Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix. Additional details for the BIOMArCS-2 Glucose trial

This supplement provides additional information on the methods and results of the BIOMArCS 2 glucose trial. BIOMArCS is an acronym for BIOMarker Study to Identify the Acute Risk of a Coronary Syndrome.

Treatment
After the prehospital diagnosis of acute coronary syndrome (ACS), using ACS-ECG software for ambulance ECGs, was established, patients were, if indicated, transported directly to the catheterization laboratory of the Medical Center Alkmaar for coronary angiography and subsequent (primary) percutaneous coronary intervention (PCI). Treatment with aspirin, heparin and clopidogrel was started prior to the PCI procedure, usually in the prehospital phase.

Glucose measurements in the intensive treatment arm
Venous whole blood samples were collected from an intravenous line at one-hour intervals, followed by three-hour intervals when values were stabilized in the target range. Blood glucose values were measured at the bedside with a Point-Of-Care testing glucometer (Accu-Chek inform device; Roche Diagnostics). This device uses a drop of venous whole blood to calculate a plasma-like glucose value in 20 to 30 seconds. In rare cases, blood could not be collected from the intravenous line, and glucose measurements were obtained from finger pricks.

Intensive insulin protocol decision rules
Perfusor adjustments were performed according to decision rules that were implemented in a bedside chart and online calculator. A new infusion rate was based upon the difference between the previous and current glucose value and the corresponding previous infusion rate. When glucose levels were outside the target range, they were measured every hour, when they were within target range, glucose levels were measured every 3 hours.
Around meals:

To compensate for the carbohydrate bolus in meals, additional subcutaneous (s.c.) insulin was given around meals. Patients started with a standard dose of 6 units s.c. insulin. According to the glucose level immediately before every meal, this standard dose was modified when necessary. Two hours after the meal a new glucose level was measured. The difference between the pre- and postprandial glucose is used to calculate a new standard dose for the next meal. In this manner, we both corrected for actual glucose levels and additionally for a patient's personal insulin sensitivity.

In case of hypoglycemia, the protocol specified the administration of concentrated lemonade (or IV 50% glucose if the patient was unconscious) and adjustment of insulin dose. Hypoglycemia was defined as mild (3.4 – 3.8 mmol/L [61 – 68 mg/dL]), moderate (2.8 – 3.3 mmol/L [50 – 60 mg/dL]) or severe (<2.8mmol/L [< 50 mg/dL]).

**Long-term patient management**

Prior to discharge, patients without previously diagnosed diabetes mellitus (DM) underwent an oral glucose tolerance test (OGTT) to evaluate glucose metabolism. Patients were classified into 3 groups: new onset DM (fasting plasma glucose (FPG) ≥126 mg/dL or 2 h post load glucose (PLG) ≥200 mg/dL), impaired glucose metabolism (FPG between 110-124 mg/dL or PLG between 140-198 mg/dL), or normal glucose metabolism (FPG <110 mg/dL and PLG <140 mg/dL).

When new onset DM was diagnosed, further management depended upon randomization group. Patients in the intensive arm were referred to an internist for further intensive glucose management with subcutaneous insulin aiming at an HbA1c level <7.0% (53 mmol/mol). Patients in the conventional arm were treated at the discretion of the internist, which usually consisted of oral treatment. Approximately 6 weeks after discharge, patients visited the internist again for further diabetes management according to diabetic state and prevailing guidelines, irrespective of the treatment arm.
Blood sample handling

We collected multiple blood samples during hospitalization. Blood samples for the primary endpoint, hsTropT72, were drawn in sodium heparin tubes (Vacutainer SST II Advance; BD and Company), centrifuged and subsequently the plasma was stored at −70°C Celsius using 1.1 mL polypropylene test tubes (Micronics BV). We aimed to complete the process from blood withdrawal to freezing within 2 h in order to minimize pre-analytic variability. Samples were stored until batch analysis took place in the central laboratory (Erasmus MC) in July 2012. For this purpose we used the Elecsys Troponin T hs assay on a Cobas 6000 analyzer (both: Roche Diagnostics).

Sample size

The mean 72 h troponin T value in patients randomized to conventional glucose management was estimated at 1.2-1.4 μg/L, with a standard deviation (SD) of 0.7-1.2 μg/L. (1) We hypothesized that intensive glucose management may reduce this value by 20-30%, resulting in troponin T values between 0.84-1.12 μg/L. In order to declare these differences statistically significant (α = 0.05, 2-sided test) with a power of 80% (β = 0.2), a total of 2× 135 = 270 patients are needed. We assumed a dropout rate of maximal 10%. Hence, the sample size was determined at a total of 300 patients.

Data quality

Several measures were taken to ensure optimal data quality. Prior to entry into the electronic case report form (CRF) data were checked for logic and consistency on an individual basis. When patient enrolment was complete, an independent monitor verified whether data from the electronic CRF, paper CRF and hospital records of 29 (10%) randomly selected patients were aligned. Any issues that appeared during this process were resolved in cooperation with the investigators, no systematic errors were found. Prior to analysis further manual edit checks were performed by the investigators to search for missing data, contradictory data entries, as well as for values that were out of the specified normal range.
Definitions

Recurrent MI was defined as a Troponin or CKMB > Upper normal limit and/or ECG showing new persistent or non-persistent ST-segment elevation >1.0 mm in two or more contiguous leads.

Given that previous values have stabilized (= Stable or decreasing values on 2 samples and 20% increase 3 to 6 hours after second sample). MI subtypes (type 1 – type 5) were recognized according to the universal definition of MI. (2)

End point adjudication

Clinical end points were adjudicated prior to analysis by two independent cardiologists who were blinded for the treatment arm. In case of disagreement a third cardiologist was asked to review the event.

Medical ethics

The design of BIOMArCS-2 Glucose was approved by the Medical Ethics Committee Noord Holland. The trial was registered in the Netherlands trial register (www.trialregister.nl): NTR 1205. All trial participants provided written informed consent. A Data Safety Monitoring Board (DSMB) conducted an interim safety analysis after the first 100 patients had completed the study protocol. The DSMB reported no safety concerns and approved the continuation of the trial.
eReferences


eTable 1a.  Glucose change and outcome by randomization arm

<table>
<thead>
<tr>
<th></th>
<th>Conventional</th>
<th>Intensive</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6hr glucose &lt;140 mg/dL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsTropT72 (ng/L)</td>
<td>1335 (445–2795)</td>
<td>1176 (573–2148)</td>
<td>.75</td>
</tr>
<tr>
<td><strong>6hr glucose ≥140 mg/dL</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>hsTropT72 (ng/L)</td>
<td>1724 (837–3238)</td>
<td>1344 (655–2646)</td>
<td>.66</td>
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<tr>
<td><strong>24hr glucose &lt;140 mg/dL</strong></td>
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<td></td>
</tr>
<tr>
<td>hsTropT72 (ng/L)</td>
<td>1156 (531–2795)</td>
<td>1216 (598–2396)</td>
<td>.76</td>
</tr>
<tr>
<td><strong>24hr glucose ≥140 mg/dL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsTropT72 (ng/L)</td>
<td>2218 (966–3700)</td>
<td>565 (262–1451)</td>
<td>.06</td>
</tr>
</tbody>
</table>

Abbreviation: hsTropT72 = high-sensitivity troponin T 72 hours after admission.
SI conversion: To convert glucose from mg/dL to mmol/L, multiply by 0.0555.
**eTable 1b.** Infarct size in patients with or without persistently elevated glucose levels (≥140 mg/dL) 6 hours after symptom onset

<table>
<thead>
<tr>
<th></th>
<th>6hr glucose ≥140 mg/dL</th>
<th>6hr glucose &lt;140 mg/dL</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsTropT72 (ng/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>1522 (779–3183)</td>
<td>1205 (517–2313)</td>
<td>.09</td>
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<tr>
<td>Conventional arm</td>
<td>1724 (837–3238)</td>
<td>1335 (445–2795)</td>
<td>.22</td>
</tr>
<tr>
<td>Intensive arm</td>
<td>1344 (655–2646)</td>
<td>1176 (573–2148)</td>
<td>.32</td>
</tr>
</tbody>
</table>

Abbreviation: hsTropT72 = high-sensitivity troponin T 72 hours after admission.
SI conversion: To convert glucose from mg/dL to mmol/L, multiply by 0.0555.
**eTable 1c. Association between glucose (change) in patients with or without a clinical event (death or second myocardial infarction)**

<table>
<thead>
<tr>
<th></th>
<th>Event (Death or repeat MI)</th>
<th>No Event (No death or repeat MI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median APG (mg/dL)</td>
<td>171 (144 to 180)</td>
<td>166 (151 to 187)</td>
<td>.49</td>
</tr>
<tr>
<td>Median glucose <em>change</em> in the first 6 hours after admission (mg/dL)</td>
<td>–5.4 (–31 to +4)</td>
<td>–41 (–65 to –13)</td>
<td>.01</td>
</tr>
<tr>
<td>Median glucose <em>change</em> in the first 12 hours after admission (mg/dL)</td>
<td>–40 (–63 to –34)</td>
<td>–52 (–74 to –31)</td>
<td>.62</td>
</tr>
</tbody>
</table>

Abbreviations: APG = admission plasma glucose; hsTropT72 = high-sensitivity troponin T 72 hours after admission; MI = myocardial infarction.

SI conversion: To convert glucose from mg/dL to mmol/L, multiply by 0.0555,
eFigure 1. Cumulative infarct size

Distribution of infarct size per treatment arm for hsTropT72 and infarct extent from myocardial perfusion scintigraphy (MPS). Thick line: Conventional arm. Thin line: Intensive arm
eFigure 2  Treatment effects

A: Effect on enzymatic infarct size for high sensitivity Troponin T 72 hours after admission (left panel) and area under the CKMB curve (right panel), logarithmic scale.

B: Effect on myocardial perfusion scintigraphy (MPS) parameters 6 weeks after the index event; left ventricular ejection fraction (LVEF) (left panel) and infarct extent (right panel)

All values are medians. Conv = conventional treatment arm; Int = intensive treatment arm