CHAPTER 3

HEBE III: A SINGLE DOSE OF ERYTHROPOIETIN IN ST-ELEVATION MYOCARDIAL INFARCTION

SUMMARY

Background
Cardioprotective effects of erythropoietin (Epo) have been shown in experimental and smaller clinical studies. We performed a prospective, multicenter, randomized trial to assess the effects of a single high dose of Epo after primary coronary intervention (PCI) for an ST-elevation myocardial infarction (STEMI).

Methods
Patients with a successful PCI for a first STEMI randomly received either standard medical care alone, or in combination with a single bolus with 60,000 IU i.v. of epoetin alfa within 3 hours after PCI. Primary endpoint was Left Ventricular Ejection Fraction (LVEF) after 6 weeks, assessed by planar radionuclide ventriculography. Pre-specified secondary endpoints included enzymatic infarct size and major adverse cardiovascular events.

Results
529 patients were enrolled in the study (Epo n=263, control n=266). At baseline (before Epo administration), groups were well-matched for all relevant characteristics. After a mean of 6.5 (±2.0) weeks, LVEF was 0.53 (±0.10) in the Epo group and 0.52 (±0.11) in the control group (p=0.41). Median area under the curve (IQR) after 72 hours for CK was 50,136 (28,212-76,664) U/L per 72 hours in the Epo group and 53,510 (33,973-90,486) U/L per 72 hours in the control group (p=0.058). More major adverse cardiac events occurred in the control than in the Epo group (27 vs.10; p=0.032).

Conclusions
A single high dose of Epo after a successful PCI for a STEMI did not improve global LVEF after six weeks. However, the observed beneficial effects on the secondary endpoints suggest a potentially relevant cardioprotective effect and a favourable clinical safety profile. (NCT00449488)
INTRODUCTION

Erythropoietin (Epo) is commonly known as an effective treatment for anaemia, partly caused by an inadequate production of endogenous Epo in patients with renal disease. Experimental studies have suggested cardioprotective effects of Epo, which might be beneficial in the setting of a myocardial infarction. In an ischemia-reperfusion model, mice lacking an Epo-receptor in the heart developed larger infarcts compared with wild type mice. In addition, a number of animal studies provided consistent evidence for a reduced infarct size and improved left ventricular function caused by Epo administration.

Two important cardioprotective mechanisms of Epo have been described that provide a rationale for potential beneficial effects of Epo during a myocardial infarction. First, Epo decreased apoptosis in ischemia-reperfusion models, potentially leading to smaller infarct sizes. Second, Epo stimulates neovascularization, through the stimulation of vascular endothelial growth factor (VEGF).

In the last two years, a few studies have investigated the effects of Epo in patients with a myocardial infarction. In a first safety and feasibility study from our group, a single high dose bolus of Epo (60,000 IU) in patients with a first ST-elevation myocardial infarction, resulted in a 200-fold increase in serum Epo levels, without hypertension, thrombotic, or other adverse events. Ferrario et al. demonstrated a significant reduction in CK-MB release in 30 myocardial infarction patients treated with Epo (33,000 IU before PCI, and at 24 and 48h after admission), but effects on left ventricular function were not reported. Binbrek et al. showed a small and non-significant effect of a single bolus of 30,000 IU Epo on infarct size in 236 myocardial infarction patients, although effects on left ventricular function were again not reported. In 138 ST-elevation myocardial infarction patients, 30,000 IU of Epo administered during the first three days did not improve left ventricular function after 6 months, although these data have as yet not been published. We present data from a multicenter, prospective, randomized trial on the effects of a single high dose of Epo in 529 patients after a successful angioplasty for a first ST-elevation myocardial infarction.
Methods

Patients and study design
This is a prospective, randomized, open-label with blinded endpoints study. A detailed design of the study has been published elsewhere. In brief, patients were eligible for the study if they had a successful primary PCI (Thrombolysis in Myocardial Infarction (TIMI) flow 2/3 after the procedure, TIMI flow 0/1 before) for a first ST-elevation myocardial infarction. Myocardial infarction was defined as described before, by a) suggestive chest pain; b) symptom onset 12 h before hospital admission or 24 h in case of ongoing ischemia; c) electrocardiogram (ECG) with ST-T segment elevation ≥1 mV in 2 or more leads or new left bundle branch block. The most important exclusion criteria were a previous myocardial infarction, haemoglobin levels >17.1 g/dL before PCI, anticipated additional revascularization within 6 weeks, a history of persistent or permanent atrial fibrillation, cardiogenic shock, and a serum creatinine > 2.5 mg/dL.

Procedures and treatment
Patients were screened at the cardiac catheterisation laboratory of the 7 participating centres. In the ambulance, patients received aspirin, clopidogrel and heparin as per protocol. Before starting the PCI procedure, an ECG was performed and standard medical treatment was initiated consisting of aspirin, heparin, clopidogrel, and possibly a glycoprotein IIb/IIIa inhibitor. Patients were asked for their oral informed consent by an independent interventional cardiologist and nurse. If they agreed, patients were immediately randomized by means of a computerized program. This system randomly assigned patients to either receive optimal standard medical care with or without a bolus of 60,000 IU i.v. of epoetin alfa (Ortho Biotech, a division of Janssen-Cilag B.V.) intravenously in 10 minutes. After randomization, patients were then transported to the cardiac care unit, where written informed consent was signed and a second ECG was acquired. Blood pressure, heart rate, and ECG were monitored according to routine clinical practice at baseline and at regular time points up until 48 hours. After 6 weeks, a clinical visit was performed, and blood samples, ECG, and blood pressure were obtained at the outpatient clinic. The research protocol was approved by the central ethics committee of the University Medical Center Groningen, and by the local ethics committees of each participating center.

Planar radionuclide ventriculography
Planar radionuclide ventriculography was performed to determine left ventricular ejection fraction (LVEF). An injection of 500 MBq of 99mTc-pertechnetate was administered to patients intravenously, 20 min after injection of 1 mg stannous chloride. Planar radionuclide ventriculography was obtained on a gamma camera. The camera head was positioned in the best septal left anterior oblique (LAO) projection. Data acquisition was done by using 64 x 64 matrices in a 15% energy window centred on the 140 KeV photopeak. Processing was performed on dedicated com-
mercially available computers. In 24 patients (12 Epo and 12 control) a gated 99mTc sestamibi was used instead of planar radionuclide ventriculography.

**Laboratory measurements**
Measurements of CK, CKMB, troponin, haemoglobin, haematocrit, creatinine, were determined at baseline (immediately before PCI), and at 3, 6, 9, 12, 18, 24, 36, 48, 72 hours and 6 weeks after the PCI procedure. At baseline and after 6 weeks, NT-proBNP measurements were performed in plasma using a commercially available electrochemiluminescent sandwich immunoassay (Elecsys proBNP, Roche Diagnostics, Mannheim, Germany). The intra- and interassay coefficients of variation were 1.2-1.5% and 4.4-5.0% respectively, with an analytical range of 5-35,000 pg/ml.

**Endpoints and blinding**
Primary endpoint of the study was LVEF assessed at 6 weeks after the primary PCI procedure by planar radionuclide ventriculography. Secondary study endpoints were the following:
- Myocardial infarct size: determined by (1) area under the marker curves and (2) peak values of serial computerized measurements of creatinine kinase (CK) and CK-MB and troponin-T.
- Incidence of a cardiovascular event within 6 weeks after PCI: defined as cardiovascular death, reinfarction (any ST-elevation or non-ST-elevation myocardial infarction), emergency re-PCI or coronary artery bypass grafting, stroke, and clear symptoms of heart failure.

All endpoints were assessed blindly. First, the primary endpoint was assessed by nuclear physicians that were blinded for the treatment arm of the patient. Second, an independent and blinded endpoint committee adjudicated all clinical endpoints. Safety data were periodically reviewed by an independent data safety monitoring committee.

**Sample size considerations**
At the start of the study, no clinical data were available on the effects of Epo on left ventricular function. We assumed an absolute LVEF increase of 3% with Epo as both feasible and clinically relevant. To demonstrate this absolute 3% improvement of LVEF after Epo treatment, with a standard deviation of 11% \(^{10,11}\), a power of 0.8 and a p-value < 0.05, 2-sided, 212 evaluable patients per group were needed. From the first 100 patients, we observed that for several reasons (Figure 1) a primary endpoint could not be obtained in 20% of patients. Therefore, we aimed to include approximately 264 patients in each group. Therefore, to obtain 424 evaluable endpoints, a total of 528 patients (264 in each group) had to be included in this study.
Statistical analysis
Statistical analysis was performed by the department of epidemiology of the University Medical Center Groningen. Summaries of quantitative continuous variables are presented as means ± standard deviation or medians and interquartile ranges (IOR) if appropriate. Categorical data are presented as absolute frequencies. Baseline patient characteristics between Epo and control were compared with use of the $\chi^2$ test or
Fisher exact test (binary variables), Students t-test (continuous variables), and the Wilcoxon rank-sum (Non-Gaussian data). To estimate the treatment effect on the primary endpoint, differences in means and corresponding 95% confidence interval (CI) were calculated based on the analysis of variance model. In addition, between-group differences were also computed using 3-way ANOVA with terms for group, time and a group × time to measurement interaction.

For the separate analyses on clinical events, the event-free survival was estimated according to the Kaplan-Meier method and compared between the 2 treatment groups by the log-rank test. For differences in NT-proBNP levels and enzymatic infarct size the Wilcoxon rank-sum test was used.

Unless stated otherwise, all secondary endpoint analyses were performed using the same patients included in the primary endpoint analysis. Secondary variables were analysed using an appropriate statistical test, depending on the nature of the variable.

Safety was assessed by summarizing incidence and type of adverse events (AE’s) during the follow-up duration. All patients were included in the safety assessment. The safety analysis focussed in particular on AE’s, serious AE’s, and mortality. No formal statistical hypothesis testing was performed. Treatment regimens were compared on the incidence of adverse drug reactions.

All analyses were done two-sided with a p-value < 0.05 indicating statistical significance. All analyses were based on intention-to-treat principle. With regard to missing data, no replacement of missing data was performed. Statistical analyses were performed using SPSS 16 (SPSS Inc, Chicago, IL).

RESULTS

Patients
Clinical characteristics of the patients are presented in table 1. At baseline, groups were well-matched for all relevant characteristics. Mean (±SD) age of the patients was 60.9 (±11.1) years and 78% were male. Median (IQR) time from onset of symptoms to start of the PCI procedure was 180 (120-270) minutes. Although baseline haemoglobin was higher in the control group, there were no differences in changes in haemoglobin over the first 48 hours between the groups.

Patients were treated according to the guidelines, and had received aspirin, clopidogrel and heparin in the ambulance as per protocol. After a successful PCI procedure (TIMI flow 2-3), patients were randomized. After randomization, immediately before Epo-infusion, a 12-lead ECG was performed. Median (IQR) cumulative ST-segment deviation (ST-elevation and ST-depression), was 5.1 (2.6-8.5) mm in the Epo group and 5.1 (2.7-8.5) mm in the control group (p = 0.88).
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Endpoints

After a mean of 6.5 (±2.0) weeks, planar radionuclide ventriculography was obtained in 448 patients (85%). Reasons for not being able to obtain the primary endpoint are described in figure 1. Mean (±SD) LVEF was 0.53 (±0.10) in the Epo group and 0.52 (±0.11) in the control group (p=0.41). Data on myocardial infarct size are presented in table 2. Overall, myocardial infarct size was slightly larger in the control group, compared with the Epo group, although
most of these differences did not reach statistical significance. Cardiovascular events within 6 weeks are presented in Table 3. Overall, 10 cardiovascular events occurred in 8 patients in the Epo group, versus 27 events in 19 patients in the control group (Log-rank for time to first event: p = 0.031). Figure 2 demonstrates Kaplan-Meier curves of cardiovascular events in the Epo and control group.

In a subgroup of 214 patients, NT-proBNP was available at baseline and follow-up. Median (IQR) NT-proBNP increased from 81 (40-394) to 380 (198-853) pg/ml in the Epo group and from 106 (45-286) to 470 (217-1010) pg/ml in the control group (p for difference in change from baseline to 6 weeks = 0.025)

### Safety analysis

Overall, 49 Serious Adverse Events (SAE’s) were reported in 40 control patients, and 33 SAE’s were reported in 29 Epo patients. Heart failure related events (7 vs. 1; p = 0.034) and reinfarctions (mainly caused by acute stent thrombosis; 7 vs. 2; p = 0.096)

### Table 2. Secondary study endpoint: enzymatic infarct size, determined by serial CK, CKMB, and troponin T measurements in 529 acute myocardial infarction patients treated with erythropoietin (Epo) or control

<table>
<thead>
<tr>
<th></th>
<th>Epo</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (±SD) Peak CK (U/L)</td>
<td>2077</td>
<td>2385</td>
<td>0.048</td>
</tr>
<tr>
<td>Median (IQR) Peak CK (U/L)</td>
<td>1750</td>
<td>1726</td>
<td>0.293</td>
</tr>
<tr>
<td>Median AUC (IQR) CK (U/L per 72h)</td>
<td>50,136 (28,212-76,664)</td>
<td>53,510 (33,973-90,486)</td>
<td>0.058</td>
</tr>
<tr>
<td>Mean (±SD) Peak CKMB (U/L)</td>
<td>246</td>
<td>275</td>
<td>0.15</td>
</tr>
<tr>
<td>Median (IQR) Peak CKMB (U/L)</td>
<td>214</td>
<td>219</td>
<td>0.955</td>
</tr>
<tr>
<td>Median AUC (IQR) CKMB (U/L per 72h)</td>
<td>5622 (3487-8204)</td>
<td>5933 (3757-8801)</td>
<td>0.16</td>
</tr>
<tr>
<td>Mean (±SD) peak Troponin T (µg/L)</td>
<td>5.56</td>
<td>5.90</td>
<td>0.47</td>
</tr>
<tr>
<td>Median (IQR) peak Troponin T (µg/L)</td>
<td>4.30 (1.94-7.89)</td>
<td>5.90 (2.00-8.00)</td>
<td>0.564</td>
</tr>
<tr>
<td>Median AUC (IQR) Troponin T (µg/L per 72h)</td>
<td>157.5 (77.6-257.4)</td>
<td>153.0 (88.3-256.3)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

### Table 3. Secondary study endpoint: incidence of cardiovascular events within six weeks after a successful PCI for a first acute myocardial infarction in erythropoietin (Epo)-treated and control patients

<table>
<thead>
<tr>
<th>Event</th>
<th>Epo</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cardiovascular events</td>
<td>10</td>
<td>27</td>
<td>0.032*</td>
</tr>
<tr>
<td>Cardiovascular death</td>
<td>1</td>
<td>2</td>
<td>0.569</td>
</tr>
<tr>
<td>Emergency re-PCI for In-stent thrombosis</td>
<td>2</td>
<td>7</td>
<td>0.288</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>3</td>
<td>2</td>
<td>0.096</td>
</tr>
<tr>
<td>Reinfarction</td>
<td>2</td>
<td>7</td>
<td>0.569</td>
</tr>
<tr>
<td>Stroke</td>
<td>1</td>
<td>2</td>
<td>0.034</td>
</tr>
<tr>
<td>Heart failure</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

*More than 1 event occurred in several patients (e.g. reinfarction after in-stent thrombosis), but the statistical analysis was reported on the first event that had occurred.
were found more frequently in the control group. Epo was well tolerated and there were no reports of malignant hypertension, seizure, or deep vein thrombosis. Only one pulmonary embolism was reported in the control group and none in the Epo group. In addition, cardiovascular deaths and strokes were comparable between both groups (table 3).

**DISCUSSION**

A single high dose i.v. bolus of Epo immediately after a successful PCI for a first ST-elevation myocardial infarction did not improve left ventricular function after 6 weeks. However, Epo tended to decrease enzymatic infarct size and resulted in significantly fewer major adverse cardiovascular events.

Four earlier smaller studies have reported on the effects of Epo in myocardial infarction patients. \(^{11-14}\) One of these studies was a pilot study \(^{11}\), two others did not report the effects on left ventricular function \(^{12,13}\), and in one (unpublished) study in 138 ST-elevation myocardial infarction patients, intravenous infusion of Epo (3.3 x 10\(^4\) IU of epoetin beta at 3 successive days) did not improve left ventricular function at 6 months. \(^{14}\) These findings are similar to our study and might be explained by a relatively preserved left ventricular function, since patients were very well treated, and received a successful primary PCI procedure shortly after onset of symptoms. Therefore, in the current setting, left ventricular function might not be the best marker of cardioprotection of Epo during a myocardial infarction.

Myocardial infarct size was measured with cardiac enzymes, which was one of the pre-specified secondary outcomes in this study. We demonstrated a modest reduction in cardiac enzymes with Epo, although most of the differences did not reach statistical significance. These results are similar to the results published by Binbrek et al, who only showed a modest and non-significant reduction of Epo treatment on myocardial infarct...
size in 236 patients who received thrombolysis. In a small study in 30 patients, Epo significantly reduced CK-MB release after an ST-elevation myocardial infarction. In a small pilot study in 51 patients with a non-ST elevation myocardial infarction, a single intravenous dose of 40,000 IU of Epo had no significant effect on enzymatic infarct size. The second pre-specified secondary outcome marker in this study was the effect of Epo on pre-defined cardiovascular events. A significant reduction in cardiovascular events was observed in the Epo-treated patients. This difference was primarily caused by significantly fewer cases of heart failure in the Epo group. Interestingly, similar findings of less heart failure events in the Epo group were reported in non-ST-elevation myocardial infarction patients. These findings are further supported by data on NT-proBNP in a subgroup of patients from the present study, which showed that the increase of NT-proBNP from baseline to 6 weeks was significantly smaller in the Epo-treated patients, further supporting the potential cardioprotective effects of Epo in ST-elevation myocardial infarction patients. The NT-proBNP results should however be interpreted with caution, since this was a secondary outcome in a subgroup of patients.

Taken together, our findings partly support the concept that Epo might have specific cardioprotective effects. With the discovery of a functional Epo receptor in the heart, it was hypothesised that Epo elicits cardioprotective effects. Exogenous stimulation of these receptors in animal models with human recombinant Epo proved to decrease infarct size and ameliorated ejection fraction. Furthermore, stimulation of the Epo receptor in the heart increases neovascularisation through increase of VEGF and endothelial progenitor cells (EPC) homing to the myocardium. In addition, anti-apoptotic effects of Epo have been described in similar animal studies. Recently, concerns have been expressed regarding safety of the use of Epo, in particular in patients with renal anaemia. In Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT), the use of Epo in patients with renal anaemia was associated with an increased risk of stroke. In addition, Epo treatment in patients with an acute ischemic stroke was associated with an increased mortality risk. It should be noted however that in each of the studies reporting potentially hazardous effects of Epo, multiple doses were used, and doses were haemoglobin targeted, in contrast to the present study, where a single dose was used. Also, in a placebo-controlled study in myocardial infarction patients treated with aspirin and clopidogrel, Epo did not alter markers of platelet and endothelial cell activation associated with thrombosis. In the present study, Epo treatment was associated with significantly fewer serious adverse effects, mainly due to fewer heart failure related events and re-myocardial infarctions due to acute stent thrombosis. Therefore, the present study indicates that the current safety concerns about Epo cannot be translated to single dose administration in ST-elevation myocardial infarction patients treated with primary angioplasty.

In conclusion, a single high dose of Epo after a successful PCI for an ST-elevation myocardial infarction did not improve LVEF after six weeks. However, the use of Epo was related to a trend towards smaller enzymatic infarct size, and significantly less major adverse cardiovascular events and a favourable clinical safety profile. These findings suggest potential cardioprotective effects of Epo in patients with an ST-elevation myocardial infarction.
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Endpoint committee: Dr. B.J. de Smet and Dr. A.F. van den Heuvel, University Medical Center Groningen, the Netherlands.
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