

# Effects of stem cell therapy on dilated cardiomyopathy

Rong Jiao, MD, Yuan Liu, MD, Wen-jian Yang, MD, Xiao-Yan Zhu, MD, Jin Li, MD, Qi-Zhu Tang, MD.

## ABSTRACT

**الأهداف:** إجراء التحليل التلوي من التجارب السريرية ودراسة تأثير العلاج بالخلايا الجذعية على تمدد عضلة القلب.

**الطريقة:** أجري بحث منهجي من المؤلفات ما بين مايو 2012م و يوليو 2013م في PubMed ، وميدلاين، ومكتبة كوكرين، وقاعدة البيانات Excerpta Medica (EMBASE) أجريت الدراسة في قسم أمراض القلب في مستشفى رنمين، جامعة ووهان، الصين. تم احتساب فرق المتوسط الموزون (WMD) للبطين الأيسر طرد جزء (LVEF) ، قطر البطين الأيسر نهاية الانبساط (LVEDD) والوفيات، وزرع القلب، واختبار مسافة 6 دقائق (6-MWT) المسافة باستخدام برنامج RevMan 5.0.

**النتائج:** قيّم 7 تجارب مع 599 من المشاركين العلاقة بين العلاج بالخلايا الجذعية ومجموعات المراقبة. بالمقارنة مع مجموعة المراقبة، حسنت مجموعة العلاج بالخلايا الجذعية LVEF (WMD: 3.98%، 95% confidence interval [CI]: 0.55-7.41%،  $p=0.02$ ) والمسافة (WMD: 132.12 m، 95% CI: 88.15-176.09 m،  $p>0.00001$ ) و خفض الوفيات و زرع القلب (odds ratio [OR]: 0.48، 95% CI: 0.29-0.80،  $p=0.005$ ) و مع ذلك اظهرت LVEDD انه لا يوجد فرق كبير بين المجموعتين (WMD: -1.53 mm، 95% CI: -1.15-0.10 mm،  $p=0.10$ ).

**الخاتمة:** أظهر هذا التحليل التلوي أن العلاج بالخلايا الجذعية يحسن وظيفة القلب ويقلل من الوفيات في مرضى تمدد عضلة القلب، والذي أشار إلى أن العلاج بالخلايا الجذعية قد تمثل خيارا جديدا لعلاج تمدد عضلة القلب.

**Objectives:** To perform a meta-analysis of clinical trials and investigate the effect of stem cell therapy on dilated cardiomyopathy.

**Methods:** A systematic literature search was carried out between May 2012 and July 2013 in PubMed, Medline, Cochrane Library, and Excerpta Medica Database (EMBASE). The study took place in the Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, China. The weighted mean difference (WMD) was calculated for left

ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDD), mortality and heart transplantation, and the 6-minute walk test (6-MWT) distance using the RevMan 5.0 software.

**Results:** Seven trials with 599 participants evaluated the association between the stem cell therapy and control groups. Compared with the control group, stem cell therapy group improved the LVEF (WMD: 3.98%, 95% confidence interval [CI]: 0.55 - 7.41%,  $p=0.02$ ) and the 6-MWT distance (WMD: 132.12 m, 95% CI: 88.15-176.09 m,  $p<0.00001$ ), and reduced mortality and heart transplantation (odds ratio [OR]: 0.48, 95% CI: 0.29-0.80,  $p=0.005$ ). However, the LVEDD showed no significant difference between the 2 groups (WMD: -1.53 mm, 95% CI: -1.15-0.10 mm,  $p=0.10$ ).

**Conclusion:** This meta-analysis demonstrated that stem cell therapy improves cardiac function and reduces mortality in dilated cardiomyopathy patients, which suggested that stem cell therapy may represent a new therapy option for dilated cardiomyopathy.

*Saudi Med J 2014; Vol. 35 (12): 1463-1468*

From the Department of Cardiology (Jiao, Liu, Li, Tang), Renmin Hospital of Wuhan University, Department of Cardiology (Jiao, Liu, Li, Tang), Cardiovascular Research Institute of Wuhan University, Wuhan, Department of Pediatrics (Zhu), and the Department of Endocrinology (Yang), Xiangyang No. 1 People's Hospital, Affiliated Hospital of Hubei University of Medicine, Hubei, China.

Received 19th May 2014. Accepted 6th October 2014.

Address correspondence and reprint request to: Dr. Qi-Zhu Tang, Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan 430060, China. Tel/Fax: +86 (278) 8041911. E-mail: qztang@whu.edu.cn

**Disclosure.** Authors have no conflict of interest, and the work was not supported or funded by any drug company.

**D**ilated cardiomyopathy (DCM) is a primary myocardial disease of unknown etiology characterized by left ventricular or biventricular dilation and impaired contractility.<sup>1</sup> Patients with DCM many have an increased myocardial mass and an altered extracellular collagen network,<sup>2</sup> which is referred to as myocardial remodeling and eventually leads to heart failure. Reversing this process to reduce its morbidity and mortality remains one of the major challenges in healthcare practice. Recent studies have focused on stem cell therapy. In animal experiments, stem cell therapy improved both cardiac function and adverse left ventricular remodeling and reduced mortality.<sup>3</sup> In clinical trials, short-term studies indicated that intra-coronary infusion of stem cells increased the left ventricular ejection fraction (LVEF)<sup>4</sup> and improved other clinical outcomes.<sup>5-7</sup> Furthermore, long-term studies produced the same results.<sup>8-10</sup> Martino et al<sup>11</sup> demonstrated the safety of bone marrow mononuclear cell transplantation, and that it is feasible in DCM patients with severe ventricular dysfunction. Previous studies have investigated the effects of stem cells on DCM. However, the sample size of these trials was small, and the conclusions were inconsistent. In most of these studies, the effect of stem cells was measured by LVEF, left ventricular end-diastolic diameter (LVEDD), the 6-minute walk test (6-MWT) distance, and the combined mortality and heart transplantation rate. The objective of this study is to perform a meta-analysis of all known clinical trials reporting the effects of stem cells on changes in LVEF, LVEDD, 6-MWT distance, and mortality and heart transplantation.

**Methods.** We systematically searched the Cochrane Library, Excerpta Medica Database (EMBASE), and PubMed databases as well as reviews and reference list articles by using the keywords DCM, dilated cardiomyopathy, dilative cardiomyopathy, idiopathic dilated cardiomyopathy, or nonischemic dilated cardiomyopathy, and stem cell or bone marrow cells, and trial without language and time limitations in the Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, China, between May 2012 to July 2013.

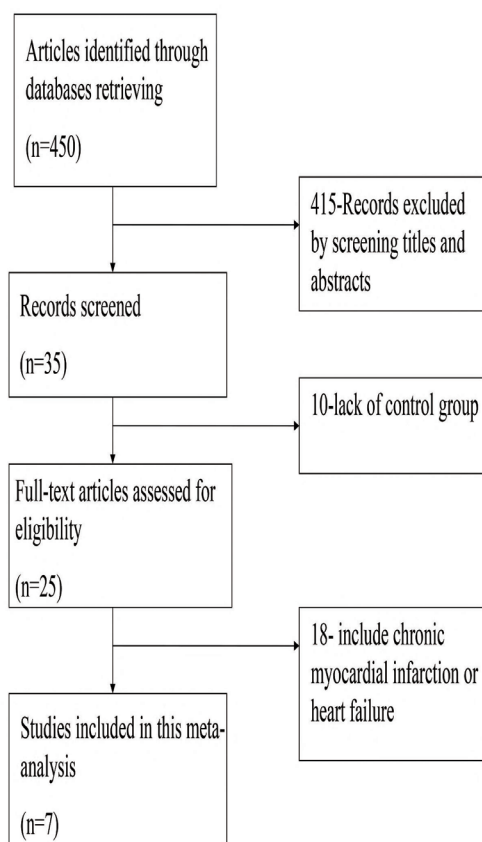
We selected completed, published, and unconfounded trials with intracoronary infusion of stem cells versus control treatments in DCM patients. The follow-up durations were more than 3 months and studies without intention-to-treat or control groups were excluded. The duplicate reports and ongoing trials were also excluded.

All trials were independently reviewed by 2 authors to identify the relevant trials meeting the inclusion criteria. Disparities were resolved by discussion. Data on the trial design, population characteristics, treatment regimen, and changes in LVEF, LVEDD, 6-MWT distance from baseline, mortality, and the combined heart transplantation rate were extracted. The primary outcome was the changes in LVEF, LVEDD, 6-MWT distance from baseline, mortality, and the combined heart transplantation rate.

Two of the investigators independently assessed the methodological quality of the included trials using the standard criteria: allocation concealment, blinding of investigators, participants and outcome assessors, use of intention to treat analysis, completeness of follow-up, and reporting of withdrawals. Each trial is scored one point with a possible score of between 0 and 5.<sup>12</sup>

We used Review Manager 5.0 software to perform the data analysis. The pooled weighted mean differences (WMDs) and the 95% confidence interval (CI) were calculated for changes in LVEF, LVEDD, and 6-MWT distance, and the odds ratios (OR) and CI were calculated for the combined mortality and heart transplantation rate using fixed or random-effect (DerSimonian and Laird)<sup>13</sup> models. The statistical heterogeneity of treatment effects between studies was formally tested with Cochran's test ( $p < 0.1$ ). The  $I^2$  statistic was also examined to measure the proportion of total variation due to heterogeneity beyond chance, and  $I^2 > 50\%$  was considered to indicate significant heterogeneity between the trials: the random effect model based on the DerSimonian and Laird<sup>13</sup> estimator was used to perform the meta-analysis. Otherwise, the fixed effect analysis of the Mantel-Haenszel model was chosen for the meta-analysis.<sup>14</sup> A funnel plot was constructed to evaluate the study bias. Continuous variables were expressed as the mean±standard deviation (SD). A  $p$ -value  $< 0.05$  was considered statistically significant.

**Results. Search results.** A total of 450 articles were identified in a combined search from PubMed, EMBASE, Cochrane Library, Clinical Trial, and meta Register of Controlled Trials (mRCT) databases and in a manual search (a search of previous studies cited in previous reviews and references listed in the identified articles); 415 citations were initially excluded at the title/abstract level (Figure 1). Among the complete articles retrieved, 10 were excluded due to lack of a control group and 18 were excluded due to the use of intra-coronary cell therapy for chronic myocardial infarction or heart failure. Eventually, 7 trials with 599 subjects were included in our meta-analysis.



**Figure 1** - Flowchart of the study selection among 415 citations initially excluded at the title/abstract level.

**Study characteristics.** The study designs, and the interventions included in the analysis are shown in Table 1. The average age of the patients included in these trials varied from 30-67 years of age. To treat patients, 3 trials used bone marrow mononuclear cells (MNCs), 2 trials used CD34 stem cells, and one trial used both bone marrow mesenchymal stem cells, and MNCs. Seven trials were randomized controlled trials. Cell injections were performed in coronary arteries, and the mean follow-up was 19.8 months (range, 3-60 months).

**Data quality.** The quality scores of the trials varied from 2-5 (maximum score). All studies were investigated the change of LVEF from baseline. Randomized allocation was used in 6 trials.<sup>5-10</sup> A total of 4 studies reported adequate details of withdrawals,<sup>8-10,15</sup> whereas the other studies did not address this issue.<sup>5-7</sup>

**Effects of stem cell therapy on DCM.** A total of 7 trials with 599 participants evaluated the association between stem cell therapy and changes in LVEF in DCM patients. In an overall pooled estimate, compared with the control group, patients who received stem cell therapy had significantly increased LVEF from baseline to follow-up (WMD: 3.98%, 95% confidence interval [CI]: 0.55-7.41%,  $p=0.02$ ; Figure 2A). This outcome showed high heterogeneity (heterogeneity test:  $I^2=93%$ ,  $p<0.00001$ ).

**Table 1** - Randomized trials reporting the influence of stem cells on dilated cardiomyopathy.

Study	Year	Number of patients	Age (year)	Male (%)	LVEF (%)	LVESD (mm)	Name of stem cell	Methods	Daily maintenance dose ( $\times 10^7$ )	Follow-up (months)	Primary outcomes	Study quality
Vrtovec et al <sup>9</sup>	2013	Test: 55 Control: 55	54±9	89.0	25.2±4.2	70±8	SC	Coronary arteries injection	113.0	60	LVEF, LVESD, exercise capacity, NT-proBNP, cardiac mortality, heart transplantation	5
Vrtovec et al <sup>8</sup>	2011	Test: 28 Control: 27	53±9	79.0	25.9±4.6	70±8	SC	Coronary arteries injection	123.0	12	LVEF, exercise capacity, NT-proBNP, cardiac mortality, heart transplantation	5
Seth et al <sup>10</sup>	2010	Test: 41 Control: 40	45±15	44.0	22.5±8.3	NA	MNC	Coronary arteries injection	168.0	28	LVEF, mortality, LVESD, mortality	5
Xiao et al <sup>6</sup>	2012	Test: 33 Control: 20	51.8±11.6	66.0	33.6±3.9	64.9±5.5	MNC MSC	Coronary arteries injection	50.0	3	LVEF, LVESD, mortality, cardiovascular events	4
Wang et al <sup>5</sup>	2006	Test: 12 Control: 12	56±11	70.8	30±9	69.3±2.2	MSC	Coronary arteries injection	1.75	6	LVEF, exercise capacity, NT-proBNP, mortality	4
Huang et al <sup>7</sup>	2006	Test: 10 Control: 8	56±11	83.3	30±13	68.6±1.5	MNC	Coronary arteries injection	57.6	6	LVEF, LVESD, exercise capacity, mortality	3
Chen et al <sup>15</sup>	2008	Test: 71 Control: 187	54±14	70.9	32.4±8.5	70.9±8.1	MNC	Coronary arteries injection	20.5	24	LVEF, LVESD, exercise capacity, mortality	2

5 and 4 represent high quality, 3 represent medium quality, 1 and 2 represent low quality. LVEF - left ventricular ejection fraction, LVESD - left ventricular end-diastolic diameter, SC - CD34<sup>+</sup> stem cell, NA - not available, MNC - bone marrow mononuclear cells, MSC - bone marrow mesenchymal stem cells, LVESD - left ventricular end-diastolic volume, NT-proBNP - N-terminal of the prohormone brain natriuretic peptide

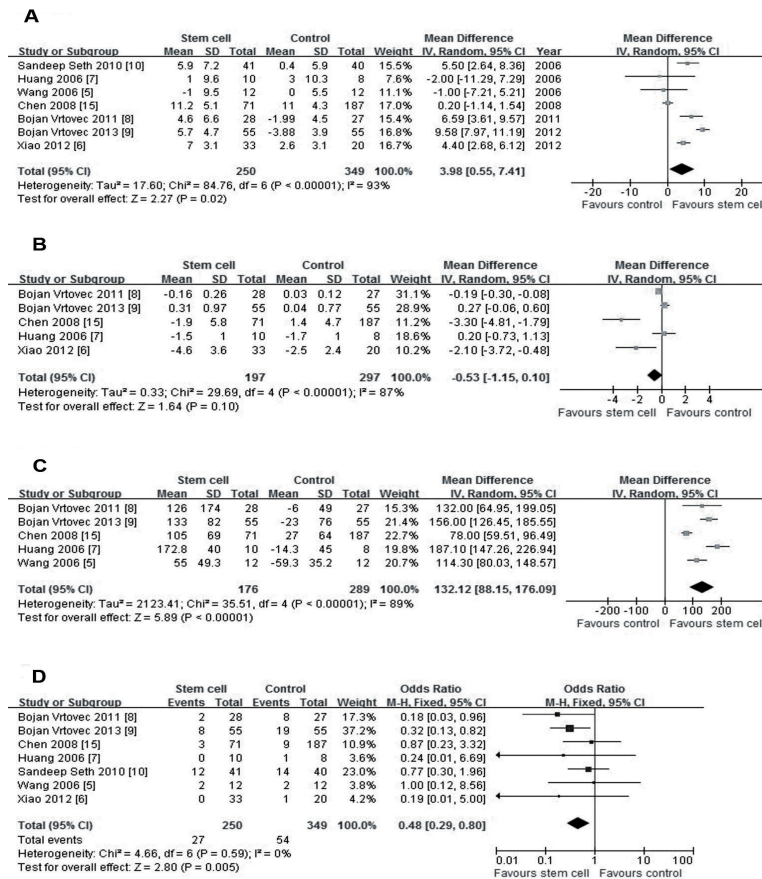
A total of 5 trials with 494 participants evaluated the association between stem cell therapy and changes in LVEDD in DCM patients. In an overall pooled estimate, compared with the control group, the stem cell therapy did not influence the LVEDD change from baseline to follow-up (WMD: -1.53 mm, 95% CI: -1.15-0.10 mm,  $p=0.10$ ; Figure 2B). High heterogeneity for this outcome was found (heterogeneity test:  $I^2=87%$ ,  $p<0.00001$ ). A total of 5 trials with 465 participants evaluated the association between stem cell therapy and changes in 6-WMT distance. In an overall pooled estimate, compared with the control group, patients who received stem cell therapy showed a greater increase in 6-WMT distance from baseline to follow-up (WMD: 132.12 m, 95% CI: 88.15-176.09 m,  $p<0.00001$ ; Figure 2C). However, the heterogeneity for this outcome was still high (heterogeneity test:  $I^2=89%$ ,  $p<0.00001$ ).

All trials evaluated the combined mortality and heart transplantation rate. In an overall pooled estimate,

the combined mortality and heart transplantation rate showed a significantly greater reduction in the stem cell therapy group (odds ratio [OR]: 0.48, 95% CI: 0.29-0.80,  $p=0.005$ ; Figure 2D). No heterogeneity was found (heterogeneity test:  $I^2=0%$ ,  $p=0.59$ ). The  $I^2$  test of heterogeneity was relatively high in LVEF ( $I^2=93%$ ), LVEDD ( $I^2=87%$ ), and 6-WMT distance ( $I^2=89%$ ). The difference in stem cell types, and the duration of follow-up in each study may have caused the high heterogeneity that could not be eliminated. Therefore, random-effect modeling was conducted using the DerSimonian and Laird<sup>13</sup> method.

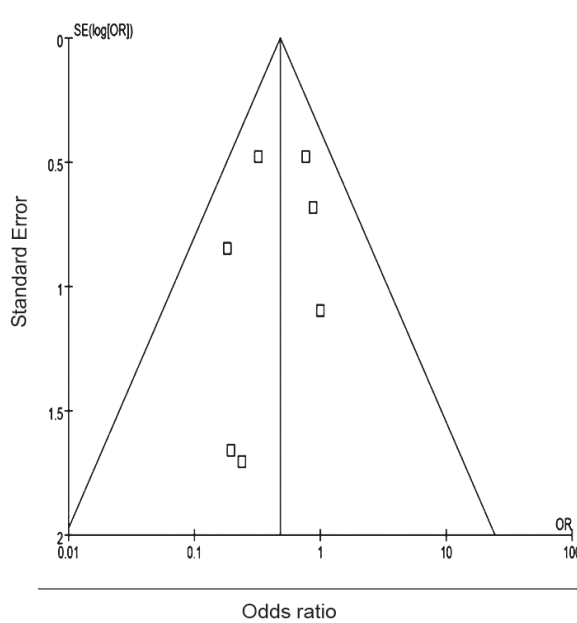
**Publication bias.** To assess the potential existence of publication bias in the effects of stem cell therapy on the combined mortality and heart transplantation rate, a funnel plot (Figure 3) indicates slight asymmetry, and therefore, a publication bias most likely exists.

**Discussion.** Our meta-analysis suggests that stem cell therapy improves left ventricular contractility and



**Figure 2** - Forrest Plot and WMD for A) LVEF, B) LVEDD, C) 6-minute walk test distance, D) mortality and heart transplantation. WMD - weight mean difference, LVEF - left ventricular ejection fraction, LVEDD - left ventricular end-diastolic diameter, OR - odds ratio, CI - confidence interval





**Figure 3** - Funnel plot of SE to evaluate publication bias for effect of stem cell therapy in mortality and heart transplantation. SE - Standard error

exercise capacity, and reduces mortality and heart transplantation in DCM patients. However, stem cell therapy has no effect on LVEDD. A total of 5 studies evaluated the association between stem cell therapy and changes in LVEDD in DCM patients.<sup>6-9,15</sup> Two of these studies,<sup>6,15</sup> reported a statistical reduction in LVEDD, but the other 3 studies<sup>7-9</sup> did not report significant differences. When we removed the maximum weight studies, the change in LVEDD was unchanged (WMD: -1.67 mm, 95% CI: -3.92-0.59 mm,  $p=0.15$ ). These indicated that stem cell therapy cannot reverse the increased LVEDD of DCM. As the heterogeneity was relatively high, further studies with longer follow-up durations are required to confirm this inconsistent result. The present analysis excluded the non-randomized controlled trials and included only randomized controlled trials with high quality making the results more dependable.

Dilated cardiomyopathy is clinically characterized by cardiac chamber dilatation and reduced systolic function which commonly results in congestive heart failure.<sup>16</sup> Cell therapy has been suggested to be a promising novel therapeutic strategy for heart diseases,<sup>17,18</sup> but the potential impact of cell therapy on DCM has not yet been fully established. Fifteen dogs with DCM were administered stem cell therapy by retrograde coronary venous delivery, and after 2 years of

follow up, the stem-cell therapy did not appear to offer any advantages.<sup>19</sup> Lin et al<sup>20</sup> found that early combined stem cell therapy and sildenafil could help to preserve left ventricular (LV) function in rats with DCM. Therefore, we performed a meta-analysis of a wide range of high-quality clinical trials with 599 participants and found that stem cell therapy improves cardiac function and reduces mortality and heart transplantation in DCM patients, which indicate that stem cell therapy may become a new method for treating DCM patients. Further studies should concentrate on more specific details such as stem cell type, dosage, injection volume, and therapy duration.

The possible mechanism whereby stem cell therapy improves cardiac function in DCM patients has not been clarified. Lin et al<sup>20</sup> showed that myogenic-like cells in the LV myocardium that were differentiated from stem cells were not sufficient to sustain cardiac function, indicating that the mechanism underlying stem cell therapy is complex. Sun et al<sup>21</sup> used bone marrow-derived stem cells to treat DCM rats and found that the therapy significantly improved LV function by limiting cellular apoptosis. The primary mechanism of action for cell therapy is now believed to act through paracrine effects that include the release of cytokines, chemokines, and growth factors that inhibit apoptosis and fibrosis, enhance contractility, and activate endogenous regenerative mechanisms through endogenous circulating or site-specific stem cells.<sup>17</sup> However, the mechanism whereby cell therapy improves cardiac function in DCM patients requires further study.

**Study limitation.** The included studies utilized small samples, which may be a limitation. The heterogeneity was relatively high for LVEF ( $I^2=93%$ ), LVEDD ( $I^2=87%$ ), and 6-MWT distance ( $I^2=89%$ ). Even when we removed any one of the studies, the  $I^2$  test of heterogeneity remained high, possibly as a result of subject characteristics, such as age, gender, concomitant diseases and baseline characteristics, study design and treatment regimen features, such as stem cell type, average number of injected cells, injected volume, and follow-up durations. Future research efforts should concentrate on larger, randomized, and placebo-controlled trials.

In conclusion, stem cell therapy improves cardiac function and reduces mortality in DCM patients, which suggests that stem cell therapy may represent a new method for treating DCM patients.

## References

1. Kaur H, Khetarpal R, Aggarwal S. Dilated cardiomyopathy: an anaesthetic challenge. *J Clin Diagn Res* 2013; 7: 1174-1176.
2. Chan BT, Abu Osman NA, Lim E, Chee KH, Abdul Aziz YF, Abed AA, et al. Sensitivity analysis of left ventricle with dilated cardiomyopathy in fluid structure simulation. *PLoS One* 2013; 8: e67097.
3. Del Corso C, Campos de Carvalho AC. Cell therapy in dilated cardiomyopathy: from animal models to clinical trials. *Braz J Med Biol Res* 2011; 44: 388-393.
4. Suarez de Lezo J, Herrera C, Romero M, Pan M, Suarez de Lezo J Jr, Carmona MD, et al. Functional improvement in patients with dilated cardiomyopathy after the intracoronary infusion of autologous bone marrow mononuclear cells. *Rev Esp Cardiol* 2013; 66: 450-457.
5. Wang JA, Xie XJ, He H, Sun Y, Jiang J, Luo RH, et al. A prospective, randomized, controlled trial of autologous mesenchymal stem cells transplantation for dilated cardiomyopathy. *Zhonghua Xin Xue Guan Bing Za Zhi* 2006; 34: 107-110.
6. Xiao WT, Gao LJ, Gao CY, Gao YJ, Dai GY, Li MW, et al. [Comparative study on the efficacy of intracoronary infusion with various types of autologous bone marrow stem cells for patients with dilated cardiomyopathy]. *Zhonghua Xin Xue Guan Bing Za Zhi* 2012; 40: 575-578. Chinese
7. Huang RC, Yao K, Li YL, Zhang YQ, Xu SK, Shi HY, et al. [Transplantation of autologous bone marrow mononuclear cells on patients with idiopathic dilated cardiomyopathy: early results on effect and security]. *Zhonghua Xin Xue Guan Bing Za Zhi* 2006; 34: 111-113.
8. Vrtovec B, Poglajen G, Sever M, Lezaic L, Domanovic D, Cernelc P, et al. Effects of intracoronary stem cell transplantation in patients with dilated cardiomyopathy. *J Card Fail* 2011; 17: 272-281.
9. Vrtovec B, Poglajen G, Lezaic L, Sever M, Domanovic D, Cernelc P, et al. Effects of intracoronary CD34+ stem cell transplantation in nonischemic dilated cardiomyopathy patients: 5-year follow-up. *Circ Res* 2013; 112: 165-173.
10. Seth S, Bhargava B, Narang R, Ray R, Mohanty S, Gulati G, et al. The ABCD (Autologous Bone Marrow Cells in Dilated Cardiomyopathy) trial a long-term follow-up study. *J Am Coll Cardiol* 2010; 55: 1643-1634.
11. Martino HF, Oliveira PS, Souza FC, Costa PC, Assuncao ESE, Villela R, et al. A safety and feasibility study of cell therapy in dilated cardiomyopathy. *Braz J Med Biol Res* 2010; 43: 989-995.
12. Moher D, Pham B, Jones A, Cook DJ, Jadad AR, Moher M, et al. Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses? *Lancet* 1998; 352: 609-613.
13. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177-188.
14. Al-Gobari M, El Khatib C, Pillon F, Gueyffier F. Beta-blockers for the prevention of sudden cardiac death in heart failure patients: a meta-analysis of randomized controlled trials. *BMC Cardiovasc Disord* 2013; 13: 52.
15. Chen Y, Gao EM, Gao CY, Xu Y, Huang KJ, Niu ZM, et al. [Effects of intracoronary autologous bone marrow mononuclear cells transplantation in patients with dilated cardiomyopathy]. *Zhonghua Xin Xue Guan Bing Za Zhi* 2008; 36: 1087-1091. Chinese
16. Sanbe A. Dilated cardiomyopathy: a disease of the myocardium. *Biol Pharm Bull* 2013; 36: 18-22.
17. Michler RE. Stem cell therapy for heart failure. *Methodist Debaquey Cardiovasc J* 2013; 9: 187-194.
18. Yip HK, Chang LT, Wu CJ, Sheu JJ, Youssef AA, Pei SN, et al. Autologous bone marrow-derived mononuclear cell therapy prevents the damage of viable myocardium and improves rat heart function following acute anterior myocardial infarction. *Circ J* 2008; 72: 1336-1345.
19. Pogue B, Estrada AH, Sosa-Samper I, Maisenbacher HW, Lamb KE, Mincey BD, et al. Stem-cell therapy for dilated cardiomyopathy: a pilot study evaluating retrograde coronary venous delivery. *J Small Anim Pract* 2013; 54: 361-366.
20. Lin YC, Leu S, Sun CK, Yen CH, Kao YH, Chang LT, et al. Early combined treatment with sildenafil and adipose-derived mesenchymal stem cells preserves heart function in rat dilated cardiomyopathy. *J Transl Med* 2010; 8: 88.
21. Sun CK, Chang LT, Sheu JJ, Chiang CH, Lee FY, Wu CJ, et al. Bone marrow-derived mononuclear cell therapy alleviates left ventricular remodeling and improves heart function in rat-dilated cardiomyopathy. *Crit Care Med* 2009; 37: 1197-1205.

## Related Articles

Han ZC, Zhang HN, Wang YZ, Lv CY, Xu ZY. Effect of the human insulin-like growth factor 1 gene transfection to human umbilical cord blood mesenchymal stem cells. *Saudi Med J* 2014; 35: 435-441.

Alajez NM. Cancer stem cells. From characterization to therapeutic implications. *Saudi Med J* 2011; 32: 1229-1234.

Ma Y, Luo M. Research of glycosylated granulocyte colony-stimulating factor on mobilizing cell regeneration of bone marrow stem cells and repairing injured myocardium. *Saudi Med J* 2010; 31: 634-639.