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Melatonin supplementation for the treatment of infantile spasms: protocol for a randomised placebo-controlled triple-blind trial

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ABSTRACT

Introduction Infantile spasms (IS) is a type of severe epileptic encephalopathy that occurs in infancy and early childhood. IS is characterised clinically by epileptic spasms, often accompanied by sleep disorder and abnormal circadian rhythm. The endogenous circadian rhythm disorder, in turn, can make spasms worse. Melatonin has also been found to have anticonvulsant and neuroprotective properties by adjusting the circadian rhythm. However, there are lack of relevant studies on controlling IS by using melatonin. This study aims to analyse the therapeutic effect of melatonin supplementation for the treatment of IS.

Methods and analysis This is a triple-blinded (trial participant, outcome assessor and the data analyst), prospective, randomised controlled trial to be conducted in the Department of Paediatrics, The First Medical Center of Chinese PLA General Hospital, Beijing, China from November 2020. Patients (n=70) aged 3 months to 2 years with IS will be recruited in this study after receiving written consent from their parents or guardians. Patients will be randomly divided into two equal groups and treated with a combination of adrenocorticotropic hormone, magnesium sulfate and either melatonin or placebo. Clinical data from the patients in the two groups before and after the treatment will be collected and compared. The primary outcome will be assessed 2 weeks later by seizure diaries and reported as the average reduced rate of spasms frequency. Secondary outcomes include the response rate (the rate of spasms-free), electroencephalogram hypsarrhythmia assessment and the psychomotor development assessment (Denver Developmental Screening Test). Sleep quality and safety will also be assessed.

Ethics and dissemination The protocol for this study was approved by the Ethics Committee of Chinese PLA General Hospital (reference number S2020-337-01) and was reported according to the Standard Protocol Items: Recommendations for Interventional Trials statement. Findings of this research will be disseminated through national and international meetings, conferences and peer-reviewed journals.

Trial registration number ChiCTR2000036208.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a prospective, randomised, controlled and triple-blinded trial.
⇒ Actigraphy sleep monitoring wristwatch can help monitor pre-treatment and post-treatment sleep quality.
⇒ The single-centre study design limits the generalisability and external validity of the results.
⇒ It is difficult to assess the seizure response directly to adjunctive melatonin supplement or not.
⇒ The main outcome is the short-term efficacy of melatonin on infantile spasm, so there is a lack of long-term follow-up.

INTRODUCTION

Infantile spasm (IS), also known as West syndrome, is an age-specific epileptic syndrome in children under the age of 2 years, which occurs in 2–5/10 000 infants and is characterised by a triad of epileptic spasms, hypsarrhythmia on electroencephalogram (EEG) and neurodevelopmental regression.[1–3] Treatment strategy for IS has not changed for the last few years and the optimal treatment of IS is still unknown.[4] At present, adrenocorticotrophic hormone (ACTH), oral corticosteroids and vigabatrin are first-line treatments for IS, but there are limited response rate and inevitable side effects.[2 5] There is an urgent need to identify alternative treatments with fewer adverse effects for children with IS.

Previous studies have suggested that melatonin had an anticonvulsant effect with a good safety profile.[6] Melatonin is an indolamine synthesised in the pineal gland from tryptophan, and releases in a circadian pattern. It can scavenge free radicles, and it is a strong antioxidant and anti-inflammatory agent,[7] which plays an important role in regulating sleep and circadian rhythm.[8] Some researchers have also suggested an anticonvulsant role of melatonin since the early 70s.[9] Numerous reports have demonstrated that exogenous melatonin may have

anticonvulsant effect both in vitro and in vivo.[10–16] The anticonvulsant property of melatonin is mediated by its effect on the neurotransmitters including N-methyl-D-aspartate (NMDA)[15] and γ-aminobutyric acid,[17–19] and also its inhibition of Ca^{2+} channels which disrupts neuronal activity[20] or induces antioxidant effect.[21]

Given melatonin's role in regulating sleep disorders, it can potentially prevent convulsion. Disrupted sleep patterns can exacerbate the severity of seizures,[22] whereas endogenous circadian rhythm disturbances caused by sleep disorders can induce various types of epilepsy.[23] And IS is often accompanied by abnormal circadian rhythms. Spasms occur in clusters throughout the day and night but rarely occur during sleep. Frequently, they occur immediately on arousal.[2 24 25]

Although the safety of long-term use of melatonin in children is lacking, existing studies have demonstrated its safety.[6 26] Therefore, as a potential anticonvulsant drug, it can be thought that melatonin may provide a new potential option for the treatment of IS.

In clinical practice, melatonin is mainly used in the treatment of sleep disorders on children with neurological and developmental problems.[6 27] Little is known about the efficacy of melatonin against epilepsy. And the existing findings on the evaluation of melatonin therapy in epilepsy are inconsistent.[28] Some reports suggest that melatonin is effective against seizures, but others show that it in fact exacerbates the condition.[29] Most human trials and studies involve relatively few patients, and most of them are neither blinded nor placebo-controlled. Given the paucity of data, the efficacy and tolerability of melatonin as add-on treatment for epilepsy remains inconclusive.[6]

Hence, we will conduct a randomised, triple-blind, placebo-controlled trial to further investigate the effect of melatonin.

OBJECTIVE

The primary objective in this study is to assess the efficacy of melatonin supplementation for the treatment of infantile spasms, by comparing the effect of melatonin with placebo on spasms control, hypsarrhythmia on EEG, change in psychomotor development and sleep quality. Other exploratory outcomes will also be assessed. For example, whether melatonin can reduce the patients' high excitability of ACTH treatment by improving sleep quality.

METHODS

Trial hypothesis

Our main hypothesis is that melatonin supplementation can improve the seizure control rate in the treatment of IS. The second hypothesis of this study is that melatonin can improve EEG hypsarrhythmia and sleep quality better than placebo, and can reduce the side effects of ACTH.

Trial design

This is a prospective, randomised, controlled and triple-blinded (trial participant, outcome assessor and the data analyst) trial with an allocation ratio of 1:1. The Consolidated Standards of Reporting Trials diagram for this study is shown in figure 1.

Trial registration and setting

This trial is already registered in the Chinese Clinical Trial Registry (ChiCTR2000036208).

This trial is being conducted at the Department of Pediatrics, The First Medical Center of Chinese PLA General Hospital, Beijing, China.

Participants

We will assess the eligibility of all patients with IS that are recruited by the Chinese PLA General Hospital. The clinical diagnosis of IS will be performed in accordance with the 2017 International League Against Epilepsy guidelines.[3] At least two paediatric neurologists will screen participants according to the inclusion and exclusion criteria. Consent to participate in this study will be obtained from parents or guardians of eligible participants.

Inclusion and exclusion criteria

Only patients with IS, aged ≥3 months and <2 years will be included in this study.

Those who fulfil any of the following criteria will all be excluded from this study. Exclusion criteria are as follows: (1) age <3 months or ≥2 years; (2) received hormone, ACTH therapy, vitamin B_6, melatonin or melatonergic drugs within 1 month; (3) the type of seizures or the type of antiseizure medications (ASMs) changed within 1 month; (4) taking sedatives or benzodiazepines drugs within 1 month; (5) bleeding or clotting disorders or taking anticoagulant; (6) allergic to melatonin; (7) diabetes mellitus; (8) incomplete medical history and (9) no interest in participating.

Patients with identified aetiology receiving epilepsy surgery and patients receiving therapies including ketogenic diet or vagus nerve stimulation will also be excluded at the data cleaning stage and will not be included in the final analysis.

Participant withdrawal

Patients who develop serious adverse events such as life-threatening infection, severe diarrhoea, hypertension, arrhythmia, electrolyte disorder and other complications after treatment with ACTH in combination with $MgSO_4$ for <2 weeks during the study period will be withdrawn from the trial and will be included in the final safety analysis, but not in the final efficacy analysis. All captured data will be stored and handled with extreme confidentiality.

Sample size

For the primary outcome variable, based on data from the pilot study of 14 patients with IS, we assume that the average reduced rate of spasms frequency (mean±SD) in

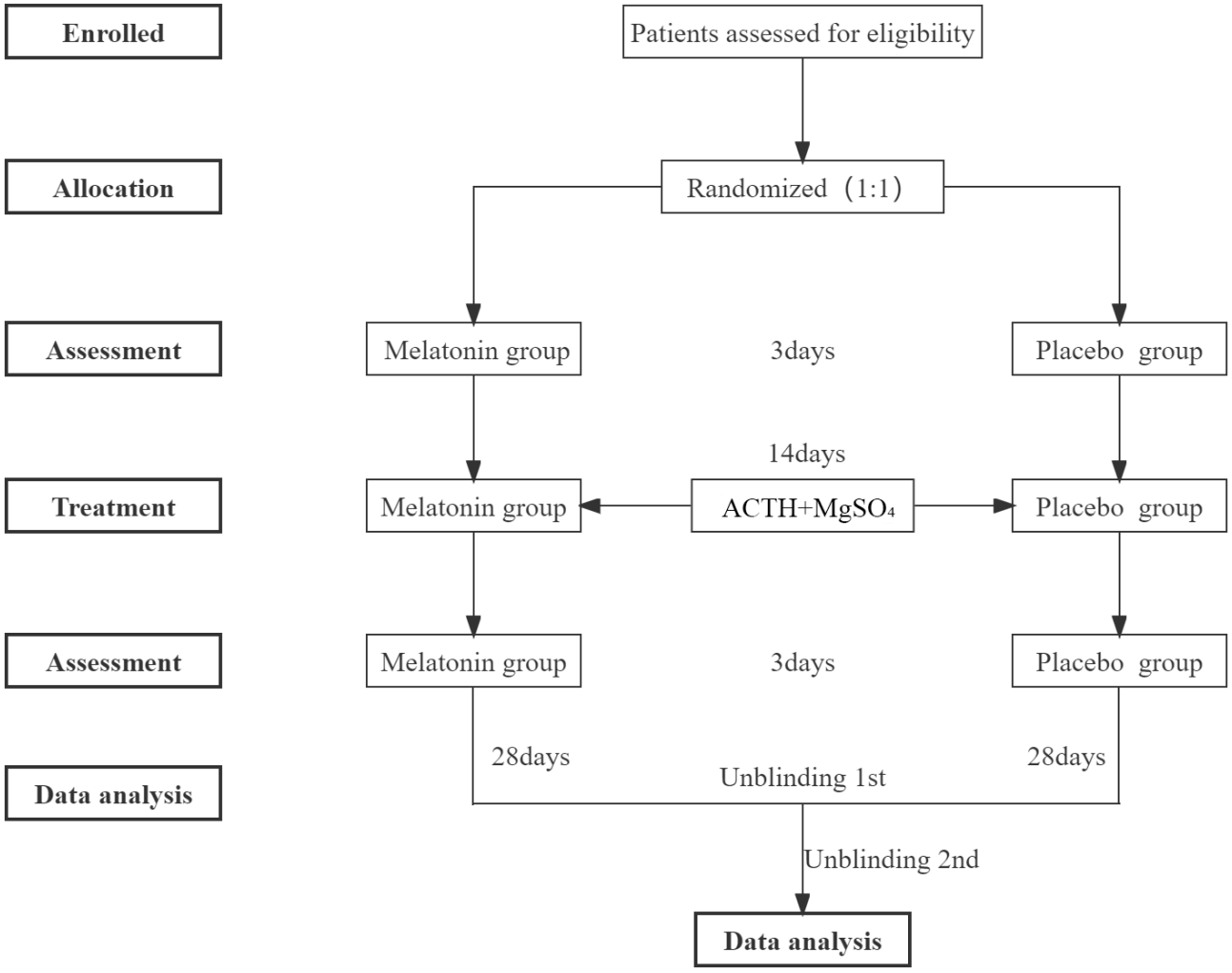

Figure 1 The Consolidated Standards of Reporting Trials diagram of the trial. ACTH, adrenocorticotropic hormone; $MgSO_4$, magnesium sulfate.

melatonin group is 80%±30% and 40%±70% in placebo group, with power 80% and significance level of 5%. This gives a sample size of 30 in each group (60 in total). The final sample is inflated to 35 in each group (70 in total) with due consideration of about 10% loss during study period.

Preparation of melatonin and placebo

Melatonin (3 mg, extended-release) tablets will be prepared at Timage Natural Bioengineering (Beijing, China). Placebo tablets identical to the melatonin tablets in shape, size, colour and packaging except for lacking the active agent will be specially prepared for the study at Timage Natural Bioengineering.

Randomisation and blinding

Patients will be equally and randomly allocated to either melatonin treatment or control group. Randomisation will be generated with a computer-generated sequence by specialised personnel not privy to the study. Participants will be numbered from 1 to 70 according to the order of recruitment. The number will remain unchanged throughout the trial. The melatonin and placebo will be well packaged and coded. The patient and study investigators will be blinded to the identity of the treatment. The trial assessor and data analyst will also be blinded. Allocation codes will be disclosed only after the entire clinical trial is completed.

Clinical assessment

Data to be collected from eligible participants will include the gender, age at enrolment, personal history, family history and medical history, including age of onset of seizure, type and aetiology of the seizure, treatment interval and history of ASMs. Clinical examination will include screening of inherited metabolic abnormalities, genetic testing, previous EEG results, neuroimaging (CT and/or MRI brain) and other clinical data. Assessment of spasms frequency, sleep quality, EEG, Denver

Developmental Screening Test (DDST) and other clinical parameters at baseline will be performed within 3 days before treatment. The assessments will be repeated within 3 days before the end of 2 weeks treatment. And then, we will have a short 28-day follow-up period, during which certain items including seizure and sleep quality will be assessed. Particularly, samples for laboratory examination will be collected on the third day of the first assessment after the children synchronise their sleep to the hospital routines, and be collected again on the last day of the treatment.

Interventions

The ASMs taken by the patients before treatment will remain unchanged. All children will be treated with a combination of ACTH and magnesium sulfate ($MgSO_4$) for 2 weeks.[30] The specific scheme is as follows: 2.5 U/kg ACTH will be dissolved in 100 mL of 5% glucose solution and administered intravenously for >6 hours. Simultaneously, 0.25 g/kg $MgSO_4$ (25%) will be dissolved in 100 mL of 5% glucose solution and administered intravenously for >5 hours. The dripping speed will be controlled by using a microinjection pump. The patients will be randomly divided into two groups. The melatonin treatment group will receive melatonin (3 mg), whereas the control group will receive placebo. Both melatonin and pacebo will be administered between 20:00 and 21:00 hours, daily, 0.5–1 hour before bedtime.

Outcome measures

The main outcome is the short-term efficacy of the melatonin treatment. The patients will be observed over 3 days[31] during the hospital stay period to minimise the influencing effect of adjustment of ASMs. The total number of spasms in a single day will be recorded by caregivers and confirmed by researchers. The scoring of each item as mentioned in table 1 will be performed independently by two independent specialists.

The primary outcome is the average reduced rate of spasms frequency, assessed as follows: (frequency at baseline–frequency after treatment)/(frequency at baseline)×100%.

Secondary outcomes include: (1) the 3-day response rate (response is defined as spasms-free for 3 days before the end of treatment) and the 28-day response rate (response is defined as spasms-free for 28 days after treatment)[32 33]; (2) EEG hypsarrhythmia assessment, categorised as 'disappearance of hypsarrhythmia (normal EEG or multifocal spike wave) or no improvement (hypsarrhythmia or modified hypsarrhythmia)',[30 32] and quantified as previously described with the Kramer score ranging from 0 (least severe) to 16 (most severe).[34] EEG assessment was performed by using EEG recordings of ≥60 min in length, including at least one awake-sleep-awake cycle; (3) the psychomotor development assessment, defined as the difference between the months of developmental delay between baseline and the last assessment. Developmental evaluation will be performed by using the DDST.[35]

Sleep quality improvement

Sleep quality will be assessed both subjectively and objectively.[36] Subjective sleep assessment will be performed using Sleep diaries, Brief Infant Sleep Questionnaire[37] and Infant Sleep Assessment Scale,[38] which will be published and adapted by the Provincial Research Working Group of Chinese Infant Sleep Assessment Scale. Objective sleep assessment will be assessed by actigraphy (Actiwatch AW64; Mini Mitter, Sunriver, Oregon, USA).[39 40]

Safety parameters

All the adverse events (event needing intervention or not disappearing) including abnormal blood parameters or other laboratory examinations, development of skin rash and other serious allergic reactions, drowsiness and other potential adverse drug reactions during the treatment will be recorded.

Data collection

Data will be collected directly into electronic case report forms or transcribed from paper forms, and crosschecked the missing data. The data analysts will be blinded to the nature of data and the type of treatment that the study participants received. The trial schedule is presented in table 1.

Data management and confidentiality

No separate data monitoring committee will be established. Each patient will be assigned a unique identification code in such a way that they cannot directly be traced back to the identity of the participant. The identifiers for the participant will be used when disseminating the findings of this research. Face sheets containing protected personal identifiable information will be removed from filled questionnaire. Data from all patients will be stored and handled with extreme confidentiality according to Chinese privacy legislation and the International Council for Harmonisation-Good Clinical Practices (ICH-GCP) regulations. Data will only be accessed by authorised persons. Data will be kept for 5 years and after that period the key files will be destroyed.

Patient and public involvement

The participants were not involved in designing, conducting or reporting this study. However, the guardians/parents of the study participants will be fully informed about the goals and designs of the research. The result of this trial will be shared with enrolled patients by telephone within 1 year after the end of the trial.

Statistical analysis

Data will be analysed using SPSS V.25.0 (IBM Statistics for Windows, Armonk, New York, USA). Parametric data will be summarised based on medians (IQR), whereas continuous and non-parametric data will be expressed as mean±SD. Categorical data will be expressed as counts (percentages). Homogeneity between variance of groups will be analysed using Levene's test. Difference between groups will be analysed using independent t-tests or

Table 1 Schedule of enrolment, interventions, assessments and data collection

	Screening	Randomisation	Assessment	Treatment	Assessment	Follow-up	Close-out
Time point		D0	D1–3	D4–17	D15–17	D18–45	
Enrolment							
Informed consent	√						
Eligibility screen	√						
Basic information	√						
Medical history	√						
Clinical examination	√						
Allocation		√					
Assessment:							
Seizure diaries*			√		√	√	
Sleep							
Sleep diaries			√		√		
BISQ			√		√	√	
ISAS			√		√	√	
Actiwatch monitor			√		√		
EEG			√		√		
Kramer scale			√		√		
DDST			√		√		
Laboratory examination							
Blood rountine			√		√		
Biochemistry			√		√		
Urinalysis			√		√		
Stool routine			√		√		
Serum melatonin (06:00 hours)			√		√		
Intervention:							
Melatonin				√			
Placebo				√			
Adverse effects					√		
Unblinding first							√
Data analysis							√
Unblinding second							√
Data analysis							√

*Seizure diaries—to be entered in eCRFs through discussion with patients and review of their seizure diary.
BISQ, Brief Infant Sleep Questionnaire; DDST, Denver Developmental Screening Tests; eCRF, electronic case report form; EEG, electroencephalogram; ISAS, Infant Sleep Assessment Scale.

univariate analysis of covariance, whereas Mann-Whitney U test will be used for non-parametric data. Differences between categorical variables will analysed using $\chi 2$ or Fisher's exact test. A logistic regression model will be established to further analyse the factors affecting the differences in efficacy.

All p values will be reported to three decimal places with those <0.001 reported as p<0.001. Statistical significance will be set at two-tailed p<0.05.

Trial status

The first participant was enrolled on 9 November 2020. Recruitment was completed in December 2021. The trial was completed in March 2022.

ETHICS AND DISSEMINATION

Potential benefits and risks

Existing evidence suggest that melatonin is unlikely to exacerbate the severity of seizures.[29] Even though long-term safety reports of melatonin use in both children and adults is lacking, the drug has not been reported to cause adverse effects.[6 41] Patients will probably benefit from

melatonin treatment, which may reduce seizures and improve sleep quality and EEG performance and so on. And we will cover the cost of managing or treating any adverse events arising from the treatment.

Ethical approval

The protocol for this study has been approved by the Ethics Committee of Chinese PLA General Hospital (reference number: S2020-337-01 on 24 September 2020), and will be conducted in line with the Helsinki Declaration, local laws and the ICH-GCP guidelines. The consent to participate in this research will be obtained from the guardians/parents of the potential participants, and a participant can be withdrawn at any time without risk of any kind.

Dissemination

The manuscript for the study findings will be drafted by the investigators. Findings of this study will be disseminated through high-impact peer-reviewed journals as well as presentation in national and international conference.

DISCUSSION

Since the initial report showed that the melatonin administration may lower the electrical amplitude of EEG recordings,[7] numerous studies had been conducted to assess the efficacy of melatonin against epilepsy.[16 42 43] But most studies on melatonin and epilepsy primarily aimed to evaluate the effect of melatonin on wake-sleep disorders, not the efficacy and tolerability of add-on melatonin as treatment for epilepsy.[6] And the available studies only used a small sample size and were neither blinded nor controlled.[29] Contradictory results have also been observed. The effect of melatonin on different types of epilepsy is yet not clearly revealed.

IS is a severe form of epilepsy, often with comorbidity sleep disorders. Patients with IS display abnormal circadian rhythm, whose spasms often occur during the day or in wakefulness state, but more frequently in the drowsiness state or shortly after waking up. ACTH is the first-line drug for IS treatment. Recent study found that ACTH combined with melatonin significantly increased the expression of circadian genes and ameliorates NMDA-induced seizures. The anticonvulsant effects of ACTH and melatonin are thought to be mediated by the expression pattern of circadian genes.[44] Accordingly, this trial was designed to assess the efficacy of melatonin in combination with ACTH in the treatment of IS. We aim to draw definite conclusions about the role of add-on melatonin in reducing spasms frequency or improving the quality of sleep or about the adverse events and so on.

This is the first prospective, randomised, controlled and triple-blinded trial to focus on the efficacy and tolerability of add-on melatonin as treatment for epilepsy. Eligible children will be treated for 2 weeks after admission and will undergo pre-treatment and post-treatment short assessments. Hospitalisation improves adherence to intervention protocols. We define the primary outcome measure as the average reduced rate of spasms frequency. All recorded seizures will be confirmed by researchers. For definite epileptic spasms, the investigator will make calculations based on the caregivers' records. For ambiguous spasms, we will ask caregiver to record a video of the patient's seizure, which will be confirmed by the researcher based on the caregiver's description and video recording. In addition, in order to reduce the impact of adjusting ASMs on treatment outcomes, we set the primary outcome of the assessment as a 3-day observation period. For a more comprehensive assessment of treatment outcomes, we also perform a 4-week assessment observation after the end of treatment. At the same time, we also calculate the response rate and assess EEG hypsarrhythmia, the psychomotor development and sleep quality. Actigraphy sleep monitoring wristwatch can help monitor pre-treatment and post-treatment sleep quality.

However, the main outcome of the study is the short-term efficacy of melatonin on IS, and there is a lack of long-term follow-up. And the single-centre study design also limits the generalisability and external validity of the results. Multicentre studies with large sample sizes may be required in the future.

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Contributors YS grafted the first manuscript. GY and TX contributed to the study conception and design of the study. JC and WF supervised EEG and sleep quality assessment. ML oversaw the randomisation of study participants, data management and analysis. JW formulated and designed the placebo and melatonin. XS and LZ participated in the formulation of the study design and protocol, drafting of the manuscript and data collection. Authorship eligibility on resulting manuscripts will follow standard guidelines.

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Disclaimer The funders did not participate in formulating the study design, data collection, analysis and interpretation of the findings.

Competing interests By partnership agreement, Timage Natural Bioengineering (Beijing, China) donated all melatonin study preparations. Dr Tianming Li is responsible for the formulation of the placebo and melatonin preparation; however, neither Dr Tianming Li, nor Timage Natural Bioengineering had any input into other aspects of study design, and will have no involvement in analysis or interpretation of results. The authors declare no conflict of interests.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained from parent(s)/guardian(s)

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