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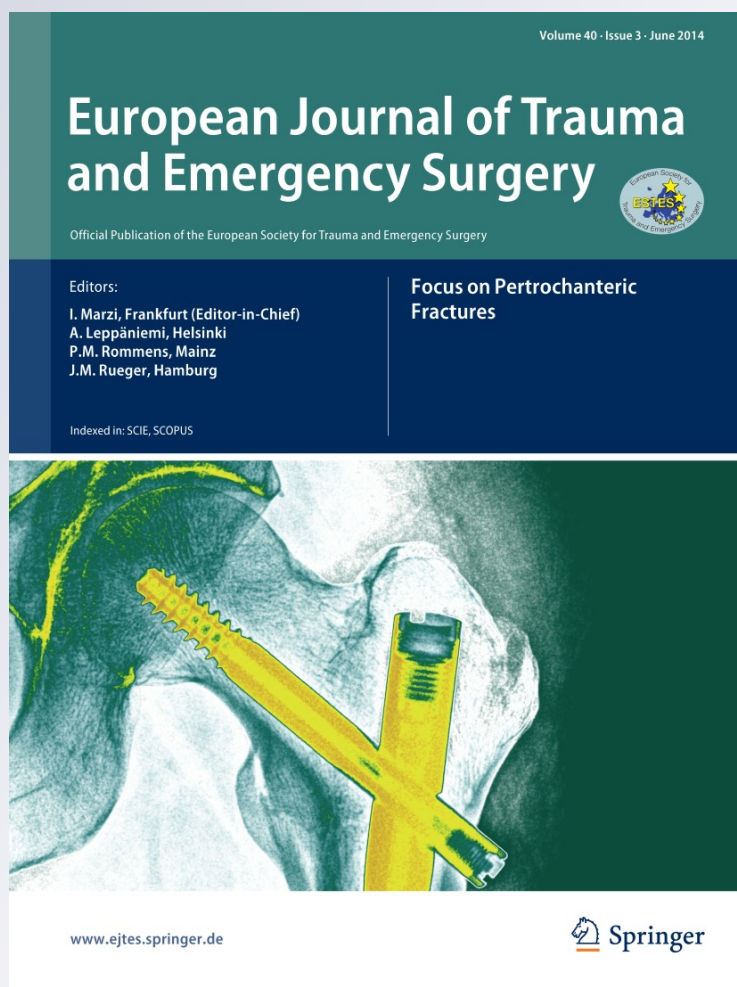
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# The prognostic value of plasma $\Delta$ -copeptin levels in patients with isolated traumatic brain injury

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## Abstract

**Purpose** Traumatic brain injury (TBI) is one of the most common causes of death among trauma patients. Earlier prediction of possible poor neurological outcomes, even upon admission to the emergency department, may help to guide treatment. The aim of this prospective study was to assess the predictive value of plasma copeptin levels for early morbidity and mortality in patients with isolated TBI. **Methods** This prospective study comprised 53 patients who were admitted to the emergency department with isolated TBI. Forty-two of these patients (group I) survived at least 1 month after the TBI; the other 11 (group II) did not. Plasma levels of copeptin were measured in these TBI patients at admission and 6 h after trauma, and were compared with those of healthy volunteers (group III).

**Results** At admission, the copeptin levels of the TBI patients (groups I and II combined) were not statistically significantly different from those of the control group (III). The copeptin levels 6 h after trauma were also not statistically significantly different from those at admission.  $\Delta$ -Copeptin levels (the difference between the copeptin level at the 6th hour after trauma and that at admission) were higher in the patients who died within a month of the TBI. Further,  $\Delta$ -copeptin levels were higher in patients who showed no improvement in the modified Rankin score when compared with patients with an improved modified Rankin score. The best cutoff point for  $\Delta$ -copeptin was 0.51 ng/ml for predicting mortality and 0.23 ng/ml for predicting improvement in the modified Rankin score. **Conclusions** Plasma  $\Delta$ -copeptin levels may help physicians predict the prognoses of patients suffering from traumatic brain injury.

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**Keywords** Copeptin ·  $\Delta$ -Copeptin · Prognostic  
value · Traumatic brain injury

## Introduction

Traumatic brain injury (TBI) is one of the most common causes of death among trauma patients [1]. Predicting a possible poor neurological outcome earlier, even upon admission to the emergency department (ED), may help to guide the physician attempting to accurately predict the clinical outcome and to determine the appropriate follow-up and treatment strategy.

Age, volume of the haematoma, presence of brain oedema, resistant hypertension, and initial Glasgow Coma Scale (GCS) score are predictors of early mortality and neurological disability in patients with TBI [1, 2].

However, a more specific and measurable predictive marker is needed.

Copeptin is synthesised in the hypothalamus with the arginine vasopressin precursor (AVP) and released into the portal circulation via the neurohypophysis [3]. Copeptin has been used as an indicator of acute stress and has received attention as a prognostic marker, mostly in emergent situations. The effects of copeptin on long-term mortality have been widely studied in patients with ischaemic stroke, cardiovascular disease, head trauma, lower respiratory tract infections, spontaneous intracerebral haemorrhage, and sepsis [3–5]. Furthermore, it has been shown that levels of copeptin are correlated with trauma severity [3, 6].

The aim of this prospective study was to assess the predictive value of plasma  $\Delta$ -copeptin (the difference between the copeptin level at the 6th hour after trauma and that at admission) for early morbidity and mortality in patients with isolated TBI.

## Methods

This prospective study was reviewed and approved by the local ethics committee before implementation. Informed consent was obtained from all patients or their families before blood samples were collected.

This prospective study comprised 53 patients who were admitted to the ED with isolated TBI from March 2012 to October 2012. Twenty-one patients with severe TBI who had a post-resuscitation GCS of  $\leq 8$  were included. Thirteen patients were classified with moderate TBI (GCS 9–13), and 19 had mild TBI (GCS  $\geq 14$ ). Exclusion criteria included the use of antiplatelet or anticoagulant medication and the presence of other systemic diseases, including uraemia, liver cirrhosis, malignancy, chronic heart or lung disease, diabetes mellitus, hyperlipidaemia, obesity and hypertension. Moreover, patients who suffered from other severe, multiple, life-threatening organ injuries such as heart contusion and shock were excluded from the study. The control group consisted of 32 healthy volunteers whose brain magnetic resonance imaging revealed no pathology and who had no neurological or systemic disease.

Blood samples were obtained at admission and 6 h later for the biochemical analysis of plasma copeptin levels following TBI. Only one fasting blood sample was taken at 8 a.m. from all volunteers in the control group.

The patients were divided into the following groups:

Group I ( $n = 42$ ): patients who survived at least 1 month after TBI

Group II ( $n = 11$ ): patients who died within 1 month after TBI

Group III ( $n = 32$ ): controls

## Clinical and radiological assessments

Upon admission to ED, all patients were evaluated and treated on the basis of the guidelines for the management of TBI [2]. Admission GCS, pupil size and reactivity, body temperature, heart rate, respiratory rate, blood oxygen saturation and blood pressure results were recorded. A routine blood analysis for sodium concentration, glucose and white blood cell count was also performed. All patients were evaluated with computed tomography (CT) as soon as the vital signs were stabilised and a neuroradiologist evaluated the CT results, and the presence of an intracranial lesion (contusion, subarachnoid haemorrhage, intracranial haematoma, epidural haematoma or subdural haematoma) or skull fracture (linear or depressed) were noted. All patients in groups I and II were treated in the neurosurgical intensive care unit.

## Determination of plasma copeptin levels

Venous blood samples were collected into EDTA-containing and aprotinin-containing tubes (Phoenix Pharmaceuticals, Burlingame, CA, USA). Blood samples were centrifuged at 1,600 rpm for 15 min, and plasma was separated and stored at  $-80^{\circ}\text{C}$  until analysis.

Measurements of copeptin were performed on an EPOCH system (BioTek Instruments, Inc., Winooski, VT, USA) using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals) in accordance with the manufacturer's instructions. The assay range of the copeptin ELISA kit was 0–100 ng/ml. Copeptin levels were expressed as ng/ml. All samples were analysed together in the same assay.

$\Delta$ -Copeptin was calculated as the difference between the copeptin level at the 6th hour ( $t_6$ ) and the level at admission ( $t_0$ ).

## Outcome assessment

The Glasgow Outcome Scale (GOS) was used to assess all patients with TBI. Outcomes of patients with severe TBI were assessed with the modified Rankin score (mRS) at admission and 1 month later. The cause of death for all patients in group II was TBI. Plasma copeptin levels were compared with the mRS and mortality ratio.

## Sample size estimation

The primary aim of this study was to compare the  $\Delta$ -copeptin levels of groups I and II. A total sample size of 45

(36 patients in group I and 9 in group II, because the allocation ratio was 4.0) was required to detect a difference of at least 0.85 (ng/ml) between these groups with a power of 85 % at the 5 % significance level. This difference of 0.85 (ng/ml) was assigned based on both a pilot study and our clinical experiments. Sample size estimation was performed using the NCSS and PASS 2000 software packages.

### Statistical analysis

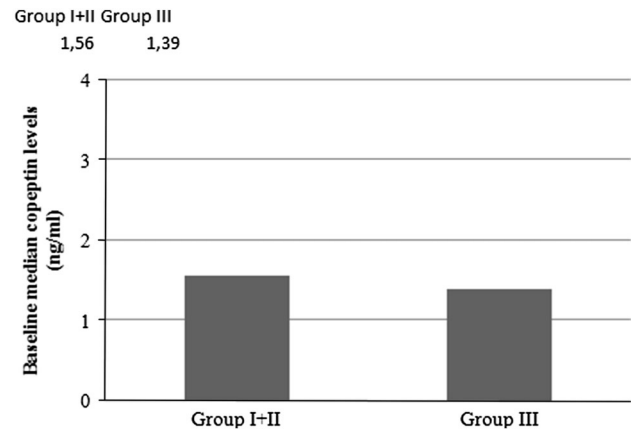
Data analysis was performed using SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to determine whether the distributions of continuous variables were normal. Data are presented as the mean  $\pm$  standard deviation or the median (range), where appropriate. The differences in the mean ages of groups I and II and the control group were compared with Student's *t* test, and the Mann–Whitney *U* or Kruskal–Wallis test was applied to compare median values, according to the number of independent groups. Nominal data were analysed by Fisher's exact test. The Wilcoxon signed-rank test was used to determine the difference between the median copeptin level at baseline and that at the 6th hour after trauma. The optimal cutoff points for  $\Delta$ -copeptin ( $t_6-t_0$ ) to determine mortality and improvement in mRS were evaluated by receiver operating characteristic (ROC) analyses, by calculating the area under the curve (AUC) and determining the sensitivity and specificity of the significance test. A *p* value of  $<0.05$  was considered statistically significant.

### Results

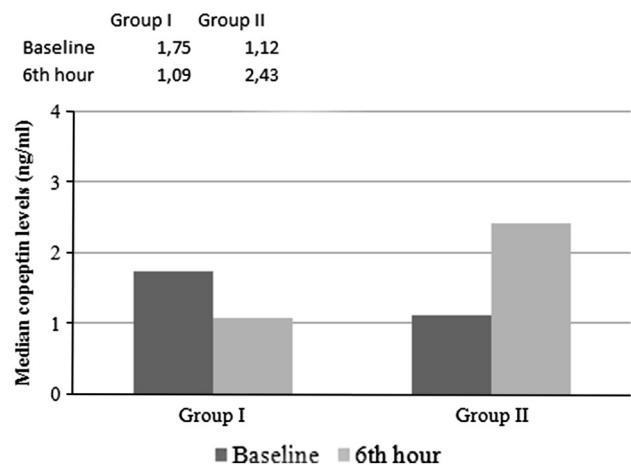
Of the 53 TBI patients, 42 survived for more than 1 month (group I), and 11 patients died within 1 month following the TBI (group II). The overall mortality rate was 20.7 %. Patients in group I ranged in age from 1 to 92 years (mean age  $24.6 \pm 24.2$  years), those in group II ranged from 2 to 80 years (mean age  $35.2 \pm 28.8$  years), and those in the control group ranged from 4 to 79 years (mean age  $26.9 \pm 22.7$  years). No significant difference was observed among the study groups in terms of mean age or gender. Furthermore, age was not determined to be a predictor of outcome among the subgroups of moderate and severe TBI.

The median copeptin level of the TBI patients at admission (groups I and II,  $n = 53$ , male/female ratio 1.30) was 1.56 ng/ml (range 0.3–9.2 ng/ml), which was higher than that for the control group (group III,  $n = 32$ , male/female ratio 1.28; 1.39 ng/ml; range 0.3–3.4 ng/ml) (Fig. 1), but this difference was not statistically significant ( $p = 0.618$ ).

A slight increase in copeptin level was observed at hour 6 (1.7 ng/ml; range 0.3–7.5 ng/ml) when compared with its



**Fig. 1** Baseline median copeptin (ng/ml) levels for the TBI patients (groups I + II) and the control group (group III)



**Fig. 2** Median copeptin (ng/ml) levels at baseline and at the 6th hour after TBI for groups I and II

level at admission (1.6 ng/ml; range 0.3–9.2 ng/ml), but this difference was not statistically significant ( $p = 0.518$ ). Median copeptin (ng/ml) levels at baseline and at the 6th hour after TBI for groups I and II are shown in Fig. 2.

$\Delta$ -Copeptin levels were significantly higher in group II than in group I ( $p < 0.001$ ). In addition,  $\Delta$ -copeptin levels were higher in patients who showed no improvement in mRS than in patients with an improved mRS ( $p < 0.001$ ) (Table 1).

The best cutoff level for  $\Delta$ -copeptin was 0.51 ng/ml for predicting mortality and 0.23 ng/ml for predicting improvement in mRS (Table 2). The best cutoff level was 0.51 ng/ml to predict patient mortality with 90.9 % sensitivity and 90.5 % specificity (AUC 0.959;  $p < 0.001$ ). The best cutoff level was 0.23 ng/ml to predict improvement in mRS with 100 % sensitivity and 90.9 % specificity (AUC 0.991;  $p < 0.001$ ) (Table 2).

**Table 1** Δ-Copeptin levels in patient groups subdivided according to mortality and mRS

Variables	Difference in copeptin (Δ-copeptin)
<b>Status</b>	
Group I	-0.1 (-6.6-1.4)
Group II	1.4 (0.3-4.4)
<i>p</i> value	<0.001
<b>mRS</b>	
Improved to better mRS	-0.3 (-6.6-0.4)
No improvement	1.4 (0.3-4.4)
<i>p</i> value	<0.001

**Table 2** Results of an ROC analysis of the use of Δ-copeptin to determine mortality and improvement in mRS

Statistic	Definition	Mortality	Improvement in mRS
AUC		0.959	0.991
<i>p</i> value		<0.001	<0.001
Best cutoff point		>0.51	>0.23
Number of cases	<i>n</i>	53	21
Sensitivity	TP/(TP + FN)	10/11 (90.9 %)	10/10 (100.0 %)
Specificity	TN/(TN + FP)	38/42 (90.5 %)	10/11 (90.9 %)
PPV	TP/(TP + FP)	10/14 (71.4 %)	10/11 (90.9 %)
NPV	TN/(TN + FN)	38/39 (97.4 %)	10/10 (100.0 %)

AUC area under the curve, TP true positive, FN false negative, TN true negative, FP false positive, PPV positive predictive value, NPV negative predictive value

Upper limit was found to be higher than 1.000

**Table 3** Copeptin levels in patients with different GOS scores at baseline and at the 6th hour after TBI

Variables	Baseline	6th hour	<i>p</i> value <sup>a</sup>	Difference
<b>GOS score</b>				
1 ( <i>n</i> : 11)	1.1 (0.4-6.2)	2.4 (0.7-7.5)	0.003	1.4 (0.3-4.4)
4 and 5 ( <i>n</i> : 42)	1.7 (0.3-9.2)	1.1 (0.3-5.6)	0.007	-0.1 (-6.6-1.4)

GOS Glasgow Outcome Scale

<sup>a</sup> Comparison between baseline and 6th-hour measurements within a GOS group

The positive predictive value for Δ-copeptin for mortality was 71.4 %, and the negative predictive value was 97.4 %. The positive predictive value for improvement in mRS was 90.9 %, and the negative predictive value was 100 % (Table 2). No significant change in copeptin level based on GCS was observed (*p* = 0.156). However, a significant difference in copeptin level was observed between patients whose GOS was 1 and those whose GOS was 4 or 5 (*p* < 0.01) (Table 3). In this study cohort, no patient had a GOS of 2 or 3.

**Discussion**

This study showed that the increase in copeptin levels 6 h after head trauma was an independent factor for predicting both mortality and clinical deterioration. Copeptin is gaining attention as a unique and useful prognostic marker that is correlated with disease severity and the degree of activation of the stress axis [7]. Copeptin has even been shown to be superior to cortisol for determining the severity of stress because cortisol has a circadian rhythm and is challenging to measure as a free hormone [8]. In the present study, Δ-copeptin was used to assess short-term mortality in terms of mRS and GCS in patients with TBI.

Recent studies have shown that copeptin is a useful predictor of short- and long-term mortality and length of hospital and intensive care unit stay in patients with acute myocardial infarction, chronic obstructive pulmonary disease, multiple trauma, sepsis, congestive heart failure and ischaemic stroke [9-14]. Zhang et al. [15] reported that copeptin levels are independent prognostic indicators for impaired nerve function 90 days after spontaneous intracerebral haematoma, independent of the volume of the haematoma, GCS, Hemphill score, white blood cell count and blood glucose level. They also reported that high copeptin levels might be a predictor of brain oedema and its severity. In another study, copeptin levels were correlated with the volume of haematoma and clinical status in patients with a spontaneous acute intracerebral haematoma [6]. An animal study showed that copeptin levels are correlated with brain oedema in an ischaemic brain model [16].

Plasma levels of copeptin and its predictive effects on head trauma have been widely studied [4, 5, 9]. Dong et al. [5] reported that copeptin levels increased following TBI when compared with those in a control group. Their study showed a significant increase in plasma copeptin levels 6 h after TBI. They concluded that high plasma levels of copeptin were correlated with high mortality. Our study demonstrated a slight difference in plasma copeptin levels between the TBI patients and the control group. Another slight difference between the plasma copeptin levels at baseline and at the 6th-hour after TBI was also demonstrated. However, these differences were not statistically significant. We believe that this lack of a significant difference in plasma copeptin levels between the groups in our study is due to the small number of patients included in it, and the fact that most of the study cohort consisted of mild TBI patients.

Westermann et al. [9] reported that initial AVP levels in patients with multiple trauma were higher than those in a control group in which head trauma had no additional effects on plasma AVP levels. Furthermore, in that study they showed the existence of a positive correlation between AVP and copeptin.

Kleindienst et al. [4] concluded that head trauma causes increased copeptin levels after 3 days. These high levels were correlated with low GCS and the presence of a midline shift and intracerebral haemorrhage. Previous studies have shown that the discriminatory effectiveness of copeptin level in terms of clinical course and outcome increases when combined with other prognostic factors such as age, GCS, and the volume of a haematoma [6, 17, 18].

Several authors have reported that older age is associated with a higher mortality in TBI patients [19, 20], which conflicts with our results, as no significant effect of age on mortality in TBI patients was seen in our study. We conclude that the small number of patients included in our study cohort is the cause of this difference from other studies.

In our study, no significant correlation was observed between GCS and  $\Delta$ -copeptin levels. However, patients with a GOS of 1 showed significantly higher plasma copeptin levels than patients with a GOS of 4 or 5.

Although mRS is generally used to assess the outcome of ischaemic stroke, it is also useful for assessing TBI [21, 22]. However, to the best of our knowledge, no study has revealed the prognostic effects of  $\Delta$ -copeptin in relation to mortality and mRS in patients with isolated TBI. Our results demonstrate that there is no significant increase in plasma copeptin levels after head trauma. However,  $\Delta$ -copeptin levels were shown to be useful for predicting both mortality and clinical improvement in terms of mRS.

On the other hand, there were some limitations of this study. The number of patients included in the study was limited and could be augmented in a subsequent study. A prolonged assessment utilising neurobehavioral tests may also be needed. Furthermore, since this study was carried out under emergency department conditions, the follow-up duration was limited to 4–6 h, making it impossible to get blood samples that could be used to assess long-time changes in copeptin levels. Further studies with a prolonged follow-up and a more detailed assessment strategy may demonstrate the prognostic value of copeptin levels in TBI patients more effectively.

## Conclusion

Our results showed that an increase in copeptin level 6 h after head trauma is an independent factor predicting both mortality and clinical deterioration. Plasma copeptin levels may help physicians in the ED predict the prognosis of patients suffering from TBI.

**Conflict of interest** None.

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