

STUDY PROTOCOL

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Influences on and prevention of self-harm behavior among the most at-risk adolescents: study protocol for the SH-MARA prospective longitudinal cohort study

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Abstract

Background Both suicidal and non-suicidal self-injuring behaviors (NSSI) are common during adolescence. In Slovenia, adolescent suicide rates are high, making suicide the leading cause of death in the year 2022 in this age group. These behaviors are influenced by a complex interplay of environmental, psychological, and genetic factors. Previous research has identified risk and protective factors mainly for suicidal behavior in adults, a notable gap in understanding these factors in adolescents remains, especially for NSSI. Notably there is an important lack of effective clinical tools or psychometric assessment methods to reliably assess the risk for either suicidal or NSSI behaviors in acutely hospitalized adolescents.

Methods and analysis The proposed study uses a mixed-method observational design consisting of a prospective longitudinal cohort component involving adolescents hospitalized for high risk of DSH, and a cross-sectional comparison with a control group of healthy adolescents recruited from primary care settings. It is aimed at identifying genetic, psychosocial, and clinical factors associated with suicidal behaviors and NSSI in adolescents. The study group is recruited from adolescents aged 12–19, admitted to the Intensive Child and Adolescent Psychiatry Unit in Ljubljana due to severe self-harm risk. Exclusion criteria include involuntary treatment, acute psychotic disorders, intellectual disability, severe physical or central nervous system illnesses and acute intoxication. The control group comprises adolescents of comparable age, recruited through regular scheduled health check-ups in Slovenia. Exclusion criteria include suicidality, severe mental disorder, a history of self-harm behavior in a first-degree relative, intellectual disability, severe physical or central nervous system illnesses and acute intoxication. Enrollment runs from February 1, 2023, to December 31, 2025. Participation is voluntary, requiring parental or guardian consent for those 14 or younger.

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Data collection involves psycho-diagnostic tools assessing demographic, psychopathology, personality traits, trauma and traumatic events, and attachment patterns. Genetic analysis of blood samples will be performed using long-read sequencing to detect DNA methylation and bioinformatic tools for further analyses. Various statistical methods will be used to identify factors potentially linked to suicidality and NSSI. Additionally, the utility of a newly developed tool for the assessment of inpatient self-harm risk (Adolescent Self-Harm Risk Scale; ASHRS) will be tested. The efficacy of the proposed scale will be assessed using data on inpatient self-harm episodes, as well as subsequent suicidal and NSSI behavior collected during follow-ups at 6 and 18 months after the initial inpatient assessment. The study has been approved by the National Medical Ethics Committee of the Republic of Slovenia (No: 0120–507/2022/3) and financed by Slovenian Research Agency grants J3-4534, J5-50176 and P3-0343.

Discussion This study represents the first longitudinal examination of psychosocial and genetic factors associated with severest suicidal and NSSI behavior in the Slovenian adolescent population. The development of the ASHRS will add to the gap in the quick assessment of inpatient self-harm risk and better inpatient safety for adolescents.

Keywords Adolescents, Deliberate self-harm, Non-suicidal self-injury, Suicidal behavior, Intensive psychiatry, Personality disorder, Traumatic experience, Genetics, Epigenetics

Background

Adolescence marks a critical developmental transition from childhood to adulthood, characterized by significant physical, cognitive, and emotional changes. These changes are driven not only by hormonal fluctuations but also by extensive brain development. Recent advancements have deepened our understanding of this period, highlighting the influence of both genetically predetermined pathways and environmental factors through epigenetic modifications. During this period, the brain undergoes accelerated changes, including extensive synaptic pruning and reformation in various areas of the cerebral grey matter, alongside delayed and gradual myelination. This developmental process follows a dorso-lateral trajectory, progressing from evolutionarily older brain regions responsible for basic functions (e.g., breathing, eating) to the younger frontal regions responsible for complex cognitive tasks (e.g., planning, reasoning) [1, 2].

As adolescents' cognitive abilities evolve from concrete to abstract thinking, they often grapple with existential questions such as "Who am I?", "What am I like?", and "Why do I exist?" [3]. This developmental stage also involves increased contemplation of mortality and death, making self-harm behavior notably prevalent [4]. In Slovenia, a country with a historically high suicide rate of 19.8 deaths per 100,000 [5], suicide remains the leading cause of death among individuals aged 10 to 19, with an average of 10 deaths per year over the past two decades [1]. Deliberate self-harm behaviors (DSH) can be categorized into suicidal behavior (with intent to die) and non-suicidal self-injuring (NSSI), defined as DSH without suicidal intent [6]. The latter includes behaviors such as cutting, burning, or hitting oneself, primarily as a means of self-soothing or releasing internal tension [7, 8].

Epidemiological data show that while suicide is rare in children under the age of 10, these behaviors become more prevalent in adolescents and young adults. The

lifetime prevalence of attempted suicide in adolescents ranges from 1 to 10% [9], with higher rates observed in females. In Slovenia, a 1996 study reported that more than 10% of adolescents had attempted suicide by the age of 19 [10], with similar rates observed in subsequent studies [11]. In a 2016 study conducted on adolescents using outpatient psychiatric services in Slovenia, 59% of adolescents reported suicidal ideation, and 21% had a history of attempted suicide [12]. In addition, in the last years, we have seen a marked increase in visits to emergency child and adolescent psychiatric services for suicidal ideation and attempted suicide which could be related to the COVID-19 pandemic [13].

The lifetime prevalence of NSSI in adults is reported at 6%, while in adolescents it averages around 19% [14, 15]. Among Slovenian secondary school students, 24% of females and 12% of males reported NSSI [11]. Higher prevalence rates are expected in clinical samples of adolescents, with some studies reporting annual rates up to 50% [16, 17]. A 2016 study at the University Psychiatric Clinic in Ljubljana found that 44% of hospitalized adolescents reported a lifetime history of NSSI [12]. Longitudinal surveys suggest that a large majority of adolescents outgrow these behaviors by adulthood, although they remain problematic during the developmental period, and are associated with poorer outcomes [18, 19].

Understanding the etiology of DSH in adolescents involves examining a complex interplay of risk and protective factors. Research has predominantly focused on suicidality, but the factors influencing NSSI are often similar due to the interconnected nature of these behaviors [20]. Risk factors for both types of behavior include mental illnesses such as depression and anxiety, family dynamics, and exposure to trauma or abuse [19]. Protective factors, on the other hand, include strong social support, coping skills, and healthy family and peer relationships. Recent studies highlight the importance of

resilience, emphasizing the need to promote resilience in adolescents to mitigate the risk of deliberate self-harm [21]. Fergusson et al., in a longitudinal study, identified factors such as family history of suicidal behavior, childhood sexual abuse, neuroticism, exploration desire, self-esteem, and peer connectedness as key influencers. Positive configurations of these factors reduced suicide risk, while negative configurations increased it [22]. Bridge et al. proposed a model where mood disorders and impulsivity/aggression increase the risk of suicidal behavior, influenced by pre-pubertal predictors and additional triggers or stressors in the absence of protective factors [9].

Biological factors also play a crucial role in understanding DSH. Studies on adolescents are considerably scarcer than studies on neurobiology of suicidal behavior in adults. Genetic research indicates that suicidal behavior is strongly familial and genetically determined. Studies involving twins and adopted children have shown that suicide and attempted suicide often occur within families independently of mental disorders [23]. Additionally, certain genetic polymorphisms, such as those in the serotonin transporter and tryptophan hydroxylase genes, have been associated with aggressive behavior in suicidal adolescents [24]. Research suggests that familial transmission of suicidal tendencies may be related to impulsive aggression, influenced by serotonin metabolism and low levels of 5-hydroxyindoleacetic acid [25].

Regarding NSSI, genetic research shows mixed results. While some studies suggest genetic influences on thoughts about NSSI, others indicate genetic factors account for significant variance in NSSI behavior, particularly in women [26, 27]. The role of specific genes, such as the serotonin transporter promoter region polymorphism (5-HTTLPR), remains inconclusive, with some studies finding associations and others not [28, 29]. Epigenetic studies have identified potential methylation changes in genes related to the hypothalamic-pituitary-adrenal axis, the neurotropic system, and the serotonin system, indicating a possible link between adverse early experiences and self-injurious behavior [30].

The proposed study aims to enhance the safety and long-term prognosis of adolescents evaluated as the most at risk of severe DSH by addressing a critical gap in current clinical practice by pursuing three primary objectives:

1. Identify the factors that influence the risk of DSH and the success of treatment in the most at-risk adolescents over an 18-month period.
2. Develop a method to more effectively identify the acute and long-term risk of adolescents with the most threatening DSH (psychiatric inpatients).

3. Develop Slovenian guidelines for more effective treatment of the most at-risk adolescents.

Methods

Design

This study is aimed at identifying the genetic, psychosocial, and clinical factors associated with NSSI and suicidality in adolescents. It uses a mixed-method observational design consisting of a prospective longitudinal cohort component involving adolescents hospitalized for high risk of DSH, followed at 6 (time 2) and 18 months post-admission, (time 3) and a cross-sectional comparison with a control group of healthy adolescents recruited from primary care settings. An overview is provided in Fig. 1 below.

Eligibility criteria

Study group

The inclusion criteria for the study group encompass adolescents aged 12–19 years who are admitted to the Intensive Child and Adolescent Psychiatry Unit of the University Psychiatric Clinic Ljubljana due to severe risk of DSH (suicidal or NSSI). Participants will be enrolled between February 1, 2023, and December 31, 2025. Eligibility for participation in the study will require treatment with their consent and voluntary participation in the study. For children aged 14 or younger, parental or guardian consent will also be required. Exclusion criteria include involuntary treatment, the presence of an acute psychotic disorder or intellectual disability, severe physical illnesses (heart disease, vascular disease or kidney disease), central nervous system disorders (encephalitis, brain injury or hemorrhage, epilepsy), and acute intoxication. We will invite all eligible adolescents over a period of 3 years, expecting a 30% rejection rate, the projected sample size is 200 participants.

Control group

The control group includes adolescents aged 12–19 years, recruited through regular health check-ups at various health centers across Slovenia. Enrollment in the study will occur between February 1, 2023, and December 31, 2025. Participation will be voluntary, and for children aged 14 or younger, parental or guardian consent will be required. Exclusion criteria for the control group include suicidality or NSSI, the presence of significant mental disorders (depression, bipolar disorder, or psychotic disorders), intellectual disability, severe physical illness (heart disease, vascular disease or kidney disease), central nervous system disorders (encephalitis, brain injury or hemorrhage, epilepsy) and acute intoxication.

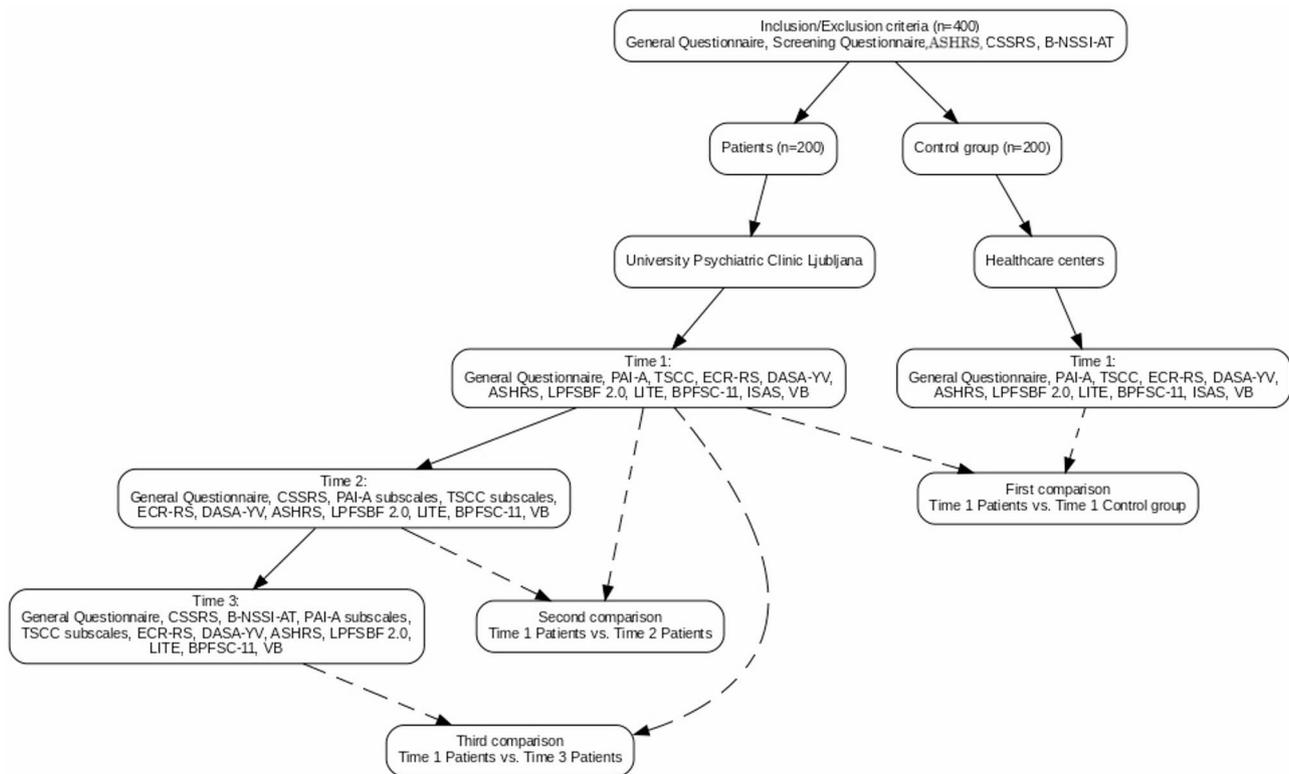


Fig. 1 Research flow diagram

Measurements

The following questionnaires and psychodiagnostics tools will be used to collect data from participants:

- General Questionnaires: These will collect general and demographic data, previous treatments, medication received, mental disorder at discharge (ICD-10), psychosocial situations (ICD-10), physical illnesses (ICD-10), COVID status (not-acquired, recovered, vaccinated), use of psychoactive substances, school performance, experience with peers, sexual orientation and identity, residence, family composition, and family history of mental disorders, history of DSH, recent DSH, and suicide attempts.
- Columbia Suicide Severity Rating Scale (CSSRS) screener: The questionnaire is scientifically supported, has the most evidence of utility and efficacy, and is internationally accepted. It has been translated into more than 100 different languages, including Slovene. It contains 2 screening questions on suicidality and 4 more specific questions, 6 items in total [31].
- The Brief Non-Suicidal Self-Injury Assessment Tool (B-NSSI-AT): This tool is used for research purposes to assess the core characteristics of NSSI (form, frequency, function) as well as the secondary characteristics of NSSI (habituation, context of NSSI, perceived impact on life and treatment). It is most useful for adolescents and young adults, who are the population most at risk for NSSI [32].
- Personality Assessment Inventory-Adolescent (PAI-A): An objective-type self-assessment questionnaire for assessing personality in adolescents. It has 264 items to be answered on a 4-point scale. 22 independent scales are obtained (Inconsistency, Rarity, Negative impression, Positive impression, Physical complaints, Anxiety, Anxiety-related disorders, Depressiveness, Mania, Paranoid, Schizophrenia, Borderline traits, Antisocial traits, Alcohol problems, Drug problems, Aggressiveness, Suicidal ideation, Stress, Lack of support, Refusal to deal, Dominance, Warmth) [33, 34].
- Trauma Symptom Checklist for Children (TSCC): The questionnaire helps to assess children's/ adolescents' experiences of various traumatic experiences, such as physical or sexual violence, peer violence, loss, witnessing violent acts, natural disasters, etc. The questionnaire consists of 6 clinical scales (Anxiety, Depression, Anger, Post-traumatic Stress Symptom, Dissociativeness (two subscales), Sexual Concerns) and 2 validity scales [35, 36].
- Experiences in Close Relationships-Relationship Structures (ECR-RS): This questionnaire is designed

to assess attachment styles across different types of relationships, including romantic partners, parents, and friends. It evaluates two key dimensions of attachment anxiety and avoidance and consists of 9 items for each relationship context, with responses rated on a Likert scale [37].

- Lifetime Incidence of Traumatic Events Questionnaire (LITE): A short checklist for screening and assessing the exposure to trauma in children and adolescents. It covers a broad range of potentially upsetting situations that can cause trauma to children and adolescents, such as a car accident, fire, death of a family member, exposure to threats, sexual assault, or witnessing violence. The questionnaire had been validated on a Slovene population of children and adolescents [38, 39].
- Level of Personality Functioning Scale-Brief Form 2.0 (LPFS-BF 2.0): A short, user-friendly instrument that gives a quick impression of the expression of personality pathology. It consists of 12 items grouped into two higher-order domains: self-functioning and interpersonal functioning. Participants are asked to rate the 12 items on a four-point Likert scale ranging from 1 (completely false) to 4 (completely true). A total score (sum of all items), a self-functioning score (sum of items 1–6) and an interpersonal functioning score (sum of items 7–12) can be calculated. Satisfactory internal consistency and promising construct validity have been demonstrated. Sensitivity to change after three months of treatment was high [40].
- Borderline Personality Features Scale (BPFSC-11): It includes borderline personality disorder (BPD) indicators such as affective instability, identity problems and negative attitudes. Responses to the items are on a 5-point Likert scale ranging from 'not at all true' to 'always true'. Studies have shown construct validity of interpretations of BPFSC-11 scores through positive associations with other measures of BPD and positive associations with measures of BPD correlates, including emotional dysregulation. The scale has been officially translated into Slovenian and will be used with the permission of the authors. It is intended for use by children and adolescents and consists of 11 items [41].
- Inventory of Statements About Self-Injury (ISAS): A self-assessment questionnaire used for research purposes to assess the basic characteristics of NSSI (form, frequency, function, time to event). It also assesses the desire to stop. The questionnaire has been previously translated into Slovene and used in a population of adolescents with self-injurious behaviour [42, 43].
- Dynamic Appraisal of Situational Aggression-Youth Version (DASA-YV): A professional assessment tool which provides a daily assessment of the risk of heteroaggression. The evaluation is efficient and takes less than five minutes. The nurse in charge of each patient or a researcher completes the DASA-YV once a day [44, 45].
- Adolescent Self-Harm Risk Scale (ASHRS): Developed for this study, this scale aims to assess and predict the risk of self-harm behavior during and after hospitalization.

Genetic methods

Genomic DNA will be isolated from 5 mL of EDTA-anti-coagulated blood using the FlexGene DNA kit (Qiagen, Germany) and stored at 4 °C until processing. Long-read sequencing will be performed on the PromethION platform (Oxford Nanopore Technologies). This state-of-the-art setup provides enhanced read accuracy and improved sensitivity for detecting base modifications, including epigenome-wide DNA methylation. Library preparation will involve a multi-step process: initial end repair and dA-tailing using the NEBNext Ultra II kit, native barcode ligation, sequencing adapter attachment, flow cell priming, and final library loading. For cost-effectiveness and to maintain individual sample integrity, each sample will be specifically barcoded and sequenced to achieve an average coverage of 10×. The resulting sequencing data will then be pooled for downstream analyses, ensuring robust detection of cohort-specific epigenetic signals.

Bioinformatics

Raw sequencing data will be processed using the latest Nanopore EPI2ME pipeline wf-basecalling, utilizing tools such as Dorado to convert raw electrical signals into high-quality nucleotide sequences and methylation patterns as Remora also integrates methylation detection, enabling direct identification of epigenetic modifications during basecalling. Variant calling will be conducted with the standard Nanopore EPI2ME pipeline wf-human-variation, aligning reads to the human reference genome (CHM13-T2T/GRCh38). Differential methylation patterns will be detected with R packages appropriate for differential methylation analyses incorporated into custom scripts. This comprehensive, integrative pipeline will merge genetic and epigenetic findings with clinical and psychosocial data, thereby providing deeper insights into the molecular factors underlying DSH in adolescents and guiding future preventive and interventional strategies.

Statistical analysis

The statistical analysis will be conducted in several stages, using both supervised and unsupervised learning

approaches to examine the clinical, psychosocial, and genetic factors associated with DSH in adolescents.

First, a binary classification model will be developed to distinguish between the study group (adolescents hospitalized due to high risk of DSH) and the control group (healthy adolescents). For this purpose, a logistic regression model will be employed. The model will include variables from all data sources: clinical assessments, questionnaire responses, and genetic/epigenetic data. Interaction terms between variables will also be included to capture the complex connection between biological and psychosocial risk factors. To prevent overfitting due to many predictors and interactions, regularization techniques such as LASSO (Least Absolute Shrinkage and Selection Operator) will be applied.

In the second step, only the data from the study group will be used to develop a model predicting the ordinal level of DSH risk, as defined by clinical evaluation and follow-up data. A proportional odds logistic regression model (ordinal logistic regression) will be used to predict these risk levels based on the same set of input variables, including interaction terms. Again, regularization methods will be used where appropriate to ensure model stability and interpretability.

In an exploratory phase of the analysis, unsupervised clustering techniques (e.g., k-means) will be applied within the study group to identify subgroups of adolescents who share similar clinical, psychosocial, and genetic profiles. Clustering will be based on baseline data and will help uncover potential subtypes within the high-risk population. The two primary models, the classification model and the ordinal risk model, will then be rerun separately within each identified cluster. The goal of this analysis is to determine whether the predictive factors for DSH differ across subgroups, which may inform future efforts toward individualized risk assessment and treatment.

All analyses will be conducted using R statistical software, and assumptions for each model will be tested. Statistical significance will be evaluated at the $p < 0.05$ level, and model performance will be assessed using standard metrics (e.g., AUC for classification, accuracy of ordinal predictions, and cross-validation methods where applicable).

Discussion

To our knowledge, this will be the first study that longitudinally examines the genetic, psychosocial, and clinical factors associated with NSSI behaviors and suicidality in adolescents aged 12–19 years in the adolescent population at highest risk. Previous research has identified various protective and risk factors, primarily in adult populations, but there is a significant gap in understanding these dynamics in adolescents, particularly with the

integration of newer epigenetic methods. Additionally, our study includes a control group of healthy adolescents, allowing us to differentiate different factors associated with normative behaviors and those indicative of underlying mental health issues. Our study also includes novel assessments consistent with state-of-the-art recommendations for the assessment of early detection of personality pathology in youth, a known correlate of NSSI [46]. The development of ASHRS as part of this research holds significant potential for clinical practice. Current tools, such as the DASA-YV, are effective for predicting hetero-aggressive behavior [44] but are less reliable for assessing self-injurious behaviors in acutely hospitalized adolescents. ASHRS aims to fill this gap, providing clinicians with a quick and practical tool for assessing and managing the risk of DSH in inpatient settings, thereby enhancing patient safety and treatment outcomes.

Study limitations

The first limitation is that our sample includes only adolescents who are admitted to our emergency intensive care services and not all adolescents who seek help for mental health issues, which can create a selection bias. However, the population sampled includes all the Slovenian adolescents, who require hospitalization because of the highest risk of DSH, thereby representing the entire and most at risk population. Furthermore, during the follow-up period, the pharmacological or psychotherapeutic treatments administered will not be the same for all patients included. The study will thereby assess correlations between the biological and psychosocial changes during the follow-up period, regardless the specific interventions used (treatment as usual). We may also expect a significant participant dropout based on the 18-month follow-up time proposed.

Abbreviations

NSSI	Non-Suicidal Self-Injury
ASHRS	Adolescent Self-Harm Risk Scale
B-NSSI-AT	Brief Non-Suicidal Self-Injury Assessment Tool
BPFSC-11	Borderline Personality Features Scale
CSSRS	Columbia Suicide Severity Rating Scale
DASA-YV	Dynamic Appraisal of Situational Aggression-Youth Version
DSH	deliberate self-harm
ECR-RS	Experiences in Close Relationships-Relationship Structures
ISAS	Inventory of Statements About Self-Injury
LITE	Lifetime Incidence of Traumatic Events Questionnaire
LPFS-BF 2.0	Level of Personality Functioning Scale-Brief Form 2.0
PAI-A	Personality Assessment Inventory-Adolescent
TSCC	Trauma Symptom Checklist for Children

Authors' contributions

Maja Drobnič Radobuljac is the principal researcher who developed the original idea of the study and the study design and will coordinate the study throughout the duration. Lana Podnar Serneć (corresponding author), Petra Tomažič, Anja Tomašević Kramer, Barbara Plemeniti Tololeski, Gorjan Tasevski, Žiga Rosenstein, Simona Klemenčič, Tadej Battelino, Blaž Vrhovšek, Tadej Lahovnik, Jernej Kovač, Carla Sharp, Barbara Jenko Bizjan, Sašo Karakatič and Maja Drobnič Radobuljac all participated in study design and in data acquisition. All the above mentioned authors will participate in the statistical

analyses. All the above authors have read and corrected the draft versions of the present manuscript and approved the final version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by The Republic of Slovenia National Medical Ethics Committee (approval reference number 0120–507/2022/3). All participants and their legal guardians (for anyone aged 15 or less) provided written informed consent prior to participation in the study.

Consent for publication

Is a part of informed consent signed by the included subjects and their parents (in subjects under the age of 15) and was a part of the ethical approval process.

Competing interests

The authors declare no competing interests.

Trial registration

The study was registered at ClinicalTrials.gov (Identifier: NCT05765864) on March 8, 2023 (<https://clinicaltrials.gov/study/NCT05765864>).

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