# Heparin Binding Protein

## An early indicator of critical illness and predictor of outcome in Cardiac Arrest

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Master's Thesis in Medicine

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## Populärvetenskaplig sammanfattning:

Hjärtstopp utanför sjukhus är vanligt i Sverige. Cirka 10 000 personer drabbas varje år och bara 5-600 överlever. Trenden är dock positiv och andelen som överlever har ökat stadigt de senaste tio åren. Viktiga anledningar till detta är adekvat hjärt-lungräddning, snabba transporter, tidig defibrillering med så kallad hjärtstartare och högkvalitativ vård på sjukhus.

De patienter som överlever till sjukhus behandlas numera med kylbehandling för att minska hjärnskador. Denna kylbehandling gör det svårt att bilda sig en uppfattning om hur patientens hjärna och nervsystem fungerar. För att kunna prognostisera och underlätta beslut om t.ex. avslutande av intensivvård används bland annat olika röntgenmetoder, registrering av hjärnbarkens elektriska aktivitet (EEG) och olika blodprover.

I denna studie har ett sådant blodprov undersökts. Provet undersökte förekomsten av äggviteämnet Heparin bindande protein (HBP) och togs på 83 patienter som drabbats av hjärtstopp och fått kylbehandling. Från tidigare studier vet man att HBP finns i vita blodkroppar och är inblandat i reglering av blodkärls genomsläpplighet och kunde förutsäga vilka patienter som skulle komma att utveckla svår blodförgiftning (sepsis) på en infektionsakut.

Nivåerna av HBP i blodet var högre än vad man kan förvänta sig hos friska personer hos nästan alla hjärtstoppspatienter. Dessutom var nivåerna vid 6 och 12 timmar efter hjärtstoppet högre hos de patienter som sedermera avled eller fick svåra hjärnskador. Det fanns också ett samband mellan nivåerna av HBP i blodet och två olika system (APACHE II och SOFA) som klassificerar hur svårt sjuka patienter på intensivvårdsavdelningar är.

Sammanfattat visade det sig alltså att HBP-nivåerna var högre hos svårt sjuka patienter och hos patienter som avled. Eventuellt skulle därför ett HBP-prov i akutskedet efter hjärtstopp kunna användas som en del i prognostiseringen för patienten.

## Abstract

**Purpose:** The purpose of this study was to investigate plasma levels of Heparin Binding Protein (HBP) in patients with induced hypothermia after cardiac arrest (CA) and study any correlation to severity of organ failure and neurological outcome.

**Methods:** This study included 84 consecutive patients with CA of mixed origin treated with hypothermia. Plasma samples from 7 different time points during the first 72 hours were collected and analysed for HBP with ELISA. Outcomes were dichotomised: a Cerebral Performance Category scale (CPC) of 1 or 2 at 6 months follow-up was considered a good outcome, a CPC of 3 or higher, a poor outcome. APACHE II-scores and SOFA-scores were retrieved from the computerized system for quality assurance for intensive care. Lactate was considered elevated at 2.5mmol/l.

**Results:** At 6 and 12 hours 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. The 6h HBP-level correlated with APACHE II and SOFA-scores and predicted lactate elevation at 24h

**Conclusions:** Elevated HBP may be considered an early indicator of organ failure and poor neurological outcome after CA. The temporal profile of HBP is suggestive of a role in the pathogenesis of hypoperfusion and shock after CA.

**Introduction** Survivors of cardiac arrest (CA) suffer from a systemic syndrome known as the post-cardiac arrest syndrome (PCAS), which is a result of whole body hypoxia and reperfusion [1]. Among patients with return of spontaneous circulation (ROSC) and treated in the intensive care unit (ICU), approximately half regain consciousness. The other half remain comatose until death. A significant number of survivors suffer permanent brain damage of different degrees [2, 3]. Cause of death after ROSC is primarily due to neurological damage (approximately 60%), and an approximate third of deaths are caused by multiple organ failure [4].Two randomized trials showed a treatment benefit with induced hypothermia[5, 6], and guidelines recommend this treatment for comatose CA-patients [7, 8]. In these patients, neurological prognosis is delayed several days due to mechanical ventilation and sedation [9]. In lieu of reliable clinical examination; electroencephalography (EEG), neuroradiology and biochemical markers are used as adjuncts in prognostication of neurological outcome [10]. An ideal biochemical prognostic marker would not only be able to differentiate between good and poor outcome, but also help anticipate severity of critical illness. Among biomarkers investigated as potentially useful are: neuron-specific enolase (NSE), S-100B, neurofilament and procalcitonin (PCT). Among these, NSE [10-12] and PCT seem to provide the most information; they appear to be of significant value at 24-48 hours after CA [13, 14]. Heparin-binding protein (HBP), also known as azurocidin or cationic antimicrobial peptide (CAP37), an antimicrobial protein stored in neutrophil granules, is a potent inflammatory mediator and inducer of endothelial leakage [15, 16]. HBP has been shown to be a potential early biomarker of circulatory failure in patients with sepsis and shock [17, 18] The pathology of PCAS involves impaired vasoregulation and endothelial leakage, [19] and is known as a sepsis-like syndrome [20]. Our hypothesis was that HBP would be elevated in patients with PCAS, independent of microbial infection.

## Materials and methods

### Data collection

Between November 2003 and September 2006, 84 consecutive patients treated with therapeutic hypothermia after CA were enrolled in a prospective trial at Lund University Hospital. Plasma samples were obtained during the first 72 hours, at 0h, 2h, 6h, 12h, 24h, 36h and 72h after CA. Due to a substantial amount of missing samples and inconsistency in the 0h and 2h-sampling, these 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 (Bio-Rad) (1/3000).

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.

APACHE II scores and SOFA-scores were retrieved from the computerized system for quality assurance for intensive care (PASIVA). Lactate levels and fluid balance day 1 were retrieved from medical records. Fluid balance was categorized as either

positive (≥5 litres) or normal (<5 litres). Lactate was considered elevated at 2.5 mmol/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. Hypothermia was initiated as soon as possible after ROSC in the emergency room or catheterization laboratory using 30 ml/kg cold saline and subsequent treatment in the ICU was performed as described earlier [27].

A cardiologist initially evaluated all patients. Urgent angiography, PCI and if necessary, circulatory support using intraaortic balloon pump counter pulsations was undertaken when indicated. Patients received hypothermia for 24 hours at 33+/-1°C and rewarming was controlled at 0.5 °C/h. Patients were sedated using propanol 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 as a basis for a decision on level of care [28].

**Statistical analysis**

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.

**Ethics**

The study was approved by the local ethics committee at the Regional Ethical Review Board at Lund University (411/2004, 223/2008) informed consent was sought from next of kin or, retrospectively, from the patient.

**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%)(Table 1). 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 7.8-11). Sixty-three patients (76%) had an out of hospital CA of cardiac origin. Forty-two patients (53%) had a good outcome and only one patient was alive at six months in the poor outcome group (CPC 3). 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 24-72 hours 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 ($p=0.010$) after CA with Bonferroni corrections (x7). 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 24 hours (2h, 6h, 12h, 24h) differed significantly between the good and poor outcome groups. There were no differences in HBP-levels from 36-72h. (Figure 2, Table 2). Further comparisons were therefore limited to the 2-24h interval. The sub-group of patients who suffered an out of hospital arrest of cardiac origin had identical results (data not shown).

There were significant correlations between the APACHE II score and the HBP-levels at 6h ($rho=0.36$) and 12h ($rho=0.30$). Day 1 SOFA-score correlated with HBP-levels at 6h ($rho=0.32$) (Table 3). When used for prediction of outcome, APACHE-II-score and day 1 SOFA-score performed similarly to HBP on ROC-analysis. (data not shown)

Patients with elevated lactate levels at 24h had a higher median 6h HBP-value than patients with normal lactate at 24h. This different was significant ($p=0.036$) (Figure 3) HBP-levels were higher among patients with a positive fluid balance in the first 24h after CA, however the significance disappeared when adjusted.

HBP-levels could not discriminate between patients with or without infections, either with the prospectively recorded definition or the retrospectively recorded definitions by Scheutz *et al* [25].

There was a significant correlation between early elevated HBP-values (2, 6 and 12 hours) and a prolonged time to ROSC. The respective correlation coefficients were 0.35, 0.42 and 0.43. An even stronger correlation (0.61) was found when comparing HBP-values at hospital admission (n=43) with time to ROSC.

## Discussion

The present study was conducted to investigate the time course of HBP and its association with critical illness and outcome at six months. We found HBP to be elevated in a majority of patients during the first 24 hours after CA. Plasma levels of HBP correlated with critical illness and patients with poor outcome had significantly higher values already at 6 and 12 hours 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 a variety of proinflammatory cytokines immediately after successful resuscitation. This increase has been found to discriminate between survivors and non-survivors [20].

Proinflammatory activity is also increased in systemic inflammation in response to infection. However in the setting of PCAS, neither CRP nor PCT discriminate between patients with or without infections [25]. HBP has been shown to predict both septic and non-septic shock, regardless of presence of infection [17, 18]. In this study HBP-levels were not higher among patients who had infections. This was thoroughly investigated using both a prospectively recorded definition, as well as two retrospective definitions [21]. Consequently it can be concluded that the elevated levels of HBP after CA represent a nonspecific inflammatory response as part of PCAS, rather than a specific response to infection.

Early elevated HBP indicated an increased likelihood for elevated lactate at 24 hours, moreover there was a tendency towards a positive fluid balance among these patients. The interval between elevated HBP and signs of hypoperfusion 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. The finding that HBP elevation preceded lactate elevation is intriguing and might have clinical relevance in early identification of critically ill PCAS-patients. HBP also correlated with APACHE-II and SOFA-scores thereby indicating an association with critical illness.

Measurement of biomarkers may be useful in prognostication of survival and neurological outcome. Studies on other biomarkers such as NSE and PCT have revealed them to be of use in predicting neurological outcome from 24-48h after CA

[11-14]. Here we show that HBP might be of predictive value at an earlier stage. This time frame is consistent with what is known from pre-clinical studies of HBP. HBP is mainly derived from neutrophils [21] and is the only granule protein in neutrophils that is stored in secretory vesicles, which are rapidly mobilized upon neutrophil activation. 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 induced hypothermia. Interestingly, the median and range of HBP in this study was lower than in previous reports of patients with severe sepsis and shock [17](Linder *et al*, submitted), where azurophilic granules also become activated. This selected and limited activation of leukocytes might hint towards the type of inflammatory response elicited by CA and subsequent reperfusion injury.

HBP had a best rho=0.61 correlation with time to ROSC, which is a well-established prognostic factor [29]. As information on time to ROSC often is unreliable, HBP might be used to elucidate latency to ROSC and quantify the burden of reperfusion injury.

In the study by Linder *et al* on HBP and septic shock a cut-off value of 15ng/ml (Figure 2) was suggested with an 88.4% positive predictive value [15]. 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.

Elevated intracranial pressure caused by cerebral edema has been known to occur to a limited extent after CA [1]. Given the capacity of HBP in increasing vascular leakage and causing edema [16] and that drugs inhibiting HBP release have shown to attenuate edema formation in bacterial meningitis [30] HBP could play a role in this process, thereby correlating with neurological outcome. Although HBP was not measured in cerebrospinal fluid (CSF) in this study, it has previously shown a high sensitivity for detecting bacterial meningitis in CSF [31].

Study limitations include a long storage time for the plasma samples. Although not likely, an effect on the results cannot be ruled out. 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 about 48 hours [32], 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. Regarding HBP's relationship to lactate elevation, there would ideally have been simultaneous sampling of lactate and HBP; this should be part of any future studies. The strengths of this prospective study on consecutive patients include serial sampling from a relatively large and well-defined patient cohort.

**Conclusions.**

An early elevation of Heparin Binding Protein was seen in a majority of patients after cardiac arrest. HBP-values correlate with severity of critical illness and may predict long-term outcome, but it is too early to suggest its use in clinical practice. The temporal profile of HBP points towards a role in the pathogenesis of endothelial leakage and shock.

**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.

**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 | 63 (54-74) | 72 (57-78) | 0.11 |
| Time to ROSC (n=77) | 15 (2-20) | 30 (7-48) | <0.01 |
| Male sex (n=57) | 32(56%) | 25 (44%) | 0.16 |
| Out-of-hospital (n=73) | 38 (52%) | 35 (48%) | 0.52 |
| Initial VT/VF (n=57) | 32 (56%) | 25 (44%) | 0.16 |
| Infection[#] in ICU | 19 (59%) | 13 (41%) | 0.26 |

Good and poor outcome - group comparisons. Age in years and time to ROSC in minutes, expressed as median with IQR in brackets. [#]Pneumonia or sepsis in the intensive care unit (ICU). VT; Ventricular tachycardia, VF; Ventricular fibrillation

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

| HBP ng/ml | Good outcome | Poor outcome | p-value |
|---|---|---|---|
| 2h[#] | 13.2 (9.7-56) | 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 (9.2-22.33) | *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) | *NS* |
| 72h | 11.2 (7.7-16.7) | 13.1 (9.5-19.6) | *NS* |

[#]Highest HBP-value within 2 hours of cardiac arrest. Comparisons of HBP-levels in patients with a good or poor outcome, Bonferroni corrections included. Inter-quartile range in brackets.

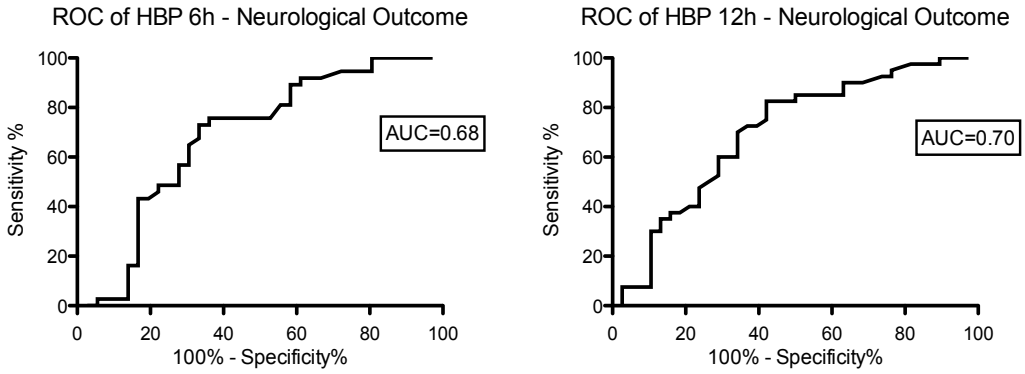


**Figure 1.** Receiver operating characteristics (ROC) curves for heparin binding protein (HBP) and discrimination between good and poor outcome (CPC1-2 versus CPC 3-5). CPC; Cerebral Performance Category.

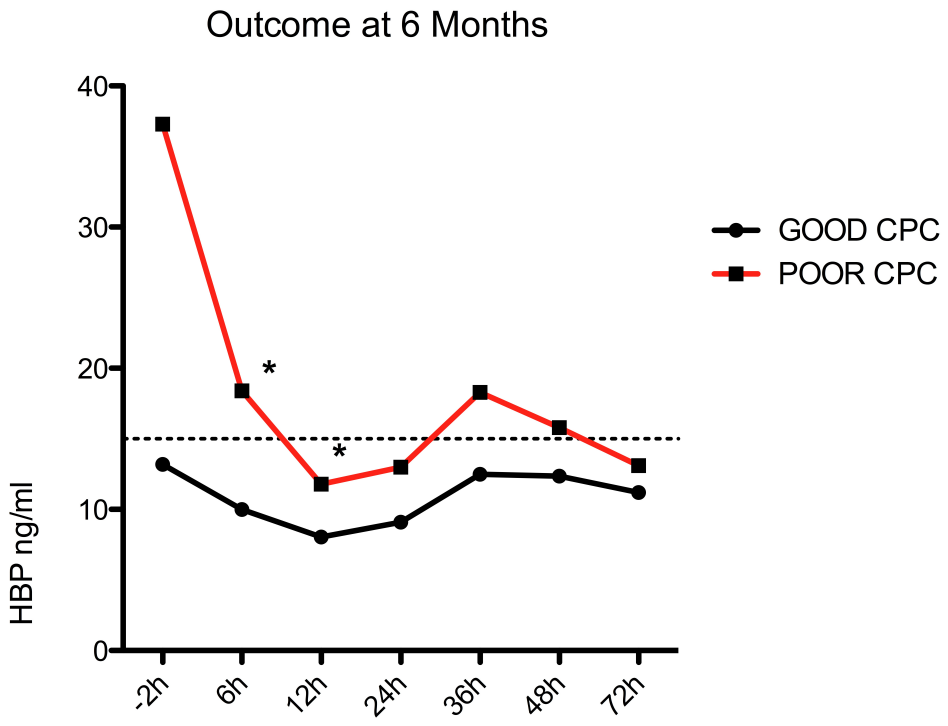


**Figure 2**. Time plot of median HBP-values at 2-72h after cardiac arrest. A Cerebral Performance Category (CPC) of 1-2 was considered good and a CPC 3-5, poor. Ticked line represents suggested cut-off value for predicting septic shock in infectious disease [17]. * $p<0.05$

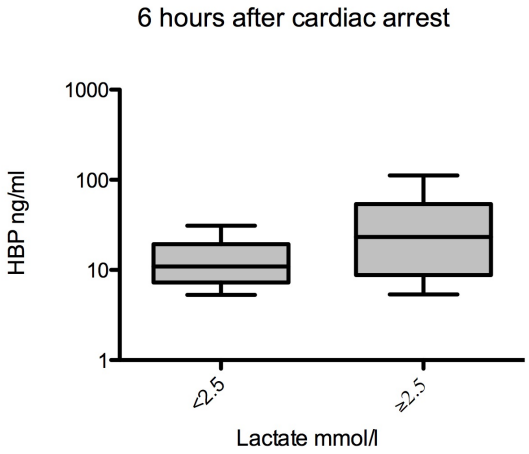


**Figure 1.** Six-hour plasma levels of HBP dichotomized by lactate at 24 hours. Range 0.1-0.9, interquartiles and median. There was a significant difference (p=0.036) in 6h HBP-levels between patients with normal (<2.5 mmol/l, n=42), and elevated (>2.5 mmol/l, n=23) lactate levels at 24h. Median HBP was 23.3 ng/ml in the elevated lactate group and 10.9 ng/ml the normal lactate group.

**Table 3. Correlation between HBP 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 | 0.092 | 0.14 |

Correlation (Spearman's rank correlation coefficient) between HBP-values and APACHE II and SOFA-scores during the first 24h after CA. *=$p<0.05$

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