Pediatric Diabetes

ISPAD International Society for Pediatric and Adolescent Diabetes

*Pediatric Diabetes 2014: 15: 100–109*
*doi: 10.1111/pedi.12072*
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## Original Article

# Effect of a single autologous cord blood infusion on beta-cell and immune function in children with new onset type 1 diabetes: a non-randomized, controlled trial

Giannopoulou EZ, Puff R, Beyerlein A, von Luettichau I, Boerschmann H, Schatz D, Atkinson M, Haller MJ, Egger D, Burdach S, Ziegler A-G. Effect of a single autologous cord blood infusion on beta-cell and immune function in children with new onset type 1 diabetes: a non-randomized, controlled trial. Pediatric Diabetes 2014: 15: 100–109.

Background: The application of autologous cord blood in children with type 1 diabetes has been found to be safe, but not to preserve beta-cell function in a previous study, which, however, had not included a control group.
Objective: To compare the changes of metabolic and immune function over time between cord blood infused children and natural controls.
Subjects and methods: Seven children with newly diagnosed type 1 diabetes underwent a single autologous cord blood infusion and 10 children were enrolled as natural controls in a non-randomized, controlled, open label intervention trial. Primary analyses were performed 1 year following cord blood infusion. Cases and controls were compared regarding metabolic [area under the curve (AUC) and peak C-peptide, insulin use, and HbA1c] and immune outcome (islet autoantibody titer and T-cell response), adjusted for age, gender, diabetes duration, and baseline levels.
Results: There were no significant adverse events related to the infusion. Metabolic and immune outcomes were not significantly different at 12 months follow-up between infused children and controls (e.g., adjusted $p = 0.244$ for AUC C-peptide, adjusted $p = 0.820$ for insulin use, adjusted $p = 0.772$ for peripheral regulatory T cells). Six-month change of AUC C-peptide correlated significantly with the number of infused CD34+ cells ($r = 0.931$, $p = 0.002$).
Conclusions: An autologous cord blood infusion does not change the natural course of metabolic and immune parameters after disease onset. However, the content of CD34+ cells in the stored blood sample might offer potential for improvement of future cell therapies.

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Key words: C-peptide – regulatory T-lymphocytes – stem cells

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Submitted 9 June 2013. Accepted for publication 30 July 2013

Type 1 diabetes is one of the most common chronic diseases in childhood, with a continuously increasing incidence especially in children under the age of 5 years (1). Regarding young individuals the only accepted strategy to date for controlling blood glucose levels is exogenous insulin administration, which is however still associated with unstable glucose levels and restricting everyday life. The persistence of endogenous insulin secretion is associated with better glycemic control and a delay in the progression toward diabetic complications as shown by The Diabetes Control and Complications Trial (2, 3). Several immunosuppressive and immunomodulatory agents have been examined in order to assess efficacy in preserving beta-cell function in settings of type 1 diabetes, but when it comes to young individuals safety remains a critical factor (4).

As a vast source of primitive hematopoietic stem cells and endothelial progenitor cells, cord blood is considered a safe treatment option for pediatric patients with various malignant and non-malignant diseases, being able to reconstitute the hematopoietic system and restore immunological function (5–7). The introduction of cord blood in the regulation of immune imbalance in various autoimmune diseases such as type 1 diabetes has gained great interest during the past years (8, 9). A critical role in the suggested mechanism plays a subpopulation of CD4+ T cells, the CD4 + CD25+ regulatory T cells (Tregs), described to manifest potent suppressor functions and maintain self-tolerance (10).

Haller et al. have shown that the application of cord blood in children with type 1 diabetes is safe, but fails to preserve beta-cell function (11). However, despite that effort, it remains unclear whether infused children demonstrate a better course of beta-cell function preservation in contrast to natural controls. In addition, it is already known that the number of infused cells, especially CD34+ cells, is associated with rate of engraftment and clinical outcome in hematopoietic disorders (6, 12–14). Here, we performed a non-randomized, open-label, controlled trial for young children with newly diagnosed type 1 diabetes in order to assess safety and clinical efficacy of a single autologous cord blood infusion. The objective of this study is to compare the changes of metabolic and immune function over time between infused children and natural controls. Furthermore, we aim to investigate the effect of CD34+ cell dose on the course of beta-cell function.

## Research design and methods

### Recruitment

The trial was conducted in compliance with the protocol used by the University of Florida (15) in the Forschergruppe Diabetes München, Technische Universität München, Germany (trial registration: clinicaltrials.gov, NCT00989547) and was approved by the local ethics committee (Technische Universität München Nr. 2022/08). Between September 2008 and March 2012, children with newly diagnosed type 1 diabetes were recruited across Germany according to the following inclusion criteria: age >1 year, disease duration <1 year, possession of a suitable autologous cord blood sample stored in the accredited, private cord blood bank Vita34 (Leipzig, Germany), laboratory results [complete blood count (CBC) and basic metabolic profile] within normal ranges and compliance with intensive insulin treatment. Cord blood samples had to meet the following selection criteria: cord and mother's blood at the time of collection were free of infectious disease markers, and viability of cord blood cells was >50%. The following exclusion criteria were applied: positive test for hepatitis B virus (HBV), hepatitis C virus (HCV), *Treponema pallidum*, human immunodeficiency virus (HIV), parvovirus B19, cytomegalovirus (CMV), human T-lymphotropic virus types 1 and 2 (HTLV-1/2), or other active infections and chronic disease requiring immunosuppressive therapy. In the German study center, an additional group of children with type 1 diabetes who fulfilled the above inclusion criteria but did not possess an autologous cord blood unit were recruited across Germany as natural controls. Type 1 diabetes diagnosis was established by clinical symptoms (polydipsia, polyphagia, polyuria, and weight loss) and presence of disease associated autoantibodies. Written informed consents were obtained from all parents/guardians.

### Autologous cord blood infusion

Cord blood was shipped to the study center using standard shipping methods for frozen cord blood cells (temperature $<-130^{\circ}C$), where it was thawed (Plasmatherm, Fa.Barkey GmBH&Co KG, Leopoldshoehe, Germany) to a maximum 37°C and washed with NaCl 0.9% twice (Sepax Biosafe Kit CS 600.1, Biosafe SA, Eysins, Switzerland). Viability, total number of nucleated cells, and CD34+ cells were determined by Vita34 technicians in the final cord blood product. Following preparation of the unit, children received an i.v. pretreatment with mannitol (2 mL/kg body weight infused in 30 min) and pethidine (Dolantin 1 mg/kg body weight infused in 30 min). An emergency schedule was calculated for each individual separately and safety parameters were assessed in blood. Washed cord blood cells (in a volume of circa 50 mL) were then infused through a peripheral intravenous line for 20–30 min. During the procedure, pulse oximetry was applied and vital

signs including blood pressure, heart and breathing rate were monitored every 15 min during and 1 h after infusion. Subjects were dismissed after a monitoring period of circa 6 h and returned the next day for a control of blood safety parameters.

### Follow-up

Children who received cord blood infusion were followed every 3 months during the first and every 6 months during the second year postinfusion, while controls were examined at months 0, 6, 12, and 24. A mixed meal tolerance test (MMTT), CBC, basic metabolic panel, HbA1c, T-cell assays, and autoantibody titer were performed at each visit. All children continued on their normal insulin regimen throughout the study, unless changes were clinically indicated. HbA1c levels below 7.5% were intended, according to current pediatric guidelines (16). According to the investigation plan for the German site, primary goal was the recruitment of 30 children (10 cases and 20 controls). Due to prolonged recruitment and limited funding, recruitment ended earlier.

### Safety parameters

Safety parameters included laboratory tests (CBC, electrolytes, serum creatinine, and urea) and documentation of adverse events and concomitant medication, all performed at each visit. Laboratory tests were performed centrally at the Institute for Clinical Chemistry of the Klinikum Schwabing, Germany, using accredited methods.

### Metabolic outcome

The primary efficacy variable was area under the curve ($AUC_{0-120\,min}$) C-peptide (AUC C-peptide) of a 2-h standard MMTT. Blood samples were drawn for the determination of C-peptide at −20, −10, 0, 15, 30, 60, 90, and 120 min after ingesting 6 mL/kg body weight of a standard oral mixed formula liquid meal (Boost Nutritional Energy Drink, Nestle Healthcare Nutrition Inc., Vevey, Switzerland). Secondary efficacy parameters were peak C-peptide after MMTT, daily insulin requirements, and HbA1c. C-peptide determination was performed at the Northwest Lipid Research Laboratories, Washington, using a two-site immunoenzymometric assay (TOSOH AIA 1800, TOSOH Bioscience Inc., South San Francisco, CA, USA). HbA1c was measured centrally at the Institute for Clinical Chemistry of the Klinikum Schwabing. On the basis of personal insulin diaries, daily insulin requirements per kilogram body weight were calculated for each subject.

### Immune outcome

Immune function was assessed by autoantibody titer and T-cell repertoire (peripheral blood Tregs, memory Tregs, recently activated CD4+ T cells and CD4+ to CD8+ T-cell ratio), performed at all visits. Islet autoantibodies to insulin (IAA), glutamate decarboxylase (GADA), protein tyrosine phosphatase-related molecules IA-2a (IA-2A) and zinc transporter 8 (ZnT8A) were determined centrally by the Forschergruppe Diabetes München using radiobinding assays as previously described (17, 18). Briefly, IAA were measured by Protein A/G radio-binding assays using [$^{125}$I]-recombinant human insulin labeled at tyrosine aa14. GADA, IA-2A, and ZnT8A were measured by Protein A radiobinding assays using [$^{35}$S]-methionine-labeled *in vitro* transcribed/translated recombinant human GAD65 (aa1-585), the intracellular portion of IA-2 (aa605-979), and COOH-terminal (aa268-369) constructs of the ZnT8 R325 (ZnT8RA) and W325 (ZnT8WA) variants, respectively. Samples were considered ZnT8A-positive if antibodies to at least one of the ZnT8 variants were found. The upper limit of normal for each assay was determined using Q–Q plots and corresponded to the 99th percentile of 836 control children. Children were considered islet autoantibody-positive when two consecutive samples collected after birth were positive. Autoantibody assays were evaluated by the Diabetes Autoantibody Standardization Program (19–21). Peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation over Lymphoprep (Axis-Shield) and freshly stained for the markers CD3, CD4, CD8, CD25, CD45RO, CD127, and FOXP3 using standard techniques. The phenotypes of examined T-cell subpopulations were defined as follows: cells were identified as peripheral blood Tregs when they were CD4+, CD25+, CD127low, and FOXP3+, memory Tregs were defined by the markers CD4+, CD25+, CD127low, FOXP3+, and CD45RO+, and recently activated CD4+ T cells by the markers CD3+, CD4+, CD8−, CD25+, and CD69+. Cells were acquired on a Becton Dickinson LSR-II flow cytometer with FACSDiva software. At least 50 000 gated CD4+ events were acquired for each sample and analyzed using FlowJo software version 7.6.3 (TreeStar Inc., Ashland, OR, USA). T-cell measurements including peripheral Tregs, memory Tregs, and recently activated CD4+ T cells, which had not been performed immediately after collection in fresh blood, were excluded from the present analysis, because samples which were stored in the meantime were found to have significantly lower T-cell numbers.

### Statistical analysis

AUC C-peptide was calculated using the formula introduced by Tai (22). Unadjusted comparisons between cases and controls were performed using the non-parametric Mann–Whitney *U* test. Linear regression was used to adjust for age, gender, type 1 diabetes duration, and baseline levels. Absolute changes of AUC C-peptide, daily insulin use, and HbA1c from baseline to 6-month follow-up were correlated with the total number of infused nucleated cells and the number of infused CD34+ cells using Pearson's correlation coefficient. Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) with $p < 0.05$ indicating significance. All data are presented as median (range). To account for potential attrition bias, we performed sensitivity analyses for all examined parameters including only those participants with complete measurements at each time point.

## Results

As of February 2013, 17 children were enrolled in the trial (Fig. 1). Of these, 7 underwent an autologous cord blood infusion and 10 were followed as controls (Table 1). Participants of the control group were significantly older compared with those of the treatment group (median age 3.02 vs. 6.60 years, $p = 0.005$). Both groups were similar regarding diabetes duration ($p = 0.813$) and baseline values of metabolic (Table 2) and immune parameters (Table 3). Primary analyses were performed 1 year following cord blood infusion. One participant withdrew consent voluntarily 6 months postinfusion, but was included in this analysis.

Table 1. Patient demographics and cord blood characteristics

| | Cases | Controls |
|---|---|---|
| N | 7 | 10 |
| Age (years) | 3.02 (1.82–5.38) | 6.60 (3.59–10.85) |
| Sex | | |
| Male | 5 | 4 |
| Female | 2 | 6 |
| Time since diagnosis (days) | 101 (73–348) | 139 (63–410) |
| Cord blood characteristics: | | |
| TNC/kg (cells $\times 10^7$) | 3.89 (0.73–5.24) | — |
| Volume (mL) | 56.3 (33.0–70.9) | — |
| Viability TNC (%) | 80.3 (73.0–87.5) | — |
| $CD34^+$ cells (cells $\times 10^6$) | 1.27 (0.43–2.27) | — |
| Amount dimethyl sulfoxide (g) | 3.4 (2.0–4.2) | — |
| Hemoglobin (mg/dL) | 124.0 (89.0–142.0) | — |

TNC, total nucleated cells.
Data are shown as median (range) and n is the number of participants.

### Safety assessment

No acute infusion-related reactions have been observed. None of the seven infused children developed fever, hypo- or hypertension, nausea, abnormalities in safety parameters or severe hypo- or hyperglycemias.

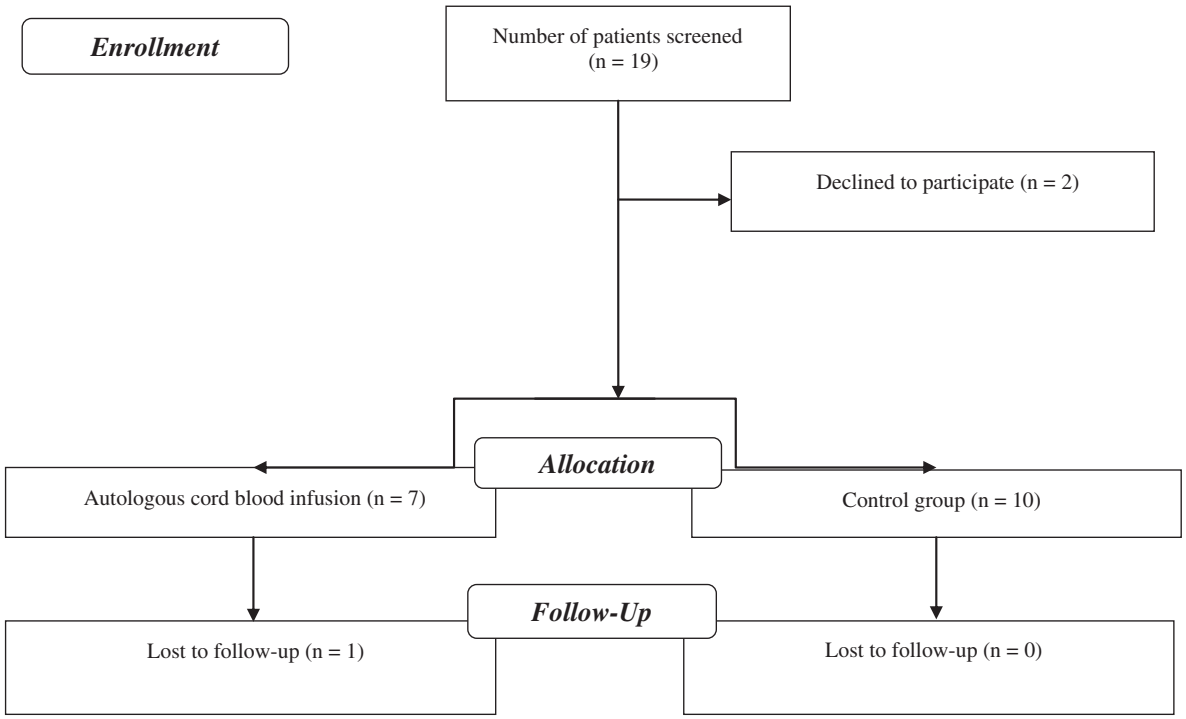


*Fig. 1.* Flow of participants.

Table 2. Metabolic outcome during 1st year of follow-up

| Metabolic outcome and time point | Cases | Controls | p value (unadjusted) | p value (adjusted) |
|---|---|---|---|---|
| AUC C-peptide (ng/mL × 120 min) | | | | |
| Baseline | 111.3 (0.0–146.3) | 162.1 (0.0–224.3) | 0.193 | 0.376 |
| Month 3 | 101.0 (0.0–144.6) | — | | |
| Month 6 | 60.6 (0.0–112.1) | 104.6 (0.0–183.9) | 0.193 | 0.915 |
| Month 9 | 25.4 (0.0–83.3) | — | | |
| Month 12 | 23.9 (0.0–107.6) | 59.3 (0.0–222.3) | 0.435 | 0.244 |
| Peak C-peptide (ng/mL) | | | | |
| Baseline | 1.15 (0.00–1.48) | 1.51 (0.0–2.33) | 0.270 | 0.415 |
| Month 3 | 0.92 (0.00–1.78) | — | | |
| Month 6 | 0.67 (0.00–1.20) | 1.02 (0.00–2.14) | 0.315 | 0.995 |
| Month 9 | 0.24 (0.00–1.00) | — | | |
| Month 12 | 0.25 (0.00–1.06) | 0.55 (0.27–2.76) | 0.524 | 0.228 |
| HbA1c (%) | | | | |
| Baseline | 7.1 (5.8–8.1) | 6.9 (5.3–7.6) | 0.740 | 0.771 |
| Month 3 | 7.4 (6.2–7.9) | — | | |
| Month 6 | 7.3 (6.5–7.7) | 7.3 (5.4–8.0) | 0.962 | 0.429 |
| Month 9 | 7.1 (6.6–7.3) | — | | |
| Month 12 | 7.2 (6.7–7.9) | 7.0 (5.8–9.1) | 0.428 | 0.525 |
| Daily insulin dose (IU/day/kg) | | | | |
| Baseline | 0.50 (0.17–1.46) | 0.41 (0.23–0.95) | 0.669 | 0.611 |
| Month 3 | 0.54 (0.31–0.98) | — | | |
| Month 6 | 0.55 (0.21–1.01) | 0.69 (0.14–0.95) | 0.270 | 0.403 |
| Month 9 | 0.57 (0.32–1.33) | — | | |
| Month 12 | 0.68 (0.34–1.45) | 0.76 (0.27–1.00) | 0.713 | 0.820 |

AUC, area under the curve.
Sample size: $n \leq 7$ for cases and $n \leq 10$ for controls. Data are shown as median (range). Unadjusted comparisons were performed by Mann–Whitney *U* test and adjustment for age, gender, type 1 diabetes duration and baseline levels was performed by linear regression.

## Metabolic outcome

Median AUC C-peptide decreased gradually in both groups (Fig. 2A), in infused children by 78.53% and in controls by 63.42% 1 year after enrollment. There were no significant differences between groups at the 6-month ($p = 0.193$ and adjusted $p = 0.915$) and 12-month visits ($p = 0.435$ and adjusted $p = 0.244$, Table 2). Median peak C-peptide decreased in similar rates over the first year (Fig. 2B, Table 2) and the changes in median peak C-peptide from baseline to months 6 and 12 were comparable in both groups (Table 2). Two children, one from each group, had undetectable levels of C-peptide (both fasting and stimulated) already at baseline.

Median daily insulin requirements remained stable during the first 6 months postinfusion in the case group but increased 12 months postinfusion by 0.18 units (Fig. 2C), i.e. 36.0% in comparison to baseline. Controls had increased insulin requirements in both follow-up examinations compared to baseline, but their 6- and 12-month levels were not significantly higher in contrast to cases (month 6: $p = 0.270$ and adjusted $p = 0.403$/month 12: $p = 0.713$ and adjusted $p = 0.820$). HbA1c peaked in both groups at month 6 and declined at month 12 (Fig. 2D); median 6- and 12-month values were not significantly different between groups (Table 2). Sensitivity analyses with complete cases and controls yielded similar results (data not shown).

## Immune function markers

Regarding changes in immune function, there were no significant differences between cases and controls in the titers of type 1 diabetes associated autoantibodies during follow-up (Table 3). Peripheral blood Tregs declined until month 12 in both groups (Table 4). Memory Tregs remained at stable levels in cases and decreased in the control group during the first year of follow-up; however, median levels of 6- and 12-month peripheral blood and memory Tregs did not differ significantly between cases and controls (Table 4). Recently activated CD4+ T cells and CD4+ to CD8+ T-cell ratio remained at similar levels at all study visits in both groups (Table 4). Sensitivity analyses with complete cases and controls yielded similar results (data not shown). Individual trajectories of metabolic and immune function parameters are presented in Figs. S1 and S2.

## Cell dose and metabolic outcome

There was a tendency that those infused children who had received more nucleated cells per kilogram showed a better course regarding AUC C-peptide preservation in the first 6 months postinfusion (Pearson's $r = 0.751$, $p = 0.052$, Fig. 3A). The absolute change of AUC C-peptide was significantly correlated with the number of infused CD34+ cells (Pearson's $r = 0.931$, $p = 0.002$,

Table 3. Immune outcome during 1st year of follow-up

| Immune outcome and time point | Cases | Controls | p value (unadjusted) | p value (adjusted) |
|---|---|---|---|---|
| IA-2A (units) | | | | |
| Baseline | 104.2 (0.0–281.9) | 105.5 (0.0–333.4) | 0.601 | 0.392 |
| Month 3 | 92.6 (0.4–403.2) | — | | |
| Month 6 | 87.1 (0.0–280.0) | 128.9 (0.0–404.7) | 0.669 | 0.327 |
| Month 9 | 94.4 (0.0–335.6) | — | | |
| Month 12 | 41.1 (0–330.9) | 54.0 (0.0–529.5) | 0.699 | 0.366 |
| GADA (units) | | | | |
| Baseline | 25.2 (3.1–297.1) | 19.2 (0.1–738.8) | 0.962 | 0.770 |
| Month 3 | 49.5 (0.0–425.2) | — | | |
| Month 6 | 8.7 (0.1–349.4) | 14.5 (0.1–348.8) | 0.887 | 0.883 |
| Month 9 | 51.1 (0.0–490.4) | — | | |
| Month 12 | 14.8 (0.1–276.1) | 47.1 (0.1–729.8) | 0.898 | 0.437 |
| IAA (units) | | | | |
| Baseline | 262.5 (121.0–958.0) | 219.0 (0.42–750.0) | 0.864 | 0.936 |
| Month 3 | 301.7 (193.0–484.4) | — | | |
| Month 6 | 340.3 (165.5–1160.9) | 191.3 (58.3–2046.9) | 0.364 | 0.900 |
| Month 9 | 331.5 (147.6–548.3) | — | | |
| Month 12 | 92.3 (62.3–415.6) | 251.0 (7.30–2419.2) | 0.364 | 0.308 |
| ZnT8RA (units) | | | | |
| Baseline | 53.2 (0.1–1594.5) | 134.5 (0.1–1763.0) | 0.887 | 0.760 |
| Month 3 | 62.2 (0.1–958.8) | — | | |
| Month 6 | 18.4 (0.1–1095.4) | 77.7 (0.1–2130.3) | 0.962 | 0.157 |
| Month 9 | 28.31 (0.1–1353.8) | — | | |
| Month 12 | 27.4 (0.1–431.2) | 167.7 (0.1–5554.0) | 0.518 | 0.289 |
| ZnT8WA (units) | | | | |
| Baseline | 148.4 (0.1–2241.6) | 100.8 (0.1–1658.0) | 0.740 | 0.314 |
| Month 3 | 59.4 (0.1–585.3) | — | | |
| Month 6 | 38.1 (0.1–1285.4) | 90.5 (0.1–2097.4) | 0.740 | 0.206 |
| Month 9 | 56.6 (0.1–1004.5) | — | | |
| Month 12 | 92.4 (10.3–829.5) | 115.7 (0.1–11663.8) | 0.797 | 0.689 |

GADA, glutamic acid decarboxylase autoantibodies; IAA, insulin autoantibodies; IA-2A, islet antigen-2 autoantibodies; ZnT8RA, zinc transporter 8 R325 autoantibodies; ZnT8WA, zinc transporter 8 W325 autoantibodies.
Sample size: $n \leq 7$ for cases and $n \leq 10$ for controls. Data are shown as median (range). Unadjusted comparisons were performed by Mann–Whitney *U* test and adjustment for age, gender, disease diabetes duration and baseline levels was performed by linear regression.

Fig. 3B). No significant correlation was found between the change in HbA1c and the total number of infused cells ($r = -0.052$, $p = 0.912$) or the number of CD34+ cells ($r = -0.178$, $p = 0.706$). Change in daily insulin requirements also did not correlate with the number of infused cells ($r = -0.026$, $p = 0.956$) or the number of CD34+ cells significantly ($r = -0.008$, $p = 0.986$). The doses of all infused nucleated and CD34+ cells for each participant are provided in Table S1.

## Discussion

The aim of this trial was to investigate the effect of a single autologous cord blood infusion close to diagnosis on the course of metabolic and immune function parameters in children with type 1 diabetes. Although there were no significant safety issues or adverse events related to infusion, this intervention did not prevent from the natural loss of residual beta-cell function, did not cause significant changes in daily insulin requirements and glucose control, and was not found to change the T-cell repertoire significantly.

The administration of autologous cord blood in newly diagnosed children with type 1 diabetes has already been proven to be safe in previous reports from Haller et al. (11), nevertheless it was unknown if infused children presented different courses in contrast to controls. In this trial, infused children showed a similar gradual decline rate of C-peptide over 1 year of follow-up as controls. Here, participants of the control group were significantly older compared with infused children. Therefore, the initial measurements of C-peptide levels at time of inclusion were considerably different between groups. Except for metabolic outcome, the present trial investigated possible changes in immune response postinfusion by analyzing the T-cell repertoire, because it is suggested that cord blood is a rich source of Tregs, which are able to suppress effector T cells and maintain self-tolerance (10). We did not observe a significant increase in Tregs after infusion in the present trial as previously reported by Haller et al. (11), while memory Tregs remained rather stable. The presence of self-reactive effector T-cells, found to be resistant to Treg suppression (23), as well

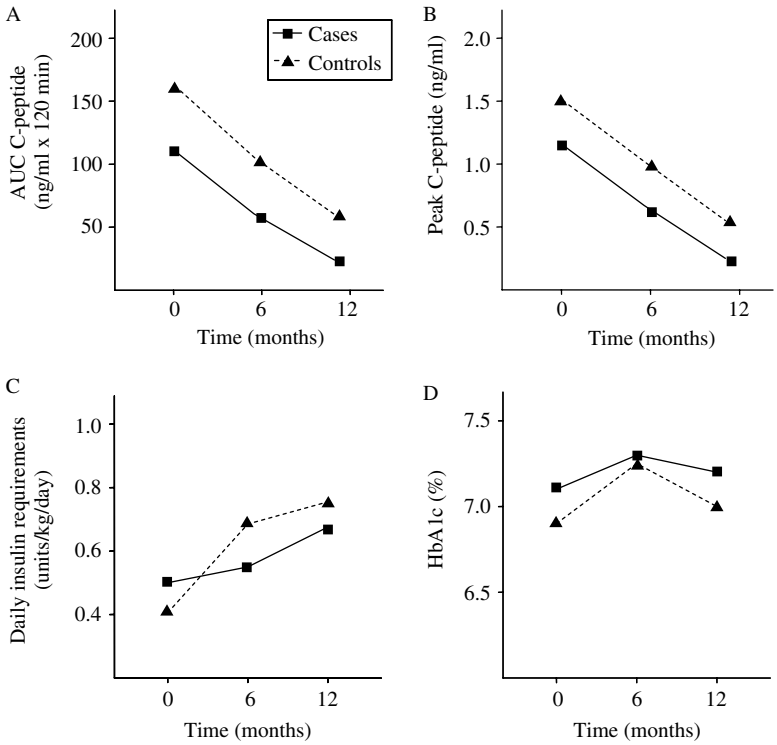


*Fig. 2*. Metabolic outcome: median AUC C-peptide (A), peak C-peptide (B), daily insulin requirements (C) and HbA1c (D) from baseline (=0) to months 6 and 12 in cases (black squares) and controls (black triangles).

as the already described impaired suppressive ability of Tregs in patients suffering from type 1 diabetes (24, 25), could act as a burden in upcoming cell therapies.

The total number of infused nucleated cells and CD34+ cells has been found to correlate with clinical outcome in other diseases (12–14); however, with regard to type 1 diabetes such findings have not been documented thus far. In the present trial the median number of infused nucleated cells per kilogram was higher in contrast to Haller's study ($3.89 \times 10^7$ vs. $1.88 \times 10^7$cells/kg, respectively) (11), which can be explained by the fact that our participants were of younger age (median age 3.02 vs. 5.10 years). Children who received a richer in stem cells cord blood sample were found to have a better preservation of residual beta-cell function, assessed by AUC C-peptide. The exact mechanism is unclear; however, it may suggest that cord blood therapy could be efficacious if enough CD34+ cells are infused. Efficacy could be due to the ability of cord blood stem cells to travel to damaged tissues and protect them from further destruction or stimulate the remaining tissue to regenerate. This has been shown by previous studies in mouse models of streptozotocin-induced pancreatic damage where infused bone marrow-derived stem cells were able to correct elevated blood glucose levels, by migrating preferentially to destroyed tissues and by stimulating beta-cell regeneration (26, 27). Alternatively, it could be via immune regulation but we did not observe a direct association between the number of Treg cells postinfusion and the number of CD34+ cells making this mode of action more unlikely. With this observation, our findings should give direction to future follow-up trials in which it may be necessary to initially define the suitable final blood product that affects clinical outcome. Improvement of cord blood harvesting procedures and infusion of *ex vivo* expanded cells (28), ensuring certainly that safe suppression of autoimmunity is achieved, should be considered.

A main strength of this study involves the recruitment of a control group, which for the first time allows for a comparison of the disease course after infusion with a 'natural' course. Furthermore, the use of the same storing and thawing procedures for all infused cord blood samples improves comparability of metabolic and immunologic measurements between individuals. Limitations of our study include the small number of participants as well as the significant age difference between cases and controls. The inclusion criteria narrowed the potential target group initially and the experimental nature of the trial in combination with the fact that many interested families had only one stored cord blood unit were inhibitory factors for participation. Parents of younger children, who did not possess a cord blood sample, decided not to participate as controls, mainly because of their will to take part in intervention trials. As a further limitation,

Table 4. T-cell repertoire during 1st year of follow-up

| Immune outcome and time point | Cases | Controls | p value (unadjusted) | p value (adjusted) |
|---|---|---|---|---|
| Peripheral blood Treg (%) | | | | |
| Baseline | 6.53 (4.20–9.10) | 6.05 (1.29–9.00) | 0.905 | 0.365 |
| Month 3 | 6.14 | — | | |
| Month 6 | 4.44 (1.46–7.11) | 5.31 (3.46–7.88) | 0.622 | 0.448 |
| Month 9 | 4.81 (2.37–8.95) | — | | |
| Month 12 | 4.30 (2.53–6.83) | 3.78 (2.41–7.30) | 0.833 | 0.772 |
| Memory Treg (%) | | | | |
| Baseline | 1.66 (0.60–2.42) | 1.53 (0.65–2.60) | 0.905 | 0.586 |
| Month 3 | 1.97 | — | | |
| Month 6 | 1.62 (0.64–4.22) | 1.48 (0.77–3.70) | 0.950 | 0.531 |
| Month 9 | 1.41 (1.03–1.94) | — | | |
| Month 12 | 1.63 (0.60–1.97) | 1.15 (0.85–3.11) | 0.943 | 0.669 |
| Recently activated CD4+ T cells (%) | | | | |
| Baseline | 0.21 (0.20–0.26) | 0.16 (0.15–0.65) | 0.167 | 0.398 |
| Month 3 | 0.32 | — | | |
| Month 6 | 0.50 (0.11–1.04) | 0.27 (0.07–0.66) | 0.145 | 0.219 |
| Month 9 | 0.20 (0.17–0.69) | — | | |
| Month 12 | 0.17 (0.06–0.53) | 0.17 (0.09–0.70) | 0.699 | 0.899 |
| CD4+ to CD8+ T cell ratio | | | | |
| Baseline | 2.22 (0.42–3.35) | 1.97 (1.22–5.83) | 0.918 | 0.860 |
| Month 3 | 2.28 (1.36–3.08) | — | | |
| Month 6 | 1.85 (1.27–4.37) | 1.97 (1.31–2.97) | 0.536 | 0.686 |
| Month 9 | 1.95 (1.63–4.30) | — | | |
| Month 12 | 1.82 (1.47–3.14) | 1.65 (1.45–3.31) | 0.529 | 0.713 |

Sample size: $n \leq 7$ for cases and $n \leq 10$ for controls. Data are shown as median (range). Unadjusted comparisons were performed by Mann–Whitney *U* test and adjustment for age, gender, disease duration and baseline levels was performed by linear regression. Peripheral blood Treg, memory Treg, and recently activated T cells are expressed in % of CD4+T cells.

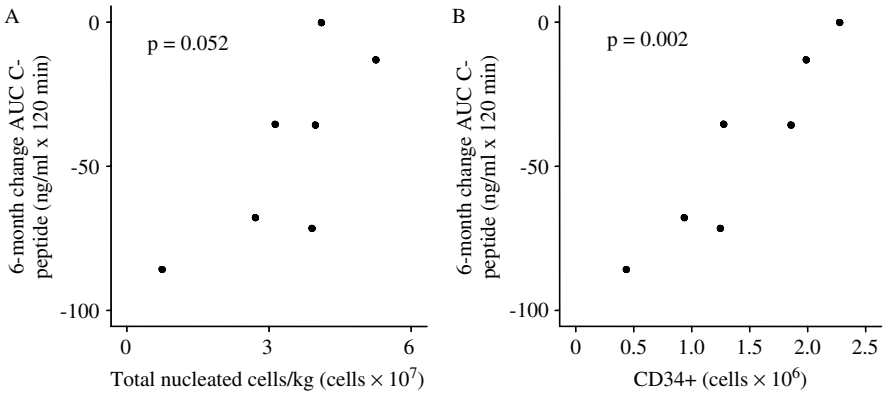


*Fig. 3*. Correlation between the absolute change of AUC C-peptide over 6 months of follow-up after autologous cord blood infusion, the total number of infused nucleated cells [(A) Pearson's r = 0.751, p = 0.052] and the number of infused CD34+ cells [(B) Pearson's r = 0.931, p = 0.002] for all seven infused children.

some T-cell measurements were taken from stored samples and could therefore not be used for statistical analysis.

In conclusion, our results show that a single autologous cord blood infusion in children with newly diagnosed type 1 diabetes is safe but does not seem to protect from the natural loss of beta-cell function during the first year postinfusion. Cell expansion techniques and probably combinational intervention with immune modulators should be considered in order to improve this approach.

## Acknowledgements

The work was funded by Juvenile Diabetes Research Foundation (JDRF), JDRF Center Grant 4-2007-1065 (subcontract Nr.UFO9121). The sponsors of the study had no role in the study design, data collection, data analysis, interpretation of data, or writing of the manuscript.

We acknowledge ongoing cord blood collaborations with University of Florida, Dr Marcovina's lab (University of Washington), and Vita34. We thank Melanie Bunk, Claudia Ramminger, Anita Gavrisan, and Anja Wosch for data collection, and expert technical assistance, the Department of Pediatrics of the Technische Universität München (staff and

nurses), and most importantly the children and families who participated in the trial.

## Conflict of interest

No potential conflicts of interest relevant to this article were reported.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Total number of infused nucleated cells and CD34+ cells for all study participants.

Figure S1. Individual courses of studied metabolic parameters (AUC C-peptide, peak C-peptide, daily insulin requirements and HbA1c) for cases and controls over the first year of follow-up.

Figure S2. Individual courses of studied immune parameters (peripheral blood Tregs, memory Tregs, recently activated T cells and CD4 to CD8 T-cell ratio) for cases and controls over the first year of follow-up.

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