



CD34⁺ Cell Transplantation Improves Right Ventricular Function in Patients with Nonischemic Dilated Cardiomyopathy

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ABSTRACT

We investigated the effects of CD34⁺ cell therapy on right ventricular (RV) function in patients with nonischemic dilated cardiomyopathy (DCM). We enrolled 60 patients with DCM who were randomized to CD34⁺ cell therapy (Stem Cells (SC) Group $n = 30$), or no cell therapy (Controls, $n = 30$). The SC Group received granulocyte-colony stimulating factor, and CD34⁺ cells were collected by apheresis and injected transendocardially. Patients were followed for 6 months. At baseline, the groups did not differ in age, gender, left ventricular ejection fraction, N-terminal probrain natriuretic peptide, or parameters of RV function. At 6 months, we found a significant improvement in RV function in the SC Group (tricuspid annular plane systolic excursion [TAPSE]: $+0.44 \pm 0.64$ cm, $p = .001$; peak systolic tissue Doppler velocity of tricuspid annulus [St]: $+1.5 \pm 2.1$ cm/s; $p = .001$; percent of fractional area change [FAC]: $+8.6\% \pm 5\%$, $p = .01$), but not in Controls (TAPSE: -0.07 ± 0.32 cm, $p = .40$; St: -0.1 ± 1.2 cm/s; $p = .44$; FAC: $-1.2\% \pm 3.2\%$, $p = .50$). On repeat electroanatomical mapping, we found an improvement in interventricular septum viability in 19 of 30 patients from the SC Group; this correlated with the improvements in RV function (13/19 in the improved septum group versus 3/11 in the remaining cohort, $p = .029$). These results suggest that patients with DCM, changes in RV function correlate with changes of viability of interventricular septum. CD34⁺ cell therapy appears to be associated with improved right ventricular function in this patient cohort. (Clinical Trial Registration Information: www.clinicaltrials.gov; NCT02248532). STEM CELLS TRANSLATIONAL MEDICINE 2018;7:168–172

SIGNIFICANCE STATEMENT

The results of this pilot clinical study demonstrate that cell therapy may improve right ventricular function in patients with nonischemic heart failure. Because the improvements in right ventricular function correlated with increased viability of interventricular septum, ventricular interdependence may represent a potential underlying mechanism for these findings.

INTRODUCTION

Nonischemic dilated cardiomyopathy (DCM) is the most frequent cause of advanced heart failure [1]. Disease progression in DCM affects both ventricles, and right ventricular (RV) dysfunction represents an important adverse prognostic factor [2, 3]. Although several clinical trials have demonstrated that left ventricular dysfunction in DCM can be partially reversed by cell therapy [4–7], no study to date investigated the effects of this approach on RV. Thus, the aim of the present study was to investigate the effects of transendocardial CD34⁺ cell therapy on RV function in patients with DCM.

MATERIALS AND METHODS

Patient Population

This study protocol represents a sub-study of a parent prospective randomized study comparing

the effects of repetitive and single-dose cell therapy in patients with nonischemic DCM (ClinicalTrials.gov number NCT02248532). Patients enrolled in the present sub-study include patients enrolled in the repetitive arm of the parent study, who received transendocardial cell therapy at baseline and again 6 months after enrollment. This design allowed for the analysis of the repetitive electroanatomical mapping, which was performed before cell injections at both time points. The study was conducted at the Advanced Heart Failure and Transplantation Centre at University Medical Centre Ljubljana between January 2014 and September 2016.

Patient inclusion criteria consisted of the following: age 18 to 70 years, diagnosis of nonischemic DCM according to European Society of Cardiology position statement [8], optimal medical management for ≥ 3 months, left ventricular ejection fraction (LVEF) $< 40\%$, and New York

Table 1. Baseline patient characteristics

Characteristics	Stem cells group (n = 30)	Control group (n = 30)	p value
Age, yr	56 ± 9	54 ± 11	.42
Male gender	27 (90)	26 (87)	.69
DCM pathogenesis			
Viral infection	25 (83)	27 (93)	.69
Familial	1 (3)	0 (0)	
Idiopathic	4 (14)	3 (10)	.69
LVEF, %	32.2 ± 9.3	31.1 ± 7.8	.39
Creatinine, μmol/L	90.6 ± 23.4	84.4 ± 19.5	.50
Diabetes	3 (10)	2 (6)	.64
NT-proBNP, pg/ml	1,525 ± 1,030	1,654 ± 987	.65
6-minute walk, m	320 ± 92	340 ± 83	.22
Medical therapy			
ACEI/ARB	30 (100)	30 (100)	1.00
Beta blockers	30 (100)	30 (100)	1.00
MRA	28 (93)	27 (90)	.64
Digoxin	2 (7)	3 (10)	.64
Loop diuretics	30 (100)	30 (100)	1.00

Values are presented as mean ± SD or number of patients (%). Abbreviations: ACEI, angiotensin convertase inhibitor; ARB, angiotensin receptor blocker; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal probrain natriuretic peptide.

Heart Association functional class III for ≥3 months before referral. Patients with acute multiorgan failure, a history of hematologic neoplasms, diminished functional capacity due to noncardiac comorbidities (chronic obstructive pulmonary disease, peripheral artery occlusive disease, morbid obesity), or pregnancy were not included. Informed consent was obtained in all patients prior to participation in the study, and the study protocol was approved by the National Medical Ethics Committee. The trial was registered with the European Medical Agency (EudraCT 002153-38).

Study Design

Patients were randomized in a 1:1 ratio to transendocardial cell therapy (SC Group) or no cell therapy (Controls). The allocation was done according to the covariate adaptive randomization strategy. At baseline, patients in the SC Group received granulocyte-colony stimulating factor (G-CSF); CD34⁺ cells were collected by apheresis and injected transendocardially in the target areas defined by electroanatomical mapping. In the Controls, no mobilization, apheresis, or cell therapy was performed. All patients were followed for 6 months, and in the SC Group electroanatomical mapping was repeated at the end of the follow-up. At the time of enrollment and at 6 months thereafter, we performed echocardiography and 6-minute walk test and measured plasma levels of N-terminal probrain natriuretic peptide (NT-proBNP).

Echocardiography

Echocardiography was performed according to European Association of Cardiovascular Imaging recommendations [9]. All images were stored and analyzed at the end of the follow-up by an independent echocardiographer who was blinded for the group allocation and timing of the recordings. RV size was obtained from

two-dimensional apical four-chamber view. RV function was assessed by tricuspid annular plane systolic excursion (TAPSE), peak systolic tissue Doppler velocity of tricuspid annulus (St), and percent of fractional area change (FAC). Improved RV function was defined as concomitant improvement in TAPSE, St, and FAC. Tissue Doppler velocity mitral annular early diastolic velocity (e') was measured on medial mitral ring, and the ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/e') was calculated for estimation of the left ventricular filling pressure.

6-Minute Walk Test and NT-proBNP Measurements

The 6-minute walk test was performed by a blinded observer according to the standard protocol [10], and NT-proBNP assays were performed at a central independent laboratory with a commercially available kit (Roche Diagnostics, Mannheim, Germany, <https://www.roche.com/>).

Peripheral Blood Stem Cell Mobilization and Collection

Stem cell mobilization and collection was performed as described previously [4]. In short, stem cells were mobilized by daily subcutaneous injections of G-CSF (10 mcg/kg daily, 5 days). Peripheral blood stem cells were collected with Amicus Separator System (Fenwal Inc., Lake Zurich, IL, <http://www.fresenius-kabi.com>), and the magnetic cell separator Clinimacs (Miltenyi Biotec, Germany, <http://www.miltenyibiotec.com>) was used for the immunomagnetic positive selection of CD34⁺ cells. Of the recovered CD34⁺ cells, 80 million were used for transendocardial injection.

Electroanatomical Mapping and Transendocardial Cell Delivery

Electroanatomical mapping was performed using the Biosense NOGA system (Biosense-Webster, Diamond Bar, California, <https://www.biosensewebster.com>). In short, electroanatomical mapping allows for point-by-point analysis of left ventricular viability and local contractility. Using this technique, maps of color-coded myocardial viability (unipolar voltage; UV) and regional myocardial contraction (linear shortening) and their corresponding bull's-eye maps, consisting of ≥150 sampling points, were generated for each patient prior to stem cell transplantation. Unipolar voltage was averaged for each of the 9 myocardial segments. Viability of the interventricular septum (IVS) was defined by averaging the measurements obtained in the septal and basal septal areas. In accordance with previous data on electroanatomical mapping in nonischemic DCM [11], transendocardial delivery of cell suspension was performed with MyoStar injection catheter targeting areas with unipolar voltage ≥8.27 mV and linear shortening <6%. Each patient received 20 injections of 0.3 mL.

Follow-Up and Endpoints

Patients were followed for 6 months. The primary endpoint was change in TAPSE. Secondary endpoints included changes in St, FAC, LVEF, NT-proBNP, and 6-minute walk test distance. In an exploratory analysis, we investigated a correlation of RV function and viability of the IVS.

Statistical Methods and Analysis

Based on our previous findings on CD34⁺ cell transplantation in DCM [4, 5], the sample size calculation for this study was based on the 90% probability that the study will detect a treatment difference with a 5% 2-sided significance level, if the true difference in TAPSE at 6 months between the SC Group and Controls is 0.40 cm

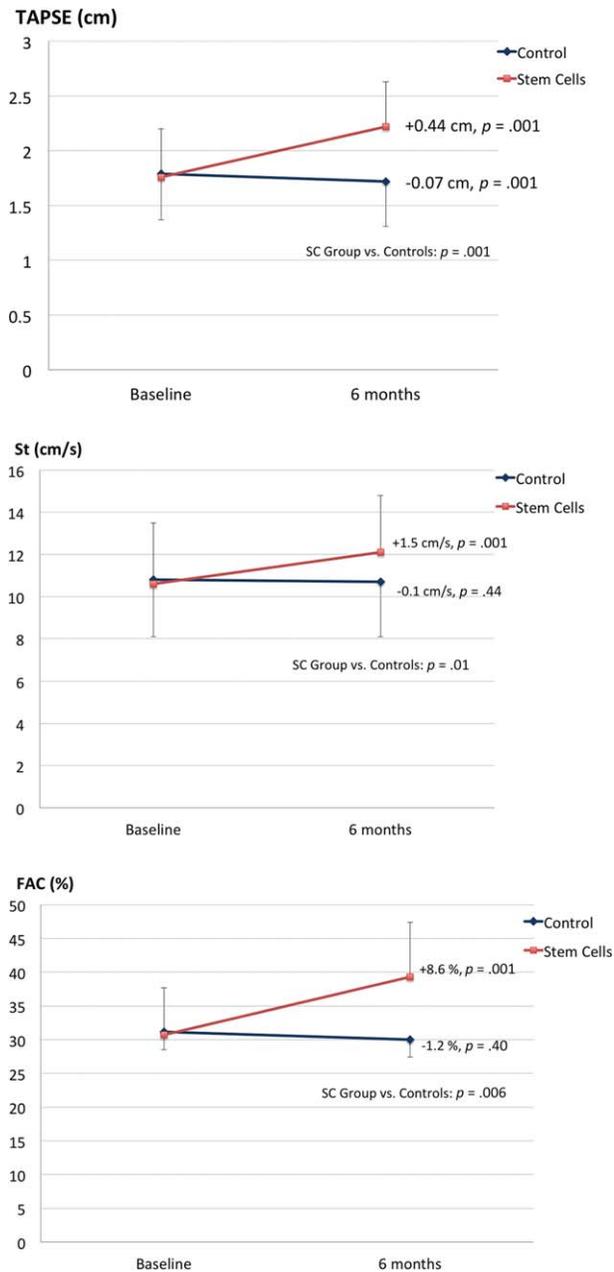


Figure 1. Changes of right ventricular function during the 6-month follow-up. We found a significant improvement in TAPSE, St, and FAC in the SC Group (red lines) but not in the Control Group (blue lines). This resulted in significantly higher values of all three parameters of RV function in the SC Group when compared with Controls. Abbreviations: FAC, percent of fractional area change; St, peak systolic tissue Doppler velocity of tricuspid annulus; TAPSE, tricuspid annular plane systolic excursion.

(assuming a standard deviation of 0.45 cm). Continuous variables are presented as means (\pm standard deviation). Categorical variables were compared with the use of the chi-square test. Differences between the SC Group and Controls were analyzed by two-way analysis of variance. The relationship of changes in TAPSE and changes in IVS viability was evaluated by Pearson's correlation coefficient. Statistical significance was assumed for p values of less than .05. All statistical analyses were performed with SPSS software (version 20.0, IBM, Armonk, NY, <https://www.ibm.com>).

RESULTS

Patient Characteristics

Of 60 patients entering the study, 1 patient from the Control group died of sudden cardiac death (14 weeks after enrollment), and 1 patient from the SC Group underwent urgent heart transplantation (20 weeks from enrollment). During follow-up, there were three hospitalizations for worsening of heart failure in the Control Group and one in the SC Group. At baseline, the SC Group and Controls did not differ in demographic and clinical parameters; all patients were treated with optimized medical management (Table 1).

Changes in Right Ventricular Function

At baseline, the two groups did not differ in parameters of right ventricular function. At 6 months, we found a significant improvement in TAPSE, St, and FAC in SC Group but not in Controls, which resulted in significantly higher values of all 3 parameters of RV function in the SC Group when compared with Controls (Fig. 1). In the SC Group, we also found a significant increase in LVEF and 6-minute walk test, as well as a decrease in NT-proBNP; however, no significant changes were present in the Control Group. E/e' , left ventricular end-diastolic dimension, and RV size did not change in any of the groups (Table 2).

On repeat electroanatomical mapping in the SC Group, we found a significant correlation between individual changes in TAPSE and changes in IVS viability (Pearson's $r = .64$, $p = .001$; Fig. 2).

DISCUSSION

This is the first clinical trial to date investigating the effects of cell therapy on RV function in DCM. In these patients, we found a significant improvement in parameters of RV function with transendocardial transplantation of cells in the left ventricle. Because the improvements in RV function correlated with increased viability of IVS, ventricular interdependence may represent a potential underlying mechanism for these findings.

In several preclinical settings it has been demonstrated that RV function may be improved by the use of cell therapies. In a murine model of RV failure, intramyocardial injection of mononuclear cells was associated with a reduction of RV fibrosis, an increase in the expression of proangiogenic markers, and a significant improvement in RV function [12]. In accordance with these findings, intramyocardial injection of bone marrow-derived mesenchymal stem cells in a swine model of RV failure resulted in increased RV fractional area of change and improved myocardial strain mechanics [13]. Cell-treated myocardium demonstrated enhanced neovessel formation and increased proliferation of cardiomyocytes and endothelial cells. In isolated rat hearts, pretreatment with mesenchymal stem cells was also associated with decreased ischemia-reperfusion injury and better functional recovery of the RV after acute pressure overload [14]. Collectively, these data suggest that cell therapy may offer an interesting novel strategy to improve RV function, possibly by improving myocardial perfusion at the cell injection sites.

Data of the present study suggest that the effects of CD34⁺ cell therapy are not limited to the left ventricle but may also be associated with improved RV function in DCM: we found a significant improvement in all parameters of RV function at 6 months after cell therapy in more than half of patients. Interestingly, there was a strong correlation between parameters of RV function and

Table 2. Changes in left ventricular function and dimensions, exercise capacity, and NTproBNP levels between baseline and 6 months

Parameter	Stem cell group (n = 30)	p value	Control group (n = 30)	p value
Δ LVEF, %	+6.9 ± 3.3	.001	+1.3 ± 7.8	.49
Δ LVEDD, cm	+0.09 ± 0.08	.81	+0.19 ± 0.06	.52
Δ E/e'	-1.2 ± 4.1	.32	+0.1 ± 2.9	.78
Δ RV size, cm	+0.05 ± 2.91	.92	+0.12 ± 3.12	.74
Δ NT-proBNP, pg/ml	-578 ± 211	.02	+167 ± 422	.70
Δ 6-minute walk, m	+57 ± 21	.03	+12 ± 18	.65

Abbreviations: Δ, change; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal probrain natriuretic peptide; RV, right ventricle; E/e', the ratio between early mitral inflow velocity (E) and mitral annular early diastolic velocity (e').

viability of IVS, suggesting that ventricular interdependence may at least partially account for the observed changes [15, 16]. Previously it has been demonstrated that measurement of endocardial UV can be used to identify left ventricular dysfunction in the absence of macroscopic scar defined with standard cardiac magnetic resonance imaging (cMRI) criteria, which suggests that unipolar electrograms could also detect a more diffuse underlying myocardial process [17]. Moreover, an inverse relation between amplitude of unipolar endocardial recordings and myocardial fibrosis burden has been described already in an experimental model of nonischemic cardiomyopathy and in an anatomic study of explanted human hearts [18, 19]. Thus, it appears that in the absence of macroscopic fibrosis, diffuse microscopic fibrosis may explain the impairment of left ventricular function in the setting of DCM. This suggests that the improvement of viability in the IVS at 6 months after cell therapy may potentially reflect the changes of diffuse microscopic fibrosis.

However, in the present study, we found an improvement in RV function also in patients who showed no improvement in IVS viability, suggesting that mechanisms other than ventricular interdependence may be of importance. There is increasing evidence that decreased myocardial perfusion also may play an important role in heart failure patients without epicardial coronary artery disease. In patients with DCM, coronary flow reserve is often impaired both globally and regionally because of impaired vasculogenesis and angiogenesis [20, 21]. In our previous clinical studies in DCM, CD34⁺ cell therapy was associated with a significant improvement in myocardial perfusion as assessed by SPECT [5, 22]. Interestingly, although the majority of changes occurred in the myocardial segments targeted with stem cell injections, some improvement in perfusion occurred also in the remote nontargeted areas, suggesting that a paracrine component may play an important role. Similarly, in accordance with this hypothesis, the improvement in RV function in patients without IVS improvement may reflect the remote paracrine effect of CD34⁺ cell therapy.

To date, clinical data on the effects of cell therapy on RV function are limited to patients with hypoplastic left heart syndrome. In these infants, cardiosphere-derived cell therapy was associated with a significant improvement in RV ejection fraction and reduced BNP levels [23]. In accordance with these findings, data from the present study suggest that cell therapy may also represent an effective therapeutic intervention to improve RV function in adult chronic heart failure population.

Study Limitations

The results of our study are subject to several limitations. The findings of the present study represent an extension of the parent study, with the primary aim to compare the effects of repetitive and single-dose cell therapy on left ventricular function in patients with nonischemic DCM. The control arm did not receive any placebo procedures. Although, to minimize the bias,

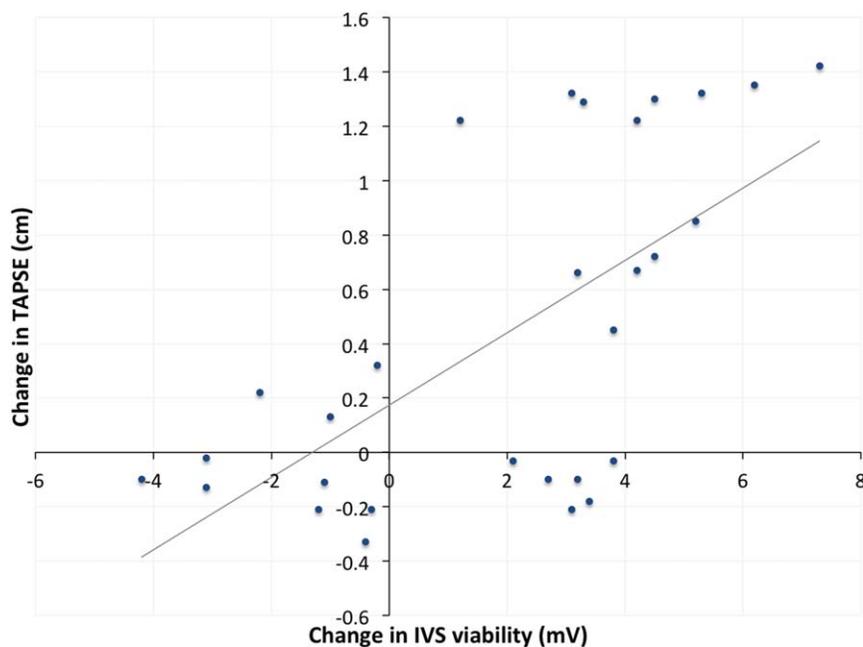


Figure 2. Correlation of changes in TAPSE with changes in IVS viability. In the SC Group, we found a significant correlation between changes in TAPSE and changes of viability of IVS within 6 months (Pearson's $r = .64$, $p = .001$). Abbreviations: IVS, interventricular septum; TAPSE, tricuspid annular plane systolic excursion.

echocardiography data were analyzed at the end of the follow-up period by an independent echosonographer, this does not fully overcome the limitations of an unblinded study. However, the echocardiography data were corroborated by the analysis of electroanatomical mapping, which is a fully automated procedure that generates data on myocardial viability using a computer algorithm performing in an operator-independent manner. Although our sample size was small, both groups of patients were well matched at baseline. Our patient population included patients with DCM, but no biopsies were performed to exclude secondary cardiomyopathies. Finally, we recognize that patients with DCM are a heterogeneous patient population and dynamic changes in IVS viability and RV function may be multifactorial. In particular, parameters of RV function also may be affected by changes in loading conditions; however, we have found no changes in LV filling pressures or RV size in our study cohort.

CONCLUSION

In patients with DCM, CD34⁺ cell therapy appears to be associated with increased IVS viability and improved RV function. Further

studies are warranted to verify our preliminary findings, better define the underlying mechanisms, and investigate whether or not this therapeutic approach could offer benefit in a broader population of patients with chronic heart failure and RV dysfunction.

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AUTHOR CONTRIBUTIONS

S.F. and B.V.: conception and design, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing; M.J., G.Z., A.C., and G.P.: conception and design, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation.

DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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