The effectiveness of a single session of mindfulness-based cognitive training on cardiac vagal control and core symptoms in children and adolescents with attention-deficit/hyperactivity disorder (ADHD): a preliminary randomized controlled trial

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Received: 27 September 2021 / Accepted: 4 May 2022 / Published online: 24 May 2022
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Abstract
This study examined the effectiveness of a mindfulness-based intervention (MBI) on Conners’ continuous performance test scores (CPTs), cardiac vagal control (CVC) assessed by vagally mediated heart rate variability (HRV), and mood in children and adolescents with ADHD. We conducted a randomized controlled trial (RCT) recruiting 70 children and adolescents (M age 11.03, SD 2.78) with a clinical diagnosis of ADHD, which were allocated to either 1 session of mindfulness cognitive training, or an active control condition and were examined at baseline, post-treatment and 4-week follow-up. See clinicaltrials.gov: NCT04316832. There was a significant main effect of time on the primary outcomes measured by CPT scores of attention-related problems (omission errors, reaction time) and hyperactivity–impulsivity (commission errors). However, time-by-group interaction did not achieve statistical significance for commission errors and hit RT, indicating that the changes over time in these outcomes were not significantly different between the MBI and Control conditions. In addition, there was a significant time-by-group interaction for omission errors. Relative to control, MBI resulted in a small \( d = 0.011 \) non-statistically significant reduction in omission errors post-treatment. Furthermore, there were no significant differences in detectability. Secondary outcomes were CVC and mood. A small treatment effect on CVC \( d = 0.37 \) was observed; there was a slight increase in vagally mediated HRV measure post-treatment. There were no significant differences in mood improvement over time between conditions. One brief session of MBI effectively enhances CVC but does not significantly improve CPT scores of attention-related problems and hyperactivity–impulsivity or mood in children with ADHD. Clinicaltrials.gov: NCT04316832.

Keywords ADHD · Children · Mindfulness · Vagal activity · Mood

Introduction
ADHD is one of the most commonly diagnosed psychiatric disorders, with an estimated worldwide prevalence between 2 and 7% in youngsters [1]. The disorder is strongly linked to poor quality of life, delinquency, addictions, gambling, educational failure, teenage pregnancy, suicide, difficulties socializing, and premature death [1]. Behavioral interventions and/or pharmacological treatment are the first-line treatment options for ADHD [2]. Although medication and behavioral therapy have shown to be effective in reducing the disorder’s core symptoms [3], about 30% of patients with ADHD would not achieve the treatment response and symptomatic remission [4]. In addition, some children can experience side effects related to medication, such as sleep...
There is evidence that MBIs could significantly reduce ADHD core symptoms [6] and may enhance HRV through increased parasympathetic modulation [7]. In addition, research has demonstrated that brief mindfulness training programs can improve cognitive functions, including attention and memory, after a single session with brief interventions of 5 min or longer [8]. Although emerging research has demonstrated MBIs to be an effective treatment for ADHD symptoms [6, 9], most of the research in this area involves extensive multi-week training; there are no clinical trials evaluating the efficacy of brief mindfulness interventions in children and adolescents with ADHD. Given that children with ADHD have trouble staying engaged in activities for long periods, they may benefit from a brief mindfulness intervention.

This RCT aimed to examine the effectiveness of a single session of mindfulness-based cognitive training for children and adolescents with ADHD aged 6–17 years. We hypothesized that children and adolescents receiving MBI would significantly improve ADHD symptoms, vagally mediated HRV, and mood relative to the control group. An exploratory secondary aim was to determine whether improvement of symptoms and CVC may be sustained for 4 weeks after the intervention.

Participants

Participants aged 6–17 years were recruited from an outpatient Child and Adolescent Psychiatric Unit and surrounding clinics via a specified referral pathway or via advertisements in the community (internet, flyers, and social media) of Cluj-Napoca city. The participants were invited to participate in a trial that aimed to investigate the effectiveness of mindfulness-based cognitive training on ADHD symptoms, autonomic dysregulation, and mood, delivered in one session. Children and adolescents with a primary diagnosis of ADHD according to DSM 5 criteria [10] were enrolled. Additional inclusion criteria included the ability to verbally communicate and write in Romanian, normal intellectual ability operationalized as an intellectual quotient above 70 based on Raven Standard Progressive Matrices tests, and no medication/agree to no medication changes (dose or type) within 3 months of trial onset. In our study, the children and adolescents did not receive any other treatment beyond ADHD medication. Exclusion criteria were as follows: comorbidities of conduct disorder or oppositional defiant disorder, the presence of a chronic disorder, and previous participation in mindfulness-based training.

Procedure

One research assistant screened potential participants consenting to participate in the study for eligibility through a brief telephone interview. Those who met the inclusion criteria were scheduled for a baseline assessment. The principal investigator, a well-trained child and adolescent psychiatrist, examined the children and made the clinical diagnosis of ADHD, according to the DSM 5 criteria. For all children, written informed consent for the evaluation and intervention was obtained from parents. Each participant was tested separately in a quiet room; the evaluation included HRV monitoring and a computer-based attention task.

Randomization, blinding, and allocation concealment

Immediately after baseline assessments, participants were randomized to either one session of MBI, or to control condition, to keep the assessors blinded. Randomization was performed by an independent research assistant using a random numbers generator, https://www.random.org/lists/. The allocation ratio was 1:1. The intervention was delivered immediately after the randomization process. The outcome assessors and the statistician performing analyses were kept blind to treatment assignments.
Intervention

The mindfulness-based intervention was delivered individually in one session. It included three short mindfulness exercises: (a) a breathing exercise that encourages the participant to focus on a slow and deliberate breath, (b) a body scan exercise that promotes the awareness of body sensations while maintaining an accepting attitude towards these sensations and helps children to relieve tension, and (c) a mindfulness attention exercise to increase moment-by-moment awareness. The psychotherapeutic content of the intervention was designed by adapting, for children, a mindfulness protocol, previously used with adults. The program was found to be effective for improving mindfulness skills and lessening general psychiatric complaints [11]. These exercises were selected because previous research has shown that they can enhance self-management of attention [11]. The instructions for the breathing, the body scan, and the mindfulness attention exercises were recorded and played to participants through external computer speakers. Approximately, 10 min were allocated for participants to engage in mindfulness training.

Control

Participants allocated to the control condition listened to the first chapter of the audiobook The Hobbit, JRR Tolkien [12]. The listening task was selected as a control because it has been used before in multiple studies as an active control condition for brief mindfulness exercises [13–15], and it requires a comparable amount of attention and concentration to the meditation task [13].

Outcome measures

Primary outcomes

The primary outcome was the change from pre- to post-treatment, pre-treatment to follow-up, and post-treatment to follow-up in CPT scores. This study employed the CPT-II [16]. Typically, the “not-X” CPT task requires the subject to hit the space bar as quickly as possible to all stimuli except the letter X, at which point they should, instead, inhibit their response. The test provides several performance measures, four of which were analyzed in this study: omission errors, commission errors, hit reaction time, and detectability. Omission errors (missed responses) and reaction time (latency response) are related to sustained attention deficits; commission errors (responding when the target is not present) are indicative of impulsive and hyperactive symptoms. Detectability reflects the subject’s ability to distinguish and detect targets and non-targets; poor detectability is considered an indicator of inattentiveness. The CPT task was performed on an IBM laptop computer [17]. Participants underwent neurocognitive testing of the clinical features through the computerized attention task at baseline, immediately after the intervention, and after four additional weeks.

Secondary outcomes

The secondary outcomes of this trial were changes in (a) cardiac vagal control and (b) mood. CVC was tracked through vagally mediated HRV indexed by frequency- (high-frequency HRV: HF-HRV) and time-domain measures (root mean square of two consecutive R-waves of the QRS signal on the electrocardiogram (RR interval) differences: RMSSD). The vagally mediated HRV measures from the time- and frequency domain are consistently used as valid markers of vagal tone in short-term analysis [18]. We used HF power in normalized units in the present study, HF (n.u.)=HF/(Total Power–VLF)×100. HF-HRV (n.u.) and RMSSD (ms) were averaged across the first 5 min of the CPT task. The mood was assessed through a Visual Analogue Scale (VAS) for four basic emotions (e.g., anxiety, sadness, anger, and worry as experienced at the moment), derived from the Present Functioning Visual Analogue Scale [19]. Participants had to rate their emotions on a 10 cm (100 mm) horizontal line with verbal descriptors at each side of the line to express the extremes of the feeling (e.g., ‘not at all’ versus ‘very much’). An Emotional Distress Summary Score (EDSS) was computed by summing the scores of the worry, sadness, anxiety, and anger items, similar to the PedsQL™ Emotional Functioning Scale [20].

Additional clinical outcomes

The ADHD Rating Scale-IV, Home version (ADHD-RS; [21]) is an 18-item questionnaire that requires the parents to rate the frequency of occurrence of ADHD symptoms as defined by the DSM-IV-TR over the previous 6 months using one of the following: 0 (Never or Rarely), 1 (Sometimes), 2 (Often), and 3 (Very often). This questionnaire was adapted for the Romanian population and demonstrated good validity and reliability coefficients [22]. The raw scores are converted into percentile scores based on the child’s gender and age. In our study, ADHD-RS total scores were used to rate the global symptom severity based on the cutoff scores for the 80th percentile established for the Romanian population [22].

Possible adverse events were assessed through a free-response question asking participants to report if they had any unpleasant experiences (difficult thoughts, emotions, and bodily sensations) during the intervention.

All participants provided demographic information such as age, gender, urban or rural residency, and education level.
and had their weight and height checked. The psychiatric comorbidities and current medication were documented.

**Data analysis**

An a priori power analysis was conducted to determine the number of required participants to identify a statistically significant condition by time interaction, using G*Power software [23]. For the repeated measures analysis of variance, power calculations were based on the assumption of large effect size [6]. A total sample size of 64 participants (e.g., 32 participants per group) was estimated (assuming a power of 0.80, alpha = 0.05 for two-sided tests). To allow for a participant dropout rate of 10%, we aimed to include 70 participants.

All statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Chicago, IL). The data were screened for missing values, outliers, and normality. The RMSSD values were natural log-transformed. Analysis of variances (ANOVs) was used to examine group differences in the distribution of all outcome measures at baseline.

Linear mixed models (LMM) were used to compare the MBI and Control groups’ change scores from pre- to post-treatment, pre-treatment to follow-up, and post-treatment to follow-up on CPT scores (primary outcomes), HF-HRV and RMSSD and EDSS scores (secondary outcomes). The data were structured in a two-level hierarchical model, with time at Level 1 nested within individuals at Level 2. All participants with at least one measurement were included in the analyses. All models were fitted using the maximum likelihood (ML) estimation, with a random intercept per subject for all outcomes, with variance components matrix for the random intercept and an autoregressive structure (AR1) of the within-subject variance–covariance matrix for the repeated measure of time. The Akaike’s information criterion (AIC) was selected to determine the appropriate statistical model. The changes in CPT scores were analyzed while controlling for baseline level of inattention and hyperactivity/impulsivity, using the raw scores of inattention, respectively hyperactivity/impulsivity from pre-treatment, to avoid using the same variables both as predictor and outcome, consistent with Stjerneklar et al. [24]. All models that included CVC-related outcomes were corrected for body mass index, whereas the EDSS baseline score was included as a control for pre-treatment levels of emotional distress in all univariate LMM analyses related to mood outcomes, given the baseline differences between groups in these two variables. The LMM analyses on CPT scores were repeated while controlling for age since there is evidence that age could moderate the effects of mindfulness on executive functioning [25]. We computed Cohen’s d as an effect size indicator based on the standardized mean difference divided by the standard deviation.

**Missing data, dropout**

There were minimal missing data at pre-treatment as the data were collected during baseline evaluation; the CVC measures were unavailable for one subject due to overall bad data signal. Post-treatment, physiological data were missing for two subjects due to no data or overall bad data signal; the CPT scores were also missing for two participants due to a computer disruption. Overall, the percentage of missing values ranged between 0 and 10.6%; the Little’s Missing Completely at Random test (MCAR) was non-significant, indicating that data were missing completely at random [26]. Missing data were handled through the LMM analyses, which account for all available data, under the missing-at-random assumption. None of the participants dropped out of the treatment; seven participants were unavailable at 4-week follow-up, six in the active control condition, and one in the intervention group.

**Results**

Between October 2020 and July 2021, 104 participants were recruited. Figure 1 presents the Consolidated Standards of Reporting Trials flow diagram of participants through the study. A total of 70 participants were enrolled in the trial and assigned to the MBI (N = 35) or Control (N = 35) groups.

The baseline clinical and demographic characteristics (Table 1) were largely comparable between conditions. At baseline, there were no significant differences between MBI and Control groups in all CPT scores, CVC- or mood-related measures except for the EDSS, F (1.69) = 11.24, p = 0.001. No adverse events during the intervention were reported.

The observed group means and standard deviations of the primary and secondary outcomes at pre-, post-treatment, and follow-up are listed in Table 2.

For the primary outcome changes in CPT scores, there was a significant main effect of time on the commission, omission errors, and hit RT. However, time-by-group interaction did not achieve statistical significance for commission errors and hit RT, indicating that the changes over time in these outcomes were not significantly different between the MBI and Control conditions. Furthermore, there were no significant differences in detectability. There were significant between-group differences in omission errors; overall, the Control group presented more errors (M = 25.72, SE = 3.45) than the intervention group (M = 13.27, SE = 3.43). In addition, there was a significant time-by-group interaction for omission errors. Estimated marginal means revealed that there was a small (d = 0.4) statistically significant increase in the number of omission errors from pre- (M = 24.52, SE = 3.91) to post-treatment (M = 34.97, SE = 3.95) in the control condition. In the intervention group, estimated
**Table 1** Clinical and demographic characteristics for all participants and groups

|                         | Allª (N=70) | MBPª (N=35) | Controlª (N=35) |
|-------------------------|-------------|-------------|-----------------|
| **Sociodemographic characteristics** |             |             |                 |
| Age (years)             | 11.03 (2.78) | 11.66 (2.68) | 10.40 (2.77)    |
| Sex (% male)            | 44 (62.9%)   | 18 (51.4%)  | 26 (74.3%)      |
| Weight (kg)             | 40.53 (13.69)| 46.24 (13.06)| 34.82 (11.95)   |
| Height (m)              | 1.47 (0.17)  | 1.53 (0.15) | 1.41 (0.17)     |
| BMIª (kg/m²)            | 18.27 (3.76) | 19.46 (3.89) | 17.04 (3.24)    |
| **Clinical characteristics** |             |             |                 |
| Psychiatric comorbidities |             |             |                 |
| Yes (%)                 | 38 (54.3%)   | 23 (65.7%)  | 15 (42.9%)      |
| No (%)                  | 32 (45.7%)   | 12 (34.3%)  | 20 (57.1%)      |
| Medication during intervention |         |             |                 |
| No change in medication (%) | 7 (10%)     | 6 (17.10%)  | 1 (2.9%)        |
| Stimulant washout (%)   | 5 (7.1%)     | 2 (5.7%)    | 3 (8.6%)        |
| No medication (%)       | 58 (82.9%)   | 27 (77.1%)  | 31 (88.6%)      |
| ADHD-RSª,c,d > 80th percentile | 23.37 (12.25) | 21.20 (12.13) | 24.04 (12.43) |

*N sample size
ªData are mean (SD) or proportions (%) unless otherwise stated
ªBody mass index
ªªADHD Rating Scale Total Score; > 80th percentile—the number (%) of the participants with raw scores higher than the 80th percentile score on ADHD-RS total score
marginal means indicated a small (d = 0.011, respectively, d = 0.015) non-statistically significant reduction in omission errors from pre- (M = 14.63, SE = 3.91) to post-treatment (M = 14.38, SE = 3.94) and 4-week follow-up (M = 10.80, SE = 3.93). Participants in the intervention group also displayed significantly fewer omission errors, (M = 14.38, SE = 3.94) post-intervention relative to control (M = 34.97, SE = 3.95). Results of the LMM analyses for the primary and secondary outcomes are presented in Table 3. The LMM analyses with age as a covariate revealed the same significant results, showing the consistency of the previous analyses. Secondary outcomes, including changes in CVC, assessed by vagally mediated HRV (HF-HRV, RMSSD), from pre- to post-treatment, pre-treatment to follow-up, and post-treatment to follow-up, indicated significant differences between the two conditions and a significant main effect of time on RMSSD values. Estimated marginal means revealed that there was a small (d = 0.37) statistically significant increase in RMSSD values from pre- (M = 3.94, SE = 0.11) to post-treatment (M = 4.18, SE = 0.11) only in the intervention group. Post-intervention the RMSSD values were significantly different between groups, and the mean difference was 0.353 (95% CI 0.048–0.658, p = 0.024, Cohen’s d = 0.58).
Furthermore, no significant differences were found for HF-HRV.

The LMM analysis of change in mood, assessed by EDSS, revealed a significant main effect of time on EDSS scores. Post hoc LMM contrasts showed that participants reported a significant decrease in EDSS score from pre- ($M = 3.42$, SE = 0.09) to post-intervention ($M = 2.80$, SE = 0.10); at the 4-week follow-up, the EDSS increased but remained significantly different from baseline ($M = 2.95$, SE = 0.10). However, the time-by-group interaction and the main effect of the group were non-significant, indicating that the decrease in this outcome post-intervention was not significantly different between the conditions.

Discussion

In this RCT, we examined the effectiveness of a single session of mindfulness-based cognitive training for improving Conners’ continuous performance test scores, vagally mediated HRV, and mood in a sample of 6- to 17-year-old children and adolescents diagnosed with ADHD. We hypothesized that CPT scores, CVC, and mood would significantly improve after the intervention. Results partially confirmed our hypothesis; there was a small positive post-treatment effect on one CVC-related measure (RMSSD). Contrary to our expectation, the results suggest that the brief MBI was ineffective for improving CPT-related scores of inattention and hyperactivity/impulsivity, neither CVC as assessed by HF-HRV, nor mood. There was a small non-statistically significant reduction in omission errors post-treatment, and several main effects of time were found, more specifically pre-post reduction in scores of commission errors and emotional distress and increase in hit RT. However, the change over time was not significantly different between conditions. This may be explained by the length of the mindfulness exercises, which was too short to induce change. Previous meta-analyses suggested that MBIs have medium to large effects on hyperactivity/impulsivity and inattention [6, 9]. Nevertheless, most of the studies included in these meta-analyses involved several weeks of training (4–12 weeks) with weekly sessions of practice and multiple modules (e.g., psychoeducation, introduction to the body scan, using the breath). Furthermore, these studies used behavioral measures, both self and other (participant’s parents or teachers) informant ratings to assess treatment effects of MBIs on ADHD symptomology, whereas we used a computerized attention test. Usually, the effect sizes for neurocognitive variables in comparable trials are typically small, even more so if an active control group is used [27]. Since the trial was powered to detect changes in ADHD symptoms as assessed by behavioral measures, we were likely underpowered for neurocognitive variables, such as CPTs. CPTs are commonly used to assess ADHD symptoms; numerous studies have shown that patients with ADHD exhibit performance deficits on the CPT, especially in the proportion of commission and omission errors [28]. Previous studies have indicated that a mindful breathing exercise (focus breathing) can immediately enhance performance on a working memory task [29] or an executive functioning task, such as the Stroop task [30]. In our study, mindfulness training did not increase the participants’ performance on the CPT task, except for the small but not statistically significant reduction in omission errors post-treatment. These results are consistent with [13] findings, showing the lack of effect of focused breathing mindfulness exercise on a simple sustained attention task, the attention task of the Toulouse-Pierron factorial battery [13]. One possible explanation for the lack of effect would be that one short mindfulness session may not have been strong enough to affect the participants’ performance on the cognitive task or that the task lacks the complexity to capture minor behavioral changes induced by a short mindfulness session. Future studies should also consider the task characteristics when investigating the efficacy of mindfulness to better understand how mindfulness interferes with cognitive processes.

Regarding the CVC, the MBI increased vagally mediated HRV assessed by time- (RMSSD) but not by frequency-domain (HF-HRV) measures. Although RMSSD and HF-HRV are highly correlated, the lack of improvement in HF-HRV might be related to measurement techniques. Usually, frequency-domain methods are recommended over time-domain methods for HRV recordings less than 5 min [31]. Our results are consistent with previous research showing that brief mindfulness interventions can enhance HRV [32, 33]. The overall effects of the MBI on HRV measures were small, in line with other studies; for a review, see Rådmark et al. [34], although the results in this area are mixed [35]. Even though the effect size was small, these results are particularly important. The reduced cardiac vagal tone, which can be influenced through specific non-pharmacological interventions such as mindfulness-based interventions, may be a promising option for children who insufficiently respond to current best practices in ADHD treatment.

As for the mood outcomes, our study has shown that a single session of MBI did not improve mood in children and adolescents with ADHD. Previous studies have shown that MBIs improve anxiety and mood symptoms, especially among patients with anxiety disorders and depression and even when these symptoms are associated with chronic medical conditions, such as cancer [36]. Moreover, brief mindfulness training interventions have been shown to be efficacious in reducing negative affectivity measures, a dimension of subjective distress [37]. In our study, the mood was operationalized similarly, as an aversive, negative, uncomfortable, or unpleasant emotional state, such
as anxiety, worry, depression, or anger. A potential reason for the inefficacy of the intervention could be related to the small sample size; a recent meta-analysis indicated that brief MBIs have an immediate and significant (albeit small) effect on decreasing negative affectivity in both nonclinical and clinical samples [37]. Another reason for the inefficacy of the MBI could be related to the type of the control program, which could hold the potential also to elicit mood changes while the outcome assessed only focused on evaluating four negative emotions.

**Strengths and limitations**

The current trial had several strengths. First, we used a customized protocol intervention for children and adolescents with ADHD, which was created to address potential psychophysiological mechanisms (autonomic dysfunction and emotion dysregulation) and symptoms. Second, this is the first trial that used a brief MBI for children and adolescents with ADHD. Most of the research in this area involves intensive multi-week training [6]. Typically, children have less developed memory and attentional capacities [38] than adults; consequently, they may benefit from shorter interventions. In the third place, the study had a strong RCT design; we recruited patients from regular mental health services, used rigorous methods for randomization and well-validated measures of ADHD, and a longitudinal assessment of treatment, which increases the generalizability of our results. Furthermore, no adverse events during the intervention were reported, and the dropout rate was low. In the fourth place, we used an active control condition where participants listened to a chapter of an audiobook. The active comparison with a well-matched control condition (groups closely resembled the intervention in terms of duration and the amount of required activity) could more accurately account for general or non-specific intervention effects [37]. Nonetheless, this study had several limitations. One notable limitation is the relatively small sample size. Even though the study was powered to detect large changes in ADHD symptoms as assessed by behavioral measures, it may have been underpowered to detect changes in neurocognitive variables, such as CPT, and the potential beneficial effects of MBI on HRV and mood. Future replication studies should consider extending the sample size to detect between-group differences adequately. Furthermore, in our study, we used multiple outcome variables to assess the same concept, e.g., omission errors, reaction time, and detectability as indicators of inattentiveness; usually, the power analyses are based on one outcome measure. Second, we did not check for different levels of engagement in mindfulness training or the participants’ mindfulness skills. Although we assessed the engagement in the intervention, the question was broad and did not differentiate between varying levels of engagement. Previous studies have shown that poor engagement could limit the intervention’s effectiveness [39]. In addition, low trait mindfulness skills could interfere with engagement in mindfulness practice [40]. Future studies need to carefully assess the level of engagement in training and baseline trait mindfulness skills to differentiate the therapeutic impact of engaging in mindfulness practices. Third, this study was conducted face-to-face while maintaining the safety measures introduced during the COVID-19 pandemic (e.g., wearing face masks and social distancing), which may have impacted the effectiveness of the intervention.

In conclusion, this is the first trial that systematically examined the potential benefits of a brief mindfulness intervention in children and adolescents with ADHD. The current findings suggest that one session of mindfulness-based training was insufficient to affect the CPT’s inattention and hyperactivity/impulsivity scores, neither vagally mediated HRV assessed by HF-HRV, nor mood. The improvement in CVC indexed by time-domain measures, the positive evaluation of the program, and the high rate of adherence suggest that this type of intervention could be easily implemented in different settings, such as the classroom. This study represents an initial attempt to deliver a more accessible and flexible mindfulness intervention for children and adolescents with ADHD.

**Author contributions** All the authors contributed to the study’s conception and design. AR: performed material preparation, data collection, and analysis. The first draft of the manuscript was written by AR as well. AD: methodology, supervision, and writing—review and editing.

**Funding** No funding was received for conducting this study.

**Availability of data and materials** The data will be provided on request.

**Code availability** Not applicable.

**Declarations**

**Conflict of interest** The authors declare no conflict of interest.

**Ethics approval** This trial was approved by the Research Ethics Board of Babeș-Bolyai University (approval number: 4171/04.03.2020). The procedures used in this study adhere to the tenets of the Declaration of Helsinki and its later amendments or comparable ethical standards.

**Consent to participate** All the participants included in the study and their parents or legal guardians provided informed consent.

**Consent for publication** Not applicable.

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