Chapter 8

# Summary and Conclusions

## SUMMARY

In the general introduction **(Chapter 1)** of this thesis, an overview is provided of the current experience with cell therapy for the treatment of cardiac disease. First, the available cell types are reviewed along with the mechanisms through which these cells may improve myocardial perfusion and function. In addition, possible routes of cell delivery are discussed and compared. Finally, the results are described of experimental and clinical studies investigating the safety, feasibility and efficacy of cardiac cell therapy. After cell therapy had been introduced into the clinical setting, most studies were performed in patients with acute myocardial infarction. Since it was supposed that bone marrow cells improved myocardial function mainly by stimulation of angiogenesis, intramyocardial injection of autologous bone marrow-derived mononuclear cells emerged as a new therapeutic option for patients with chronic myocardial ischemia due to severe coronary artery disease. Initial clinical studies demonstrated the safety and feasibility of this approach, and suggested improvements in myocardial perfusion and left ventricular (LV) function after intramyocardial bone marrow cell injection. Therefore, in the current thesis, the efficacy of bone marrow cell injection was further investigated in a randomized, double-blinded clinical trial and a long-term follow-up study. In addition, the functional effects of bone marrow cell injection were explored using several imaging modalities such as myocardial innervation imaging, magnetic resonance imaging and echocardiographic speckle tracking strain analysis.

In **Chapter 2,** the results are described of a randomized, placebo controlled, double-blinded trial investigating intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia. This trial had been initiated after the findings of several of non-randomized clinical studies, one of which was performed in the Leiden University Medical Center, had indicated the safety and feasibility of intramyocardial bone marrow cell injection and had suggested beneficial effects on myocardial perfusion and function. Fifty patients with severe angina pectoris despite optimal medical therapy and stress-inducible myocardial ischemia were randomized to either bone marrow cell injection of placebo treatment. Primary end point was the summed stress score, a 17-segment score for stress myocardial perfusion assessed by Tc-99m tetrofosmin SPECT. Secondary endpoints included LV ejection fraction, Canadian Cardiovascular Society (CCS) class, and Seattle Angina Questionnaire quality-of-life score. After 3-months follow-up, the

summed stress score improved from 23.5±4.7 to 20.1±4.6, P<0.001) in the bone marrow cell group, compared with a decrease from 24.8±5.5 to 23.7±5.4, P=0.004) in the placebo group. However, when the 2 groups were compared, the improvement in summed stress score was significantly greater in bone marrow cell–treated patients as compared with placebo-treated patients (treatment effect, −2.44;95% CI, −3.58 to −1.30; *P*<0.001). In addition, the increase in LV ejection fraction was larger in the bone marrow cell group as compared to the placebo group 3±5% vs. -1±3%, P=0.03). Furthermore, improvements in CCS class and quality-of-life score were significantly greater in bone marrow cell–treated patients than in placebo-treated patients (*P*=.03 and *P*=.04, respectively). In conclusion, intramyocardial bone marrow cell injection was associated with improvements in myocardial perfusion compared with placebo in patients with chronic myocardial ischemia. In addition, improvements were observed in LV function, anginal complaints and quality-of-life.

After completion of the randomized trial, patients which were initially treated with placebo were offered to enter the cross-over phase of the trial to receive intramyocardial bone marrow cell injection. The results of this cross-over phase are described in **chapter 3**. Similar to the main study, the effect of bone marrow cell injection on myocardial perfusion and function was evaluated using SPECT and MRI at baseline and 3 months follow-up. Moreover, Canadian Cardiovascular Society (CCS) angina score and quality of life were evaluated at baseline, 3 and 6 months. Tc-99m tetrofosmin SPECT revealed an improvement in summed stress score from 24.7±4.5 to 21.9±3.9 (P<0.01) after bone marrow cell injection, whereas summed stress score improved from 24.6±4.8 to 23.8±5.0 (P=0.02) after placebo injection. When both treatments were compared, the improvement was significantly larger after bone marrow cell injection (P=0.03).
LV ejection fraction did not significantly change after both treatments. CCS angina score and quality of life improved significantly after BMC injection as compared to placebo treatment (P=0.01 and P=0.02, respectively). Thus, this study demonstrated improvements in myocardial perfusion and anginal symptoms after intramyocardial BMC injection as compared to placebo treatment in the same patients, thus confirming the results of the previous randomized trial in an intra-patient comparison.

Diastolic dysfunction is often present in patients with coronary artery disease and is an important prognostic factor. Therefore, in **chapter 4**, we evaluated the effect of

intramyocardial bone marrow bone marrow cell injection on diastolic function in patients with chronic myocardial ischemia. In a substudy of the randomized trial, diastolic function was evaluated before and 3 months after bone marrow cell injection using standard echocardiography, strain analysis and MRI-derived transmitral flow measurements in 50 patients with chronic myocardial ischemia.
In bone marrow cell-treated patients, filling pressure estimate E/E' ratio improved from 14±5 at baseline to 12±4 at 3 months in the bone marrow cell group, which was significantly different form placebo-treated patients, in which no improvement was observed (13±4 vs. 13±5, P=0.008 between groups). Furthermore, the E/A peak flow ratio as assessed by MRI showed a significant increase in the bone marrow cell group as compared to the placebo group (+0.16±0.25 vs. -0.04±0.21, P=0.01), which was mainly related to an improvement in the early (E) peak flow rate in the bone marrow cell group (from 407±96 mL/sec to 468±110 mL/sec, P=0.009 as compared to placebo group). Thus, the results of this study show that intramyocardial bone marrow cell injection is associated with beneficial effects on myocardial relaxation and filling pressures in patients with chronic myocardial ischemia. It may be hypothesized that these improvements in diastolic function are secondary to enhanced myocardial perfusion,

In **chapter 5**, the effect of intramyocardial bone marrow cell injection on myocardial sympathetic nerve function was evaluated in patients with chronic myocardial ischemia. Experimental studies have suggested that bone marrow cell transplantation may improve nerve function through increasing neural blood flow. Since myocardial sympathetic nerve function has been established as a prognostic factor, we chose to evaluate the changes in sympathetic nerve function in a substudy of the randomized trial. In this study, 16 patients (64±8 years, 13 men) underwent early and late iodine-123 metaiodobenzylguanidine (MIBG) imaging before treatment and at 3 months follow-up, additionally to the study protocol. No improvements were observed in the global parameters early H/M ratio (P=0.40), late H/M ratio (P=0.43), and cardiac washout rate (P=0.98). However, regional myocardial sympathetic nerve function, as assessed by late 123-I MIBG SPECT defect score, showed a trend to improvement in the bone marrow cell group (from 31.0±7.1 at baseline to 28.1±14.9 after 3 months follow-up) as compared to the placebo group (from 33.6±8.5 to 34.5±9.8, P=0.055 between groups). This trend was predominantly determined by a substantial improvement (≥5 points) in 3 bone marrow cell-treated patients. Furthermore, these improvements were not related to myocardial

perfusion or cell injection sites, but it must be noted that these 3 patients all had diabetes and a high 123-I MIBG/perfusion mismatch at baseline. Thus, the findings of this study suggest that intramyocardial bone marrow cell injection may have a therapeutic effect on diabetic cardiac autonomic neuropathy

To further investigate the effects of intramyocardial bone marrow cell injection, we evaluated the effect of bone marrow cell injection on global strain and LV dyssynchrony in patients with post-infarction heart failure **in chapter 6**. In 15 patients, 10.1±1.1 intramyocardial bone marrow cell injections, targeted to in the infarction border zone, were performed. At baseline and at 3 months, LV ejection fraction (Tc-99m tetrofosmin gated SPECT), echocardiographic global strain (automated function imaging) and LV dyssynchrony (speckle tracking analysis) were assessed. After 3 months, LV ejection fraction had increased from 23±8% at baseline to 27±9% at 3 months follow-up (P=0.02). In patients with a substantial improvement in LV ejection fraction(>5%), LV dyssynchrony decreased from 173±64 ms to 116±64 ms (P=0.01). In patients without a substantial improvement in LV ejection fraction, LV dyssynchrony remained unchanged (155±67 ms vs. 177±81 ms; P=NS). There was an good correlation between the improvement in LV ejection fraction and the reduction in LV dyssynchrony (r=-0.77; P<0.01). In parallel, global strain improved from -8.7±4.6% to -10.6±4.5% (P<0.01) patients with a substantial improvement (>5%) in LV ejection fraction, where as no improvement was observed in patient without a significant improvement in LV ejection fraction. (-6.6±4.9% vs. -6.4±4.5%; P=NS). There was a significant relation between the increase in LV ejection fraction and the improvement in global strain (r=0.84; P<0.01). In conclusion, bone marrow cell injection improves LV systolic function in patients with severe post-infarction heart failure. The improvement in LV ejection fraction was related to a reduction in LV dyssynchrony and an increase in global strain.

The results of a long term follow-up study are presented in **chapter 7**. In this study, we evaluated the long-term safety and clinical benefit of intramyocardial bone marrow cell injection in 25 patients with chronic myocardial ischemia. Quality of life and clinical status according to the Canadian Cardiovascular Society (CCS) classification were assessed at baseline and at 3-, 6-, and 12 months follow-up, and after 4 years. In addition, data of clinical events were obtained by interviews and review of hospital records.

At 4 years follow-up, mortality was 12% (3/25 patients). No ventricular arrhythmias or sudden cardiac death were documented. Two patients died of a non-cardiac cause and the third patient died because of progressive heart failure at 3.7 years follow-up for which this patient was hospitalized 3 times previously. Eight other hospital admissions occurred for acute coronary syndrome. One percutaneous coronary revascularization procedure was performed and one patient received a biventricular implantable cardioverter-defibrillator. After an initial improvement after 3, 6, and 12 months follow-up, CCS class and quality of life were still significantly improved at 4 year follow-up (P=0.045 and P=0.030, respectively). At an individual level, 7 of 22 patients had an improved CCS class at 4 year follow-up as compared to baseline, accompanied by an increase in quality of life of 15±8% at 4 year follow-up, whereas the other 15 patients had similar (n=13) or worsened (n=2) CCS class, corresponding with a 2±7% increase in quality of life. Thus, after clinical improvement in the first 12 months of follow-up, 2 out of 3 patients had worsening complaints at long term follow-up. Therefore, it may be considered to perform a repeat bone marrow cell injection in selected patients, aiming to stimulate neovascularization and improve myocardial perfusion.

## CONCLUSIONS

- Although the mechanisms by which bone marrow cells may improve myocardial perfusion are not completely understood, experimental studies have suggested that bone marrow cells improve myocardial perfusion and function through differentiation in vascular and cardiac cell types, and secretion of paracrine factors which promote angiogenesis, exert cytoprotective effects, recruit resident cardiac stem cells or alter mechanical properties of myocardial (scar) tissue.

- The findings of a randomized, placebo controlled, double-blinded trial indicate that intramyocardial injection of autologous bone marrow-derived mononuclear cells is associated with beneficial effects on anginal complaints, myocardial perfusion, and LV systolic function.

- Intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia results in enhanced LV diastolic function, as evidenced by improvements in parameters of myocardial relaxation and filling pressures. It may be hypothesized that these improvements are secondary to enhanced myocardial perfusion.

- In patients with chronic myocardial ischemia, intramyocardial bone marrow cell injection is not associated with changes in global parameters of myocardial sympathetic nerve function. However, a substantial improvement in regional myocardial sympathetic nerve function in 3 patients with diabetes and a high innervation/perfusion mismatch suggests a therapeutic effect of bone marrow cell injection on diabetic cardiac autonomic neuropathy.

- In patients with severe post-infarction heart failure, intramyocardial bone marrow cell injection into the border zone of the infarcted myocardium improves LV ejection fraction. This improvement in LV ejection fraction was related to a reduction in LV dyssynchrony and an increase in global strain.

- During long term follow-up, an important number of patients develops an increase in anginal complaints, which is probably related to progression of atherosclerosis. Therefore, a repeat bone marrow cell injection may be considered in selected patients, aiming to stimulate new vessel formation resulting in enhanced myocardial perfusion.

## FUTURE PERSPECTIVES

Intramyocardial bone marrow cell injection has emerged as a promising new treatment strategy for the treatment of patients with chronic myocardial ischemia. Nonrandomized clinical studies have demonstrated the safety and feasibility of this approach, and suggested a beneficial effect on clinical and functional parameters. To date, 3 small or medium-sized randomized trials have confirmed these findings by demonstrating significant improvements in clinical parameters, although evaluation of myocardial perfusion and LV systolic function in these studies yielded variable results. In parallel, 2 randomized studies investigating bone marrow cell injection in patients with ischemic heart failure yielded discordant results, with 1 study demonstrating improvements in myocardial perfusion, LV function and clinical parameters, whereas the multicenter FOCUS-trial only observed improvements in LV systolic function without improvements in myocardial perfusion or clinical parameters. These differences may be explained by a variety of factors, such as study design, use of imaging modalities, and differences between cell type, cell number and cell isolation protocols. Therefore, as pointed out below, a number of fundamental and practical issues have to be addressed in future studies.

**Fundamental questions to be answered**

Although cardiac cell therapy has been introduced into the clinical setting for over 10 years, some fundamental questions still remain unanswered. First, the mechanisms by which bone marrow cells can improve myocardial perfusion and LV function are not fully understood. Second, cell dose, cell type and cell isolation protocols have been pragmatically chosen until now, since limited comparative data are available. Third, studies comparing delivery routes yielded contradicting results, leaving the optimal delivery route to be addressed.

Thus, future studies should further unravel the precise mechanisms by which bone marrow cells may improve myocardial perfusion and function. Furthermore, comparative studies are necessary to establish the most advantageous cell type, cell dose, and isolation protocol for each patient group. Finally, more comparative studies will be necessary to determine the optimal delivery method for each situation, taking into account factors such as anatomical location of target site, the presence of homing signals and the used cell type.

**Potential Directions for Future Research in Patients with Chronic Ischemic Heart Disease**

In the future, the effect of bone marrow cell injection should be further evaluated in larger phase III trials. In addition to the optimization of cell type, dose, and isolation protocols, the effectiveness of bone marrow cell injection should be further explored. For example, future studies will have to investigate whether the observed improvements are sustained over longer periods of follow-up. In addition, predictors of response may be identified, since these may provide directions for future experimental research and contribute to effective patient selection. Finally, future studies will have to assess whether the observed improvements in anginal complaints and myocardial perfusion result in improved survival and/or a reduced number of hospitalizations, because such data are crucial to determine the cost-effectiveness of this novel treatment modality in its current form.

**Innovative Strategies for Cardiac Cell Therapy**

Further innovations which may possibly enhance the efficacy of cell therapy are under investigation. Beside identification of promising cell types strategies have been developed to augment cell engraftment and function, such as ischemic preconditioning or pharmacologic stimulation. Furthermore, the combination of gene therapy and cell therapy holds great potential. Transduction of genes into therapeutic cells may improve survival, retention and angiogenic function. On the other hand, stem cells may be used as vectors for local delivery of drugs or cytokines. Finally, the use of biomaterials is a promising technique to improve cell retention and survival, which may augment the efficacy of delivery methods. These innovations may contribute to the development of effective cell-based treatment strategies for a variety of diseases, extending the spectrum of therapeutic options for patients who have exhausted conventional therapies.