Effects of therapeutic hypothermia and kinetics of serum protein S100B after cardiopulmonary resuscitation

BY ÁKOS MÉREI, BÁLINT JÁNOS NAGY, GÁBOR LÁSZLÓ WOTH, NÓRA ZSIDÓ, JÁNOS LANTOS, DIANA MÜHL

Abstract

Introduction. Post-resuscitation care is regulated by international guidelines. A milestone of these is the application of therapeutic hypothermia (TH). The aims of our study were: to determine the 30-day-mortality for our patients, to monitor the efficacy and effects of TH, and to investigate serum protein S100B – as an early prognostic marker.

Materials and Methods. In our study, 57 patients, treated after cardiopulmonary resuscitation (CPR) on a multidisciplinary intensive care unit, were included. Patients were divided into groups who received and who didn’t receive TH. 30-day-mortality was determined as an endpoint. Effects of TH were monitored using statistical analysis according to clinical parameters and laboratory tests. Serum protein S100B levels were measured with ELISA technique on 20 randomised patients at admission and the 1st, 3rd and 5th day after CPR.
Results. Total 30-day-mortality was 74%. TH did not reduce the 30-day-mortality (73% vs. 74%, p>0.05). We found a significant correlation between TH and serum lactate concentration after admission (oh, p=0.006) and at 12 (p=0.045) and 36 (p=0.049) hours after CPR. On the 3rd (p=0.005) and 4th (p=0.043) day after CPR, as a result of TH, platelet count was significantly higher compared to normothermic samples. There was no significant difference in protein S100B levels between the normothermic and TH group and protein S100B levels did not correlate with 30-day-mortality.

Conclusion. Despite recommendations of international guidelines, we cannot prove the beneficial effect of TH, or a correlation of protein S100B levels with a positive outcome.

Keywords: cardiac arrest, cardiopulmonary resuscitation, post-resuscitation care, therapeutic hypothermia, protein S100B

Introduction

Ischaemic heart disease is the leading cause of mortality worldwide. In Europe cardiovascular disorders are responsible for 40% of total mortality. In developed countries the major cause of out-of-hospital sudden death is cardiac arrest with a survival rate of 5-35%. After cardiopulmonary resuscitation (CPR) the rate of the return of spontaneous circulation (ROSC) is 25-50%, but the chance that patients survive without neurological deficit is less than 10%. (1) CPR is regulated by international guidelines. It is well known that post-resuscitation therapy basically determines the neurologic outcome. (2–4)

Hypothermia is the state, when body core temperature is below 36°C. Hypothermia can be classified by its effects on the human body (mild 32-34°C, moderate 28-32°C, deep <28°C). (5) Since the 1950s, moderate hypothermia has been used in cardiac surgery to prevent cerebral ischaemia. It was also applied after cardiac arrest (CA), but has been stopped due to unclear benefits. (6) Therapeutic hypothermia (TH) after ROSC has been recommended by the International Liaison Committee on Resuscitation (ILCOR) since 2003 and by the European Resuscitation
Council (ERC) since 2005. Since 2010 ERC has recommended the use of TH after CPR (32-34°C core temperature for 12-24 hours). TH could be beneficial following primary cerebral injury by preventing further neuronal damage. (5,7) After ROSC one of the major causes of mortality is cerebral injury (68% of out-of-hospital, 23% of in-hospital cases). (4) Former attempts to determine the level of cerebral injury and prognosis, within the first 24 hours with clinical neurological or electrophysiological methods were unreliable. Biochemical markers, such as neuron specific enolase (NSE) and protein S100B (PS100B), may indicate the level of cerebral injury and prognosis in an early phase. (8,9)

In our study the aims were to investigate the survival rate of patients after CPR, ROSC, to determine the efficiency of TH in our practice, to monitor the effects of TH and to measure the level of PS100B as an early predictor of prognosis.

**Materials and Methods**

**Patient selection**

Our study was carried out in accordance with the ethical guidelines of the 2005 Declaration of Helsinki, and permission was obtained from the Institutional Scientific and Human Research Ethics Committee of the University of Pécs. Patients (n=57) treated on the multidisciplinary intensive care unit (ICU) of University of Pécs between June 2009 and February 2012 after CPR, ROSC were included in the study. In our patient group the cause of CA and the initial cardiac electrical activity was heterogeneous (table 1A) Patients with chronic disorders were not excluded (table 1B). A written informed consent was acquired after providing detailed information about the study design and blood sampling to the closest relative of unconscious patients according to national law. In the case of each patient, CPR, defibrillation, drug administration were carried out under the advanced life support recommendation of the Hungarian Resuscitation Committee which complies with the ERC guideline. (10) Different types of initial cardiac rhythm were not excluded. After ROSC ICU therapy was started at the earliest time. To reach the recommended level of TH, 30ml/kg of cold (4°C) 0.9% Saline infusion and additional external cooling (cooler blocks
over great blood vessels) were used. TH was maintained for 24 hours. Core temperatures of the patients were measured at the upper oesophagus. During ICU therapy regular laboratory blood tests (including blood gas parameters, lactate levels, blood cell count, electrolyte levels, C-reactive protein and organ function specific parameters) were taken, blood samples were collected via an arterial cannula, which were inserted at the time of ICU admission according to local protocol. To compare the general condition of patients, the New Simplified Acute Physiology Score (SAPSII) was measured in each case. Patients were separated according to 30-day-mortality. To monitor patient’s early neurological state, the Glasgow Coma Scale (GCS) was used. Investigation of long-term neurological outcome and functional capacity of the survivors was not the aim of the study.

Protein S100B assays

20 randomised patients were chosen to determine PS100B. Blood samples were collected within 2 hours after CPR, intensive care unit admission (1st sample) and after 24, 72 and 120 hours (2nd, 3rd, 4th samples). Native blood samples were centrifuged (1500g, 10min) and stored at –80°C until analysis. PS100B quantitative measurement was performed by sandwich enzyme immunoassay according to manufacturer’s instructions (RD192090100R, BioVendor – Laboratornímedicína a.s., Brno, Czech Republic). The concentrations of PS100B (ng/l) were determined spectrophotometrically at 450nm absorption wavelength in comparison with the standard curve.

Statistical analysis

For statistical analysis SPSS v21 for Windows (IBM SPSS Statistics) was used. Data were expressed as minimum, maximum and median. Non-parametric data were analysed with Mann-Whitney U test. In calculations, p<0.05 was considered significant.

Results

Patient mortality rate

Among the 57 patients (female: 19, male: 38), the overall mean age was 62 years, and there was no difference between the two genders (female:
64y, male: 61y). The duration of CPR was between 1 and 50 minutes (mean: 14.5 minutes). After CPR, TH was applied on 22 patients. The total 30-day-mortality was 74%. There was no difference in the 30-day-mortality between the two genders and the age of the patients did not influence the 30-day-mortality. In our study, TH did not reduce the 30-day-mortality (73% vs. 74%). Neither the duration of CPR nor the initial cardiac electrical activity (ventricular fibrillation, pulseless electrical activity or asystole) influenced the mortality (73% vs 71% vs 78%). SAPSII values were not altered between survivors and non-survivors. Glasgow Coma Scale values on ICU admission were significantly related to 30-day-mortality (p=0.047). To differentiate patients, a GCS cut-off-point of 6 was established. In the GCS<6 group the mortality was significantly higher than in GCS≥6 group (table 2).

**Effects of TH**

In the TH group, serum lactate concentration after ICU admission (oh, p=0.006) and at 12 (p=0.045) and 36 (p=0.049) hours after ROSC was significantly higher compared to NT samples. The median serum lactate concentration after TH was more than twice that in the normothermic (NT) group, at the first measurement (6.3 mmol/l vs 2.8 mmol/l). In both groups, lactate concentrations reached normal range after 12 hours and stayed within this range in the further samples (figure 1). Medians of serum bicarbonate (HCO$_3^-$) in the TH group showed an increasing tendency in the first 96 hours after ROSC. In NT patients, HCO$_3^-$ levels increased to normal serum range until the 12$^{th}$ hour, however, in the TH group elevation was more sustained (normalisation at 36$^{th}$ hour). After that, HCO$_3^-$ concentrations rose to higher than normal range (from the 48$^{th}$ hour in NT group vs. 72$^{th}$ hour in TH group (figure 2). On the 3$^{rd}$ (p=0.005) and 4$^{th}$ (p=0.043) days after ROSC, as a result of TH, platelet count was significantly higher compared to NT samples. In the TH group, median platelet count was higher in each measurement, although neither in NT nor after TH, could higher-than-normal levels be measured (figure 3).

**Protein S100B levels**

Levels of serum PS100B measured in the 4$^{th}$ samples showed a
significant decrease compared to the 1\textsuperscript{st} (p=0.001) and 2\textsuperscript{nd} (p=0.019) samples (figure 4). Among non-survivors, in the 1\textsuperscript{st} and 2\textsuperscript{nd} samples, a tendency to higher serum levels was observed, which disappeared until the 3\textsuperscript{rd} and 4\textsuperscript{th} samples, however the difference was not significant between survivors and non-survivors (figure 5). There was no significant difference in PS100B levels between the NT and TH group (figure 6), but among survivors a marked lower tendency in the TH group occurred (figure 7).

**Discussion**

**Patients’ mortality rate**

The incidence of out-of-hospital cardiac arrest (CA) is approximately 350,000 per year in Europe. (11) Corner stones of management are: early recognition, early basic life support, early defibrillation (if necessary) and early post-resuscitation therapy. After CA global anoxia occurs. The extent of cellular injury is determined by the duration and rate of hypoxia, individual cellular resistance and the extracellular environment. (4)

Cerebral dysfunction which develops after CA can be completely reversible within the first 5 minutes. After this period, ROS production and hypoxia-generated calcium-ion movement causes excitatory neurotransmitter (e.g. glutamate) release leading to further neuronal injury. Most hypoxia-sensitive neurons are located in the basal ganglia, hippocampus and cerebellum. (11) After CA, central nervous system dysfunction may manifest at variable levels of cognitive dysfunction: myoclonus, convulsions, coma and brain death. Cerebral injury may occur via microcirculation- and autoregulation disturbances, hyperpyrexia, hypoxia, hypercarbia or hyperglycaemia. (8)

According to the ERC guideline, published in 2010, TH improves neurological outcome after CPR, consequently a reduction in total mortality has been described in several publications. (12–16) In contrast, in the present study TH did not alter 30-day-mortality. This might have been influenced by the low number of patients and the efficiency of the
cooling method in our unit, although in a recent randomised study Nielsen et al. (17) found that after out-of-hospital cardiac arrest of presumed cardiac cause, hypothermia at a targeted temperature of 33°C did not confer a benefit as compared with a targeted temperature of 36°C. In paediatric practice, TH and consequent microcirculatory impairment was associated with a higher mortality rate. (18) In contrast with Arrich et al., (19) in the present study 30-day-mortality was not influenced by the gender and age of the patients. The duration of CPR, and initial cardiac activity did not significantly influence 30-day-mortality in our study, but earlier findings reported elevated mortality rate after non-shockable rhythms (20) and the duration of CPR as an independent predictor of mortality. (21,22) It is well known that the original ILCOR guideline recommended TH after ventricular fibrillation but for other rhythms the „may also be beneficial“ statement was used. (6) However, over recent years, TH, after non-shockable rhythms, was found to improve outcomes. (23) A GCS<6 consciousness on ICU admission was related to significantly higher mortality rates compared to the GCS≥6 group. Hassan et al. (24) found the same relationship between GCS score and mortality. In recent years „Full Outline of UnResponsiveness“ (FOUR) score has been widely used, which can eliminate the incorrect assignment of verbal function in intubated patients (25,26) but positive correlation between BIS values and GCS scores was also found, and BIS values may predict the post-resuscitative outcome. (27)

**Effects of TH**

Today TH after ROSC is recommended by international guidelines. Since 2010 ERC recommends the use of TH after CPR (32-34°C core temperature for 12-24 hours) as a beneficial method after primary cerebral injury by preventing further neuronal damage. (5,7)

In the present study, serum lactate levels in the TH group were significantly higher than in the NT group on ICU admission and in the 12th and 36th hours after ROSC. In contrast, Bernard et al. (11) measured a significant difference just in the 18th and 24th hour after ROSC. This alteration may be due to the duration of applied TH (12h vs 24h in our practice). In parallel, we found a prolonged normalisation of serum lactate levels in the TH group, which can be explained by decreased
lactate-clearance caused by TH. In the present study, lower normalization tendency of serum bicarbonate concentrations was measured in the TH group compared to the NT group, which was associated with a longer elevation to normal values. This may be influenced by a decreased dissociation rate of carbonic acid at lower temperatures. (4,28) Contrary to an earlier publication (29) – where cold intravenous fluid volume was applied to reach TH, as a result a lower platelet count was measured – we found higher thrombocyte counts in the TH group. In an attempt to find an explanation, no completely acceptable reasons were found, further investigation is required.

**Protein S100B levels**

PS100B is a small acidic protein, a member of the calcium-binding protein family. PS100B is synthesized by astrocytes, oligodendrocytes and Schwann-cells. This protein is the biochemical marker of the integrity of the blood-brain-barrier (BBB). After BBB dysfunction, the PS100B level increases in the cerebrospinal fluid and serum. High PS100B levels correlate with poor survival. (30) Compared to NSE (a dimer enzyme (2-phosph-D-glycerate-dehydrogenase), synthesized by neuroectodermal-origin-cells, also a serum marker of cerebral injury), PS100B was found to be more reliable as an early predictor of poor neurological outcome after CA. (31,32)

We measured decreasing levels of PS100B, from the 1st to 4th samples, that can comply with the relative short half-life (120min) of this protein in the serum. (30) Several human investigations have found that PS100B is a good early predictor of mortality after ROSC (31–34) however, complete agreement cannot be found. In the present investigation there was no significant alteration in PS100B between the survivor and non-survivors groups, based on 30-day-mortality. Our result can be compared to the findings of Song et al. (35) Among 151 patients who experienced out-of-hospital resuscitation, they found that higher levels of PS100B at the start of CPR were significantly associated with lower survival on admission, but the PS100B levels were not different for 1-month survival rates between survivors and non-survivors. We could not measure differences in PS100B levels in the TH and NT groups which might be influenced by the low number of cases, and the same mortality observed
Conclusions

Despite recommendations of international guidelines, efficacy of TH in the ICU after CPR has become questionable in recent years. Results of different trials may be conflicting. Such a tendency can be recognised in connection with the clinical use of PS100B, as an early predictor of mortality and neurological outcome after CA, ROSC. Despite a lack of evidence, the dominance of findings in support seems to be strong. In our study we could not prove the beneficial effect of TH, or a relationship between PS100B levels and positive outcome, although the low case number was a limitation of the present investigation.

Acknowledgements

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References


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**Figure 1.** Serum lactate concentrations of normothermic (NT-grey boxes) and therapeutic hypothermia (TH-striped boxes) groups. Data are expressed as median and inter-quartile range (IQR; standard 25\textsuperscript{th}-75\textsuperscript{th} percentile and 5\textsuperscript{th} and 95\textsuperscript{th} confidence interval). *p<0.05 NT vs. TH

**Figure 2.** Serum bicarbonate concentrations of normothermic (NT-grey boxes) and therapeutic hypothermia (TH-striped boxes) groups. Data are expressed as median and inter-quartile range (IQR; standard 25\textsuperscript{th}-75\textsuperscript{th} percentile and 5\textsuperscript{th} and 95\textsuperscript{th} confidence interval).
**Figure 3.** Thrombocyte count of normothermic (NT-grey boxes) and therapeutic hypothermia (TH-striped boxes) groups. Data are expressed as median and inter-quartile range (IQR; standard 25<sup>th</sup>-75<sup>th</sup> percentile and 5<sup>th</sup> and 95<sup>th</sup> confidence interval). *p<0.05

**Figure 4.** Serum protein S100B levels in study patients. Data are expressed as median and inter-quartile range (IQR; standard 25<sup>th</sup>-75<sup>th</sup> percentile and 5<sup>th</sup> and 95<sup>th</sup> confidence interval). *p<0.05, ns: non-
Figure 5. Serum protein S100B levels among non-survivors (NS – grey boxes) and survivors (S – striped boxes) Data are expressed as median and inter-quartile range (IQR; standard 25th–75th percentile and 5th and 95th confidence interval). *p<0.05, ns: non-significant
Figure 6. Serum protein S100B levels in normothermic (NT – grey boxes) and therapeutic hypothermia (TH – striped boxes) groups. Data are expressed as median and inter-quartile range (IQR; standard 25th-75th percentile and 5th and 95th confidence interval). *p<0.05, ns: non-significant
Figure 7. Serum protein S100B levels in normothermic (NT – grey boxes) and therapeutic hypothermia (TH – striped boxes) groups among survivors. Data are expressed as median and inter-quartile range (IQR; standard 25\textsuperscript{th}-75\textsuperscript{th} percentile and 5\textsuperscript{th} and 95\textsuperscript{th} confidence interval).
*p<0.05, ns: non-significant
Table I.A: Causes of cardiac arrest in the patient group (n=57)

<table>
<thead>
<tr>
<th>Cause of CA</th>
<th>Patient number</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>27</td>
<td>47.3%</td>
</tr>
<tr>
<td>Hypokalaemia</td>
<td>7</td>
<td>12.4%</td>
</tr>
<tr>
<td>Hypovolemia</td>
<td>5</td>
<td>8.7%</td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>3</td>
<td>5.2%</td>
</tr>
<tr>
<td>Acute decompensation of dilated cardiomyopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intoxication</td>
<td>3</td>
<td>5.2%</td>
</tr>
<tr>
<td>Acidosis</td>
<td>2</td>
<td>3.5%</td>
</tr>
<tr>
<td>Subarachnoid haemorrhage with cerebral oedema</td>
<td>2</td>
<td>3.5%</td>
</tr>
<tr>
<td>Hyperkalaemia</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td>Thyrotoxic crisis</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td>Mechanical irritation (by guide wire of central venous catheter)</td>
<td>1</td>
<td>1.8%</td>
</tr>
</tbody>
</table>
Table 1.B: Prevalence of chronic disorders in the patient group (n=57)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Patient number</th>
<th>Prevalence</th>
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</thead>
<tbody>
<tr>
<td><strong>Cardiovascular disorders</strong></td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>35</td>
<td>61.4%</td>
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<tr>
<td>Ischaemic heart disease</td>
<td>14</td>
<td>24.6%</td>
</tr>
<tr>
<td>Dilated</td>
<td>10</td>
<td>17.5%</td>
</tr>
<tr>
<td>cardiomyopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>6</td>
<td>10.8%</td>
</tr>
<tr>
<td>Atrial flutter</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td>Third degree atrioventricular block</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td>16</td>
<td>28%</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic haemodialysis</td>
<td>4</td>
<td>7%</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>3.5%</td>
</tr>
<tr>
<td><strong>Pulmonary disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic pulmonary obstructive disease</td>
<td>16</td>
<td>28%</td>
</tr>
<tr>
<td>Asthma bronciale</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td>Silicosis</td>
<td>2</td>
<td>3.5%</td>
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</table>
Table 2: 30-day-mortality depending on Glasgow Coma Scale at intensive care unit admission

<table>
<thead>
<tr>
<th>GCS at ICU admission</th>
<th>Died</th>
<th>Survived</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>&lt;6</td>
<td>35 (80%)</td>
<td>9 (20%)</td>
<td>44</td>
</tr>
<tr>
<td>≥6</td>
<td>7 (58%)</td>
<td>6 (42%)</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>42 (74%)</td>
<td>15 (26%)</td>
<td>57</td>
</tr>
</tbody>
</table>

GCS: Glasgow Coma Scale, ICU: intensive care unit.