Intracoronary Injection of CD34⁺-Cells in Chronic Ischemic Heart Failure: 7 Years Follow-Up of the DanCell Study

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Key Words

Ischemic Heart Failure · Stem Cell Transplantation · Mortality

Abstract

Objectives: Seven years ago, the DanCell study was carried out to test the hypothesis of improvement in left ventricular ejection fraction (LVEF) following repeated intracoronary injections of autologous bone marrow-derived stem cells (BMSCs) in patients suffering from chronic ischemic heart failure. In this post hoc analysis, the long-term effect of therapy is assessed. **Methods:** 32 patients [mean age 61 (SD ± 9), 81% males] with systolic dysfunction (LVEF 33 ± 9%) received two repeated intracoronary infusions (4 months apart) of autologous BMSCs ($1,533 \pm 765 \times 10^6$ BMSCs including $23 \pm 11 \times 10^6$ CD34⁺ cells and $14 \pm 7 \times 10^6$ CD133⁺ cells). Patients were followed for 7 years and deaths were recorded. **Results:** During follow-up, 10 patients died (31%). In univariate regression analysis, the total number of BMSCs, CD34⁺ cell count and CD133⁺ cell count did not significantly correlate with survival (hazard ratio: 0.999, 95% CI: 0.998–1.000, $p = 0.24$; hazard ratio: 0.94, 95% CI: 0.88–1.01, $p = 0.10$, and hazard ratio: 0.96, 95% CI: 0.87–1.07, $p = 0.47$, respectively).

After adjustment for baseline variables in multivariate regression analysis, the CD34⁺ cell count was significantly associated with survival (hazard ratio: 0.90, 95% CI: 0.82–1.00, $p = 0.04$). **Conclusions:** Intracoronary injections of a high number of CD34⁺ cells may have a beneficial effect on chronic ischemic heart failure in terms of long-term survival.

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Introduction

Morbidity and mortality remains high in patients suffering from chronic ischemic heart failure [1]. Stem cell therapy, in which various types of autologous stem cells are injected either into coronary arteries or directly into the infarcted myocardium, has been proposed to improve myocardial function [2, 3]. Some hypothesized mechanisms of the beneficial effects of stem cell therapy on the myocardium include stem cell-induced stimulation of neovascularization in the infarcted area and enhanced regeneration of myocytes. The latter may be caused by (1) differentiation from mononuclear cells to cardiac myocytes, (2) cytokine-induced growing and increase of residual viable myocytes, especially within the border zone

of the infarcted area, (3) stimulation of intrinsic myocardial stem cells, or (4) induction of cell fusion between transplanted bone marrow cells and resident myocytes [4].

The use of stem cell treatment in patients with chronic ischemic heart failure has been investigated increasingly over the last decade. Although data are conflicting and likely subjected to publication bias, a recent systematic review found that bone marrow-derived stem cells (BMSCs) improved left ventricular ejection fraction (LVEF) modestly, reduced the infarct size in patients with ST segment elevation myocardial infarction, and decreased cardiac remodeling in patients with ischemic heart disease compared with conventional therapy. These benefits seem to persist when observed in long-term follow-up >12 months, except for LVEF which persisted >24 months. Also, BMSC transplantation may possibly reduce mortality, decrease recurrence of myocardial infarction, and stent thrombosis in patients with ischemic heart disease [2].

In the DanCell study, conducted 7 years ago, patients with severe ischemic heart failure received two intracoronary infusion of autologous BMSCs. No significant improvement could be demonstrated either with contrast-enhanced 2D echocardiography or with 2D speckle tracking [5, 6]. However, 12 months of follow-up revealed an unexpected trend of improved survival in the treated patients. In this post hoc study we sought to explore this more with an extended follow-up.

Methods

Patients

In 2007, 87 patients with symptomatic ischemic heart failure were screened for inclusion in the DanCell study [5]. The inclusion criteria were stable symptomatic patients [New York Heart Association (NYHA) functional class II/III and/or Canadian Heart Association Classification of Angina (CCS) class II/III], aged 18–75 years, LVEF <45%, on optimal medical therapy, and not amenable for further coronary revascularization. The exclusion criteria were noncardiac serious diseases expected to reduce the patient's short-term survival, recent (<6 months) myocardial infarction, permanent atrial fibrillation, or an implanted pacemaker. Of the 87 patients screened, 14 patients were mildly symptomatic, 13 had preserved systolic function, 8 had a serious comorbidity, and 17 patients were unwilling to participate. The remaining 35 patients were included in the study, but 3 patients did not complete the protocol (1 patient died before the first treatment, 1 patient withdrew consent, and 1 patient experienced dissection of the right coronary artery during the first pretreatment coronary angiography). Thus, 32 patients completed the study. The protocol was approved by the Regional Scientific Ethical Committee for Southern Denmark (VF-20050025), and the study was registered at Clinical-

Trials.gov (NCT00235417) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

Stem Cell Treatment

The patients were treated twice with the stem cells, retrieved a few hours before each infusion. Under local anesthesia the bone marrow was aspirated from the iliac crest and resuspended with heparin (2,500 IE/ml), and BMSCs were isolated by Lymphoprep (Axis-Shield, Oslo, Norway). The CD34⁺ and CD133⁺ cell counts were measured with flow cytometry. Prior to this, the isolated cell populations were stained with anti-CD34 FITC (clone 8G12; Becton Dickinson, Franklin Lakes, N.J., USA), anti-CD133 PE (clone AC133; Miltenyi Biotec, Bergisch Gladbach, Germany) and propidium iodide, respectively. Acquisition was performed on a FACS Calibur and subsequent data analysis was carried out with CellQuest 3.3 software (Becton Dickinson). Cells were infused within 7 h of bone marrow aspiration. At the first treatment, 139 ± 35 ml of bone marrow were harvested and at the second treatment 163 ± 3 ml were harvested. Subsequently, $647 \pm 382 \times 10^6$ BMSCs with an average of $9.7 \pm 6.3 \times 10^6$ CD34⁺ cells and $5.1 \pm 3.0 \times 10^6$ CD133⁺ cells were infused at the first treatment, and at the second treatment $889 \pm 361 \times 10^6$ cells with an average of $14.1 \pm 7.3 \times 10^6$ CD34⁺ cells and $8.5 \pm 4.2 \times 10^6$ CD133⁺ cells were given. Intracoronary cell infusion was done using the stop-flow technique, with an over-the-wire balloon catheter placed in the target vessel supplying the area with the most extensive regional wall motion abnormalities. The balloon was inflated for 2 min, while 5 ml of the cell suspension was infused distally into the vessel. This was repeated 4 times, interrupted by 3 min of reflow. Target vessels for intracoronary BMSC infusion were the left anterior descending artery ($n = 21$), left circumflex artery ($n = 4$), and right coronary artery ($n = 9$).

Follow-Up

The patient files were reviewed via both regional and national IT record systems in December 2012 and January 2013, thus ensuring that any contact to Danish hospitals would be noticed. Rehospitalization was defined as admittance to a hospital not including outpatient consultations. This system covers all admissions, thus a complete follow-up for all patients was possible. The primary endpoint in the follow-up was death, with secondary endpoints being the composite of death and readmission for congestive heart failure.

Statistical Analysis

Continuous variables are presented as means $\pm$ SD. Categorical variables are listed as n (%). Parametric statistics are used on normally distributed variables and log-transformed NT-proBNP. Cox regression analysis was used to evaluate the prognostic significance of the following variables: age, log (NT-proBNP), hemoglobin, creatinine, LVEF, BMSC count, CD34⁺ cell count, and CD133⁺ cell count. Variables with $p < 0.20$ in the univariable Cox regression analysis were included in the multivariable Cox regression model. Variable selection was performed in the multivariable Cox regression analysis as a stepwise backward elimination method, each time excluding the one variable with the highest p value according to Wald statistics. The final model included age and variables with $p < 0.05$. Also, the population was divided in two equal groups (high and low CD34⁺ cell therapy group) through the me-

Table 1. Baseline demographic and laboratory characteristics of the patients (n = 32)

Age, years	61.3±8.6
Females	6 (19)
Blood pressure, mm Hg	
Systolic	122±23.7
Diastolic	72.3±12.7
Heart rate, beats/min	65.7±11.6
BMI	28.9±4.8
Serum creatinine, mg/dl	1.4±0.4
Plasma NT-proBNP, pg/ml	1,948.2±2,550.0
LVEF, %	33.5±9.2
<i>Risk factors</i>	
Hypertension	13 (41)
Diabetes mellitus	9 (28)
Smoking	
Active	10 (31)
Never	5 (16)
Previous	17 (53)
Family history of coronary disease	14 (44)
Medical history	
Previous CABG	8 (25)
Time since most recent MI – months	
Mean ± SD	62±68.1
Median, range	27 (6–276)
Extent of coronary artery disease	
1 vessel	7 (22)
2 vessels	8 (25)
3 vessels	17 (53)

Values are expressed as n (%) or mean ± SD, unless otherwise indicated. CABG = Coronary artery bypass graft; MI = myocardial infarction.

dian of the number of injected CD34⁺ cells, and unadjusted mortality rate, readmission rate, and the combined endpoint (mortality or readmission rate) plotted as Kaplan-Meier curves was compared using log-rank statistics. All analyses were performed using Stata SE 11 (StataCorp LP, College Station, Tex., USA).

Results

A total of 32 patients completed the study protocol with two intracoronary injections of BMSCs 4 months apart. Table 1 lists the baseline characteristics of the patients. Overall the patients received $1,533 \pm 664 \times 10^6$ BMSCs with an average of $23.0 \pm 11.5 \times 10^6$ CD34⁺ cells and $14.4 \pm 6.5 \times 10^6$ CD133⁺ cells.

During 7 years of follow-up, 10 patients died (31%), 12 patients were admitted due to heart failure, and the composite endpoint (death and/or readmission) was seen in

Table 2. Prognostic significance of various variables concerning mortality at follow-up

	Hazard ratio	95% CI	p
Age (per year)	1.09	(0.99–1.19)	0.07
LVEF (per 1%)	0.85	(0.76–0.96)	0.01
Cell count (per 10^6 cells)	0.999	(0.998–1.000)	0.24
CD34 ⁺ cell count (per 10^6 cells)	0.94	(0.88–1.01)	0.10
CD133 ⁺ cell count (per 10^6 cells)	0.96	(0.87–1.07)	0.47
log NT-proBNP (per 1 log, pg/ml)	1.89	(1.21–2.96)	0.005
Hemoglobin (per 1 mmol/l)	0.84	(0.41–1.76)	0.65
Serum creatinine (per 1 mg/dl)	1.01	(1.00–1.02)	0.19

Univariable Cox regression analyses.

Table 3. Variables retained in the multivariable Cox regression models

	Hazard ratio	95% CI	p
LVEF (per 1%)	0.87	(0.78–0.97)	0.01
Creatinine (per 1 mg/dl)	1.03	(1.00–1.05)	0.03
CD34 ⁺ cell count (per 10^6 cells)	0.90	(0.82–1.00)	0.04
Age (per year)	1.06	(0.95–1.19)	0.29

17 patients (53%). Twelve patients received an implantable cardioverter-defibrillator after cell treatment, and 4 of these (33%) died during the follow-up. The 12 implantable cardioverter-defibrillators were implanted 14–72 months after the first treatment.

Univariable predictors of all-cause mortality at follow-up are presented in table 2. LVEF and log (NT-proBNP) did provide prognostic information, but age and CD34⁺ cell count did not reach significance. The number of BMSCs and CD133⁺ cells was not associated with mortality. In multivariate analyses with backward selection, CD34⁺ cell count, creatinine, and LVEF provided independent prognostic information (table 3). The hazard ratio for the CD34⁺ cell count was 0.90 (95% CI: 0.82–1.00, p = 0.04); therefore, decreasing the injected CD34 cell count by 10^6 increases the mortality risk by 10%.

The patients were divided into two groups based on median number of CD34⁺ cells received. The high CD34⁺ cell therapy group (n = 16) received a total average of $32 \pm 9 \times 10^6$ CD34⁺ cells compared with $15 \pm 5 \times 10^6$ CD34⁺ cells in the low CD34⁺ cell therapy group (n = 16). The two groups were comparable in terms of mean age (61 ± 8 and

Fig. 1. The included patients were divided into two equal groups through the median of the number of injected CD34⁺ cells (high- and low-CD34⁺ cell therapy group) and unadjusted Kaplan-Meier curves displays percent event-free survival. Events were death (a), readmission (b), and death or readmission (c).

62 ± 9 years, $p = 0.76$) and LVEF (33 ± 8 and 33 ± 10%, $p = 0.93$), respectively. There was a trend toward decreased mortality and morbidity in the high CD34⁺ cell therapy group: mortality (3 vs. 7 patients, $p = 0.10$ by log-rank test and $p = 0.07$ if adjusted for age), readmission rate (5 vs. 7 patients, $p = 0.25$ by log-rank test), and the combined endpoint (death or readmission; 7 vs. 10, $p = 0.15$ by log-rank test) was nonsignificantly lower among those who received the highest amount of BMSCs (fig. 1a–c).

Discussion

To the best of our knowledge the present stem cell study has the longest published duration of follow-up, and with 7 years of follow-up we suggest that intracoronary injection of CD34⁺ stem cells is safe and may have beneficial long-term effects on clinical outcome.

Previously, improvement in survival was suggested in two prospective trials. Pokushalov et al. [7] performed a randomized control trial with 109 patients (55 treated and 54 controls) in which stem cells were given intramyocardially. The follow-up time was 12 months, and 21 died in the control group while only 6 died in the intervention group. Further, in a nonrandomized study with 391 patients (191 treated and 200 controls), stem cells were injected intracoronarily. During the 60 months of follow-up, 32 died in the control group compared to 7 in the treated group [4]. This is supported by our findings since a beneficial effect on mortality in patients treated with a high number of stem cells was seen. Interestingly, this effect did not appear to be dependent on the total number of BMSCs or the number of CD133⁺ cells. While the positive effect on mortality in the two prior studies was seen after 12 months and 5 years, respectively, in our study it persisted for at least 7 years, suggesting a sustained effect.

Importantly, mortality was the primary endpoint in neither of the trials mentioned above, thus interpretation has to be made with great caution.

When comparing the DanCell study to other studies similar in design and demographics, no significant increase in LVEF was found in DanCell, while other studies have been able to show this [4, 7–11]. In DanCell, the evaluation of LVEF was performed in a blinded setting, thus excluding bias. In contrast to all other studies, the patients in our study underwent two treatments with stem cell injections. This ‘dual injection’ technique in itself does not necessarily warrant a superior outcome; however, our number of injected cells exceeds the number injected in any other study manifold: the average total injected BMSCs in DanCell was $1,536 \times 10^6$ as opposed to averages spanning $41\text{--}205 \times 10^6$ in the other studies. It is plausible that the number of patients in DanCell was too small to detect an improvement in LVEF, a type 2 error. However, the LVEF only increased 1.1% (hardly clinically relevant), and in the majority of the other studies fewer patients were included. Another reason could be that the patients in DanCell served as their own control as opposed to a randomized design, thus compromising our ability to demonstrate a significant difference in LVEF between two groups, where the control group could be thought to experience a decrease in LVEF.

Although no significant improvement in LV function could be detected, neither with conventional echocardiography nor with subsequent speckle tracking analysis, this post hoc analysis suggests a possible improvement in clinical outcome. Yet, LVEF is a surrogate marker for outcome, and the question is whether changes in LVEF are a valid measure for detection of an effect of stem cell therapy. Parallel to this, other treatments of heart failure, like ACE-inhibitors and β -blockers, do have a substantial impact on mortality but a modest effect on LVEF.

While the DanCell study used intracoronary administration of stem cells, another route of injection is directly into the myocardium. Our findings suggest a positive coherence between the amount of cells injected and mortality, and this trend is supported by other studies, regardless of the route of injection. In the two randomized controlled trials using intramyocardial injection of stem cells, improvement in LVEF was shown [7, 11] and one showed improvement in mortality [7]. The rest of the comparable studies all used intracoronary administration, and these also seem to have a positive outcome [4, 8–10]. On the basis of the current limited data, a recent meta-analysis

found that intramyocardial injection may be superior to intracoronary infusion in patients with LV systolic dysfunction [3]. Further studies, however, are required to determine the best administration route.

The type of injected stem cells varies between studies. In the DanCell study, BMSCs were used with known quantities of CD34⁺ and CD133⁺ cells. In general BMSCs have been the preferred choice and in some of the studies the number of CD34⁺ and CD133⁺ cells has been specified [8, 9, 11]. As an alternative to BMSCs, cardiac stem cells have been extracted during coronary artery bypass graft surgery from the right atrium [12]. Subsequently these cells were administered either intramyocardially or intracoronarily. This has proven to be feasible, with improvement in both global and regional LV function.

Future of Stem Cells

After examining the recent data, it is still not obvious whether treatment with stem cells holds a place in the upcoming management of ischemic heart disease. First of all, more research is needed. We do not know enough about stem cells, i.e. where they are, how they should be pretreated, and how they should be administered. Secondly, large-scale trials are warranted. Additionally, the outcome of stem cell therapy may be dependent on the number of stem cells injected, but other factors might also be important, such as LVEF and creatinine in our study. Age, gender, and time from infarction to treatment are probably other important predictors of successful treatment.

Limitations

As this is a post hoc analysis of a study with a neutral primary endpoint and low power, it can only be considered hypothesis-generating at best. Death as the endpoint, however, is unambiguous, not subject to investigator interpretation, and is of direct clinical relevance to patients. We therefore consider the result of interest. Our study also has several other limitations, the most important being the absence of blinding and a placebo group. Additionally, the population was small, and subgroup analyses, e.g. differences in outcome among gender, different age groups, and LVEF, were difficult to perform. However, the number of cells used did exceed the number of cells in the comparable studies – by a factor of three to the study that used the second most cells. Furthermore, this study focused only on ischemic heart failure due to infarction, while the effect by stem cells on heart failure due to other causes is an area which demands more research.

Conclusion

This small post hoc analysis suggests that patients might benefit from intracoronary stem cell injections in terms of long-term clinical outcome. The power of the study, however, was low and future larger randomized studies with extended follow-up are warranted.

Acknowledgements

We are indebted to the dedicated nursing and technical staff at the Heart Failure Clinic, the Cardiac Catheterization Laboratory, the Coronary Care Unit and the Department of Clinical Immunology of Odense University Hospital.

Funding

The study was supported in part by a grant from County of Fynen (Denmark) and the Danish Heart Foundation.

Conflict of Interest

No relationship.

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