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

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Abstract

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

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

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

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

Background

Survivors of cardiac arrest (CA) suffer from a systemic disease, known as the post-cardiac arrest syndrome (PCAS), which is a result of whole body ischaemia and reperfusion.[1] Approximately half of the patients with return of spontaneous circulation (ROSC) who are treated in the intensive care unit (ICU) regain consciousness, a significant number of whom have brain damage of various degrees.[2, 3] The other half remain comatose until death which in a majority of patients is due to brain injury, while an approximate third of deaths are caused by cardiac failure or other causes.[4, 5] Current guidelines recommend the use of a standardized protocol, including induced hypothermia for out-of-hospital CA of cardiac origin and emphasize emergency coronary care, including angiography and percutaneous coronary intervention, when indicated.[1, 6] As a result, survival rates have improved.[7] In patients remaining in coma, evaluation of prognosis is delayed and intricate due to sedation and prolonged mechanical ventilation.[8] In lieu of reliable clinical examinations; electroencephalography (EEG), neuroradiology and biochemical markers are increasingly used as adjuncts in prognostication of neurological outcome.[9] An ideal prognostic marker should be easy to interpret, inexpensive and able to differentiate between good and poor outcome. Potentially useful prognostic biomarkers are neuron-specific enolase (NSE), S-100B, neurofilament and procalcitonin (PCT). Among these, NSE[10-12]and PCT[13, 14] seem to provide the most information at 24-48h after CA, while early prognostic biomarkers are scarce. Heparin-binding protein (HBP), also known as azurocidin or cationic antimicrobial protein (CAP37), a multifunctional protein stored in neutrophil granules, is an inflammatory mediator and a powerful inducer of endothelial leakage.[15, 16] HBP has been shown to be an early biomarker of circulatory failure in patients with severe sepsis and shock.[17-19] PCAS has been described as a sepsis-like syndrome,[20] involving impaired vasoregulation and endothelial leakage.[1] Our hypothesis was that HBP would be elevated early in patients with PCAS and be associated with severity of critical illness and with outcome, independent of microbial infection.

Patients and Methods

Data collection

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

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

The concentration of HBP was determined by ELISA.[21] Samples were coded and the researcher performing the analysis was blinded to patient data at the time. Briefly, a mouse monoclonal antibody directed against human HBP (2F23A)[22] was used at 1.0 µl/ml. Patient plasma samples were diluted 1/40 and each plate also contained calibration samples of known concentration of recombinant human HBP.[23] Plates were incubated with a polyclonal rabbit antiserum towards human HBP diluted 1/7000[22] and peroxidase-conjugated antibody against rabbit IgG(1/3000) (Bio-Rad, Berkeley, CA, USA).

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

interviewing other caregivers and family members.

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

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

Study population

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

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

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

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

Statistical methods

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

Results

Eighty-four patients were included and one was excluded due to lack of all samples. The final study included 83 patients with CA of mixed origin, including cardiac and non-cardiac causes. Fifty-seven patients were men (69%). The median time from cardiac arrest to ROSC was 20 minutes (IQR 14-30). Median APACHE II score was 30 (IQR 26-32), median SOFA score day 1 was 9 (IQR 8-11). Sixty-three patients (76%) had an out of hospital CA of cardiac origin. Forty-two patients (51%) had a good outcome and only one patient was alive at six months in the poor outcome group (CPC 3). In 40 deceased patients (48%), the cause of death was classified as brain injury in 29 patients, cardiac disorder in 8 and other causes in 3. [5] Patient characteristics, dichotomized by good and poor outcome are shown in table 1. A total of 557 samples out of a theoretical maximum of 664 were analyzed. Missing early samples were largely due to transfer of patients between hospitals, or wards. The main reasons for missing samples between 24-72h were patients dying, or leaving the ICU.

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

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

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

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

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

Discussion

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

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

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

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

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

There is an increasing interest in biomarkers as adjuncts in prognostication of survival and neurological outcome after CA. NSE and PCT can predict neurological outcome starting at 24-48h after CA with acceptable sensitivity and specificity.[10-14] Here we show that HBP may predict outcome earlier, which is consistent with pre-clinical studies of HBP.[16] HBP is mainly derived from neutrophils[21] and is the only granule protein in neutrophils that is released from both secretory vesicles and azurophilic granules. Secretory vesicles release HBP rapidly upon neutrophil activation, while azurophilic granules are mobilized slowly. The fall in HBP in the first 12h after CA is thus likely due to complete initial secretion from secretory vesicles, though it may be compounded by reduced leukocyte function due to hypothermia. Interestingly, the median and range of HBP after CA was lower than in previous reports of patients with severe sepsis and shock (analyzed in the same lab),[17, 19] where azurophilic granules most likely also are activated. We hypothesize that a limited activation of leukocytes after CA might hint towards a specific inflammatory response elicited by CA and reperfusion injury.

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

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

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

Conclusions.

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

Abbreviations

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

Potential conflicts of interest

None.

Author's contributions

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

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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*

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

Table 2. Temporal profile of heparin-binding protein, dichotomized by good and 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)	*NS*
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)	*NS*
36h	12.5 (8.4-21.1)	18.3 (10.8-30.8)	*NS*
48h	12.4 (8.6-25.1)	15.8 (9.9-24.0)	*NS*
72h	11.2 (7.7-16.7)	13.1 (9.5-19.6)	*NS*

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

Table 3. Correlation between heparin-binding 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

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

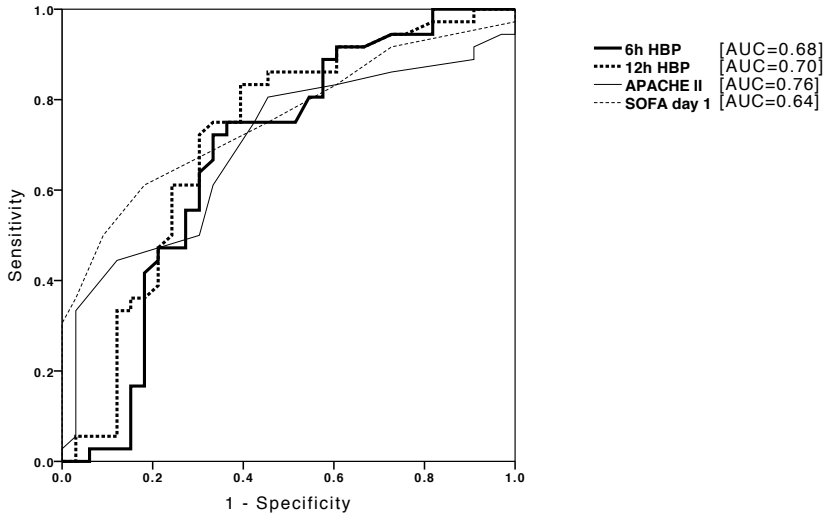

Fig. 1 Receiver operating characteristics (ROC) curves for heparin binding protein (HBP), APACHE II and SOFA day 1, for discrimination between good and poor outcome (CPC1-2 vs. CPC 3-5, 6 months after cardiac arrest). CPC; Cerebral Performance Category. AUC ; Area Under Curve

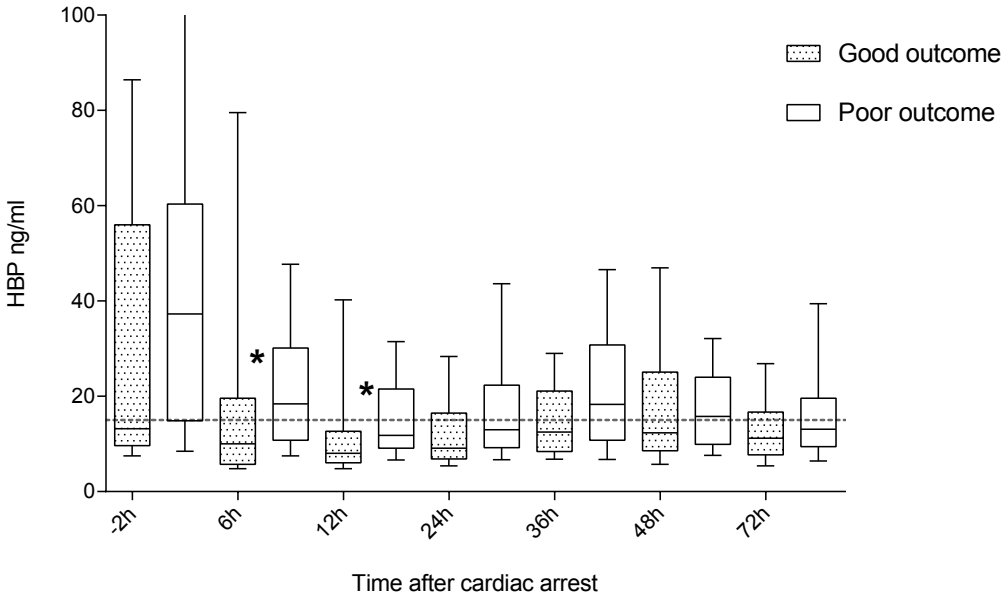

Fig. 2 Time plot of heparin-binding protein (HBP) at 2-72h after cardiac arrest. A Cerebral Performance Category (CPC) of 1-2 was considered good and a CPC of 3-5, poor. Ticked line represents suggested cut-off value for predicting severe sepsis in infectious disease.[17] Boxes represent inter-quartile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

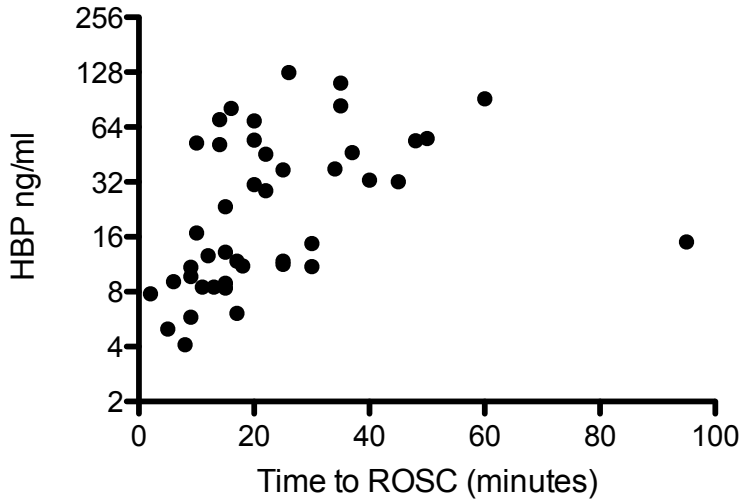

Fig. 3 Correlation between levels of heparin-binding protein (HBP) on admission to hospital and time to return of spontaneous circulation (ROSC).
Correlation coefficient 0.61 (n=43, p<0.001)

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