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# The Impact of Umbilical Cord Mesenchymal Stem Cells on Motor Function in Children with Cerebral Palsy: Results of a Real-world, Compassionate use Study

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**Abstract**
The aim of this study was to analyze the impact of human umbilical cord-derived MSCs (hUC-MSCs) on motor function in children with cerebral palsy (CP). The study enrolled 152 children with CP who received up to two courses of five hUC-MSCs injections. Children's motor functions were assessed with the Gross Motor Function Measure (GMFM), 6-Minute Walk Test (6-MWT), Timed Up and Go test (Up&Go test), and Lovett's test, and mental abilities were assessed with the Clinical Global Impression (CGI) scale. Data collected at visit 1 (baseline) and visit 5 (after four injections) were analyzed retrospectively. After four hUC-MSCs administrations, all evaluated parameters improved. The change in GMFM score, by a median of 1.9 points (IQR: 0.0–8.0), correlated with age. This change was observed in all GFMCS groups and was noticed in all assessed GMFM areas. A median increase of 75 m (IQR: 20.0–115.0) was noted on the 6-MWT, and this correlated with GMFM score change. Time on the Up&Go test was reduced by a median of 2 s (IQR: −3 to − 1) and the change correlated with age, GMFM score at baseline, and the difference observed on the 6-MWT. Results of Lovett's test indicated slight changes in muscle strength. According to the CGI, 75.5% (96/151) of children were seriously (level VI) or significantly ill (level V) at the 1$^{st}$ visit, with any improvement observed in 63.6% (96/151) of patients at the 5$^{th}$ visit, 23.8% (36/151) with improvement (level II) or great improvement (level I). In conclusion, the application of hUC-MSCs generally enhanced functional performance, but individual responses varied. The therapy also benefited children with high level of disability but not to the same extent as the initially less disabled children. Although younger patients responded better to the treatment, older children can also benefit. **Trial Registration** 152/2018/KB/VII and 119/2021/KB/VIII. Retrospective registration in ClinicalTrials: ongoing.

**Keywords** Wharton's Jelly · Mesenchymal Stromal Cells · WJ-MSCs · UC-MSCs · Nervous System

## Introduction

Cerebral palsy (CP), a group of permanent disorders affecting movement ability and posture maintenance caused by non-progressive impairments in the developing brain, remains the most common motor disability in childhood [1, 2]. The severity and degree of clinical manifestations correspond to the timing of the damage to the developing central nervous system (CNS). As there is no cure for cerebral palsy, patients undergo physical, mental, social, and other therapies to improve their condition [3]. However, the purpose of such treatment is not to cure the disease but to relieve pain, manage symptoms, and maximize patients' independence, improving their wellbeing. The nervous tissue in the brain and the spinal cord have almost no spontaneous regenerative capacity, so the brain or spinal cord damage is largely permanent. The recent emergence of therapy based on mesenchymal stem cells (MSCs) engenders hope for more effective therapeutics that can potentially replace the damaged tissue in the CNS [4, 5]. Other studies suggest that instead of replacing dying neurons, stem cells might ameliorate the damaged

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microenvironment via acting on paracrine pathway [6, 7]. The preliminary results of studies concerning the use of stem cells in various therapies are encouraging due to their excellent potential for tissue healing [8]. MSCs from human umbilical cord (hUC-MSCs) appear to be a promising tool for CP cell-based therapy [9, 10].

The umbilical cord core of connective embryonic tissue, known as Wharton's Jelly (WJ), is extremely rich in stem cells [11]. Their easy accessibility, low immunogenicity, and immunosuppressive potential rank them as superior to other types of stem cells [12]. These cells are being developed into medicinal products for application in therapeutic settings. The first case of successful autologous stem cell intervention to treat CP was reported in 2009, in a 2.5-year-old patient with hypoxic-ischemic brain damage as a result of cardiac arrest [13]. Several studies have demonstrated that human hUC-MSCs (also called WJ-MSCs) could enhance motor function in children with CP [14–16]. A phase 1 clinical study showed that infusion of intravenous allogeneic umbilical cord-derived mesenchymal stem cells (CLV-100) was safe and well-tolerated [17]. Other recent randomized clinical trials have confirmed that hUC-MSCs are safe and their use might improve clinical and imaging outcomes [18], especially when combined with rehabilitation [19]. MSCs therapies are not currently licensed for marketing. Despite being not authorized, they have a therapeutic index, as evidenced in many preclinical and clinical trials [18, 20]. Advanced Therapy Medicinal Product (ATMP) intervention is allowed under Polish law in compliance with European Medicines Agency approval [21, 22] on the basis of hospital exemption cases. Such treatment is considered to be the last therapeutic option, when all previous treatments have failed. Patients who have run out of all available options of medical intervention are granted a hospital exemption and qualified for a therapeutic experiment that is strictly regulated by the state authorities [23]. In children with a high risk of CP, the intervention should start as soon as possible at a critical developmental plasticity window to achieve better outcomes [24]. Therefore, there is an increasing need to shift MSCs interventions (classified as ATMP) from hospital exemption niches to first-line treatment of patients with CP, since the currently available treatments do not provide satisfactory clinical outcomes. However, this therapeutic option still requires studies to direct the choice of the best source of stem cells and to confirm treatment efficacy and safety.

In our previous paper, we described subjective benefits in quality of life and self-sufficiency after Wharton's Jelly administration [25]. The aim of this paper was to analyze the impact of hUC-MSCs administration on motor function in children with CP assessed with well-known motor scales and tests.

## Materials and Methods

### Wharton's Jelly MSCs – Preparation and Administration

Umbilical cords were collected from neonates delivered vaginally or via Cesarean section. The mother's health status was confirmed through the medical history and verified with a medical questionnaire. Informed consent was obtained from mothers, who voluntary donated the tissue, before the procedure.

The hUC-MSCs were obtained as previously described [25, 26]. Preparation of hUC-MSCs was in compliance with Good Manufacturing Practice and was conducted under the control of the Chief Pharmaceutical Inspectorate. The umbilical cords were processed within 48 h following delivery. Briefly, the umbilical cords were washed in saline solution supplemented with an antibiotic-antimycotic mixture (Gibco™), aseptically dissected, and cleaned from any visible blood vessels. The Wharton's Jelly was then fragmented into 2 $cm^3$ pieces, placed into 6-well plates coated with MAC Attachment solution (Biological Industries Ltd.), and cultured in NutriStem® XF serum-free medium (Biological Industries Ltd.) supplemented with NutriSteam$^R$XF Supplement Mix (Biological Industries Ltd.) enriched with antibiotic-antimycotic solution (Gibco™). After 1–2 h of incubation at 37 °C and 5% $CO_2$, non-adherent cells were removed. The attached fibroblast-like cells were cultured for about 2–3 weeks, and when confluency reached 90%, they were subcultured and reseeded at a density of $1.2 \times 10^4$ cells/$cm^2$ into 75 $cm^2$ culture flasks (BD). The number of passages did not exceed five. The cell number was estimated using a hemocytometer chamber after detaching cells with trypsin solution (Biological Instruments Ltd.). To determine the specificity of hUC- MSCs, immunophenotyping was performed in accordance with published criteria [27]. Briefly, when the cells showed about 60–80% confluency, they were detached with trypsin, and stained with fluorochrome-conjugated antibodies against markers distinct for MCS-negative (CD14–, CD34– CD45–, and HLA-DR-FITC), and MCS-positive (CD73–, CD90–, CD105–, and HLA ABC-PE) phenotypes. As a control, mouse anti-IgG1-FITC and anti-IgG1-PE were used to verify the specificity of the antibodies. The analysis was performed with a BD FACSCalibur™ flow cytometer (BD Bioscience). The obtained MSCs met the Chief Pharmaceutical Inspectorate's requirements for a medicinal product suitable for human administration in terms of: the unit final volume (5 mL), number of MSCs in an individual container (from $1 \times 10^6$ to $5 \times 10^7$ cells), cell vitality ($\geq 90\%$), morphology (fibrous-like), immunophenotype, microbiological purity, and absence of endotoxins. The cells at desired densities were resuspended in 10% v/v

DMSO solution (WAK-Chemie GmbH) supplemented with 5% human albumin (CSL Behring™), transferred into freezing containers, and stored in the vapor phase of liquid nitrogen for cryopreservation. Before administration, the cells were placed in a water bath and thawed at 37 °C. The viability of post-thawed MSCs was estimated with Trypan Blue staining and compared with a reference sample. Neither the proliferation rate nor other indicators of cellular senescence were monitored.

This preparation was given to patients in up to two treatment courses. Each course included five intravenous or intrathecal stem cell injections given at visits that took place every 2 months. Depending on the patient's body mass, different cell doses were administered (10, 20, 30, or $40 \times 10^6$ MSCs per injection) to provide a final dose of $1 \times 10^6$ MSCs/kg. The first hUC-MSCs dosage was administrated on the 1st visit, and the last one was given on the 5th visit.

Patients were evaluated at each visit before receiving the next dose of hUC-MSCs. The complete data collected at visit 1 (baseline) and visit 5 (after four stem cell injections) were used for statistical analysis.

## Patients

This retrospective study involved data from patients with CP who were treated with hUC-MSCs infusions as part of a therapeutic experiment conducted between May 2018 and August 2021. The patients were approved to participate in a medical therapeutic experiment during neuronal examination performed by a medical doctor. Patients' parents or legally acceptable representative signed the informed consent form including permission to use their medical records, assurance of confidentiality of the patient's identity, and agreement to publish the results of the study.

The following information was obtained from patients' medical records: sex, age, weight, comorbidities, and the results of motor function tests.

The patients received the stem cell therapy on a compassionate use basis within the legal framework of a therapeutic medical experiment (hospital exemption) and the product has been classified as an ATMP by the European Medicines Agency Committee for Advanced Therapies [28]. The procedure was approved by the Bioethics Committee at the Regional Chamber of Physicians and Dentists in Lublin, Poland (152/2018/KB/VII and 119/2021/KB/VIII). The study adhered to the ethical principles that have their origin in the Declaration of Helsinki.

## Evaluated Parameters

### Gross Motor Function Classification System

At baseline (1st visit), motor skills were assessed in all patients with the Gross Motor Function Classification System (GMFCS). This is a five-level grading system providing a clear description of a child's current motor function. The higher the level, the worse the motor abilities [29]. GMFCS was used as a grouping variable to evaluate changes in children's motor abilities after treatment relative to the baseline level. At visits 1 and 5 patients' motor and mental abilities were assessed with the tests described below.

## Gross Motor

### Gross Motor Function Measure

Changes in motor function over time were assessed with the Gross Motor Function Measure (GMFM). The evaluation contains 88 items that explore five areas of a child's motor ability: lying down and rotation; sitting; crawling on hands and knees; standing; and walking, running, and jumping. Items are ordered in terms of difficulty and each item is scored from 0 to 100 [30].

### 6-Minute Walk Test

Functional mobility was rated with the 6-Minute Walk Test (6-MWT), which enables assessment of the number of meters a child walks in 6 min [31].

### Timed Up and Go Test

Patients' mobility was also evaluated with the Timed Up and Go test (Up&Go test). This test involves the measurement of the time (in s) it takes the patient to get up from a chair, walk a distance of 3 m, turn, come back and sit down again on the chair [32].

### Clinical Global Impression Test

The Clinical Global Impression (CGI) scale was applied to quantify the patient's mental condition before and after the medical intervention. It comprises two questions about severity (CGI-S) and improvement (CGI-I), each of which is scored from I to VII (in the case of CGI-S, I means normal and VII means extremely ill; in the case of CGI-I, I means very much improved and VII means very much worse) [33]. At visit 1, the CGI-S was applied, and the CGI-I was performed at visit 5.

#### Lovett's Test

Muscle strength on Lovett's scale was assessed for the biceps brachii, quadriceps femoris, and gluteus maximus muscles. Outcomes were scored from 0 (lack of muscle contraction) to 5 (normal muscle strength).

### Data Processing and Statistical Analysis

Statistical analysis was performed with Statistica 13.0 (Tibco). All variables were summarized with descriptive statistics (median, range, interquartile range [IQR]). Continuous variables before and after treatment were compared with the Wilcoxon test. The Mann-Whitney U test was used to compare results for binarily defined subgroups. Differences in changes in GMFM between subgroups distinguished on the basis of GMFCS at baseline were compared using the Kruskal-Wallis test. Spearman's R was used to assess correlation between continuous and ordinal variables; the Kendall Tau test was also used for ordinal variables. The level of statistical significance was set at 0.05.

## Results

### Patient Characteristics

We analyzed the medical records of 152 children with CP who received hUC-MSCs injections. Patients characteristics at baseline is showed in Table 1.

**Table 1** Patients' baseline characteristics

| Variable | Value |
|---|---|
| Age [years], median (IQR) | 5.0 (3.0–8.0) |
| Sex | |
| Male | 84 (55.3%) |
| Female | 68 (44.7%) |
| Body mass at the first hUC-MSCs injection [kg] | |
| Range | 7–56 |
| Median [IQR] | 15.85 (12.–22) |
| Comorbidities | |
| Epilepsy, n (%) | 75 (49.3) |
| Other Comorbidities, n (%) | 15 (10) |
| Autism spectrum disorder | 1 |
| Intellectual disability | 2 |
| Genetic defect | 4 |
| Premature birth | 2 |
| Hydrocephalus | 3 |
| Encephalopathy | 1 |
| Microcephaly | 1 |
| Congenital cytomegalovirus | 1 |
| GMFCS levels at baseline, n (%) | |
| I | 16 (10) |
| II | 15 (10) |
| III | 14 (9) |
| IV | 33 (22) |
| V | 74 (49) |

### Comparison of Results Obtained Before and After hUC-MSCs Therapy

#### Gross Motor Function Measure

This test was performed in all patients ($n = 152$). The median change in GMFM from baseline to visit 5 (after the fourth hUC-MSCs injection) was 1.9 points ($p < 0.000001$) (Table 2). The change in GMFM score correlated significantly with patient age (Spearman's $R = -0.38$, $p < 0.05$) (Fig. 1A), but was unrelated to gender ($p = 0.40$) nor the presence of epilepsy ($p = 0.17$). A statistically significant change was seen across all evaluated areas of GMFM (Table 3).

The change in GMFM score between visit 1 and 5 was observed in all GMFCS groups, as presented in Table 2 and at Fig. 2. Fig. 3 presents the relationship between GMFM at 1st visit and changes in Up&Go outcomes from 1st to 5th visit.

#### 6-Minute Walk Test

This test was conducted on a subgroup of 26 children (mainly with GMFSC I and II) who were able to meet the test conditions. In this subpopulation, the median increase in functional mobility assessed with the 6-MWT was 75 m (Table 4) and a statistically significant improvement ($p = 0.000058$) was achieved. The increase in 6-MWT outcomes correlated with GMFM score change (Spearman's $R = 0.47$, $p < 0.05$), but was not related to GMFM at baseline, age (Fig. 1C), gender, or epilepsy ($p > 0.05$).

#### Timed Up and Go Test

This test was conducted on a subgroup of 26 children who were able meet the test conditions. For this subgroup, the median change was 2 s (Table 5) and this improvement was statistically significant ($p = 0.000027$). This improvement correlated with age (Spearman's $R = 0.46$, $p < 0.05$) but not with epilepsy ($p = 0.56$) or gender ($p = 0.48$). The improvement in the Up&Go test correlated ($p < 0.05$) with GMFM at the 1st visit (Spearman's $R = 0.66$) and the difference in the 6-MWT (Spearman's $R = -0.50$), but not with the difference in the total GMFM. However, there was a statistically significant ($p < 0.05$) correlation with the difference in two subscales of this assessment: lying (Spearman's $R = -0.45$) and sitting (Spearman's $R = -0.48$).

**Table 2** Changes in GMFM (points) before and after treatment in children with cerebral palsy in the total population and in subgroups defined by baseline GMFCS level

| Difference in total GMFM between 1st and 5th visit | General group $n=152$ | GMFCS level at baseline | | | | |
|---|---|---|---|---|---|---|
| | | I $n=16$ | II $n=15$ | III $n=14$ | IV $n=33$ | V $n=74$ |
| $p$ value[a] | n/a | 0.001872 | 0.071190 | 0.018604 | 0.000014 | 0.00000 |
| $p$ value[b] | | 0.35 | | | | |
| Median | 1.9 | 2.9 | 1.0 | 6.4 | 2.0 | 1.7 |
| Minimum | −9.0 | −0.4 | −2.0 | −9.0 | −1.0 | −3.4 |
| Maximum | 34.4 | 34.4 | 18.8 | 16.4 | 20.2 | 12.0 |
| Lower quartile | 0 | 0.3 | −0.4 | 0.0 | 0.4 | 0.2 |
| Upper quartile | 5.0 | 4.9 | 4.4 | 9.8 | 5.6 | 4.2 |

*GMFCS* Gross Motor Function Classification System, *GMFM* Gross Motor Function Measure, *n* number, *n/a* non-applicable

[a]For comparison between GMFM before and after treatment in each group

[b]For comparison between all groups

### Clinical Global Impression Test

Results of this test were available for 151 patients. At the 1st visit, most children were found to be seriously ill (level VI, $n=70$, 46.4%) or significantly ill (level V, $n=44$, 29.1%); 18 (11.9%) patients were moderately ill (level IV). A few patients were mildly ill (level III, $n=9$, 6.0%), hardly ill (level II, $n=7$, 4.6%), or among the sickest patients (level VII, $n=3$, 2.0%).

At visit 5, most patients achieved minimal improvement (level III, $n=60$, 39.7%) or no change (level IV, $n=54$, 35.8%). Improvement (level II) was achieved by 28 (18.5%) patients and great improvement (level I) by eight (5.3%) patients. Minimally worse health status (level V) was noticed in one (0.7%) patient. None of the patients achieved a worse or significantly worse condition. Association between baseline CGI and CGI after treatment is presented in Table 6.

The recorded improvements did not correlate with gender nor with epilepsy diagnosis. However, baseline measurements significantly correlated with baseline GMFCS (Spearman's $R=0.61$, $p<0.05$; Kendal Tau 0.55, $p<0.05$). Additionally, the differences in GMFM score between the 1st and 5th visit significantly correlated with CGI results obtained on the 5th visit (Spearman's $R-0.28$, $p<0.05$; Kendall Tau $-0.22$, $p<0.05$).

### Lovett's Test

This test was performed in 63 patients. Descriptive statistics indicated very slight changes in muscle strength (Table 7). Muscle strength for biceps brachii improved by one unit in two (1.3%) patients; three (2.0%) children had a one-unit change in the quadriceps femoris muscle, and one (0.7%) patient had an change of two units in the quadriceps femoris muscle. The difference between baseline and visit 5 was close to significant ($p=0.068$). Gluteus maximus muscle strength improved by one unit for eight (5.2%) children and the improvement was statistically significant ($p=0.01$).

Surprisingly, improvement in muscle strength did not correlate with change in GMFM score, Up&Go, or 6MWT, but there was a moderate correlation between increased strength of quadriceps femoris and gluteus maximus (Spearman's $R=0.49$, $p<0.05$) and biceps brachii (Spearman's $R=0.32$, $p<0.05$).

## Discussion

As CP is a long-life disorder [2, 34], patients, their families, and health care providers are in dire need of therapeutic interventions to curb disease burden and improve patients' quality of life and well-being. The implementation of human MSCs creates new avenues for more effective CP treatment [12, 35]. In our previous research, we showed the positive effect of hUS-MSCs injections on patients' quality of life and self-sufficiency [25].

Here we report significant improvement in functional mobility and global functioning after the 4th dose of hUC-MSCs in pediatric patients. We noticed 1.9-point change in GMFM score and observed increase across all areas of assessment. Moreover, the upward shift in GMFM in all five subgroups (according to GMFCS) suggests that the impact of hUC-MSCs injections on patients' gross motor functions occurs in children with all disability level. Similar outcomes were reported in an open-label randomized phase II study that assessed motor function in patients with CP receiving allogeneic umbilical cord blood infusions [20]. The results of a meta-analysis of randomized controlled trials demonstrated a significant increase in GMFM scores and comprehensive function assessment (CFA) with human MSCs therapy in children with CP [10]. Subgroup analysis revealed considerable benefit 3, 6, and 12 months from the treatment.

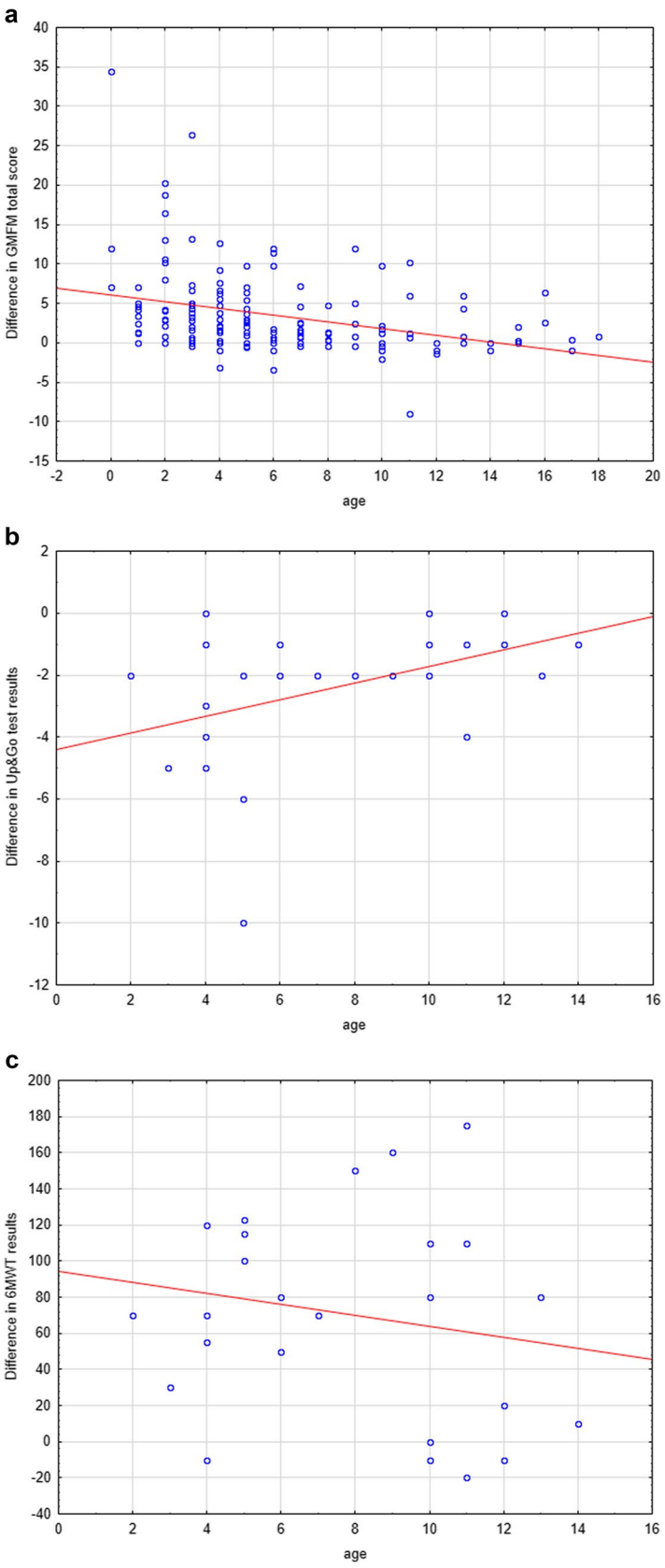


**Fig. 1** **a**-**c**. Scatter plot: relationship between patient age and changes from 1st to 5th visit in GMFM (**A**), Up&Go (**B**), and 6-MWT (**C**) tests. 6-MWT, 6-Minute Walk Test; GMFM, Gross Motor Function Measure; Up&Go, Timed Up and Go test

**Table 3** Changes in GMFM areas of assessment (points) in children with cerebral palsy before and after treatment

| Difference in GMFM between 1st and 5th visit | Areas of assessment | | | | |
|---|---|---|---|---|---|
| | Lying down and rotation | Sitting | Crawling on hands and knees | Standing | Walking, running, and jumping |
| *p*-value[a] | < 0.000001 | < 0.000001 | < 0.000001 | 0.000003 | 0.000035 |
| Median | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| Minimum | –19.0 | –7.0 | –7.0 | –10.0 | –50.0 |
| Maximum | 41.0 | 40.0 | 62.0 | 67.0 | 52.0 |
| Lower quartile | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Upper quartile | 8.0 | 7.0 | 3.0 | 0.0 | 0.0 |

*GMFM* Gross Motor Function Measure

[a]For comparison between GMFM areas of assessment before and after treatment

This meta-analysis suggested that human MSCs therapy is not only effective, but also safe for children with CP. Clinical trial outcomes [36] confirmed the safety of the intervention and revealed significant improvement in mobility in children aged 2–5 years. In our study of patients with CP and a median age of 5.0 years (ranging from 0 to 18 years) who received the treatment as compassionate use, we observed significant change in GMFM scores. However, more pronounced effects were found in younger children than in older ones, in all GMFCS subgroups. The GMFM results are in agreement with those of the Timed Up&Go test [32], which also correlated with age. However, in a randomized control trial, the impact of hUC-MSCs transplantation on GMFM scores was not age-related [37]. Some researchers suggested that the effects of such therapy could be related to the patient's maturity since the patient's resident stem cells reservoir is age-dependent [38]. Indeed, age-dependent neuroprotection following the administration of umbilical cord-derived stem cells was reported in animal models [39, 40]. Further, in children with CP, initiation of treatment at a younger age translates into better outcomes. However, in some cases, the disorder is not diagnosed until approximately 2 years of age [41]. Unfortunately, there are currently no official guidelines or formal recommendations advising the optimal timing of stem cell therapy [38], although clinical trials showed that earlier intervention is more effective.

The aim of using stem cell therapy in the treatment of patients with CP is to promote the recovery of damaged cells, increase the odds for their survival, and soften the consequences of damage. Human MSCs are multipotent cells with the ability to self-renew and differentiate upon proper stimulation in in vitro settings into several cell lineages of mesodermal phenotype [42]. MSCs have been demonstrated to differentiate into adipocytes, osteoblasts, chondrocytes, and neurons [43]. Although these mechanism has been observed in vitro, its relevance in clinical settings still needs to be confirmed. Certainly, benefits of therapy with MSCs are associated with the secretion of factors involved in the regulation of immune and inflammatory responses, stimulation of angiogenesis, and provision of neurotrophic functions [44, 45]. Their therapeutic effect could also be related to the migration of these cells to sites of injury or disease [46]. This hypothesis is supported by the outcomes of a randomized controlled trial in which the administration of hUC-MSCs enhanced gross motor and cognitive functions in children with CP [19]. The improvements observed in this clinical trial were most visible at 6 months after hUC-MSCs transplantation and lasted up to 12 months. Fu et al. also observed increase in GMFM score and fine motor function measure scores in children 6 months after treatment [9]. Another course of transplantation further enhanced gross and fine motor functions and the improvement displayed a linear upward trend. It seems that the long-lasting benefits of hUC-MSCs are associated with their paracrine effects [47]. Moreover, Gu et al. suggested that functional improvement triggered by hUC-MSCs transplantation might be the consequence of improved cerebral metabolism [19]. The release of active molecules, including growth factors, neurotrophic signals, angiogenic mediators, and anti-inflammatory agents, that stimulate the recovery of damaged brain tissue, is suggested to be responsible for the favorable effects of stem cells in CP [48, 49]. MSCs also play roles in neuroprotection, immunoregulation, and neurodifferentiation [48, 50]. MSCs could modulate the immune system to protect the CNS, inhibiting the damaging effects of possible autoreactive responses [51], but they also secrete neuroprotective factors, triggering innate repairing mechanisms [5]. Stem cells have been demonstrated to effectively cross the blood-brain barrier [52]. MSCs have also been suggested to transdifferentiate into brain cells, resulting in cell replacement, but this has not been confirmed. However, stem cells were found to promote nerve recovery through an immunomodulatory function or nerve repair strategy [5].

In this study, we observed a moderate correlation between gross motor function improvement and increased muscle strength, Up&Go test or and 6-MWT after hUC-MSCs

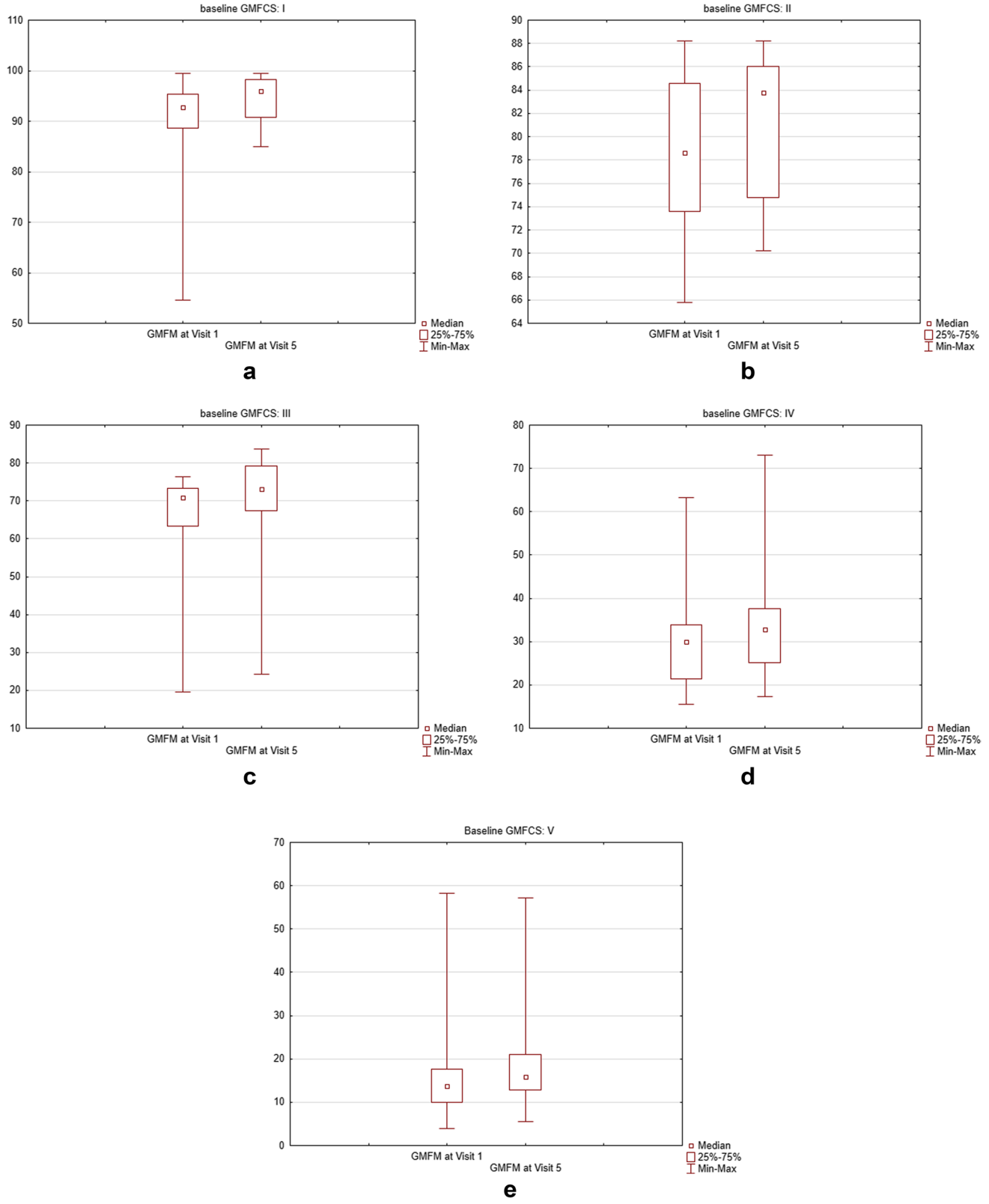


**Fig. 2** Outcomes of GMFM at 1st and 5th visit in subgroups defined by baseline GMFCS. GMFCS, Gross Motor Function Classification System; GMFM, Gross Motor Function Measure

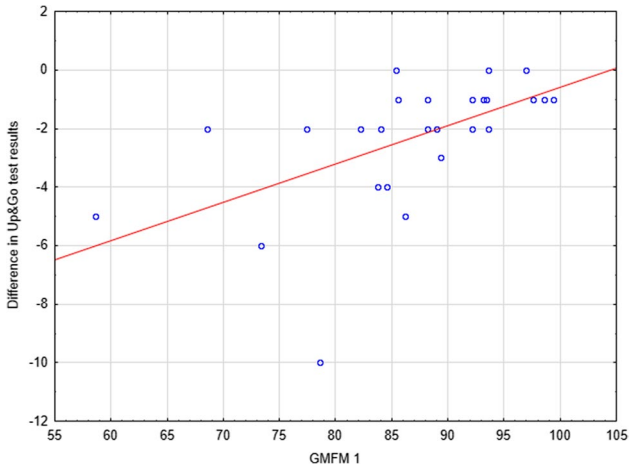


**Fig. 3** Scatter plot: relationship between GMFM at 1st visit and changes in Up&Go outcomes from 1st to 5th visit. GMFM, Gross Motor Function Measure; Up&Go, Timed Up and Go test

therapy. This observation suggests that peripheral neuronal cells and neuromuscular junctions are the plausible therapeutic targets, rather than the brain or muscle cells [53]. Indeed, a recent study in an animal model showed the potential of UC-MSCs extracellular vesicles [49, 54, 55] in enhancing Schwann cell proliferation after peripheral nerve injury [56]. The role of mitochondria derived from MSCs should not be overlooked [57, 58]. In our study, the muscle strength improvement seen in a limited number of children could also result from physiotherapy [59]. Moreover, we noticed a statistically significant improvement in 6-MWT and Timed Up&Go. CGI assessment also revealed significant change, which is in agreement with our previously published observations [60].

By now there are 426 registered clinical trials worldwide investigating the therapeutic potential of MSCs (https://www.clinicaltrials.gov/). So far, most studies have shown positive outcomes with no serious adverse effects [18, 20, 42, 61]. However, clinical evidence concerning the benefits of hUC-MSCs in the treatment of CP is still limited since data are inconclusive. The clinical outcome of hUC-MSCs therapy could depend on many factors. Zhang et al. have demonstrated that the quality and long-term effectiveness of MSCs depend on the vitality of infused cells and their homing ability [62]. It appears that in vitro expansion of MSCs is associated with the loss of some of their natural characteristics; therefore, strategies that can at least maintain (or better, promote) the biological activities and therapeutic efficacy of MSCs are needed [35]. Zhang et al. [62] also found that hUC-MSCs at various passages could have different therapeutic effects. In the study by Zhao et al., hUC-MSCs at a higher passage number (P15) displayed higher apoptosis and adipogenic differentiation potential in vitro as well as reduced cell proliferation capacity and lower osteogenic and

**Table 4** Changes in 6-Minute Walk Test (m) in children with cerebral palsy before and after treatment

| | Before treatment (1st visit) | After treatment (5th visit) | Difference |
|---|---|---|---|
| Valid n | 27 | 27 | 26 |
| Median | 120.0 | 200.0 | 75.0 |
| Minimum | 10.0 | 32.0 | −20.0 |
| Maximum | 400.0 | 420.0 | 175.0 |
| Lower quartile | 100.0 | 160.0 | 20.0 |
| Upper quartile | 200.0 | 275.0 | 115.0 |

*n* number

**Table 5** Changes in Timed Up and Go test in children with cerebral palsy before and after treatment

| | Before treatment (1st visit) | After treatment (5th visit) | Difference |
|---|---|---|---|
| Valid n | 26 | 27 | 26 |
| Median | 12.0 | 10.0 | −2.0 |
| Minimum | 9.0 | 8.0 | −10.0 |
| Maximum | 22.0 | 17.0 | 0 |
| Lower quartile | 10.0 | 9.0 | −3 |
| Upper quartile | 13.0 | 11.0 | −1 |

n, number

chondrogenic differentiation potential [63]. In our current and previous studies, our patients received ≥ 90% viable cells from a maximum of the 5th passage. Kim et al. [64] suggested that the use of a small population of human umbilical cord blood-derived MSCs (UCB-MSCs) could result in higher cell growth and lower senescence compared with large or non-isolated populations. Moreover, they implied that the use of a small population of UCB-MSCs offers an effective way to enhance the efficacy of cell therapy.

Successful translation of research concerning MSCs into efficient clinical therapies is hindered by numerous factors, including the heterogeneity of studied populations and their characteristics, donor-related issues, differences in protocols for isolation, in vitro expansion, and premodification, differences in methods and sites of cell delivery, use of various drugs and chemicals, and differences in MSCs dosing, as well as cell homing [65]. Therefore, standardized methodologies are needed (i.e., selection of patients, therapeutic cell dose, treatment regime, optimal timing, experimental MSCs handling) to obtain comparable results as well as to maximize the efficacy and safety of stem cell therapy [66]. Better understanding of differences between MSCs obtained from diverse sources could improve the selection of the best treatment option [35]. The efficacy of MSCs transplantation might be restricted by their limited replicative lifespan [67]. So far, studies and clinical trials have demonstrated

**Table 6** Association between CGI at baseline and CGI after treatment

| CGI after treatment | n=150 | Baseline CGI | | | | | |
|---|---|---|---|---|---|---|---|
| | | II | III | IV | V | VI | VII |
| I | | 0 | 0 | 1 | 2 | 5 | 0 |
| | | 0.00% | 0.00% | 5.56% | 4.65% | 7.14% | 0.00% |
| II | | 1 | 2 | 3 | 12 | 10 | 0 |
| | | 14.29% | 22.22% | 16.67% | 27.91% | 14.29% | 0.00% |
| III | | 2 | 5 | 9 | 15 | 29 | 0 |
| | | 28.57% | 55.56% | 50.00% | 34.88% | 41.43% | 0.00% |
| IV | | 4 | 2 | 5 | 14 | 25 | 3 |
| | | 57.14% | 22.22% | 27.78% | 32.56% | 35.71% | 100.00% |
| V | | 0 | 0 | 0 | 0 | 1 | 0 |
| | | 0.00% | 0.00% | 0.00% | 0.00% | 1.43% | 0.00% |

Color intensity correlates with severity of disease (baseline CGI) or degree of improvement (CGI after treatment)

*CGI* Clinical Global Impression, *n* number

**Table 7** Changes in Lovett's scale in children with cerebral palsy before and after treatment

| | | Before treatment (1st visit) | After treatment (5th visit) | Difference |
|---|---|---|---|---|
| Biceps brachii | Valid n | 62 | 63 | 62 |
| | Median | 4.0 | 4.0 | 0.0 |
| | Minimum | 3.0 | 3.0 | 0.0 |
| | Maximum | 103.0 | 103.0 | 1.0 |
| | Lower quartile | 4.0 | 4.0 | 0.0 |
| | Upper quartile | 5.0 | 5.0 | 0.0 |
| Quadriceps femoris | Valid n | 62 | 63 | 62 |
| | Median | 4.0 | 4.0 | 0.0 |
| | Minimum | 2.0 | 3.0 | 0.0 |
| | Maximum | 5.0 | 5.0 | 2.0 |
| | Lower quartile | 3.0 | 4.0 | 0.0 |
| | Upper quartile | 4.0 | 4.0 | 0.0 |
| Gluteus maximus muscles | Valid n | 63 | 63 | 62 |
| | Median | 3.0 | 3.0 | 0.0 |
| | Minimum | 1.0 | 1.0 | 0.0 |
| | Maximum | 5.0 | 5.0 | 1.0 |
| | Lower quartile | 3.0 | 3.0 | 0.0 |
| | Upper quartile | 4.0 | 4.0 | 0.0 |

*n* number

the safety of hUC-MSCs, including in terms of immunology due to their low expression of major histocompatibility complex class I (MHC-I) and MHC-II [68]. The goal of future research is to increase the ability of MSCs to migrate and promote neurogenesis and angiogenesis with better efficacy. Moreover, since there are some safety concerns regarding the possible teratogenic/neoplastic potential of MSCs and risk of transmission of infectious diseases, further research in this field is necessary.

Our study has several limitations. The first is the lack of a control group and the fact that this study was not randomized. The non-randomized scheme is associated with the fact that this therapy is expensive and not many parents can afford it, which could cause bias. However, in case of such diseases, the use of placebo or the administration of a therapy that is not yet approved would be unethical. Despite the absence of a control group, we are able to estimate the extent of improvement compared to the literature data. A systematic review conducted by Eggenberger [69] revealed that in four controlled studies in the control group, an improvement of 5–13% was observed compared to the baseline, while in the cell therapy group, the improvement

ranged from 15 to 34%. In our analysis, comparably good results were obtained for children above the third quartile, with individual outliers reaching the upper ranges of values for the clinical trial average. The explanation for this phenomenon may be twofold. Firstly, cells are not a chemical compound, so the reproducibility of results is lower than in the case of pharmaceutical substances. The specific characteristics of a particular donor, in addition to tissue origin, can have a significant impact on secretory properties. Due to individual differences among donors, not all children received identical products. For example, it is known that MSCs obtained from cords of older mothers, despite identical markers, differed in proliferation and differentiation potential [70].

Secondly, studies conducted in real-life conditions often do not achieve as good results as clinical trials, which is why post-marketing observational studies are conducted. Compassionate use involves application in real-life conditions, without strict inclusion and exclusion criteria, so slightly lower efficacy is to be expected. However, in connection with the above, there should not be a significant weakening of effectiveness when introduced into the treatment of a broad population, unlike in clinical trials.

CGI is not as widely used as the GMFM scale, and due to its nature, it is subject to the subjectivity of assessment. However, because of this, although not a gold standard in CP, it is less sensitive to validation issues in a specific condition than other scales. Despite being less popular, it has been utilized in previous studies evaluating the effectiveness of botulinum toxin [71–74], acupuncture [75], and hippotherapy in children with CP [76], as well as pallidal stimulation in young adults [77]. Finally, to strengthen the value of the GCI score, we performed an analysis that demonstrated a significant correlation between CGI results and objective measures. Considering the irreversibility of CP, one may question the significance of a slight improvement in a child's intellectual functioning, which remains disabled after therapy. However, based on parents' reports collected through open-ended questions assessing changes observed during therapy, it appears that improvement considered minor by a healthy individual, such as a researcher, is subjectively very significant from the perspective of a caregiver of a disabled child. This is because it can translate into a relatively significant relief in caring for a dependent child, thanks to the acquisition of skills undervalued by healthy individuals, such as the ability to leave the house due to an increase in the child's tolerance to associated stimuli. Subjectively assessed impact on quality of life was reported in our previous work [25]. The next limitation of our paper is mixed route of administration. When planning the medical experiment, the optimal route of administration was uncertain, as there were no studies comparing both routes in the target group. However, it was known that both routes yielded positive results in animal studies. Intrathecal administration had the advantage of bypassing the blood-brain barrier, but it also posed the risk of post-lumbar puncture headaches. Therefore, a cautious approach was taken, and the initial administration was always intravenous for safety reasons. While literature reports generally indicated a high level of safety for MSC administrations [78, 79], we believed that an excess of caution would not harm and that if individual-specific side effects were to occur, it was preferable for them to take place in the circulation rather than the central nervous system. After empirically confirming safety in specific cases, we administered cells intrathecally to children whose parents preferred this route, particularly if parents of some patients expressed concerns about their child undergoing lumbar puncture. In the presence of any factors favoring a change in the route of administration, even minor ones or those motivated by non-medical considerations such as parental preferences, we altered the route. This decision was based on the understanding that cells primarily act through a paracrine effect, making the route of administration less critical. The dosing method and the analysis of the collected data that we applied may not be optimal. We quantitatively analyzed only the change between the $1^{st}$ and $5^{th}$ administrations, without considering repeated measurements. As we knew from previous data, achieving improvement was not possible before receiving a total dose of less than 3 million cells per kilogram of body mass, which the patient reaches at visit 3 or 4. However, we did not want to administer the smallest effective dose all at once to avoid exposing the patient to the risk of pulmonary embolism. The dosing scheme applied is the same as the one used in our pilot study planned in 2013, as it proved to be effective and safe. It aligns with the framework later described by Eggenberger in the systematic review [69]. The dose-effect correlation we observed was also demonstrated by three other authors cited in this systematic review. Medical therapeutic experimentation is not a phase 1 clinical trial, and its aim was not to establish optimal dosing. There is still an urgent need for dosing optimization and standardization, as the significant variability in administration methods limits drawing conclusions from meta-analyses.

## Conclusions

In conclusion, the application of hUC-MSCs enhanced the functional performance of patients, but the individual responses varied. The therapy also benefited children with high level of disability but not to the same extent as the initially less disabled children. Although younger patients responded better to the treatment, older children can benefit too.

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**Authors Contributions** MCh-K designed the study, recruited the patients, performed all medical procedures, collected and interpreted data, and edited the first draft of the paper prepared by the medical writing agency (see: Acknowledgments). IZ-M performed statistical analysis, interpreted data, and edited the first draft of the paper prepared by the medical writing agency (see: Acknowledgments). Dariusz Boruczkowski interpreted data, supervised the project, and edited the first draft of the paper.

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**Data Availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Code Availability** Not applicable.

## Declarations

**Ethics Approval and Consent to Participate** The procedure was approved by the Bioethics Committee at the Regional Chamber of Physicians and Dentists in Lublin, Poland (152/2018/KB/VII and 119/2021/KB/VIII). The study adhered to the ethical principles that have their origin in the Declaration of Helsinki. All participants' parents signed an informed consent form before the procedure.

**Consent for Publication** Individual cannot be identified and therefore consent for publication is not required.

**Conflict of Interests** Izabela Zdolińska-Malinowska and Dariusz Boruczkowski are employees of Polski Bank Komórek Macierzystych (FamiCord Group), Warsaw, Poland.

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