ST-segment elevation myocardial infarction (STEMI) is still high in Western countries and Japan [1, 2]. Furthermore, in the chronic stage after MI, heart failure can develop due to left ventricular (LV) remodeling [3]. To date, most clinically tested agents that induce cardioprotection have failed to reduce infarct size in clinical settings [4]. Thus, novel pharmaceutical interventions to improve the clinical outcomes of patients with STEMI are urgently needed. Animal studies show that the intravenous administration of erythropoietin (EPO), a glycoprotein hormone consisting of 165 amino acid residues [5], at the onset of reperfusion reduces the myocardial infarct size and prevents cardiac remodeling, with enhanced neovascularization in the heart after MI [6, 7]. Several proof-of-concept studies have been performed to clarify the cardioprotective effects of EPO in patients with STEMI. The administration of high-dose EPO (60,000–99,000 IU) did not improve left ventricular ejection fraction (LVEF) or reduce infarct size [8–10]. Regarding secondary endpoints, the use of EPO has been associated with a trend toward an increase in major adverse cardiovascular events in 2 studies [8, 10] and significantly fewer events in a third study [9]. In contrast, low-dose EPO is likely to be cardioprotective, according to small clinical trials [11–13]. Platelet activation by high-dose EPO [14] and the existence of an optimal dose for limiting infarct size [15] may explain the dose-dependent discrepancy of EPO-induced cardioprotection. Importantly, pilot studies showed that low-dose EPO is associated with improved left ventricular function without major adverse cardiovascular events [11, 12]. Furthermore, our post-hoc analysis revealed that EPO administration was highly associated with improved LV function in STEMI patients with a low LV ejection fraction (LVEF) (<50 %) (Fig. 1). Therefore, we have started a double-blind, placebo-controlled, randomized, multicenter clinical trial (EPO-AMI-II) to clarify the safety and efficacy of low-dose EPO in STEMI patients with a low LVEF (<50 %). The protocol was submitted to the Evaluation System of Investigational Medical Care of the Ministry of Health, Labour and Welfare of Japan and was approved under the Japanese governmental health insurance system on 1 August 2011.

## Methods

### Study objects

The objectives of this study are to evaluate whether a single bolus administration of EPO prevents ischemia-reperfusion injury dose-dependently and to estimate the optimum clinical dose of EPO in patients with STEMI after successful PCI by analyzing the improvement in LVEF between the acute and chronic stages.

### Study design

EPO-AMI-II is an ongoing multicenter, prospective, randomized, double-blind, placebo-controlled, dose-finding study in patients presenting with a first STEMI. After a successful PCI, patients will be randomly assigned to receive either an intravenous bolus dose of epoetin-beta (EPO) (6,000 or 12,000 IU) or placebo on top of standard medical care (Fig. 2). This trial was registered at the UMIN Clinical Trials Registry as UMIN000005721.

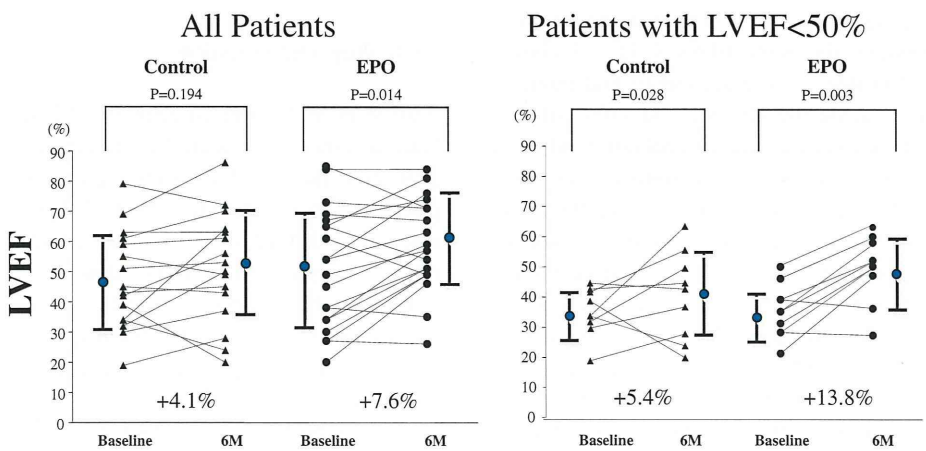


**Fig. 1** Post-hoc analysis of the EPO-AMI-I results. Panel **a** shows the LVEF between the acute and chronic stages in all patients in the EPO-AMI-I study. EPO, but not saline, administration significantly increased LVEF at 6 months after an MI. Panel **b** shows the LVEF between the acute and chronic stages in patients with LVEF <50 % in the EPO-AMI-I study. Both saline and EPO significantly increased LVEF at 6 months after an MI. The improvement of LVEF did not significantly differ between the saline- and EPO-treated groups. See the abbreviation definitions in the text

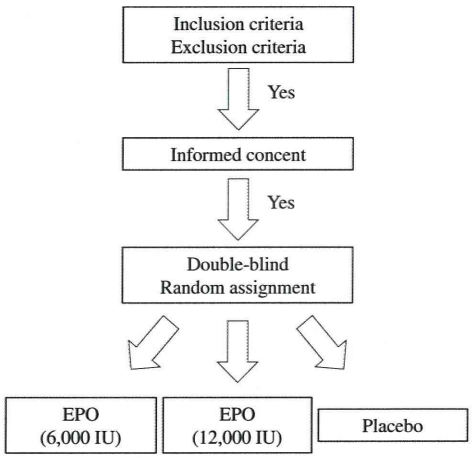


**Fig. 2** Study flow chart

## Patients

Consecutive patients with diagnostic signs and symptoms of an acute MI who satisfy the study inclusion and exclusion criteria (Table 1). After successful PCI, patients will be asked for written informed consent, and if they agree, will be assigned according to a pre-defined central web-based randomization system to receive EPO or placebo on top of optimal standard medical care. Patients will receive the study drug within 6 h after PCI. The patient, the attending physician, and the staff performing SPECT and the clinical follow-up will be unaware of the assigned treatment.

## End points

The primary end point of this study is to evaluate the LVEF improvement between the acute (days 4–7) and chronic stages (6 months) (Table 2). The secondary end points of this study are to evaluate the efficacy and safety of EPO treatment. The efficacy is evaluated by analyzing indices of cardiac function 6 months after EPO administration. These are calculated with electrocardiogram-gated single-photon emission computed tomography (SPECT) and include LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESD), LVEDV index, LVESV index, regional wall motion score, % uptake at resting, and defect size. The survival ratio, cardiovascular events (defined as cardiac death, stroke, nonlethal myocardial infarction, admission due to worsening of heart failure or unstable angina, revascularization, and onset of heart failure symptoms), and NT-ProBNP at the 6-month follow-up will also be analyzed to evaluate the efficacy of EPO treatment (Table 2). The safety is based on the incidence of major adverse events, clinical laboratory test data and vital signs.

**Table 1** Inclusion and exclusion criteria

| |
|---|
| Inclusion criteria |
| 1. Patients with first-time myocardial infarction |
| 2. Patients with ST-elevation acute myocardial infarction (AMI) who have successful reperfusion by PCI within 12 h after the symptom onset |
| 3. Patients whose ejection fraction at enrollment is <50 % on UCG or LVG |
| 4. Age: over 20 years old, under 80 years old |
| 5. Patients who agreed with participation to the trial in writing |
| Exclusion critetia |
| 1. Patients with significant stenotic lesions in non infarct-related artery which require revascularization |
| 2. Patients who resulted in obviously impaired reperfusion |
| 3. Patients with Killip class III or IV, or cardiogenic shock at admission |
| 4. Patients with advanced renal or hepatic dysfunction (Cre more than 2 mg/dl, or T-Bil more than 3 mg/dl) |
| 5. Patients with blood pressure more than 140/90 mmHg after PCI |
| 6. Hematocrit more than 54 % on admission |
| 7. Patients who exhibit atrial fibrillation after PCI |
| 8. Patients who have been diagnosed with malignant hypertension |
| 9. Patients who have previously received treatment with EPO |
| 10. Patients who received a blood transfusion in the last 3 months |
| 11. Patients who are or have been diagnosed with cancer in the past 5 years |
| 12. Patients who are complicated with severe infection such as pneumonia or sepsis |
| 13. Patients who are contraindicated to aspirin or thienopyridine derivatives |
| 14. Women who are pregnant, breastfeeding, or have a possibility for pregnancy |
| 15. Patients whom researchers judged that they are not appropriate to participate this trial |

## Study drug administration

Prior to or at the time of primary PCI, standard antithrombotic treatments for acute MI are administered. Within 6 h after PCI, the enrolled patients are randomly assigned to placebo or an Epo dose (6,000 or 12,000 IU). Active drug or placebo is diluted in 10 mL of saline and administered intravenously over 1 min. The double-blind administration is ensured by a subject identification code unknown to physicians, nurses and patients. Drug or placebo is prepared under medical supervision according to instructions contained in predefined packages provided by the EPO-AMI-II organization. Standard treatment, including beta-blockade, lipid-lowering therapy, and angiotensin-converting enzyme inhibition or angiotensin-II receptor blockade, is additionally prescribed. EPO and placebo are kind gifts of Chugai Pharmaceutical Co. Ltd (Tokyo, Japan).

**Table 2** Primary and secondary end points

| |
|---|
| Primary end point |
| The improvement of left ventricular ejection fraction at the chronic phase (the mean of differences between LVEF value at 4–7 days and that at 6 months after administration) |
| Secondary end point |
| [Efficacy] |
| 1. Indexes of cardiac function 6 months after administration of epoetin-beta, which are calculated with cardiac scintigraphy (LVEDV, LVESV, LVEDVI, LVESVI, regional wall motion score, ischemia and defect size (SRS (Summed rest Score), SDS (Summed difference Score), %Defect Size, %uptake at resting)) |
| 2. Survival ratio |
| 3. Cardiac event ratio (Cardiac death, stroke, nonlethal myocardial infarction, admission due to worsening of heart failure or unstable angina, revasculization, onset of heart failure symptoms (typical dyspnea at rest or during exercise, pulmonary congestion or pretibial edema) |
| 4. NT-ProBNP 6 months after administration |
| [safety] |
| 1. Adverse events |
| 2. Laboratory test data |
| 3. Vital signs (blood pressure, pulse rate) |

## Clinical and laboratory measures

Blood pressure, heart rate, and ECG are monitored at regular intervals until discharge (Fig. 3). Major adverse events (as defined above) are recorded during hospitalization and up to 6 months thereafter. At 4–7 days after admission and at 6 months, cardiac SPECT is also performed to evaluate cardiac function.

## Quantification of LV function and infarct size

We will perform ECG-gated 99mTc-MIBI SPECT 4–7 days after PCI as the baseline measurement and at the 6-month follow-up. The 99mTc-MIBI (600–740 MBq) is administered at baseline and at the 6-month follow-up. SPECT image acquisition is performed 60 min after the 99mTc-MIBI injection. ECG-gated SPECT is performed after the administration of 99mTc-MIBI at rest. In ECG gating, SPECT data divided into 16 equal intervals are analyzed using Quantitative Gated SPECT software (Cedars-Sinai Medical Center, Los Angeles, CA, USA), which is also used to calculate EDVI, ESVI and LVEF. Pharmacologic stress tests are performed with non-gated 99mTc-MIBI SPECT. Adenosine (Adenoscan; DAIICHI SANKYO, Tokyo, Japan) is administered at a rate of 0.72 mg/kg for 6 min. The 99mTc-MIBI is injected 3 min after the start of adenosine infusion. The non-gated SPECT image is used to assess the severity of myocardial perfusion abnormalities, and regional uptake and the infarct area are calculated using Quantitative Perfusion SPECT software (Cedars-Sinai Medical Center). Regional uptake is assessed by applying a 17-segment model of the left ventricle according to the standard myocardial segmentation of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Regional uptake is expressed as the mean uptake count in these segments. Defects at less than the threshold of 60 % of peak counts are identified as infarcted myocardium, and the infarct area is expressed as a percentage of the entire left ventricle involved. SPECT data will be analyzed in a blinded fashion by the SPECT Core Center members with the assistant of nuclear medicine special radiological technologist at MICRON Co., Ltd (Molecular Imaging CRO Network, Tokyo, Japan.). Finally, the analyzed data will be evaluated by an independent RI assessment committee.

## Adverse events and additional safety assessments

An independent data safety monitoring board (DSMB) will receive real-time clinical information and will perform interim safety and efficacy analyses at 33 %, 66 % and 100 % recruitment. There are no formal (statistical) rules for stopping treatment due to safety reasons in this study. The DSMB recommendations are based on a clinical assessment of the frequency, and the nature of the serious adverse events and their relation to the investigational treatment.

## Sample size calculation

Based on the results of our pilot study in STEMI patients with LVEF <50 % (LVEF improvement in the EPO-II group: 13.80±9.85 %, $n$=11, and in the placebo group: 5.44±14.80 %, $n$=9) (Fig. 1), the difference in LVEF improvement between the EPO (12,000 IU) treatment group and the placebo group is estimated to be 4.42 % with a common standard deviation of 14.33 %. As a result, the effect size is estimated to be 0.31 [16]. To demonstrate the

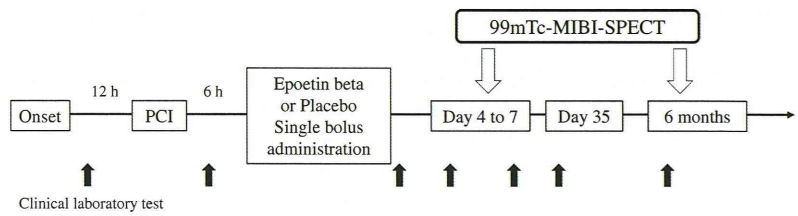


**Fig. 3** Study schedule

treatment difference with a power of 0.85 and a 1-sided alpha of 0.025, 190 patients per group will be needed. However, because we plan to perform two interim analyses, we will need 193 patients per group [17]. Taking into account several patients dropping out, the total sample size to be recruited will be 200 patients in each treatment group, i.e., 600 patients will be recruited in this study.

### Interim analysis

There will be two formal interim analyses on the safety and efficacy of the primary end point: after 198 and 396 randomized patients are enrolled and followed up for 6 months. For the interim analyses on efficacy, the DSMB will evaluate the primary end point using the Lan-DeMets method with the O'Brien-Fleming spending function. Asymmetric stopping boundaries are planned, with early termination of the study recommended in the event of evidence of overwhelming benefit (2-sided $P<.001$ favoring EPO) or substantive harm (2-sided $P<.01$ against EPO) once sufficient events have accrued.

### Statistical analysis

Data will be analyzed based on an intention-to-treat principle. The efficacy end point is LVEF improvement. The null hypothesis, that all treatment groups will have the same mean LVEF improvement, will be tested against the alternative hypothesis, that the mean LVEF improvement in the treatment groups will increase in the order of placebo, EPO (6,000 IU) and EPO (12,000 IU), according to the contrast test with a contrast coefficient (−1, 0, 1) based on the t-statistic. The contrast test will be evaluated based on a 1-sided significance level of 0.025. The secondary efficacy end point of OS in each group will be analyzed by the Kaplan-Meier method and compared using the log-rank test. Cardiovascular events and NT-ProBNP at the 6-months follow-up will be analyzed by a nonparametric test (e.g., Wilcoxon rank sum test). Safety analyses will be performed to summarize the adverse events in each treatment group. The baseline characteristics of the study patients will be summarized using frequencies and percentages for categorical variables and using means with standard deviations for continuous variables.

### Current status

EPO-AMI-II began enrolling patients in December 2011. As of May 15, 2012, the application for the Evaluation System of Investigational Medical Care is ongoing, and 14 of 24 eligible centers have been approved. Completion of study enrollment is targeted for September 30, 2014. Allowing for the 6-month follow-up of the final randomized patient, trial completion is anticipated by March 2015.

## Discussion

We have started the EPO-AMI-II study to clarify the safety and efficacy of low-dose EPO in the improvement of LVEF in STEMI patients with a low LVEF (<50 %). EPO-AMI-II is a multicenter, prospective, randomized, double-blind, placebo-controlled, dose-finding study in patients with their first STEMI.

### Randomized clinical studies to clarify the effects of low-dose EPO in patients with STEMI

Therapies that can reduce myocardial damage and augment neovascularization in the heart after an MI may be beneficial in patients with STEMI. Experimental studies demonstrate that the intravenous administration of EPO at the onset of reperfusion reduces myocardial infarct size and prevents cardiac reverse remodeling, with enhanced neovascularization in the heart after an MI [6, 7]. Recently, proof-of-concept studies using high-dose EPO have reported inconsistent cardioprotection results from EPO in patients with STEMI (Table 3). The use of high-dose EPO at the time of reperfusion for an acute MI to salvage the myocardium or to improve LV function will not be further pursued in any newly initiated study.

In contrast, low-dose EPO is likely to be cardioprotective in small clinical trials [11–13]. Potential mechanisms to explain the dose-dependent discrepancy of EPO in cardioprotection may be attributable to platelet activation and the existence of an optimal dose for limiting infarct size. Platelet activation by a high dose of EPO [14] and the existence of an optimal dose for limiting infarct size [15] may explain the dose-dependent discrepancy of EPO-induced cardioprotection. Because EPO has structural similarity with thrombopoietin, high-dose EPO increases platelet production and reactivity, which leads to an increased risk of thrombosis and cardiovascular events. Additionally, a dose response curve of the bioactivity of cytokines does not necessarily appear to be guided by a sigmoid function. Positive intracellular signal of cytokine receptors via serial chain reaction of protein tyrosine kinases is typically interfered by automated circuit reaction of protein tyrosine phosphatase such as SHP1 to avoid overdoing of growth and inflammation [18]. In fact, administration of high-dose EPO lost its cardioprotective activity in rat and mouse coronary ischemia/reperfusion models [15, 19]. The rationale for EPO treatment for

**Table 3** Overview of randomized controlled studies investigating the effects of EPO in patients with acute myocardial infarction

| Trial | Dose of EPO | Primary outcome | Result | Cardiovascular event |
|---|---|---|---|---|
| REVIVAL-3 | 33,333 IU×3 (0, 24, 48 h) | LV EF | No change | Increase (not significant) |
| HEBE-III | 60,000 IU | Infarction size | No change | Decrease (significant) |
| REVEAL | 60,000 IU | Infarction size | No change | Increase (not significant) |
| EPOC-AMI | 6,000 IU×3 (day 0, 2, 4) | LV EF | Improve | No change |
| EPO-AMI-I | 12,000 IU | LV EF | Improve | No change |
| EPO-AMI-II | 6,000 or 12,000 IU | LV EF | | |

patients with STEMI lies in the low-dose EPO trials, although these have only been small clinical trials to date.

Protocol of EPO-AMI-II study

On the basis of a post-hoc analysis of our pilot study (EPO-AMI-I) and a recent proposal from workshops [20–22], we have modified the protocol for the EPO-AMI-II study. First, we created new inclusion criteria to include patients with an LVEF <50 %. Only patients who have large myocardial infarcts can receive benefits from any adjunctive therapy [23, 24]. Consistently, the post-hoc analysis of the EPO-AMI-I study revealed that STEMI patients with an LVEF <50 % received large benefits from EPO administration11 (Fig. 1). When patients with significant stenotic lesions in non-infarct-related arteries that required revascularization were excluded, more than 90 % of STEMI patients who met the inclusion and exclusion criteria presented with a proximal left anterior descending artery in the EPO-AMI-I study. This type of STEMI patient will receive more benefit from adjunctive therapy [23, 24]. Second, we have shortened the therapeutic time window from the onset of chest pain to reperfusion time (from 24 h to 12 h), which will also result in a shorter time window between EPO administration and the onset of chest pain. For example, in rats with a permanent coronary occlusion, EPO does not effectively reduce myocardial infarct size when administered ≥24 h after the MI [25]. These protocol modifications of the EPO-AMI-I study will improve the efficacy and safety of low-dose EPO in patients with STEMI.

Safety of EPO in STEMI patients

In the EPO-AMI-I (12,000 IU) and EPOC studies (6,000 IU × 3) in which low-dose EPO was administered, the risk of cardiovascular events was not increased [11, 12]. When high-dose EPO was administered, the results were inconsistent. In the REVEAL study, the subanalysis showed that EPO (60,000 IU) had a higher incidence of serious adverse events, although the authors noted that the analysis was based on a very small number of events. Conversely, in the HEBEIII study, the subanalysis revealed that EPO showed a trend toward a reduction of enzymatic infarct size and significantly reduced the incidence of the combined endpoint (cardiovascular death, myocardial infarction, in-stent thrombosis, unstable angina and heart failure). In the REVIVAL study, EPO (33,000 IU × 3) showed a trend toward an increased rate of serious adverse effects. Their meta-analysis showed that the administration of EPO appeared to be safe for patients with acute STEMI [26]. For the safety of patients in the EPO-AMI-II study, a report system for serious adverse events has been established, and the clinical research coordinator will often visit the hospitals that participate in this study. Recently, the post-hoc analysis suggested the association of high-dose EPO with the restenosis of the culprit lesion, although no significant differences in late lumen loss between the EPO and placebo groups were observed [27, 28]. Additionally, no significant difference in late lumen loss was found when low-dose EPO was used [11, 12].

Quantification of LV function and infarct size

In the EPO-AMI-II study, we are only including patients with a first STEMI because ECG-gated SPECT allows for no distinction between previous and new infarcts. The primary end point of this study is to evaluate the improvement of LVEF between the acute and chronic stages (Table 2). In the chronic stage, ECG-gated SPECT with adenosine allows for the evaluation of the residual myocardial ischemia. One alternative evaluation method is cardiac magnetic resonance imaging, which may be able to assess the at-risk area and the final infarct size, but this technique remains to be validated for quantification [29].

## Conclusions

Because the randomized control trials conducted to date have used high-dose EPO and demonstrated heterogeneous results, the EPO-AMI-II study will clarify the safety and efficacy of low-dose EPO in STEMI patients with LV dysfunction in double-blind, placebo-controlled, multicenter studies (Appendix).

**Acknowledgments** We sincerely thank Drs. Akira Myoi and Yasutaka Hayashida (Medical Center for Translational Research Osaka University Hospital) and Dr. Yoichi Yamamoto (Center for Clinical Investigation and Research, Osaka University Hospital) for advice on conducting the EPO-AMI-II study. We also thank Dr. Hiroyuki Uesaka for statistical aspects of the study design. We thank Hiromi Umezome, Sakiko Ichijo (Center for Clinical Investigation and Research, Osaka University Hospital) for their assistants and Sugako Mitsuoka for her excellent secretarial work. The management of this study is supported by Grants-in-Aid from the Ministry of Health, Labour and Welfare of Japan, a Japanese Circulation Society Grant for Translational Research 2010 and the Support and Training Program for Translational Research at Osaka University.

**Disclosures** None.

## Appendix

**Principle Investigators:**

Aizawa Y, Komuro I.

**Steering Committee:**

Higo S, Ito H, Kato K, Kobayashi N, Ozawa T, Minamino T, Suzuki H. Tobaru T, Toba K.

**Data Management:**

Higo S, Takahara S (Medical Center for Translational Research Osaka University Hospital, Suita, Japan)

**Statistical Analysis:**

Yamamoto K, Hamasaki T (Department of Biomedical Statistics, Osaka University Graduate School of Medicine, Suita, Japan)

**SPECT Core Center:**

Ishida Y (Kansai Rosai Hospital Cardiovascular Center, Amagasaki, Japan), Sakata Y, (Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan), Yoshimura N (Department of Radiology, Niigata University Medical and Dental Hospital, Niigata, Japan)

**Data and Safety Monitoring Board:**

Fujio Y (Laboratory of Clinical Science and Biomedicine, Graduate School of Pharmaceutical Sciences, Osaka University, Japan), Hasegawa S (Department of Cardiology, Osaka Kosei Nenkin Hospital, Osaka, Japan), Seki Y (Department of Internal Medicine (Hematology), Niigata Prefectural Shibata Hospital, Japan). Sugimoto T (Department of Mathematical Sciences, Hirosaki University Graduate School of Science and Technology, Japan)

**EPO-AMI-II Investigators:**

Chiba-Nishi General Hospital, Chiba, Japan.
Yoshida T, Kuramochi M
Dokkyo University School of Medicine, Tochigi, Japan;
Ishimitsu T, Kobayashi N
Fujisawa City Hospital, Fujisawa, Japan
Yano H, Himeno H
Higashi-Osaka City General Hospital, Osaka, Japan
Kijima Y, Ichikawa M
Kansai Rosai Hospital, Amagasaki, Japan;
Uematsu M, Watanabe T
Kanto Rosai Hospital, Kawasaki, Japan
Namiki A, Shibata M
Kokura Memorial Hospital, Kitakyushu, Japan
Yokoi H, Nomura A
National Cerebral and Cardiovascular Center, Suita, Japan
Yasuda S, Kotani J
Niigata University Medical and Dental Hospital, Niigata, Japan;
Toba K, Ozawa T
Nippon Medical School, Tokyo, Japan
Mizuno K, Yasutake M
Nozaki Tokushukai Hospital, Osaka, Japan
Okutsu M, Sumitsuji S
Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan;
Ito H, Kusano K
Osaka General Medical Center, Osaka, Japan;
Fukunami M, Yamada T
Osaka National Hospital, Osaka, Japan;
Yasumura Y, Hirooka K
Osaka Police Hospital, Osaka, Japan;
Ueda Y, Hirata A
Osaka Rosai Hospital, Sakai, Osaka, Japan;
Nishino M, Makino N
Osaka University Graduate School of Medicine, Suita, Japan;
Komuro I, Minamino T
Sakakibara Heart Institute, Fuchu, Japan
Tobaru T, Asano R
Senri Critical Care Medical Center, Osaka Saiseikai Senri Hospital, Suita, Japan
Ito N, Doi, Y
Shonan Kamakura General Hospital, Kamakura, Japan
Saito S, Tobita K
Showa University Fujigaoka Hospital, Yokohama, Japan;
Suzuki H, Wakabayashi K
Showa University School of Medicine, Tokyo, Japan
Kobayashi Y, Koba S
St. Marianna University School of Medicine, Kawasaki, Japan
Yoneyama Y, Akashi Y
Tachikawa General Hospital, Nagaoka, Niigata, Japan;
Aizawa Y, Sato M

## References

1. Lloyd-Jones D, Adams RJ, Brown TM, et al. Executive summary: heart disease and stroke statistics–2010 update: a report from the American Heart Association. Circulation. 2010;121:948–54.

2. Takii T, Yasuda S, Takahashi J, et al. Trends in acute myocardial infarction incidence and mortality over 30 years in Japan: report from the MIYAGI-AMI Registry Study. Circ J. 2010;74:93–100.
3. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation. 2000;101:2981–8.
4. Minamino T. Cardioprotection from ischemia/reperfusion injury: basic and translational research. Circ J. 2012;76:1074–82.
5. Jelkmann W. Erythropoietin: structure, control of production, and function. Physiol Rev. 1992;72:449–89.
6. Hirata A, Minamino T, Asanuma H, et al. Erythropoietin just before reperfusion reduces both lethal arrhythmias and infarct size via the phosphatidylinositol-3 kinase-dependent pathway in canine hearts. Cardiovasc Drugs Ther. 2005;19:33–40.
7. Hirata A, Minamino T, Asanuma H, et al. Erythropoietin enhances neovascularization of ischemic myocardium and improves left ventricular dysfunction after myocardial infarction in dogs. J Am Coll Cardiol. 2006;48:176–84.
8. Ott I, Schulz S, Mehilli J, et al. Erythropoietin in patients with acute ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention: a randomized, double-blind trial. Circ Cardiovasc Interv. 2010;3:408–13.
9. Voors AA, Belonje AM, Zijlstra F, et al. A single dose of erythropoietin in ST-elevation myocardial infarction. Eur Heart J. 2010;31:2593–600.
10. Najjar SS, Rao SV, Melloni C, et al. Intravenous erythropoietin in patients with ST-segment elevation myocardial infarction: REVEAL: a randomized controlled trial. JAMA. 2011;305:1863–72.
11. Ozawa T, Toba K, Suzuki H, et al. Single-dose intravenous administration of recombinant human erythropoietin is a promising treatment for patients with acute myocardial infarction—randomized controlled pilot trial of EPO/AMI-1 study. Circ J. 2010;74:1415–23.
12. Taniguchi N, Nakamura T, Sawada T, et al. Erythropoietin prevention trial of coronary restenosis and cardiac remodeling after ST-elevated acute myocardial infarction (EPOC-AMI): a pilot, randomized, placebo-controlled study. Circ J. 2010;74:2365–71.
13. Opie LH. Erythropoietin as a cardioprotective agent: down but not out. Heart. 2011;97:1537–9.
14. Vaziri ND. Thrombocytosis in EPO-treated dialysis patients may be mediated by EPO rather than iron deficiency. Am J Kidney Dis. 2009;53:733–6.
15. Baker JE, Kozik D, Hsu AK, Fu X, Tweddell JS, Gross GJ. Darbepoetin alfa protects the rat heart against infarction: dose–response, phase of action, and mechanisms. J Cardiovasc Pharmacol. 2007;49:337–45.
16. Uesaka H. A personal view of methods of sample size estimation for a clinical trial. Jpn J Biom. 2003;24:17–41.
17. Jennison C, Turnbull BW. Group sequential methods with applications to clinical trials. Boca Raton: Chapman & Hall/CRC; 2000.
18. Zhang J, Somani AK, Siminovitch KA. Roles of the SHP-1 tyrosine phosphatase in the negative regulation of cell signalling. Semin Immunol. 2000;12:361–78.
19. Kanellakis P, Pomilio G, Agrotis A, et al. Darbepoetin-mediated cardioprotection after myocardial infarction involves multiple mechanisms independent of erythropoietin receptor-common beta-chain heteroreceptor. Br J Pharmacol. 2010;160:2085–96.
20. Hausenloy DJ, Baxter G, Bell R, et al. Translating novel strategies for cardioprotection: the Hatter Workshop Recommendations. Basic Res Cardiol. 2010;105:677–86.
21. Schwartz Longacre L, Kloner RA, Arai AE, et al. New horizons in cardioprotection: recommendations from the 2010 National Heart, Lung, and Blood Institute Workshop. Circulation. 2011;124:1172–9.
22. Downey JM, Cohen MV. Why do we still not have cardioprotective drugs? Circ J. 2009;73:1171–7.
23. Piot C, Croisille P, Staat P, et al. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. N Engl J Med. 2008;359:473–81.
24. Botker HE, Kharbanda R, Schmidt MR, et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. Lancet. 2010;375:727–34.
25. Moon C, Krawczyk M, Paik D, Lakatta EG, Talan MI. Cardioprotection by recombinant human erythropoietin following acute experimental myocardial infarction: dose response and therapeutic window. Cardiovasc Drugs Ther. 2005;19:243–50.
26. Li J, Xu H, Gao Q, Wen Y. Effect of erythropoiesis-stimulating agents in acute ST-segment elevation myocardial infarction: a systematic review. Eur J Clin Pharmacol. 2011;68:469–77.
27. Stein A, Mohr F, Laux M, et al. Erythropoietin-induced progenitor cell mobilisation in patients with acute ST-segment-elevation myocardial infarction and restenosis. Thromb Haemost. 2012;107:769–74.
28. Minamino T, Toba K, Higo S, Nakatani D, Ozawa T. Erythropoietin, progenitor cells and restenosis. A critique of Stein et al. Thromb Haemost. 2012;107:1193.
29. Carlsson M, Ubachs JF, Hedstrom E, Heiberg E, Jovinge S, Arheden H. Myocardium at risk after acute infarction in humans on cardiac magnetic resonance: quantitative assessment during follow-up and validation with single-photon emission computed tomography. JACC Cardiovasc Imaging. 2009;2:569–76.

European Journal of Cardio-Thoracic Surgery 42 (2012) 551–556
doi:10.1093/ejcts/ezs032 Advance Access publication 9 February 2012

**ORIGINAL ARTICLE**

# Risk factors for post-transplant low output syndrome†

Tomoyuki Fujita[a,*], Koichi Toda[a], Masanobu Yanase[b], Osamu Seguchi[b], Yoshihiro Murata[b], Hatsue Ishibashi-Ueda[c], Junjiro Kobayashi[a] and Takeshi Nakatani[b]

[a] Department of Cardiovascular Surgery, National Cerebral and Cardiovascular Center, Osaka, Japan
[b] Department of Transplantation, National Cerebral and Cardiovascular Center, Osaka, Japan
[c] Department of Pathology, National Cerebral and Cardiovascular Center, Osaka, Japan

* Corresponding author. Department of Cardiovascular Surgery, National Cerebral and Cardiovascular Center, 5-7-1 Fujishirodai, Suita City, Osaka, 565-8565, Japan. Tel: +81-6-6833-5012; fax: +81-6-6872-7486; e-mail: tomofujita@nifty.com (T. Fujita).

Received 31 August 2011; received in revised form 13 December 2011; accepted 22 December 2011

## Abstract

**OBJECTIVES**: Due to a serious heart donor shortage, the criteria for acceptance for transplantation have been expanded. This study assesses donor-related factors associated with postoperative low output syndrome (LOS) and long-term survival of recipients.

**METHODS**: From 1999 to February 2011, 36 heart transplantations were performed at our institute, of which 28 donor hearts (78%) were considered to be marginal due to high inotropic requirement ($n = 11$), recent episode of cardiac arrest ($n = 11$), female to male transplantation ($n = 11$), reduced left ventricular contraction ($n = 6$), old age ($n = 6$), small donor heart ($n = 5$), donor–recipient size mismatch ($n = 2$), ventricular hypertrophy ($n = 2$) or prolonged ischaemic time ($n = 1$). St Thomas solution ($n = 6$) and Celsior ($n = 30$) were used for preservation. Ischaemic damage in post-transplant cardiac patients was graded by perioperative ischaemic myocardial injury (PIMI) score (scores 0–3).

**RESULTS**: The donor age was 39 ± 11 years old, which was not significantly different to that of the recipients. 50% of the donors were female. Thirty-three donors (92%) required catecholamine at an average of 8.0 ± 5.2 μg/hg/min and echocardiogram findings showed that left ventricular ejection fraction was 65 ± 10%. All recipients survived during the perioperative period (one patient died from sepsis at 4 years after transplantation) for a 10-year survival rate of 95%. Severe primary graft dysfunction was observed in two patients who required intra-aortic balloon pumping or veno-arterial extra-corporeal membrane oxygenation, and five other patients showed postoperative LOS (cardiac index < 2.2 l/min/m$^2$). Left ventricular diastolic diameter smaller than 36 mm ($P = 0.0002$), high inotropic requirement ($P = 0.0089$) and left ventricular ejection fraction less than 55% ($P = 0.0383$) were related to post-transplant LOS. All patients recovered cardiac function and were discharged from the intensive care unit after an average of 6 days. Although preservation with Celsior was not related to LOS, it had relationships with lower CKMB level ($P = 0.0013$) and lower PIMI score ($P = 0.0054$).

**CONCLUSIONS**: Cautious donor selection is essential when the donor heart has a small ventricular diameter or requires a high level of inotropic support. However, long-term survival in recipients with marginal donor hearts can be anticipated with adequate treatment.

**Keywords:** Heart transplantation • Marginal donor • Primary graft dysfunction



## INTRODUCTION

Primary graft dysfunction (PGD) is a life-threatening condition that is a major cause of early mortality after cardiac transplantation. As the number of acute rejection cases has reduced, PGD has become the leading cause of death in the first 30 days after transplantation (39% of deaths) and continues to remain prominent throughout the post-transplant period [1]. This condition is defined as severe dysfunction of the cardiac allograft without any obvious anatomic or immunologic cause, and is characterized by low output syndrome (LOS), which requires high-dose inotropic or mechanical support [2]. The incidence of PGD is reported to be greater than 20% [3, 4]. Although veno-arterial extracorporeal membrane oxygenation (VA-ECMO) has been found to improve outcomes in PGD patients, mortality remains high [5]. Despite the high incidence and high mortality rate, the mechanisms underlying PGD are uncertain. Consequently, donor selection has not been established, because the predicted risk factors are uncharacterized. On the other hand, due to a serious donor shortage in Japan, acceptance of marginal donors is essential. Although extended donor acceptance criteria were recently reported to not compromise clinical outcome, donor-related risk factors for post-transplant PGD remain uncertain [4, 6, 7]. In the present study, we evaluated donor-related factors associated with postoperative LOS and long-term survival.

†Presented at the 25th Annual Meeting of the European Association for Cardio-Thoracic Surgery, Lisbon, Portugal, 1–5 October 2011.

© The Author 2012. Published by Oxford University Press on behalf of the European Association for Cardio-Thoracic Surgery. All rights reserved.

## Methods

### Recipients

Between May 1999 and February 2011, 36 patients underwent heart transplantation at our institution (Table 1). They were comprised of 27 males (75%) and 9 females, with ages ranging from 14 to 60 years old (mean 38 ± 11 years). Causes of refractory heart failure were dilated cardiomyopathy in 32 (89%), dilated phase of hypertrophic cardiomyopathy in 2 (6%), ischaemic cardiomyopathy in 1 (3%) and restrictive cardiomyopathy in 1 (3%). Six recipients (14%) were rated as New York Heart Association (NYHA) class IV, with inotropic support and left ventricular assist devices (LVADs) employed in the remaining 30 recipients (86%), which were comprised of 25 Nipro (previously Toyobo)-extracorporeal LVAD, 2 Heart Mate VE, 1 Novacor, 1 EVAHEART and 1 Jarvik 2000 devices. The support periods ranged from 3 to 57 months (mean 30 ± 13 months).

### Donors

According to criteria proposed by Laks *et al.* [7] and Wittwer and Wahlers [8], we considered marginal donors as those as those who were older than 50 years of age, those requiring high inotropic support (intravenous infusion of more than 10 μg/kg/min of inotropic agents or epinephrine) and those affected by left ventricular hypertrophy (echocardiographic diagnosis of left ventricular septum thickness >13 mm and left posterior wall thickness >13 mm), small left ventricular diameter (echocardiographic diagnosis of left ventricular diameter < 36 mm) or reduced left ventricular contraction [echocardiographic diagnosis of left ventricular ejection fraction (LVEF) less than 55%]. Prolonged ischaemic time (>4 h) and recipient/donor ratio for body weight less than 0.8 were also considered marginal parameters. Informed consent was obtained from all recipients. Donor–recipient matching criteria were based on blood group compatibility, morphological criteria and clinical conditions of the recipients.

**Table 1:** Recipient demographics

| Variables | |
|---|---|
| Male/female | 27/9 (75% males) |
| Age in years | 38 ± 11 (14–60) |
| Indications for heart transplantation | |
| Dilated cardiomyopathy | 32 (89%) |
| Dilated phase of hypertrophic cardiomyopathy | 2 (6%) |
| Ischaemic cardiomyopathy | 1 (3%) |
| Restrictive cardiomyopathy | 1 (3%) |
| Mechanical circulatory support (MCS) | 30 (86%) |
| Nipro (Toyobo)-extracorporeal LVAD | 25 (72%) |
| HeartMate VE | 2 (6%) |
| EVAHEART | 1 (3%) |
| Novacor | 1 (3%) |
| Jarvik 2000 | 1 (3%) |
| Support period of MCS in months | 30 ± 13 (3–57) |
| Status one | 36 (100%) |

LVAD: left ventricular assist device; MCS: mechanical circulatory support.

### Cardioplegia solution for harvesting

After the aorta of the donor heart was cross-clamped, cold cardioplegia solution was infused via the aortic root. St Thomas solution was used in the first six cases and Celsior in the subsequent 30 cases [9]. The total amount of Celsior was 2–3 l, based on donor heart size.

### Operative technique for implantation

A modified bicaval anastomosis technique was applied, as previously reported [10]. Care was taken to avoid cardiac re-warming, with ice slush generously poured into the pericardial cavity. Before releasing the cross-clamp, terminal blood cardioplegia solution was given for 10 min.

### Immune suppression

A conventional triple drug immune-suppressive therapy was administered to the entire population of recipients. Methylprednisolone (0.5 + 0.5 g) was given intra-operatively before aortic cross-clamp removal, followed by three 125-mg pulses during the first postoperative day. Prednisone was commenced orally following extubation. The induction immunotherapy course, when required, was initially muromonab-CD3 (Orthuclon OKT3), while basiliximab was given in more recent years [11, 12]. Cyclosporine A (Neoral®, Novartis International AG, Basel, Switzerland) or tacrolimus hydrate (Prograf®, Astellas) combined with mofetil mycophenolate (Cellcept®, Roche) was commenced on the second postoperative day [12, 13]. Tacrolimus hydrate was given as first-line treatment beginning in 2007.

### Follow-up examinations

The entire patient population in this study had immediate postoperative evaluations. Epicardial two-dimensional (2-D) echocardiogram findings were obtained daily for the first week and then weekly for the first month. Furthermore, 2-D colour flow Doppler echocardiogram examinations of the four subcostal and apical chambers were performed. Right heart catheterization through a Swan-Ganz catheter was obtained within the first 48 h. A right ventricular flow-directed pulmonary artery catheter was inserted and advanced into the pulmonary artery until wedge pressure was determined. Measurements of pulmonary artery pressure, wedge pressure, central venous pressure, cardiac index and cardiac output were recorded. Graft dysfunction was defined as a cardiac index lower than 2.2 l/min/$m^2$ with adequate medical therapy, or the requirement of intra-aortic balloon pumping (IABP) and/or VA-ECMO.

### Pathological examinations

Pathological examinations including rejection surveillance were performed using endomyocardial biopsies, which were carried out after 1, 2, 3, 5, 7 and 11 weeks, then after 4.5, 6, 9 and 12 months. Specimens were stained with Masson's trichrome and

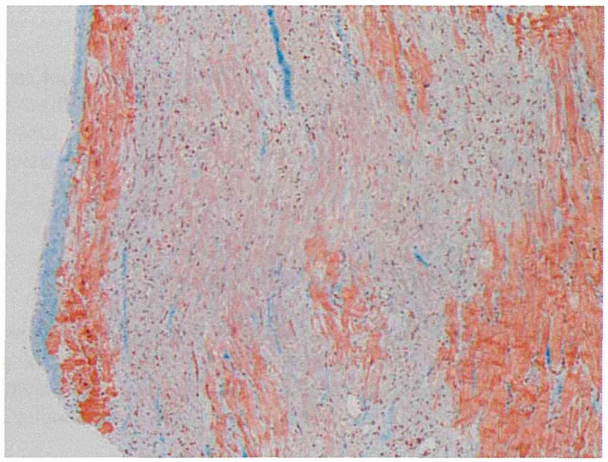

**Figure 1:** Representative biopsy specimen obtained from right side ventricular septum. Zonal myocardial necrosis with marginal granulation change is shown. The histology was consistent with a PIMI score of 3. Masson's trichrome stain, original magnification ×100.

haematoxylin–eosin. Diagnosis of acute cardiac rejection was made according to the International Society for Heart and Lung Transplantation (ISHLT) criteria [14]. Perioperative ischaemic myocardial injury (PIMI) was also assessed using PIMI score (0–3), as described by Fyfe *et al.* [15], with a score of 0 considered to be no evidence of coagulative myocyte necrosis (CMN), 1 showing mild or focal CMN, 2 showing moderate or multifocal CMN and 3 showing severe or confluent CMN (Fig. 1).

## Statistical analysis

Data are shown as the mean ± standard deviation. Categorical variables were analysed by a $\chi^2$-test when all expected cell frequencies were greater than or equal to 5. Comparisons of values between patients were done using a Mann–Whitney *U*-test for independent samples. All analyses were performed using the statistical software package JMP 8 (SAS Institute, Inc., Cary, NC, USA). Differences were considered significant with a *P*-value less than 0.05%. Actuarial survival was estimated using the Kaplan–Meier method.

# RESULTS

## Survival

No perioperative deaths occurred and only one patient died (sepsis) up to 4 years after transplantation, for an overall 10-year actuarial survival rate of 95% (Fig. 2). There was no haemodynamically compromised antibody-mediated rejection observed.

## Primary graft dysfunction

The patient baseline characteristics are shown in Table 2. The age of the donors was 39 ± 11 years, which was not significantly different than the recipient age. Although half of the donors were female and 31% of the cases were female to male transplantation, the donor–recipient weight ratio was 1.2.

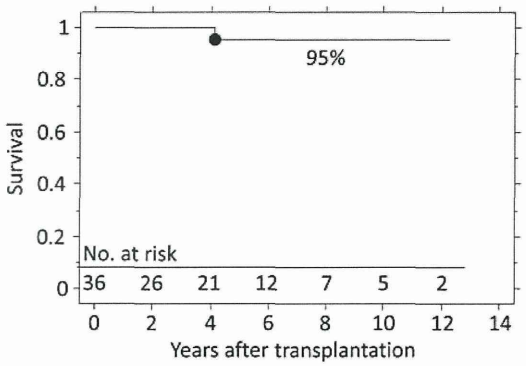


**Figure 2:** Kaplan–Meier survival estimates after transplantation.

**Table 2:** Donor demographics

| Variables | |
|---|---|
| Male/female | 18/18 (50%) |
| Age in years (year-old) | 39 ± 11 |
| Causes for brain death | |
| Atraumatic intracranial bleeding | 22 (61%) |
| Hypoxic encephalopathy | 10 (28%) |
| Head trauma | 3 (8%) |
| Brain malignancy | 1 (3%) |
| Weight ratio (donor/recipient) | 1.2 |
| Catecholamine usage | 33 (92%) |
| Dose of catecholamine (μg/kg/min) | 8.0 ± 5.2 |
| Echocardiographic data | |
| LVDd (mm) | 42 ± 6 |
| LVDs (mm) | 27 ± 6 |
| IVS (mm) | 10 ± 2 |
| PWth (mm) | 10 ± 2 |
| LVEF (%) | 65 ± 10 |
| Ischaemic time (min) | 209 ± 21 |

LVDd: left ventricular diastolic diameter; LVDs: left ventricular systolic diameter; IVS: interventricular septum; PWth: posterior wall thickness; LVEF: left ventricular ejection fraction.

Echocardiography revealed cardiac function was maintained with LVEF of 65 ± 10%, which was supported by 8.0 ± 5.2 μg/kg/min of catecholamine. The average ischaemic time was 209 ± 21 min and there was only one case that exceeded 240 min.

PGD was observed in seven patients (19%). Five patients showed a low cardiac index less than 2.2 l/min/m$^2$ with adequate postoperative medical treatment. One patient required VA-ECMO and IABP, and one required IABP. The patient who required VA-ECMO showed severely reduced LV contraction soon after transplantation and estimated LVEF assessed by intraoperative transoesophageal echocardiography was 10%. However, weaning from VA-ECMO on day 1 and from IABP on day 4 was successful with maximum inotropic support, including epinephrine, though LVEF was 47% after 1 week and 48% after 1 month. On the other hand, the patient who required IABP was weaned successfully on day 2, and cardiac function recovered quickly to an LVEF of 72% after 1 week. The other five patients had uncomplicated postoperative courses.

The causes of PGD were assessed and statistical analysis showed that it was significantly associated with high-dose