Transcoronary infusion of cardiac progenitor cells in hypoplastic left heart syndrome: 3-year follow-up of the TICAP trial

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Abstract

Objectives: Our aim was to assess midterm safety and clinical outcomes of intracoronary infusion of cardiosphere-derived cells (CDCs) following staged palliation in patients with hypoplastic left heart syndrome (HLHS).

Methods: In this prospective controlled study, fourteen consecutive patients with HLHS who were undergoing 2- or 3-staged surgical palliations were assigned to receive intracoronary CDC infusion 1 month after the cardiac surgery (n=7), followed by 7 patients allocated to a control group with standard care alone. The primary endpoint was to assess the procedural feasibility and safety and the secondary endpoint was to evaluate the cardiac function and heart failure status through 36-month follow-up.

Results: No complications including tumor formation were reported within 36 months after CDC infusion. Echocardiogram showed a significantly greater improvement in right ventricular ejection fraction (RVEF) in infants receiving CDCs than in controls at 36 months (+8.0 ± 4.7% vs. +2.2 ± 4.3%, P=0.03). These cardiac function improvements resulted in reduced brain natriuretic peptide levels (P=0.04), lower incidence of unplanned catheter interventions (P=0.04), and higher weight-for-age Z score (WAZ) (P=0.02) at 36 months compared with those of controls. As independent predictors of treatment responsiveness, absolute changes in RVEF at 36 months were negatively correlated with age, WAZ, and RVEF at CDC infusion (age: r=-0.91, P=0.005; WAZ: r=-0.88, P=0.009; EF: r=-0.90, P=0.006).

Conclusions: Intracoronary CDC infusion after staged procedure is safe and improves RVEF in patients with HLHS, which persists during 36-month follow-up. This therapeutic strategy may merit somatic growth enhancement and reduce the incidence of heart failure.

Abbreviations and Acronyms

HLHS = hypoplastic left heart syndrome

CDCs = cardiosphere-derived cells

RVEF = right ventricular ejection fraction

EDV = end-diastolic volume

ESV = end-systolic volume

Ea = effective arterial elastance

Ees = end-systolic elastance

cMRI = cardiac magnetic resonance imaging

UCG = ultrasonic echocardiography

Ultramini Abstract

Intracoronary infusion of autologous CDCs is safe in infants with HLHS after staged surgical procedures during 3-year observation. CDC infusion could improve right ventricular function-from 3 through 36 months of observation. Patients treated by CDCs showed an increase in somatic growth and reduced heart failure status in midterm follow-up.

Central Message

This is the first clinical trial to report the midterm safety and efficacy of cardiac progenitor therapy in congenital heart disease.

Perspective

These preliminary results suggest that cardiac progenitor cells in younger age of children may secrete specific effectors to stimulate progenitor cell growth for myocardial repair. Cardiac fibrosis in congenital heart disease may be alternative therapeutic target assessed by advanced cardiac imaging technologies to understand the mechanisms for cardiac function improvement after cell therapy.

Among congenital heart lesions, hypoplastic left heart syndrome (HLHS) is associated with the highest mortality.[1] As management strategies for neonates and infants with HLHS have markedly evolved in recent years,[2] an increasing number of patients with HLHS have survived to Fontan completion. Outcomes of patients undergoing Fontan procedures have also dramatically improved as a variety of modifications have been introduced, including lateral tunnel and extracardiac conduit variations.[3] However, circulation created after Fontan operation exhibits a number of physiological limitations that have negative effects on organ system development and functionality, such as arrhythmia, thromboembolism, protein-losing enteropathy (PLE), plastic bronchitis, and liver fibrosis.[4] Delayed somatic growth and development are also major concerns in single ventricle physiology.[5] The prevalence of systolic and diastolic ventricular dysfunction continues to increase after Fontan operation, particularly in those with morphologic right ventricles,[6] and impaired ventricular function itself has been reported as an independent risk factor of an adverse outcome after Fontan procedure.[7] Moreover, a recent study has shown that HLHS was strongly associated with adverse events and late failure.[8]

In 2011, we conducted the TICAP (Transcoronary Infusion of CArdiac Progenitor cells in patients with single ventricle physiology) trial and demonstrated the feasibility of the manufacture and intracoronary delivery of cardiosphere-derived cells (CDCs) in patients with HLHS following staged

palliation. This study showed that patient-derived CDCs were safe and effective for improving right ventricular function, heart failure status, and somatic growth during 18 months of follow-up.[9] However, whether these effects could persist at 3-year follow-up and whether CDC infusion has an impact on clinical outcomes after Fontan operation remained unknown. Here, we report the 36-month endpoint results of the TICAP trial, including cardiac function analysis and evaluation of midterm safety and outcomes after Fontan operation.

Methods

Study Design and Patient Population

The detailed study protocol was reported previously.[9] In this non-randomized, prospective controlled study, 14 consecutive patients with HLHS were assigned to receive intracoronary CDC infusion 1 month after the cardiac surgery (n=7), followed by 7 patients allocated to a control group with standard care alone. The eligible criteria was the patients under the age of 6 years old with HLHS undergoing stage-2 or -3 palliations. Patients undergoing stage 1 reconstruction were not included due to the difficulty in coronary angiogram. Exclusion criteria were cardiogenic shock, history of pacemaker implantation, intractable arrhythmia, repeated infections, and advanced hepatic or renal insufficiency. The primary endpoint was to assess the procedural feasibility and safety and the secondary endpoint was to evaluate the right ventricular ejection fraction (RVEF) ~~cardiac function~~ and heart failure status from the baseline through mid-term follow-up. In patients allocated to receive CDCs, the atrial tissues were obtained during staged surgical palliation for autologous cell production. Clinical mid-term follow-up was scheduled at 24, 30, and 36 months. The data include 3 years of follow-up on all of the patients in both CDC-treated and control groups. The study protocol of the TICAP trial was approved in December 2010 by the Ethics Committee of Okayama University and followed the Guidelines on Clinical Research Using Human Stem Cells issued by the Ministry of Health, Labour and Welfare, Japan. The study was performed in accordance with the Declaration of

Helsinki with written informed consent from all parents of eligible patients. This study is registered with ClinicalTrials.gov (NCT01273857).

Cell Processing and Infusion

Atrial tissues were excised, minced, and digested with 0.4% type II collagenase and 0.01% DNAse. Obtained cells were then plated at ultra-low culture dishes to generate cardiospheres in DMEM/F12 media supplemented with 10% autologous serum, 20 ng/mL epidermal growth factor (Sigma), and 40 ng/mL basic fibroblast growth factor (Promega). Manufactured CDCs were verified by immunostaining, flow cytometry, and gene expression analysis to confirm the mesenchymal phenotype as previously reported.[9] When the specified cell dose ($3.0x10^5$ cells per kilogram of body weight) was achieved, CDCs were selectively infused into each coronary artery 1 month after surgical operations. During CDC infusion, targeted coronary artery was inflated by occlusion balloon catheter Iiguman type C (Fuji Systems Corporation) for 2 min and the cells diluted in 1mL for each coronary artery were infused through the distal site of the balloon.

Data Acquisition and Analysis

Demographics, patient characteristics, preoperative assessment, and surgical characteristics were recorded from the medical records. In reporting preoperative assessment, the latest records of heart catheterization and echocardiography were used. Because all of the staged palliations in both groups were performed by the same surgeon (S.S.) during follow-up, the same surgical strategies and

techniques were used.

Clinical outcomes including adverse events and catheter interventions were also collected by reviewing the medical records and through telephone interviews. Tumor formation was assessed using the serum levels of tumor markers such as CEA and CA19-9, as well as carefully evaluated by echocardiography and cardiac magnetic resonance imaging (cMRI). Data of hemodynamic status were obtained by heart catheterization of the final follow-up performed from 30 to 36 months of follow-up.

Cardiac function was evaluated by echocardiography at 24, 30, and 36 months of follow-up. Efficacy was also assessed by cMRI study at 36 months of follow-up in both groups. Detailed methods of cardiac function analysis were performed as previously reported.[9] In short, using echocardiography, RVEF, end-systolic volume (ESV), and end-diastolic volume (EDV) were calculated by the monoplane ellipsoid approximation method. Tricuspid valve annulus diameter was measured by the annulus diameter in the apical 4-chamber view and Z score was calculated based on body surface area (BSA). The same as in our previous study,[9] cMRI scans were performed on a Philips 1.5 Tesla Achieva Scanner (Philips Healthcare, Netherlands) under general anesthesia. RVEF, RVEDV, and RVESV were calculated from a stack of short-axis cine images using the disk summation methods after tracing endocardial contours in end-diastole and end-systole. Right ventricular wall mass was calculated by tracing endocardial and epicardial contours. Mean arterial pressure, right ventricular systolic and end-diastolic pressure (RVEDP), RVEDV, RVESV, mean pulmonary arterial (PA) pressure, pulmonary

vascular resistance index (PVRI), and cardiac index were measured by catheterization study. The approximations of stiffness of the RV chamber and ventriculoarterial coupling (Ea/Ees) were calculated as previously reported.[9]

Heart failure status was evaluated by serum brain natriuretic peptide (BNP) levels and New York University Heart Failure Index (NYUPHFI).[10] For assessment of somatic growth at each follow-up, weight-for-age Z scores were calculated using the Center for Disease Control normative population data.[11] Quality of life including the condition of physical, mental, and social well-being of the patients was assessed using The Infant Toddler Quality of Life Questionnaire™ (ITQOL-SF47).[12] To identify stressful aspects of parent-child interaction, Abidin's Parenting Stress Index Short Form was used.[13]

Statistical Analysis

Continuous variables are expressed as mean ± SD. The categorical data are given as numbers or proportions. For continuous variables, the normality of data in the control group and the CDC-treated group was tested using the Shapiro-Wilk test, and comparisons were performed using the 2-tailed Student's *t* test or Mann–Whitney *U* test, as appropriate. Categorical variables were compared using Fisher's exact test. The incidences of catheterization interventions occurring during the follow-up period were compared between the two groups using Poisson regression models. Time to first adverse event or catheterization intervention was estimated using the Kaplan-Meier method and compared with the log-rank test. For comparison between the two groups by cMRI, 2-way ANOVA

was used to analyze the categorical independent variables between groups and the time interaction term within a group. To investigate predictors of cardiac functional efficacy, bivariate Pearson's correlation and simple linear regression analyses were performed. The analyses were performed using SPSS software, version 22 (IBM). The study funding source had no role in study design, data collection, data analysis and interpretation, or in the preparation of the manuscript.

Results

Patient Characteristics

All of the 14 patients enrolled for this study were followed up for 36 months. The basic characteristics of the patients in both groups are outlined in Table 1. There was no significant difference in age or body weight at baseline. No difference were seen in potential risk factors, such as history of cardiac arrest, induction of extracorporeal membrane oxygenation (ECMO), restrictive atrial septal defect for which balloon atrial septectomy was necessary, sinusoid arteries, aortic size, RV function before 1st palliation, genetic syndromes, extracardiac malformation, and prematurity. The morphology of all of the patients was HLHS, and mitral atresia combined with aortic atresia was the most common subtype in both groups. One HLHS variant was included in each group. All of the patients had undergone initial palliation including Norwood operation or bilateral pulmonary artery banding. One patient in the controls and 2 patients in the CDC-treated group underwent Norwood operation after bilateral pulmonary artery banding. In Norwood operation, Sano shunt (right ventricle-pulmonary artery conduit) was used in all of the patients apart from one in the controls who underwent Norwood in another hospital. Stage-2 palliation was completed in 2 patients in the controls and 4 patients in the CDC-treated group before study enrollment. No significant difference was seen in preoperative hemodynamic data between the two groups. For staged palliative operation during which atrial tissues were obtained for cardiac progenitor cell production, 2 patients in the controls and 4

patients in the CDC-treated group underwent total cavopulmonary connection. Stage-2 palliation was performed in the rest of the patients, including the Norwood-Glenn procedure in one patient in each group. There was no difference in cardiopulmonary bypass time and aorta cross clamp time between the two groups.

Safety and Adverse Events

No complications associated with cell infusion including sustained myocardial ischemia and ventricular arrhythmia and prolonged infection were reported within 36 months. No evidence of tumor formation was detected by echocardiography and cMRI, and serum levels of tumor markers such as CEA and CA19-9 remained within their normal ranges 3 years after cell infusion (Figure 1A). We assessed the late adverse events in both groups (Table 2). One patient in the controls developed heart failure (New York Heart Association class III-IV) because of prolonged impaired ventricular function and had a history of cardiopulmonary resuscitation. Another patient in the controls had a history of prolonged chest tube drainage and finally developed protein-losing enteropathy. No adverse events occurred in the CDC-treated group. The adverse-event-free survival curve showed no significant difference between the two groups (Figure 1B).

Clinical Outcomes and Hemodynamic Status

Among 8 patients who underwent stage-2 palliation, 7 had undergone Fontan operation during the follow-up period (Table 3). One patient in the controls mentioned before still remained in Glenn

circulation because of impaired ventricular function and poor weight gain. Fenestration was created in all patients. Catheterization study at final follow-up revealed no differences in mean PA pressure, RVEDP, and PVRI. However, cardiac indices in the CDC-treated group were significantly higher than in the controls (3.6 ± 0.6 vs. 4.9 ± 0.9 L/min/m^2, *P*=0.02).

Catheter Intervention

The incidence rates of catheter interventions during follow-up are shown in Table 2. The incidence rate of any type of unintended catheter intervention was significantly lower in the CDC-treated group than in the controls (0.52 vs. 1.43/person-year, *P*=0.04). No difference was seen between the two groups in terms of the incidence rate of each type of intervention, including coil embolization for aortopulmonary collateral artery (APCA) and balloon angioplasty (Table 2). However, freedom of APCA coil embolization was markedly worse in controls than in the CDC-treated group, whereas there was no significant difference in freedom from any catheter intervention (Figures 1C and D).

Cardiac Functional Analyses

Pooled data of echocardiography and cMRI-measured parameters in the two groups are shown in Table E1. None of the parameters at baseline differed between the CDC-treated group and the controls. In cMRI study, improvement of RVEF at 36 months from baseline was significantly greater in the CDC-treated group than in controls (41.8 ± 5.2 vs. 32.2 ± 9.4%, *P*=0.04, Figure 2A). The absolute changes in RVEF at 36 months from baseline were found to be greater in the CDC-treated group than

in the controls (+5.7 ± 4.6 vs. -2.2 ± 4.4%, *P*=0.04). Although the indices of RV volumes (RVEDVI and RVESVI) and right ventricular wall masses corrected by $BSA^{1.3}$ and EDV were reduced compared with those at baseline, no difference was seen between the two groups at 36 months (Figures 2B and C, and Table E1).

In echocardiographic assessment, the absolute changes in both RVEF and fractional area change at 36 months from baseline were significantly greater in the CDC-treated group than in the controls (+8.0 ± 4.7 vs. +2.2 ± 4.3%, *P*=0.03, and +5.4 ± 4.1 vs. +0.8 ± 2.7 %, *P*= 0.03; Figures 2D and E). As shown in Figure 2F, the absolute changes in tricuspid valve diameter from baseline to 36 months also significantly differed between the groups. To investigate diastolic function and mechanical efficiency, we assessed the stiffness of the systemic right ventricle and ventriculoarterial coupling (Ea/Ees) at final follow-up (Figures 2G and H). The CDC-treated group showed significantly decreased RV chamber stiffness and Ea/Ees compared with the controls (*P*=0.02 and *P*=0.001, respectively).

Somatic Growth and Heart Failure Status

Body weight correlated by Z score was analyzed to assess somatic growth during the follow-up period. The Z scores for weight at 36 months of follow-up were significantly higher in the CDC-treated group than in the controls (-1.6 ± 1.2 vs. -3.5 ± 1.5, *P*=0.02, Figure 3A). The CDC-treated group showed lower NYU PHFI scores and BNP levels, indicating better functional status, than the controls in the midterm (*P*=0.02 and *P*=0.04, respectively; Figures 3B and C). There was no significant

difference between the groups in terms of quality of life assessed by ITQOL-SF47 and parenting stress in mothers (Figure E1).

Predictors of Efficacy

We next investigated independent predictors of cardiac functional efficacy of CDC infusion (Figures 3D-F). Younger age was strongly associated with greater improvement of right ventricular ejection fraction measured by echocardiography (r=-0.91, P=0.005). We also found that lower weight-for-age Z score (WAZ) and reduced ejection fraction at infusion were the predictors of cardiac functional improvements 36 months after CDC infusion (r=-0.88, P=0.009, and r=-0.90, P=0.006, respectively).

Discussion

Since the first case of autologous mononuclear bone marrow cell (BMC) transplantation for acute myocardial infarction was reported in 2001, numerous clinical studies using various types of stem cell have been performed to investigate the effects of stem cell therapy, mainly in adult patients with ischemic heart disease.[14] Recent meta-analyses of intracoronary application of BMC revealed modest but significant improvement of left ventricular function after myocardial infarction and a reduced incidence of death and major adverse events, although these benefits were mixed or absent in several studies.[15] Among these numerous reports, limited studies have reported long-term outcomes after BMC administration for ischemic heart disease and dilated cardiomyopathy.[16-22] Five of these studies have shown long-term benefits in left ventricular function and quality of life;[16, 17, 20-22] however, the other reports revealed no evidence of sustained improvement of left ventricular performance.[18, 19] These controversies over the effect of non-cardiac progenitors such as BMCs led to the investigation of new cell types including CDCs.

Recent studies have shown that, among various cell types, CDCs exhibit a balanced profile of paracrine factor secretion and the greatest myocardial regeneration potential.[23, 24] In clinical trials, cardiac progenitors showed improvement of left ventricular ejection fraction in the SCIPIO trial and the reduction in scar mass in the CADUCEUS trial in patients with ischemic heart disease,[25, 26] whether the benefit for ventricular function or regeneration after cardiac progenitor infusion could be

maintained in long-term has remained unknown.

The clinical outcome of Fontan patients who require mechanical circulatory support as a bridge to heart transplantation is still poor; in addition, the survival after transplantation was the worst among patients with congenital heart disease.[27] Under these circumstances, clinical studies of cell therapy for pediatric patients have been limited to case reports, including one case of HLHS, until the initial results of the TICAP trial were reported recently.[28, 29] To our knowledge, this study is the first on the midterm outcomes of CDC infusion in pediatric patients.

In this 3-year follow-up study, no side effects including tumor formation were observed during 36 months of follow-up. Beneficial effects such as improvement of RV function with reduced tricuspid annulus diameter could be observed at 36 months. It has been demonstrated that CDCs in younger children are present at a much larger number in the heart and have stronger regenerative ability than those in adults.[30] Interestingly, greater improvement in cardiac function was detected in patients with a younger age or a low weight-for-age Z score, which also supports this hypothesis. Because recent report has suggested that growth hormone-releasing hormone may stimulate cardiac stem cell self-renewal to support myocardial repair, targeting the age-dependent CDC-secreted effectors could be a novel therapeutic approach.[31]

Although ejection fraction is a valuable measurement to determine ventricular function, up to 50% of patients with heart failure have a preserved ejection fraction and most of them show diastolic

dysfunction. Increased ventricular stiffness is a distinct finding in those patients.[32] Ventricular diastolic stiffness also affects morbidity, particularly the duration of pleural effusions after Fontan operation.[33] In our study, ventricular stiffness, that could be associated with myocardial fibrosis, was lower in the CDC-treated group at the final follow-up, indicating that the beneficial effect of cell transfusion on diastolic function may improve ventriculoarterial coupling, leading to superior mechanical efficiency compared with that in the control group.[34]

In our midterm follow-up study, no late adverse events were observed in the CDC-treated group, whereas several complications were seen in the control group. Although the difference in the number of patients was not significant, the relatively good clinical course of the CDC-treated group may have been due to the improved ventricular function as well as better diastolic function. Moreover, sustained improvement in cardiac function resulted in significant reduction of heart failure symptoms and better somatic growth in the midterm. Importantly, we found that the reactivity of CDC infusion was greater in patients with lower cardiac function. When we look at the patients in detail, the CDC-treated patient with the lowest right ventricular ejection fraction at baseline within the group showed a remarkable increase in ejection fraction after CDC infusion and improved cardiac function was preserved at 36 months of follow-up (37.9% at baseline to 47.4% at 3 months and 53.2% at 36 months). This patient underwent Fontan operation 27 months after bidirectional Glenn procedure and has been doing well without heart failure symptoms during midterm follow-up. In contrast, the patient in the controls with

the lowest RVEF at baseline showed no improvement in RVEF during the follow-up period (39.3% at baseline to 38.0% at 3 months and 32.2% at 36 months) and has not completed the Fontan operation yet because of heart failure. These data indicate that CDC infusion may have the potential to become an alternative medicine or bridge to transplantation for severe heart failure in patients with single ventricle physiology.

As no studies have yet reported impaired ventricular function as a risk factor of catheter intervention or APCA coil embolization, the mechanisms underlying the lower incidence of catheter interventions and higher degree of freedom from APCA coil embolization in the CDC-treated group are not clear. It could be anticipated that a lower incidence of catheter intervention may result in better quality of life for patients and greater reduction in medical costs. Further studies are necessary to elucidate these clinically remarkable observations.

Study Limitations

The study was a non-randomized and small phase 1 study, not powered to assess efficacy in a definitive manner. The cardiac interventionists were not blinded to allocation of CDC infusion because of the lack of placebo infusion in control group. There could be imbalance in pre-registration covariates between the groups to affect the clinical outcomes of primary interest that may need to be adjusted. The efficacy prediction was assessed by bivariate analysis with limited variables in small sample size, thus the results should be interpreted with caution. Although the morphology of all of the

patients was HLHS, there was a mixture of patients after Fontan operation and bidirectional Glenn procedure. Most of the patients after bidirectional Glenn procedure underwent Fontan operation during the follow-up period, which could also have affected outcome measures including right ventricular volume, and the incidences of catheter intervention and coil embolization. In cMRI study, we did not assess myocardial fibrosis by late gadolinium enhancement, suggested by a recent study as a potential contributor to ventricular dysfunction in patients with single ventricle physiology, which remains to be elucidated in future phase 2 clinical trials.[35]

Conclusions

This is the first clinical trial of midterm outcomes of CDC infusion in pediatric patients with HLHS. The results of our study demonstrate the safety and feasibility of intracoronary infusion of autologous CDCs in infants with HLHS. Improved ventricular function was maintained during 36 months of observation, which resulted in improvement of heart failure status in the midterm. Larger phase 2 studies focusing on changes in cardiac function, myocardial fibrosis, and quality of life and clinical event rates are warranted to confirm these effects of CDC administration in patients with single ventricular physiology.

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Conflict of Interest Statement

The authors have nothing to disclose with regard to commercial support.

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Table 1. Baseline characteristics

	controls	CDCs
Demographics		
Male	5 (71%)	4 (57%)
Age at surgery (years)	1.5 ± 1.7	2.1 ± 1.2
Weight at surgery (kg)	6.8 ± 3.8	9.2 ± 3.8
Patient characteristics		
Morphology		
Hypoplastic left heart syndrome	7 (100%)	7 (100%)
Mitral atresia/aortic atresia	3 (43%)	3 (43%)
Mitral stenosis/aortic atresia	1 (14%)	2 (29%)
Mitral atresia/aortic stenosis	0	1 (14%)
Mitral stenosis/aortic stenosis	2 (29%)	0
Variant (DORV, MA, hypo LV, IAA, HAA)	1 (14%)	1 (14)
Restrictive ASD	0	1 (14%)
Sinusoidal arteries	0	1 (14%)
Aortic size before 1st palliation (mm)	3.3 ± 1.0	3.6 ± 2.0
RVEF before 1st palliation (%)	53.9 ± 3.8	50.4 ± 5.2
Previous cardiac surgical procedures		
Cardiac catheter intervention	5 (71%)	5 (71%)
Norwood operation	6 (86%)	6 (86%)
Modified Blalock-Taussig shunt	1 (14%)	0
Sano shunt	5 (71%)	6 (86%)
Bilateral pulmonary artery banding	2 (29%)	3 (43%)
Bidirectional Glenn procedure	2 (29%)	4 (57%)
Cardiac arrest	0	0
ECMO	0	0
Genetic syndrome	0	0
Extracardiac malformation	0	0
Prematurity	1 (14%)	0
Preoperative assessment		
Oxygen saturation (%)	84.9 ± 7.9	87.8 ± 9.9
Tricuspid regurgitation >mild	1 (14%)	0
Mean PA pressure (mmHg)	11.1 ± 1.5	9.9 ± 2.8

RVEDP (mmHg)	6.5 ± 2.1	5.7 ± 1.2
PVRI (Wood unit•m^2)	1.6 ± 0.5	1.3 ± 0.3
Cardiac index (L/min/m^2)	5.1 ± 0.8	4.5 ± 2.3
Surgical characteristics		
Norwood-Glenn procedure	1 (14%)	1 (14%)
Bidirectional Glenn procedure	4 (57%)	2 (29%)
Total cavopulmonary connection	2 (29%)	4 (57%)
Concomitant procedures performed		
None	3 (43%)	3 (43%)
Arch augmentation	1 (14%)	1 (14%)
Tricuspid valve repair	1 (14%)	1 (14%)
Atrial septectomy	1 (14%)	0
PA patch augmentation	1 (14%)	3 (43%)

DORV, double-outlet right ventricle; *MA*, mitral atresia; hypo *LV*, hypoplastic left ventricle; *IAA*, interrupted aortic arch; *HAA*, hypoplastic aortic arch; *ASD*, atrial septal defect; *RVEF*, right ventricular ejection fraction; *ECMO*, extracorporeal membrane oxygenation; *PA*, pulmonary artery; *RVEDP*, right ventricular end diastolic pressure; *PVRI*, pulmonary vascular resistance index

Table 2. Clinical outcomes and catheter intervention during 36-month follow-up

	controls	CDCs	*P* value
Adverse events			
Late failure	2	0	.23
NYHA class III-IV	1	0	.50
PLE/plastic bronchitis	1	0	.50
Takedown	0	0	
Transplant	0	0	
Death	0	0	
Cardiopulmonary resuscitation	1	0	.50
Supraventricular tachycardia	0	0	
Pacemaker implantation	0	0	
Prolonged chest tube drainage	1	0	.50
Cirrhosis	0	0	
Thromboembolic events	0	0	
Stroke	0	0	
Tumor formation	0	0	
Any type of unplanned catheter intervention (N/person-year)	1.43	0.52	.04
APC coils (N/person-year)	0.86	0.24	.10
Balloon angioplasty (N/person-year)	0.57	0.29	.25
Pulmonary artery (N/person-year)	0.43	0.14	.17
Aortic arch (N/person-year)	0.05	0.05	
Fenestration (N/person-year)	0.10	0.05	.53

NYHA, New York Heart Association; *PLE*, protein-losing enteropathy; *APC*, aortopulmonary collateral

Table 3. Hemodynamics by catheterization study at final follow-up and Fontan operative characteristics

	controls	CDCs	*P* value
Hemodynamics			
Mean PA pressure (mmHg)	11.9 ± 1.9	12.1 ± 1.3	.75
RVEDP (mmHg)	7.2 ± 1.7	6.6 ± 1.1	.47
PVRI (Wood unit•m^2)	2.1 ± 0.7	1.8 ± 0.4	.31
Cardiac index (L/min/m^2)	3.6 ± 0.6	4.9 ± 0.9	.02
Fontan operation performed	6	7	.50
Age at Fontan (years)	3.0 ± 0.8	3.0 ± 0.6	.97
Interval between bidirectional Glenn and Fontan (years)	2.5 ± 0.8	2.4 ± 0.4	.77
Follow-up period after Fontan (months)	20.1 ± 13.0	24.9 ± 14.2	.63
Fontan type (extracardiac conduit)	6	7	
Creation of fenestration	6	7	
Concomitant procedures performed			
None	2	2	.66
Arch augmentation	0	2	.27
Tricuspid valve repair	3	1	.22
Atrial septectomy	0	0	
PA patch augmentation	1	3	.34

PA, pulmonary artery; *RVEDP*, right ventricular end-diastolic pressure; *PVRI*, pulmonary vascular resistance index. Final follow-up catheterization studies were performed from 30 to 36 months of follow-up.

Table E1. Pooled data of echocardiography- and cMRI-measured parameters in control and CDC-treated groups

	controls	CDCs	*P* value
cMRI			
Right ventricular ejection fraction (%)			
Baseline	33.2 ± 4.9	36.1 ± 7.5	.50
18 months	31.5 ± 6.8	40.4 ± 7.6	.04
36 months	32.2 ± 9.4	41.8 ± 5.2	.04
RVEDVI (mL/BSA$^{1.3}$)			
Baseline	160.4 ± 48.6	139.0 ± 43.4	.48
18 months	150.2 ± 41.7	112.2 ± 31.4	.08
36 months	131.8 ± 26.4	101.1 ± 30.2	.08
RVESVI (mL/BSA$^{1.3}$)			
Baseline	105.7 ± 34.3	91.6 ± 37.5	.55
18 months	103.8 ± 36.5	67.9 ± 23.6	.049
36 months	90.6 ± 27.4	58.9 ± 18.8	.03
Wall mass/BSA$^{1.3}$ (g/ BSA$^{1.3}$)			
Baseline	62.0 ± 30.4	69.9 ± 31.0	.69
18 months	40.1 ± 14.2	41.3 ± 14.5	.88
36 months	40.0 ± 8.4	35.9 ± 14.3	.55
Wall mass/EDV (g/mL)			
Baseline	0.40 ± 0.15	0.49 ± 0.08	.23
18 months	0.36 ± 0.12	0.35 ± 0.08	.62
36 months	0.29 ± 0.03	0.36 ± 0.13	.25
Echocardiography			
Right ventricular ejection fraction (%)			
Baseline	46.7 ± 4.4	46.9 ± 4.6	.93
24 months	48.9 ± 6.9	54.4 ± 2.0	.07
30 months	49.4 ± 6.1	54.1 ± 2.3	.13
36 months	48.9 ± 7.8	54.9 ± 2.1	.03
Fractional area change (%)			
Baseline	33.6 ± 3.3	34.2 ± 4.4	.78
24 months	34.1 ± 4.3	39.4 ± 2.2	.01
30 months	33.9 ± 4.5	39.2 ± 2.2	.02
36 months	34.4 ± 5.1	39.5 ± 3.3	.046

Tricuspid inflow E/A ratio			
Baseline	1.5 ± 0.4	1.9 ± 0.8	.35
24 months	1.7 ± 0.3	1.5 ± 0.7	.39
30 months	1.7 ± 0.3	1.7 ± 0.6	.53
36 months	1.6 ± 0.4	1.6 ± 0.6	.87
Tricuspid valve annulus diameter (Z score)			
Baseline	2.0 ± 0.3	2.1 ± 0.5	.80
24 months	1.9 ± 0.6	1.4 ± 0.4	.08
30 months	1.9 ± 0.6	1.3 ± 0.3	.048
36 months	2.0 ± 0.6	1.3 ± 0.3	.03

RVEDVI, right ventricular end-diastolic volume index; *RVESVI*, right ventricular end-systolic volume index; *BSA*, body surface area; *EDV*, end-diastolic volume

FIGURE LEGENDS

Figure 1. Safety and adverse events observed in TICAP trial during 3-year follow-up.

(A) Serum levels of tumor markers, CEA and CA19-9, were assessed at 36 months after CDC infusion. Data are shown as mean ± SD. (B) Kaplan-Meier adverse-event-free survival curve. (C) Freedom for coil embolization for aortopulmonary collateral artery. (D) Freedom for any unintended catheter intervention. Confidence interval limits (95%) are shown as dotted lines.

Figure 2. cMRI and echocardiographic analyses in controls and CDC-treated group at 36 months of follow-up.

Right ventricular ejection fraction (A), right ventricular end-diastolic volume indexed to $BSA^{1.3}$ (B), and right ventricular end-systolic volume indexed to $BSA^{1.3}$ (C) measured by cMRI in controls and the CDC-treated group at baseline and 36 months of follow-up are shown. Absolute changes in right ventricular ejection fraction (D), fractional area change (E), and tricuspid valve annulus diameter assessed by Z score (F) measured by echocardiography (UCG) at 36 months of follow-up are shown. Ventricular stiffness (G) and ventriculoarterial coupling (H; Ea/Ees) measured by catheterization study at final follow-up from 30 to 36 months are demonstrated. Data are shown as mean ± SD.

Figure 3. Somatic growth, functional status, and clinical outcomes during 3-year observation of TICAP trial.

Weight for age corrected by Z score (A), New York University Pediatric Heart Failure Index (NYU PHFI) scores (B), and serum brain natriuretic peptide (BNP) levels (C) in controls and CDC-treated group at 36 months follow-up are shown. Data are shown as mean ± SD. Correlation between changes in right ventricular ejection fraction and age at infusion (D), weight for age (E), and right ventricular ejection fraction at infusion (F) are shown. *WAZ*, weight-for-age Z score.

Figure E1. Parenting stress and quality of life at final follow-up.

Parenting Stress Index (A) and score of each scale from The Infant Toddler Quality of Life Questionnaire™ (B) are shown. Data are shown as mean ± SD.

Figure 1

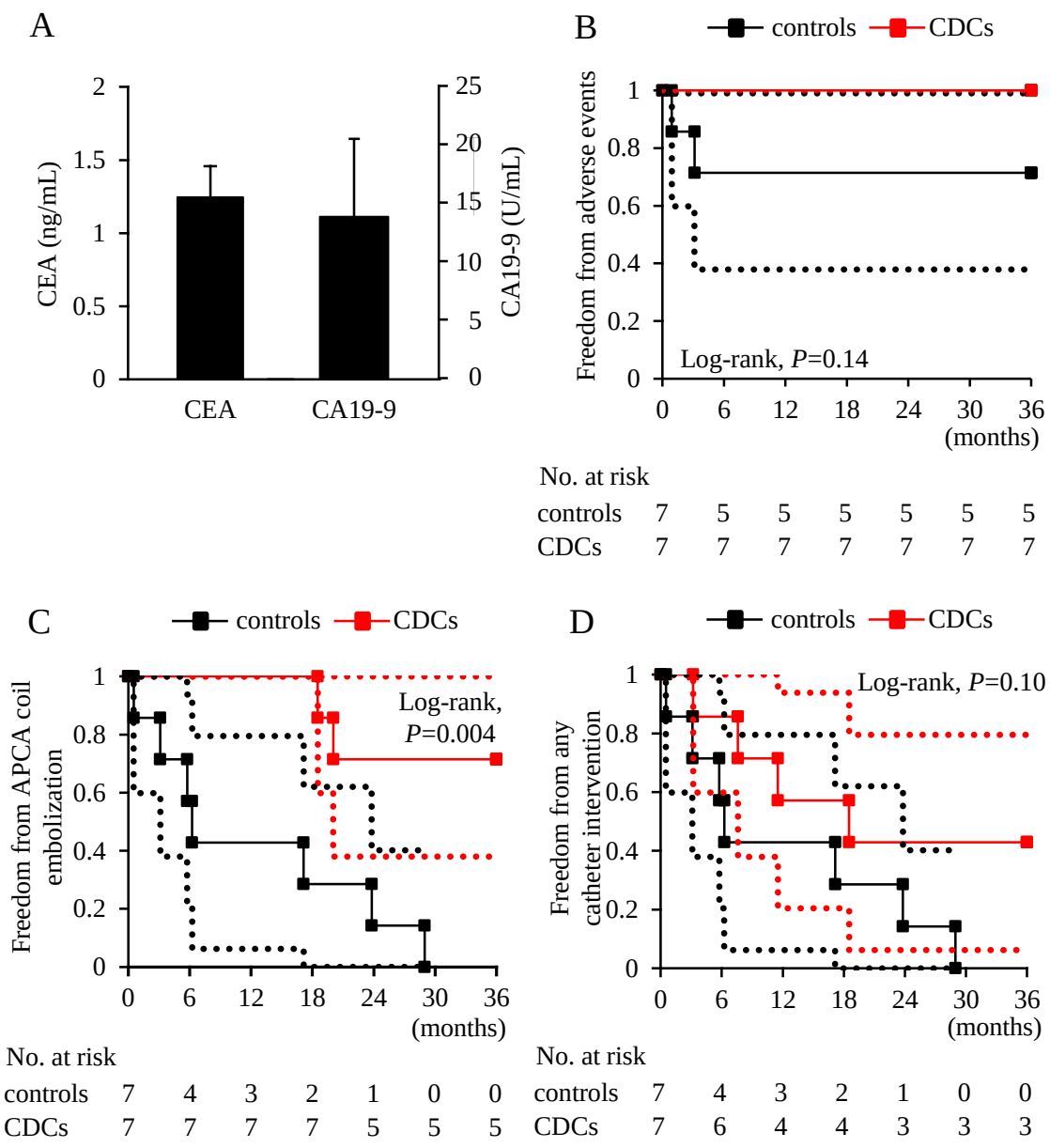

A
CEA (ng/mL)
CA19-9 (U/mL)
CEA
CA19-9
B
controls
CDCs
Freedom from adverse events
Log-rank, P=0.14
(months)
No. at risk
controls 7 5 5 5 5 5 5
CDCs 7 7 7 7 7 7 7
C
controls
CDCs
Freedom from APCA coil embolization
Log-rank, P=0.004
(months)
No. at risk
controls 7 4 3 2 1 0 0
CDCs 7 7 7 7 5 5 5
D
controls
CDCs
Freedom from any catheter intervention
Log-rank, P=0.10
(months)
No. at risk
controls 7 4 3 2 1 0 0
CDCs 7 6 4 4 3 3 3

Figure 2

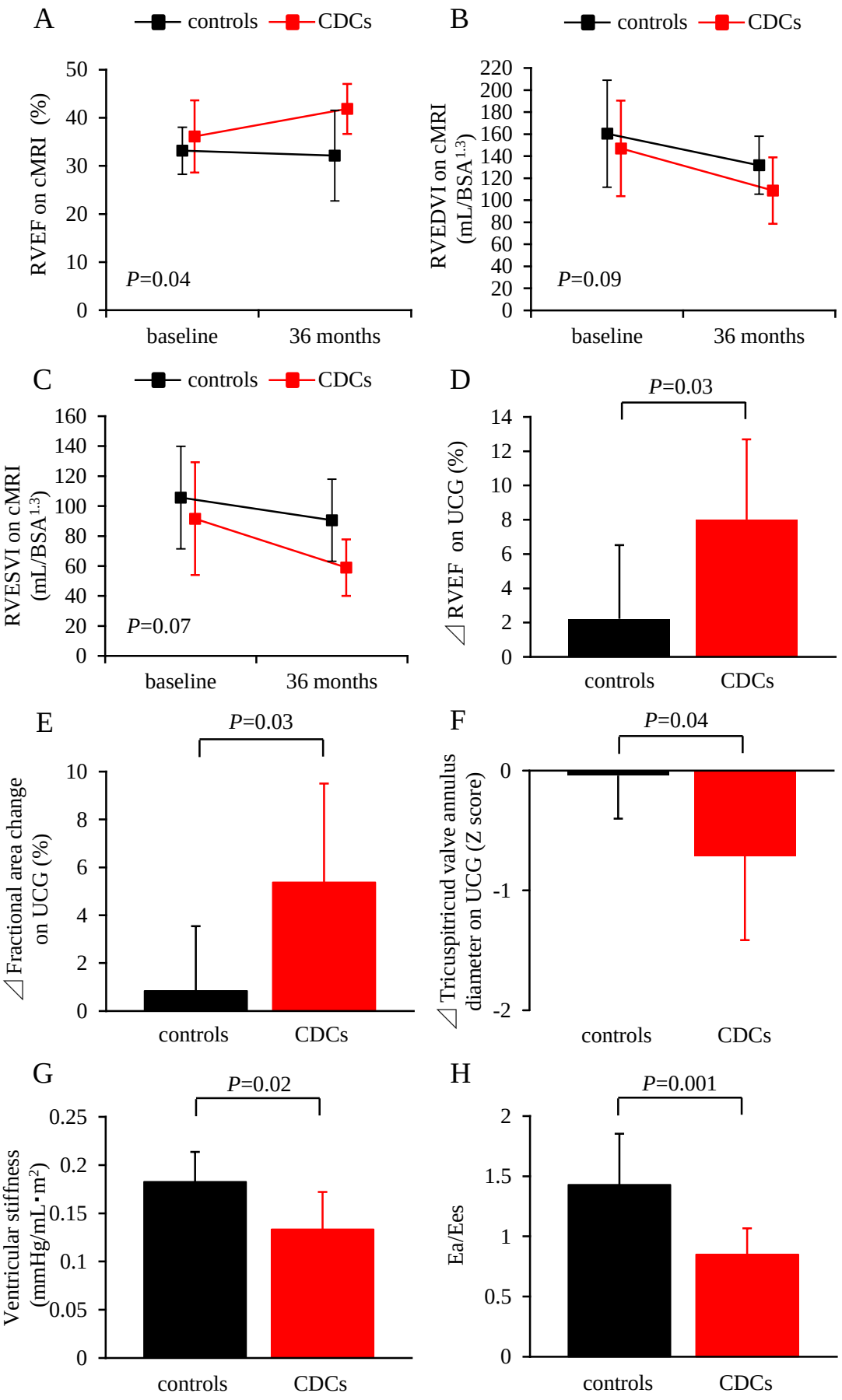

A
controls
CDCs
RVEF on cMRI (%)
50
40
30
20
10
0
P=0.04
baseline
36 months
B
controls
CDCs
RVEDVI on cMRI (mL/BSA1.3)
220
200
180
160
140
120
100
80
60
40
20
0
P=0.09
baseline
36 months
C
controls
CDCs
RVESVI on cMRI (mL/BSA1.3)
160
140
120
100
80
60
40
20
0
P=0.07
baseline
36 months
D
P=0.03
△RVEF on UCG (%)
14
12
10
8
6
4
2
0
controls
CDCs
E
P=0.03
△Fractional area change on UCG (%)
10
8
6
4
2
0
controls
CDCs
F
P=0.04
△Tricuspitricud valve annulus diameter on UCG (Z score)
0
-1
-2
controls
CDCs
G
P=0.02
Ventricular stiffness (mmHg/mL·m2)
0.25
0.2
0.15
0.1
0.05
0
controls
CDCs
H
P=0.001
Ea/Ees
2
1.5
1
0.5
0
controls
CDCs

Figure 3

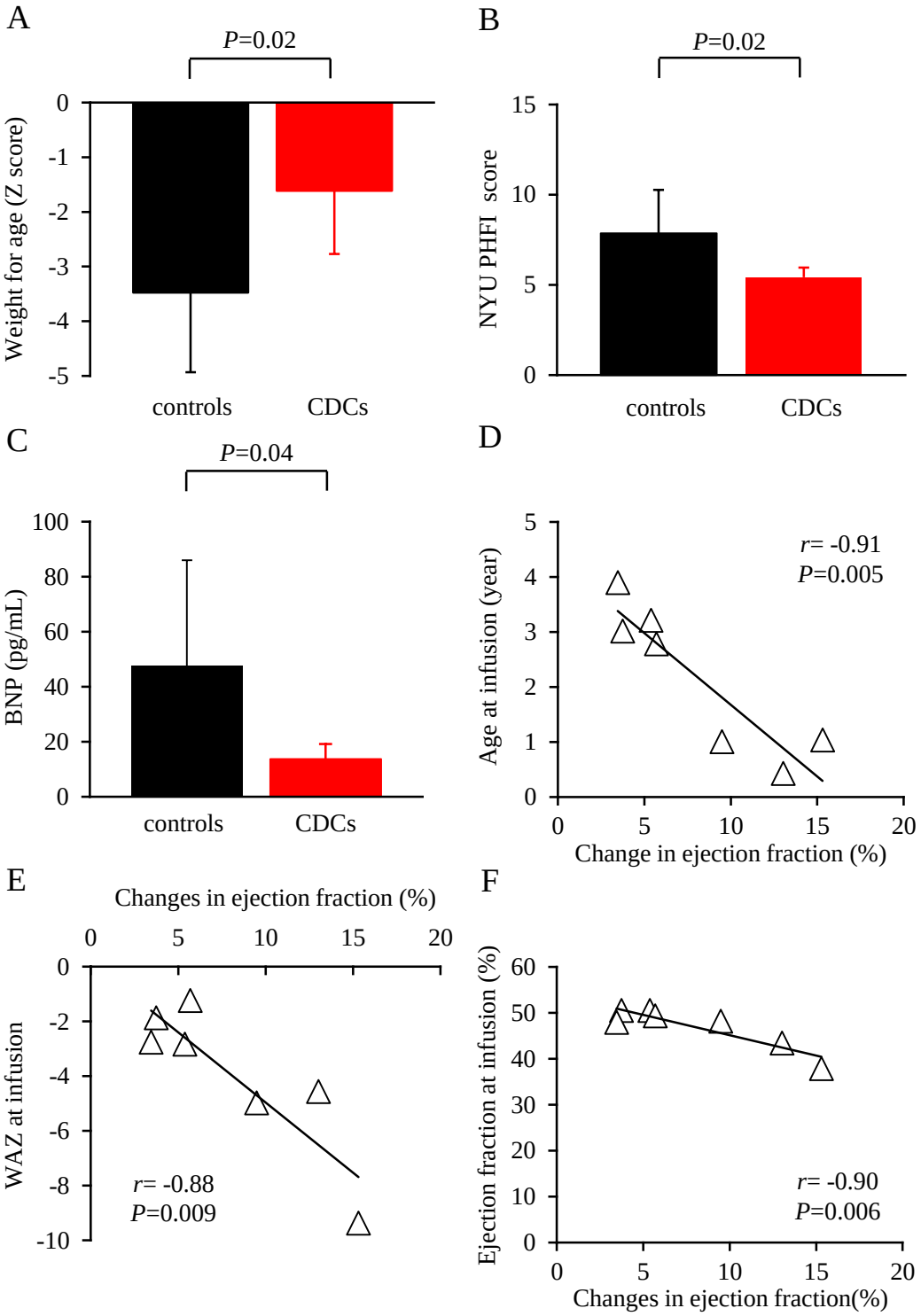
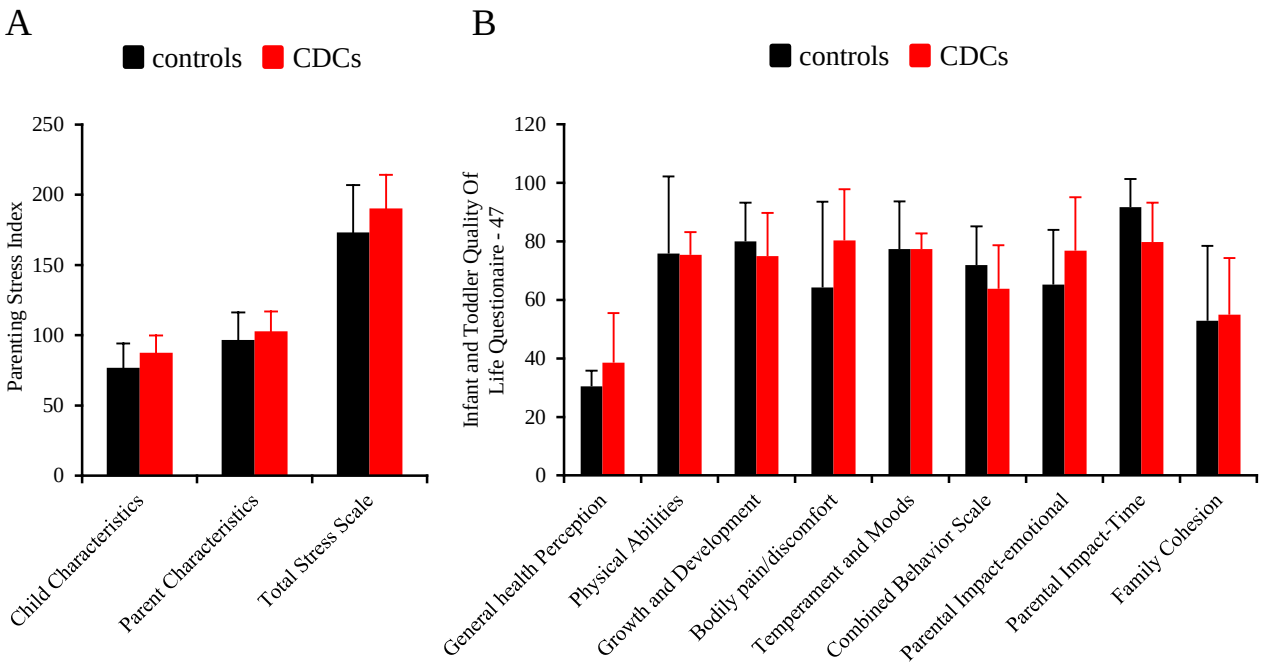

A
P=0.02
Weight for age (Z score)
0
-1
-2
-3
-4
-5
controls
CDCs
B
P=0.02
NYU PHFI score
15
10
5
0
controls
CDCs
C
P=0.04
BNP (pg/mL)
100
80
60
40
20
0
controls
CDCs
D
r= -0.91
P=0.005
Age at infusion (year)
5
4
3
2
1
0
0
5
10
15
20
Change in ejection fraction (%)
E
Changes in ejection fraction (%)
0
5
10
15
20
WAZ at infusion
0
-2
-4
-6
-8
-10
r= -0.88
P=0.009
F
Ejection fraction at infusion (%)
60
50
40
30
20
10
0
0
5
10
15
20
Changes in ejection fraction(%)
r= -0.90
P=0.006

Figure E1


A
controls
CDCs
Parenting Stress Index
250
200
150
100
50
0
Child Characteristics
Parent Characteristics
Total Stress Scale
B
controls
CDCs
Infant and Toddler Quality Of Life Questionaire - 47
120
100
80
60
40
20
0
General health Perception
Physical Abilities
Growth and Development
Bodily pain/discomfort
Temperament and Moods
Combined Behavior Scale
Parental Impact-emotional
Parental Impact-Time
Family Cohesion