto-immune	Adaptive	ANA			
Oligo-articular JIA	Arthritis affecting one to 4 joints during the first 6 months of disease. Two subcategories are recognized: 1. Persistent oligoarthritis: Affecting not more than 4 joints throughout the disease course; 2. Extended oligoarthritis: Affecting a total of more than 4 joints after the first 6 months of disease. Exclusions: a, b, c, d, e (see below the table)		Auto-immune	Adaptive	ANA	Imbalance between inflammatory Th1/Th17 and Treg cells	TNF-alfa, IL-17, IFN-gamma	Inhibition of T-cell proliferation, rarely anti-TNF therapy is needed

Table 1.1.2.1. *Continued*

1	2	3	4	5	6	7	8	9
Enthesitis related JIA	Arthritis and enthesitis, or arthritis or enthesitis with at least two of the following: 1. The presence of or a history of sacroiliac joint tenderness and/or inflammatory lumbosacral pain 2. The presence of HLA-B27 antigen 3. Onset of arthritis in a male over 6 years of age 4. Acute (symptomatic) anterior uveitis 5. History of ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative. Exclusions: a, d, e (see below the table).	Peripheral arthritis and enthesitis, or arthritis or enthesitis, plus ≥ 3 months of inflammatory back pain and sacroiliitis on imaging, or arthritis or enthesitis plus 2 of the following: (1) sacroiliac joint tenderness; (2) inflammatory back pain; (3) presence of HLA-B27 antigen; (4) acute (symptomatic) anterior uveitis; and (5) history of a spondyloarthritis in a first-degree relative.	Auto-immune	Adaptive	ANA in some cases	HLA-B27 is involved in the presenta-tion of unidentified arthritogenic peptide caused T-cells activation and induction of endoplasmic reticulum stress	TNF-alfa, IL-17, IL-23	Block of TNF-alfa

Table 1.1.2.1. *Continued*

1	2	3	4	5	6	7	8	9
Psoriatic JIA: Early-onset Late-onset	Arthritis and psoriasis, or arthritis and at least two of the following: 1. Dactylitis; 2. Nail pitting or onycholysis; 3. Psoriasis in a first-degree relative. Exclusions: b, c, d, e (see below the table).	Splits to other groups, such as Early-onset ANA-positive JIA or enthesitis-related JIA.	Auto-immune Autoinflammatory	Adaptive Innate	ANA for early-onset None for late-onset	Autoinflammatory activation at the synovial-entheseal complex. Autoimmune processes in extraarticular tissues	IL-17, IL-23	Inhibition of T-cell proliferation, block of TNF-alfa
Undifferentiated JIA	Arthritis that fulfils criteria in no category or in two or more of the above categories.	**Unclassified JIA:** Arthritis for ≥6 weeks and fits >1 disorder 1∼4.	Auto-immune or autoinflammatory					
		Other JIA: Arthritis for ≥ 6 weeks Does not fit criteria for disorders 1 to 4.	Auto-immune or autoinflammatory					

Possible exclusions for each category: a. Psoriasis or a history of psoriasis in the patient or first-degree relative. b. Arthritis in an HLA-B27-positive male beginning after the 6th birthday. c. Ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis, or a history of one of these disorders in a first-degree relative. d. The presence of IgM rheumatoid factor on at least two occasions, at least three months apart. e. The presence of systemic JIA in the patient.

Abbreviations: ADA2 – adenosine deaminase 2; ANA – antinuclear antibodies; anti-CCP – anti-cyclic citrullinated peptides; anti-MCV – antibodies to mutated citrullinated vimentin; HLA-B27 – human leucocyte antigen B27; IFN-gamma – interferon gamma; ILAR – the International League of Associations for Rheumatology; IL – interleukin; JIA – juvenile idiopathic arthritis; LRG – leucine-rich α2 glycoprotein; PRINTO – Paediatric Rheumatology International Trials Organisation; RF – rheumatoid factor; TNF – tumour necrosis factor.

The most distinct subtype in terms of clinical features and pathogenesis is systemic JIA, which is driven by autoinflammatory mechanisms due to abnormal activation of the innate immune system, and eventually can progress to autoimmune pathway activation [61]. Specific major and minor diagnostic criteria have been developed to capture its diverse clinical manifestations [60]. A recent consensus also – recognized its similarity to adult Still's disease [62].

Most other forms of JIA arise from innate immune activation, leading to dysregulation of adaptive immunity, causing antibody production and joint inflammation [63] (Table 1.1.2.1). Among those, RF-positive polyarthritis is associated with the poorest long-term prognosis, and oligoarticular JIA tends to have the most favourable outcomes [64–66]. Notably, some JIA subtypes, particularly enthesitis related arthritis (ERA) and psoriatic JIA, show a strong genetic predisposition. Genetic factors are estimated to contribute up to one-third of the risk for JIA [6, 67]. The human leukocyte antigen (HLA) region within the major histocompatibility complex on chromosome 6 contains several well-established risk alleles, particularly HLA B27 [68]. The presence of this antigen is included in the ERA diagnostic criteria both in the ILAR and PRINTO classifications (Table 1.1.2.1).

1.1.3. JIA etiopathogenesis

The aetiology of JIA is not yet fully understood; however, it is known as a multifactorial disease involving a complex interplay between genetic predispositions and environmental triggers. Genetic factors, particularly variations in human leukocyte antigen (HLA) alleles, play a significant role in the susceptibility to JIA, with distinct subtypes associated with specific genetic markers [7, 63]. Non-HLA-associated genes have also been implicated in immune system dysregulation [69]. A recent study by Kim et al. found that JIA demonstrates greater polygenicity compared to other autoimmune diseases [70], possibly due to the broader clinical spectrum encompassed by the JIA classification. Similarly, a study done by Barnes et al. identified distinct gene expression profiles in peripheral blood mononuclear cells across different JIA subtypes [71]. These profiles were also influenced by age at disease onset, with early-onset oligoarthritis (up to 6 years of age) showing increased expression of B-cell activation genes [72]. Genetic studies confirm that inherited susceptibility contributes to immune dysregulation, involving both innate and adaptive immune responses, which together sustain chronic inflammation [7, 63, 73].

A variety of immune cells, including monocytes, macrophages, neutrophils, and natural killer cells, contribute to disease activity [7, 63, 73]. For

instance, synovial monocytes enhance T-cell activation via the IL-6/JAK/STAT pathway, while innate cells release inflammatory proteins such as S100A8/9/12 and signal through Toll-like receptor/IL-1 pathways [74]. The adaptive immune response features activated CD4+ T-cells, particularly Th1 and Th17 subsets, as well as regulatory T cells (Tregs) whose Foxp3 expression is regulated by RUNX1 and IL-2 signaling [63, 75]. B-cells, through autoantibody production and cytokine release, further amplify the inflammatory response. In conjunction with aberrant synoviocyte proliferation, this leads to synovial membrane thickening [7]. The synovium, a membrane lining the joint cavities, tendons, and bursae, normally consists of an intimal lining with two or three layers of fibroblast-like synoviocytes (FLS) and macrophage-like synoviocytes embedded in a dense extracellular matrix, along with a sub-lining layer of loose connective tissue, blood vessels, lymphatics, fibroblasts, collagen fibres, nerve fibres, and minimal leucocytes [76]. In JIA, proinflammatory cytokine overproduction results in synovial hypertrophy and hypervascularization, which can be visualized using MSUS. The Power (or Colour) Doppler of MSUS assesses vascular flow within the hypertrophic synovium – also known as pannus – providing insight into inflammation intensity [28]. The pannus promotes secretion of additional proinflammatory cytokines, such as IL-1 and TNF-α, which in turn stimulate osteoclast activity, increasing cartilage degradation and promoting the formation of bone erosions [77, 78].

Evidence of T-cell activation is found in both oligoarticular and polyarticular JIA patients [79, 80]. Inflammation is believed to arise from an imbalance between pro-inflammatory Th1/Th17 cells and anti-inflammatory Tregs. A reduction in Tregs is inversely correlated with an increase in the Th17 cells with naïve T-cell differentiation driven by IL-1β via the NF-κB pathway [80–82]. Interestingly, the NF-κB pathway is also critical to Treg-mediated suppression of other immune cells [83]. A recent study by Quilis et al. found that higher Treg counts were associated with lower ESR levels in JIA patients [84].

Monocytes and macrophages, key effector cells in inflammation, contribute to chronic inflammation via multiple signalling cascades, including IL-6/JAK/STAT axis [74], TNF-α, IFN-γ, GM-CSF [85], NF-κB pathways [86]. The canonical NF-κB pathway is activated by proinflammatory stimuli such as TNF-α, IL-1β, lipopolysaccharide (LPS), triggering receptor-mediated signal transduction through bridging proteins, leading to NF-κB nuclear translocation and transcriptional regulation of inflammation-related genes [87, 88]. Additionally, cross-talk among inflammation pathways modulates various immune functions [87, 89]. NF-κB also activates the NLRP3 inflammasome, a key regulator of innate immune responses [90].

Activated monocytes and macrophages produce an array of proinflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-12, IL-18, and IL-23 [91–93]. While the relative levels of these cytokines vary across JIA subtypes [93–95], their interactions with immune and stromal cells, including synovial fibroblasts, chondrocytes, and osteoclasts play a central role in disease pathogenesis [7,75]. Importantly, elevated cytokine levels persist even during clinical remission, suggesting that remission represents a state of compensated inflammation and subclinical signs of inflammation may still be present [93].

Despite significant advances in understanding the molecular and cellular mechanisms of JIA, its precise aetiology remains unclear. Continued research is essential to the complex interactions between genetic, epigenetic, and environmental factors underlying JIA pathogenesis.

1.1.4. JIA diagnostics

The diagnosis of JIA is a multifaceted process that hinges on a combination of clinical evaluation, laboratory investigations, and imaging techniques. The ILAR classification criteria (Table 1.1.2.1) [49] remain central to diagnostic decision-making. It's essential to emphasize that JIA is a diagnosis of exclusion, necessitating the exclusion of other conditions that may mimic its presentation.

Laboratory testing plays a key role in assessing the inflammatory burden and autoimmune status. Commonly used markers include C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), which reflect systemic inflammation. Additionally, antinuclear antibodies (ANA), RF, and anti-cyclic citrullinated peptide (anti-CCP) antibodies aid in identifying autoimmune involvement [96]. HLA-B27 testing is often performed when specific subtypes of JIA are suspected [96]. Emerging biomarkers such as IL-6, IL-18, and S100 proteins are increasingly recognized for their diagnostic value, especially in systemic JIA [97–99].

While imaging techniques are not included in current classification criteria, they are crucial for differentiating JIA from other pathologies and for evaluating cartilage damage due to chronic inflammation. Conventional radiography is widely available and can identify structural abnormalities and growth disturbances, though it typically reflects a later stage of disease progression [100, 101]. Due to the high cartilage content in the paediatric skeleton, early erosions may not be detectable radiographically [101]. Musculoskeletal ultrasound offers a non-invasive, radiation-free, and cost-effective method for the early detection of synovitis and joint inflammation, even before clinical symptoms appear [24, 25, 43, 44]. Meanwhile, magnetic

resonance imaging (MRI) is increasingly recognized as a gold standard for early detection of JIA, offering detailed visualization of synovium, cartilage, bone marrow, cortical bone, and soft tissues [101]. MRI is particularly valuable for assessing hard-to-access joints, such as the temporomandibular and sacroiliac joints [102]. However, its use is often limited by cost, availability, and the need for sedation in younger children. Therefore, the selection of imaging modality depends on the patient's age, clinical presentation, and institutional resources.

1.1.5. JIA treatment and outcomes

Initial treatment for a patient with JIA often begins with low-dose or non-aggressive therapies, with escalation based on treatment response over time [103]. Contemporary pharmacological options include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, both systemic and intra-articular, and disease-modifying antirheumatic drugs (DMARDs), subdivided into synthetic DMARDs (e.g., methotrexate, sulfasalazine), biological DMARDs (e.g., anti-TNF, anti-IL1, anti-IL6 agents), and the newest small molecule DMARDs (e.g., JAK inhibitors) [104, 105].

Among biological DMARDs, anti-TNF therapy has significantly improved the ability to achieve clinically inactive disease (CID) and favourable long-term outcomes [104, 106, 107]. In the absence of effective treatment, patients face complications, including permanent disability [108]. Recently, up to 50% of JIA patients receive anti-TNF agents, enabling more to achieve remission, prevent joint destruction, and improve quality of life [10, 104–106, 109, 110]. However, a recent systematic review revealed that the overall disease burden remains substantial, with fewer than 50% of patients achieving remission after a decade of disease [14]. Moreover, less than 30% reach an inactive disease status within a 2-year follow-up [48, 64]. Disease flare or loss of CID are common during long-term follow-up [26, 44, 46, 65, 111–113].

Several factors are associated with the higher risk of JIA reactivation, including delayed achievement of clinically inactive disease, female sex, early-onset arthritis, symmetric joint involvement, hip or ankle involvement, and frequency of intra-articular injections [48, 65, 66, 114, 115]. Notably, RF positivity has been considered a poor prognostic factor associated with more aggressive disease, increased joint damage, prolonged disease activity, and a higher likelihood of requiring multiple biological DMARDs [14, 65, 66, 116–118]. A recent study by Inoue et al. demonstrated a persistent need for DMARD therapy in young adults following transition from paediatric care,

especially in RF-positive patients [119]. These findings raise critical questions for patients in clinical remission: when and how should disease-modifying drugs be tapered or discontinued to minimize long-term side effects while maintaining disease control [9, 110]? Addressing these challenges remains a priority in the management of JIA.

1.1.6. JIA disease activity evaluation

Regular evaluation of disease activity and joint damage is a fundamental aspect of the clinical assessment of children with JIA. Disease activity monitoring is included in treatment decisions and guides long-term follow-up [12, 113, 120, 121]. Although disease heterogeneity and methodological differences in outcome measures present challenges, several validated clinical scales are widely used in both clinical practice and research [122]. In addition, several inflammatory proteins have been suggested as biomarkers for disease activity [97, 123], and some imaging modalities are increasingly used to evaluate joint involvement and detect residual inflammation signs [24, 44].

1.1.6.1. Clinical scales for disease activity evaluation in JIA

Some of the earliest clinical outcome measures for JIA were developed under the American College of Rheumatology (ACR) framework, including the “Paediatric (Pedi) 30“, “Pedi 50“, and “Pedi 70”, resembling clinically significant change in disease activity [124]. These scales are composed of six core items, which are thought to be the key JIA activity indicators, and those are: 1) physician’s global assessment of disease activity; 2) parent/patient’s assessment of overall well-being; 3) functional ability; 4) number of joints with active arthritis; 5) number of joints with limited range of motion; and 6) ESR. For instance, Pedi 30 requires at least a 30% improvement from baseline in three of any six variables, with no more than one variable worsening by over 30% [124]. However, these scales have limitations in reflecting real-time disease activity. For example, joint limitation may result from damage rather than active inflammation [125]. To address these limitations, the Juvenile Arthritis Disease Activity Scale (JADAS) was developed in 2009 as a more dynamic and practical tool for assessing disease activity [15]. Multiple versions of JADAS are available, based on the number of joints assessed: JADAS-71, which includes up to 101 joint evaluation; JADAS-27, which includes up to 57 joints; and JADAS-10, which includes up to 40 joints for assessment. All versions share four components: 1) physician’s global assessment of disease activity; 2) parent/patient assessment of overall well-being; 3) active joint count (swollen and/or painful/tender joints); 4) ESR. All

variants of JADAS were validated in multiple JIA cohorts [15] and are widely adopted in both clinical practice and research [10, 126, 127]. A version incorporating CRP instead of ESR showed equivalent reliability [128]. Additionally, McErlane et al. demonstrated that a short clinical variant of JADAS (cJADAS), omitting the laboratory parameters, provides a reliable alternative in settings where blood tests are unavailable [129]. The use of JADAS has increased substantially following the establishment of cut-off values for different disease activity states in oligo- and polyarthritis [130]. A definition of clinically meaningful improvement based on the JADAS10 was also presented, which was shown to outperform the Pedi ACR scores [131].

For several JIA subtypes with distinct clinical features, such as spondyloarthritis or systemic JIA, specific tools have been developed. The Juvenile Spondyloarthritis Disease Activity scale (JSpADA) addresses axial involvement and enthesitis, incorporating assessment of back mobility, active enthesitis count, and presence of uveitis [132]. For systemic JIA, which is characterized by fever, rash, and systemic inflammation rather than joint symptoms, the Systemic JADAS (sJADAS) is used, with recently validated cut-offs for different disease activity states [19, 60, 133].

Although clinical decisions are made primarily by the physician's evaluation of disease activity, the parent/patient perception of disease course can improve the general assessment, especially during long-term follow-up. The most comprehensive tool for parent/patient-reported outcomes is the Juvenile Arthritis Multidimensional Assessment Report (JAMAR) [134, 135], which includes evaluation of morning stiffness, disease activity, rating of disease status and course, proxy- or self-assessment of joint involvement and extraarticular symptoms, medication side effects, therapeutic adherence, and patient satisfaction with the outcome of the illness – domains often missing from other health-related questionnaires [136].

Despite the practicality, comprehensiveness, and simplicity of JADAS and other clinical scales, some limitations remain. Extra-articular manifestation (e.g., uveitis, rashes) are typically not included. Moreover, spine segments (cervical, thoracic, lumbar) are usually counted as a single joint, underrepresenting disease severity in axial forms, and sacroiliac joints, which lack visible swelling and are difficult to assess clinically, are also excluded. Additionally, ESR and CRP also have some limitations; JADAS-71 correlates only moderately with the ESR overall, and this correlation varies markedly by subtypes [129]. These issues highlight the need for complementary tools – especially imaging and novel biomarkers – to fully assess JIA disease activity.

1.1.6.2. Imaging for JIA disease activity evaluation

A range of imaging modalities can be used to assess joint involvement in JIA, including conventional radiology (CR), ultrasound, magnetic resonance imaging (MRI), and computed tomography (CT). However, not all are suitable for disease activity evaluation in JIA.

CR remains a first-line tool to exclude trauma or orthopaedic conditions, which are more common in children than JIA. While it can detect structural damage and growth disturbances [100, 125, 137], but it has limited utility in early disease and is not validated in paediatric population [138].

CT offers detailed visualization of bone, but involves high radiation exposure and is less accessible in paediatric settings [139]. MRI, by contrast, provides a comprehensive view of the joint and is especially valuable for evaluating the temporomandibular joint (TMJ) and axial skeleton, including the sacroiliac joints [140–144]. Whole-body MRI has been proposed as a promising tool for assessing overall disease burden in JIA [145, 146], however, its use is constrained by high cost, limited availability, long scanning times, and the need for sedation in younger children.

MSUS has emerged as the most patient-friendly and clinically informative imaging modality in paediatric patients. It enables the identification and differentiation of synovitis, tendinitis, bursitis, and enthesitis in all paediatric age groups. MSUS has proven to be more sensitive than clinical examination in detecting signs of joint inflammation, sometimes resulting in the reclassification of JIA subtypes [25, 147]. Importantly, studies have also demonstrated that MSUS is also capable of identifying subclinical inflammation – inflammatory changes not detectable through clinical assessment or conventional biomarkers such as CRP and ESR [23, 25, 45, 148].

Subclinical inflammation has been reported across all JIA subtypes and in both active and remission phases of the disease [23, 26, 44, 46, 112, 147–149]. Although it has been proposed as a risk factor for disease flare after anti-rheumatic therapy tapering or discontinuation, current evidence remains inconclusive [26, 44, 46, 112]. Recent collaborative efforts have focused on standardizing MSUS protocols and the definition of synovitis across different joints in both JIA patients and healthy children [24, 28–30]. Nevertheless, longitudinal data describing MSUS findings over the course of the disease remain scarce. Given the relatively low rates of long-term remission and the frequent need for treatment intensification or reintroduction after remission (REM), there is growing interest in using MSUS to monitor subclinical inflammation and guide clinical decisions in JIA management.

1.2. Biomarkers in JIA

1.2.1. Conventional biomarkers

The general definition of a biomarker, according to the U.S. Food and Drug Administration (FDA) and the National Institutes of Health (NIH) Biomarker Working Group, is "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention" [150]. However, more precise definitions have been proposed for specific applications in medicine. Several categories of biomarkers are recognized, including diagnostic, monitoring, predictive, prognostic, and multicomponent biomarkers [150, 151]. Several of these have been applied in chronic diseases to capture different aspects of conditions more effectively. Moreover, a single molecule may fulfil multiple biomarker roles, depending on the clinical context [151, 152].

In paediatrics, ideal biomarkers should be non-invasive, easy to perform, reproducible, cost-effective, and reflect disease activity with high sensitivity and specificity [31, 152]. Although conventional inflammatory markers such as ESR and CRP are easy to measure, they reflect systemic inflammation rather than joint-specific activity. Therefore, biomarkers that are more closely associated with the underlying pathophysiological mechanisms of JIA are of particular interest both for diagnosis and monitoring.

Due to the complex pathophysiology of JIA, no specific diagnostic biomarker has been universally identified. However, several routinely measured biomarkers provide valuable information regarding disease progression or extra-articular damage. For instance, RF is used to distinguish RF-positive and RF-negative polyarthritis, indicating a worse prognosis and increased risk of long-term joint damage [48, 65]. ANA positivity is strongly associated with the development of chronic uveitis, guiding ophthalmologic screening and follow-up strategies [153–155]. Anti-CCP antibodies, though infrequent in JIA, are associated with erosive joint disease [156, 157]. Notably, these autoantibodies are not suitable for monitoring JIA disease activity.

More promising candidates for disease activity monitoring in JIA include members of the S100 protein family, particularly S100A8, S100A9, and S100A12 [123]. These proteins, produced primarily by neutrophils and monocytes, are found at high concentrations in inflamed tissue [158]. Moreover, S100 proteins regulate calcium homeostasis, enzyme activities, energy metabolism, cell growth and differentiation, and also mediate inflammatory responses and recruit inflammatory cells to sites of tissue damage [159, 160]. Several studies have demonstrated that elevated levels of S100A8/A9 and S100A12 in serum and synovial fluid correlate strongly with disease activity in JIA [93, 161].

A recent study by Angeles-Han et al. detected S100A12 together with other inflammatory proteins in tear fluid, suggesting potential for non-invasive monitoring of JIA-associated uveitis [162]. However, the predictive utility of these biomarkers is still contradictory. For example, in a study by Hinze et al., S100A12 showed only moderate predictive value for disease flare in 137 JIA patients in REM on anti-TNF therapy [123]. In contrast, Brunner et al. demonstrated that both S100A8/A9 and S100A12 could predict treatment response to abatacept, suggesting a potential role in personalized treatment strategies [163]. These findings underscore the need to refine biomarker panels further to support individualized disease management, improve early diagnosis, and prevent long-term disability. Given the multi-factorial pathogenesis of JIA, emerging fields such as epigenetics offer promising opportunities for identifying novel molecular targets and enhancing our understanding of this complex paediatric condition.

1.2.2. Novel biomarkers – microRNA

Epigenetic regulation encompasses alterations in gene expression, related to environmental factors, without changes in the sequence of the bases in the DNA [164]. Among the key mediators for epigenetic control are microRNAs (miRNAs) – a class of small, non-coding RNAs that play an essential role in post-transcriptional gene regulation [165]. Although miRNAs constitute only ~3% of the human genome, they regulate approximately 90% of genes [166].

miRNAs are typically ~22 nucleotides in length and function by binding to complementary sequences on informational RNA, thereby inhibiting translation or inducing messenger RNA (mRNA) degradation [166]. MiRNAs are generated endogenously with miRNA genes located throughout the genome, including protein-coding and non-coding regions, transposable DNA sequences, and repetitive genome elements [167].

MiRNA biogenesis is a multi-step process essential for the post-transcriptional regulation of gene expression. It begins with the transcription of primary miRNAs (pri-miRNAs) by RNA polymerase II, which are then processed in the nucleus by the endoribonuclease Drosha into precursor miRNAs (pre-miRNAs) [168]. These pre-miRNAs are exported to the cytoplasm and further cleaved by Dicer, another endoribonuclease, to produce mature miRNA duplexes having a guide strand (functional) and the passenger strand (usually degraded) (Fig. 1.2.2.1). The mature miRNA is incorporated into the RNA-induced silencing complex (RISC), which targets specific mRNAs for translational repression or degradation, thereby modifying protein expression [34, 169] (Fig. 1.2.2.1).

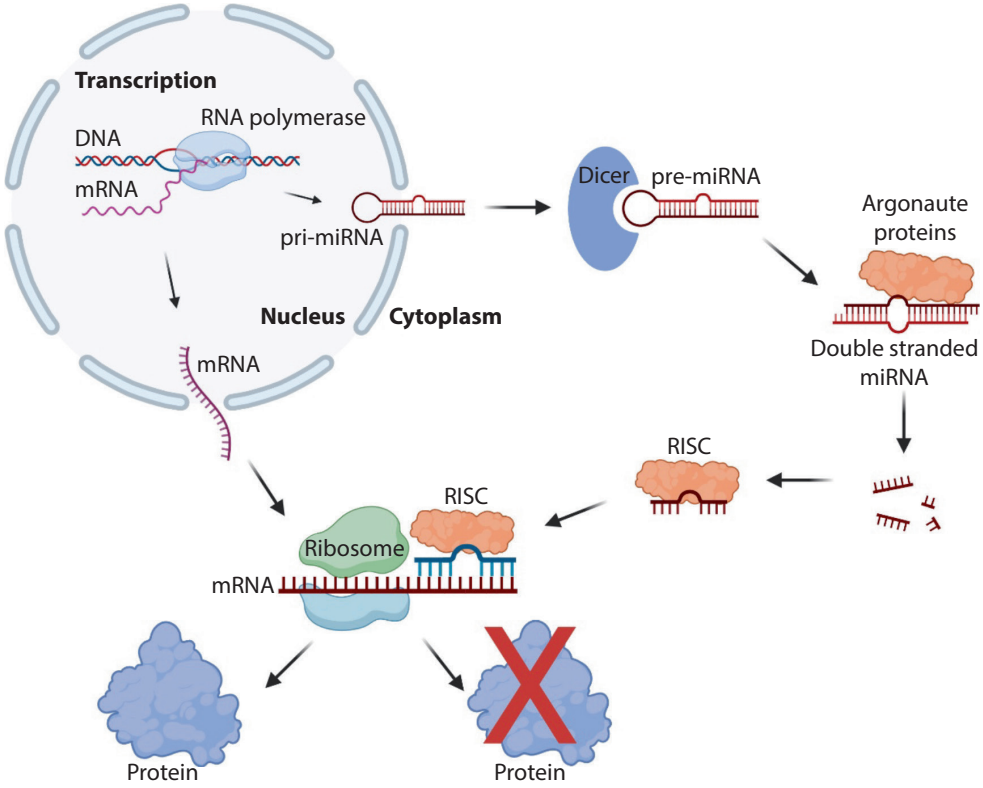


Fig. 1.2.2.1. *Simplified scheme of miRNA role in gene expression*

A hairpin primary miRNA (pri-miRNA) is synthesised in the nucleus and transported to the cytoplasm through an Exportin-5 protein. An enzyme called Dicer trims the pre-miRNA and removes the hairpin loop, leaving a double stranded miRNA duplex molecule. One of the strands joins a group of proteins (Argonaute), meanwhile, another strand, known as the passenger strand, is degraded. Eventually, miRNA-protein complex (RISC) is formed. MiRNA nucleotides function by binding to complementary sequences on messenger RNA (mRNA) nucleotides and in such a way blocking the ribosome and modifying protein expression (*Figure created by Ausra Snipaitiene using BioRender.com*).

Both strands of the miRNA duplex may be functionally active and incorporated into RISC, providing a flexible system for modulating gene expression in a switch-like fashion [170]. Aberrant strand selection has been implicated in the pathogenesis of various disease, including autoimmune and autoinflammatory disorders.

In addition to canonical pathways, non-canonical miRNA biogenesis pathways exist, some of which are Drosha-, and/or Dicer-independent [168]. These atypical pathways can be used for creating molecules that efficiently bypass both RNase III enzymes to generate functional miRNA [171].

The biogenesis pathway of miRNAs is regulated at multiple levels, including transcriptional, post-transcriptional, and post-translational modifications, which are crucial for maintaining the balance of miRNA and target mRNA levels [172, 173]. One of the processes controlling miRNA levels is targeted degradation of miRNA (TDMD), where specific targets can impact the ratio of miRNA strands [174]. This regulation is vital for various biological processes and is often disrupted in diseases, highlighting the importance of understanding miRNA biogenesis in both health and disease contexts [169, 175].

Extracellular miRNAs are usually located in the vesicles (exosomes, microvesicles, and apoptotic bodies) [176] or bound to the various proteins (AGO2, high-density lipoprotein, nucleophosmin-1) [177, 178]. Each miRNA has a specific number of target genes it can regulate [168]. Some miRNAs play a significant role in controlling the standard and functional aspects of the innate and adaptive immune system [179, 180].

1.2.2.1. miR-16

MiR-16 is a small non-coding RNA molecule located on chromosome 13q14 (miRBase, available at: *https://mirbase.org/hairpin/MI0000070?acc=MI0000070*). It plays a multifaceted role in inflammation, targeting various immune-related pathways and molecules, including those involved in T-cell metabolism and activation [181] (Fig. 1.2.2.1.1). Liang et al. have demonstrated that miR-16 can suppress the activation of inflammatory macrophages in atherosclerosis via the mitogen-activated protein kinase (MAPK) and NF-κB signalling pathways, thereby suppressing the secretion of key pro-inflammatory factors, such as IL-6 and TNF-α, both of which are well-known mediators in the pathogenesis of JIA [182]. Other studies have shown that the absence of miR-16 leads to increased NF-κB pathway activation in T-cells, promoting immune activation [183]. Similarly, in monocyte, miR-16 modulates NF-κB signalling through regulation of the silencing mediator for retinoid and thyroid hormone receptor (SMRT) [184]. In a mouse model of lung inflammation, Yang et al. revealed the ability of miR-16 to reduce inflammation by directly targeting Toll-like receptor 4 (TLR4) and inhibiting the activation of NLRP3 inflammasome [185]. In the context of myocardial injury, Wang et al. reported that the upregulation of miR-16 attenuated cell apoptosis and inflammatory response by reducing the expression of dedicator of cytokinesis 2 (DOCK2) [186]. Data in inflammatory bowel diseases (IBD) demonstrate the other side of miR-16 effects. Using a mouse model of IBD, Chen et al. found that inhibiting miR-16 can significantly decrease the expression of IL-1β, IL-6, and TNF-α, thereby alleviating inflammation and

disease activity [187]. Moreover, Tian et al. demonstrated that miR-16 can promote activation of the NF-κB signalling pathway in ulcerative colitis by inhibiting the expression of A2aAR protein at the post-transcriptional level [188]. Furthermore, miR-16 was found to activate $CD4^+$ T cells and shift macrophage polarization from the anti-inflammatory M2 phenotype to a pro-inflammatory M1 phenotype by downregulating programmed cell death ligand 1 (PD-L1) in murine peritoneal macrophages [189] (Fig. 1.2.2.1.1). Clinical studies support these mechanistic findings. Several investigations have identified a positive correlation between miR-16 and disease activity and severity in the clinical setting of IBD patients [190, 191]. In rheumatoid arthritis (RA), miR-16 is thought to contribute to the imbalance between Th17 and Treg cells, potentially through regulation of retinoid-related orphan receptor γ-t (RORγt) and forkhead box P3 (FoxP3) expression [192]. These variable effects of miR-16 across different inflammatory conditions highlight its context-specific roles, which are likely influenced by the surrounding cellular and molecular environment. Also, the possibility of miR-16 acting through multiple targets coordinately to regulate the same biological process [193, 194] underlines miR-16's potential as a therapeutic target for managing different inflammatory conditions. Importantly, these findings emphasize the need for further research in paediatric populations, where data on miR-16 remain scarce.

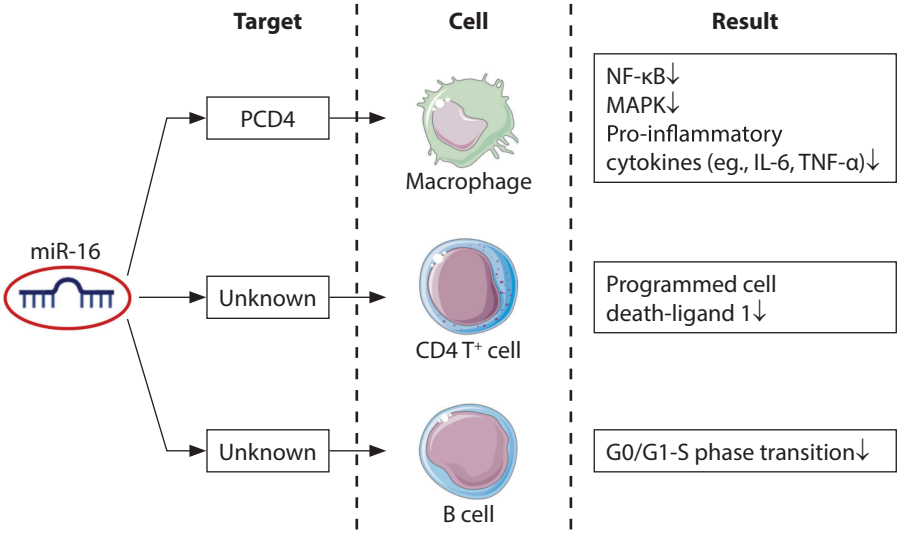


Fig. 1.2.2.1.1. *Main roles of miR-16 in autoimmunity*

Abbreviations: PDC4 – programmed cell death 4; MAPK – mitogen-activated protein kinase (*Figure created by Ausra Snipaitiene using BioRender.com*).

1.2.2.2. miR-146a

MiR-146a is located on chromosome 5q33.3 (miRBase, available at: *https://mirbase.org/hairpin/MI0000477*) and functions as a key regulator in numerous inflammatory diseases [180]. It exists in two isoforms – miR-146a-5p and miR-146a-3p – which exhibit opposing roles in inflammation: miR-146a-5p generally exerts an anti-inflammatory effect, whereas miR-146a-3p can have a pro-inflammatory effect [195]. Both act through the NF-κB pathway, which can be partly cell-specific [87, 196] (Fig. 1.2.2.2.1). Nakasa et al. identified that 146a-5p is upregulated in synovial tissues of RA patients compared to healthy controls, and its expression level is stimulated by inflammatory cytokines such as TNF-α and IL-1β [197]. Similarly, Stanczyk et al. found increased levels of miR-146a in the peripheral blood mononuclear cells (PBMCs), synovial fibroblasts, and synovial fluid in RA patients [198]. MiR-146a predominantly acts as a molecular brake on inflammation, modulating the NF-κB signalling pathway and cytokine production through the Toll-like receptor (TLR) [199, 200], especially in monocytes. An *in vitro* study revealed that miR-146a expression in monocytes gradually increases after 4 hours of lipopolysaccharide (LPS) stimulation, peaking at a 35-fold increase over 24 hours [201]. Interestingly, TNF-α levels were inversely correlated with miR-146a amounts, underscoring its role in LPS-induced immune tolerance [201]. Beyond innate immunity, miR-146a modulates adaptive immune responses. It has been implicated in regulating Tregs suppressor function [202, 203], especially by influencing the STAT1 activation threshold, which is critical for Treg-mediated control of Th1 responses and associated autoimmunity [204]. Curtale et al. further demonstrated that miR-146a is induced upon T-cell receptor (TCR) stimulation in human primary T lymphocytes, with low expression in naïve T cells, suggesting its role in modulating adaptive immunity [205]. Furthermore, Li et al. demonstrated miR-146a's capabilities of significantly suppressing extracellular-matrix-associated proteins such as matrix metalloproteinase (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) in human knee joint chondrocytes [206]. Few experimental models revealed the therapeutic utility of miR-146a in reducing NF-κB-driven inflammation, joint destruction, and bone damage [197, 207, 208]. These effects can be supported by miR-146a's role in the modulation of activation-induced cell death (AICD) [205]. All the mentioned research results above suggest miR-146a as a possible novel diagnostic and therapeutic target for autoimmune diseases such as JIA.

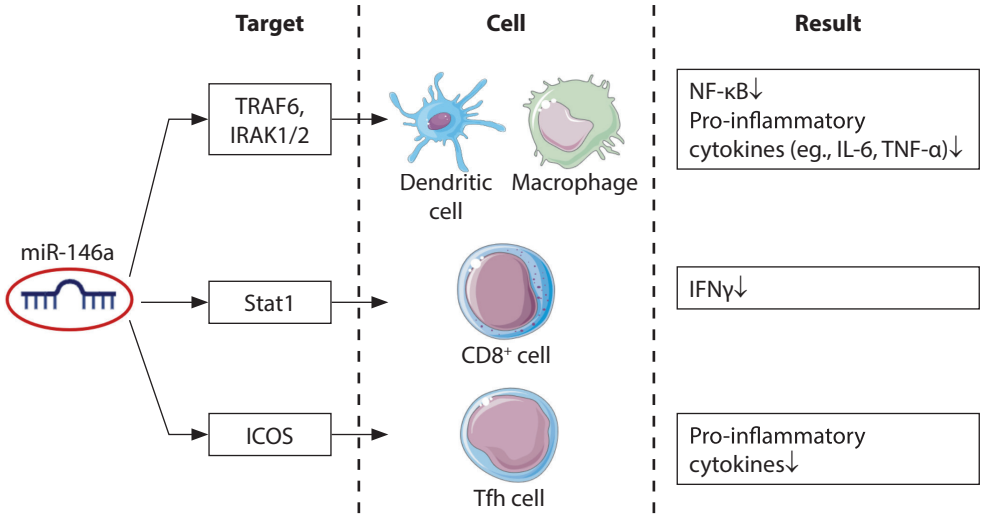


Fig. 1.2.2.2.1. *Main roles of miR-146a in autoimmunity*

Abbreviations: ICOS – inducible T cell Co-stimulator; IRAK1/2 – interleukin-1 receptor-associated kinase 1 or 2; Stat1 - signal transducer and activator of transcription 1; TRAF6 – factor associated with the TNF receptor 6 (*Figure created by Ausra Snipaitiene using BioRender.com*).

1.2.2.3. miR-155

miR-155 is a potential pro-inflammatory microRNA located on chromosome 21 (miRBase, available at: *https://mirbase.org/hairpin/MI0000681*) and encoded within a B-cell integration cluster (BIC) gene. Its expression is induced by pro-inflammatory stimuli, such as LPS and TNF-α, and it plays a critical role in immune regulation and chronic inflammation [209, 210]. It plays an essential role in arthritis by regulating the polarization of macrophages through suppressor of cytokine signalling 1 (SOCS1), interleukin 13 receptor α1 (IL-13Rα1), and CCAAT-enhancer-binding proteins (C/EBP)-β [211]. MiR-155 overexpression prevents monocyte polarization into M2-like macrophages and increases cytokine production [212, 213] (Fig. 1.2.2.3.1). Inhibition of miR-155 in RA patient-derived synovial macrophages has been shown to reduce TNF-α production [214]. Beyond arthritis, murine models of ulcerative colitis have revealed that miR-155 deficiency alters macrophage polarization and can be used as a treatment target [215]. Elmesmari et al. identified that miR-155 promotes inflammatory cell recruitment into RA synovial fluid by regulating chemokine production and pro-inflammatory chemokine receptor expression [216]. Additionally, miR-155 enhances monocyte survival by inhibiting spontaneous apoptosis, thereby sustaining chronic joint inflammation [217]. Furthermore, miR-155 has been identified as a regulatory component in T helper cell differentiation and the germinal centre reaction to produce an optimal T-cell-dependent antibody response

[218]. miR-155 interacts with several key regulatory molecules, including Treg-specific transcription factor FoxP3 [219], AID-mediated Myc-IgH [220], and Src homology 2-containing inositol phosphatase-1 (SHIP-1) [219, 221–223], further expanding its role in immune dysregulation.

Although the described pathways and experimental murine models identify miRNAs as promising therapeutic targets for autoimmune diseases, several limitations prevent their clinical implications. This is primarily due to their pleiotropic activity, as miRNAs often regulate multiple targets across various cell types, raising concerns about off-target effects and immune dysregulation [224]. Additionally, early trials in oncology have identified several limitations in miRNA-based therapies, including delivery challenges, organ-specific accumulation, and liver toxicity [225, 226]. Nevertheless, miR-155 holds promise as a diagnostic and monitoring biomarker, given its upregulation in inflammatory states, role in disease progression, and mechanistic relevance across autoimmune conditions, including JIA.

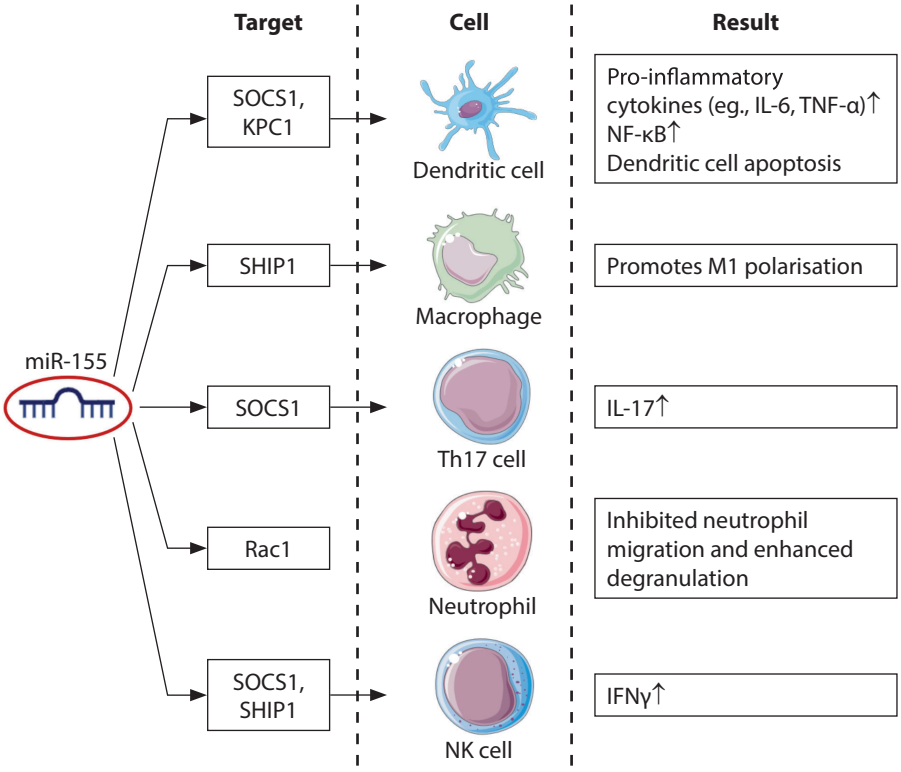


Fig. 1.2.2.3.1. *Main roles of miR-155 in autoimmunity*

Abbreviations: KPC1 – KIP1 ubiquitination-promoting complex; SHIP1 - Src homology 2-containing inositol phosphatase-1; SOCS1 – Suppressor of Cytokine Signalling 1; Rac1 – Ras-related C3 botulinum toxin substrate 1 (*Figure created by Ausra Snipaitiene using BioRender.com*).

2. MATERIALS AND METHODS

A prospective single-centre study was conducted from January 2021 to March 2023 at the Hospital of Lithuanian University of Health Sciences Kauno klinikos, enrolling all consecutive patients diagnosed with JIA (excluding systemic JIA) at the Department of Paediatrics and further followed up in the Department of Rheumatology if the patient reached 18 years of age during the study period.

2.1. Ethics statement

Permission to conduct this study was obtained from the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-100; 26-Oct-2020). Before participation, parents or legal guardians of the enrolled children, as well as participants aged 12 years and older, provided written informed consent after receiving comprehensive information about the study. This study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines.

2.2. Personal Contribution

The implementation of this scientific study has required primary author's involvement in every stage (conceptualization, methodology, literature analysis, data collection, organization of timely patient's visits, clinical evaluation of the patients and joint ultrasound performance for every patient according to approved protocol, blood and urine sample preparation for further analysis according to approved protocol, data analysis and publication of the results, writing the original draft and editing). All issues arising were discussed and solved with the help of the scientific consultant.

2.3. Study population

2.3.1. JIA patients

We managed to include 31 patients diagnosed with JIA and followed up them every 3 months for one year, involving clinical examinations, laboratory tests, and MSUS assessments at each visit. **Inclusion criteria were as follows:**

1) age at first study visit between 2 and 18 years;
2) children diagnosed with JIA according to the International League of Associations for Rheumatology (ILAR) classification criteria [49] and receiving treatment with NSAIDs and/or methotrexate (MTX) and/or glucocorticoids (GCC) and/or bDMARDs.

Exclusion criteria:

1) systemic form of JIA;
2) any other chronic diseases, including autoimmune, allergic, and others.

2.3.2. Control group

This study group included 22 age- and sex-matched healthy controls (HC) without signs of inflammation, infection, or chronic diseases. Twenty-one serum samples and 22 urine samples were collected from this group for miRNA analysis and comparison with JIA samples.

2.4. Study design

The overarching scientific project was divided into two distinct studies, each designed to investigate different aspects of disease, and biomarker development in JIA.

2.4.1. Study I: Systematic Musculoskeletal Ultrasound (MSUS) assessment

Study I involved a prospective, longitudinal evaluation of patients with JIA, conducted at 3, 6, 9, and 12 months post-inclusion. At each time point, MSUS assessments were performed and correlated with clinical disease activity indicators and conventional laboratory biomarkers of JIA (Fig. 2.4.1.1). The objective was to enhance understanding of disease progression, detect subclinical joint inflammation, and evaluate treatment efficacy over time. This study focused on monitoring JIA patients every three months, aiming to capture both clinically evident disease activity and subclinical inflammatory changes detectable only via imaging. The integration of MSUS into routine follow-up provided valuable insights into disease dynamics beyond conventional clinical evaluation.

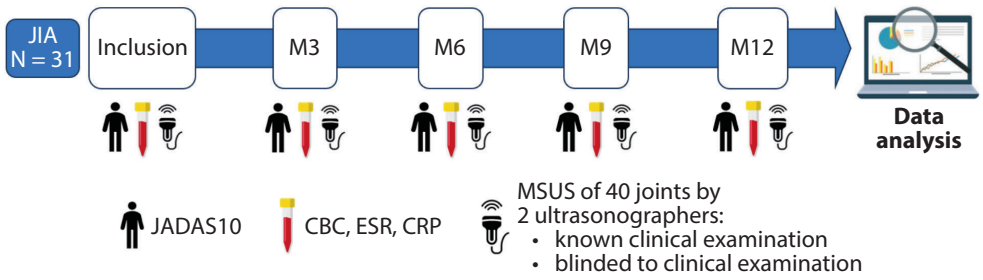


Fig. 2.4.1.1. *Study I structure*

Abbreviations: CBC – complete blood count; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; JIA – juvenile idiopathic arthritis; JADAS10 – juvenile arthritis disease activity score of 10 joints; M – month from study inclusion; MSUS – musculoskeletal ultrasound.

2.4.2. Study II: analysis of miRNA diagnostic potential in serum and urine

Study II focused on the prospective analysis of miRNA in both serum and urine samples of JIA patients, conducted at baseline (inclusion) and after 12 months of follow-up (Fig. 2.4.2.1). The primary aim was to evaluate the diagnostic potential of miRNAs and their associations with clinical disease activity measures and MSUS findings during both active and remission phases of JIA.

JIA patients were stratified into active and remission groups based on standardized disease activity criteria. The levels of selected miRNAs were then compared across these groups and also contrasted with healthy control (HC) samples. This allowed to identify potential biomarker candidates in serum and urine which could reflect disease status or subclinical inflammation.

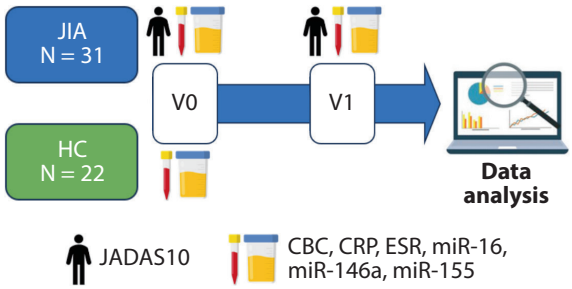


Fig. 2.4.2.1. *Study II structure*

Abbreviations: CBC – complete blood count; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; HC – healthy control; JIA – juvenile idiopathic arthritis; JADAS10 – juvenile arthritis disease activity score of 10 joints; V0 – baseline visit; V1 – 12-month visit from the inclusion into the study.

2.5. Data collection

Demographic data, including age and sex, JIA subtype according to ILAR classification, and disease duration, were collected during inclusion in the study. Information regarding current and prior medications used for JIA treatment was also documented. In addition, the most recent examination regarding uveitis related to JIA was reviewed retrospectively. To ensure accurate patient classification according to ILAR, serological and genetic markers were recorded, including the presence or absence of RF, ANA, and HLA-B27.

2.5.1. Clinical disease activity evaluation

Patients were clinically evaluated every three months by a paediatric rheumatologist (Aušra Šnipaitienė), certified in joint examination by the Paediatric International Trials Organisation (PRINTO). Disease activity parameters included:

1. Active joint count (AJC), defined as a joint with the presence of swelling or, if no swelling was present, of pain on motion, or limited range of motion;
2. Patient global assessment of disease activity (PaGA), recorded using a visual analogue scale of 10 (where 0 = no activity and 10 = maximum activity);
3. Physician global assessment of disease activity (PhGA), also recorded using a visual analogue scale of 10 (where 0 = no activity and 10 = maximum activity).

At each visit, JIA disease activity was calculated using the validated clinical juvenile arthritis disease activity scale of 10 joints (JADAS10). According to the established JADAS10 scale cut-offs [15], patients during the first visit were classified into active disease (ACT, n = 23) and remission (REM, n=8) groups.

Additionally, all clinical signs of inflammation were scored as either 0 (absent) or 1 (present) and quantified as the sum variable (ClinSUM) with a total score range of 0-8. Each variable was weighted equally to minimize the redundant scoring of disease severity [227].

2.5.2. Conventional inflammatory parameters

Routine laboratory inflammatory markers, including complete blood count (CBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), were assessed at each 3-month follow-up visit for JIA patients, in accordance with the disease monitoring protocol.

The HC group underwent single blood tests at inclusion into the study.

2.6. Musculoskeletal ultrasound assessment protocol

MSUS of 40 joints was performed by two independent examiners at each visit:

1. A senior ultrasonographer (Rimantas Uktveris) with over 30 years of expertise in paediatric MSUS, blinded to clinical findings;
2. A paediatric rheumatologist (Aušra Šnipaitienė), who completed the European Alliance of Associations for Rheumatology (EULAR) intermediate course of paediatric MSUS and has six years of experience in daily clinical MSUS practice.

Gray scale (B-mode) and Power Doppler (PD) ultrasonographic evaluations were performed using an Affinity 70G (Philips) and ACUSON Sequoia™ (Siemens Healthineers) systems, equipped with a linear transducers with a frequency range from 5 to 18 Mega Hertz (MHz) for grayscale and up to 12.5 MHz for PD. A pulse repetition frequency of 500–900 MHz with a low-wall filter was used, adjusting the gain to eliminate signals on or below the bone surface.

Both B-mode and PD images were acquired and scored from 0 to 3 for each of the 40 joints, following the guidelines outlined by the Outcome Measures in Rheumatology Clinical Trials (OMERACT) group [29, 228, 229] (Table 2.6.1). Results for each joint were registered in a specific standardized data spreadsheet (Supplement A). Subclinical synovitis was defined as synovitis detected exclusively on MSUS, without corresponding clinical signs.

Table 2.6.1. *Synovitis grading definitions in children in B-mode and colour/power Doppler. Adapted from [229]*

Grade	B mode (GS)	Colour/Power Doppler (PD)
0	**No** synovial effusion or hypertrophy	**Absence** of colour/power PD signal within synovial hypertrophy
1	**Mild** joint recess enlargement/capsular distension due to synovial effusion or hypertrophy	**Up to 3** single PD signals within the area of synovial hypertrophy
2	**Moderate** joint recess enlargement/capsular distension due to synovial effusion or hypertrophy	**More than 3** single PD signals but **less than 30% of the area** of synovial hypertrophy
3	**Severe** joint recess enlargement/capsular distension due to synovial effusion or hypertrophy	PD signals registered in **more than 30% of the area** of synovial hypertrophy

Abbreviations: GS – grey scale; PD – power doppler.

Joint groupings for analysis included:

1) small hand joints (SHJ), comprising MCP (metacarpophalangeal) and PIP (proximal interphalangeal) joints;
2) ankle complex: tibiotalar, talonavicular, subtalar joints;
3) all MTPs (metatarsophalangeal joints);
4) hips, knees, wrists, and elbows: analysed individually.

Inter-rater reliability was calculated using Cohen's kappa.

2.7. Sample collection for miRNA analysis

Blood and urine samples were collected from JIA patients at baseline (visit V0) and 12-month follow-up (visit V1). Patients were categorized into three groups:

1) ACT-REM (n=14): active disease at V0, remission at V1;
2) ACT-ACT (n=7): active disease at V0 and remaining active disease at V1;
3) REM-REM (n=10): remission at V0 and remaining remission at V1.

2.7.1. Blood sample collection for miRNA analysis

Blood samples were collected into 3.5 mL serum separator vacutainer tubes from JIA and HC participants. Samples were centrifuged within 4 hours of blood collection at 3000 ×g for 10 minutes at room temperature. The serum layer was then aliquoted into 1 mL portions and stored at –80 °C until further analysis.

2.7.2. Urine sample collection for miRNA analysis

Morning urine (up to 100 mL) was collected from JIA patients and HC participants into sterile containers. The urine samples were centrifuged within 4 hours of collection at 3000 ×g for 15 min at room temperature. The resulting urine sediments were washed twice with phosphate-buffered saline (PBS), centrifuged under the same conditions twice, resuspended in PBS, aliquoted into 1 mL portions in cryovials, and stored at –80 °C until further use.

2.7.3. Selection of target miRNAs

Target miRNAs were selected by two independent researchers who have done a literature review in January 2020 using the National Library of Medicine database (*https://pubmed.ncbi.nlm.nih.gov/*). Studies exploring the role of miRNAs in JIA were included. However, data on miRNAs in paediatric rheumatology, particularly JIA, are scarce and highly variable. Therefore, manuscripts describing research of miRNAs role in adult rheumatic disease were also reviewed. Three miRNAs – miR-16, -146a, and

-155 – were selected based on their known involvement in inflammatory and autoimmune conditions.

2.7.4. RNA extraction

RNA extraction was performed at the Vilnius University Life Sciences Centre, Institute of Biosciences. Total RNA was extracted from 200 µL of serum and urine sediments using the miRNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. During the cell lysis step, 5 µL of synthetic cel-miR-39 (Qiagen) was spiked into each sample as an internal control for RNA extraction and further reaction efficiency. The purified RNA was eluted in 60 µL RNase-free water, and its concentration and quality were quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). RNA samples were then stored at –80 °C until further analysis.

2.7.5. cDNA synthesis and quantitative reverse transcription PCR (RT-qPCR)

Copy DNA (cDNA) synthesis of miR-16, -146a, -155, and cel-miR-39 was performed using TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific; assay IDs: 000391, 000468, 467534_mat, and 000200, respectively). cDNA reactions were conducted in a total volume of 7.5 µL, comprising 3.5 µL of reverse transcription mix (100 mM dNTPs with dTTP, 50 U/µL MultiScribe™ Reverse Transcriptase, 10× Reverse Transcription Buffer, and 20 U/µL RNase Inhibitor), 1.5 µL of miRNA-specific stem-loop primers, and 2.5 µL of total RNA (Table 2.6.1.5.1). The reaction mixes were incubated under the following thermal conditions: 30 min at 16 °C, 30 min at 42 °C, and 5 min at 85 °C (Table 2.6.1.5.2). The synthesized cDNA was used immediately or stored at –20 °C until use.

Table 2.6.1.5.1. *Amounts of substances used for 1 RT-qPCR*

Substance	Volume per 1 reaction (µL)
Reverse transcription mix:	3.5
• 100 mM dNTPs with dTTP	0.075
• 50 U/µL MultiScribe™ Reverse Transcriptase	0.5
• 10× Reverse Transcription Buffer	0.75
• 20 U/µL RNase Inhibitor	0.095
• RNAse free water	2.08
miRNA-specific stem-loop primers	1.5
Total RNA	2.5
Total amount of the mix	**7.5**

Table 2.6.1.5.2. *Copy DNA synthesis thermal conditions*

Cycle	Temperature (°C)	Time (min)
Primer annealing	16 °C	30
cDNA synthesis	42 °C	30
Enzyme deactivation	85 °C	5
Finishing reaction	4 °C	∞

For miRNA quantification, 1.33 µL of cDNA was further amplified in triplicate using 10 µL final volume RT-qPCR reactions, each consisting of 2× TaqMan Universal PCR Master Mix II, 20× TaqMan™ MicroRNA Assay (both from Applied Biosystems, Thermo Fisher Scientific), and RNase-free water. Reactions were run using ViiA7™ Real-Time PCR System, with data analysis performed using ViiA7 Software v1.2 (Applied Biosystems, Thermo Fisher Scientific).

2.8. Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. Continuous variables were reported as mean ± standard deviation (SD) if the data were normally distributed, or as the median and interquartile range (IQR) if not. Categorical variables were expressed as counts and percentages (%). To compare normally distributed variables, Student's t-test was applied (paired t-test for paired samples), while the Mann-Whitney U test was used for non-normally distributed data. Fisher's exact test was used for the statistical testing of categorical variables.

RT-qPCR run data preprocessing and statistical analysis were performed using GenEx v.6.0.1 (MultiD Analyses AB, Göteborg, Sweden). A relative quantification method was used, where Cq values underwent normalization using the spiked-in cel-miR-39 and global miRNA expression levels. The normalized Cq values were then converted into relative quantities, $\log_2$-transformed, and subsequently used for statistical analysis.

Cohen's kappa value was used for evaluating inter-rater reliability, with subsequent cut-offs: 1) below 0.20 indicating poor reliability, 2) 0.21–0.40 representing fair reliability, 3) 0.41–0.60 showing moderate reliability, 4) 0.61–0.80 indicating good reliability, and 5) 0.81–1 representing excellent reliability.

Associations between clinical variables, MSUS signs of subclinical synovitis, and miRNA levels were evaluated by Spearman's correlation. Logistic regression analysis and odds ratio (OR) with 95% confidence intervals (CI) were used to evaluate multivariate associations.

The effectiveness of biomarkers in differentiating JIA patients from healthy controls (HC) was evaluated by calculating the area under the receiver operating characteristic (ROC) curve (AUC). Combinations of biomarkers for ROC analysis were generated by using logistic regression to enhance diagnostic accuracy. Univariate and multivariate Cox regression analyses were performed to identify clinico-pathological variables and genetic markers related to ACT JIA patient remission. Only characteristics of ACT JIA cases at baseline (V0) were analysed, performing multivariate analysis with variables with a p value ≤ 0.1 in the univariate analysis. The remission prediction was also evaluated by performing Kaplan-Meier analysis.

The statistical testing was performed using GraphPad Prism 10.2.0 (GraphPad Software, Boston, MA, USA) and IBM SPSS Statistics version 29.0 software (SPSS Inc., Chicago, IL, USA) for Windows. A p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1. General characteristics of the participants

The study included a total of 31 patients with Juvenile Idiopathic Arthritis (JIA), with a median age of 13.4 years, ranging from 3 to 17 years old, and 87% of these patients were female. For comparison, 22 healthy controls (HC) matched by gender were also part of the study, consisting of 18 females (82%) and four males (18%), with a median age of 13.1 years, ranging from 4 to 17 years. None of the HC participants showed any signs of inflammation or had been diagnosed with chronic illnesses.

At the initial visit, the median duration of JIA was 9 months, with a range of 5 to 17 months. Nearly 42% of the patients had been experiencing the condition for over a year, whereas only nine patients, accounting for 29%, had been affected for less than half a year (Table 3.1.1).

Table 3.1.1. *Clinical characteristics of JIA patients at baseline visit*

Characteristics	Total (n = 31)
Gender:	
Female, n (%)	27 (87.1)
Male, n (%)	4 (12.9)
ILAR classification:	
Oligoarthritis, n (%)	13 (41.9)
RF negative polyarthritis, n (%)	9 (29.0)
RF positive polyarthritis, n (%)	1 (3.2)
Enthesitis-related arthritis, n (%)	8 (25.8)
ANA positive, n (%)	19 (61.3)
HLA B27 positive, n (%)	10 (32.3)
Disease duration:	
< 6 months, n (%)	9 (29.0)
6–12 months, n (%)	9 (29.0)
> 12 months, n (%)	13 (41.9)

Table 3.1.1. *Continued*

Characteristics	Total (n = 31)
Treatment:	
None, n (%)	3 (9.7)
NSAIDs, n (%)	13 (41.9)
Prednisone, n (%)	1 (3.2)
Intraarticular injections of steroids, n (%)	2 (6.5)
MTX only, n (%)	12 (38.7)
Sulfasalazine, n (%)	2 (6.5)
Anti-TNF only, n (%)	6 (19.4)
MTX + anti-TNF, n (%)	7 (22.6)

Abbreviations: ANA – antinuclear antibodies; anti-TNF – tumour necrosis factor inhibitors; HLA B27 – human leucocyte antigen B27; ILAR – International League of Associations for Rheumatology; MTX – methotrexate; n – number; NSAIDs – non-steroidal anti-inflammatory drugs; RF – rheumatoid factor.

The most common JIA subtype in the study cohort was oligoarticular JIA, observed in 41.9% of patients. This was followed by RF-negative polyarthritis and enthesitis-related JIA, which was observed in 9 and 8 patients, respectively (Fig. 3.1.1). Only one patient in the entire cohort was diagnosed with RF-positive polyarticular JIA. Antinuclear antibodies (ANA) were detected in two-thirds of the patients, while human leucocyte antigen B27 (HLA-B27) was present in one-third of the patients (Table 3.1.1).

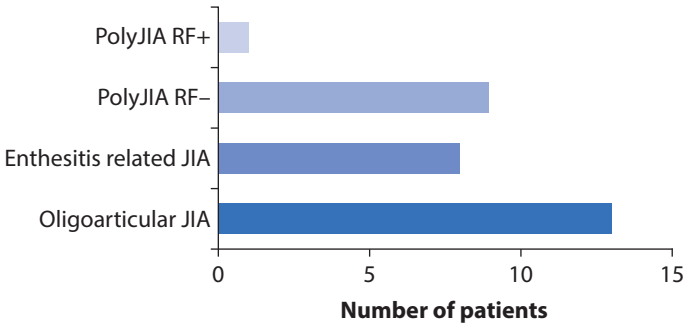


Fig. 3.1.1. *Number of patients with different subtypes of JIA in total cohort*

Abbreviations: JIA – juvenile idiopathic arthritis; PolyJIA – polyarticular JIA; RF+ – rheumatoid factor positive; RF– – rheumatoid factor negative.

Patients received treatment based on established protocols tailored to their specific JIA subtypes. The majority, accounting for 80.6%, were administered methotrexate and/or a tumor necrosis factor (TNF) inhibitor, specifically adalimumab. Furthermore, 61.9% of the patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs) (Table 3.1.1).

At the time of enrolment, 21 patients, accounting for 67.7%, were experiencing active JIA (ACT) according to the Wallace criteria, whereas 10 patients, or 33.3%, were in remission (REM) (Table 3.1.2). Those in the REM group had been diagnosed with JIA at a younger age than ACT patients ($p < 0.05$, Table 3.1.2).

Table 3.1.2. *Comparison of different cohort characteristics in patients based on disease activity at the baseline visit*

Characteristics	ACT (n = 21)	REM (n = 10)	p value
Gender:			
Female, n (%)	19 (90.5)	8 (80)	0.577
Male, n (%)	2 (9.5)	2 (20)	
Age:			
Age at baseline, mean yrs (SD)	13.87 (4.1)	12.47 (4.7)	0.406
Age of JIA diagnosis, mean yrs (SD)	12.91 (4.1)	8.97 (5.0)	**0.025**
Disease duration, median months (IQR)	6 (3–11)	23.5 (15.5–75.8)	**0.002**
Disease duration:			
< 6 months, n (%)	9 (42.9)	0 (0.0)	**< 0.001**
6–12 months, n (%)	9 (42.9)	0 (0.0)	
> 12 months, n (%)	5 (23.8)	8 (80)	
Clinical features:			
Joint swelling, n (%)	16 (76.2)	1 (10)	**< 0.001**
Joint pain, n (%)	21 (100)	1 (10)	**< 0.001**
Limited range of motion, n (%)	8 (38)	0 (0)	**0.042**
Morning stiffness, n (%)	6 (28.6)	0 (0.0)	–
Uveitis, n (%)	0 (0.0)	0 (0.0)	–
ClinSUM, median (IQR)	3 (0–3)	0 (0)	**0.005**
Disease activity scales:			
JADAS10, median (IQR)	11 (7–13)	0 (0)	**< 0.001**
PhGA, median (IQR)	4 (3–5)	0 (0–1)	**< 0.001**
PaGA, median (IQR)	4 (3–6)	0 (0)	**< 0.001**

Table 3.1.2. *Continued*

Characteristics	ACT (n = 21)	REM (n = 10)	p value
Complete blood count:			
WBC × 10^9/L, mean (SD)	6.59 (1.7)	5.74 (1.7)	0.202
Lymph × 10^9/L, median (IQR)	2.3 (1.6–2.7)	1.8 (1.6–3.8)	0.639
Neu × 10^9/L, mean (SD)	3.59 (1.2)	2.65 (0.95)	**0.036**
Mon × 10^9/L, median (IQR)	0.6 (0.4–0.7)	0.5 (0.38–0.6)	0.293
Other laboratory tests:			
CRP mg/L, median (min–max)	5 (5–32)	5 (5–17.3)	0.803
ESR mm/hour, median (min–max)	6.5 (2–23)	7.0 (2–16)	0.968

Abbreviations: ACT – active group of JIA; ANA – antinuclear antibodies; CRP – C reactive protein; DA – disease activity; ESR – erythrocyte sedimentation rate; HLA B27 – human leucocyte antigen B27; IQR – interquartile range; JADAS10 – juvenile arthritis disease activity score 10; Lymph – lymphocytes; Mon – monocytes; n – number; Neu – neutrophiles; PhGA – physician global assessment of DA VAS (0: no disease activity – 10: maximum disease activity); PaGA – patient/parent global assessment of DA VAS (0: very good – 10: very poor); REM – remission group of JIA; RF – rheumatoid factor; SD – standard deviation; VAS – Visual Analogue Scale, WBC – white blood cells, yrs - years. Statistically significant differences between groups are marked in bold ($p < 0.05$ was considered significant).

Regarding the clinical features, joint pain was reported by all ACT patients (100%), followed by joint swelling (76.2%) (Table 3.1.2, Fig. 3.1.2). Morning stiffness was reported by six patients (28.6%), while no cases of uveitis were observed in the cohort. The total sum of the clinical features (ClinSUM) was significantly higher in ACT patients, while patients in REM had no clinical signs of JIA ($p = 0.005$, Table 3.1.2).

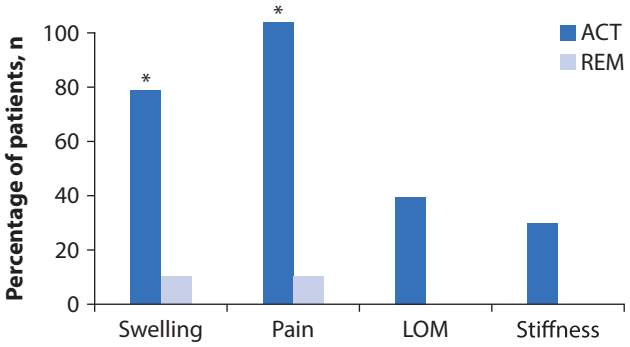


Fig. 3.1.2. *Clinical signs in ACT and REM groups at baseline visit*

Significant differences are marked with * ($p < 0.001$).

In the group of ACT patients, 76.2% were found to have high disease activity based on the JADAS10 criteria, 19.1% showed moderate activity, and just one patient exhibited low disease activity (Table 3.1.2). Negative correlation was seen between disease activity and disease duration: the shortest disease duration was associated with higher disease activity, and all REM patients had a disease duration of more than 1 year ($r_s = –0.775$, $p < 0.001$, Table 3.1.3).

Table 3.1.3. *Disease activity according to disease duration at baseline visit*

Total (n = 31)	**Disease duration**		
Disease activity	**Up to 6 months**	**6 months – 1 year**	**More than 1 year**
High, n (%)	9 (29)	6 (19.4)	1 (3.2)
Moderate, n (%)	0	2 (6.5)	2 (6.5)
Low, n (%)	0	1 (3.2)	0
Inactive/REM, n (%)	0	0	10 (32.3)

Abbreviations: y – years; mo – months; n – number; REM – remission.

There was no statistically significant difference between the physician global assessment (PhGA) and patient/parent assessment (PaGA) of disease activity using the visual analogue scale (VAS) in the ACT group (median value 4 in both, $p = 0.694$, Table 3.1.2). As expected, the JADAS10 value negatively correlated with disease duration ($r_s = –0.727$, $p < 0.001$).

In terms of inflammation markers used in daily clinical practice like ESR and CRP, only four patients (12.9%) showed a slight increase in CRP at their first appointment (Table 3.1.2). There were no notable differences in ESR or CRP levels between the ACT and REM groups (Table 3.1.2). The CBC analysis indicated that neutrophil counts were significantly higher in ACT JIA patients compared to those in the REM group ($p = 0.036$, Table 3.1.2). Nonetheless, all measurements for both groups were within the age-appropriate normal range. No other CBC parameters showed significant differences between the groups (Table 3.1.2).

Throughout the follow-up period, the majority of symptoms subsided, with just 12.9% of patients still experiencing joint pain, and joint swelling reduced to under 10%. Additionally, both limited range of motion (LOM) and morning stiffness had completely disappeared (Table 3.1.4).

Table 3.1.4. *Frequency of patients with pathological clinical findings, description of outcome measures, disease activity and treatment at each visit*

Characteristic	M0 visit (n = 31)	M3 visit (n = 31)	M6 visit (n = 31)	M9 visit (n = 31)	M12 visit (n = 31)
Clinical features:					
Joint swelling, n (%)	17 (54.8)	14 (45.2)	8 (25.8)	6 (19.4)	3 (9.7)
Joint pain, n (%)	21 (67.7)	17 (54.8)	11 (35.5)	7 (22.6)	4 (12.9)
Limited range of motion, n (%)	17 (54.8)	3 (9.7)	0	0	0
Morning stiffness, n (%)	6 (19.4)	7 (22.6)	2 (6.7)	0	0
Uveitis, n (%)	0	0	0	0	0
Disease activity (DA):					
Remission, n (%)	8 (25.8)	10 (32.3)	15 (48.4)	24 (77.4)	24 (77.4)
Low DA, n (%)	3 (9.7)	5 (16.1)	3 (9.7)	0	5 (16.1)
Moderate DA, n (%)	4 (12.9)	7 (22.6)	7 (22.6)	6 (19.4)	1 (3.2)
High DA, n (%)	16 (51.6)	9 (29)	6 (19.4)	1 (3.2)	1 (3.2)
JADAS10, median (IQR)	7 (1–12)	3 (0.5–9)	2 (0–5)	0 (0–1)	0 (0–1)
PhGA, median (IQR)	3 (1–5)	1 (0–4)	1 (0–2)	0 (0–0.5)	0 (0–0.5)
PaGA, median (IQR)	3 (0–5)	1 (0–3)	0 (0–2)	0 (0–1)	0 (0–1)
ClinSUM, median (IQR)	2 (0–3)	1 (0–2)	0 (0–1)	0 (0–1)	0 (0–1)
Treatment:					
None, n (%)	2 (6.7)	1 (3.2)	3 (9.7)	4 (12.9)	4 (12.9)
NSAIDs, n (%)	13 (41.9)	8 (25.8)	3 (9.7)	1 (3.2)	1 (3.2)
Prednisone, n (%)	1 (3.2)	0	0	0	0
Intraarticular injections of steroids, n (%)	2 (6.5)	0	0	0	0
MTX, n (%)	22 (71)	24 (77.4)	24 (77.4)	22 (71)	18 (58)
Sulfasalazine, n (%)	2 (6.5)	2 (6.5)	1 (3.2)	1 (3.2)	1 (3.2)
Anti-TNF, n (%)	11 (35.5)	15 (48.4)	17 (54.8)	16 (51.6)	16 (51.6)
MTX + anti-TNF, n (%)	7 (22.6)	10 (32.3)	13 (42)	12 (38.7)	12 (38.7)

Abbreviations: anti-TNF – tumour necrosis factor inhibitors; DA – disease activity; IQR – interquartile range; JADAS10 – juvenile arthritis disease activity score 10; PhGA – physician global assessment of DA VAS (0: no disease activity – 10: maximum disease activity); PaGA – patient/parent global assessment of DA VAS (0: very good – 10: very poor); MTX – methotrexate; n – number; NSAIDs – non-steroidal anti-inflammatory drugs; VAS – Visual Analogue Scale.

Most children (77.4%) achieved clinical remission on medication during the study time, and only 7 patients displayed signs of active disease (Table 3.1.4, Fig. 3.1.3). The majority of the patients (51.6%) reached remission while using anti-TNF or MTX (58%). Additionally, a notable portion of patients were on a combination therapy of MTX and anti-TNF at their last visit (38.7%).

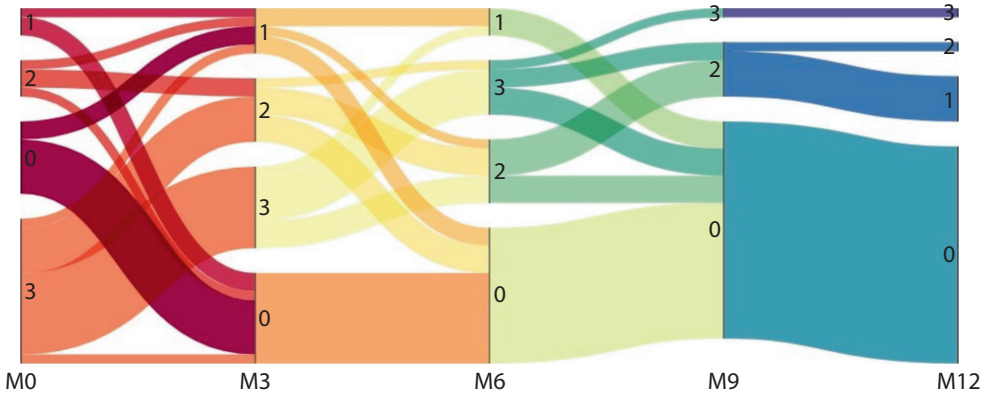


Fig. 3.1.3. *Patient proportion according to disease activity in different study visits*

Numbers in the figure represent the disease activity level according to JADAS10 as follows: 3 – high disease activity, 2 – moderate disease activity, 1 – low disease activity, 0 – remission. Abbreviations: M0 – baseline visit; M3 – 3 months from inclusion into the study; M6 – 6 months from inclusion into the study; M9 – nine-month visit from inclusion into the study; M12 – twelve-month visit from inclusion into the study.

3.2. Study I: Systematic Musculoskeletal Ultrasound (MSUS) assessment

At each visit 42 joints per patient were evaluated for subclinical synovitis in MSUS by two independent researchers. Cogen's kappa was used for inter-rater reliability evaluation. Agreement was highest for elbows, wrists, and hips (kappa = 1.0), and lowest for MTPs (kappa = 0.890) (Table 3.2.1).

Table 3.2.1. *Inter-rater reliability of MSUS evaluation for different joints*

Area	Total number	kappa value
Elbows	31	1
Wrists	31	1
Small hand	31	0.918
Hips	31	1
Knees	31	0.926
Ankles	31	0.912
MTPs	31	0.890

Abbreviations: MTPs – metatarsophalangeal joints.

3.2.1. Links between clinical outcome measures and subclinical synovitis in MSUS

At each visit several clinical outcome measures were evaluated for all patients. A positive correlation was found between the cumulative value of all clinical signs (ClinSUM) and synovitis detected by MSUS at M0, M3, M6 and M9 visits (M0 r_s = 0.65; M3 r_s = 0.38; M6 r_s = 0.47; M9 r_s = 0.42, $p < 0.05$). However, by the M12 visit, when most of the patients had maintained clinical remission for more than six months, this correlation weakened significantly (r_s = 0.28; $p > 0.05$). A similar trend was identified between JADAS10, active joint count (AJC) and MSUS examination findings (Fig. 3.2.1.1).

		MSUS synovitis, months				
		0	3	6	9	12
Clinical features; laboratory markers	ClinSUM	0.65*	0.38*	0.47*	0.42*	0.28
	Joint Pain	0.64*	0.29	0.24	0.28	0.15
	Joint Swelling	0.45*	0.42*	0.47*	0.36	0.22
	JADAS10	0.49*	0.31	0.48*	0.46*	0.28
	PhGA	0.53*	0.33	0.46*	0.35	0.33
	PaGA	0.52*	0.34	0.43*	0.37*	0.35*
	LOM	0.15	0.29	NA	NA	NA
	AJC	0.41*	0.20	0.52*	0.47*	0.31
	ESR	–0.03	–0.26	–0.42	–0.21	–0.21
	CRP	0.21	0.31	–0.18	–0.07	–0.06

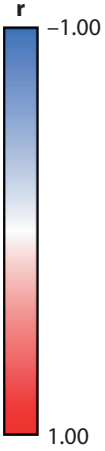


Fig. 3.2.1.1. *The correlation between the number of patients with clinical symptoms and the sum score of synovitis signs in MSUS*

The Spearman correlation coefficients are color-coded according to the scale bar, with red signifying a positive correlation, blue indicating a negative correlation, and white indicating no correlation. The results showing significant correlations are indicated as follows: *$p \leq 0.050$, **$p \leq 0.010$, ***$p \leq 0.001$. Abbreviations: AJC – active joint count; ClinSUM – sum of all clinical signs; JADAS10 – juvenile arthritis disease activity scale 10; ESR – erythrocyte sedimentation rate; CRP – C reactive protein; LOM – limited range of motion; NA – non applicable as there were no LOM registered for any patient; PaGA – patient/parent global disease activity; PhGA – physician's global disease activity; r – Spearman's correlation coefficient.

In the initial three visits, a detailed examination of individual arthritis symptoms, like joint swelling, showed significant positive correlations with MSUS (M0 $r_s = 0.45$; M3 $r_s = 0.42$; M6 $r_s = 0.47$; $p < 0.05$), whereas no correlation was observed during the M12 visit ($p = 0.492$) (Fig. 3.2.1.1). Interestingly, joint pain showed positive correlation only in the first visit (M0 $r_s = 0.64$, $p < 0.05$), and LOM had no correlation with MSUS in any visit (Fig. 3.2.1.1).

Furthermore, no link was observed between MSUS and blood inflammation indicators (ESR and CRP) during any of the follow-up appointments (Fig. 3.2.1.1).

Although there was a consistently strong correlation between PaGA and PhGA during the follow-up period ($r_s > 0.895$; $p < 0.001$), both of these outcome measures showed only weak correlations with MSUS, with values reaching up to $r_s = 0.53$ ($p < 0.05$; Fig. 3.2.1.1). Notably, the association of PaGA with MSUS remained statistically significant at the M12 visit ($r_s = 0.35$, $p < 0.05$), whereas PhGA did not show any correlation starting from the M9 visit ($p = 0.094$).

Furthermore, the relationship between clinical assessments and MSUS for various joints lacked a uniform pattern. For some joints, for example subtalar, hips and elbows, it was impossible to conduct correlation analysis due to the absence of clinically observable signs of arthritis (Table 3.2.1.1).

Table 3.2.1.1. *Correlation between the presence of MSUS synovitis and the presence of clinical arthritis signs for different joints during the study period*

Joints	M0 visit		M3 visit		M6 visit		M9 visit		M12 visit	
	r_s	p value	r_s	p value	r_s	p value	r_s	p value	r_s	p value
Elbows	–0.062	0.745	NA	–	NA	–	NA	–	NA	–
Wrists	0.361	0.050	0.230	0.222	0.447	0.013	NA	–	NA	–
MCPs	0.408	0.025	0.650	< 0.001	0.286	0.125	NA	–	NA	–
PIPs	0.847	< 0.001	–0.043	0.823	0.833	< 0.001	0.557	0.001	NA	–
Hips	0.274	0.142	NA	–	NA	–	NA	–	NA	–
Knees	0.520	0.003	0.451	0.012	0.354	0.055	0.515	0.004	NA	–
Ankles	0.098	0.608	0.280	0.134	0.604	< 0.001	0.079	0.679	0.03	0.721
Subtalar	NA	–	NA	–	NA	–	NA	–	NA	–
MTPs	0.347	0.061	0.088	0.645	0.282	0.130	0.777	< 0.001	NA	–

Abbreviations: MSUS – musculoskeletal ultrasound; MCPs – metacarpophalangeal joints; PIPs – proximal interphalangeal joints; MTPs – metatarsophalangeal joints; M0 – baseline visit; M3 – 3 months from inclusion into the study; M6 – 6 months from inclusion into the study; M9 – nine-month visit from inclusion into the study; M12 – twelve-months visit from inclusion into the study; NA – not applicable as there were no clinical signs detected.

3.2.2. Longitudinal assessment of subclinical synovitis signs in MSUS

At the M0 visit, subclinical synovitis was observed in 4.6% of all joints (Table 3.2.2.1, Fig. 3.2.2.1) and was present in 80.6% of patients (Table 3.2.2.2). Over the course of the follow-up, the overall percentage of joints with subclinical synovitis dropped to 1.8% (Table 3.2.2.1, Fig. 3.2.2.1); nevertheless, a significant portion of patients (51.6%) continued to be affected, with the majority belonging to the REM group (Table 3.2.2.2).

At the M0 visit, subclinical synovitis signs on MSUS were detected predominantly in the knees and ankles, each constituting 16.1% of all joints. This was followed by the subtalar joint at 8.1%, elbows and wrists both at 6.5%, proximal interphalangeal joints (PIP) at 4.2%, MTPs at 3.2%, and MCPs at 1.3% (Table 3.2.2.1, Fig. 3.2.2.2 and 3.2.2.3). At the M12 visit, subclinical synovitis remained mostly in knees (8%), ankles (8%), wrists (6.5%), elbows (3.2%), and subtalar joints (3.2%) (Table 3.2.2.1, Fig. 3.2.2.2 and 3.2.2.3).

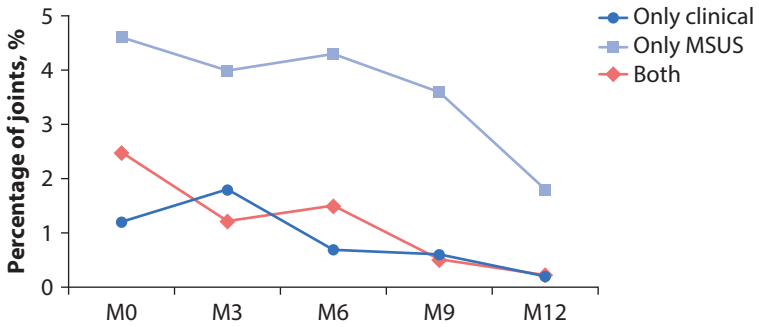


Fig. 3.2.2.1. *Clinical and MSUS inflammation sign changes during the study period*

Table 3.2.2.1. *Clinical and MSUS signs of inflammation in different joints at each visit*

Joints examined in total (n)	M0 visit				M3 visit				M6 visit				M9 visit				M12 visit			
	Joints with arthritis by PhE only (%)	Joints with synnovitis by MSUS only (%)	Joints with inflammation on both PhE and MSUS (%)	Total joints affected (%)	Joints with arthritis by PhE only (%)	Joints with synnovitis by MSUS only (%)	Joints with inflammation on both PhE and MSUS (%)	Total joints affected (%)	Joints with arthritis by PhE only (%)	Joints with synnovitis by MSUS only (%)	Joints with inflammation on both PhE and MSUS (%)	Total joints affected (%)	Joints with arthritis by PhE only (%)	Joints with synnovitis by MSUS only (%)	Joints with inflammation on both PhE and MSUS (%)	Total joints affected (%)	Joints with arthritis by PhE only (%)	Joints with synnovitis by MSUS only (%)	Joints with inflammation on both PhE and MSUS (%)	Total joints affected (%)
Elbows (n = 62)	1 (1.6)	4 (6.5)	0	**5 (8.1)**	0	3 (4.8)	0	**3 (4.8)**	0	6 (9.7)	0	**6 (9.7)**	0	8 (12.9)	0	**8 (12.9)**	0	2 (3.2)	0	2 (3.2)
Wrists (n = 62)	1 (1.6)	4 (6.5)	3 (4.8)	**8 (12.9)**	2 (3.2)	1 (1.6)	1 (1.6)	**4 (6.5)**	1 (1.6)	4 (6.5)	3 (4.8)	**8 (12.9)**	0	5 (8.1)	0	**5 (8.1)**	0	4 (6.5)	0	4 (6.5)
MCPs (n = 310)	0	4 (1.3)	3 (0.97)	**7 (2.3)**	3 (0.97)	3 (0.97)	1 (0.3)	**7 (2.3)**	5 (1.6)	7 (2.3)	1 (0.3)	**13 (4.2)**	0	4 (1.3)	0	**4 (1.3)**	0	2 (0.7)	0	2 (0.7)
PIPs (n = 310)	1 (0.3)	13 (4.2)	11 (3.6)	**25 (8.1)**	9 (2.9)	9 (2.9)	0	**18 (5.8)**	1 (0.3)	10 (3.2)	6 (1.9)	**17 (5.5)**	6 (1.9)	2 (0.7)	1 (0.3)	**9 (2.9)**	2 (0.7)	0	1 (0.3)	3 (0.97)
Hips (n = 62)	3 (4.8)	0	1 (1.7)	**4 (6.5)**	4 (6.5)	0	0	**4 (6.5)**	0	0	0	**0**	0	1 (1.6)	0	**1 (1.7)**	0	0	0	0
Knees (n = 62)	1 (1.7)	10 (16.1)	10 (16.1)	**21 (33.9)**	3 (4.8)	7 (11.3)	8 (12.9)	**18 (29)**	2 (3.2)	8 (12.9)	4 (6.5)	**14 (22.6)**	0	11 (17.7)	4 (6.5)	**15 (24.2)**	0	5 (8)	1 (1.6)	6 (9.7)
Ankles (n = 62)	4 (6.5)	10 (16.1)	2 (3.2)	**16 (26.8)**	1 (1.6)	10 (16.1)	4 (6.5)	**15 (24.2)**	0	7 (11.3)	4 (6.5)	**13 (21.7)**	2 (3.2)	7 (11.3)	1 (1.6)	**10 (16.1)**	1 (1.6)	5 (8)	1 (1.6)	7 (11.3)

Table 3.2.2.1. *Continued*

Joints examined in total (n)	**M0 visit**				**M3 visit**				**M6 visit**				**M9 visit**				**M12 visit**			
	Joints with arthritis by PhE only (%)	**Joints with synnovitis by MSUS only (%)**	**Joints with inflammation on both PhE and MSUS (%)**	**Total joints affected (%)**	**Joints with arthritis by PhE only (%)**	**Joints with synnovitis by MSUS only (%)**	**Joints with inflammation on both PhE and MSUS (%)**	**Total joints affected (%)**	**Joints with arthritis by PhE only (%)**	**Joints with synnovitis by MSUS only (%)**	**Joints with inflammation on both PhE and MSUS (%)**	**Total joints affected (%)**	**Joints with arthritis by PhE only (%)**	**Joints with synnovitis by MSUS only (%)**	**Joints with inflammation on both PhE and MSUS (%)**	**Total joints affected (%)**	**Joints with arthritis by PhE only (%)**	**Joints with synnovitis by MSUS only (%)**	**Joints with inflammation on both PhE and MSUS (%)**	**Total joints affected (%)**
Subtalars (n = 62)	0	5 (8.1)	0	**5 (8.3)**	0	4 (6.5)	0	**4 (6.5)**	0	4 (6.5)	0	**4 (6.5)**	0	3 (4.8)	0	**3 (4.8)**	0	2 (3.2)	0	2 (3.2)
MTPs (n = 310)	3 (0.97)	10 (3.2)	3 (0.97)	**16 (5.2)**	1 (0.3)	15 (4.8)	2 (0.65)	**18 (5.8)**	0	10 (3.2)	1 (0.3)	**11 (3.6)**	0	6 (1.9)	1 (0.3)	**7 (2.3)**	0	4 (1.3)	0	4 (1.3)
Total (n = 1302)	**14 (1.2)**	**60 (4.6)**	**33 (2.5)**	**107 (8.2)**	**23 (1.8)**	**52 (4)**	**16 (1.2)**	**91 (7)**	**9 (0.7)**	**56 (4.3)**	**19 (1.5)**	**86 (6.6)**	**8 (0.6)**	**47 (3.6)**	**7 (0.5)**	**62 (4.8)**	**3 (0.2)**	**24 (1.8)**	**3 (0.2)**	**30 (2.3)**

Abbreviations: MCP – metacarpophalangeal joints; MSUS – musculoskeletal ultrasound; MTPs – metatarsophalangeal joints; M0 – baseline visit; M3 – three months visit; M6 – six months visit; M9 – nine-month visit; M12 – twelve months visit from inclusion into the study; n – number; PhE – physical examination; PIP – proximal interphalangeal joints.

Table 3.2.2.2. *Proportion of patients during all study visits based on disease activity with persisting MSUS synovitis signs*

Disease activity	M0 visit MSUS synovitis, n (%)	M3 visit MSUS synovitis, n (%)	M6 visit MSUS synovitis, n (%)	M9 visit MSUS synovitis, n (%)	M12 visit MSUS synovitis, n (%)
High	13 (41.9)	8 (25.8)	5 (16.1)	1 (3.2)	1 (3.2)
Moderate	4 (12.9)	5 (16.1)	6 (19.4)	2 (6.5)	1 (3.2)
Low	1 (3.2)	4 (12.9)	1 (3.2)	4 (12.9)	2 (6.5)
Inactive/REM	7 (22.6)	7 (22.6)	5 (16.1)	14 (45.2)	12 (38.7)
Total	**25 (80.6)**	**24 (77.4)**	**17 (54.8)**	**21 (67.7)**	**16 (51.6)**

Abbreviations: MSUS – musculoskeletal ultrasound; M0 – baseline visit; M3 – 3 months from inclusion into the study; M6 – 6 months from inclusion into the study; M9 – 9 months from inclusion into the study; M12 – 12 months from inclusion into the study.

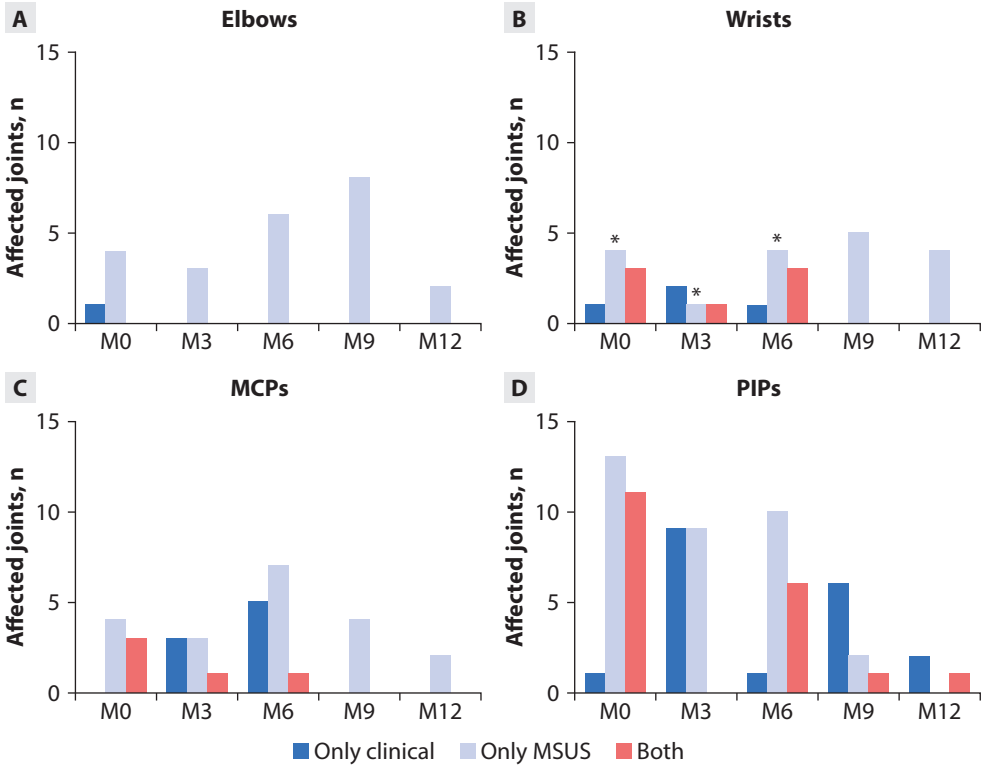


Fig. 3.2.2.2. *The number of joints exhibiting both clinical and MSUS signs of inflammation in various regions of the arm during each study visit*

* marks a statistically significant ($p < 0.05$) difference between groups in each visit.

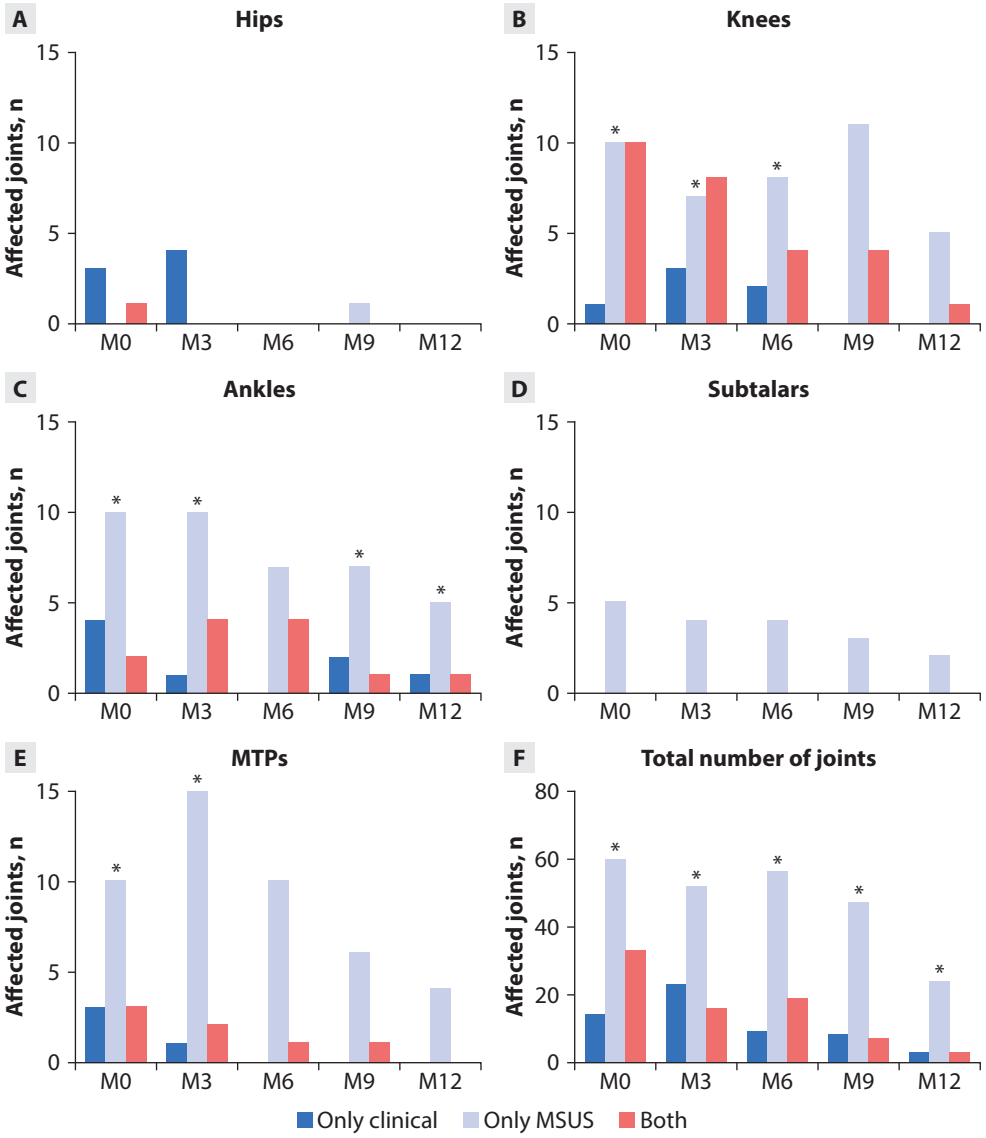


Fig. 3.2.2.3. *The number of joints exhibiting both clinical and MSUS signs of inflammation in various regions of the leg at each study visit (A–E) and total number of affected joints (F)*

* marks a statistically significant ($p<0.05$) difference between groups in each visit.

Throughout the study period, statistically significantly more inflammation signs were seen in MSUS than in clinical examination (Fig. 3.2.2.3 F). In conclusion, despite clinical joint inflammation signs diminishing rapidly during the study period, subclinical synovitis in MSUS was evident in a significant portion of patients even after 12 months of follow-up (Table 3.2.2.2).

3.3. Study II: Analysis of miRNA diagnostic potential in serum and urine

3.3.1. Associations of miRNA levels with JIA

In this study involving 31 patients with JIA, miR-16, -146a, and -155 levels were measured in 29 serum and 31 urine samples collected at the initial visit (V0). Additionally, 21 serum and 22 urine samples were analyzed from 22 healthy controls (HCs). The comparison of miRNA levels between JIA patients and HC showed that JIA patients had significantly lower serum levels of miR-16 and higher levels of miR-155 ($p < 0.001$ and $p = 0.002$, respectively) (Fig. 3.3.1.1 A). In urine samples, JIA patients exhibited a notably lower level of miR-146a ($p = 0.032$, Fig. 3.3.1.1 B). Although miR-146a levels in the serum were also lower in JIA patients compared to HCs, this difference did not reach statistical significance ($p > 0.05$).

Subgroup analysis comparing ACT and REM JIA patients with HC demonstrated significantly lower serum miR-16 levels in both ACT and REM groups compared to HC ($p < 0.001$ and $p = 0.020$, respectively) (Fig. 3.3.1.1 C). At the same time, miR-155 amounts were higher only in the ACT vs. HC ($p = 0.006$) (Fig. 3.3.1.1 C). However, there were no notable differences of serum miRNA levels observed between ACT and REM groups.

Urinary miRNA levels showed distinct patterns between ACT and REM groups. In REM patients, miR-16 levels were significantly higher, while miR-146a levels were markedly lower compared to those in ACT group patients ($p = 0.013$ and $p = 0.007$, respectively) (Fig. 3.3.1.1 D). Additionally, when compared to HC, REM patients presented increased miR-16 levels ($p = 0.031$) and decreased miR-146a levels ($p = 0.002$) (Fig. 3.2.1.1 D). No significant changes in miRNA amounts were observed between ACT JIA patients and HC subjects.

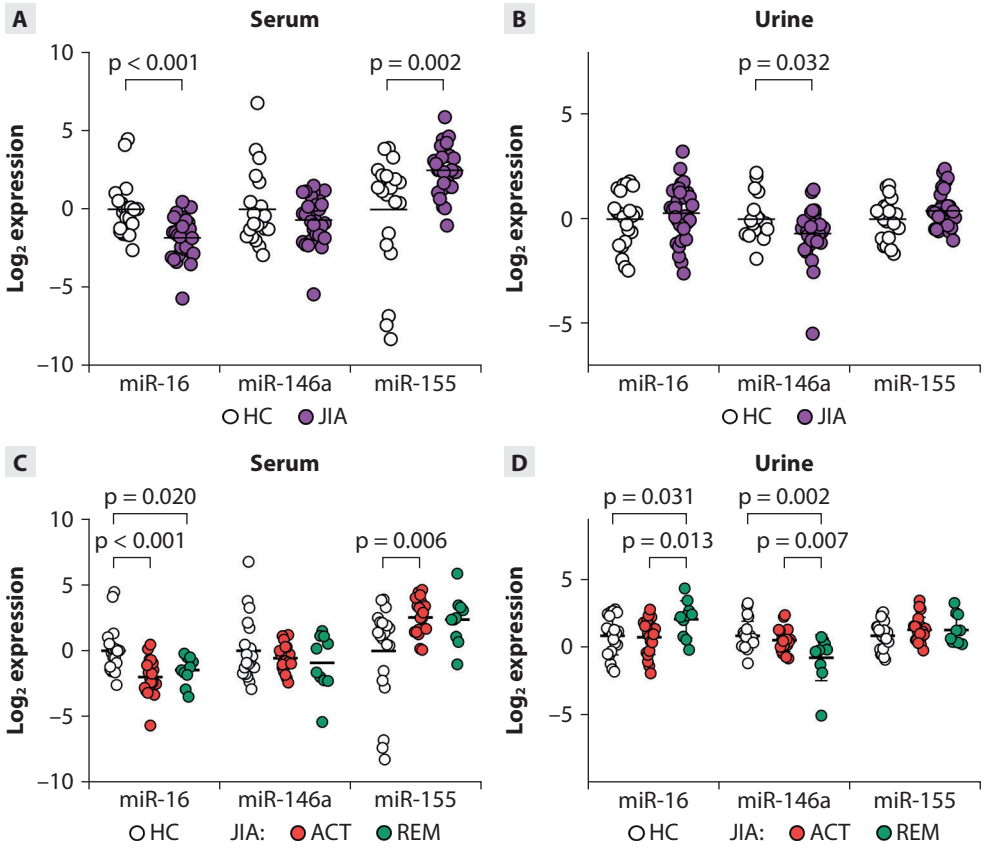


Fig. 3.3.1.1. *Comparison of miRNA levels:* ***(A)*** *serum and* ***(B)*** *urine samples collected from juvenile idiopathic arthritis (JIA) patients and healthy controls (HC);* ***(C)*** *serum and* ***(D)*** *urine miRNA levels according to disease activity at the baseline visit*

The white dots represent the HC group, the purple dots represent the total JIA sample group, the red dots represent the active (ACT) disease group, and the green dots represent the remission (REM) group. Lines depict the average miRNA values.

Additionally, an analysis of miRNA levels across various clinical forms of JIA was conducted. The study found that none of the miRNAs analyzed could differentiate between the clinical courses of oligoarthritis, polyarthritis, or enthesitis-associated JIA in either serum or urine samples ($p > 0.05$). Likewise, no variations in miRNA levels were observed when comparing ANA or HLA-B27 positivity ($p > 0.05$).

3.3.2. Correlation analysis of miRNA levels with JIA clinical variables and MSUS

Correlation analysis between selected serum miRNAs and clinical or laboratory parameters yielded diverse but predominantly weak associations (Fig. 3.3.2.1). Serum miR-16 statistically significantly correlated with white blood cell (WBC) count ($r_s = 0.316$, $p = 0.007$), lymphocyte count ($r_s = 0.369$, $p = 0.001$), PCT ($r_s = –0.281$, $p = 0.016$), and MPV ($r_s = 0.272$, $p = 0.020$). MiR-146a exhibited a significant albeit weak correlation with haemoglobin (Hgb) ($r_s = –0.300$, $p = 0.01$), platelet count ($r_s = 0.241$, $p = 0.04$), platelet biomarkers (PDW $r_s = –0.258$, $p = 0.027$; PCT $r_s = 0.278$, $p = 0.017$), and CRP ($r_s = –0.276$, $p = 0.048$). Furthermore, serum miR-155 had a weak positive correlation with PaGA ($r_s = 0.307$, $p = 0.027$), although no significant associations were found between miR-155 and laboratory markers. In contrast, a moderate negative correlation was detected between miR-155 and miR-146a ($r_s = –0.550$, $p < 0.001$), while a strong negative correlation was noted between miR-155 and miR-16 ($r_s = –0.619$, $p < 0.001$). Finally, none of the serum miRNAs demonstrated significant associations with disease duration.

There were no associations found between miRNA levels and any clinical or laboratory parameters in the urine samples. Nonetheless, strong negative correlations were identified between urine miR-16 and other miRNAs (miR-146a $r_s = –0.711$, $p = 0.001$, and miR-155 $r_s = –0.609$, $p < 0.001$).

From miRNAs examined, only urine miR-146a had a moderately negative correlation with signs of inflammation in MSUS at V1 visit ($r_s = –0.62$; $p < 0.05$). No correlation was identified between other miRNAs, both in serum and urine samples, and MSUS at the baseline of follow-up visits.

Correlation analysis of miRNAs in different bodily fluids revealed a weak positive correlation between miR-155 levels in serum and urine ($r_s = 0.245$, $p = 0.040$), as well as a weak negative correlation between serum miR-16 and urine miR-155 ($r_s = –0.259$, $p = 0.029$) (Fig. 3.3.2.1).

		Serum			Urine		
	miRNA	**-16**	**-146a**	**-155**	**-16**	**-146a**	**-155**
Clinical features	SJC	–0.24	–0.15	0.27	0.00	–0.13	0.07
	TJC	–0.13	–0.08	0.19	0.11	–0.10	–0.02
	JADAS10	–0.16	–0.15	0.22	0.09	–0.13	0.05
	PhGA	–0.18	–0.14	0.24	0.16	–0.16	–0.01
	PaGA	–0.15	–0.26	0.31*	0.00	–0.01	0.03
	Disease dur.	0.12	–0.07	0.06	0.17	–0.26	0.06
Laboratory markers	WBC	0.32**	–0.05	–0.15	0.05	0.14	–0.17
	Neu	0.20	0.05	–0.17	–0.01	0.16	–0.15
	Lymph	0.37**	–0.19	–0.09	0.09	0.08	–0.18
	Mon	–0.11	–0.15	0.18	–0.09	0.16	–0.03
	Hgb	0.06	–0.30**	0.19	0.08	–0.12	0.05
	PLT	–0.19	0.24*	–0.01	0.01	–0.10	0.08
	PDW	0.07	–0.26*	0.14	–0.10	0.07	0.12
	PCT	–0.28*	0.28*	0.06	0.01	–0.06	0.05
	MPV	0.27*	–0.02	–0.12	–0.02	0.17	–0.11
	ESR	–0.08	–0.15	0.15	0.13	–0.02	–0.19
	CRP	–0.01	–0.28*	0.21	0.15	–0.16	–0.05
Serum	miR-16		–0.18	–0.62***	0.14	0.02	–0.26*
	miR-146a			–0.55***	–0.10	0.04	0.04
	miR-155				–0.03	–0.11	0.25*
Urine	miR-16					–0.71***	–0.61***
	miR-146a						–0.02
	miR-155						

Fig. 3.3.2.1. *Correlation of clinical symptoms, inflammation markers, and patient- and physician-reported measures with miRNAs in serum and urine*

The Spearman correlation coefficients are color-coded according to the scale bar, with red signifying a positive correlation, blue indicating a negative correlation, and white indicating no correlation. The results showing significant correlations are indicated as follows: *$p \leq 0.050$, **$p \leq 0.010$, ***$p \leq 0.001$. Abbreviations: SJC – swollen joint count; TJC – tender joint count; JADAS10 – juvenile arthritis disease activity scale of 10 joints; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; Hgb – haemoglobin; Lymph – lymphocytes; Mon – monocytes; Neu – neutrophils; PaGA – patient/parent global disease activity; PCT – plateletcrit; PDW – platelet distribution volume; PhGA – physician's global disease activity; PLT – platelet count; WBC – white blood cells; dur. – duration; r – correlation coefficient.

3.3.3. Diagnostic potential of miRNAs

Serum miR-16 with a 72% sensitivity and 71% specificity (AUC 0.81; 95% CI: 0.70–0.93; $p < 0.001$) showed to be statistically significant to distinguish between JIA patients and HC (Fig. 3.3.3.1 A). Serum miR-155 also demonstrated significant diagnostic ability for identifying JIA, with a sensitivity of 62% and specificity of 71% (AUC 0.73, 95% CI: 0.59–0.87; $p = 0.005$). When serum miR-16 was combined with miR-155, the diagnostic precision improved slightly, with enhanced specificity and a slightly increased AUC (0.82 vs 0.81, respectively; $p = 0.002$) (Fig. 3.3.3.1 A). In urine, miR-146a was the only miRNA capable of differentiating JIA patients from HC, achieving a sensitivity of 65% and a specificity of 64% (AUC 0.68; 95% CI: 0.53–0.82; $p < 0.05$) (Fig. 3.3.3.1 B). The remaining miRNAs that were analysed did not demonstrate statistically significant diagnostic capabilities ($p > 0.05$).

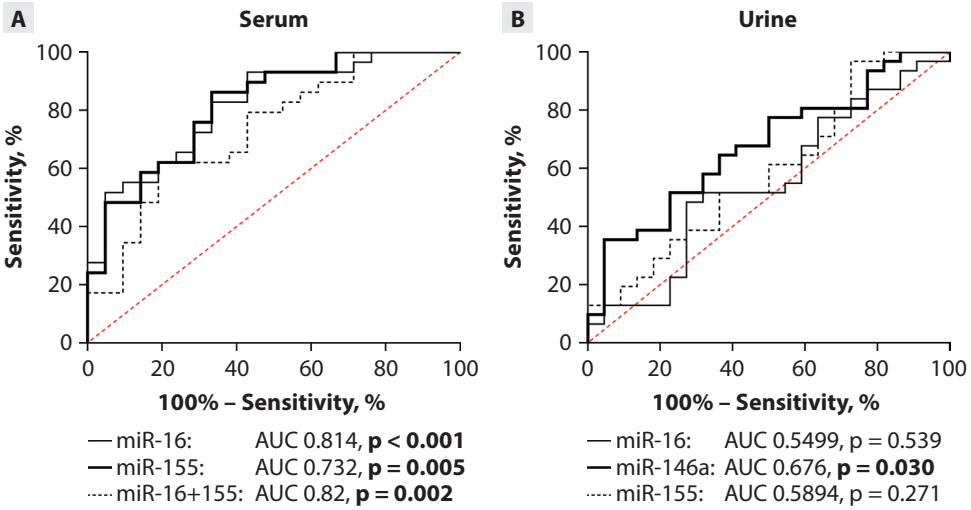


Fig. 3.3.3.1. *Receiver operating characteristic (ROC) curve analysis of circulating miRNAs for distinguishing JIA patients from healthy controls (HC):* ***(A)*** *miR-16, miR-155, and their combination in serum;* ***(B)*** *miR-16, miR-146a, and miR-155 in urine*

AUC – area under the ROC curve.

3.3.4. Changes of miRNA levels during the follow-up period

To assess miRNA alterations during the follow-up period, JIA patients were categorized into three groups, as outlined in the methods section. Paired V0 and V1 samples for longitudinal miRNA level analysis were available from 23 serum and 23 urine samples (74%, 23/31). Patients transitioning from active disease to remission (ACT-REM group) exhibited significantly lower levels of serum miR-16 ($p = 0.021$) and higher levels of miR-155 ($p = 0.009$) during the active phase of the disease (Fig. 3.3.4.1 A). Conversely, no significant differences in serum miRNA levels were detected between V0 and V1 in the samples of patients who remained in the active disease state through all study period (ACT-ACT group) (Fig. 3.3.4.1 B). Importantly, patients in remission at both V0 and V1 (REM-REM group) demonstrated significantly lower serum miR-146a levels at the V0 visit compared to the V1 visit ($p = 0.004$) (Fig. 3.3.4.1 C). No significant changes in the levels of the selected miRNAs were observed within any group in the paired urine sample miRNA analysis.

To evaluate the relationships between demographic and clinico-pathological factors, genetic markers, and the remission status of ACT JIA cases at V0, both univariate and multivariate Cox proportional hazards ratio analyses were conducted. In the univariate analysis, it was observed that lower PhGA and JADAS scores, along with elevated serum miR-146a levels, were inclined to be linked with REM; however, none of these variables achieved statistical significance ($p > 0.05$; Table 3.3.4.1). Multivariate analysis, using variables with $p \leq 0.1$ (highlighted in bold), did not identify any factors that predicted remission (Table 3.3.4.1). Kaplan-Meier survival analysis of the same variables, specifically JADAS10, PhGA, and serum miR-146a, indicates that higher serum miR-146a levels were significantly associated with JIA remission ($p = 0.040$, Fig. 3.3.4.1 D).

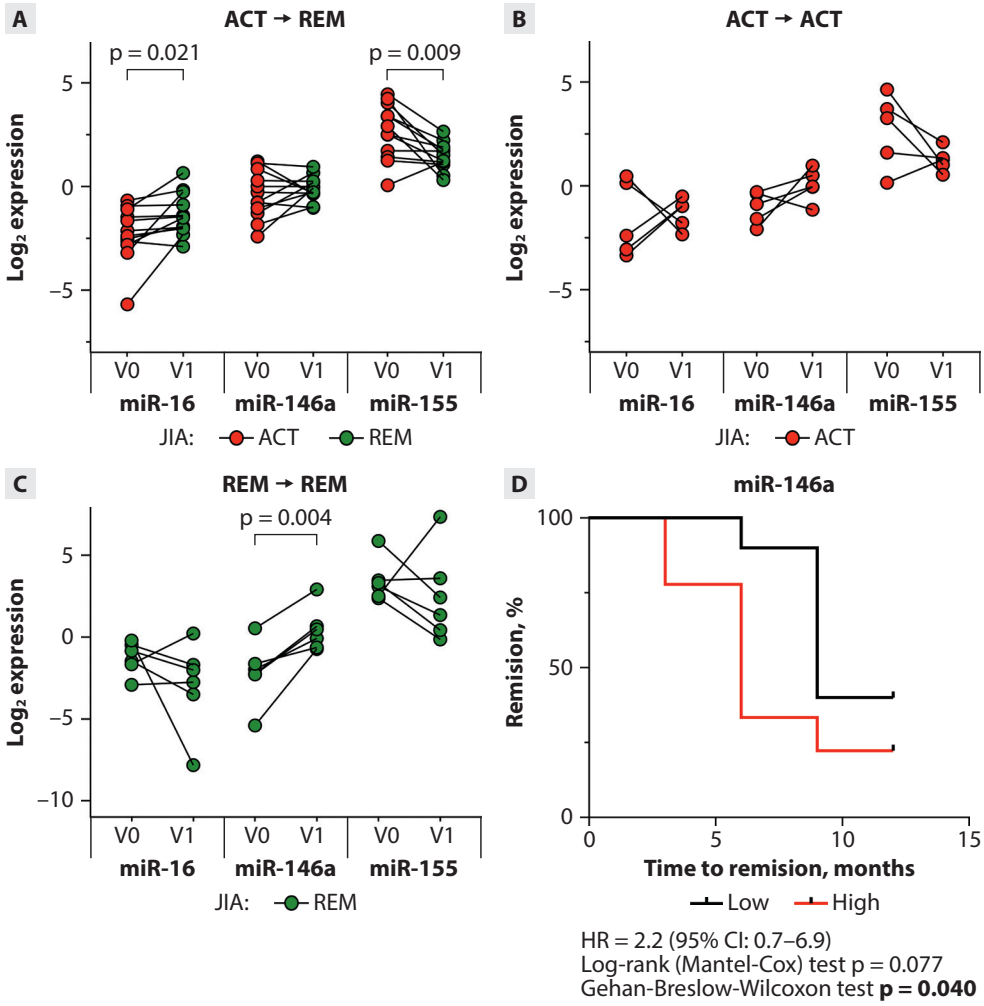


Fig. 3.3.4.1. *Comparison of miRNA levels between paired serum samples of JIA patients' in different groups according to the course of the disease:* ***(A)*** *ACT patients in V0 who reached REM at V1,* ***(B)*** *patients who stayed ACT during the entire study period,* ***(C)*** *patients who were in REM during the entire study period.* ***(D)*** *Kaplan-Meier analysis of remission prediction for JIA patients according to serum miR-146a values*

The red dots represent patients with active (ACT) disease, and the green dots represent patients in remission (REM). HR – hazard ratio.

Table 3.3.4.1. *Univariate and multivariate Cox proportional hazards regression analyses of demographic, clinical-pathological and genetic variables for remission prediction in JIA patients*

Covariate		Univariate		Multivariate	
		HR (95% CI interval)	**p-value**	**HR (95% CI interval)**	**p-value**
Demographic and clinico-pathological	Gender	0.52 (0.07–3.95)	0.527		
	Age at diagnosis	0.98 (0.85–1.13)	0.821		
	Active joint count	0.76 (0.51–1.12)	0.168		
	PaGA	0.80 (0.60–1.07)	0.136		
	PhGA	0.73 (0.52–1.04)	**0.081**	0.95 (0.18–4.85)	0.947
	JADAS10	0.90 (0.79–1.02)	**0.100**	0.94 (0.52–1.67)	0.827
	CRP	1.01 (0.92–1.10)	0.819		
	ESR	1.03 (0.93–1.13)	0.608		
Serum	miR-16	0.79 (0.54–1.16)	0.231		
	miR-146	1.62 (0.93–2.83)	**0.091**	1.45 (0.78–2.70)	0.238
	miR-155	1.0 (0.69–1.45)	0.998		
Urine	miR-16	0.88 (0.56–1.39)	0.578		
	miR-146	0.98 (0.53–1.83)	0.960		
	miR-155	1.51 (0.70–3.26)	0.302		

Abbreviations: PaGA – patient/parent global disease activity; PhGA – physician's global disease activity; JADAS10 – juvenile arthritis disease activity scale of 10 joints; ESR – erythrocyte sedimentation rate; CRP – C reactive protein; HR – hazard ratio. The variables included in the multivariate analysis are in bold.

4. DISCUSSION

Juvenile idiopathic arthritis (JIA), although rare, remains one the most frequent chronic rheumatological conditions affecting children, necessitating regular assessment of disease activity and timely therapy adjustment across all types of JIA [3, 230]. Despite substantial progress, early diagnostics and longitudinal monitoring still pose clinical challenges, and discrepancies across clinical disease activity scales emphasize the need for additional biomarkers to support decision-making in JIA management [11].

In this prospective cohort of JIA patients, we show that (i) subclinical synovitis in musculoskeletal ultrasound (MSUS) persists in a substantial proportion of children even after 12 months of follow-up, (ii) correlations between clinical measures and MSUS are the strongest in early stage and wane as clinical remission is achieved, and (iii) selected circulating and urinary miRNAs – particularly serum miR-16 and miR-155, and urinary miR-146a – exhibit diagnostic or monitoring potential. To our knowledge, this is the first JIA study to demonstrate that urine is a viable biofluid for exploring miRNA biomarkers, thereby broadening non-invasive options for paediatric populations.

4.1. Study I: Systematic Musculoskeletal Ultrasound (MSUS) assessment

Although a range of clinical tools are utilized to assess JIA activity [16], there is growing evidence pointing to the role of ultrasound [23, 43–46]. In our prospective study, which included a 12-month follow-up, we evaluated disease activity in 31 JIA patients using both clinical examination and MSUS. During the first 6 months, several clinical outcome measures were strongly aligned with MSUS findings. However, by the end of the 12 months, most patients still showed signs of inflammation on MSUS, even though they had achieved clinical remission.

Subclinical inflammation signs in MSUS among JIA patients have been recognized for several years [25, 43–45, 149, 231]. However, research has predominantly focused on cases that are either newly diagnosed or in short-term remission [23, 25, 231], often without employing validated MSUS definitions for paediatric population. In our study, we applied the OMERACT group definitions and criteria for MSUS specifically for children [29, 228, 229]. At the baseline visit, only 25% of our participants met the Wallace criteria for clinical remission [47], and were considered to have inactive disease according to JADAS10 cut-offs [16]. As expected, patients with a longer history of the disease were more frequently classified as inactive.

Despite this, the majority still showed signs of synovitis on MSUS. Similar observations have been made in a few prior studies. De Lucia et al.'s reported that 22.7% of clinically inactive patients and 0.98% of scanned joints had abnormal MSUS findings [26]. Loredo et al. noted that 11.8% of JIA patients in remission exhibited subclinical inflammation on MSUS over a year [148]. Bugni Miotto e Silva at al. found a higher prevalence of subclinical synovitis in 36 JIA patients (median duration: 1.9 years), with 41.7% of patients and 3.1% of joints affected [45].

The relationship between clinically active JIA and inflammation signs in ultrasound is well-established. Nonetheless, a significant number of patients with active JIA also exhibit signs of subclinical synovitis on MSUS. According to a recent study by Vega-Fernandez et al., up to 30% of joints of clinically active patients had signs of subclinical inflammation on MSUS [23]. Other studies have reported fewer cases of subclinical synovitis within the active JIA group [147, 148, 231]. The association between ACJ and MSUS signs of inflammation in active or newly diagnosed JIA is both well-recognized and strong [147, 148, 232]. In our patient cohort, half of the individuals demonstrated high disease activity as per JADAS10 criteria at the initial assessment, with a significant number showing subclinical synovitis on MSUS. Furthermore, MSUS revealed inflammation in most patients who were considered to be in JIA remission. Our study also found a robust positive association between ACJ and MSUS at the start and during the early follow-up periods (M3 and M6). However, this association weakened during the remission phase at the final follow-up (M12), suggesting ongoing synovitis signs on MSUS. Similar observations were reported in a recent study by Vega-Fernandez et al., which examined active JIA patients after 3 months of follow-up [23].

Several previous multi-joint scanning studies have documented the distribution of variations in inflammation signs across different joints [45, 147, 149, 231]. Knees, ankles, and wrists are the joints most susceptible to subclinical inflammation signs on MSUS [45, 147, 149, 231]. Our scanned joint complex revealed a similar pattern, with knees and ankles being prominent throughout the entire follow-up period. Additionally, the correlation between MSUS and clinical signs in the knee joint was significantly moderate at the baseline visit and three months later ($r_s = 0.52$, $p = 0.003$ and $r_s = 0.45$, $p = 0.012$, respectively), supporting findings from several other studies [147, 233].

Clinical indicators of arthritis are fundamental in diagnosing JIA, categorizing its types, and assessing disease activity. Initial research on MSUS in JIA patients highlighted varying correlations between clinical signs and MSUS. Magni-Manzoni et al. reported a weak correlation between MSUS

and symptoms like tenderness, pain during joint movement, or LOM, with only swelling showing a moderate to strong correlation with MSUS [25]. In our research, clinical signs such as joint pain and swelling demonstrated a moderate to strong correlation with MSUS within the first six months of follow-up. Notably, we observed no correlation between MSUS and LOM at any visit, underscoring the necessity of a comprehensive clinical evaluation of the patient.

At present, multiple clinical outcome tools are employed to assess JIA disease activity, including various adaptations of JADAS (such as cJADAS, JADAS10, JADAS27, etc.) that incorporate different clinical metrics [16]. Several research teams have identified a significant correlation between JADAS and MSUS in patients with active JIA [147]. In our study, we also observed a significant, moderate positive correlation between JADAS10 and inflammatory signs in MSUS during the first 6 months of follow-up. Nevertheless, no correlation was observed at the M9 and M12 visits, when most patients were in clinical remission, mainly due to ongoing signs of synovitis in MSUS. Similarly, a recent study by Licciardi et al. described a significant correlation between JADAS27 and MSUS in the active JIA group, but not in those in remission [147].

Conflicting results are provided in the literature regarding the association between other clinical disease activity metrics, such as the global disease activity assessments by physicians and patients/parents, and MSUS. Bugni Miotto e Silva et al. examined patients with a median remission duration of 1.9 years and found no connection between synovitis detectable by MSUS and clinical disease activity evaluation by PhGA or PaGA [45]. Additionally, a recent study by Nguyen et al. highlights that clinical outcome measures do not consistently change throughout the disease course [106]. Even though there was a higher percentage of inactive JIA patients according to cJADAS and PhGA, there was minimal or no improvement observed in PaGA [106]. Interestingly, in our patient cohort, we found that the association between PaGA and MSUS remained positive after 9 months of follow-up, even though clinical signs of inflammation had subsided; however, no correlation was observed between MSUS and PhGA. These observations suggest that patients' or parents' perceptions of disease activity might be more sensitive to subclinical inflammation. A similar trend of persistently high PaGA scores was observed in a prospective multicenter study involving more than 1000 JIA patients [234]. Researchers identified older age and enthesitis-related JIA as potential risk factors for prolonged elevation of PaGA. Additionally, a longitudinal, population-based study by Rypdal et al. highlighted PaGA as a key factor linked to higher JADAS10 values [113]. Our study suggested that

subclinical synovitis detected through MSUS could be a contributing factor for the sustained elevation of PaGA.

From a clinical standpoint, our findings reinforce the value of MSUS as a complementary tool within treat-to-target strategies for JIA. As subclinical synovitis often persists despite apparent clinical remission, relying solely on clinical scores may risk underestimating inflammatory activity and, consequently, undertreatment or premature tapering of therapy. Evidence from adult RA indicates that residual power Doppler activity predicts disease flare and radiographic progression [235, 236], and similar tendencies are emerging in paediatric cohorts [23, 46]. Our results suggest that integrating MSUS into longitudinal monitoring could help identify children with ongoing subclinical inflammation, guide more cautious medication withdrawal, and support shared decision-making with families when discrepancies arise between PaGA and physician-based assessments. In future clinical pathways, combining MSUS with molecular biomarkers such as miRNAs may further refine disease stratification, enabling more individualized and biologically informed care for children with JIA.

4.2. Study II: analysis of miRNA diagnostic potential in serum and urine

Given their regulatory roles in immune pathways, miRNAs are attractive candidates for minimally invasive biomarkers in chronic paediatric inflammation [32, 34]. In this single-center, prospective study on JIA, we explored the diagnostic and monitoring capabilities of serum and urine miRNAs, specifically miR-16, -146a, and -155. Utilizing RT-qPCR, our findings indicated that circulating miRNAs can accurately differentiate JIA patients from healthy controls, demonstrating high sensitivity and specificity. Furthermore, our longitudinal analysis highlighted a strong association between miRNAs and both disease activity and remission, suggesting their potential as biomarkers for JIA disease course monitoring. Importantly, this study is the first to establish that urine can be utilized as an additional biofluid for miRNA research in JIA.

Examination of serum miRNAs highlighted the notable diagnostic capability of serum miR-16, demonstrating significant sensitivity and specificity. Additionally, our study found reduced serum miR-16 levels in JIA patients, both during active disease (ACT) and remission (REM), compared to healthy study participants. Importantly, JIA patients experiencing clinically active disease had significantly lower serum miR-16 levels than those in remission. A longitudinal analysis of paired serum samples revealed an increase in miR-16 levels over time, suggesting its potential as a novel diagnostic biomarker

for JIA. These observations align with studies in adults with rheumatoid arthritis (RA), which also reported reduced miR-16 levels in the disease's early stages [237]. Similarly, Raggi et al. discovered downregulation of miR-16 in the synovia and plasma of JIA patients [33]. Nevertheless, there are inconsistencies – studies by Ma et al. [238] and McAlpine et al. [239] identified contrasting changes in miR-16 levels in plasma samples from JIA patients compared to HC, while Demir et al. reported no variation in miR-16 levels between JIA patients in ACT and REM states and HC [240]. It is likely that those heterogeneous results may be influenced by the different biofluids analysed, technical platforms, and patient populations. In addition to its role in rheumatology, extensive cancer studies have shown that miR-16 plays a crucial role in influencing cell growth and invasion, encouraging cell death, and inhibiting the progression of the cell cycle [193, 241]. On a mechanistic level, miRNAs affect immune function by impacting T-cell metabolism and activation [181]. Research by Marcais et al. revealed that the absence of miR-16 can initiate T-cell activation by stimulating the nuclear factor-kappa B (NF-κB) pathway [183], which is a key regulator of the inflammatory response [242, 243]. Furthermore, Zhou et al. demonstrated that miR-16 targets a silencing mediator for retinoid and thyroid hormone receptor (SMRT), modulating NF-κB-regulated inflammatory responses in human monocytes [184]. While we did not observe a link between miR-16 and the overall lymphocyte count, more refined phenotyping could clarify its role in T-cell and monocyte subsets in JIA. Considering previously published data, changes in miR-16 levels may reflect a restored balance in T-cell regulation, which may help reduce inflammation and highlight the potential application of miR-16 in clinical settings for tracking the progression of JIA.

In our research, we also focused on miR-155, another miRNA associated with inflammation. We discovered that JIA patients had higher serum levels of miR-155 compared to healthy controls. Moreover, paired serum sample analysis revealed higher miR-155 levels during the active phase, which decreased over time with treatment. This trajectory indicates that miR-155 may function as a marker of inflammatory burden and therapeutic response. These results are consistent with earlier research on JIA, which found elevated miR-155 levels in plasma when compared to HC, with this increase remaining during the disease's inactive phase [240, 244]. Raggi et al. conducted a study on the oligoarthritis form of JIA, revealing higher miR-155 expression in both synovial fluid and plasma compared to HC [33]. Similarly, Nziza et al. showed that miR-155 could distinguish between JIA synovial fluid and joint fluid samples from septic arthritis patients [37]. Collectively, these studies identify miR-155 as a significant pro-inflammatory miRNA that intensifies inflammation. Various mechanisms by which miR-155 influences

inflammation have been identified, including its interactions with molecules like the Treg-specific transcription factor FoxP3 [219], AID-mediated myc IgH [220], and Src homology 2-containing inositol phosphatase-1 (SHIP-1), among others [219, 221–223]. Research by Kurowska-Storalska et al. revealed that suppressing miR-155 in RA synovial CD14+ cells led to a decrease in TNF-α production [214]. Paoletti et al. found that the overexpression of miR-155 interferes with monocyte polarization into M2-like macrophages in adult RA patients' samples, leading to increased cytokine production [212]. Although direct studies in JIA macrophages are lacking, their established role in JIA pathogenesis suggest that miR-155 may contribute to shared pathogenic pathways across autoimmune arthritis [86, 245]. Our study supports the potential involvement of similar RA pathogenetic pathways in JIA, highlighting the diagnostic potential of miR-155 and its relevance in monitoring the disease progression. However, further data are needed, including long-term studies with serial sample collection, to validate these findings.

Among the three molecules studied, miR-146a presented a complex picture. In our researche miR-146a, the most extensively researched miRNA in rheumatic conditions [180], showed an increase in serum levels during the follow-up period in JIA patients who maintained remission throughout the study, indicating possible long-term treatment effectiveness. Moreover, higher initial serum miR-146a levels in ACT JIA patients were indicative of JIA remission. Nonetheless, we did not detect a significant difference in serum miR-146a levels between JIA patients and HC. Similarly, miR-146a levels did not differ between the similar groups in the research done by Kamiya et al. [246]. On the other hand, Ma et al. [238] observed elevated miR-146a levels in plasma, which showed high sensitivity in distinguishing JIA patients from HC. Interestingly, JIA patients from our cohort had reduced miR-146a levels in urine compared to HC, suggesting the involvement of unidentified mechanisms in miRNA biogenesis. Additionally, miR-146a levels were significantly higher in urine of active JIA patients compared to their remission state. This is the first demonstration that miR-146a in urine can distinguish JIA patients from HC with high sensitivity and specificity and can differentiate between active and inactive disease phases, adding a novel, child-friendly dimension to biomarker development. Although there is inconsistent data regarding miR-146a in blood, numerous experimental models propose that miR-146a serves as a negative-feedback regulator through the Toll-like receptor (TLR) within the NF-κB inflammatory pathway [200]. Animal research has indicated that miR-146a can act as a therapeutic agent, effectively preventing joint and bone damage in arthritis models [197, 207]. Moreover, Mann et al. discovered a cross-regulation between miR-146a and

miR-155 in macrophage inflammatory responses through the NF-κB pathway, underscoring the pivotal role of miR-155 in promoting inflammation [247]. Our study further supports these findings by identifying a negative correlation between miR-155 and both miR-16 and miR-146a. In summary, our findings generated from different bodily fluids suggest that miR-146a may be a valuable biomarker for forecasting the progression of the disease in patients with JIA.

Interestingly, most research on JIA has concentrated on miRNA alterations in blood [33, 214, 238, 240, 246]. To the best of our knowledge, this study is pioneering in its systematic evaluation of urinary miRNAs in chronic joint disease, such as JIA. There is no data published regarding urine miRNA importance in RA patients. The exploration of urine as a source of miRNAs has been extensively pursued in cancer research and is gaining traction in paediatric studies, particularly for kidney-related conditions such as nephrotic syndrome [248] and lupus nephritis [42, 249]. Nevertheless, there is a notable lack of data on urinary miRNA levels in conditions that are not primarily associated with the kidneys [40, 250]. Data remain scarce for non-renal inflammatory diseases, but our results, together with evidence from proteins involved in bone and cartilage degradation [251] or glycosaminoglycans [252] in JIA, suggest that urine may serve as a valuable, non-invasive reservoir of disease-related molecular signals. Research in RA patients' urine has predominantly focused on proteomic studies [253]. Validation in large, multicenter cohorts will be critical to establish urinary miRNAs as reliable clinical biomarkers in JIA.

The findings of this study suggest that circulating and urinary miRNAs could complement existing clinical and imaging tools within treat-to-target strategies for JIA. Serum miR-16 and miR-155 showed promise as diagnostic and monitoring biomarkers, reflecting active inflammation and dynamic treatment response, while urinary miR-146a provided a novel, non-invasive marker that could distinguish between active disease and remission. Because conventional clinical scores and inflammatory markers such as ESR and CRP often fail to capture low-grade or residual activity, miRNA signatures could provide an additional molecular layer of precision in disease evaluation.

In practice, incorporating miRNA profiling may help address key challenges in JIA management, including prediction of flares, individualized adjustment of therapy, and safe tapering of immunosuppressive or biologic medications. For example, persistently elevated miR-155 despite apparent clinical remission might signal ongoing subclinical inflammation and caution against rapid drug withdrawal, whereas rising miR-146a levels could indicate durable control of disease activity. Furthermore, because urine sampling is simple, painless, and repeatable, urinary miRNAs could be particularly useful

in paediatric populations, reducing the need for frequent blood draws and improving adherence to longitudinal monitoring.

Looking ahead, integration of miRNA-based biomarkers with clinical indices (e.g., JADAS), and imaging tools such as MSUS, could pave the way for personalized disease activity scores and risk stratification models. Such composite approaches have the potential to transform JIA management from population-based protocols to individualized, biologically informed care, optimizing outcomes while minimizing treatment burden.

5. LIMITATIONS AND STRENGTHS OF THE STUDY

This study has several limitations that should be acknowledged. First, the sample size was relatively small and derived from a single centre, which may limit the generalizability of the findings across different populations and JIA subtypes. Moreover, the study cohort was also predominantly female, reflecting the epidemiology of JIA, but potentially reducing applicability to male patients. However, considering the uncommon nature of JIA and the 12-month observation period, our patient group is either comparable to or exceeds the size of some earlier single-center study cohorts on MSUS or miRNAs [23, 33, 37, 45, 46, 147, 149, 239, 240, 246, 254]. Another limitation is the inclusion of various JIA clinical subgroups, which reduces the homogeneity of the cohort. Furthermore, employing various scales to measure JIA disease activity, like JADAS [238] or active joint count [239], might lead to inconsistencies. We utilized the validated JADAS10 tool, applying specific cut-offs for oligo and polyarticular JIA, to ensure that all non-systemic JIA types were included. Furthermore, this method offers a comprehensive perspective on the MSUS synovitis signs across various JIA groups in remission.

One more limitation of the imaging part of the study is the lack of independent validation cohort or imaging comparator such as MRI, limiting the observation of long-term disease trajectories. However, the systematic evaluation of 40 joints per patient using patient-friendly imaging modality (MSUS) ensured comprehensive coverage, while the use of standardized OMERACT paediatric definitions strengthened methodological rigor and comparability with international research in the paediatric population done so far [23, 26, 44, 45]. Moreover, the prospective longitudinal design with regular 3-month follow-up visits over 12-month period allowed for detailed monitoring of disease dynamics, in contrast to many previous studies that relied on cross-sectional assessments [23, 44, 45, 148]. Furthermore, the simultaneous real-time evaluation of all children by two ultrasonographers on the same day offers an added benefit of enhancing inter-rater reliability. Our study confirmed that, even with differences in specialties and ultrasound expertise, MSUS is a trustworthy imaging method for assessing inflammation in JIA patients, provided that the appropriate guidelines are followed.

Another major strength lies in the innovative integration of imaging and molecular biomarkers. We chose not to include joint aspiration samples, which would have indicated miRNA alterations at the inflammation site, as this invasive procedure is not necessary for all JIA patients. Furthermore, ethical considerations would have prevented us from comparing these

samples with those from HC. Notably, this is the first study to demonstrate the feasibility of urinary miRNA profiling in JIA, providing a novel, non-invasive, and child-friendly approach to biomarker discovery. As discussed, the variations in miRNA changes observed across most studies could be attributed to factors like the inclusion of different JIA cohorts, such as the focus on polyarticular JIA in Kamiya et al. [246] compared to the predominance of oligoarthritis in our study and Raggi et al. [33]. Moreover, different biofluids used (e.g., plasma samples by Ma et al. [238] vs. extracellular vesicles by Raggi et al. [33] vs. serum in our study and Demir et al. [240]), and methods for miRNA identification may influence the results. However, by examining serum and urine miRNAs in parallel, the study was able to capture differential biomarker dynamics and show that distinct miRNA patterns, particularly miR-16, miR-155, and miR-146a, are associated with disease activity and remission. The combined analysis of clinical outcomes, MSUS findings, and circulating biomarkers provides a multidimensional view of JIA activity that goes beyond conventional clinical or laboratory measures.

Finally, this study contributes to the broader international context of paediatric rheumatology research. It directly addresses unmet needs highlighted by organizations such as EULAR, ACR, and PRINTO, which have emphasized the importance of developing objective biomarkers and advanced imaging tools for improving treat-to-target strategies in JIA. The demonstration that urine can serve as a reliable biofluid for molecular monitoring has global relevance, particularly in paediatric settings where repeated blood draws or advanced imaging may be challenging. By using standardized methodologies and linking biological mechanisms with clinical observations, the study provides a foundation for future multi-centre, international research aimed at refining precision medicine approaches in JIA.

CONCLUSIONS

1. A considerable number of JIA patients continue to display subclinical inflammation on MSUS, even after achieving clinical remission for six months or more, despite the resolution of all other clinical disease outcome measures.
2. Serum concentrations of miR-16 and miR-155 offer a highly specific and sensitive method for distinguishing patients with JIA from those who are healthy.
3. The significant miRNA changes were determined in urine samples demonstrating the effectiveness of urine as a non-invasive biofluid for miRNA analysis in children with JIA. MiR-146a was identified as a promising diagnostic biomarker in urine.
4. Low correlation levels were found between some of the miRNAs and conventional inflammatory markers (complete blood count and C-reactive protein). No correlation was evident with erythrocyte sedimentation rate, and only miR-146a in urine showed negative correlation with MSUS findings.
5. Notable alterations in miRNA levels were observed during the disease course, especially when comparing the clinically active phases to the remission stages of JIA. Additionally, the potential of miR-146a as indicator for predicting remission in patients with JIA was determined.

RECOMMENDATIONS

Currently, the criteria for determining remission or a clinically inactive disease state in JIA do not take imaging results into account [16, 47]. However, the findings of the present study suggest that ultrasound results offer valuable insights into disease activity and should be integrated into routine clinical practice for the evaluation and management of JIA patients. By including the assessment of subclinical inflammation through MSUS as an additional criterion for defining JIA remission, healthcare providers could enhance treatment decision-making and support personalized care for JIA patients throughout the entire course of the disease.

MiRNA can be detected in different bodily fluids of JIA patients, including urine. Some of the miRNA in serum and urine can be used for JIA diagnostics and monitoring. To deepen our comprehension of miRNAs' involvement in JIA, further investigation is necessary. This includes evaluating the long-term prognostic significance of miRNA changes and exploring how treatments affect miRNA behavior in JIA. It is also vital to confirm these results in larger, independent groups. Additionally, examining the differences in miRNA expression between autoimmune and non-autoimmune conditions related to joint damage, such as paediatric orthopedic diseases, is important to pinpoint miRNAs unique to autoimmune processes. Despite growing evidence on the importance of miRNAs in JIA pathogenesis, many questions remain that require further investigation.

Further research efforts should concentrate on developing methods for sample collection that are either minimally invasive or non-invasive, such as utilizing urine samples, to minimize stress for patients during their regular appointments with paediatric rheumatologists. Furthermore, employing non-invasive sampling techniques would facilitate longitudinal research and enhance patient adherence, ultimately leading to a more thorough understanding of miRNA dynamics in JIA. To enhance the consistency of future studies, it is important to work with larger JIA cohorts, standardize the technical procedures for miRNA detection, and explore additional biomarkers linked to miRNA pathways.

SANTRAUKA

SANTRUMPOS

ACT	–	aktyvi liga
AJC	–	aktyvių sąnarių skaičius (angl. *active joint count*)
ANA	–	antinukleariniai antikūnai
Anti-TNF	–	naviko nekrozės faktoriaus blokatoriai
AT-kPGR	–	atvirkštinės transkripcijos kiekybinė polimerazės grandininė reakcija
BKT	–	bendras kraujo tyrimas
bLMARV	–	biologiniai ligą modifikuojantys antireumatiniai vaistai
ClinSUM	–	bendras klinikinių požymių suminis balas
CRB	–	C-reaktyvus baltymas
DNR	–	dezoksiribonukleininė rūgštis
ENG	–	eritrocitų nusėdimo greitis
EULAR	–	Europos reumatologijos asociacijų aljansas (angl. *European Alliance of Associations for Rheumatology*)
GKK	–	gliukokortikoidai
HC	–	kontroliniai asmenys (angl. *healthy controle*)
IL	–	interleukinas
ILAR	–	tarptautinė reumatologijos asociacijų lyga (angl. *the International League of Associations for Rheumatology*)
IQR	–	interkvartilinis diapazonas
JADAS	–	juvenilinio artrito ligos aktyvumo balas (angl. *Juvenile Arthritis Disease Activity Score*)
JIA	–	juvenilis idiopatinis artritas
kDNR	–	kopijinė DNR
Leuk	–	leukocitai
Limf	–	limfocitai
LSMU	–	Lietuvos sveikatos mokslų universitetas
M	–	mėnuo nuo įtraukimo į studiją
mėn.	–	mėnuo
miRNR	–	mikroribobukeininė rūgštis
MKF	–	metakarpofalanginiai sąnariai
Mon	–	monocitai
MTF	–	metatarsofalanginiai sąnariai
MTX	–	metotreksatas
N (arba N)	–	skaičius
Neu	–	neutrofilai
NVNU	–	nesteroidiniai vaistai nuo uždegimo
OMERACT	–	reumatologijos klinikinių tyrimų išeičių rodiklių darbo grupė (angl. *Outcome Measures in Rheumatology Clinical Trials*)
PaGA	–	paciento bendras ligos aktyvumo vertinimas (angl. patient global assessment of disease activity)
PD	–	galios dopleris (angl. *Power Doppler*)
PI	–	pasikliautinasis intervalas (CI, angl. *confidence interval*)
PIF	–	priksimaliniai interfalanginiai sąnariai

PhGA – gydytojo bendras ligos aktyvumo vertinimas (angl. *physician global assessment of disease activity*)
PRINTO – tarptautinė vaikų reumatologinių tyrimų organizacija (angl. *Paediatric International Trials Organisation*)
proc. – procentai
REM – remisija
RF – reumatoidinis faktorius
RNR – ribonukleininė rūgštis
SN – standartinis nuokrypis
SRS – smulkieji rankų sąnariai
ŠS – šansų santykis (OR, angl. *odds ratio*)
TNF – naviko nekrozės faktorius (angl. *tumor necrosis factor*)
UG – ultragarsinis tyrimas
V0 – pirmas vizitas (įtraukimas į tyrimą)
V1 – 12 mėnesių po įtraukimo į tyrimą
ŽLA B27 – žmogaus leukocitų antigenas B27

ĮVADAS

Juvenilinis idiopatinis artritas (toliau – JIA) yra vaikams iki 16 metų amžiaus pasireiškianti lėtinė uždegiminė sąnarių liga, kuriai būdinga įvairi klinikinė išraiška [1, 2]. Naujausiuose tyrimuose atskleidžiami skirtumai tarp įvairių JIA potipių (įskaitant oligoartritą, poliartritą ir su entezitu susijusį artritą) patogenezės, genetinio polinkio ir epidemiologijos [3–6]. Išsamūs tyrimai parodė, kad JIA yra daugiaveiksnė liga, apimanti įvairius patogenetinius kelius, kuriuos siekiama paveikti pritaikant tikslinį gydymą medikamentais, tokiais kaip naviko nekrozės faktoriaus (angl. tumor necrosis factor – TNF) blokatoriai, interleukino (IL)-1 ir IL-6 blokatoriai ir kiti [7]. Taikant biologinę terapiją, pastaraisiais metais JIA išeitys žymiai pagerėjo [8–10]. Tačiau JIA diagnozė vis dar grindžiama klinikiniais kriterijais ir reikšminga pacientų dalis nepasiekia ilgalaikės remisijos [11–13]. Daugeliui sergančiųjų liga paūmėja, kai anksčiau pažeistuose sąnariuose atsinaujina uždegimas arba plinta į kitus sąnarius, o kai kuriems išsivysto sunkios komplikacijos [13, 14], todėl reikalingi tikslesni ligos eigos stebėjimo būdai ir optimalios gydymo strategijos.

Ligos aktyvumo įvertinimas išlieka pagrindinis kriterijus koreguojant JIA skiriamą gydymą. Šiuo metu klinikinėje praktikoje ligos aktyvumui vertinti plačiai naudojami keli skirtingi JIA pacientų populiacijoje išbandyti ir patvirtinti klinikiniai įrankiai [15, 16]. Dažniausiai naudojamas – juvenilinio artrito ligos aktyvumo balas (angl. *Juvenile Arthritis Disease Activity Score* – JADAS) [17]. Tačiau tokie įrankiai turi trūkumų, pavyzdžiui, reikalingos korekcijos, vertinant pacientus, kuriems nustatyti skirtingi JIA potipiai [16, 18–20]. Iššūkių kelia ir jaunas pacientų amžius, lemiantis ribotą paciento bendradarbiavimą (įskaitant gebėjimą vertinti skausmą), taip pat su

amžiumi susijęs sąnarių hipermobilumas bei praeinantys sąnarių sutrikimai vaikams (pvz., trumpalaikis sinovitas). Be to, tyrimai atskleidė, kad skirtingiems gydytojams atliekant tam pačiam pacientui klinikinį artrito vertinimą, vertinimo rezultatų sutapimas yra menkas ar vidutinis, taip pat pabrėžiami įvairūs veiksniai, turintys įtakos gydytojų bendram JIA ligos aktyvumo vertinimui [21, 22]. Daugiacentrė studija, atlikta Shoop-Worrall ir bendraautorių, parodė reikšmingus neatitikimus tarp skirtingų klinikinio ligos aktyvumo vertinimo sistemų, kai jos pritaikomos tiems patiems pacientams [11]. Šie rezultatai pabrėžia tikslesnių, standartizuotų metodų ir biožymenų poreikį vertinant ligos aktyvumą bei siekiant užtikrinti geresnę pacientų gyvenimo kokybę.

Pastaraisiais metais vaikų reumatologijos srityje analizuojami keli skirtingi JIA ligos aktyvumo stebėjimo metodai. Vieni skiria daugiau dėmesio neinvazinėms technikoms, tokioms kaip raumenų ir skeleto sistemos ultragarsinis tyrimas (toliau – UG), kiti tyrinėja naujos kartos biožymenų, įskaitant mikroRNR (toliau – miRNR), potencialą. Sąnarių UG yra neinvazinis, apšvitos neturintis, santykinai nebrangus ir pacientui draugiškas vaizdinis tyrimas, leidžiantis įvertinti pacientų sąnarių būklę realiu laiku [23–26]. Keletas atliktų tyrimų pabrėžia subklinikinio sinovito vertinimo ultragarsu svarbą apibrėžiant JIA potipius ir nustatant uždegimo apimtų sąnarių skaičių [23, 25, 27]. Stengiamasi standartizuoti UG atlikimo ir uždegimo vertinimo skirtinguose sąnariuose protokolus tiek sergantiems JIA, tiek sveikiems vaikams [24, 28–30]. Tačiau skirtingais JIA ligos periodais ilgalaikio stebėjimo duomenų apie UG požymius trūksta.

Kita sparčiai besivystanti vaikų reumatologijos tyrimų šaka yra cirkuliuojančių biožymenų paieškos. Pastaraisiais metais tyrinėjama daugybė molekulių, įskaitant genetinius faktorius, antikūnus ir uždegiminius baltymus [31]. Nesant nustatyto specifinio JIA biožymens, dėmesys atkreiptas į galimus epigenetinio reguliavimo veiksnius. MiRNR – mažos, nekoduojančios ribonukleorūgštys – yra pagrindiniai veiksniai, reguliuojantys po transkripcijos vykstančius procesus ir dalyvaujantys kituose biologiniuose procesuose, tokiuose kaip angiogenezė, ląstelių augimas, diferenciacija, uždegimas ir imuninis atsakas, kurie visi yra ypač svarbūs įvairių lėtinių ligų, įskaitant ir JIA, išsivystymui vaikų amžiuje [32–34]. Šios molekulės laikomos perspektyviais biožymenimis dėl stabilumo įvairiuose kūno skysčiuose, atsparumo RNazių poveikiui, įvairių aplinkos pH toleravimo ir gebėjimo išlikti taikant pasikartojančius užšaldymo-atšildymo ciklus [35, 36]. Daugelyje studijų, tiriančių miRNR JIA pacientų populiacijose, nustatomi jų pokyčiai kraujo plazmoje, serume ar sąnariniame skystyje [37–39]. Didžioji šių tyrimų dalis miRNR koncentracijas nustatė ligos pradžioje arba tada, kai buvo tiriamas

atsakas į gydymą, tačiau duomenų apie ilgalaikius miRNR pokyčius yra mažai.

Jaunesniems vaikams, kuriems reikalingas intensyvesnis būklės stebėjimas ir dažnesnis jos vertinimas, neinvaziniai tyrimų metodai yra priimtinesni nei pakartotiniai kraujo tyrimai. Šlapimo ėminys dėl patogaus surinkimo būdo, didelio mėginio tūrio ir nesudėtingų pakartotinių tyrimų atlikimo gali būti naudojamas klinikiniuose tyrimuose naujų biožymenų paieškai. Nustatyta, kad šlapime aptinkamos miRNR yra patikimi biožymenys keletui ligų, pasireiškiančių skirtingų amžiaus grupių vaikams – nuo kūdikystės iki paauglystės [40–42]. Tačiau iki šiol nebuvo paskelbta studijų apie iš šlapimo išskiriamų miRNR, kaip potencialių JIA biožymenų, reikšmę.

Atlikto tyrimo svarba ir naujumas

Ankstyva JIA diagnostika ir ilgalaikis pacientų monitoravimas išlieka rimtu iššūkiu klinikinėje praktikoje. Skirtingų klinikinių ligos aktyvumo skalių neatitikimai [11] pabrėžia papildomų biožymenų ar vaizdinių tyrimų poreikį gydant pacientus, sergančius JIA. Tam tikros biologinės miRNR savybės ir šių molekulių dalyvavimas įvairiuose biologiniuose procesuose lemia su miRNR siejamus lūkesčius vertinant lėtinį uždegimą [32, 34]. Be to, atsiranda vis daugiau įrodymų, kad ultragarsas gali būti naudojamas kasdienėje klinikinėje praktikoje kaip pacientui palankus tyrimo būdas, suteikiantis vertingos informacijos apie ligos aktyvumą [23, 43–46].

Atliktame tyrime nustatyta, kad nepaisant JIA klinikinės remisijos, trunkančios 6 mėnesius ar daugiau, reikšmingai pacientų daliai sąnarių UG išlieka subklinikinio uždegimo požymiai. Šiuo metu JIA remisijos ar neaktyvios ligos apibrėžime nėra įtrauktų vaizdinių tyrimų rezultatų [16, 47]. Tačiau atsirandant vis daugiau duomenų apie sąnarių UG svarbą, ultragarsinio tyrimo rezultatai turėtų būti vertinami kartu su klinikiniais uždegimo žymenimis, taip sudarydami visapusišką ligos aktyvumo vertinimą. Taip pat ateityje UG duomenys gali būti įtraukti kaip papildomas kriterijus apibūdinti JIA remisiją.

Mūsų žiniomis, tarptautinėje tyrėjų bendruomenėje tai pirmasis tyrimas, analizuojantis ilgalaikius miRNR pokyčius per 12 mėnesių kartu su UG stebimais uždegimo požymiais. Tyrimo rezultatai atskleidė serumo miRNR diagnostinį potencialą sergant JIA, be to, šis tyrimas yra pirmasis, kuriame šlapimas naudojamas kaip alternatyvus organizmo skystis miRNR paieškai JIA pacientų populiacijoje. Tyrimas pabrėžia miRNR svarbą JIA patogenezėje ir galimą šių molekulių panaudojimą ilgalaikiam pacientų stebėjimui.

1. TYRIMO TIKSLAS IR UŽDAVINIAI

Tyrimo tikslas

Įvertinti per 12 mėnesių sąnarių ultragarsiniame tyrime išliekančius subklinikinius sinovito požymius JIA segantiems vaikams ir išanalizuoti serumo bei šlapimo miRNR diagnostinį potencialą, kai yra sergama JIA, bei miRNR pokyčių ryšį su klinikiniais ir UG stebimais sąnarių uždegimo požymiais skirtingais JIA ligos periodais – aktyvios ligos ir remisijos metu.

Tyrimo uždaviniai

1. Išanalizuoti UG požymių sąsajas su klinikiniais ligos aktyvumo požymiais pacientams, sergantiems JIA.
2. Įvertinti serumo miRNR (miR-16, miR-146a ir miR-155) reikšmę, diagnozuojant JIA, ir pokyčius skirtingais ligos aktyvumo periodais.
3. Ištirti miRNR kiekius bei jų pokyčius JIA sergančiųjų šlapimo mėginiuose skirtingais ligos aktyvumo momentais, ir įvertinti jų, kaip neinvazinių ligos biožymenų, galimybes.
4. Išanalizuoti miRNR kiekio koreliaciją su tradiciniais uždegimo žymenimis (bendro kraujo tyrimo rodikliais, C-reaktyviu baltymu, eritrocitų nusėdimo greičiu) ir UG uždegimo požymiais skirtingais ligos aktyvumo periodais.
5. Išanalizuoti miRNR pokyčius per 12 mėnesių, įvertinant jų koncentracijos kitimą pagal ligos eigą.

2. DARBO METODIKA

Perspektyvusis tyrimas buvo vykdomas nuo 2021 m. sausio iki 2023 m. kovo mėnesio Lietuvos sveikatos mokslų universiteto ligoninės Kauno klinikų Vaikų ligų klinikoje, įtraukiant visus pacientus, kuriems diagnozuotas JIA (išskyrus sisteminį JIA), ir kurie buvo toliau stebimi Reumatologijos klinikoje, jei pacientui tyrimo metu sukako 18 metų.

2.1. Pacientų grupės

JIA pacientai

Į tyrimą įtrauktas 31 pacientas, kuriam diagnozuotas JIA. Pacientai buvo toliau stebimi kas 3 mėnesius vienerius metus laiko. Kiekvieno vizito metu buvo atliekamas klinikinis ištyrimas, laboratoriniai tyrimai ir sąnarių UG pagal patvirtintą protokolą.

Pacientų įtraukimo kriterijai:

1. Amžius pirmo vizito metu nuo 2 iki 18 metų.
2. Pacientai, kuriems diagnozuotas JIA pagal Tarptautinės reumatologijos asociacijų lygos (angl. *the International League of Associations for Rheumatology* – ILAR) klasifikacijos kriterijus [49] ir skiriamas gydymas nesteroidiniais vaistais nuo uždegimo (NVNU) ir / arba metotreksatu (MTX), ir / arba gliukokortikoidais (GKK), ir / arba biologiniais ligą modifikuojančiais antireumatiniais vaistais (bLMARV).

Atmetimo kriterijai:

1. Sisteminė JIA forma.
2. Jei pacientui diagnozuota bet kokia kita lėtinė liga, įskaitant autoimunines, alergines ir kitas.

Kontrolinė grupė

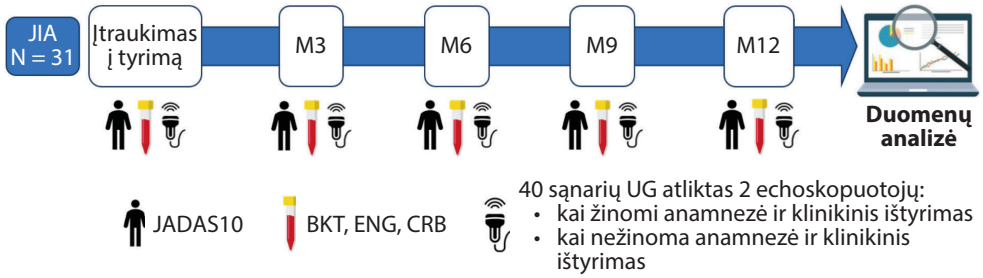
Į šį tyrimą buvo įtraukti 22 sveiki vaikai, atitinkantys tiriamųjų grupę pagal amžių bei lytį ir neturintys jokių uždegimo, infekcijos ar lėtinių ligų požymių. Šioje grupėje buvo surinktas 21 kraujo mėginys ir 22 šlapimo mėginiai, skirti išanalizuoti miRNR ir palyginti su JIA sergančiųjų mėginiais.

2.2. Tyrimo planavimas

Visas tyrimas buvo padalintas į du atskirus tyrimus.

Tyrimas Nr. 1: Sisteminis sąnarių vertinimas ultragarsu

Šio tyrimo metu kas 3 mėnesius vaikams, sergantiems JIA, perspektyviai buvo vertinamas klinikinis JIA ligos aktyvumas ir subklinikiniai uždegimo požymiai UG tyrime.

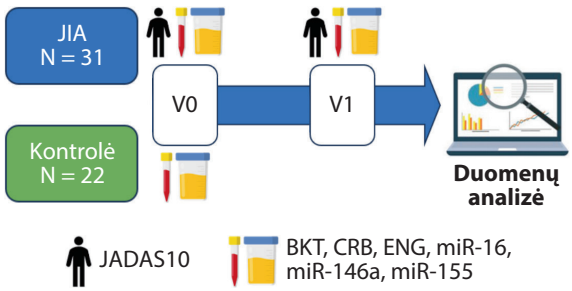


2.2.1 pav. *Tyrimo Nr.1 struktūra*

Santrumpos: BKT – bendras kraujo tyrimas; CRB – C-reaktyvus baltymas; ENG – eritrocitų nusėdimo greitis; JIA – juvenilinis idiopatinis artritas; JADAS10 – juvenilinio artrito ligos aktyvumo skalė; M – mėnuo nuo įtraukimo į studiją; UG – sąnarių ultragarsinis tyrimas.

Tyrimas Nr. 2: MiRNR diagnostinių galimybių analizė serumo ir šlapimo mėginiuose

Šio tyrimo metu buvo analizuojami miRNR pokyčiai, atsiradę JIA sergančiųjų serume ir šlapime įtraukimo į studiją metu ir praėjus 12-kai mėnesių. JIA pacientai buvo suskirstyti į aktyvios ligos ir remisijos grupes pagal klinikinį ligos aktyvumą. miRNR kiekiai buvo palyginti su kontrolinės grupės serumo ir šlapimo mėginiuose nustatytomis miRNR koncentracijomis.



2.2.2 pav. *Tyrimo Nr. 2 struktūra*

Santrumpos: BKT – bendras kraujo tyrimas; CRB – C-reaktyvus baltymas; ENG – eritrocitų nusėdimo greitis; JIA – juvenilinis idiopatinis artritas; JADAS10 – juvenilinio artrito ligos aktyvumo skalė; V0 – pirmas vizitas (įtraukimas į tyrimą); V1 – 12 mėnesių po įtraukimo į tyrimą.

2.3. Duomenų rinkimas

Pirminio vizito metu pas gydytoją vaikų reumatologą buvo surinkti demografiniai paciento duomenys, įskaitant amžių ir lytį, taip pat surinkta informacija apie JIA tipą (pagal ILAR klasifikaciją), ligos trukmę ir JIA gydymui vartojamus vaistus. Be to, buvo retrospektyviai peržiūrėti paciento medicininėje dokumentacijoje pateikti paskutinės oftalmologo konsultacijos įrašai dėl galimo uveito, susijusio su JIA, požymių. Tiksliam pacientų priskyrimui ILAR klasifikacijos grupei buvo surinkti duomenys apie teigiamą ar neigiamą reumatoidinio faktoriaus (toliau – RF) tyrimo rezultatą, antinuklearinių antikūnų (toliau – ANA) ir žmogaus leukocitų antigeno B27 (toliau – ŽLA B27) tyrimų rezultatus.

2.3.1. Klinikinio ligos aktyvumo vertinimas

Kas 3 mėnesius pacientų klinikinė būklė buvo vertinama vaikų reumatologo (Aušros Šnipaitienės), turinčio standartizuoto sąnarių vertinimo sertifikatą, išduotą Tarptautinės vaikų reumatologinių tyrimų organizacijos (angl. *Paediatric International Trials Organisation* – PRINTO). Kiekvieno vizito metu vertinti ligos aktyvumo požymiai: 1) aktyvių sąnarių skaičius (angl.

active joint count – AJC), kuris apibrėžiamas kaip sąnarių skaičius su išliekančiu patinimu arba, jei patinimo nėra, skausmu judesio metu, arba ribota judesio amplitude, 2) paciento bendras ligos aktyvumo vertinimas (angl. *patient global assessment of disease activity* – PaGA) naudojant 10 taškų vizualinę skalę (kur 0 = liga neaktyvi, o 10 = maksimalus ligos aktyvumas), ir 3) gydytojo bendras ligos aktyvumo vertinimas (angl. *physician global assessment of disease activity* – PhGA), naudojant 10 taškų vizualinę skalę (kur 0 = liga neaktyvi, o 10 = maksimalus ligos aktyvumas).

JIA ligos aktyvumas buvo vertinamas kiekvieno vizito metu, naudojant patvirtintą klinikinio juvenilinio artrito ligos aktyvumo skalę 10-čiai sąnarių (angl. *clinical juvenile arthritis disease activity scale of 10 joints* – JADAS10). Pagal JADAS10 skalės ribas [15] pirminio vizito metu pacientai buvo suskirstyti į dvi grupes: aktyvi liga (ACT, n = 23) ir remisija (REM, n = 8).

Visiems klinikiniams uždegimo požymiams buvo skiriami balai: 0, kai klinikinis požymis nebuvo stebimas, arba 1, kai klinikinis požymis buvo stebimas. Šie balai buvo naudojami suskaičiuoti bendrą klinikinių požymių suminį balą (toliau – ClinSUM) (bendros sumos ribos 0–8), kiekvienam pacientui sukuriant papildomą visapusišką kintamąjį tolesnei analizei. Norint sumažinti perteklinį simptomo sunkumo įvertinimą [227], kiekvienas kintamasis buvo vertinamas tokiu pat svoriu (yra arba nėra).

2.3.2. Įprastiniai uždegimo biožymenys

Kievieno vizito metu kas 3 mėnesius JIA pacientams pagal ligos stebėjimo protokolą buvo atliekami rutininiai laboratoriniai tyrimai, skirti uždegimui vertinti, įskaitant bendrą kraujo tyrimą (toliau – BKT), eritrocitų nusėdimo greitį (toliau – ENG) ir C reaktyvų baltymą (toliau – CRB).

Kontrolinės grupės tiriamiesiems kraujo tyrimai buvo atliekami vieną kartą, įtraukimo į mokslinį tyrimą metu.

2.3.3. Sąnarių ultragarsinio tyrimo atlikimo protokolas

Kiekvieno vizito metu JIA sergantiems pacientams buvo atliekamas 40-ties sąnarių UG tyrimas. Tyrimą atliko du echoskopuotojai: echoskopuotojas (Rimantas Uktveria), turintis daugiau kaip 30 metų patirtį vaikų sąnarių echoskopijose, kuris nežinojo klinikinio paciento ištyrimo rezultatų, ir gydytoja vaikų reumatologė (Aušros Šnipaitienės), baigusi Europos reumatologijos asociacijų aljanso (angl. *European Alliance of Associations for Rheumatology* – EULAR) organizuojamus vaikų sąnarių UG kursus pažengusiems bei turinti šešerius metus kasdienės UG praktikos su pediatriniais pacientais. UG buvo atliekamas Affinity 70G (Philips) ir ACUSON Sequoia™ (Siemens Healthineers) echoskopais. Tyrimo metu vertinti pilkos

skalės (toliau – B-mode) ir galios Doplerio (toliau – PD) vaizdai, naudojant linijinius daviklius, kurių dažnio diapazonas nuo 5 iki 18 megahercų (toliau – MHz) pilkoje skalėje ir iki 12,5 MHz galios Doplerio režime. Norint pašalinti Doplerio signalus žemiau kaulinio paviršiaus, buvo naudojamas pulsinės bangos dažnis nuo 500 iki 900 MHz su žemu filtru (angl. *low-wall filter*) ir koreguojama skvarba (angl. *gain*). Gauti pilkos skalės ir PD vaizdai buvo vertinami nuo 0 iki 3 kiekvienam iš 40-ties sąnarių pagal Reumatologijos klinikinių tyrimų išeičių rodiklių (angl. *Outcome Measures in Rheumatology Clinical Trials* – OMERACT) grupės sudarytas rekomendacijas [29, 228, 229]. Kiekvieno sąnario vertinimo rezultatai buvo registruojami specialioje formoje (Supplement A). Subklinikinis sinovitas apibrėžtas kaip uždegimo požymiai, kurie buvo stebimi išskirtinai tik sąnarių UG tyrime.

Statistinės analizės metu kai kurie sąnariai buvo suskirstyti grupėmis:

1) smulkieji rankų sąnariai (toliau – SRS), susidedantys iš metakarpo–falanginių (toliau – MKF) ir proksimalinių interfalanginių (toliau – PIF) sąnarių;
2) čiurnos sąnariai (talokruralinis, talonavikulinis ir subtaliarinis sąnariai);
3) visi metatarsofalanginiai (toliau – MTF) sąnariai.

Klubų, kelių, riešų ir alkūnių sąnariai buvo vertinami atskirai.

Rezultatų patikimumas tarp skirtingų vertintojų, kai vertinami pakitimai pilkoje skalėje ir PD, buvo didžiausias vertinant alkūnių, riešų ir klubų sąnarius (kappa = 1), o didžiausi skirtumai rasti vertinant MTF sąnarius (kappa = 0,890).

2.3.4. Mėginių paruošimas miRNR analizei

Kraujo ir šlapimo mėginiai miRNR analizei buvo renkami pirminio vizito (V0) metu ir po 12-kos mėnesių (V1 vizitas). Pacientai buvo suskirstyti į tris grupes pagal ligos eigą stebėjimo periodu: 1) pacientai, kuriems JIA buvo aktyvus V0 vizito metu ir kurie pasiekė remisiją V1 vizite (ACT-REM; n = 14), 2) pacientai, kurių JIA išliko aktyvus per visą stebėjimo laikotarpį (ACT-ACT; n = 7), ir 3) pacientai, kurių JIA buvo remisijoje V0 vizito metu ir išliko neaktyvus per visą stebėjimo laikotarpį (REM-REM; n = 10).

2.3.4.1. Kraujo mėginių paruošimas miRNR analizei

V0 ir V1 vizitų metu JIA sergančių pacientų kraujo mėginiai buvo renkami į 3,5 ml serumą atskiriančius vakuuminius mėgintuvėlius. Taip pat įtraukimo į studiją metu kraujo mėginiai buvo surinkti iš kontrolinės grupės tiriamųjų. Visi serumą atskiriantys vakuuminiai mėgintuvėliai per 4 val. nuo kraujo paėmimo buvo centrifuguojami 3000 × g 10 minučių kambario

temperatūroje. Paskui serumo sluoksnis buvo išpilstytas po 1 ml ir saugomas –80 °C temperatūroje iki tolesnės analizės.

2.3.4.2. Šlapimo mėginių paruošimas miRNR analizei

Rytinio šlapimo mėginiai (iki 100 ml) buvo renkami iš JIA pacientų ir kontrolinės grupės tiriamųjų į sterilius indelius. Šlapimo mėginiai per 4 val. nuo surinkimo buvo centrifuguojami 3000 × g 15 min. kambario temperatūroje. Likusios šlapimo nuosėdos buvo plaunamos du kartus su fosfatiniu buferiniu tirpalu (toliau – PBS), centrifuguojamos tokiomis pat sąlygomis ir pakartotinai ištirpintos PBS. Paskui buvo išpilstytos po 1 ml į šaldymo mėgintuvėlius, kurie buvo saugomi –80 °C temperatūroje iki tolesnės analizės.

2.3.4.3. Tikslinių miRNR pasirinkimas

2020 m. sausio mėnesį Nacionalinės bibliotekos medicinos duomenų bazėje (angl. *National Library of Medicine database*: *https://pubmed.ncbi.nlm.nih.gov/*) buvo atlikta literatūros analizė tikslinėms miRNR pasirinkti. Analizę atliko du tyrėjai. Į analizę įtrauktos studijos, kuriose aprašomas miRNR vaidmuo JIA metu. Dėl ribotų ir gana prieštaringų duomenų apie miRNR reikšmę JIA, buvo įtraukti ir tyrimai, nagrinėjantys miRNR vaidmenį suaugusiųjų reumatinių ligų metu. Remiantis literatūros duomenimis apie miRNR reikšmę uždegiminėms ir reumatologinėms būklėms, tokioms kaip JIA, perspektyvaus, ilgalaikio stebėjimo tyrimo metu pasirinktos analizuoti trys miRNR (miR-16, -146a ir -155).

2.3.4.4. RNR išskyrimas

Vilniaus universiteto Gyvybės mokslų centre, Biologijos mokslų institute buvo atlikti genetiniai tyrimai. Visuminė RNR buvo išskirta iš 200 µl serumo ir šlapimo nuosėdų, naudojant miRNR rinkinius miRNeasy Mini Kit (Qiagen, Valencija, CA, JAV) pagal gamintojo protokolą. Ląstelių lizės metu į kiekvieną mėginį buvo įlašinta 5 µl sintetinio cel-miR-39 (Qiagen), taip užtikrinant vidinę RNR ekstrakcijos ir tolesnių reakcijų efektyvumo kontrolę. Išgryninta RNR buvo išplauta 60 µl RNazės neturinčiame vandenyje, o jos koncentracija ir kokybė buvo įvertintos naudojant „NanoDrop™ 2000“ spektrofotometrą („Thermo Fisher Scientific“, Vilmingtonas, Delaveras, JAV). RNR mėginiai buvo laikomi –80 °C temperatūroje iki tolesnės analizės.

2.3.4.5. Kopijinės DNR sintezė ir kiekybinė atvirkštinė transkripcija, naudojant polimerazės grandininę reakciją (AT-kPGR)

MiR-16, -146a, -155 ir cel-miR-39 kopijinės DNR (toliau – kDNR) sintezė buvo atlikta naudojant TaqMan™ MicroRNA atvirkštinės transkripcijos rinkinį („Applied Biosystems“, „Thermo Fisher Scientific“, rinkinių ID:

000391, 000468, 467534_mat ir 000200, atitinkamai). Kopijinės DNR reakcijos atliktos 7,5 µl bendroje talpoje, kurią sudarė 3,5 µl atvirkštinės transkripcijos mišinio, 1,5 µl miRNR-specifinių pradmenų ir 2,5 µl visos RNR. Reakcijos mišiniai buvo inkubuojami 30 min. 16 °C temperatūroje, 30 min. 42 °C ir 5 min. 85 °C temperatūroje. Susintetinta kDNR buvo panaudota nedelsiant, arba laikoma –20 °C temperatūroje.

MiRNR kiekybiniam nustatymui 1,33 µl kDNR buvo toliau amplifikuota tris kartus AT-kPGR metu, naudojant 10 µl galutinio mėginio tūrio, kurį sudarė 2× TaqMan Universal PCR Master Mix II, 20× TaqMan™ MicroRNA Assay (abu iš „Applied Biosystems“, „Thermo Fisher Scientific“) ir vanduo be RNazių. Reakcijos buvo atliktos naudojant „ViiA7™“ realaus laiko PGR sistemą, o duomenų analizė – „ViiA7“ programinę įrangą v1.2 („Applied Biosystems“, „Thermo Fisher Scientific“).

2.4. Statistinė analizė

Duomenų normalumas buvo įvertintas naudojant Šapiro-Viko (angl. *Shapiro-Wilk*) testą. Tolydieji kintamieji buvo pateikti kaip vidurkis ± standartinis nuokrypis (toliau – SN), jei duomenys buvo normaliai pasiskirstę, arba kaip mediana ir interkvartilinis diapazonas (toliau – IQR), jei ne. Kategoriniai kintamieji buvo išreikšti kiekiais ir procentais (toliau – proc.). Norint palyginti normaliai pasiskirsčiusius kintamuosius, buvo taikomas Stjudento t testas (porinis t testas porinėms imtims), o Mano–Vitnio (angl. *Mann–Whitney*) U testas – duomenims, kurie nebuvo normaliai pasiskirstę. Kategorinių kintamųjų statistiniam patikrinimui buvo naudojamas Fišerio tikslusis testas.

AT-kPGR duomenų apdorojimas ir statistinė analizė atlikta naudojant „GenEx v.6.0.1“ („MultiD Analyses AB“, Geteborgas, Švedija). Buvo naudojamas santykinio kiekybinio įvertinimo metodas, kai Cq vertės buvo normalizuotos naudojant įterptus cel-miR-39 ir visuminius miRNR raiškos lygius. Normalizuotos Cq vertės konvertuotos į santykinius dydžius, o vėliau log2 reikšmės panaudotos statistinei analizei.

Dviejų nepriklausomų ekspertų UG tyrimo rezultatų vertinimo tarpusavio patikimumas buvo įvertintas naudojant Coheno kapa vertę: ribos mažesnės nei 0,20 rodo prastą patikimumą, 0,21–0,40 – patenkinamą, 0,41–0,60 – vidutinį, 0,61–0,80 – gerą, o 0,81–1 – puikų patikimumą.

Klinikinių duomenų, subklinikinio sinovito požymių UG ir miRNR kiekio koreliacijos buvo analizuojamos naudojant Spirmeno (angl. *Spearman*) koreliacijos analizę. Daugiamatėms sąsajoms įvertinti atlikta logistinė regresinė analizė ir šansų santykio (toliau – ŠS) skaičiavimai su 95 proc. pasikliautinaisiais inter–valais (toliau – PI).

Biožymenų diagnostinės galimybės atskirti JIA sergantiems pacientams nuo kontrolinės grupės buvo įvertintos nustatant plotą po charakteristikų kreive (toliau – ROC). Biožymenų deriniai ROC analizei buvo gauti naudojant logistinę regresiją, siekiant padidinti diagnostinį tikslumą. Atlikta vienmatė ir daugiamatė Cox regresinė analizė, siekiant nustatyti klinikinius kintamuosius ir genetinius žymenis, susijusius su ACT JIA pacientų remisija. Pradinio įvertinimo metu (V0) analizuotos tik ACT JIA atvejų charakteristikos, atliekant daugiamatę analizę su kintamaisiais, kurių vienmatėje analizėje $p \leq 0,1$. Remisijos prognozė taip pat buvo įvertinta atliekant Kaplano–Mejerio (angl. *Kaplan–Meier*) analizę.

Statistiniai skaičiavimai atlikti naudojant „GraphPad Prism 10.2.0“ („GraphPad Software“, Bostonas, Masačusetsas, JAV) ir „IBM SPSS Statistics“ 29.0 versijos programinę įrangą („SPSS Inc.“, Čikaga, Ilinojus, JAV), skirtą „Windows“. p reikšmė < 0,05 buvo laikoma statistiškai reikšminga.

3. REZULTATAI

3.1. Dalyvių bendrosios charakteristikos

Iš viso tyrime dalyvavo 31 JIA sergantis pacientas. Tiriamųjų amžiaus mediana buvo 13,4 metų (nuo 3 iki 17 metų), iš kurių 87 proc. buvo moteriškos lyties. Palyginimui, tyrime dalyvavo 22 sveiki kontroliniai asmenys (toliau – HC), lyčių požiūriu atitinkantys JIA pacientus: 18 mergaičių (82 proc.) ir keturis berniukus (18 proc.), kurių amžiaus mediana buvo 13,1 metų (nuo 4 iki 17 metų). Nė vienas iš HC dalyvių neturėjo uždegimo požymių ar diagnozuotos lėtinės patologijos.

Vidutinė ligos trukmė įtraukimo į studiją metu buvo 9 mėnesiai (nuo 5 iki 17 mėn.). Beveik pusei (41,9 proc.) įtrauktų pacientų JIA buvo diagnozuotas daugiau kaip prieš vienerius metus ir tik 9 pacientai (29 proc.) sirgo mažiau nei 6 mėnesius (3.1.1 lentelė).

Oligoartritas buvo dažniausiai nustatytas JIA tipas tyrimo imtyje (41,9 proc.). RF-neigiamas poliartritas ir su entezitu susijęs JIA buvo atitinkamai nustatyti 9 ir 8 pacientams. Šioje imtyje tik vienam pacientui buvo diagnozuotas RF-teigiamas poliartritas. Antinukleariniai antikūnai (toliau – ANA) rasti dviem trečdaliams įtrauktų pacientų, o ŽLA B27 – vienam trečdaliui imties (3.1.1 lentelė).

Pacientai buvo gydomi pagal patvirtintas gydymo taktikas, priklausomai nuo JIA tipo. Dauguma pacientų (80,6 proc.) vartojo metotreksatą ir / arba naviko nekrozės faktoriaus blokatorių (toliau – anti-TNF) – adalimumabą.

Taip pat 61,9 proc. pacientų buvo skiriami nesteroidiniai vaistai nuo uždegimo (toliau – NVNU) (3.1.1 lentelė).

3.1.1 lentelė. *Klinikinės įtrauktų JIA pacientų charakteristikos pirmo vizito metu*

Charakteristika	Iš viso (n = 31)
Lytis:	
Mergaitės, n (proc.)	27 (87,1)
Berniukai, n (proc.)	4 (12,9)
ILAR klasifikacija:	
Oligoartritas, n (proc.)	13 (41,9)
RF-neigiamas poliartritas, n (proc.)	9 (29,0)
RF-teigiamas poliartritas, n (proc.)	1 (3,2)
Su entezitu susijęs artritas, n (proc.)	8 (25,8)
Teigiami ANA, n (proc.)	19 (61,3)
Teigiamas ŽLA B27, n (proc.)	10 (32,3)
Ligos trukmė:	
< 6 mėnesiai, n (proc.)	9 (29,0)
6–12 mėnesių, n (proc.)	9 (29,0)
> 12 mėnesių, n (proc.)	13 (41,9)
Gydymas:	
Jokio, n (proc.)	3 (9,7)
NVNU, n (proc.)	13 (41,9)
Prednizolonas, n (proc.)	1 (3,2)
Intrasąnarinės steroidų injekcijos, n (proc.)	2 (6,5)
Tik MTX, n (proc.)	12 (38,7)
Sulfasalazinas, n (proc.)	2 (6,5)
Tik anti-TNF, n (proc.)	6 (19,4)
MTX + anti-TNF, n (proc.)	7 (22,6)

Santrumpos: ANA – antinukleariniai antikūnai; anti-TNF – naviko nekrozės faktoriaus blokatoriai; ŽLA B27 – žmogaus leukocitų antigenas B27; ILAR – Tarptautinė reumatologijos asociacijų lyga (angl. *International League of Associations for Rheumatology*); MTX – metotreksatas; n – skaičius; NVNU – nesteroidiniai vaistai nuo uždegimo; proc. – procentai; RF – reumatoidinis faktorius.

Įtraukimo į tyrimą metu dvidešimt vienas pacientas (67,7 proc.), remiantis Wallace'o kriterijais, turėjo aktyvų JIA (toliau – ACT), o 10 pacientų (33,3 proc.) buvo remisijoje (toliau – REM) (3.1.2 lentelė). REM grupės pacientams JIA buvo diagnozuotas jaunesniame amžiuje nei ACT pacientams ($p < 0,05$, 3.1.2 lentelė).

3.1.2 lentelė. *Skirtingų pacientų grupių pagal ligos aktyvumą įtraukimo į studiją metu palyginimas*

Charakteristika	ACT (n = 21)	REM (n = 10)	p reikšmė
Lytis:			
Mergaitės, n (proc.)	19 (90,5)	8 (80)	0,577
Berniukai, n (proc.)	2 (9,5)	2 (20)	
Amžius:			
Amžius įtraukimo į studiją metu, vidurkis metais (SN)	13,87 (4,1)	12.47 (4,7)	0,406
Amžius, kai diagnozuotas JIA, vidurkis metais (SN)	12,91 (4,1)	8,97 (5,0)	**0,025**
Ligos trukmė, mediana mėnesiais (IQR)	6 (3–11)	23,5 (15,5–75,8)	**0,002**
Ligos trukmė:			
<6 mėnesiai, n (proc.)	9 (42,9)	0 (0,0)	**< 0,001**
6–12 mėnesių, n (proc.)	9 (42,9)	0 (0,0)	
>12 mėnesių, n (proc.)	5 (23,8)	8 (80)	
Klinikiniai požymiai:			
Sąnarių patinimas, n (proc.)	16 (76,2)	1 (10)	**< 0,001**
Sąnarių skausmas, n (proc.)	21 (100)	1 (10)	**< 0,001**
Riboti judesiai per sąnarius, n (proc.)	8 (38)	0 (0)	**0,042**
Rytinis sustingimas, n (proc.)	6 (28,6)	0 (0,0)	–
Uveitas, n (proc.)	0 (0,0)	0 (0,0)	–
ClinSUM, mediana (IQR)	3 (0–3)	0 (0)	**0,005**
Ligos aktyvumo skalės:			
JADAS10 mediana (IQR)	11 (7–13)	0 (0)	**< 0,001**
PhGA mediana (IQR)	4 (3–5)	0 (0–1)	**< 0,001**
PaGA mediana (IQR)	4 (3–6)	0 (0)	**< 0,001**
Bendras kraujo tyrimas:			
Leuk × 10^9/l vidurkis (SN)	6,59 (1,7)	5,74 (1,7)	0,202
Limf × 10^9/l, mediana (IQR)	2,3 (1,6–2,7)	1,8 (1,6–3,8)	0,639
Neu × 10^9/l, mediana (SN)	3,59 (1,2)	2,65 (0,95)	**0,036**
Mon × 10^9/l, mediana (IQR)	0,6 (0,4–0,7)	0,5 (0,38–0,6)	0,293
Kiti laboratoriniai tyrimai:			
CRB mg/l, mediana (min–max)	5 (5–32)	5 (5–17.3)	0,803
ENG mm/val., mediana (min–max)	6,5 (2–23)	7,0 (2–16)	0,968

Santrumpos: ACT – aktyvaus artrito pacientų grupė; ANA – antinukleariniai antikūnai; CRB – C-reaktyvus baltymas; ENG – eritrocitų nusėdimo greitis; IQR – interkvartilinis diapazonas; JADAS10 – juvenilinio artrito ligos aktyvumo skalė 10; Leuk – leukocitai; Limf – limfocitai; Mon – monocitai; n – skaičius; Neu – neutrofilai; PhGA – gydytojo bendras ligos aktyvumo vertinimas vaizdinėje analoginėje skalėje (0: liga neaktyvi – 10: maksimalus ligos aktyvumas); PaGA – paciento/tėvų bendras ligos aktyvumo vertinimas vaizdinėje analoginėje skalėje (0: labai gerai – 10: labai blogai); REM – remisijos pacientų grupė; SD – standartinis nuokrypis. Statistiškai reikšmingas skirtumas tarp grupių paryškintas tamsiai juodai ($p < 0,05$ laikoma statistiškai reikšminga).

Iš klinikinių požymių sąnarių skausmą išsakė visi ACT grupės pacientai (100 proc.), taip pat didelei daliai stebėtas sąnarių patinimas (76,2 proc.) (3.1.2 lentelė). Rytiniu sąnarių sustingimu skundėsi 6 pacientai (28,6 proc.), o uveito nebuvo rasta nė vienam į tyrimą įtrauktam pacientui. Bendra klinikinių požymių suma (toliau – ClinSUM) buvo reikšmingai didesnė ACT grupės pacientams, o remisijoje esantys pacientai neturėjo jokių klinikinių JIA simptomų ($p = 0,005$, 3.1.2 lentelė).

ACT pacientų grupėje 76,2 proc. ligos aktyvumas pagal JADAS10 skalės kriterijus buvo didelis, 19,1 proc. – vidutinis ir tik vienas pacientas turėjo mažą ligos aktyvumą (3.1.2 lentelė). Stebėta neigiama koreliacija tarp ligos aktyvumo ir ligos trukmės: trumpiausiai sergantys pacientai turėjo didžiausią ligos aktyvumą, o visi REM grupės pacientai sirgo JIA ilgiau kaip vienerius metus ($r_s = –0,775$, $p < 0,001$, 3.1.3 lentelė).

3.1.3 lentelė. *Ligos aktyvumas pagal ligos trukmę įtraukimo į studiją metu*

Iš viso (n = 31)	**Ligos trukmė**		
Ligos aktyvumas	**Iki 6 mėn.**	**6 mėn. – 1 m.**	**Daugiau kaip 1 m.**
Didelis, n (proc.)	9 (29)	6 (19,4)	1 (3,2)
Vidutinis, n (proc.)	0	2 (6,5)	2 (6,5)
Mažas, n (proc.)	0	1 (3,2)	0
Neaktyvi / remisija, n (proc.)	0	0	10 (32,3)

Santrumpos: m. – metai; mėn. – mėnesiai; n – skaičius; proc. – procentai.

Nenustatytas statistiškai reikšmingas skirtumas tarp PhGA ir PaGA, vertinant ligos aktyvumą vaizdinėje analoginėje skalėje ACT grupėje (abiejų mediana 4, $p = 0,694$). Kaip ir tikėtasi, JADAS10 reikšmė neigiamai koreliavo su ligos trukme ($r_s = –0,727$, $p < 0,001$).

Iš įprastinių uždegiminių žymenų, tokių kaip ENG ir CRB, tik CRB buvo nežymiai padidėjęs įtraukimo į tyrimą metu. Šių biožymenų skirtumo tarp ACT ir REM pacientų grupių nenustatyta (3.1.2 lentelė). Analizuojant bendro kraujo tyrimo duomenis, ACT grupėje nustatytas reikšmingai didesnis neutrofilų skaičius, palyginus su REM ($p = 0,036$, 3.1.2 lentelė). Tačiau visi tyrimų rezultatai abiejose grupėse pagal amžių buvo normos ribose ir nė vienas iš bendro kraujo tyrimo parametrų nesiskyrė tarp grupių (3.1.2 lentelė).

Viso pacientų stebėjimo metu dauguma simptomų išnyko, tik sąnarių skausmas liko 12,9 proc. pacientų, o sąnarių patinimas stebėtas mažiau nei 10 proc. pacientų. Riboti judesiai ir rytinis sustingimas per stebėjimo laikotarpį visiškai išnyko.

Dauguma pacientų (77,4 proc.) per tyrimo laikotarpį vartodami vaistus pasiekė remisiją ir tik 7 pacientams išliko aktyvios ligos požymių. 51,6 proc.

pacientų pasiekė remisiją vartodami anti-TNF, o 58 proc. – vartodami MTX. Reikšminga dalis pacientų (38,7 proc.) paskutinio tyrimo vizito metu vartojo abu medikamentus (MTX + anti-TNF).

3.2. Tyrimas Nr. 1: Sisteminis sąnarių vertinimas ultragarsu

Du nepriklausomi tyrėjai kiekvieno vizito metu ultragarsu įvertino po 40 sąnarių kiekvienam pacientui dėl galimų subklinikinio sinovito požymių. Tyrėjų atliktų UG tyrimų tarpusavio patikimumui įvertinti buvo apskaičiuota Koheno kapa (angl. *Cohen's kappa*). Didžiausias sutarimas buvo vertinant alkūnių, riešų ir klubų sąnarius (kappa = 1,0), o mažiausias – metatarsofalanginius sąnarius (kappa = 0,890).

3.2.1. Klinikinių rodiklių ryšys su UG stebimais subklinikinio sinovito požymiais

Kiekvieno vizito metu visiems pacientams buvo įvertinti keli klinikiniai rodikliai. M0, M3, M6 ir M9 vizitų metu teigiama koreliacija nustatyta tarp suminio klinikinių simptomų balo ir UG stebimo sinovito (M0 $r_s = 0,65$; M3 $r_s = 0,38$; M6 $r_s = 0,47$; M9 $r_s = 0,42$, $p < 0,05$). Tačiau ši koreliacija reikšmingai sumažėjo ($r_s = 0,28$; $p > 0,05$) paskutinio vizito (M12) metu, kai daugelis pacientų buvo klinikinėje remisijoje ilgiau nei 6 mėnesius. Panašus polinkis buvo stebimas ir tarp JADAS10, aktyvių sąnarių skaičiaus (toliau – AJC) ir UG tyrimo radinių.

Taip pat analizuotas atskirų artrito simptomų ryšys su UG stebimais pakitimais. Pirmųjų trijų vizitų metu nustatyta teigiama koreliacija tarp sąnarių patinimo ir UG uždegimo požymių (M0 $r_s = 0,45$; M3 $r_s = 0,42$; M6 $r_s = 0,47$; $p < 0,05$), tačiau nerasta ryšio nuo M9 vizito ($p = 0,492$). Tuo tarpu sąnarių skausmas koreliavo su UG radiniais tik pirmo vizito metu (M0 $r_s = 0,64$, $p < 0,05$), o riboti sąnarių judesiai nekoreliavo su UG duomenimis nei vieno vizito metu.

Tiriamuoju laikotarpiu nenustatyta jokio ryšio tarp UG stebimo subklinikinio sinovito ir kraujo uždegiminių rodiklių (ENG ir CRB).

Nepaisant išliekančios stiprios koreliacijos tarp PaGA ir PhGA viso tyrimo metu ($r_s > 0,895$; $p < 0,001$), abu klinikiniai parametrai silpnai koreliavo su UG radiniais (r_s iki 0,53; $p < 0,05$). Įdomu, kad PaGA koreliacija su UG duomenimis išliko statistiškai reikšminga ir M12 vizito metu ($r_s = 0,35$, $p < 0,05$), kai PhGA nebekoreliavo su UG nuo M9 vizito ($p = 0,094$).

Nenustatyta pastovios koreliacijos tarp skirtingų sąnarių klinikinių uždegimo požymių ir UG tyrimo duomenų. Keletui sąnarių koreliacijos neapskaičiuotos, kadangi nebebuvo jokių klinikinių artrito požymių.

3.2.2. Ilgalaikis subklinikinio sinovito požymių UG tyrime vertinimas

Įtraukimo į tyrimą metu daugeliui pacientų (80,6 proc.) subklinikinis sinovitas buvo stebimas 4,6 proc. sąnarių. Stebėjimo metu bendras sąnarių skaičius, kuriuose nustatyti sinovito požymiai, sumažėjo iki 1,8 proc., tačiau pacientų, kuriems UG stebėti sinovito požymiai, skaičius išliko didelis (51,6 proc.), nepaisant to, kad daugelis buvo pasiekę remisiją (3.2.2.1 lentelė).

3.2.2.1 lentelė. *Pacientų, kuriems stebimi išliekantys subklinikiniai sinovito požymiai UG tyrime, skaičius pagal ligos aktyvumą skirtingų vizitų metu*

Ligos aktyvumas	M0 vizito metu stebėtas UG sinovitas, n (proc.)	M3 vizito metu stebėtas UG sinovitas, n (proc.)	M6 vizito metu stebėtas UG sinovitas, n (proc.)	M9 vizito metu stebėtas UG sinovitas, n (proc.)	M12 vizito metu stebėtas UG sinovitas, n (proc.)
Didelis	13 (41,9)	8 (25,8)	5 (16,1)	1 (3,2)	1 (3,2)
Vidutinis	4 (12,9)	5 (16,1)	6 (19,4)	2 (6,5)	1 (3,2)
Mažas	1 (3,2)	4 (12,9)	1 (3,2)	4 (12,9)	2 (6,5)
Neaktyvu/REM	7 (22,6)	7 (22,6)	5 (16,1)	14 (45,2)	12 (38,7)
Iš viso	**25 (80,6)**	**24 (77,4)**	**17 (54,8)**	**21 (67,7)**	**16 (51,6)**

Santrumpos: UG – ultragarsinis tyrimas; M0 – pirmas tyrimo vizitas; M3 – 3 mėnesiai nuo įtraukimo į tyrimą; M6 – 6 mėnesiai nuo įtraukimo į tyrimą; M9 – 9 mėnesiai nuo įtraukimo į tyrimą; M12 – 12 mėnesių nuo įtraukimo į tyrimą; n – skaičius; proc. – procentai; REM – remisija.

M0 vizito metu subklinikinis sinovitas UG dažniausiai buvo stebimas kelių ir čiurnų sąnariuose (16,1 proc. abiems), rečiau subtaliariniuose (8,1 proc.), alkūnių (6,5 proc.), riešų (6,5 proc.), proksimaliniuose interfalanginiuose (toliau – PIF) (4,2 proc.), MTF (3,2 proc.) ir MKF (1,3 proc.) sąnariuose. O M12 vizito metu, subklinikinio sinovito požymiai daugiausia išliko keliuose (8 proc.), čiurnose (8 proc.), riešuose (6,5 proc.), alkūnėse (3,2 proc.) ir subtaliariniuose sąnariuose (3,2 proc.).

Viso tyrimo metu statistiškai reikšmingai daugiau uždegimo požymių sąnariuose buvo stebima UG tyrime nei klinikinio ištyrimo metu. Nors stebėjimo laikotarpiu klinikiniai uždegimo požymiai greitai išnyko, subklinikinis sinovitas UG buvo stebimas reikšmingam pacientų skaičiui net po 12 mėnesių nuo įtraukimo į tyrimą.

3.3. Tyrimas Nr.2: MiRNR diagnostinių galimybių analizė serumo ir šlapimo mėginiuose

3.3.1. miRNR kiekio ryšys su JIA

Įtraukimo į studiją metu (V0) miR-16, -146a ir -155 kiekis analizuotas 29 serumo ir 31 šlapimo mėginiuose, surinktuose iš 31 JIA paciento, ir 21 serumo bei 22 šlapimo mėginiuose, surinktuose iš 22 kontrolinių asmenų. Palyginamojoje miRNR kiekio analizėje tarp JIA pacientų ir kontrolinės grupės nustatyti statistiškai reikšmingai mažesni miR-16 ir didesni miR-155 kiekiai JIA sergančiųjų serume ($p < 0,001$ ir $p = 0,002$, atitinkamai). O šlapime nustatyti reikšmingai mažesni miR-146a kiekiai JIA pacientų grupėje ($p = 0,032$). miR-146a kiekis serume taip pat buvo mažesnis JIA nei sveikų asmenų grupėje, tačiau skirtumas nebuvo statistiškai reikšmingas ($p > 0,05$).

Palyginus ACT ir REM grupių JIA pacientus su kontroline grupe, nustatyti reikšmingai mažesni miR-16 kiekiai serume tiek aktyvios ligos, tiek remisijos grupėse ($p < 0,001$ ir $p = 0,020$, atitinkamai). Tuo metu miR-155 kiekis buvo didesnis tik ACT grupės tiriamųjų serume, palyginus su kontroline grupe ($p = 0,006$). Tačiau serume reikšmingų miRNR skirtumų, lyginant ACT ir REM grupes tarpusavyje, nenustatyta.

Skirtumai tarp aktyvaus artrito ir remisijos grupių rasti tiriant miRNR šlapime. REM grupėje nustatyti reikšmingai didesni miR-16 kiekiai, o miR-146a koncentracija buvo ženkliai mažesnė nei ACT pacientų grupėje ($p = 0,013$ ir $p = 0,007$, atitinkamai). Taip pat, palyginti su sveikais tyrimo dalyviais, REM pacientų miR-16 kiekis buvo didesnis ($p = 0,031$), o miR-146a mažesnis ($p = 0,002$). Reikšmingų miRNR pokyčių tarp aktyvių JIA pacientų ir sveikų tiriamųjų nenustatyta.

Palyginus miRNR kiekius tarp skirtingų JIA klinikinių formų, nenustatyta nė vienos iš analizuotų miRNR skirtumų tarp oligoartrito, poliartrito ir su entezitu susijusio JIA nei serumo, nei šlapimo mėginiuose ($p > 0,05$). Taip pat skirtumų nerasta palyginus JIA pacientus, kuriems nustatyti teigiami ANA ar ŽLA B27 ($p > 0,05$).

3.3.2. MiRNR koreliacijos su JIA klinikiniais ir UG duomenimis

Tyrimo metu nustatyta silpna koreliacija tarp tirtų serumo miRNR koncentracijų, klinikinių ir laboratorinių parametrų. Serumo miR-16 statistiškai reikšmingai koreliavo su leukocitų kiekiu ($r_s = 0,316$, $p = 0,007$), limfocitų skaičiumi ($r_s = 0,369$, $p = 0,001$), trombokritu ($r_s = –0,281$, $p = 0,016$), ir vidutiniu trombocitų tūriu ($r_s = 0,272$, $p = 0,020$). miR-146a koreliacija su hemoglobinu ($r_s = –0,300$, $p = 0,01$), trombocitų skaičiumi ($r_s = 0,241$, $p = 0,04$), trombocitų žymenimis (trombocitų pasiskirstymo tūriu (angl. PDW) $r_s = –0,258$, $p = 0,028$ ir trombokritu $r_s = 0,278$, $p = 0,017$) ir CRB

(r_s = –0,276, p = 0,048) buvo reikšminga, tačiau silpna. O miR-155 silpnai teigiamai koreliavo tik su PaGA (r_s = 0,307, p = 0,027), tačiau jokio ryšio nenustatyta su laboratoriniais rodikliais. Tiriant miRNR tarpusavio ryšius, nustatyta vidutinė neigiama koreliacija tarp serumo miR-155 ir miR-146a (r_s = –0,550, p < 0,001), ir stipri neigiama koreliacija tarp miR-155 ir miR-16 (r_s = –0,619, p < 0,001). Nė viena iš tirtų miRNR nebuvo susijusi su JIA ligos trukme.

Nenustatyta reikšmingos koreliacijos tarp šlapimo miRNR ir klinikinių ar laboratorinių rodiklių. Tačiau stipriai neigiamas ryšys aptiktas tarp šlapimo miR-16 ir kitų miRNR (miR-146a r_s = –0,711, p = 0,001, ir miR-155 r_s = –0,609, p < 0,001).

Iš visų tirtų miRNR tik šlapimo miR-146a vidutiniškai neigiamai koreliavo su UG stebimais uždegimo požymiais V1 vizito metu (r_s = –0,62; p < 0,05). Koreliacijos nenustatyta tarp kitų miRNR tiek serumo, tiek šlapimo, ir UG duomenų nei įtraukimo į studiją, nei pakartotinio vizito metu.

3.3.3. miRNR diagnostinės galimybės

Gauti statistiškai patikimi rezultatai, rodantys, kad miR-16 serume gali atskirti JIA sergančius nuo sveikų tiriamųjų su 72 proc. jautrumu ir 71 proc. specifiškumu (AUC 0,81; 95 proc. PI: 0,70–0,93; p < 0,001). Serumo miR-155 taip pat jautriai (62 proc.) ir specifiškai (71 proc.) gali atskirti sergančius JIA (AUC 0,73, 95 proc. PI: 0,59–0,87; p = 0,005). Naudojant šias dvi miRNR kartu, diagnostinis tikslumas šiek tiek pagerėja, padidėjus specifiškumui ir plotui po kreive (0,82 ir 0,81, atitinkamai; p = 0,002). Šlapimo mėginiuose tik miR-146a galėjo diferencijuoti sergančius JIA pacientus nuo sveikų tyrimo dalyvių (jautrumas 65 proc. ir specifiškumas 64 proc.; AUC 0,68; 95 proc. PI: 0,53–0,82; p < 0,05). Likusios miRNR šlapime nebuvo reikšmingos diagnostikai (p > 0,05).

3.3.4. miRNR kiekių pokyčiai pacientų stebėjimo laikotarpiu

miRNR pokyčiai stebėjimo metu buvo vertinami suskirsčius JIA pacientus į tris grupes pagal ligos eigą, kaip aprašyta tyrimo metoduose. Ilgalaikiams miRNR pokyčiams įvertinti buvo analizuojami 23 serumo ir 23 šlapimo poriniai V0 ir V1 mėginiai (74 proc., 23 / 31). Pacientams, kurie stebėjimo laikotarpiu iš aktyvios ligos pasiekė remisiją (ACT-REM grupė), aktyvios ligos metu serume nustatyti reikšmingai mažesni miR-16 kiekiai (p = 0,021) ir didesni miR-155 kiekiai (p = 0,009). Tačiau pacientams, kuriems liga liko aktyvi (ACT-ACT), reikšmingų miRNR koncentracijų skirtumų tarp V0 ir V1 vizitų nenustatyta. Įdomu, kad pacientų, kurie viso tyrimo metu liko remisijoje (REM-REM grupė), serume nustatytas 6 kartus mažesnis miR-146a kiekis V0 vizito metu nei pakartotino vizito metu (V1)

(p = 0,004). Reikšmingų skirtumų tarp grupių poriniuose šlapimo mėginiuose nė vienai miRNR nenustatyta.

IŠVADOS

1. Nustatyta, kad nemažai daliai JIA sergančių pacientų išlieka subklinikinio uždegimo požymiai UG tyrime net po 6 mėnesių ar ilgesnės klinikinės remisijos, nors klinikiniai ligos aktyvumo rodikliai išnyksta.
2. MiR-16 ir miR-155 serume gali atskirti JIA sergančius pacientus nuo sveikų kontrolinių asmenų su dideliu specifiškumu ir jautrumu.
3. Šlapimas gali būti naudojamas kaip neinvaziškai surenkamas organizmo skystis miRNR analizei JIA sergantiems vaikams, o miR-146a šlapime yra perspektyvus diagnostinis biožymuo.
4. Koreliacija tarp kai kurių miRNR ir įprastinių uždegimo žymenų bendrame kraujo tyrime ir CRB yra silpna. Koreliacijos tarp ENG ir miRNR nenustatyta. Su UG tyrimo duomenimis koreliavo tik miR-146a šlapime (neigiamai).
5. JIA metu pastebimi reikšmingi miRNR kiekių pokyčiai, ypač tarp kliniškai aktyvios ligos ir remisijos stadijų, o miR-146a yra JIA remisijos predikcinis veiksnys.

REKOMENDACIJOS

Ultragarsinis sąnarių tyrimas suteikia vertingos informacijos apie JIA ligos aktyvumą ir turėtų būti integruotas į įprastinę klinikinę praktiką, vertinant ir gydant JIA sergančius pacientus. Įvertinus subklinikinius sąnarių uždegimo požymius, stebimus UG tyrime, ir įtraukus juos kaip papildomą kriterijų į sprendimų dėl gydymo taktikos priėmimą, būtų lengviau įgyvendinti individualios JIA pacientų priežiūros koncepciją, atsižvelgiant į kintančią lėtinio uždegimo eigą.

JIA sergantiems pacientams miRNR gali būti tiriamos skirtinguose organizmo skysčiuose, įskaitant ir neinvaziniu būdu surenkamus, tokius kaip šlapimas. Tam tikros miRNR gali būti naudojamos JIA diagnostikai ir ligos eigos monitoravimui.

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LIST OF PUBLICATIONS

1. **Šnipaitienė A.**, Snipaitiene K., Šlegerytė A., Buragaite-Staponkiene B., Baranauskaitė A., Jarmalaitė S., Jankauskaitė L. (2025). The utility of miR-16, miR-146a and miR-155 in serum and urine for juvenile idiopathic arthritis diagnostics and monitoring. Pediatric Rheumatology, 23(1), 1–13. https://doi.org/10.1186/s12969-025-01136-w
2. **Šnipaitienė A.**, Šlegerytė A., Uktveris R., Šileikienė R., Jakučionis P., Baranauskaitė A., Jankauskaitė L. (2024). The importance of ultrasound examination in care of juvenile idiopathic arthritis patients: 9 months follow-up study. Frontiers in Pediatrics, 12, 1–9. https://doi.org/10.3389/fped.2024.1414384

LIST OF SCIENTIFIC CONFERENCES

1. Šnipaitienė A., Jakučionis P., Uktveris R., Šileikienė R., Baranauskaitė A., Jankauskaitė L. (2024). Correlation of muscoskeletal ultrasound and juvenile idiopathic arthritis disease outcome measures: A 9-months of follow-up study. International Health Sciences Conference for All (IHSC for All) "Precision Medicine": Abstract Book 2024: [March 25-26, 2024, Kaunas] Edited by Ignas Lapeikis, Livija Petrokaitė, 481–483. https://smd.lt/uploads/publications/IHSCforAll2024.pdf
2. Šnipaitienė A., Uktveris R., Baranauskaitė A., Jankauskaitė L. (2024). Evaluation of muscoskeletal ultrasound inter-reader reliability in juvenile idiopathic arthritis patients. International Health Sciences Conference for All (IHSC for All) "Precision Medicine": Abstract Book 2024: [March 25–26, 2024, Kaunas] Edited by Ignas Lapeikis, Livija Petrokaitė, 480-481. https://smd.lt/uploads/publications/IHSCforAll2024.pdf
3. Jakučionis P., Šnipaitienė A., Uktveris R., Šileikienė R., Baranauskaitė, A., Jankauskaitė L. (2024). Incidence of subclinical synovitis in juvenile idiopathic arthritis patients with different disease activity levels. Medicina: Abstracts of the International Scientific Conference on Medicine Organized Within the Frame of the 82nd International Scientific Conference of the University of Latvia [5 April 2024, Riga, Latvia] Editor-in-Chief Edgaras Stankevičius, 60(Suppl. 1), 91–91. https://medicina.lsmuni.lt/wp-content/uploads/pdf/Abstracts_2024-1-Medicina.pdf
4. Šnipaitienė A., Šlegerytė A., Juškevičiūtė I., Skerbaitė B., Buragaitė-Steponkienė B., Šnipaitienė K., Baranauskaitė A., Jarmalaitė S., Jankauskaitė L. (2023). Urinary mirnas as non-invasive biomarkers for juvenile idiopathic arthritis. Pediatric Rheumatology: Proceedings of the 29th European Paediatric Rheumatology Congress: Netherlands, 28 September-1 October 2023: Meeting Abstracts, 21(S2), 213-213.

5. Šnipaitienė A., Šnipaitienė K., Juškevičiūtė I., Skerbaitė B., Baranauskaitė A., Jarmalaitė S. (2022). Circulating mirnas as non-invasive biomarkers for juvenile idiopathic arthritis disease activity monitoring. Pediatric Rheumatology: Proceedings of the 28th European Paediatric Rheumatology Congress (PReS 2022): Prague, Czech Republic. 20-23 September 2022, 20(Suppl. 2), 58-58. https://doi.org/10.1186/s12969-022-00729-z

SUPPLEMENTS

Supplement A. *Spreadsheet for MSUS data recording during visits*

Right/Left					Date	Patient Visit 1 2 3 4				
B mode					**Joint**	**Power Doppler**				
0	1	2	3	NA	Elbow: *Humeroradial?* *Humeroulnar?* *Posterior reccess?*	0	1	2	3	NA
0	1	2	3	NA	Wrist: *Radiocarpal?* *Midcarpal?*	0	1	2	3	NA
0	1	2	3	NA	MCP I	0	1	2	3	NA
0	1	2	3	NA	MCP II	0	1	2	3	NA
0	1	2	3	NA	MCP III	0	1	2	3	NA
0	1	2	3	NA	MCP IV	0	1	2	3	NA
0	1	2	3	NA	MCP V	0	1	2	3	NA
0	1	2	3	NA	PIP I	0	1	2	3	NA
0	1	2	3	NA	PIP II	0	1	2	3	NA
0	1	2	3	NA	PIP III	0	1	2	3	NA
0	1	2	3	NA	PIP IV	0	1	2	3	NA
0	1	2	3	NA	PIP V	0	1	2	3	NA
0	1	2	3	NA	Hip ..... mm	0	1	2	3	NA
0	1	2	3	NA	Knee	0	1	2	3	NA
0	1	2	3	NA	Ankle (TT)	0	1	2	3	NA
0	1	2	3	NA	Subtalar joint	0	1	2	3	NA
0	1	2	3	NA	Talonavicular joint	0	1	2	3	NA
0	1	2	3	NA	MTP I	0	1	2	3	NA
0	1	2	3	NA	MTP II	0	1	2	3	NA
0	1	2	3	NA	MTP III	0	1	2	3	NA
0	1	2	3	NA	MTP IV	0	1	2	3	NA
0	1	2	3	NA	MTP V	0	1	2	3	NA

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2002–2008	Medical doctor, Master's degree, Kaunas University of Medicine, Kaunas, Lithuania

Work experience:

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2024–present	Head of The Center for Rare Paediatric Cardiology and Rheumatology Diseases of Kauno klinikos
2019–present	Lecturer for medical studies at Department of Paediatrics, Kauno klinikos
2019–present	Paediatric Cardiologist at Department of Paediatrics, Kauno klinikos
2018–present	Paediatric Rheumatologist at Department of Paediatrics, Kauno klinikos

International experience:

2020/2021	7th EULAR / PRES Online Course in Paediatric Rheumatology 2020/2021 with 11 modules. Knowledge exam passed (2021 06 22).
2019 June	"EULAR/PReS intermediate MSUS Course", Madrid, Spain

2018 September	Basic musculoskeletal ultrasound course in rheumatological disorders in children organised by Portuguese Rheumatology Association (endorsed by Paediatric Rheumatology European Organisation), Lisabona, Portugal
2018 February–April	Paediatric rheumatology clinical internship in Istituto Giannina Gaslini (EULAR center of excellence in paediatric rheumatology from 2008), Genova, Italy

International projects:

2025–present	Clinical trial "Toward personalized medicine to guide drug withdrawal in children with juvenile idiopathic arthritis in clinical remission: a randomized clinical trial comparing early versus late drug withdrawal combining imaging and multi-Omics (Re-JIA)" – investigator
2022–present	COST Action: CA21168 – Improving outcome of Juvenile Inflammatory Rheumatism via universally applicable clinical practice strategies (JIR-CliPS) – main investigator in Lithuania, project management comittee member and grant coordinator of the project
2022–2024	PReS international examination creation "Pediatric Rheumatology Knowledge Based Exam"
2022–2024	Clinical observational trial "Applicability of standardized ultrasound examination to estimate disease activity in combination with JADAS and inflammation markers in JIA patients – DAISY study" – main investigator in Lithuania
2021–2023	"MERITA PROJECT: A METADATA REGISTRY FOR THE ERN RITA" – main investigator in Lithuania

Memberships:

2021–present	PReS "EMErging RheumatoloGists and rEsearchers" (PReS EMERGE)
2019–present	Paediatric rheumathology international trials organization (PRINTO)
2018–present	Paediatric Rheumatology European Society (PReS) active member (involved in 3 working groups)

PADĖKA

Norėčiau nuoširdžiai padėkoti savo mokslinei vadovei prof. dr. Astai Baranauskaitei – už patikėjimą mokslu vaikiškose ligose, už pasitikėjimą ir tikėjimą iki pat paskutinės dienos ir už ypač greitus atsakymus, kai jų reikėjo.

Taip pat dėkoju doc. Rimai Šileikienei už palaikymą nuo pat pirmo nedrąsaus pasvarstymo apie mokslą ir prof. Rimantui Kėvalui už palaikymą, vaikų reumatologijos ateities viziją ir suteiktą galimybę suderinti darbą ir studijas.

Atskiras ačiū Rimantui Uktveriui, patikėjusiam tyrimo prasmingumu ir kantriai echoskopavusiam visus tyrimo dalyvius.

Didelis dėkui prof. Sonatai Jarmalaitei už idėjos palaikymą nuo pat pradžių ir už galimybę atlikti tyrimus jos vadovaujamoje laboratorijoje. Taip pat didelis ačiū visai jos komandai, prisidėjusiai prie projekto įgyvendinimo.

Ypatingas dėkui Kristinai Šnipaitienei už visokeriopą pagalbą viso mokslinio kelio metu, pradedant idėjos tobulinimu ir laboratoriniais darbais, baigiant patyrusios rankos pridėjimu prie informatyvaus ir estetiško rezultatų atvaizdavimo. Tavo dėka ambicingi tikslai tapo realybe.

Didžiulis ačiū prof. Linai Jankauskaitei – už draugystę, palaikymą, tikėjimą, paskatinimą, mokslines diskusijas, SPSS vingrybių atskleidimą, nenustygstamą smalsumo skatinimą, platų žvilgsnį ir perspektyvos matymą. Esu dėkinga už puoselėjamą draugystę.

Nuoširdus ačiū tyrime sutikusiems dalyvauti mažiems ir dideliems pacientams ir jų šeimų nariams, už kantrybę ilgų echoskopijų metu, už naujas pažintis ir nuoširdų bendravimą. Taip pat dėkoju Andželikai Šlegerytei ir Pauliui Jakučioniui, reikšmingai prisidėjusiems prie nuobodaus duomenų rinkimo ir tai atlikusiems greitai ir kruopščiai.

Ačiū visiems artimiesiems, draugams ir kolegoms už tikėjimą ir palaikymą, už priminimą nenuleisti rankų ir eiti iki galo. Ypatingas ačiū vyrui Viliui, kuris visokeriopai stengėsi sudaryti sąlygas šiam mokslui įvykti, o vaikams – už kantrybę ir netikėtus vizitus pas mamą į darbą. Jūs esate ir liksite mano įkvėpimas ir stiprybė.

Tai, kas pradžioje atrodė neįgyvendinama, visų jūsų dėka tapo realybe!