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Efficacy and safety of allogeneic umbilical cord blood mononuclear cells therapy in children with autism spectrum disorder and immune dysregulation: a single-center, double-blinded, randomized, placebo-controlled trial

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Abstract

Background: Immune-inflammatory dysregulation is increasingly implicated in the pathogenesis of autism spectrum disorder (ASD). Umbilical cord blood mononuclear cells (UCB-MNCs), with their immunomodulatory effects, represent a promising therapeutic avenue by potentially alleviating neuroinflammation. This study aimed to evaluate the safety and efficacy of multiple intravenous infusions of allogeneic UCB-MNCs in children with ASD and peripheral immune dysregulation.

Methods: In this single-center, double-blind, randomized, placebo-controlled

trial (RCT), 34 children (aged 3 to 8 years) diagnosed with ASD were randomly assigned to receive intravenous infusion of allogeneic UCB-MNCs (3×10^8 cells per infusion) (n=17) or placebo (n=17) four times with 1-week interval, alongside their existing rehabilitation therapy, and followed up for 13 weeks. The primary endpoint were the Total score of the Social Responsiveness Scale-2 (SRS-2). Additional endpoints were changes of the five domains of the SRS-2, the Vineland Adaptive Behavior Scales-3 (Vineland-3), the Childhood Autism Rating Scale (CARS), Aberrant Behavior Checklist (ABC), Swanson, Nolan, and Pelham Rating Scale-IV (SNAP-IV), Self-Rating Anxiety Scale (SAS) and safety.

Results: At final follow-up, the UCB-MNCs group demonstrated a significantly greater reduction in the SRS-2 Total scores (LS Mean Difference = -6.03, 95% confidence interval [CI]: -11.93 to -0.14, $P = 0.045$) and Social Cognition domain (LS Mean Difference = -9.95, 95% confidence interval [CI]: -15.90 to -4.00, $P = 0.002$) scores compared to the placebo group. No major allogeneic UCB-MNCs transplantation-related adverse events occurred.

Conclusion: Allogeneic UCB-MNCs transplantation demonstrated preliminary safety and potential efficacy in improving specific aspects of symptoms in children with ASD and peripheral immune dysregulation.

This study was registered on ChiCTR.org.cn (Identifier: ChiCTR2400082762). Date registered 12 April 2024. https://www.chictr.org.cn/index.html.

Key words: Autism Spectrum Disorder, Umbilical Cord Blood Mononuclear Cells, Immune Inflammation, Randomized Controlled Clinical Trial, Cell Therapy

Background and objectives

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder, primarily characterized by persistent deficits in social communication and interaction, along with restricted and repetitive patterns of behavior[1]. Its prevalence has been steadily increasing globally[2-4]. Notably, a report from the U.S. Centers for Disease Control and Prevention (CDC) in April 2025 revealed that the prevalence of ASD among 8-year-old children has risen to

3.22%[5]. The first national epidemiological survey in China in 2020 indicated that the prevalence of ASD among children aged 6 to 12 years was 0.7%[6], underscoring the growing public health significance of this disorder.

While the pathogenesis of ASD remains incompletely elucidated, studies indicate it arises from genetic, environmental, and other factors[7, 8]. Mounting evidence highlights neuroinflammation and immune dysregulation as potential central pathological features [3, 9]. Abnormal activation of microglia and astrocytes, along with elevated levels of proinflammatory cytokines such as IL-1, IL-6, and TNF-α [10-13], have been consistently observed in individuals with ASD. These findings not only highlight the role of neuroinflammation in ASD pathogenesis but also suggest that immunomodulatory interventions may represent a promising therapeutic strategy.

Currently, treatment of ASD relies primarily on behavioral interventions, as no specific pharmacotherapies address core symptoms[14, 15]. This treatment gap imposes substantial burdens on affected families and society at large[2, 3, 16, 17]. Given the established role of immune dysregulation in ASD, immunomodulatory therapy targeting this pathological pathway has become a potential new research direction[18]. Stem cells have garnered extensive attention for neurological disease treatment, owing to their inherent regenerative, anti-inflammatory, and neuroprotective properties[19]. Among these, umbilical cord blood mononuclear cells (UCB-MNCs) have attracted interest due to their immunomodulatory, anti-inflammatory, and neuroprotective properties[19-22].

In the treatment of ASD, stem cells may be derived from the patient’s own body or from an allogeneic source. Although autologous UCB-MNCs carry no immune risks, their availability depends on storage at birth, and their functions may be affected by the child’s own immune status[23-25]. In contrast, allogeneic UCB-MNCs offer an “off-the-shelf” advantage, making them suitable for children without autologous cord blood storage. Additionally, cells derived from healthy donors may exhibit superior

immunomodulatory functions[26, 27]. However, allogeneic sources also face challenges such as HLA matching restrictions, the risk of immune rejection, and the accessibility of public umbilical cord blood bank resources. A systematic review[28] indicated that current data have not identified safety concerns associated with allogeneic umbilical cord blood infusion for the treatment of neurological disorders, even in cases of inconsistent HLA matching or without the use of immunosuppressive agents. UCB-MNCs have certain advantages. Bone marrow (BM) is a widely used source of mesenchymal stem cells (MSCs) in clinical trials[29]. However, the isolation of MSCs from BM and adipose tissue (AD) is problematic due to the invasive collection process, which may cause harm to donors. Although UCB-MSCs are easy to isolate, the success rate of cell isolation is unstable[30]. UCB-MSCs have been more extensively investigated in clinical translational research for certain diseases (such as Graft-versus-Host Disease [GVHD] and osteoarticular diseases), with the development of multiple standardized therapeutic protocols, and they indeed exhibit a lower risk of GVHD[31, 32]. UCB-MNCs, as a naturally occurring mixed cell population in umbilical cord blood, can be directly used for clinical infusion without in vitro expansion and culture[33]. At the same time, the incidence and severity of GVHD associated with UCB-MNCs remain relatively low, as they contain fewer T cells characterized by immature function[34]. The present study aims to supplement the efficacy and safety data of UCB-MNCs in children with ASD complicated with immune disorders.

Previous studies using UCB-MNCs or other stem cell types in ASD have shown preliminary promise, but their conclusions are limited by open-label designs, small sample sizes, and lack of placebo controls[26, 35-41]. To address these limitations, we conducted a single-center, double-blinded, randomized, placebo-controlled trial to evaluate the efficacy and safety of repeated intravenous infusions of UCB-MNCs in children with ASD and evidence of peripheral immune dysregulation.

Methods

Study design

This prospective, single-center, randomized, double-blinded, placebo-controlled trial was performed at the First Medical Center of Chinese PLA General Hospital (PLAGH; Beijing, China). The study protocol was approved by the Medical Ethics Committee of Chinese PLA General Hospital (approval number: S2023-681-02) and conformed to the Declaration of Helsinki guidelines. All participants' caregivers provided written informed consent before recruitment. This study was registered on ChiCTR.org.cn (identifier: ChiCTR2400082762).

Participants

The patients were all recruited prospectively from the outpatient departments of the PLAGH. A total of 55 individuals underwent screening for enrollment in the study. Among them, 16 were excluded due to failure to meet the criteria (10 had received other treatments within half a year, 3 did not meet the criteria for immune dysregulation, and 3 had immunodeficiency), and 3 declined to participate. In accordance with the inclusion and exclusion criteria, a total of 36 subjects were enrolled and randomized. All 36 subjects received four infusions, and 34 completed the final follow-up. Therefore, 34 children were ultimately included in the efficacy analysis (17 in each group) (FIGURE 1).

Inclusion criteria

Patients who met the following inclusion criteria were qualified for enrollment: (1) diagnosed with ASD as specified in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) [33]; (2) aged 3 to 8 years, with no restriction on gender; (3) with laboratory evidence of peripheral immune dysregulation, defined as having at least one serum cytokine (including IL-1, IL-2, IL-6, IL-8, or TNF-α) level exceeding 1.5 times the upper limit of the normal reference range; (4) guardian who can fully understand the study's informed consent and voluntarily sign it.

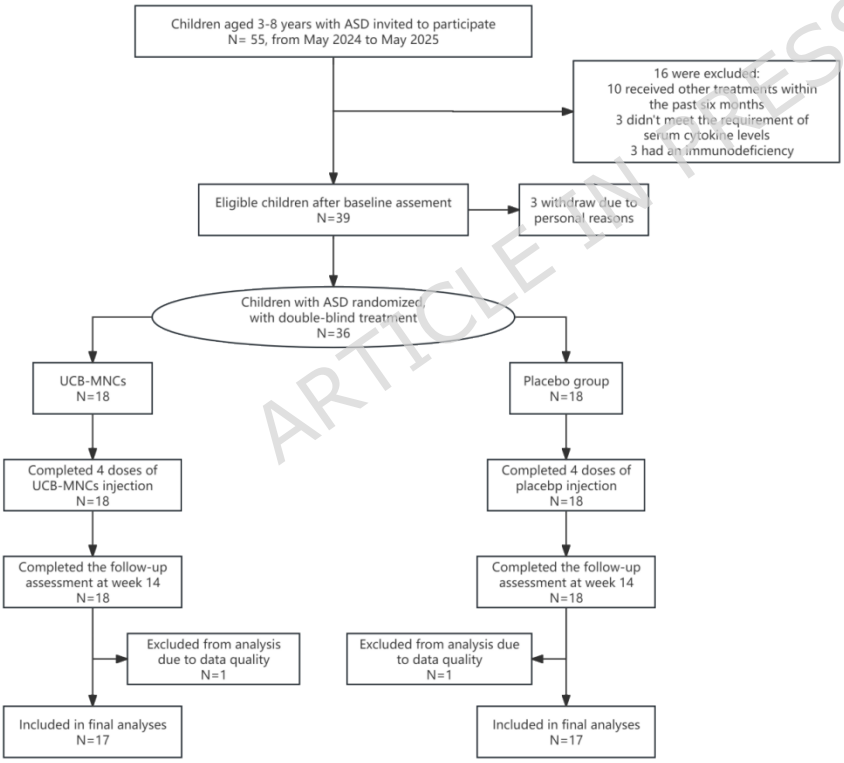
Exclusion criteria

Patients were excluded from this study if they (1) have clinical evidence of

active infection within the preceding four weeks; (2) have concurrent tumors or a history of tumors (including hematological tumors and solid tumors); (3) have immunodeficiency diseases or abnormal functioning of major organs; (4) have a history of severe allergic reactions or allergies to blood products; (5) have autism caused by epilepsy, cerebrovascular diseases, or traumatic brain injury, as well as mental disorders other than autism and ASD; (6) failure to meet inpatient or follow-up requirements, severe cognitive/motor impairment precluding assessments, receipt of new ASD intervention during enrollment, or refusal/withdrawal of consent.

All subjects were informed that they could withdraw from the trial freely at any time.

FIGURE1 Participant flowchart.



UCB-MNCs preparation and Infusion

UCB-MNCs characterization

UCB-MNCs were provided by the Shandong Provincial Cord Blood Hematopoietic Stem Cell Bank, sourced from multiple donors. All products strictly complied with Good Manufacturing Practice (GMP) standards. Prior

to infusion, the following tests were conducted: cell viability (viable cells ≥ 90%), pathogen detection (negative results for bacterial endotoxin, bacterial culture, fungi, mycoplasma, etc.), and flow cytometry phenotypic detection (CD34+ cell positive rate ≥ 0.05%). Each patient received an intravenous infusion once a week for four weeks. Patients in the experimental group were infused with 3×10^8 cells per infusion, while those in the control group were infused with saline of the same volume.

UCB-MNCs infusion

Intravenous injection is a non-invasive administration method for systemic infusion via blood vessels, featuring simple operation and repeatable dosing as well as enabling the systemic distribution of stem cells/drugs. However, it is associated with issues such as circulatory dilution, low homing efficiency to target tissues and poor ability to cross the blood-brain barrier (BBB), which may easily lead to insufficient effective concentrations in the central nervous system (CNS) and trigger immune clearance and off-target effects. Although cells may be retained in organs such as the heart, liver, kidneys or lungs, studies have demonstrated that intravenously infused cells can exert therapeutic effects by secreting brain-targeting factors[42]. Based on the above considerations, intravenous infusion was ultimately chosen as the administration route for UCB-MNCs to balance safety and treatment feasibility. Regarding the dosage, it was confirmed in[43] that the total nucleated cell dose of autologous UCB-MNCs administered via intracoronary infusion was $644.9\pm424.6\times10^6$ cells, and this dosage regimen exhibited good safety.

During the infusion process, the doctor supervised the whole procedure. At first, the infusion rate was set to a slow pace (20 to 30 drops per minute) and maintained for 10 minutes. If no adverse reactions occurred in the patient, the infusion rate was increased to 60 to 70 drops per minute, and the infusion was completed within 30 minutes. After the infusion, the patient stayed under medical supervision for two hours to observe and monitor for any potential adverse events (AEs) or serious AEs (SAEs). During the infusion treatment,

the patient's original routine medical care (such as behavioral intervention and rehabilitation training) was continued.

Measurements of outcomes

Primary Efficacy Outcome Measure

The Social Responsiveness Scale, Second Edition (SRS-2) is a quantitative tool for assessing the severity of autistic traits and monitoring intervention response, allowing detection of subtle longitudinal changes in symptom severity over time[44]. It comprises 65 items that evaluate social behavior deficits across five domains: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Restricted Interests and Repetitive Behavior, providing insights into social skills including these key dimensions. Each item is rated on a 4-point Likert scale (1 = Not True, 2 = Sometimes True, 3 = Often True, and 4 = Almost Always True). All SRS-2 assessments are completed in paper-and-pencil form by parents or primary caregivers under the guidance of a qualified clinician, with a completion time of 15 to 20 minutes. Raw data from completed rating forms is processed via PAR's official desktop scoring software (requiring a Rockey USB authorization key), which generates the total raw score and raw scores for the five subdomains. These raw scores are then converted into age-normed scores. Clinicians use these scores to compare an individual's social responsiveness to the normative sample. ASD symptom severity is categorized based on the total T-score, where higher scores indicate greater social functioning impairments: scores below 60 fall within the normal range; 60 to 65 denote mild impairment; 66 to 75 denote moderate impairment; and scores above 75 indicate severe impairment. Elevated T-scores are associated with more pronounced social deficits and autistic behaviors.

Secondary Efficacy Outcome Measures

The Vineland Adaptive Behavior Scales, Third Edition (Vineland-3)[45] is a comprehensive caregiver-report questionnaire designed to assess adaptive behavior across the entire lifespan, from birth to 90 years of age. It evaluates an individual's capacity for independent functioning and environmental

adaptation across 4 adaptive domains and 11 subdomains, including Communication Skills, Daily Living Skills, Social Skills, and Motor Skills, as well as the overall Adaptive Behavior Composite score. The scale consists of 458 items, each rated on a 3-point scale based on the frequency of independent behavioral performance (without prompts or assistance) in daily life (0 = Never, 1 = Sometimes, and 2 = Usually or Frequently). The Communication domain assesses auditory, receptive, expressive, and written abilities. The Daily Living Skills domain evaluates age-appropriate performance in daily tasks, including Personal, Domestic, and Community. The Socialization domain reflects functional performance in social contexts, with three subdomains: Interpersonal Relationships, Play and Leisure, and Coping Skills. The Adaptive Behavior Composite score is derived from the three core domains of Communication, Daily Living Skills, and Socialization. Since no official Chinese version of the Vineland-3 is available, the scale was translated into Chinese for parent/caregiver completion. The entire assessment process was facilitated by experienced clinicians to ensure accurate understanding and proper completion of the paper-based form. Raw responses were entered into the Pearson Q-global online scoring platform, which generated standardized scores algorithmically. In this study, standard scores were uniformly used for analysis, with lower scores indicating more severe impairments in adaptive behavior. A higher score indicates stronger abilities in independent living and social adaptation.

The Autism Behavior Checklist (ABC) is one of the most commonly used ASD assessment instruments in Mainland China. The ABC addresses 57 atypical behaviors (scored 1 to 4) related to five areas: sensory behaviors, relating behaviors, body and object use behaviors, language behaviors, and social self-help behaviors[46, 47]. This caregiver-reported questionnaire was completed by the patients' parents or caregivers throughout the study. Higher scores indicate more severe symptoms.

In contrast, the Childhood Autism Rating Scale (CARS) is a 15-item clinician-administered behavioral rating scale used to diagnose ASD and categorize

overall symptom severity, with a maximum score of 60[48]. Scores ranging from 30 to 36 indicate mild-to-moderate severity, while scores greater than 36 signify severe symptoms[49]. This scale was administered by qualified healthcare professionals across all study assessments. A higher score indicates more typical and severe autistic symptoms.

The Swanson, Nolan, and Pelham Rating Scale-IV (SNAP-IV) was employed to evaluate the dimensions of attention deficit and hyperactivity-impulsivity in children with ASD. A higher score suggests more prominent symptoms in the corresponding dimension.

The Self-Rating Anxiety Scale (SAS), developed by Zung, is a concise clinical instrument designed to evaluate individuals' subjective symptoms of anxiety[50]. It is applicable to adults with anxiety symptoms and has broad clinical applicability. Previous international research has validated that the SAS can effectively reflect the subjective feelings of individuals with anxiety tendencies seeking psychological help. The scale consists of 20 items, each rated on a 4-point Likert scale ranging from 1 (none, or a little of the time) to 4 (most, or all of the time). The raw score was calculated as the sum of all item scores. Raw scores were transformed to standard scores using a conversion factor of 1.25. For clinical interpretation, standard scores of 50–59 represented mild anxiety, 60–69 represented moderate anxiety, and scores ≥ 70 represented severe anxiety. A higher total standard score indicates a higher level of anxiety. This study was completed by the parents of the children under the supervision of the researchers. A higher total score indicates a higher level of anxiety.

Safety Outcome Measures

AEs and SAEs were defined in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, issued by the National Institutes of Health and the National Cancer Institute. All SAEs and unexpected SAEs were reported to the relevant regulatory authorities. AEs were classified into Grades 1 to 5 based on severity, with Grade 1 indicating mild symptoms (no intervention required) and Grade 5 indicating a fatal

outcome.

After infusion, the children's vital signs were monitored. If a subject was asymptomatic and showed no allergic reactions or unstable vital signs, they were discharged after the observation period. Meanwhile, caregivers were requested to record any observed changes in the child's behavior, irritability, or language ability. This supplemented data from a daily living perspective, reflecting the treatment's impact on the child's daily performance. Researchers documented in detail any post-infusion AEs (e.g., nausea, allergic reactions), including information such as symptom characteristics, onset time, and duration, to fully grasp safety issues during the treatment process.

Safety assessments, including a complete blood count (CBC) and liver/kidney function tests, were conducted at baseline, after the completion of all treatments (Week 4), and at the time of unblinding (Week 13 at final follow-up).

Laboratory parameters

Blood Routine, Biochemical Indexes, and Serum Cytokine Screening

All serum cytokine, blood routine, and biochemical indexes of the subjects in this study were derived from peripheral venous blood collected by the Clinical Research Center of our hospital in accordance with a unified Standard Operating Procedure (SOP). The serum cytokine assay was performed as a pre-enrollment screening. Only subjects who met the inclusion criteria based on cytokine levels proceeded to blood routine and biochemical testing. All tests were conducted by the Clinical Laboratory of PLAGH (the only designated testing institution) using the same detection platform, reagent batches, and operating protocols to ensure consistency and comparability of results. The specific procedures are as follows:

1. Pre-enrollment Serum Cytokine Screening

After excluding candidates with active infection in the past 4 weeks, blood samples were collected for serum cytokine detection. For each candidate, 3 mL of blood was collected using serum separation tubes (disposable vacuum

tubes from a uniform manufacturer). Immediately after collection, samples were placed in a 4℃ constant-temperature transport box and delivered to the laboratory within 30 minutes. Cytokine levels (including IL-1, IL-2, IL-6, IL-8, and TNF-α) were measured using a standardized immunoassay platform. Only candidates whose cytokine profiles met the pre-defined enrollment criteria (at least one serum cytokine level exceeding 1.5 times the upper limit of the normal reference range) were allowed to proceed to the subsequent research and treatment.

2. Blood Routine and Biochemical Indexes

Blood samples were collected from all subjects between 5:00 and 7:00 a.m. after ≥8 hours of fasting and in a resting state. For each subject, 2 mL of blood was collected for routine tests using EDTA-K2 anticoagulant tubes, and 3 mL for biochemical tests using serum separation tubes (both were disposable vacuum tubes from the same manufacturer). Immediately after collection, samples were placed in a 4℃ constant-temperature transport box and delivered to the laboratory for testing within 30 minutes. Blood routine tests included leukocyte count, erythrocyte count, hemoglobin (Hb), and platelet count (PLT). Biochemical tests consisted of liver function parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transferase (GGT), as well as renal function parameters, including serum creatinine (Scr), blood urea nitrogen (BUN), and serum uric acid (SUA).

3. Quality Control and Result Confirmation

Tests were performed by professionally qualified laboratory staff in compliance with the SOP. Before daily testing, the analyzers were calibrated and quality control was conducted; sample testing commenced only after quality control passed.

4. Data Collection and Compilation

Test results were automatically transmitted to the Hospital Information Management System (HIMS) to avoid manual entry errors. The research team uniformly extracted the raw data, which were then double-checked by

two independent researchers before compilation and archiving, ensuring the accuracy of data used for statistical analysis.

Taken together, all hematological and serum biochemical indices were assayed under a unified and rigorous experimental environment. Stringent standardization was applied to sample acquisition, pretreatment procedures, detection platforms, and quality control protocols. This standardized approach effectively guarantees the robustness and scientific rigor of the laboratory test results reported herein.

Calculation of sample size for specific objectives

Based on the results of a previous RCT on intestinal flora therapy for ASD[51], the mean SRS-2 scores of the experimental group and the control group were estimated to be 70 and 80, respectively (with a standard deviation of 10). Setting the test power at 0.8, the significance level at 0.05 (one-tailed), and the dropout rate at 10%, the calculation showed that 15 cases were required for each group. For the sample size calculation based on the Vineland-3 Adaptive Behavior Composite score, with a superiority margin set at 11 points and a dropout rate set at 20%, 18 cases were needed for each group. After comprehensive consideration, 18 cases were finally included in both the control group and the experimental group.

Randomization method

A simple randomization method was employed to allocate participants to the two groups. The randomization codes were generated by an independent statistician, external to the study team, utilizing SPSS software (Version 24.0). Personnel not involved in the clinical trial assigned codes to UCB-MNCs / placebos based on random numbers to create a blinding schedule, which was affixed as labels to product packaging. The blinding schedule was prepared in duplicate and stored separately by the principal investigator and the reagent supplier. The blinding process was supervised and quality controlled. After completion, the blinding schedule was sealed, and a coding process document was archived. UCB-MNCs / placebos were distributed to clinical center per centralized system-assigned numbers. Randomization

results were recorded in triplicate on dedicated forms, stored by responsible personnel. All codes and corresponding medication numbers were irrevocable post-allocation.

To maintain blinding, UCB-MNCs and placebo (saline) were identical in appearance, odor, and texture; infusion preparations and equipment were covered with light-shielding bags by designated personnel.

Statistical analysis

Categorical Variables : Compared using the Chi-square test (χ^2) or Fisher's exact test.

Continuous Variables : Presented as Mean ± SD or Median (IQR). Mixed Model for Repeated Measures (MMRM) were used to evaluate changes in SRS, Vineland-3, ABC, CARS, SAS (parent-reported), and SNAP-IV scores at baseline, Week 4, and Week 13. All statistical analyses were performed using JASP Software (Version 0.95.10.0) , R software (Version 4.4.2) and GraphPad Software (Version 10.2). Statistical significance was set at $p < 0.05$.

Results

Patient Characteristics at Baseline

Among the 34 children included in the analysis, there were 5 females (14.7%) and 29 males (85.3%).

The UCB-MNCs group and the control group were well balanced in terms of baseline characteristics, with no statistically significant differences in SRS-2, Vineland-3, CARS, ABC, SAS, SNAP-IV scores (TABLE 1) or serum cytokines (SUPPLEMENT TABLE1) , indicating good homogeneity between the two groups.

TABLE1 Patient characteristics at baseline.

Characteristics	UCB-MNCs group （n=17）	Control group （n=17）	*P*
Age (mean ± SD)	5.75 ± 1.29	5.78 ± 1.63	0.533
Sex N(%)			
Male	15 (88.2%)	14 (82.4%)	
Female	2 (11.8%)	3 (17.6%)	
SRS-2 (mean ± SD)			
Total score	77.59 ± 12.57	77.76 ± 8.57	0.966
Social Awareness	69.12 ± 11.65	72.53 ± 9.77	0.427
Social Cognition	76.65 ± 12.14	73.88 ± 8.25	0.360

Social Communication	76.94 ± 11.18	78.88 ± 7.50	0.676
Social Motivation	71.59 ± 10.85	69.24 ± 8.92	0.379
Restricted Interests and Repetitive Behavior	74.82 ± 15.11	75.76 ± 13.06	0.522
Vineland-3 (mean ± SD)			
Adaptive Behavior Composite	64.12 ± 9.87	61.41 ± 10.09	0.523
Communication Domain	60.29 ± 19.57	57.71 ± 22.37	0.501
Daily Living Skills Domain	71.47 ± 9.37	67.71 ± 9.82	0.269
Socialization Domain	55.71 ± 13.01	52.29 ± 12.97	0.489
ABC (mean ± SD)	52.71 ± 24.88	52.06 ± 26.92	0.993
CARS (mean ± SD)	33±9.5	33.71 ± 7.40	0.858

SUPPLEMENT TABLE1 Patient serum cytokins at baseline.

Serum cytokines	**UCB-MNCs group (n=17)**	**Control group (n=17)**	***P***	**reference range**
IL-1	5.00 (5.00, 5.00)	5.00 (5.00, 5.00)	>0.999	0-5
IL-2	831.00 (637.50, 889.50)	737 .00(618.00, 931.00)	0.689	223-710
IL-6	0.00 (0.00, 2.11)	2.05 (0.00, 2.81)	0.136	0-5.9
IL-8	18.50 (9.62, 45.05)	11.40 (9.45, 36.15)	0.786	0-62
TNF-α	14.70 (12.55, 18.55)	15.80 (13.40, 17.75)	0.779	0-8.1

CBC and Blood Biochemical Parameters

At Week 13, compared with baseline, there were no significant differences in serum alanine transaminase, aspartate transaminase, γ-glutamyl transpeptidase, uric acid, white blood cell count, red blood cell count, platelet count, or hemoglobin levels ($P > 0.05$, TABLE 2). In the UCB-MNCs group, serum creatinine decreased significantly compared with baseline, with a statistically significant difference ($P = 0.013$), while in the control group, blood urea nitrogen decreased significantly ($P = 0.023$).

TABLE2 Biochemical profile comparison between baseline and post-intervention in two groups.

Parameter		baseline, N=17[1]	Week 13, N=17[1]	*P*
Alanine Aminotransferase(ALT)	UCB-MNCs	14.55±5.59	12.87±5.39	0.124[2]
	Control	12.54±3.19	13.50±5.03	0.438[2]
Aspartate Aminotransferase(AST)	UCB-MNCs	26.65±4.90	25.05±4.16	0.091[2]
	Control	24.73±4.50	26.08±4.69	0.136[2]
γ-Glutamyl Transferase(GGT)	UCB-MNCs	11.01±3.06	11.49±3.38	0.298[2]

Serum Creatinine(Scr)	Control	9.3(8.8,11)	9.8(8.6,11)	0.653[3]
	UCB-MNCs	39.35±5.58	36.89±4.64	0.013[2]
Blood Urea Nitrogen(BUN)	Control	37.78±3.84	36.77±3.93	0.155[2]
	UCB-MNCs	4.40±1.39	4.02±0.95	0.243[2]
Serum Uric Acid(SUA)	Control	5.05±1.11	4.35±0.92	0.023[2]
	UCB-MNCs	246.11±55.13	242.34±59.31	0.761[2]
Leukocyte Count	Control	252.48±63.16	237.28±60.57	0.346[2]
	UCB-MNCs	7.92±2.67	7.21±2.26	0.135[2]
Erythrocyte Count	Control	7.91±1.22	7.90±2.51	0.988[2]
	UCB-MNCs	4.72±0.37	4.71±0.40	0.943[2]
Hemoglobin(Hb)	Control	4.73±0.24	4.66±0.26	0.333[2]
	UCB-MNCs	126.59±8.41	127.29±7.65	0.639[2]
Platelet Count(PLT)	Control	129.71±5.45	128.59±8.02	0.571[2]
	UCB-MNCs	314(291,389)	322(293,358)	0.552[3]
	Control	316(303,327)	331(254,336)	0.478[3]

[1]Median; Mean ± SD
[2]Paired t-test
[3]Wilcoxon signed-rank

Clinical Outcomes

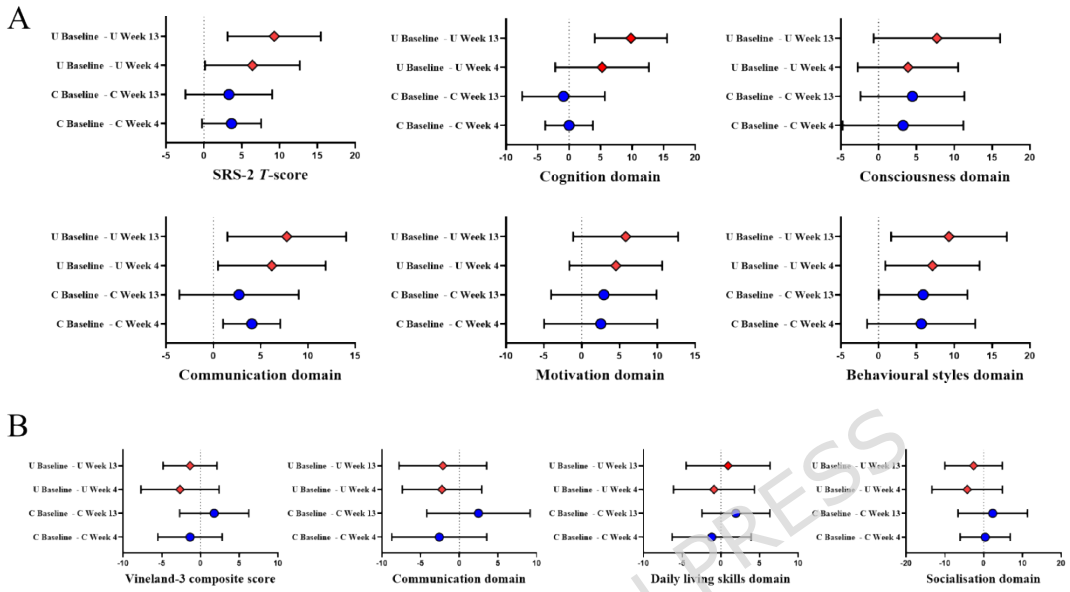
The MMRM results showed that the difference in the reduction of SRS-2 Total scores (LS Mean Difference = -6.03, 95% confidence interval [CI]: -11.93 to -0.14, P = 0.045) between the two groups were significiant. (TABLE 3; FIGURE 2A).

TABLE3 Changes in SRS-2 scores between two groups from baseline to Week 4 and Week 13.

SRS-2	Time point	LS Mean Difference	SE	95% CI		*P*
Total score	Week 4 vs Baseline	-2.80	2.52	-7.93	2.33	0.275
	Week 13 vs Baseline	-6.03	2.89	-11.93	-0.14	**0.045**
Social Awareness domain	Week 4 vs Baseline	-1.88	3.45	-8.92	5.15	0.589

Social Cognition domain	Week 13 vs Baseline	-4.47	3.61	-11.83	2.88	0.225
	Week 4 vs Baseline	-4.48	2.80	-10.18	1.22	0.119
	Week 13 vs Baseline	-9.95	2.92	-15.90	-4.00	**0.002**
Social Communication domain	Week 4 vs Baseline	-2.22	2.29	-6.89	2.45	0.340
	Week 13 vs Baseline	-5.16	3.14	-11.56	1.23	0.110
Social Motivation domain	Week 4 vs Baseline	-1.20	3.38	-8.09	5.70	0.726
	Week 13 vs Baseline	-2.08	3.17	-8.54	4.39	0.517
Restricted Interests and Repetitive Behavior domain	Week 4 vs Baseline	-1.80	2.80	-7.51	3.91	0.525
	Week 13 vs Baseline	-3.74	3.08	-10.02	2.54	0.234

FIGURE2
A: Comparison of SRS-2 Scale Scores in Children with ASD Before and After Treatment between two groups (n=17 per group at each time point).
B: Comparison of Vineland-3 Scale Scores in Children with ASD Before and After Treatment between two groups (n=17 per group at each time point).
Post-hoc tests were performed between groups using the Bonferroni multiple comparison test.



The label on the left side of the Y-axis represents the methods for calculating differences between two groups, from top to bottom: Week 13 minus baseline in UCB-MNCs group, Week 4 minus baseline in UCB-MNCs group, Week 13 minus baseline in Control group, and Week 4 minus baseline in Control group. Values on the X-axis represent intergroup differences; red dot: mean difference in UCB-MNCs group; blue dot: mean difference in Control group; and black line: 95% confidence interval. The dashed line corresponds to a score of 0. If the 95% confidence interval of the difference does not cross the dashed line, the difference between the time points is considered statistically significant.

For secondary outcomes, the difference in the reduction of SRS-2 Social Cognition domain scores (LS Mean Difference = -9.95, 95% confidence interval [CI]: -15.90 to -4.00, P = 0.002) between the two groups were significiant. (TABLE 3; FIGURE 2A). Regarding the Vineland-3 scale, no statistically significant differences were observed in the Adaptive Behavior Composite, Communication Domain, Daily Living Skills Domain, and Socialization Domain before and after treatment (SUPPLEMENT TABLE2).

At Week 4 compared with baseline, the Play and Leisure subdomain scores in the UCB-MNCs group increased significantly, with a statistically significant difference between the two groups (LS Mean Difference = 1.98, 95% confidence interval [CI]: 0.05 to 3.91, P = 0.045, SUPPLEMENT TABLE2). At

Week 4 compared with baseline, changes in the Attention Deficit and Hyperactivity Disorder domain scores of the SNAP-IV showed a statistically significant difference between the two groups (LS Mean Difference = -0.51, 95% confidence interval [CI]: -0.96 to -0.06, *P* = 0.029, SUPPLEMENT TABLE2).

SUPPLEMENT TABLE2 Changes in scores between two groups from baseline to Week 4 and Week 13.

		LS Mean Difference	SE	95% CI		*P*
Vineland-3						
Adaptive Behavior Composite score	Week 4 vs Baseline	1.21	2.33	-3.53	5.96	0.606
	Week 13 vs Baseline	3.04	2.03	-1.10	7.17	0.144
Communication domain	Week 4 vs Baseline	-0.15	2.82	-5.90	5.60	0.957
	Week 13 vs Baseline	4.79	2.97	-1.27	10.85	0.117
Daily living skills domain	Week 4 vs Baseline	0.18	2.62	-5.16	5.51	0.946
	Week 13 vs Baseline	1.47	2.45	-3.52	6.47	0.552
Socialization domain	Week 4 vs Baseline	5.14	3.93	-2.86	13.15	0.200
	Week 13 vs Baseline	5.44	4.14	-3.00	13.87	0.198
Receptive subdomain	Week 4 vs Baseline	0.92	0.83	-0.77	2.60	0.276
	Week 13 vs Baseline	1.68	0.84	-0.03	3.39	0.054
Expressive subdomain	Week 4 vs Baseline	-0.60	0.55	-1.72	0.52	0.287
	Week 13 vs Baseline	0.52	0.77	-1.04	2.09	0.500
Written subdomain	Week 4 vs Baseline	-0.06	0.88	-1.85	1.73	0.949
	Week 13 vs Baseline	0.59	0.49	-0.41	1.59	0.239
Personal subdomain	Week 4 vs Baseline	0.26	0.50	-0.75	1.28	0.601
	Week 13 vs Baseline	-0.03	0.52	-1.09	1.03	0.953
Domestic subdomain	Week 4 vs Baseline	0.43	0.71	-1.02	1.87	0.552
	Week 13 vs Baseline	0.90	0.63	-0.39	2.19	0.166
Community subdomain	Week 4 vs Baseline	-0.16	0.64	-1.47	1.15	0.804

Interpersonal Relationships subdomain	Week 13 vs Baseline	0.90	0.59	-0.31	2.11	0.140
	Week 4 vs Baseline	-0.01	0.63	-1.30	1.28	0.990
Play and leisure subdomain	Week 13 vs Baseline	0.35	0.67	-1.01	1.70	0.608
	Week 4 vs Baseline	1.98	0.95	0.05	3.91	**0.045**
Coping Skills subdomain	Week 13 vs Baseline	1.04	0.88	-0.75	2.83	0.245
	Week 4 vs Baseline	0.97	0.94	-0.95	2.89	0.309
CARS	Week 13 vs Baseline	1.62	1.08	-0.57	3.81	0.142
	Week 4 vs Baseline	-2.12	1.30	-4.76	0.53	0.112
ABC score	Week 13 vs Baseline	-3.24	1.64	-6.58	0.10	0.057
	Week 4 vs Baseline	-3.10	5.85	-15.03	8.84	0.600
Sensory domain	Week 13 vs Baseline	-9.57	6.07	-21.95	2.82	0.125
	Week 4 vs Baseline	0.49	1.77	-3.11	4.09	0.784
Relating domain	Week 13 vs Baseline	-1.69	2.00	-5.77	2.39	0.405
	Week 4 vs Baseline	-0.50	1.69	-3.96	2.95	0.768
Body domain	Week 13 vs Baseline	-2.62	1.97	-6.63	1.38	0.192
	Week 4 vs Baseline	-1.95	1.51	-5.02	1.12	0.205
Language domain	Week 13 vs Baseline	-1.66	1.65	-5.02	1.71	0.323
	Week 4 vs Baseline	-0.79	2.19	-5.26	3.67	0.719
Social and self-help domain	Week 13 vs Baseline	-2.27	2.04	-6.42	1.89	0.275
	Week 4 vs Baseline	0.14	1.46	-2.86	3.13	0.925
SAS (mother)	Week 13 vs Baseline	-0.86	1.36	-3.66	1.93	0.533
	Week 4 vs Baseline	-2.29	3.14	-8.69	4.12	0.473
SAS (father)	Week 13 vs Baseline	3.42	2.75	-2.19	9.03	0.223
	Week 4 vs Baseline	-0.27	2.34	-5.05	4.52	0.911
SNAP-IV	Week 13 vs Baseline	-0.97	2.43	-5.93	3.99	0.692
Attention deficit domain	Week 4 vs Baseline	-0.51	0.22	-0.96	-0.06	**0.029**
	Week 13 vs Baseline	0.10	0.26	-0.43	0.63	0.702

Hyperactivity disorder domain	Week 4 vs Baseline	-0.23	0.14	-0.53	0.06	0.118
	Week 13 vs Baseline	-0.24	0.18	-0.61	0.14	0.205

AE and SAEs

In the UCB-MNCs group, four children (23.5%) experienced transient excitement after treatment, and one had transient low-grade fever. In the control group, two children developed transient excitement after treatment, one had mild infiltration developed at the infusion site, and three presented with transient low-grade fever (TABLE 4).

TABLE4 Overview of adverse events in the UCB-MNCs and placebo groups.

Characteristics	UCB-MNCs (n=17)	Placebo (n=17)
Any adverse events, n(%)	6 (8.8%)	8 (11.8%)
Withdrawal or unmasking, n(%)	0	0
Total adverse events(frequency)	7 (10.3%)	9 (13.2%)
Adverse events possibly related to UCB-MNCs or placebo (frequency)	6 (8.8%)	6 (8.8%)
Adverse events severity (frequency)		
Level 1	6 (8.8%)	5 (7.4%)
Level 2	1 (1.5%)	4 (5.9%)
Level 3	0	0
Level 4	0	0
Level 5	0	0
Outcome for adverse events (frequency)		
Adverse event leading to death	0	0
Resolved or improved	7 (10.3%)	9 (13.2%)
Resolved with sequelae	0	0
Not resolved during follow-up or unknown course	0	0

Discussion

To our knowledge, this study is the first RCT investigating allogeneic UCB-MNCs for the treatment of ASD. In Dawson et al.' study[37], autologous (n=56) and allogeneic (n=63) UCB infusions were administered. Our study is the first to utilize allogeneic MNCs as the sole intervention, providing a basis for the compositional optimization of subsequent cell therapies. While both our study and previous research hypothesize immunomodulation as the therapeutic mechanism, we specifically enrolled children with peripheral blood evidence of immune dysregulation. Considering that previous animal experiments and clinical studies have shown that multiple infusions yield more potent anti-inflammatory and immunomodulatory effects[52-55], we

administered four infusions at one-week intervals, as opposed to a single infusion. Furthermore, no prophylactic medications were administered in this study, enabling a clear and unbiased assessment of infusion-related adverse events. In this study, the results demonstrated that allogeneic UCB-MNCs treatment was both safe and potentially clinically beneficial, as evidenced by week 13 outcomes: the UCB-MNCs group had lower Total scores and Social Cognition domain scores on the SRS-2 than the control group. As for iochemical profile comparison between baseline and post-intervention in two groups, the changes in creatinine and urea nitrogen were within the normal clinical reference ranges, and their clinical significance for the safety of UCB-MNCs therapy requires further investigation.

UCB-MNCs are directly isolated from UCB, which is a readily available source. They have low Human Leukocyte Antigen (HLA) expression levels, and the risk of graft-versus-host disease during allogeneic infusion is significantly lower than that of allogeneic bone marrow-derived (BM) cells[56]. In our study, AEs in the experimental group (e.g., transient excitement, low-grade fever) were mostly mild and reversible, mostly classified as CTCAE Grade 1. In previous studies on intravenous infusion of allogeneic UCB-MNCs[40], only mild adverse reactions such as transient low-grade fever were observed. In previous studies investigating intrathecal infusion of autologous BM-MNCs for ASD, reported AEs included procedure-related reactions such as post-puncture headache, nausea, vomiting, and local pain at the puncture site, as well as treatment-related reactions including transient or persistent increased hyperactivity and seizures, all of which were manageable and controllable[35, 39, 57-59]. Intrathecal injection can bypass the BBB directly and achieve extensive distribution in the CNS by virtue of cerebrospinal fluid circulation, with high local concentrations and direct action. Nevertheless, it carries the risk of puncture-related complications, fails to realize precise targeting of specific brain regions, and imposes stringent requirements on the mastery of operational techniques, administration dosage and infusion rate. To date, there are no direct clinical

comparative studies on the therapeutic effects of intravenous versus intrathecal administration. In combination with the findings of this study, intrathecal administration is an invasive procedure with low acceptance among parents in pediatric settings. Its operational complexity not only prolongs the procedure duration but also increases the risk of adverse reactions including spinal canal infection and meningeal irritation, thereby compromising the safety of subjects. Given that the pediatric population enrolled in this study has low tolerance to invasive procedures, the impact of the aforementioned risks is more pronounced. In Dawson et al.' study[37], autologous (n=56) and allogeneic (n=63) UCB infusions were administered. Both infusion modalities were safe and well-tolerated, with no product-related deaths, GVHD, or other SAEs observed. Allogeneic UCB infusion was associated with a higher risk of infusion reactions, specifically, severe reactions were only reported in the allogeneic group, potentially linked to HLA matching disparities or higher cell doses. The incidence of psychiatric AEs and SAEs was slightly higher in the allogeneic group, but these differences were not statistically significant, and most events were unrelated to the treatment itself. Autologous UCB exhibited a milder adverse event profile, with an absence of severe infusion reactions and immune-related specific reactions. These findings indicate that UCB-MNCs have a low acute safety risk in children. However, we have not yet conducted long-term safety studies, and follow-up research will be performed in the future.

To date, a total of seven clinical trials have investigated the use of MNCs for treating ASD, including two trials using UCB-MNCs[40, 60] and five using BM-MNCs[39, 41, 57, 58, 61]. Additionally, there are four case reports on BM-MNC therapy for ASD[62-65]. These trials, all open-label studies, primarily evaluated how MNCs affect neurocognitive, behavioral, and other ASD-related symptoms. Lv et al.[40] enrolled 37 children with ASD, dividing them into three groups: the UCB-MNCs monotherapy group, the UCB-MNCs + UCB-MSCs combination group, and the rehabilitation control group. At 24 weeks after treatment, compared with the control group, both treatment

groups showed statistically significant differences in CARS scores, ABC scores, and Clinical Global Impression Scale (CGI) assessments ($P < 0.05$), suggesting that UCB-MNCs improve ASD in children. Similar therapeutic effects have also been observed with other administration routes. Zhang et al. [60]Enrolled twenty children with ASD who received intravenous infusion of UCB-MNCs. After treatment, the CARS score decreased from 39.00±6.85 to 34.50±4.21. Sharma et al.[58] conducted an open-label, non-randomized clinical study involving the intrathecal transplantation of BM-MNCs combined with neurorehabilitation in 254 ASD patients. After a mean follow-up of 7.5 months, the patients showed improvements in 11 core symptoms including eye contact, attention, hyperactivity, and social interaction. All the above studies indicate that MNCs may improve the clinical symptoms of children with ASD. However, given that all these studies were open-label, the subjectivity of clinical scale assessments and the expectations of guardians regarding new therapies might have further amplify the placebo effect[51], limiting the credibility of the findings regarding the effectiveness of this treatment method. In our study, an RCT design was adopted to avoid the inherent limitations of the aforementioned methods. Our results also demonstrated the preliminary effectiveness of UCB-MNCs for ASD, as reflected by improvements in core symptoms such as cognition and communication as well as a trend toward improvement in some adaptive behaviors and symptoms related to attention.

Previous studies have suggested that the pathogenesis of ASD may be associated with immune inflammation. For example, Nour-Eldine et al.[66] analyzed 75 case-control studies and found that the levels of IL-6, IL-17, TNF-α, and IL-1β in the plasma and serum of ASD patients were significantly elevated, with increases also observed in the levels of IFN-γ, RANTES, and IL-8. This indicates that immune inflammation may be involved in the development of ASD. Liu et al.[67] employed two-sample Mendelian randomization and mediation analysis and identified nine inflammatory factors associated with ASD, including four negatively correlated factors (IL-

7, IL-2, IL-2β, IL-18-R1) and five positively correlated factors (TNF-α, natural killer cell receptor 2B4, Fms-related tyrosine kinase 3 ligand, T-cell surface glycoprotein CD5, TNF-related apoptosis-inducing ligand). A team led by Professor Sheng Ding from Tsinghua University's School of Pharmaceutical Sciences published a groundbreaking study in the journal *Immunity*. It revealed that transcription factor MEF2C, a key immune checkpoint, inhibits excessive microglial activation via regulating the p21-CDK2-RB-NFκB pathway to alleviate inflammation. It also confirmed that CDK2 is a potential target for the treatment of neurological diseases associated with excessive microglial activation[68]. Li, Meng et al demonstrated that low-dose IL-2 (LdIL-2) intervention in BTBR mice alleviates the core symptoms of ASD by improving immune imbalance (especially the dysregulation of Th/Treg subsets) and neuroinflammation[69]. MNCs possess immunomodulatory effects. Shahaduzzaman et al.[70] found that UCB-MNCs can improve the abnormal elevation of proinflammatory cytokines by regulating the activity of immune cells and inflammatory responses. Additionally, Chen et al.[71] demonstrated that UCB-MNCs can modulate inflammation-related pathways and improve the immune microenvironment. Madaric et al.[72] confirmed that BM-MNC therapy can ameliorate inflammation and oxidative stress by reducing proinflammatory cytokines, increasing antioxidant markers, and decreasing oxidative damage. To date, the mechanism underlying MNC therapy for ASD remains unclear. We hypothesize that it may exert effects through immunomodulation, and we plan to investigate this mechanism using blood proteomics and metabolomics in future studies.

Despite the findings of this study, it still has limitations. First, this study is an exploratory one with a small sample size. Although the sample size meets the requirement of statistical power, it may not be sufficient to detect all clinically relevant small-to-moderate effects. Thus, the results still need to be validated in a larger sample. Second, the study results rely on subjective scale assessments. Although a rigorous RCT design was adopted, subjective bias may still exist, leading to minor differences in the results. Hence, future

studies should incorporate more objective biological markers. Third, this study's follow-up period is short, so the long-term efficacy and safety remain unclear. We plan to address this limitation through long-term follow-up studies in the future. Fourth, all participants were recruited from a single medical center and received a single-dose treatment, which may affect the study results and limit their generalizability. Multicenter studies with various dosage regimens can be conducted in the future to improve this. Furthermore, due to the cognitive characteristics of the study population—most children scored below 80 on the Wechsler Intelligence Scale at baseline, with only one patient achieving a score of 105-stratified analysis by intellectual disability status was not performed. Finally, although this study found that UCB-MNCs may improve clinical symptoms of ASD, their underlying mechanism remains unclear. We hypothesize that UCB-MNCs may exert effects through immunomodulation, and we will investigate this mechanism using blood proteomics and metabolomics in future studies.

Conclusion

This study preliminarily demonstrated that multiple intravenous infusions of UCB-MNCs show good short-term safety and preliminary efficacy in improving specific aspects of symptoms (Total score and Social Cognition in SRS-2) in children with ASD and elevated cytokine levels. Although the sample size of this study was small, this limitation was partially compensated for by a rigorous RCT design. In the future, larger-scale, multicenter studies with longer follow-up periods and the inclusion of objective biological markers are needed to further verify the efficacy and safety of UCB-MNCs therapy, as well as to explore its mechanism of action. Only with such additional evidence can UCB-MNCs therapy potentially provide a more effective and reliable treatment option for a subset of patients with ASD.

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Author Contributions

Ziyan Zhang, Guanglei Wang, and Fang Han wrote the first draft and contributed equally to this work (co-first authors). Ziyan Zhang, Peili Hu, Xinyun Yao and Chunhua Sun performed data acquisition. Ziyan Zhang and Guanglei Wang performed data analysis. Ziyan Zhang, Fang Han and Yang Guang contributed to the study conception and design. All authors helped revise the manuscript for crucial intellectual content and approved the final version for publication.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethical Committee of the First Medical Center of Chinese PLA General Hospital on 14th March 2024 (Approval No. S2023-681-02, Public title: Clinical study on multiple intravenous infusions of umbilical cord blood mononuclear cells to improve the therapeutic effect of autistic children with high expression of serum inflammatory factors) and conformed to the Declaration of Helsinki

guidelines. All participants' guardians provided written informed consent before recruitment. This study was registered on ChiCTR.org.cn on 7th April 2024 (Identifier: ChiCTR2400082762, Public title: Clinical study on multiple intravenous infusions of umbilical cord blood mononuclear cells to improve the therapeutic effect of autistic children with high expression of serum inflammatory factors).

Consent for publication

Informed written consent was obtained from all patients for publication of this report.

Competing interests

The authors declare that they have no competing interests.

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