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Published in:
Resuscitation

DOI:
[10.1016/j.resuscitation.2013.01.006](https://doi.org/10.1016/j.resuscitation.2013.01.006)

2013

[Link to publication](#)

Citation for published version (APA):

Dankiewicz, J., Linder, A., Annborn, M., Rundgren, M., & Friberg, H. (2013). Heparin-binding protein: An early indicator of critical illness and predictor of outcome in cardiac arrest. *Resuscitation*, *84*(7), 935-939.
<https://doi.org/10.1016/j.resuscitation.2013.01.006>

Total number of authors:
5

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1 **Heparin Binding Protein: an early indicator of critical**
2 **illness and predictor of outcome in Cardiac Arrest**

3

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19

20 Word count abstract: 242

21 Word count: 2606

22

23

24 Keywords: cardiac arrest
25 outcome
26 neutrophils
27 biomarker
28 heparin binding protein
29 HBP
30 infection
31 hypothermia
32 post cardiac arrest syndrome

33

34

35 **Abstract**

36 **Aim:** To investigate plasma levels of the neutrophil-borne Heparin Binding Protein
37 (HBP) in patients with induced hypothermia after cardiac arrest (CA), and to study
38 any association to severity of organ failure, incidence of infection and neurological
39 outcome.

40 **Methods:** This study included 84 patients with CA of mixed origin who were treated
41 with hypothermia. Plasma samples from 7 time points during the first 72h after return
42 of spontaneous circulation (ROSC) were collected and analysed for HBP with an
43 ELISA. Outcomes were dichotomised: a Cerebral Performance Category scale (CPC)
44 of 1-2 at 6 months follow-up was considered a good outcome, a CPC of 3-5, a poor
45 outcome. Patient data, including APACHE II and SOFA-scores were retrieved from
46 the computerized system for quality assurance for intensive care.

47 **Results:** At 6h and 12h after CA, plasma levels of HBP were significantly higher
48 among patients with a poor outcome. A receiver operated characteristics (ROC)-
49 analysis yielded respective areas under curve (AUC) values of 0.68 and 0.70. This
50 was similar to APACHE II and SOFA-score AUC values. There was a significant
51 correlation between early elevated HBP-values and time to ROSC. HBP-levels were
52 not higher in patients with infections at any time.

53

54 **Conclusions:** Elevated HBP is an early indicator of organ failure and poor
55 neurological outcome after CA, independent of microbial infection, and should be
56 further evaluated in prospective trials. The temporal profile of HBP is suggestive of a
57 role in the pathogenesis of critical illness after CA.

58

59 **Background**

60

61 Survivors of cardiac arrest (CA) suffer from a systemic disease, known as the post-
62 cardiac arrest syndrome (PCAS), which is a result of whole body ischaemia and
63 reperfusion.¹ Approximately half of the patients with return of spontaneous
64 circulation (ROSC) who are treated in the intensive care unit (ICU) regain
65 consciousness, a significant number of whom have brain damage of various degrees.²
66 ³ The other half remain comatose until death which in a majority of patients is due to
67 brain injury, while an approximate third of deaths are caused by cardiac failure or
68 other causes.^{4, 5} Current guidelines recommend the use of a standardized protocol,
69 including induced hypothermia for out-of-hospital CA of cardiac origin and
70 emphasize emergency coronary care, including angiography and percutaneous
71 coronary intervention, when indicated.^{1, 6} As a result, survival rates have improved.⁷
72 In patients remaining in coma, evaluation of prognosis is delayed and intricate due to
73 sedation and prolonged mechanical ventilation.⁸ In lieu of reliable clinical
74 examinations; electroencephalography (EEG), neuroradiology and biochemical
75 markers are increasingly used as adjuncts in prognostication of neurological
76 outcome.⁹ An ideal prognostic marker should be easy to interpret, inexpensive and
77 able to differentiate between good and poor outcome. Potentially useful prognostic
78 biomarkers are neuron-specific enolase (NSE), S-100B, neurofilament and
79 procalcitonin (PCT). Among these, NSE¹⁰⁻¹² and PCT^{13, 14} seem to provide the most
80 information at 24-48h after CA, while early prognostic biomarkers are scarce.
81 Heparin-binding protein (HBP), also known as azurocidin or cationic antimicrobial
82 protein (CAP37), a multifunctional protein stored in neutrophil granules, is an
83 inflammatory mediator and a powerful inducer of endothelial leakage.^{15, 16} HBP has
84 been shown to be an early biomarker of circulatory failure in patients with severe
85 sepsis and shock.¹⁷⁻¹⁹ PCAS has been described as a sepsis-like syndrome,²⁰ involving
86 impaired vasoregulation and endothelial leakage.¹ Our hypothesis was that HBP
87 would be elevated early in patients with PCAS and be associated with severity of
88 critical illness and with outcome, independent of microbial infection.

89

90 **Patients and Methods**

91

92 ***Data collection***

93 Between June 2003 and March 2007, 84 patients treated with induced hypothermia
94 after CA were enrolled in a prospective trial at Lund University Hospital. The study
95 was approved by the Regional Ethical Review Board at Lund University (411/2004,
96 223/2008), and informed consent was sought from next of kin or, retrospectively,
97 from the patient.

98 EDTA plasma samples were obtained during the first 72h, on hospital admission, at
99 2h, 6h, 12h, 24h, 36h, 48h and 72h after CA. Due to missing samples in the admission
100 and 2h-sampling, these two categories were combined and the highest value was used.
101 The samples were centrifuged and frozen (-70°C) immediately after collection. After
102 the end of the study, samples were thawed once, centrifuged at 4000 rpm for 5
103 minutes, aliquoted and refrozen (-70°C) for later analysis.

104 The concentration of HBP was determined by ELISA.²¹ Samples were coded and the
105 researcher performing the analysis was blinded to patient data at the time. Briefly, a
106 mouse monoclonal antibody directed against human HBP (2F23A)²² was used at 1.0
107 µl/ml. Patient plasma samples were diluted 1/40 and each plate also contained
108 calibration samples of known concentration of recombinant human HBP.²³ Plates
109 were incubated with a polyclonal rabbit antiserum towards human HBP diluted
110 1/7000²² and peroxidase-conjugated antibody against rabbit IgG(1/3000) (Bio-Rad,
111 Berkeley, CA, USA).

112 Epidemiological data, and CA-data were collected prospectively, as was data on
113 incidence of infection. Infection was considered present at the discretion of the
114 treating physician, as described earlier.²⁴ A secondary definition of infection was
115 based on the extended and restricted definitions previously used by Scheutz *et al.*²⁵
116 The extended definition included patients with clinical evidence of infection,
117 receiving antibiotics (with or without documented positive bacterial cultures). The
118 restricted definition included only patients with microbiological confirmation of
119 bacterial growth. Time to ROSC was estimated by examining records obtained from
120 the Emergency Medical Technicians (EMT) and by interviewing them, as well as

121 interviewing other caregivers and family members.

122 APACHE II-scores and SOFA-scores were retrieved from the computerized system
123 for quality assurance for intensive care (PASIVA) (Otimo Data AB, Kalmar, Sweden).
124 Lactate levels and fluid balance 24h after CA were retrieved from medical records.
125 Fluid balance was categorized as either positive (≥ 5 litres) or normal (< 5 litres).
126 Lactate was considered elevated above 2.5mmol/l which is the cut-off value at the
127 local laboratory.

128 An intensivist evaluated all patients at discharge from the ICU, and at hospital
129 discharge. Six months later, a neurologist assessed the patients using the Cerebral
130 Performance Categories scale: CPC 1: good cerebral performance, CPC 2: moderate
131 cerebral disability, independent, CPC 3: severe cerebral disability, conscious but
132 dependent, CPC 4: coma, CPC 5: death.²⁶ A CPC score of 1-2 at 6 months was
133 considered a good outcome and a CPC of 3-5 was considered a poor outcome.

134 *Study population*

135 All cardiac arrest patients, regardless of location of arrest or initial rhythm, with
136 return of spontaneous circulation (ROSC) and with sustained unconsciousness
137 ($GCS \leq 7$), were considered for induced hypothermia. Exclusion criteria for
138 hypothermia treatment were terminal disease, intracerebral hemorrhage, aortic
139 dissection or major trauma.

140 A cardiologist initially evaluated all patients. Urgent angiography, PCI and if
141 necessary, circulatory support using intraaortic balloon pump (IABP) counter
142 pulsations was undertaken when indicated. Hypothermia was induced with cold saline
143 (30ml/kg) and maintained with either an external (CritiCool, MTRE Advanced
144 Technologies Ltd., Israel or Arctic Sun, Bard Medical Inc., Louisville, USA) or an
145 intravenous device (Icy Cath, Zoll Medical Corp., Chelmsford, USA). A bladder
146 probe was used for temperature measurements. Patients received hypothermia for 24h
147 at $33 \pm 1^\circ\text{C}$ and rewarming was controlled at 0.5°C/h . Patients were sedated using
148 propofol 2-4 mg/kg/h and fentanyl 1-3 $\mu\text{g/kg/h}$.²⁷

149 In patients remaining comatose, full intensive care was provided at least 3 days after
150 normothermia, at which time a clinical neurological evaluation was performed. In

151 addition, somatosensory evoked potentials (SSEP), amplitude-integrated
152 electroencephalogram (aEEG) and diffusion weighted magnetic resonance
153 tomography (DW-MRI) were added in many patients as a basis for a decision on level
154 of care.⁹

155 **Statistical methods**

156 Frequency comparison was done by Fischer's exact test. The non-parametric Mann-
157 Whitney U-test was used for comparing the good and poor outcome groups, as the
158 data was not normally distributed. Bonferroni corrections were used for multiple
159 comparisons. The discriminatory ability of HBP was calculated by receiver operating
160 characteristic (ROC) analysis, corresponding area under curve (AUC)-values were
161 calculated. For correlations between continuous variables the Spearman rank
162 correlation was used. A two-tailed p-value was used, $p < 0.05$ was considered
163 significant. The Software GraphPad Prism version 5.0 was used for all calculations.
164 All values are medians, unless otherwise stated. Distributions are expressed as inter-
165 quartile ranges.

166

167 **Results**

168

169 Eighty-four patients were included and one was excluded due to lack of all samples.
170 The final study included 83 patients with CA of mixed origin, including cardiac and
171 non-cardiac causes. Fifty-seven patients were men (69%). The median time from
172 cardiac arrest to ROSC was 20 minutes (IQR 14-30). Median APACHE II score was
173 30 (IQR 26-32), median SOFA score day 1 was 9 (IQR 8-11). Sixty-three patients
174 (76%) had an out of hospital CA of cardiac origin. Forty-two patients (51%) had a
175 good outcome and only one patient was alive at six months in the poor outcome group
176 (CPC 3). In 40 deceased patients (48%), the cause of death was classified as brain
177 injury in 29 patients, cardiac disorder in 8 and other causes in 3.⁵ Patient
178 characteristics, dichotomized by good and poor outcome are shown in table 1. A total
179 of 557 samples out of a theoretical maximum of 664 were analyzed. Missing early
180 samples were largely due to transfer of patients between hospitals, or wards. The main
181 reasons for missing samples between 24-72h were patients dying, or leaving the ICU.

182 Patients with a poor outcome had significantly higher HBP-levels as early as 6h
183 ($p=0.049$) and 12h ($p=0.01$) after CA with Bonferroni corrections (x7) (Table 2). The
184 ROC-analysis yielded corresponding AUC-values of 0.68 and 0.70, respectively
185 (Figure 1). Without the Bonferroni correction, all four values in the first 24h (2h, 6h,
186 12h, 24h) differed between the good and poor outcome groups, but there were no
187 differences in HBP-levels from 36-72h. (Figure 2). Further comparisons were
188 therefore limited to the 2-24h interval. The sub-group of patients with an out of
189 hospital arrest of cardiac origin ($n=63$) had identical results to the entire CA cohort
190 (data not shown).

191 There were significant correlations between the APACHE II-score and the HBP-
192 levels at 6h ($\rho=0.36$, $p=0.01$) and 12h ($\rho=0.30$, $p=0.04$). Day 1 SOFA-score
193 correlated with HBP-levels at 6h ($\rho=0.32$, $p=0.01$). SOFA minus CNS subgroup of
194 the SOFA-score had a similar correlation ($\rho=0.30$, $p=0.01$). When used for
195 prediction of outcome, APACHE II-score and day 1 SOFA-score performed similarly
196 to HBP on ROC-analysis (Figure 1).

197 Patients with elevated lactate levels at 24h had a higher median 6h HBP-value than
198 patients with normal lactate at 24h. This difference was significant ($p=0.04$). HBP-
199 levels at 6h were higher among patients with a positive fluid balance in the first 24h
200 after CA, however the significance disappeared when Bonferroni adjusted ($p=0.09$).
201 Patients who had a circulatory SOFA-score of 4 (high dose vasopressor or
202 levosimendan infusion) at 12h also had a significantly higher median 6h HBP-values
203 than those with a circulatory SOFA-score of 1-3 ($p=0.03$).

204 HBP-levels were not higher among patients with infections at any time point. This
205 held true for all three definitions of infection ($p>0.05$ for all).

206 There was a significant correlation between early elevated HBP-values and time to
207 ROSC. The strongest correlation ($\rho=0.61$) was found for HBP-values at hospital
208 admission ($n=43$, $p<0.001$) (Figure 3). There was also a significant correlation
209 between time to ROSC and outcome, $\rho=0.57$ ($p<0.001$).

210

211

212 **Discussion**

213

214 The present study was conducted to investigate the time course of HBP, its
215 association with critical illness, infections and with outcome at six months after CA.
216 We found HBP to be elevated in a majority of patients during the first 24h after CA.
217 Plasma levels of HBP correlated with severity of critical illness, and patients with
218 poor outcome had significantly higher values at 6h and 12h after CA, indicating its
219 potential as a novel early prognostic marker.

220 Cardiac arrest has been described as a sepsis-like syndrome²⁰ with elevations of
221 several proinflammatory cytokines immediately after successful resuscitation. This
222 inflammatory response has been found to distinguish between survivors and non-
223 survivors.²⁰

224 Discrimination between the inflammatory response to infection and the
225 proinflammatory activity inherent to PCAS cannot be made with either CRP or
226 PCT.²⁵ As HBP has been shown to predict severe sepsis it could be hypothesized that
227 HBP-elevation in this study was due to the presence of infection.^{17, 18} This was
228 thoroughly investigated using both a prospectively recorded definition, as well as two
229 retrospective definitions²⁵ showing that HBP was not higher among patients with
230 infection. This suggests that elevated levels of HBP after CA represent a nonspecific
231 inflammatory response as part of PCAS, rather than a specific response to infection.
232 There may indeed be several factors that affect the inflammatory activity in the CA
233 patient in the ICU, such as use of IABP and vasopressors, but most will probably be
234 concealed by the massive, systemic inflammation caused by PCAS.

235 Early elevated HBP indicated an increased likelihood for elevated lactate at 24h, with
236 a tendency towards a positive fluid balance during the same time period. The interval
237 between elevated HBP and elevated lactate, although speculative, might reflect the
238 role of HBP in the pathogenesis of critical illness as HBP has been shown to increase
239 vascular permeability causing edema and hypoperfusion.^{16, 28} Early elevated HBP also
240 correlated with time to ROSC, SOFA-score day 1 and APACHE-II, indicating an
241 association with impact of the initial insult and consequent severity of critical illness.
242 The best correlation between HBP and time to ROSC was at admission ($\rho=0.61$)
243 (Figure 3). Time to ROSC is a well-established prognostic factor,^{29, 30} but its accuracy

244 is unreliable, therefore HBP might be used to elucidate latency to ROSC and thus
245 quantify the burden of reperfusion injury.

246 There is an increasing interest in biomarkers as adjuncts in prognostication of survival
247 and neurological outcome after CA. NSE and PCT can predict neurological outcome
248 starting at 24-48h after CA with acceptable sensitivity and specificity.¹⁰⁻¹⁴ Here we
249 show that HBP may predict outcome earlier, which is consistent with pre-clinical
250 studies of HBP.¹⁶ HBP is mainly derived from neutrophils²¹ and is the only granule
251 protein in neutrophils that is released from both secretory vesicles and azurophilic
252 granules. Secretory vesicles release HBP rapidly upon neutrophil activation, while
253 azurophilic granules are mobilized slowly. The fall in HBP in the first 12h after CA is
254 thus likely due to complete initial secretion from secretory vesicles, though it may be
255 compounded by reduced leukocyte function due to hypothermia. Interestingly, the
256 median and range of HBP after CA was lower than in previous reports of patients
257 with severe sepsis and shock (analyzed in the same lab),^{17, 19} where azurophilic
258 granules most likely also are activated. We hypothesize that a limited activation of
259 leukocytes after CA might hint towards a specific inflammatory response elicited by
260 CA and reperfusion injury.

261 In the study by Linder *et al* on HBP and severe sepsis, a cut-off value of 15ng/ml was
262 suggested (Figure 2) with an 88.4% positive predictive value for severe sepsis.¹⁷ The
263 proinflammatory state inherent to PCAS probably necessitates a different reference
264 range. However, a relevant cut-off point could not be established in this study.

265 Study limitations include a long storage time in the freezer for the plasma samples
266 and although not likely, given the stability of HBP, an effect on the results cannot be
267 ruled out. The time of storing prior to centrifugation is crucial but was not measured
268 and may indeed have affected the results since HBP is released from granules in
269 neutrophils. A prospective trial on HBP should take this into account. The
270 interpretation of changes in HBP-values is also limited by lack of understanding for
271 HBP's kinetics, especially excretion and degradation. Although elevated HBP-levels
272 after serious burns dropped to almost normal values after 48h,³¹ less is known about
273 HBP's dynamics in prolonged critical illness such as after CA. This made the
274 sometimes relatively large variances in a single patient difficult to interpret, especially

275 without precise knowledge of when adverse events occurred in the ICU. The strengths
276 of the study include its prospective design and serial sampling from a relatively large
277 and well-defined patient cohort.

278 **Conclusions.**

279 An early elevation of HBP was seen in a majority of patients after cardiac arrest,
280 independent of infection. Early elevation of HBP correlates with time to ROSC,
281 severity of critical illness and is indicative of long-term neurological outcome. The
282 temporal profile of HBP points towards a role in the pathogenesis of post-cardiac
283 arrest syndrome.

284

285
286

287 Abbreviations

288 HBP, heparin-binding protein; WBC, white blood cells; SIRS, systemic inflammatory
289 response syndrome; ICU, Intensive Care Unit; SOFA, Sepsis-related Organ Failure
290 Assessment; APACHE, Acute Physiology and Chronic Health Evaluation, ROSC;
291 Return of spontaneous circulation, CA; Cardiac arrest.

292

293 Potential conflicts of interest

294 None.

295

296 Author's contributions

297 JD participated in the study design, analyzed data, performed statistical analysis and
298 wrote parts of the manuscript. AL participated in the study design, performed the
299 HBP analysis, analyzed data and wrote parts of the manuscript. MA participated in
300 the study design, included patients and collected data. MR participated in the study
301 design and collected data. HF participated in the study design, analyzed data and
302 wrote parts of the manuscript. All authors read and approved the final manuscript.

303

304 Acknowledgements

305 This project was supported in part by the Swedish Research Council (projects 7480
306 and 13413), the Royal Physiographic Society, Lund, the Swedish Government Funds
307 for Clinical Research (ALF), the University Hospital in Lund, Regional Research
308 Support Region Skåne, Hans-Gabriel and Alice Trolle-Wachtmeisters Foundation,
309 ThureCarlsson Foundation, Hansa Medical AB, Laerdal Foundation and the

310 Foundations of Greta and Johan Kock, Alfred Österlund, and Torsten and
311 RagnarSöderberg.

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315 **Table 1. Patient characteristics (n=83), dichotomized by good and poor outcome**

	Good outcome n=42 (51%)	Poor outcome n=41 (49%)	p-value
Age(n=83)	63 (54-74)	72 (57-78)	0.11
Time to ROSC(n=78)	15 (2-20)	30 (20-45)	<0.001
Time to hypothermia (n=81)	225 (149-122)	190 (122-225)	0.02
APACHE II (n=83)	28 (25-31)	31 (28-35)	0.02
SOFA day 1 (n=82)	9 (7-9)	11 (9-13)	<0.001
Male sex (n=57)	32 (56%)	25 (44%)	0.16
Out-of-hospital(n=73)	38(52%)	35 (48%)	0.52
Initial VF(n=57)	32 (56%)	25 (44%)	0.16
Coronary disease (n=83)	12 (43%)	16 (57%)	0.36
Congestive heart failure (n=83)	7 (50%)	7 (50%)	1.00
Infection [#] in ICU (n=83)	19 (59%)	13 (41%)	0.26
Initial motor-GCS 1-2(n=78)	18 (37%)	31(63%)	<0.001

316

317 **Good and poor outcome -group comparisons. Age in years and time in minutes, expressed as**
318 **median with interquartile range in brackets. VF; Ventricular fibrillation. GCS; Glasgow Coma**
319 **Scale. [#]Pneumonia or sepsis in the intensive care unit (ICU).**

320

321
322

323 **Table 2. Temporal profile of heparin-binding protein, dichotomized by good and**
324 **poor outcome**

Time after cardiac arrest	Good outcome	Poor outcome	p-value
2h [#]	13.2 (9.7-56.0)	37.3 (14.9-60.4)	<i>NS</i>
6h	10.0 (5.8-19.6)	18.4 (10.8-30.2)	0.049
12h	8.0 (6.0-12.7)	11.8 (9.1-21.6)	0.021
24h	9.1 (6.9-16.5)	13.0 (9.2-22.3)	<i>NS</i>
36h	12.5 (8.4-21.1)	18.3 (10.8-30.8)	<i>NS</i>
48h	12.4 (8.6-25.1)	15.8 (9.9-24.0)	<i>NS</i>
72h	11.2 (7.7-16.7)	13.1 (9.5-19.6)	<i>NS</i>

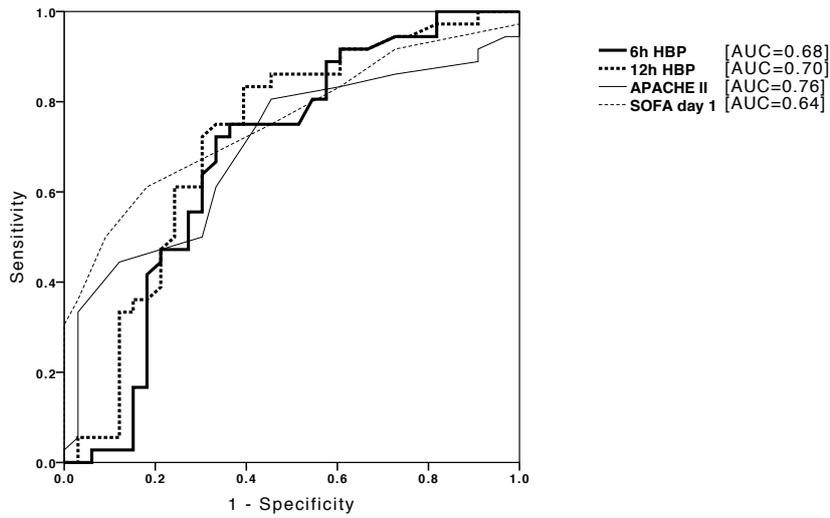
325 All values are ng/ml. [#]Highest value within 2h of cardiac arrest. Comparisons of heparin-binding
326 proteins in patients with a good or poor outcome, Bonferroni corrections (x7) included. Inter-
327 quartile range in brackets. *NS*: Not significant

328

329 **Table 3. Correlation between heparin-binding**
330 **protein and critical illness-scores.**

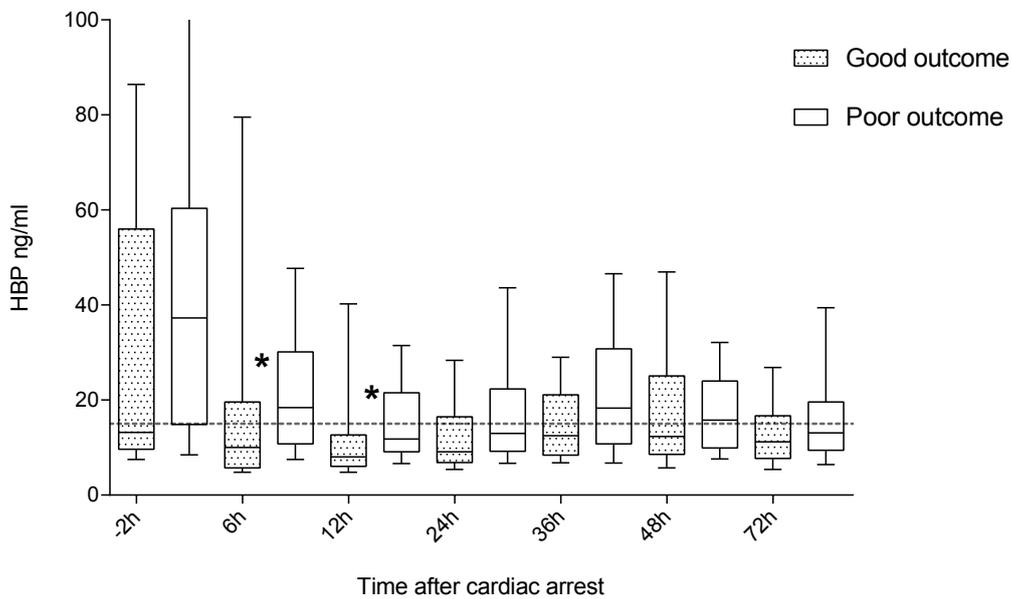
Time	APACHE-II score	SOFA-score day 1
2h	0.13	0.24
6h	0.36*	0.32*
12h	0.30*	0.27
24h	0.09	0.14

331 **Correlation (Spearman's rank correlation coefficient) between**
332 **heparin-binding protein and APACHE II and SOFA-scores**
333 **during the first 24h after Cardiac Arrest. *=p<0.05**
334
335



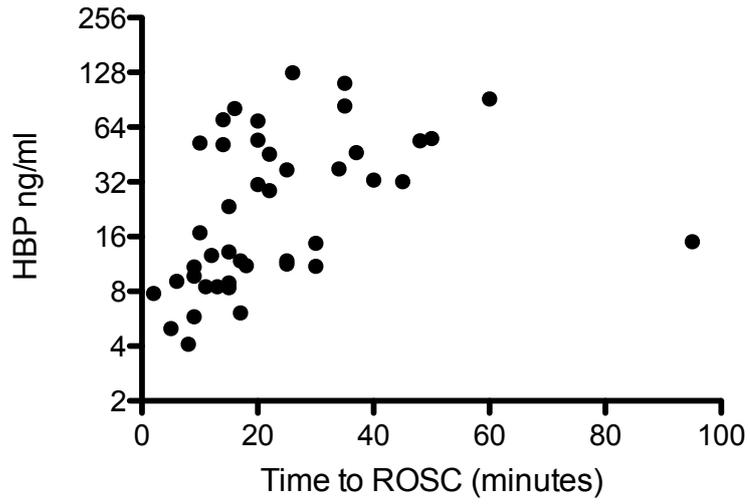
336
 337 Fig. 1 Receiver operating characteristics (ROC) curves for heparin binding protein (HBP),
 338 APACHE II and SOFA day 1, for discrimination between good and poor outcome (CPC1-2 vs.
 339 CPC 3-5, 6 months after cardiac arrest). CPC; Cerebral Performance Category. AUC ; Area
 340 Under Curve

341



342

343 Fig. 2 Time plot of heparin-binding protein (HBP) at 2-72h after cardiac arrest. A Cerebral
 344 Performance Category (CPC) of 1-2 was considered good and a CPC of 3-5, poor. Ticked
 345 line represents suggested cut-off value for predicting severe sepsis in infectious disease.¹⁷
 346 Boxes represent inter-quartile range with a line at the median, whiskers 0.1-0.9. Values and
 347 inter-quartile range in Table 2. * p<0.05



348
349 | Fig. 3 Correlation between levels of heparin-binding protein (HBP) on admission to hospital
350 | and time to return of spontaneous circulation (ROSC).
351 | Correlation coefficient 0.61 (n=43, p<0.001)
352

- 356 1. Nolan JP, Neumar RW, Adrie C, Aibiki M, Berg RA, Bottiger BW, et al. Post-
357 cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and
358 prognostication. A Scientific Statement from the International Liaison Committee
359 on Resuscitation; the American Heart Association Emergency Cardiovascular
360 Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the
361 Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on
362 Clinical Cardiology; the Council on Stroke. *Resuscitation*. 2008;79:350-79.
- 363 2. Longstreth WT, Jr., Inui TS, Cobb LA, Copass MK. Neurologic recovery after
364 out-of-hospital cardiac arrest. *Ann Intern Med*. 1983;98:588-92.
- 365 3. Cronberg T, Lilja G, Rundgren M, Friberg H, Widner H. Long-term neurological
366 outcome after cardiac arrest and therapeutic hypothermia. *Resuscitation*.
367 2009;80:1119-23.
- 368 4. Laver S, Farrow C, Turner D, Nolan J. Mode of death after admission to an
369 intensive care unit following cardiac arrest. *Intensive Care Med*. 2004;30:2126-8.
- 370 5. Dragancea I, Rundgren M, Englund E, Friberg H, Cronberg T. The influence of
371 induced hypothermia and delayed prognostication on the mode of death after
372 cardiac arrest. *Resuscitation*. 2012.
- 373 6. Deakin CD, Morrison LJ, Morley PT, Callaway CW, Kerber RE, Kronick SL, et al.
374 Part 8: Advanced life support: 2010 International Consensus on
375 Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science
376 with Treatment Recommendations. *Resuscitation*. 2010;81 Suppl 1:e93-e174.
- 377 7. Herlitz J. Report from the Swedish Cardiac Arrest Registry; 2011. www.hlr.nu.
378 Swedish Resuscitation Council; 2012.
- 379 8. Rossetti AO, Oddo M, Logroscino G, Kaplan PW. Prognostication after cardiac
380 arrest and hypothermia: a prospective study. *Ann Neurol*. 2010;67:301-7.
- 381 9. Cronberg T, Rundgren M, Westhall E, Englund E, Siemund R, Rosen I, et al.
382 Neuron-specific enolase correlates with other prognostic markers after cardiac
383 arrest. *Neurology*. 2011;77:623-30.
- 384 10. Rundgren M, Karlsson T, Nielsen N, Cronberg T, Johnsson P, Friberg H.
385 Neuron specific enolase and S-100B as predictors of outcome after cardiac arrest
386 and induced hypothermia. *Resuscitation*. 2009;80:784-9.
- 387 11. Oksanen T, Tiainen M, Skrifvars MB, Varpula T, Kuitunen A, Castren M, et al.
388 Predictive power of serum NSE and OHCA score regarding 6-month neurologic
389 outcome after out-of-hospital ventricular fibrillation and therapeutic
390 hypothermia. *Resuscitation*. 2009;80:165-70.
- 391 12. Bouwes A, Binnekade JM, Kuiper MA, Bosch FH, Zandstra DF, Toornvliet AC,
392 et al. Prognosis of coma after therapeutic hypothermia: a prospective cohort
393 study. *Ann Neurol*. 2012;71:206-12.
- 394 13. Fries M, Kunz D, Gressner AM, Rossaint R, Kuhlen R. Procalcitonin serum
395 levels after out-of-hospital cardiac arrest. *Resuscitation*. 2003;59:105-9.
- 396 14. Stammet P, Devaux Y, Azuaje F, Werer C, Lorang C, Gilson G, et al. Assessment
397 of procalcitonin to predict outcome in hypothermia-treated patients after cardiac
398 arrest. *Crit Care Res Pract*. 2011;2011:631062.

- 399 15. Linder A, Soehnlein O, Akesson P. Roles of heparin-binding protein in
400 bacterial infections. *J Innate Immun.* 2010;2:431-8.
- 401 16. Gautam N, Olofsson AM, Herwald H, Iversen LF, Lundgren-Akerlund E,
402 Hedqvist P, et al. Heparin-binding protein (HBP/CAP37): a missing link in
403 neutrophil-evoked alteration of vascular permeability. *Nat Med.* 2001;7:1123-7.
- 404 17. Linder A, Christensson B, Herwald H, Bjorck L, Akesson P. Heparin-binding
405 protein: an early marker of circulatory failure in sepsis. *Clin Infect Dis.*
406 2009;49:1044-50.
- 407 18. Chew MS, Linder A, Santen S, Ersson A, Herwald H, Thorlacius H. Increased
408 plasma levels of heparin-binding protein in patients with shock: a prospective,
409 cohort study. *Inflamm Res.* 2012;61:375-9.
- 410 19. Linder A, Akesson P, Inghammar M, Treutiger CJ, Linner A, Sundén-Cullberg J.
411 Elevated plasma levels of heparin-binding protein in intensive care unit patients
412 with severe sepsis and septic shock. *Crit Care.* 2012;16:R90.
- 413 20. Adrie C, Adib-Conquy M, Laurent I, Monchi M, Vinsonneau C, Fitting C, et al.
414 Successful cardiopulmonary resuscitation after cardiac arrest as a "sepsis-like"
415 syndrome. *Circulation.* 2002;106:562-8.
- 416 21. Tapper H, Karlsson A, Morgelin M, Flodgaard H, Herwald H. Secretion of
417 heparin-binding protein from human neutrophils is determined by its
418 localization in azurophilic granules and secretory vesicles. *Blood.* 2002;99:1785-
419 93.
- 420 22. Lindmark A, Garwicz D, Rasmussen PB, Flodgaard H, Gullberg U.
421 Characterization of the biosynthesis, processing, and sorting of human
422 HBP/CAP37/azurocidin. *J Leukoc Biol.* 1999;66:634-43.
- 423 23. Rasmussen PB, Bjorn S, Hastrup S, Nielsen PF, Norris K, Thim L, et al.
424 Characterization of recombinant human HBP/CAP37/azurocidin, a pleiotropic
425 mediator of inflammation-enhancing LPS-induced cytokine release from
426 monocytes. *FEBS Lett.* 1996;390:109-12.
- 427 24. Nielsen N, Sunde K, Hovdenes J, Riker RR, Rubertsson S, Stammet P, et al.
428 Adverse events and their relation to mortality in out-of-hospital cardiac arrest
429 patients treated with therapeutic hypothermia. *Crit Care Med.* 2011;39:57-64.
- 430 25. Schuetz P, Affolter B, Hunziker S, Winterhalder C, Fischer M, Balestra GM, et
431 al. Serum procalcitonin, C-reactive protein and white blood cell levels following
432 hypothermia after cardiac arrest: a retrospective cohort study. *Eur J Clin Invest.*
433 2010;40:376-81.
- 434 26. Jennett B, Bond M. Assessment of outcome after severe brain damage. *Lancet.*
435 1975;1:480-4.
- 436 27. Rundgren M, Rosen I, Friberg H. Amplitude-integrated EEG (aEEG) predicts
437 outcome after cardiac arrest and induced hypothermia. *Intensive Care Med.*
438 2006;32:836-42.
- 439 28. Herwald H, Cramer H, Morgelin M, Russell W, Sollenberg U, Norrby-Teglund
440 A, et al. M protein, a classical bacterial virulence determinant, forms complexes
441 with fibrinogen that induce vascular leakage. *Cell.* 2004;116:367-79.
- 442 29. Nielsen N, Hovdenes J, Nilsson F, Rubertsson S, Stammet P, Sunde K, et al.
443 Outcome, timing and adverse events in therapeutic hypothermia after out-of-
444 hospital cardiac arrest. *Acta Anaesthesiol Scand.* 2009;53:926-34.
- 445 30. Skrifvars MB, Varghese B, Parr MJ. Survival and outcome prediction using the
446 Apache III and the out-of-hospital cardiac arrest (OHCA) score in patients treated

447 in the intensive care unit (ICU) following out-of-hospital, in-hospital or ICU
448 cardiac arrest. Resuscitation. 2012;83:728-33.
449 31. Johansson J, Lindbom L, Herwald H, Sjoberg F. Neutrophil-derived heparin
450 binding protein--a mediator of increased vascular permeability after burns?
451 Burns. 2009;35:1185-7.
452
453