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Effects of Transendocardial CD34$^+$ Cell Transplantation on Diastolic Parameters in Patients with Nonischemic Dilated Cardiomyopathy

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ABSTRACT

We sought to evaluate the physiological background and the effects of CD34$^+$ cell transplantation on diastolic parameters in nonischemic dilated cardiomyopathy patients (DCM). We enrolled 38 DCM patients with NYHA class III and LVEF < 40% who underwent transendocardial CD34$^+$ cell transplantation. Peripheral blood CD34$^+$ cells were mobilized by G-CSF, collected via apheresis, and injected transendocardially in the areas of myocardial hibernation. Patients were followed for 1 year. At baseline, estimated filling pressures were significantly elevated (E/e′ ≥ 15) in 18 patients (Group A), and moderately elevated (E/e′ < 15) in 20 patients (Group B). The groups did not differ in age (54 ± 9 years vs. 52 ± 10 years; $p = .62$), gender (male: 85% vs. 78%; $p = .57$), or LVEF (31 ± 7% vs. 34 ± 6%; $p = .37$). When compared to Group B patients in Group A had more segments with myocardial scar (4.9 ± 2.7 vs. 2.7 ± 2.9; $p = .03$), myocardial hibernation (2.2 ± 1.6 vs. 0.9 ± 1.1; $p = .02$), and longer average local relaxation time on electroanatomical mapping (378 ± 41 ms vs. 333 ± 34 ms, $p = .01$). During follow-up there was an improvement in diastolic parameters in Group A (E/e′: from 24.3 ± 12.1 to 16.3 ± 8.0; $p = .005$), but not in Group B (E/e′: from 10.2 ± 3.7 to 13.2 ± 9.1; $p = .19$). Accordingly, in Group A, we found an increase in 6-minute walk distance (from 463 ± 83 m to 546 ± 91 m; $p = .03$), and a decrease in NT-proBNP (from 2140 ± 1743 pg/ml to 863 ± 836 pg/ml; $p = .02$). In nonischemic DCM, diastolic dysfunction appears to correlate with areas of myocardial scar and hibernation. Transendocardial CD34$^+$ cell transplantation may improve diastolic parameters in this patient cohort. STEM CELLS TRANSLATIONAL MEDICINE *2017;6:1515–1521*

SIGNIFICANCE STATEMENT

This is the first study to date investigating changes in diastolic function after cell therapy in patients with cardiomyopathy. Our results suggest that diastolic dysfunction is especially pronounced in patients with more myocardial scarring. In this patient cohort, cell application appears to be associated with improvement in diastolic parameters.

INTRODUCTION

Diastolic dysfunction and elevated left ventricular filling pressures are significant predictors of survival and severity of symptoms in patients with heart failure with either preserved (HFpEF) or reduced (HFrEF) ejection fraction [1, 2]. Although the underlying mechanisms for diastolic dysfunction are not clearly defined, they appear to involve multiple intracellular, extracellular, neurohumoral, and immunologic pathways [3, 4]. Recently, a new paradigm for the pathophysiology of diastolic dysfunction in HFpEF has been proposed: here, the primary changes in coronary microvascular endothelial inflammation are thought to result in diastolic dysfunction attributable to secondary alterations in cardiomyocyte function and extracellular matrix [5].

In patients with HFrEF ventricular remodeling impairs not only systolic function but also diastolic filling. In patients with dilated cardiomyopathy (DCM), left ventricular filling pressures are elevated because of slow relaxation, reduced elastic recoil, and impairment of filling from the ventricular dilatation itself [6]. Furthermore, similarly to patients with HFpEF, DCM patients have been found to have microvascular derangements that could significantly contribute to impaired diastolic function [7]. Our group has recently demonstrated that DCM can be partially reversed by either intracoronary or intramyocardial application of autologous CD34$^+$ cells [8, 9]. Although the exact nature of the beneficial course taken by CD34$^+$ cells in

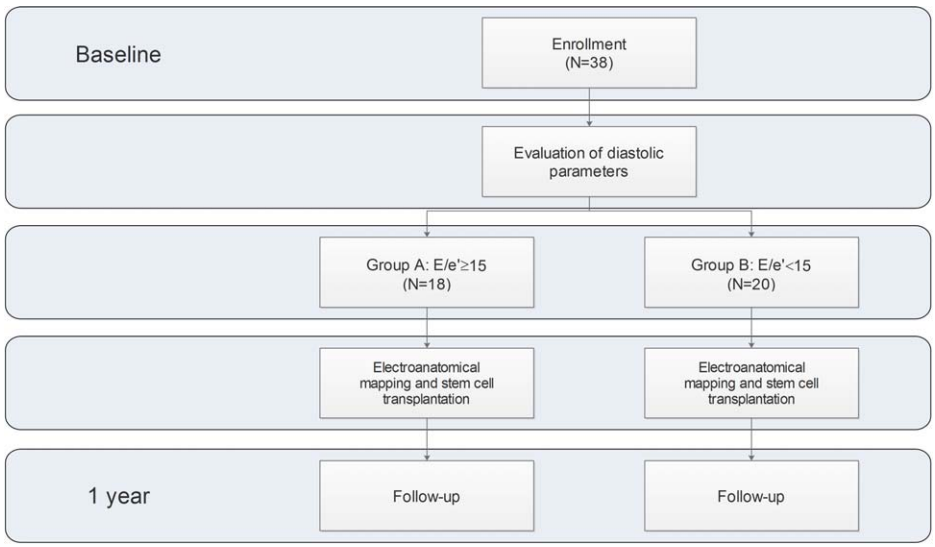

Figure 1. Flow chart of the study design. At baseline, we analyzed parameters of diastolic function in all patients and classified them in two groups according to the presence of diastolic dysfunction, defined by elevated estimated filling pressures ($E/e' \geq 15$). In all patients, peripheral blood CD34$^+$ cells were mobilized by G-CSF, collected via apheresis, and injected transendocardially in the areas of electromechanical mismatch on electro-anatomical mapping. Patients were followed for 1 year.

ischemic conditions is undefined, it is thought to involve either the direct incorporation of injected cells into the newly developing vasculature or the production and secretion of angiogenic cytokines that support an ischemia-induced angiogenic response [10]. In accordance with this hypothesis, we found an improvement in regional myocardial perfusion at the cell injection sites in patients with DCM [11].

Thus, it appears that CD34+ cell application affects ventricular remodeling and left ventricular systolic function in DCM by affecting myocardial microcirculation. Since the changes in microcirculation have also been shown to alter myocardial relaxation [5], the aim of the current study is to investigate the potential association between CD34$^+$ cell therapy and parameters of diastolic function in DCM by the use of a novel electroanatomical imaging algorithm.

Materials and Methods

Patient Population

This is a substudy of a larger registry investigating the predictors of response to CD34$^+$ cell therapy in patients with nonischemic dilated cardiomyopathy (clinicaltrials.gov ID: NCT02445534). The study used an open-label study design and was conducted at the Advanced Heart Failure and Transplantation Center in Ljubljana, Slovenia, between May 1st, 2013 and January 1st, 2014. Patient inclusion criteria included the following: age 18–65 years old, diagnosis of DCM according to the European Society of Cardiology position statement [12], optimal medical management for at least 6 months, left ventricular ejection fraction (LVEF) $<$40%, and New York Heart Association functional Class III on stable medical therapy for at least 3 months before referral. Patients with acute multiorgan failure, atrial fibrillation, cardiac resynchronization therapy, or a history of hematologic neoplasms were excluded. Informed consent was obtained from all patients before participation in the study, and the study protocol was approved by the National Medical Ethics Committee.

Study Design

At baseline, we analyzed parameters of diastolic function in all patients and classified them in two groups according to the presence of diastolic dysfunction, defined by elevated estimated filling pressures ($E/e' \geq 15$) [4]. In all patients, peripheral blood CD34$^+$ cells were mobilized by G-CSF, collected via apheresis, and injected transendocardially in the areas of hibernation on electroanatomical mapping. At the time of enrollment, and at 1, 3, 6 months, and 1 year thereafter we performed detailed clinical evaluation, echocardiography, 6-minute walk test, and measured plasma levels of NT-proBNP. At the end of the study, postprocessing of electro-anatomical mapping was performed to analyze parameters of local diastolic function. A flowchart of the study design and timelines is presented in Figure 1.

Peripheral Blood Stem Cell Mobilization and Collection

Patients underwent stem cell mobilization and collection as described previously [8]. In short, peripheral blood stem cells were mobilized by daily subcutaneous injections of G-CSF (10 mcg/kg, divided b.i.d.) for 5 days. Peripheral blood stem cells were then collected with Miltenyi cell separator (Miltenyi Biotech, Germany) and the magnetic cell separator Isolex 300i (Nexell Therapeutics Inc., CA) was used for the immunomagnetic positive selection of the CD34$^+$ cells. All recovered CD34$^+$ cells were then used for transendocardial injection.

Electro-Anatomical Mapping and Transendocardial Cell Delivery

Electro-anatomical mapping was performed using the Biosense NOGA system (Biosense-Webster, Diamond Bar, CA) as previously described [13]. For each patient, color-coded unipolar voltage and linear shortening (LLS) maps and their corresponding 9-segment "bull's-eye" maps, consisting of at least 150 sampling points were generated. In accordance with previous studies in nonischemic DCM [14, 15], hibernating segments were defined as areas with average unipolar voltage $\geq$8.27 mV and average LLS $<$6%. Scarred

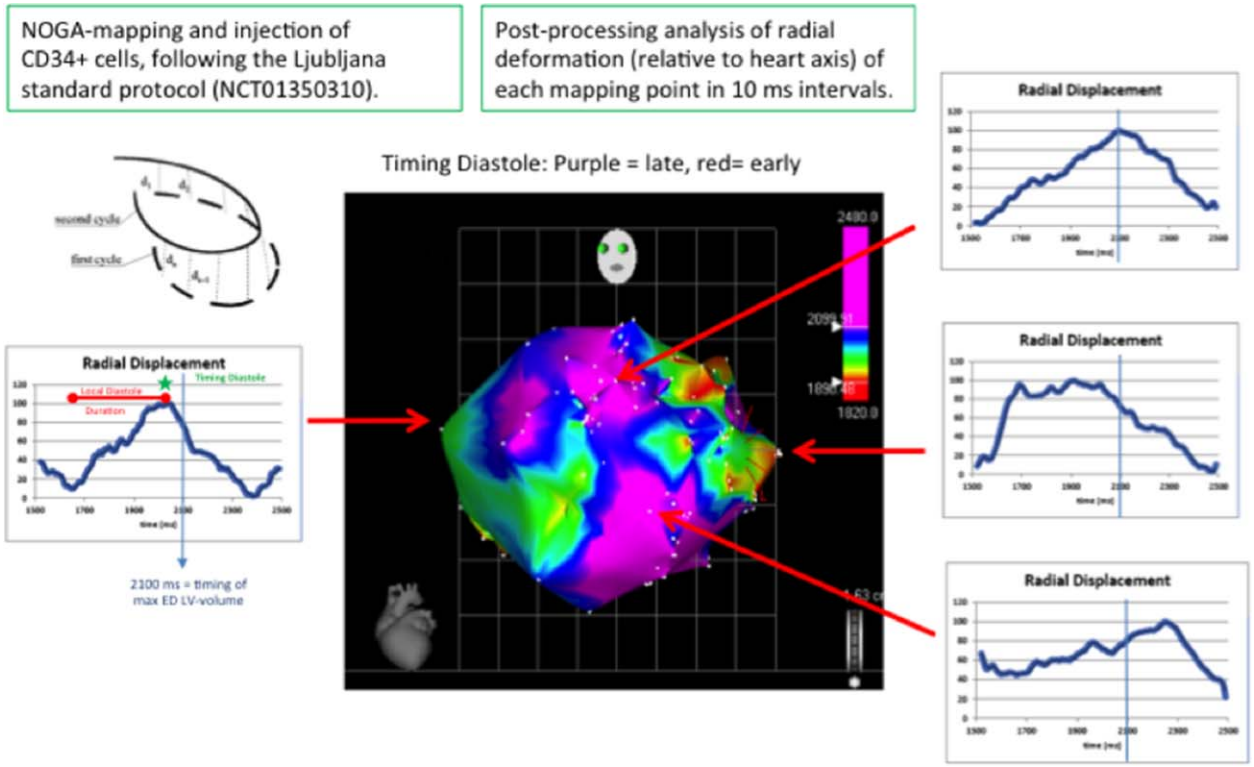

Figure 2. Analysis of local diastolic function. Exemplary postprocessing analysis of local diastolic function by measuring radial deformation (relative to heart axis) of each mapping point in 10 ms intervals. The 3D color-coded map displays the timing of diastole (red areas represent early diastole, purple areas represent late diastole).

myocardium was defined as areas with unipolar voltage $<$8.27 mV and LLS $<$6%, and normal myocardium was defined as areas with unipolar voltage $\geq$8.27 mV and LLS $\geq$6%. Local diastolic function was assessed off-line by measuring local ventricular relaxation times at each of the sampling points (Fig. 2).

Target area for cell delivery was defined as the myocardial segments with unipolar voltage potentials $\geq$8.27 mV, and LLS $<$6% [13, 14]. Transendocardial delivery of cell suspension was performed with MyoStar (Biosense Webster) injection catheter. Each patient received 20 injections of stem cell suspension (0.3 ml each).

Echocardiography, 6-Minute Walk Test, and NT-proBNP Measurement

The echocardiogram data were recorded and analyzed at 1 year by an independent echosonographer who was blinded to both the randomization and timing of the recordings. Analysis of left ventricular dimensions, and diastolic function was performed in accordance with American Society of Echocardiography guidelines [16] using Xcelera 4,0 workstation (Philips, Einthoven, The Netherlands). Average of three consecutive cycles were used for volume and velocity measurements. Diastolic function assessment included four chambers view left atrial volume (LAV), and mitral valve PW Doppler velocities: E, A, and E wave deceleration time (DT). In addition, tissue Doppler velocity è was measured on medial mitral ring and E/è was calculated for estimation of the left ventricular filling pressure. Elevated left ventricular filling pressure was defined as E/e' $\geq$ 15.

All NT-proBNP assays were performed by a central independent laboratory blinded to the clinical data, using a commercially available kit (Roche Diagnostics, Mannheim, Germany). In all patients, the 6-minute walk test was performed by a blinded observer according to the standard protocol [17].

Follow-Up and End Points

The primary end-point was the change in E/e' at 1 year after cell therapy. Secondary endpoints included changes in 6-minute walk test distance, and NT-proBNP levels.

Statistical Methods and Analysis

Based on our previous findings on transendocardial injection in nonischemic heart failure [8, 9] and the findings of studies investigating the effects of intracoronary stem cell therapy on diastolic function in ischemic heart failure [18], the sample size calculation for this study was based on the 90% probability that the study will detect a treatment difference with a 5% two-sided significance level, if the true difference in E/e' between baseline and 1 year is 1.8 (assumed standard deviation: 6.7).

Continuous variables were expressed as mean $\pm$ SD and categorical variables were expressed as a number and a percentage. Continuous variables were explored for normal distribution with the Shapiro-Wilk test. Differences within the groups were analyzed using a *T* test for continuous variables with correction for unequal variance when appropriate, and with chi-square or Fisher exact test when appropriate. Intergroup differences were analyzed with one-way ANOVA. A value of $p < .05$ was considered significant. All statistical analyses were performed with SPSS software, version 20.0 (SPSS Inc. Chicago, IL).

Table 1. Baseline patient characteristics

	Group A ($N = 18$)	Group B ($N = 20$)	p
Age, y	53 ± 9	52 ± 11	.98
Male gender	11 (64)	16 (80)	.87
Creatinine, mmol/l	86 ± 22	82 ± 17	.62
Sodium, mmol/l	137 ± 3	139 ± 3	.85
Hemoglobin, g/ml	14.1 ± 10.0	13.8 ± 8.1	.58
Bilirubin, μmol/l	18.2 ± 10.1	18.1 ± 11.3	.96
NT-proBNP, pg/ml	2052 ± 1608	735 ± 1183	.01
Blood pressure, mmHg	110 ± 12	112 ± 14	.52
6-MWT, m	463 ± 83	456 ± 101	.99
Therapy			
RAAS inhibitors	18 (100)	20 (100)	1.00
Beta-blockers	18 (100)	20 (100)	1.00
Loop diuretics	9 (50)	8 (40)	.54
MRA	18 (100)	20 (100)	1.00

Values are presented as mean ± SD or number of patients (percent). 6-MWT, 6-minute walk test; RAAS, renin-angiotensin-aldosterone; MRA, mineralnocorticoid receptor antagonist.

Results

Baseline Patient Characteristics

Of 38 patients enrolled in the study, 18 (46%) displayed elevated baseline left ventricular filling pressures (E/e' ≥ 15; Group A), and in 20 patients (64%) E/e' was <15 (Group B). The groups did not differ in age, gender, renal and liver function, blood pressure, exercise capacity, or medical therapy; with the exception for NT-pro BNP levels, which were significantly higher in Group A. (Table 1). On baseline echocardiography we have found no significant intergroup difference in LVEF, left ventricular end-diastolic volume (LVEDV), or LAV; however, patients in Group A displayed significantly higher E/A ratio and a longer DT, confirming a higher grade of diastolic dysfunction in this patient cohort. (Table 2). The number of injected CD34$^+$ cells was 92.6 ± 45.8 × 10^6 in Group A, and 139.2 ± 85.3 × 10^6 in Group B ($p = .08$).

Correlation of Parameters of Diastolic Dysfunction with Electroanatomical Mapping

An exemplary electroanatomical map performed before stem cell injection is presented in Figure 3. When compared to Group B patients in Group A had more segments with myocardial scar (4.9 ± 2.7 vs. 2.7 ± 2.9; $p = .03$) and myocardial hibernation (2.2 ± 1.6 vs. 0.9 ± 1.1; $p = .02$) on electroanatomical mapping. At baseline, patients with Group A also displayed significantly longer average local relaxation time when compared to patients from Group B (Fig. 5). During electroanatomical mapping, we recorded one episode of sustained ventricular tachycardia in Group A; in Group B no serious adverse events were recorded.

Effects of Cell Therapy on Parameters of Diastolic Function

During follow-up, one patient from Group B died of pump failure, but we recorded no deaths in Group A. Overall, there was no significant change in E/e' within 1 year after cell therapy (−1.4 ± 10.8, $p = .47$). However, we found a significant intergroup difference: in Group A, we have a found a significant continuous improvement in E/e', which persisted throughout the study period (−8.06; $p = .005$). In contrast, we found no significant changes of E/e' in Group B (+3.14; $p = .19$) (Fig. 4). This resulted in improved E/e' at 1 year in 17 of 18 patients (94%) from Group A, and only in 8 of 20 patients (40%) from Group B ($p = .001$). At 1 year, we also found a trend of decrease in E/A ratio and grade of diastolic dysfunction in Group A (E/A: −0.5 ± 1.2, $p = .16$; grade: −0.4 ± 0.4, $p = .05$), but not in Group B (E/A: +0.2 ± 0.7, $p = .72$; grade: +0.1 ± 0.5, $p = .45$). We have found no significant changes in LAV in any of the groups (−5.1 ± 6.2 ml in Group A, $p = .76$; +4.5 ± 6.0 ml in Group B, $p = .82$). Of note, the average local relaxation time measured by electro-anatomical mapping at the sites of cell injection was significantly longer in Group A than in Group B (Fig. 5).

Table 2. Baseline echocardiographic characteristics

	Group A ($N = 18$)	Group B ($N = 20$)	p
LVEF, %	32 ± 7	33 ± 10	.76
LVEDV, ml	186 ± 90	226 ± 84	.16
LAV, ml	95 ± 57	85 ± 46	.61
DT, ms	202 ± 63	155 ± 57	.02
E/A	2.4 ± 1.9	1.2 ± 0.6	.01
E/e'	24 ± 12	12 ± 5	.001
Diastolic dysfunction grade	2.3 ± 0.6	1.7 ± 0.6	.002

Values are presented as mean±SD or number of patients (percent). LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LAV, left atrial volume; DT, deceleration time.

Effects of Cell Therapy on Left Ventricular Systolic Function, Exercise Capacity, and NT-proBNP

Within 1 year we have found no significant difference in LVEF changes, or blood pressure between the groups (LVEF: +4.1 ± 3.2% in Group A vs.+3.8 ± 5.2% in Group B, $p = .41$; blood pressure: +4.5 ± 3.0 mmHg in Group A vs. +2.1 ± 5.0 mmHg in Group B, $p = .48$). However, stem cell therapy was associated with more pronounced increase in 6-minute walk distance in Group A (+48 ± 27 m, $p = .03$), than in Group B (+28 ± 33 m, $p = .23$). NT-proBNP levels decreased only in Group A (−1277 ± 1344 pg/ml; $p = .02$ vs. +127 ± 1313 pg/ml in Group B; $p = .77$), which led to comparable NT-proBNP levels in both groups at the end of the study (775 ± 1,329 pg/ml vs. 862 ± 1,320 pg/ml, $p = .76$).

Discussion

This is the first study to date investigating changes in diastolic function after CD34$^+$ cell therapy in patients with nonischemic DCM. Our results suggest that diastolic dysfunction in DCM is especially pronounced in patients with more myocardial scarring and hibernation. In this patient cohort, transendocardial CD34$^+$ cell application appears to be associated with improvement in ventricular filling pressures. These effects are associated with improved exercise capacity and decrease in NT-proBNP levels, independently from the changes in left ventricular systolic function.

Diastolic dysfunction, as assessed by the mitral filling pattern and tissue Doppler imaging, has been shown to be present in >90% of patients with heart failure, regardless of ejection fraction. When compared to HFpEF patients, the diastolic dysfunction in patients with HFrEF appears to be more pronounced, with up to 60% of patients displaying moderate or severe impairment in diastolic parameters [19, 20]. In accordance with these findings, we found evidence of elevated estimated ventricular filling pressures (E/e' > 15) in nearly 50% of patients with nonischemic DCM and LVEF < 40%. Of note, our data on estimated ventricular filling pressures (E/e') correlated well with the indices of local diastolic function obtained by electro-anatomical mapping, which further

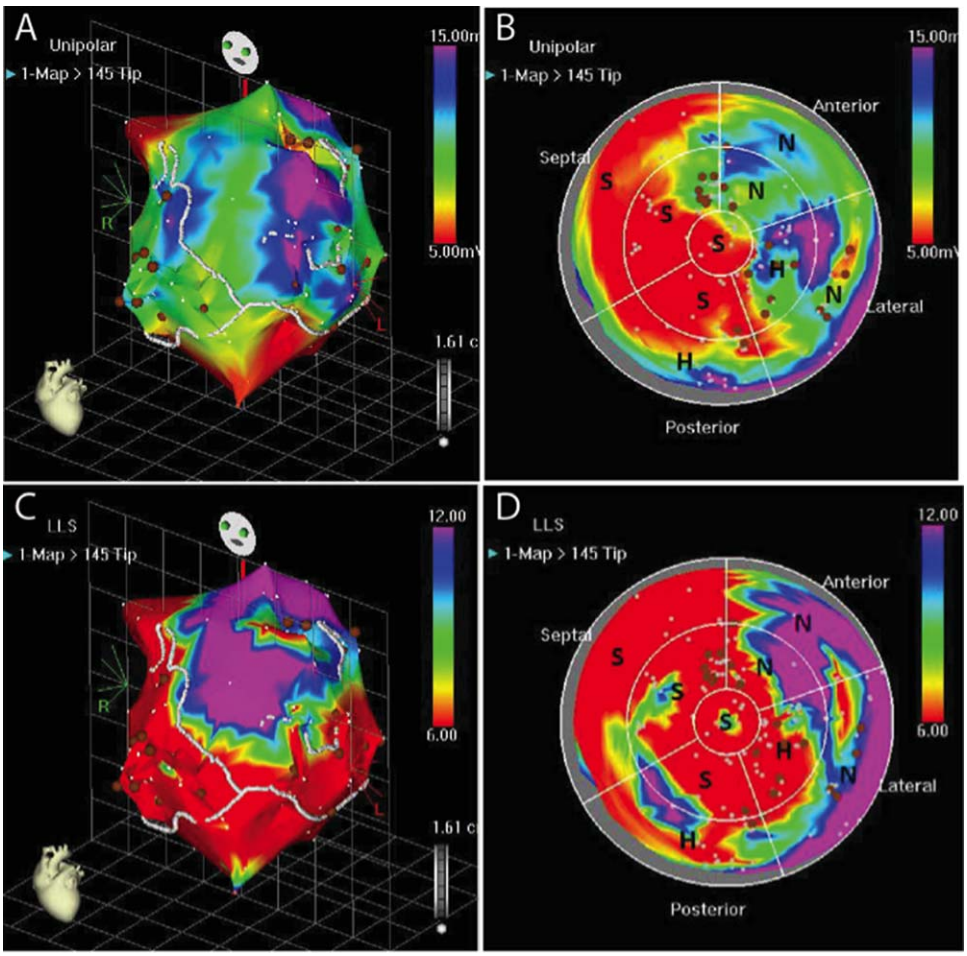

Figure 3. Analysis of scar and hibernation. Exemplary 3D **(A, C)** and corresponding 2D **(B, D)** quantitative polar maps of a patient with nonischemic DCM, showing unipolar voltage and linear shortening. Segments with predominance of high unipolar voltage and high LLS (purple, blue, or green color on both panels) are defined as normal myocardium (N); segments with predominance of low unipolar voltage and low LLS (red and yellow color on both panels) are defined as scarred myocardium (S); and segments with a predominance of high unipolar voltage (purple, blue, or green on left panel) and low LLS (red or yellow on right panel) are defined as hibernating myocardium (H). Abbreviation: LLS, linear shortening.

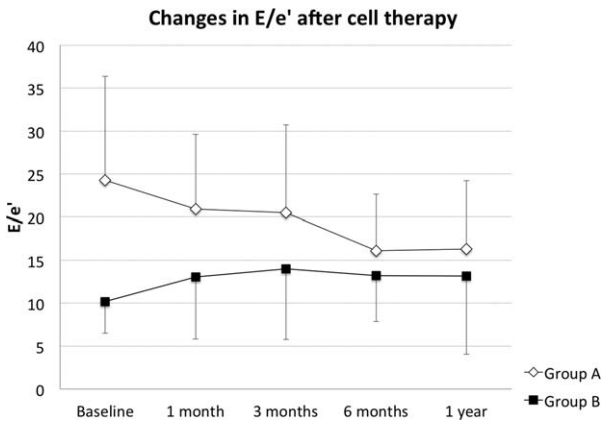

Figure 4. The effects of $CD34^{+}$ cell therapy on estimated left ventricular filling pressures in patients with nonischemic DCM. Within 1 year, we have found a significant improvement in estimated left ventricular filling pressures (measured by E/e') only in patients who displayed elevated filling pressures at baseline ($E/e' \geq 15$). In the remaining cohort, no significant change in E/e' was found.

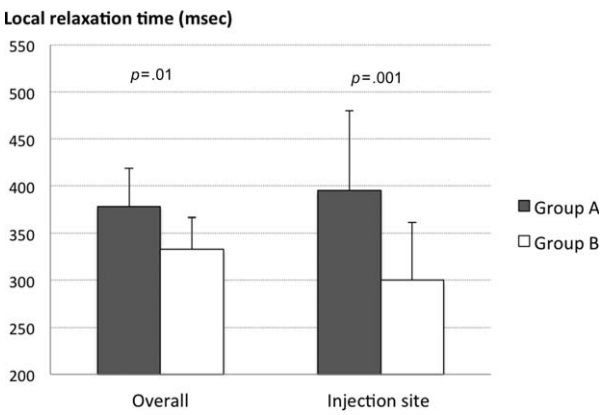

Figure 5. Differences in local diastolic function in patients in with higher (Group A) and lower (Group B) E/e'. The average local relaxation time measured by electro-anatomical mapping was significantly higher in patients with higher E/e'. This difference was even more pronounced when analyzing local relaxation time at the sites of cell injections.

validates the use of E/e' as a clinically useful index when estimating diastolic dysfunction in DCM patients.

In our cohort, impaired diastolic function also correlated well with electro-anatomical changes in the myocardium, with patients with more severe diastolic dysfunction displaying larger areas of myocardial scar. This is in accordance with the studies using cardiac magnetic resonance imaging (cMRI) in DCM, where the myocardial segments with scar have been associated with more pronounced diastolic dysfunction [21], and the extent of myocardial scarring has been shown to be a valid predictor of adverse left ventricular remodeling [22]. Furthermore, in the present study, we found an association between diastolic dysfunction and myocardial hibernation. Although the association between impaired myocardial perfusion and diastolic dysfunction has been well studied in patients with ischemic heart failure [23], data in nonischemic DCM are scarce and controversial. However, in a recent study investigating the explanted hearts of patients with heart failure undergoing heart transplantation, histological and molecular evidence of myocardial hibernation was comparable in both ischemic heart failure and nonischemic DCM [24]. Therefore, the pilot data generated by our study may represent an important background for further studies focusing on the impact of myocardial hibernation on diastolic dysfunction in nonischemic DCM.

By the guidance of electroanatomical mapping we were able to perform the transendocardial cell injections specifically in the areas of hibernating myocardium. Although there was no overall improvement in parameters in diastolic dysfunction at 1 year after cell therapy, we found a favorable response to treatment in DCM patients who displayed elevated E/e' before cell transplantation. This was corroborated with complementary changes in exercise capacity and NTproBNP levels, but not with the changes in LVEF. This is accordance with findings of other studies in heart failure suggesting that changes in systolic and diastolic function in DCM generally do not occur in parallel [19]. Interestingly, patients who demonstrated an improvement in E/e' received cell injections in the areas with more pronounced local diastolic dysfunction, as defined by prolonged local diastolic time. This suggests that patient selection and proper delivery strategies may be of paramount importance when trying to improve diastolic function in DCM using CD34$^+$ cell therapy.

To date, there have only been a few clinical trials investigating the effects of cell therapy on diastolic function, focusing only on patients with ischemic heart disease. In a study of 24 patients with refractory angina [25], transendocardial injection of bone-marrow mononuclear cells was associated with a significant improvement in parameters of diastolic function, measured by echocardiography and cMRI at 3 months after therapy. Similarly, in 32 patients with ischemic heart failure and LVEF$<$40%, they found a significant improvement in diastolic parameters at 1 year after intracoronary application of bone marrow mononuclear cells [18]. Of note, in this study, the greatest improvement was seen in patients who received the largest amount of CD34$^+$ cells. Our results are in accordance with these findings and suggest that cell therapy may indeed improve diastolic function in heart failure and that further research in this field is warranted.

Study Limitations

The results of our study are subject to several limitations. For instance, our patient population included patients with DCM, but no biopsies were performed to exclude secondary cardiomyopathies, though we obtained careful clinical history, detailed echocardiography, and coronary angiogram in all patients. Our sample size was small, which makes the study underpowered to be definitive, but the groups of patients were well matched at baseline. The absence of follow-up electroanatomical mapping makes it difficult to directly compare the effects of injections placed in the areas of worse diastolic dysfunction versus alternative areas. Therefore, we opted to calculate mean local diastolic function at the site of cell injections in each patient and correlated it with the changes in diastolic function after cell therapy. Patients were enrolled in our study based on NYHA functional assessment, without 6-minute walk test distance being a part of our inclusion criteria. However, despite the relatively high baseline 6-minute walk test distance, we were able to demonstrate an improvement in exercise capacity after cell therapy. Finally, we recognize that patients with DCM are a heterogeneous patient population and dynamic changes in ventricular function may be multifactorial.

Conclusions

In nonischemic DCM, elevated estimated ventricular filling pressures appear to correlate with myocardial hibernation and impaired local diastolic parameters on electroanatomical mapping. In these patients, transendocardial CD34$^+$ cell transplantation may be associated with improved diastolic function. Further studies are warranted to better define the underlying mechanisms and to investigate whether or not a similar approach might be beneficial in a broader heart failure population, including patients with HFpEF.

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Author Contributions

M.B.: Conception and design, Provision of study material or patients, Collection and/or assembly of data, Data analysis and interpretation; M.K.: Conception and design, Provision of study material or patients, Collection and/or assembly of data, Data analysis and interpretation; G.P.: Conception and design, Provision of study material or patients, Collection and/or assembly of data, Data analysis and interpretation; M.S.: Conception and design, Provision of study material or patients, Collection and/or assembly of data, Data analysis and interpretation; G.Z.: Conception and design, Provision of study material or patients, Collection and/or assembly of data, Data analysis and interpretation; S.F.: Conception and design, Provision of study material or patients, Collection and/or assembly of data, Data analysis and interpretation; M.C.: Conception and design, Provision of study material or patients, Collection and/or assembly of data, Data analysis and interpretation; P.C.: Data analysis and interpretation, Manuscript writing, Final approval of manuscript; F.H.: Data analysis and interpretation, Manuscript writing, Final approval of manuscript; B.V.: Conception and design, Provision of study material or patients, Collection and/or assembly of data, Data analysis and interpretation, Manuscript writing.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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