III. 研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Minamino T, Toba K, Higo S, Nakatani D, Hikoso S, Umegaki M, Yamamoto K, Sawa Y, Aizawa Y, Komuro I.	Design and Rationale of Low-Dose Erythropoietin in Patients with ST-Segment Elevation Myocardial Infarction (EPO-AMI-II Study): A Randomized Controlled Clinical Trial.	*Cardiovasc Drugs Ther.*	26(5)	409-416	2012
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Ⅳ. 研究成果の刊行物・別刷

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Design and Rationale of Low-Dose Erythropoietin in Patients with ST-Segment Elevation Myocardial Infarction (EPO-AMI-II Study): A Randomized Controlled Clinical Trial

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Abstract
Purpose The development of novel pharmaceutical interventions to improve the clinical outcomes of patients with acute ST-segment elevation myocardial infarction (STEMI) is an unmet medical need worldwide. In animal models, a single intravenous administration of erythropoietin (EPO) during reperfusion improves left ventricular (LV) function in the chronic stage. However, the results of recent proof-of-concept trials using high-dose EPO in patients with STEMI are inconsistent. In our pilot study, low-dose EPO after successful percutaneous coronary intervention (PCI) improved the LV ejection fraction (EF) and did not trigger severe adverse clinical events in patients with STEMI. One possible reason for this discrepancy is the dose of EPO used.
Methods and results 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 patients with STEMI. STEMI patients who have a low LVEF (<50 %) will be randomly assigned to intravenous administration of placebo or EPO (6,000 or 12,000 IU) within 6 h after successful PCI. The primary endpoint is the difference in LVEF between the acute and chronic phases (6 months), as measured by single-photon emission computed tomography. The patient number needed for EPO-AMI-II is 600. The study will stop when superior efficacy or futility is detected by an interim analysis. This study has been approved by the Evaluation System of Investigational Medical Care.
Conclusions EPO-AMI-II study will clarify the safety and efficacy of low-dose EPO in STEMI patients with LV dysfunction in a double-blind, placebo-controlled, multicenter study. (247 words)

Key words Erythropoietin · Low-dose · Acute myocardial infarction · LV dysfunction

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Despite improved clinical outcomes by early reperfusion with thrombolysis and primary percutaneous coronary intervention (PCI) with stenting, the mortality of patients with

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.

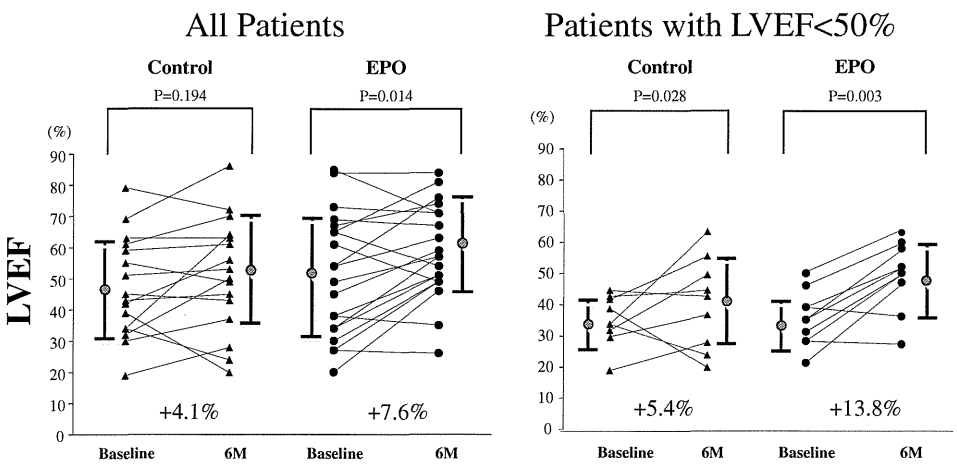


Fig. 1 Post-hoc analysis of the EPO-AMI-I results. Panel **a** shows the LVEF between the acute and chronic stages in all patients in the EPO-AMI-I study. EPO, but not saline, administration significantly increased LVEF at 6 months after an MI. Panel **b** shows the LVEF between the acute and chronic 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

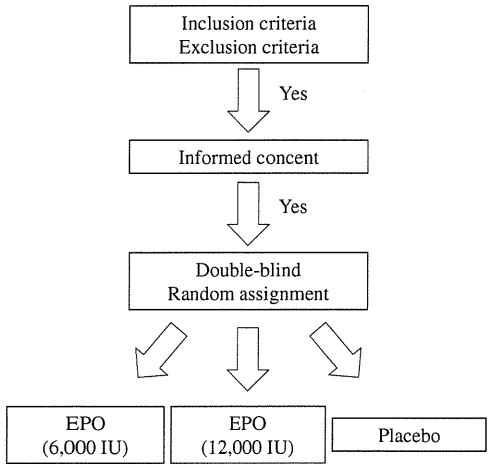


Fig. 2 Study flow chart

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.

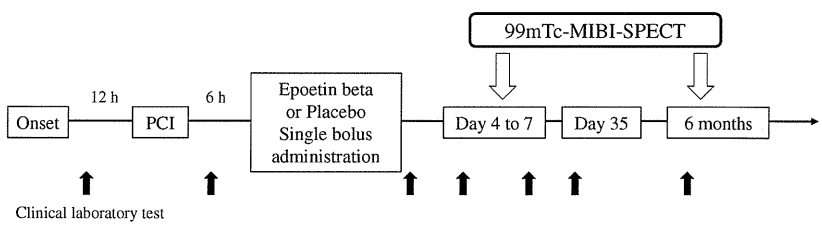
Adverse events and additional safety assessments

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).

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Discloures None.

Appendix

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Estimation of left atrial blood stasis using diastolic late mitral annular velocity

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Aims Diastolic late mitral annular velocity (a′) measured by transthoracic echocardiography (TTE) is reported to represent left atrial (LA) pump function and the severity of LA remodelling. The purpose of this study is to investigate the association between a′ and LA blood stasis in patients with non-valvular paroxysmal atrial fibrillation.

Methods and results We enrolled 138 consecutive patients with non-valvular paroxysmal atrial fibrillation who had spontaneous sinus rhythm at the time of echocardiography. Using TTE, a′ was determined as an average of tissue Doppler velocities measured at septal and lateral mitral annuli. Transoesophageal echocardiography was also performed on the same day as TTE, and spontaneous echo contrast (SEC) and LA appendage flow velocity were examined. Spontaneous echo contrast was observed in 21 (15%) patients. Patients in the lowest quartile of a′ ($\leq$6.4 cm/s) demonstrated SEC more frequently (44 vs. 6%, $P < 0.0001$) and had lower LA appendage flow velocity (39 ± 13 vs. 53 ± 16 cm/s, $P < 0.0001$) than those in the other quartiles. Receiver-operating characteristic curve analysis showed that the best cut-off value of a′ was 7.0 cm/s for the prediction of SEC with a sensitivity of 80%, specificity of 81%, and predictive accuracy of 80%. Multivariate analysis revealed that decreased a′ (OR = 0.61, $P = 0.0026$) was independently associated with SEC.

Conclusion Decreased a′ may be a useful parameter for the estimation of LA blood stasis in patients with paroxysmal atrial fibrillation.

Keywords Left atrium • Thrombus • Blood stasis • Mitral annular velocity • Spontaneous echo contrast

Introduction

In patients with atrial fibrillation, left atrial (LA) thrombus sometimes causes a systemic thromboembolism including stroke, and estimation of the risk for thromboembolism is essential in clinical practice. The risk of a thromboembolic event is often assessed using the CHA2S2-VASc score.[1] Furthermore, transoesophageal echocardiographic parameters, spontaneous echo contrast (SEC), and LA appendage flow velocity are also thought to provide important information about the risk of LA thrombus formation and thromboembolic event.[2–5] However, transoesophageal echocardiography is semi-invasive and not easily performed. A transthoracic echocardiographic method to reliably predict the risk of LA thrombus formation would be useful in the management of patients with atrial fibrillation.

Previous studies reported that advanced atrial remodelling increases the risk of SEC and thromboembolic events.[6,7] In addition, tissue Doppler diastolic late mitral annular velocity (a′) measured by transthoracic echocardiography is thought to represent LA pump function, and depressed a′ has been reported to be associated with advanced LA remodelling in patients with paroxysmal atrial fibrillation.[8–11] Therefore, we hypothesized that decreased a′ during sinus rhythm can be used as an estimation of SEC and LA blood stasis in patients with paroxysmal atrial fibrillation.

In order to investigate this hypothesis, we performed both transthoracic and transoesophageal echocardiography during

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sinus rhythm in patients with non-valvular paroxysmal atrial fibrillation.

Methods

Study patients

From April 2009 to October 2010, we enrolled consecutive patients with symptomatic drug-refractory paroxysmal atrial fibrillation scheduled for catheter ablation. We excluded patients without spontaneous sinus rhythm at the time of transoesophageal and transthoracic echocardiography. We also excluded patients that had symptomatic atrial fibrillation episodes within 2 days prior to echocardiography to avoid the influence of atrial stunning after conversion of atrial fibrillation to sinus rhythm.[12] Other exclusion criteria were significant mitral stenosis, mitral regurgitation, prior catheter ablation, prior cardiac surgery, and poor echocardiographic images. The study protocol was approved by the hospital's Ethics Committee and informed consent for the participation in this study was obtained from all patients.

Anti-arrhythmic drugs were discontinued five half-lives before echocardiography. Anticoagulation therapy was initiated based on the discretion of the attending physician according to international guidelines. We calculated the CHA2DS2-VASc score to evaluate the clinical background associated with the risk of thromboembolism.[1] About 1 week before echocardiographic study, 64-detector contrast-enhanced computed tomography was performed. Pre-atrial contraction LA volume was calculated at the onset of *P*-wave on electrocardiogram excluding the pulmonary veins and appendage, and was indexed to body surface area.

Transoesophageal echocardiography

We performed transoesophageal echocardiography 1 or 2 days prior to catheter ablation, using an SSD-6500 system (Aloka, Tokyo, Japan) equipped with a 5.0 MHz multiplane transoesophageal probe. Left atrial appendage images were obtained both in the basal short-axis view with a transverse scan and in LV-LA two chamber view with a vertical scan. Left atrial thrombus was defined as a well-circumscribed, echogenic mass that was of different texture than the LA wall and had a uniform consistency.[13,14] Spontaneous echo contrast was defined as a dynamic smoke-like signal that swirled slowly in a circular pattern within the LA and appendage, with appropriate gain settings to distinguish SEC from echoes due to excessive gain.[2,3] Left atrial appendage flow velocity was measured by placing the pulsed Doppler sample volume at the outlet of the appendage cavity more than 1 cm away from the LA wall.

Transthoracic echocardiography

Transthoracic echocardiography was performed on the same day as transoesophageal echocardiography, using a Sonus iE33 system (Philips, Andover, MA, USA). Left ventricular ejection fraction was assessed from the apical four-chamber view using Simpson's method. Left ventricular mass (grams) was calculated as $0.80 \times (1.04 \times [\{\text{septal wall thickness in diastole} + \text{left ventricular end-diastolic dimension} + \text{left ventricular posterior wall thickness in diastole}\}^3 - \text{left ventricular end-diastolic dimension}^3]) + 0.6$ and was indexed to height[1,7].[15,16] Peak velocities of diastolic early (E) and late (A) transmitral Doppler flow were measured by apical four-chamber view with the sample volume placed at the tip of the mitral leaflets. Tissue Doppler velocities during early (e′) and late (a′) diastole were measured with a colour tissue Doppler technique at the basal septal and lateral mitral annuli on the apical four-chamber image. Diastolic function was graded according to the recommendation by the Amerian Society of Echocardiography and the European Association of Echocardiography.[17] The mean values of five consecutive heart beats from septal and lateral measures were used. Interobserver variability of a′ (6%) was calculated in 12 randomly selected patients as the difference in two measurements in the same patients by two different observers divided by the mean value.

Statistics

Continuous values were expressed as the mean ± SD unless otherwise indicated. Categorical data were presented as absolute values and percentages. Tests for significance were conducted using a *t*-test or repeated measures analysis of variance to compare continuous variables, and a χ^2 test or Fisher's exact test for categorical variables. Univariate and multivariate logistic regression analyses were used to determine the factors that were associated with the presence of SEC. Variables with a *P* value ≤ 0.05 in the univariate model were included in the multivariate analysis. For the prediction of SEC, receiver-operating characteristic curves were constructed for different cut-off values of a′. The area under each curve was determined along with the 95% CI using the bootstrap method. All analyses were conducted using SPSS for Windows, version 15.0.

Results

Patient characteristics

Of the 180 patients with paroxysmal atrial fibrillation scheduled for ablation during the study period, 138 patients were included in this study. Reasons for exclusion were as follows: atrial fibrillation at the time of echocardiography (four patients), symptomatic atrial fibrillation episodes within 2 days prior to echocardiography (nine patients), prior history of ablation (23 patients), or cardiac surgery (two patients), severe mitral valve disease (three patients) and poor echocardiographic image (one patient). Left atrial thrombus was observed in two of 138 (1%) patients, and SEC was detected in 21 (15%) patients on transoesophageal echocardiography. Clinical characteristics stratified according to the presence or absence of SEC are shown in *Table 1*. Heart failure was more often present in patients with than without SEC, and other clinical risk factors for thromboembolism, such as high age, hypertension, diabetes mellitus, and previous history of thromboembolism, tended to be more frequent in patients with SEC. Therefore, the CHA2DS2-VASc score was significantly higher in patients with SEC. In addition, LA volume and its index measured by computed tomography were significantly larger in patients with SEC. In contrast, there was no difference between patients with and without SEC in the frequency of anticoagulation therapy, haematocrit level, and international normalized ratio of prothrombin time.

Echocardiography

The echocardiographic parameters are listed in *Table 2*. Patients with SEC had higher left ventricular mass index, lower e′ and a′, and higher E/e′. In addition, LA appendage flow velocity was significantly lower in patients with SEC.

There were two patients with LA thrombus and both were on anticoagulation therapy (international normalized ratio of prothrombin time was 2.3 ± 0.1). Spontaneous echo contrast was observed in 2/2 (100%) patients with LA thrombus.