Hypocalcemia as a prognostic factor of mortality and morbidity in moderate and severe traumatic brain injury and its role with Protein S-100 B

Dissertation

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ABSTRACT

Introduction
Traumatic brain injury (TBI) is responsible for significant mortality following accidents and is treated using diverse therapeutic strategies. The effects of TBI can be severe and result in severe disability and/or death. Despite advances in technology and knowledge about its pathophysiology, there are few prognostic factors to reliably predict outcome. The hypothesis of this study is that serum hypocalcaemia (defined as <2.1 mmol/L [8.5 mg/dL]) and serum ionised calcium (defined as <1.10 mmol/L [4.5 mg/ dL]) are prognostic factors for mortality and morbidity (defined as Glasgow Outcome Score ≤3) following moderate/severe TBI.

Material and Methods
Based on a previous study showing that calcium plays a role in early mortality after moderate/severe TBI, in the **first part of this study**, we retrospectively analysed the available data from n=99 patients treated in our institution from January 2004 to December 2012 . A case-control study design was used. The clinical records were assessed, emphasising the clinical status and radiological findings (CT) on admission and at 12 and 36 hours after TBI. Laboratory results were evaluated on days 0, 3 and 7. In addition ionised calcium serum levels were assessed. On this time of period of nine years, the protocol patient-management was the same.

In the **second part of the study**, a prospective cohort of n=61 patients who sustained TBI from January 2014 to December 2015 was analysed. The clinical status, vital parameters, radiological findings (CT) on admission as well as 12 and 36 hours after TBI was investigated. Laboratory results on day 0, 3 and 7.

Furthermore, according to the hypothesis that proinflammatory proteins and molecules may be the cause of hypocalcaemia after TBI, we decided to include serum protein S-100B (a marker of neuronal damage) and interleukin 6 (a pro-inflammatory cytokine) levels on admission, day 3 and 7 after TBI.

Prospective patient recruitment at the Evangelical Hospital in Oldenburg, Germany was performed with the permission of the ethical committee of Carl von Ossietzky University with the permit number Drs.21/4/2014.

Results
In the **first (retrospective) part** we found a significant difference in ionised serum calcium levels on the third day after admission between patients with Glasgow Outcome Score (GOS) ≤3 (group 1) and >3 (group 2) (p=0.008). According to this result, lower calcium levels were associated with worse outcomes (GOS ≤3).

The final logistic regression model included absent pupillary reactivity, low ionised serum calcium on day three (<1.10 mmol/L) and hyponatremia on day seven after TBI.

An odds ratio (OR) of 3.03 was calculated, with a lower limit of 1.32 and a higher limit of 9.14 (p=0.004), for the association between death/disability and low ionised serum calcium (<1.10 mmol/L) on the third day following TBI.

In analogy, in the **second (prospective) part** we found a statistically significant difference (p=0.009) in ionised serum calcium, protein S-100B (p=0.002), Interleukin 6 (IL-6) (p=0.007) and haemoglobin (p=0.011) levels on the third day after admission between the groups with GOS ≤3 and GOS >3 (death/disability). A statistically significant difference was also observed in haemoglobin levels on the third and seventh day (p=0.011, p=0.020, respectively) and potassium on day seven (p=0.039) between the group1 and group 2.

Significant factors included age, absent pupillary reactivity and ionised serum calcium, as well as protein S-100B and IL-6 levels on day three after TBI. These variables were associated with a GOS ≤3 (death/disability) at discharge.

A relative risk (RR) of 3.14 was calculated, with a lower limit of 2.49 and a higher limit of 3.78 (p=0.05) for the association between death/disability and low ionised serum calcium (<1.10 mmol/L) on the third day following TBI.

Discussion

We expected that non-ionised serum calcium (serum calcium), as seen in previous studies, would be a significant predictive factor regarding TBI outcome. Surprisingly, this was not the case. Rather, in both retrospective and prospective parts of our study, it remained non-significant. In contrast, a more specific calcium measurement, i.e. ionised serum calcium, significantly predicted mortality/morbidity. The overall results between the two patient collectives (retrospective and prospective) were strikingly similar, and supported each other.

The proposed pathophysiological mechanism for this epiphenomenon probably results from calcium depletion due to increased chelation by pro-inflammatory molecules/proteins released by injured neurons into the extracellular space after direct trauma. This leads to a decrease in calcium levels in the intracellular space with consequent Ca^{2+} release from the sarcoplasmic reticulum, thereby activating caspases and resulting in cell death (apoptosis). Moreover, concomitant ongoing metabolic acidosis due to impaired post-traumatic intracranial blood vessel regulation contributes to cellular hypo-oxygenation with consequent mitochondrial dysregulation, further disturbing the fine balance by which intracellular calcium activates caspases and intracellular pathways leading to programed cellular death. Lactate is also produced during this process because of disturbances in the aerobic mitochondrial use of ATP, with further exacerbation of hypocalcaemia through calcium-lactate chelation. S-100B protein plays an important role in this proposed mechanism because of its properties as a calcium binder.

Conclusion

Our findings suggest that hypocalcaemia is a marker of the severity of brain damage, probably as a result of various pathological mechanisms, including direct mechanical trauma, neuro-inflammation, altered vessel autoregulation and hypoxia. The ionised serum calcium levels can be useful for the prediction of mortality and disability in patients following moderate/severe TBI.

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LIST OF ABREVIATIONS

ATLS:	Advanced Trauma Life Support
CA:	Cornu ammonis (Ammon's horn)
Ca++(i):	Ionized Calcium
Ca2+:	Calcium
CBF:	Cerebral Blood Flow
CI:	Confidence Intervals
CT:	Computer Tomography
DAI:	Diffuse Axonal Injury
Fig.:	Figure
FAST	Focus Assessment Sonography in Trauma
GCS:	Glasgow Coma Scale
GOS:	Glasgow Outcome Score
HCO3:	Bicarbonate
ICU:	Intensive Care Unit
IL-6:	Interleukin-6
ISS:	Injury Severity Score
MAP:	Mean Arterial Pressure
kD:	Kilo Dalton (standard unit that is used for indicating mass on an atomic or molecular scale)
mg/dl:	Milligram/Deciliter
Mg2+:	Magnesium
mmol/l:	Millimole/Litre
mV:	Millivolts
pH:	Measure of the acidity or basicity of an aqueous solution
PO_4^{3-}	Phosphate
RR:	Relative Risk
SD:	Standard Deviation
Tab:	Table
TNFα:	Tumor Necrosis Factor alpha

INTRODUCTION

Traumatic brain injury (TBI) is one of the most common disorders within the vast field of neurology. The incidence of TBI in Germany is approximately 332 per 100,000 people in comparison to 182 per 100,000 people for strokes [1-3]. The effects of TBI can result in severe disability or death [4-8] (Fig. 1) and have an important social and economic impact [9]. The annual direct and indirect costs of TBI amount to roughly 2.5 billion Euros [2, 9].

Fig. 1. Young patient after severe traumatic brain injury on mechanical ventilation requiring 24 hour nursing attention.

Despite advances in technology and increasing knowledge about its pathophysiology, there are few predictors for outcome after TBI, with MRI findings being the most important.

MRI performed within the first eight days after head injury has been found to be a reliable predictor of death and moderate/severe disability for patients in a coma and on ventilation following TBI, depending on the location of the lesion [9]. Apart from imaging signs, other easy to assess early predictors of outcome are still missing. As such, there is an on-going effort to identify biological markers that are closely related to clinical symptoms in order to better predict the outcome after TBI. The recent adoption of high throughput technologies and a change in focus from the identification of single to multiple markers has fostered new optimism in this direction [10-13]. Different markers have been studied, particularly bivalent cations such as magnesium (Mg^{2+}) and calcium (Ca^{2+}) [10-13]. Of these, calcium seems to play a more important role in TBI[10,14,15].

1. Calcium in homeostasis.

Calcium is essential for living organisms, in particular in cell physiology, where the movement of Ca^{2+} ions into and out of inducible organelles (such as the sarcoplasmic reticulum) controls many cellular processes. As a major component required for the mineralization of bone and teeth, calcium is the most abundant metal by mass in many animals.

Blood plasma calcium in the human body appears in three forms: 50% free or ionized, 40% bound to plasma proteins and 10% bound to small anions HCO_3^-, citrate, lactate and phosphate (PO_4^{3-}); playing this anion (phosphate) an important role in calcium homeostasis.

2. Hypocalcaemia.

Hypocalcemia is a hydro-electrolytic disorder characterized by a serum calcium level <2.1 mmol/L (8.5 mg/dL), which evokes pathophysiological effects. The same occurs as result of a decrease in the ionized calcium fraction, with normal levels between 1.10 mmol/l and 1.40

mmol/l. The main causes of hypocalcemia are: hyperparathyroidism, acute pancreatitis, vitamin D deficiency, treatment with hydrochlorothiazide (diuretic) and hypoalbuminemia.

At a neuronal level, a decrease in ionized calcium leads to greater neuronal excitability when intracellular calcium is released from sarcoplasmic reticulum. Thus, the excitatory threshold is lowered from 65 to -20 mV, possibly leading to the loss of the inhibitory mechanisms and triggering neurological symptoms such as seizure.

3. General pathophysiology of TBI.

The pathogenesis of TBI can be subdivided in two types. The first (primary injury) occurs by direct damage; the event can be classified as either impact or non-impact, depending on whether the head makes direct contact with an object. A penetrating injury to the brain occurs from the impact of a sharp object that forces hair, skin, bone and fragments of the object into the brain. Objects traveling at a low rate of speed through the skull and brain can ricochet within the skull, which widens the area of damage (Fig. 2). A "through-and-through" injury occurs if an object enters the skull, goes through the brain, and exits the skull. Through-and-through traumatic brain injuries include the effects of penetration injuries, plus additional shearing, stretching and rupture of brain tissue. A non-impact injury occurs when the skull encounters a force such as blast waves or rapid acceleration and deceleration [14-16], as seen in diffuse axonal injury (DAI). The second type of injury (secondary injury) is seen in hypoxia and ischemia/reperfusion injuries, requiring surgical interventions such as decompressive hemicraniectomy performed due to post-traumatic brain edema or craniotomy with evacuation of an epidural or acute subdural hematoma (Fig. 3) inducing a host defense response in some cases [17-23].

The tissue surrounding a traumatic brain lesion is often susceptible to hypoxia and/or ischemia due to the reduction in cerebral blood flow (CBF) through altered autoregulation of intracerebral vessels [19-23].

Traumatic brain injury produces several cellular changes, such as gliosis and dysregulation of inhibitory/excitatory processes, with potential epileptic foci [21,23].

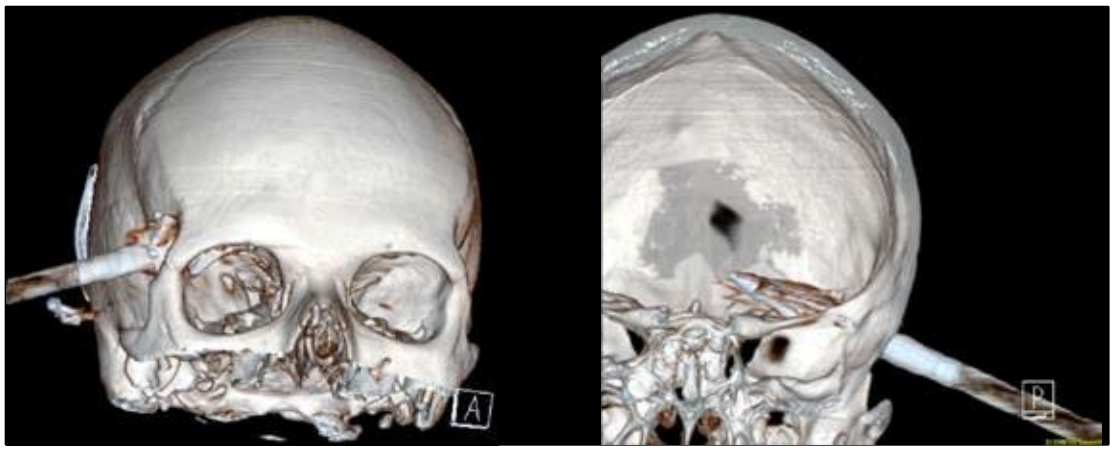

Fig. 2. Penetrating traumatic brain injury with a shear-blunt object passing through the temporal bone with direct injury to brain tissue.

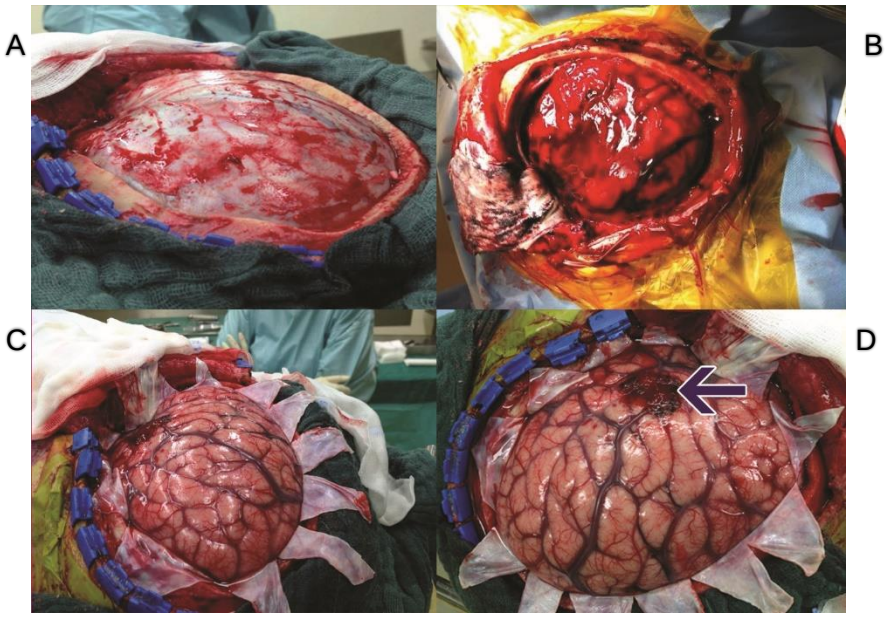

Fig. 3. Surgical procedures in traumatic brain injury. A) Decompressive hemicraniectomy performed due to brain edema. The dura mater is still closed but appears taut because of increased intracranial pressure. B) Craniotomy with acute subdural hematoma. C) Decompressive hemicraniectomy with star-shaped opened dura mater in order to reduce intracranial pressure. D) Craniectomy and dura mater opening showing a frontal contusion (indicated by an arrow). (Courtesy T. Schmidt).

4. The role of calcium in TBI.

At the cellular level after TBI, transmembrane inflow of calcium and outflow of potassium following traumatic deformation of the cellular membrane have been demonstrated; these are accompanied by the release of excitatory neurotransmitters, such as glutamate [24]. This increase in intracellular calcium (evident in acute ischemia) plays a role in driving apoptotic processes, mainly due to the inhibition of mitochondrial enzymatic processes and lipase activation [24-32].

Calcium is crucial for cerebral vessel autoregulation. In TBI mainly the small arteries, arterioles, which lose their ability to either dilate or contract depending on perfusion due to impaired calcium metabolism in the smooth muscle cells within the vessel walls.

The sudden calcium influx leads to the malfunctioning of factors FXa and FIXa in platelets, evoking hypo-coagulability with subsequent hemorrhage. In addition, procoagulant microparticles and microvesicles (such as fibrinogen) are released [33].

Other studies have evaluated neuronal death and basal free intracellular Ca^{2+} in acutely isolated rat CA3 hippocampal neurons using the Ca^{2+} indicator Fura-2 at day 7 and day 30 after moderate central fluid percussion injury. They showed that repercussion leads to a decline in memory skills, suggesting a chronic phase of neuronal death due to calcium neuronal depletion[34].

In humans, TBI survivors often suffer from post-traumatic syndrome with deficits in learning and memory skills. Calcium seems to influence post-traumatic learning and memory deficit. However, the role of long-term changes in neuronal Ca^{2+} function in surviving neurons and the potential impact on TBI-induced cognitive impairment is still poorly understood [35].

5. Factors regulating hypocalcemia in TBI.

Two main factors contribute to hypocalcemia in TBI. Mainly, hypocalcemia results from calcium depletion due to increased chelation to pro-inflammatory molecules/proteins released by injured neurons into the extracellular space after direct trauma. This leads to a decrease in calcium levels in the intracellular space with consequent Ca^{2+} release from the sarcoplasmic reticulum, thereby activating caspases and resulting in cellular death (apoptosis) [24-33, 36, 37]. Moreover, concomitant on-going metabolic acidosis due to impaired post-traumatic intracranial blood

vessel regulation contributes to cellular hypo-oxygenation with consequent mitochondrial dysregulation, further disturbing the fine balance by which intracellular calcium activates caspases and intracellular pathways leading to programmed cellular death. Lactate is also produced during this process because of disturbances in the aerobic mitochondrial use of ATP, with further exacerbation of hypocalcemia through calcium-lactate chelation. These two processes together lead to a decrease in intracellular calcium with the activation of apoptotic pathways (Fig. 4,5).

The abovementioned pathophysiological mechanisms regarding disturbances in the fine balance between calcium, activating caspases and intracellular pathways leading to programed cellular death have been demonstrated in canine models [33, 37].

6. Protein S-100B in TBI

S100B protein, a multi-gene calcium binding protein with a low molecular weight of 9 to 13 kDa, is found in the neuroglia and is released into the extracellular space in acute neuronal damage. It has gained importance as a marker together with clinical parameters such as the grade of trauma and associated lesions for the evaluation of brain injury following trauma. Different subtypes of S100 protein are found in cardiac, thyroid gland, kidney, glial and musculoskeletal cells. The subtype used in this study as a diagnostic tool in TBI is the B subtype found in glial cells.

Some studies have shown this protein to be a marker of neuronal injury with important roles in neuronal death, together with calcium. In patients with mild TBI, a high serum level of S100B protein was found in 28% of cases, which all showed damage by CT scan, mainly diffuse axonal injury correlating with poor results in neuropsychological tests [4,15].

Another important aspect of this glial damage protein is its implementation in tracking the occurrence of post-TBI hypoxia[56]. In TBI, the expression of S100B protein is upregulated and therefore it may be responsible for hypocalcemia due to its ability to bind calcium [4,15].

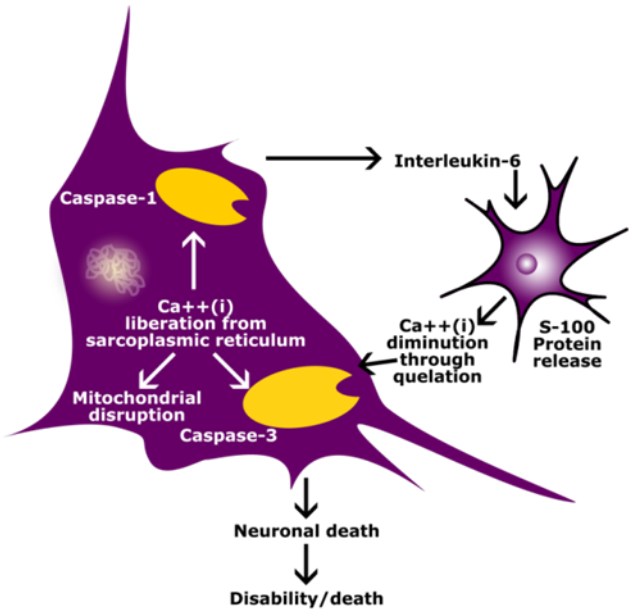

Fig. 4. Representation at the cellular level of the proposed pathophysiological mechanism after TBI in which calcium may be involved.

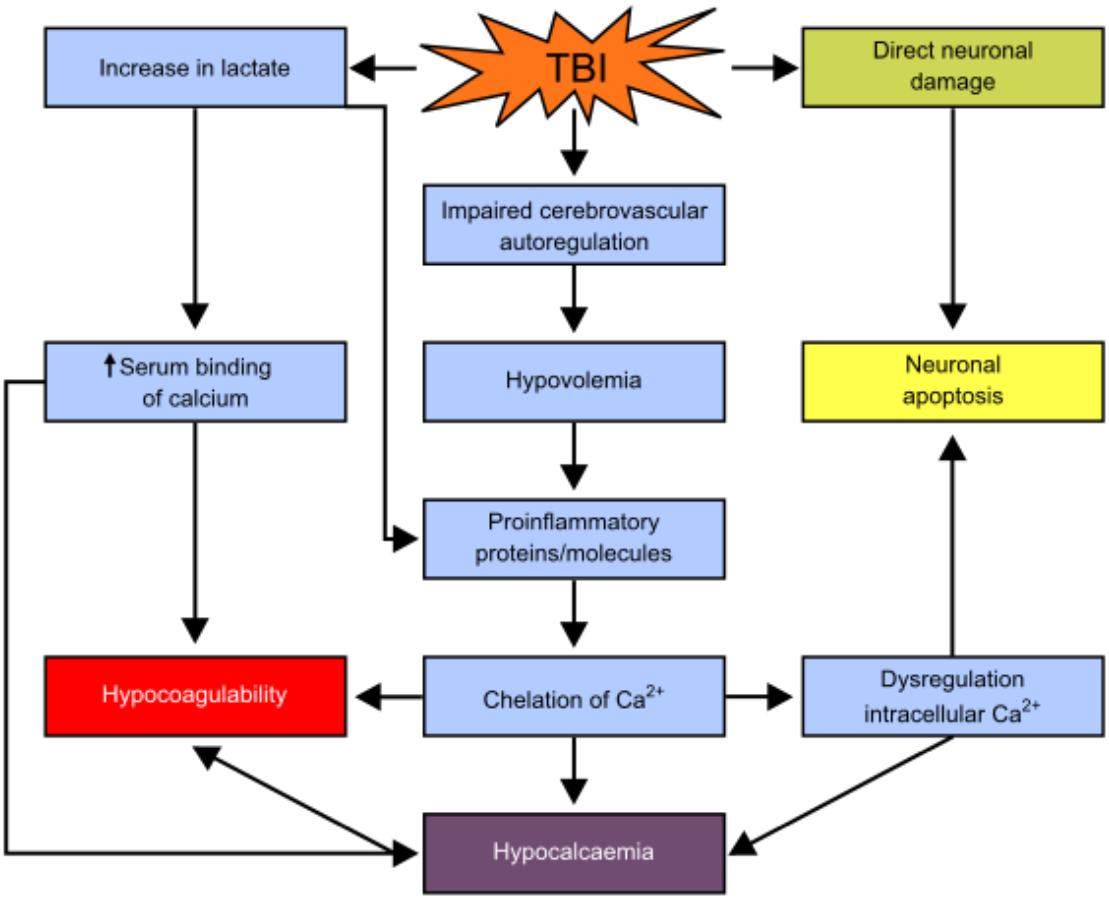

Fig. 5. Proposed pathophysiological mechanism.

OBJECTIVES

Based on a survey of the literature[10-15, 38], it seems reasonable to study calcium as well as S100 and IL-6-levels as a prognostic marker after moderate/severe TBI.

Therefore, in the **first part** we conducted a retrospective study with calcium only, in the **second part** a prospective study adding S100 and IL-6.

A preliminary study performed on a Mexican patient population in 2010 compared statins (cholesterol-lowering drugs) as inductors of anti-inflammatory effect, promoting recovery after moderate/severe TBI defined as scoring a Glasgow Coma Scale (GCS) 9-13 and < 8 points respectively (Table1) [38]. The results showed that patients with a lower serum calcium level on day 3 died earlier in comparison with those patients that had a normal serum calcium level on day 3 after TBI. This led to the development of an ambispective study including a total of 122 Mexican patients suffering from moderate/severe TBI, which showed that hypocalcemia on day 3 seemed to be a reliable predictor of mortality after TBI, reaching significant levels (p-value 0.026) [11]. However, morbidity was not assessed.

Changes in human serum calcium levels after TBI could indicate the severity of TBI, based on the hypothesis that calcium homeostasis is impaired by pro-inflammatory mediators liberated after trauma with subsequent neuronal loss.

In the **first part** of the study the objective of this thesis was to evaluate whether serum hypocalcemia (defined as <2.1 mmol/L [8.5 mg/dL]) and serum ionized hypocalcemia (defined as <1.10 mmol/L [4.5 mg/dL]) are correlated with mortality and morbidity (defined as a Glasgow Outcome Score ≤3) in the early phase of moderate/severe TBI.

We addressed the following questions:

1. Are lower serum levels of calcium and ionized calcium on day 3 reliable predictors of mortality/morbidity in moderate/severe TBI?

2. Does hypocalcemia correlate with the severity of mortality/morbidity in moderate/severe TBI?

The **second part** of this thesis served to clarify the following questions:

3. Can any other clinical, chemistry or blood cellularity variables be identified as prognostic factors regarding moderate/severe TBI?

4. Could pro-inflammatory proteins/molecules such as S100B protein and IL-6 be related to the pathogenesis in hypocalcemia on day three for mortality/morbidity after moderate/severe TBI?

MATERIALS AND METHODS

1. Materials.

As part of a follow-up study[11], results were retrospectively analyzed in a case-control study design, adding ionized calcium serum levels. The clinical records contained the clinical status upon admission (Glasgow Coma Scale, mean arterial pressure, heart rate, respiratory rate), as well as radiological findings (CT) upon admission, 12 and 36 hours after trauma and routine laboratory results on day 0, 3 and 7 from enrolled patients who sustained a traumatic brain injury from January 2004 to December 2012 and were treated at the Department of Neurosurgery of the University of Oldenburg. The assessment of the Glasgow Outcome Score (GOS) and the Glasgow Coma Scale (GCS) as outcomes predicting death/disability upon discharge was taken over from the discharge medical letter.

Based on this retrospective study, we investigated a prospective cohort of patients regarding mortality and morbidity after moderate/severe TBI, using the same protocol. In this manner, we prospectively analyzed, in an observational cohort study design, the clinical status upon admission (Glasgow Coma Scale, mean arterial pressure, heart rate, respiratory rate) radiological findings (CT) upon admission, 12 and 36 hours after trauma and routine laboratory results on day 0, 3 and 7 from the enrolled patients who sustained a traumatic brain injury from January 2014 to December 2015 and were treated at the Department of Neurosurgery University of Oldenburg, Evangelical Hospital.

The Glasgow Coma Scale (GCS) was assessed by the neurosurgeon on duty on admission of the patient to the emergency room as well as blood sampling upon admission. Within less than 24 hours, the GCS upon admission as well as the blood sampling were confirmed. Subsequent

blood sampling 3 and 7 days after TBI was performed in the same manner. Sampling of S100B protein (a marker of neuronal damage) and interleukin (IL)-6 (a pro-inflammatory cytokine) was included on admission, as well as on day 3 and 7 after TBI. Routinely, bivalent cations such as magnesium (Mg^{2+}) and multivalent anions such as Phosphates (PO_4^{3-}) were analyzed.

All patients gave informed consent signed by a legal representative or in case of reaching a GCS of 15 points (Table 1) while hospital stay, in less than 3 days, it was signed by themselves. Patient recruitment at the Evangelical Hospital, Oldenburg, Germany was performed with the permission from the Ethics Committee of Carl von Ossietzky University with the number Drs.21/4/2014.

1.1 Patients.

Our patient collective included from January 2004 to December 2012 and January 2014 to December 2015 treated at the regional trauma center at the Evangelical Hospital, Oldenburg for TBI. These patients were treated according to the Brain Trauma Guidelines[39]. Multiple traumatic injuries according to Injury Severity Score (ISS) equal to or greater than 16 points classified as polytrauma [40], were treated according to the severity of these injuries. The time between the accident and arrival at the emergency room, the mechanism of injury and pre-admission care were documented.

1.1.1 Inclusion criteria for both (retrospective and prospective) parts of the study.

Patients meeting the following criteria were included:

- Age: 16 to 87 years.
- GCS from 3 to 13 points.
- Cranial computed tomography (CCT) upon admission.

- Calcium and ionized calcium measurements taken on the day of TBI, as well as on days 3 and 7.

1.1.2 Exclusion criteria for both (retrospective and prospective) parts of the study.

The following findings were cause for a patient to be excluded:

- TBI older than 3 days.
- Intake of medication or diseases affecting calcium metabolism.
- Lesions in the brainstem as an isolated finding.
- Previous treatment at another department.
- Pregnancy.
- Hyperphosphatemia (>1.32 mmol/L).
- Hypomagnesemia (<0.61 mmol/L).
- Alcoholism.
- Hypoalbuminemia.
- Disability prior to TBI.
- Transfusion of plasma or blood derivates.
- Septic shock.

1.2 Clinical scores.

The GCS is used to assess the level of consciousness after a head injury. In hospitals, it is also used for monitoring chronic patients in intensive care [41]. The scale was published in 1974 [41] by Graham Teasdale and Bryan J. Jennett, professors of neurosurgery at the University of Glasgow's Institute of Neurological Sciences at the city's Southern General Hospital. The GCS is used to assess the status of the central nervous system, as it was designed for. The initial indication for the use of GCS is the serial assessment of patients with traumatic brain injury and coma for at least 6 hours in the neurosurgical ICU setting (Table 1).

The Glasgow Outcome Score (GOS) is a scale used to assess patients with brain injuries, such as cerebral trauma, and to subdivide these patients into groups that allow for standardized descriptions of the objective degree of recovery (Table 2). The first description of this scale was performed based on the GCS in 1975 by Jennett and Bond [42]. The GOS applies to patients with brain damage, allowing an objective assessment of their recovery in five categories. This provides a prediction of the long-term course of rehabilitation and return to work and everyday life (Table 2). A scale ≤3 predicts severe disability with a permanent need for help with daily living, incompatible with employment and self-sufficiency and with the cost-benefit problems that this entails [42]. For this reason, as well as for clinical and socio-economic reasons, the cut-off of our patients with GOS ≤3 (group 1) was defined as death/disability and GOS >3 (group 2) defined survivors.

Tab. 1: Glasgow Coma Scale.

Eye opening	Spontaneously	4
	To speech	3
	To pain	2
	NEVER	1
Best verbal response	Oriented	5
	Confused	4
	Inappropriate words	3
	Incomprehensible sounds	2
	NONE	1
Best motor response	Obeys commands	6
	To localized pain	5
Flexion to pain	Withdrawal	4
	ABNORMAL	3
	Extension to pain	2
	None	1
TOTAL		**3-15**

Tab. 2: Glasgow outcome Score.

1. Death	Severe injury or death without recovery of consciousness.
2. Persistent vegetative state	Severe damage with prolonged state of unresponsiveness and a lack of higher mental functions.
3. Severe disability	Severe injury with permanent need for help with daily living.
4. Moderate disability	No need for assistance in everyday life, employment is possible but may require special equipment.
5. Low disability	Slight impairment with minor neurological and psychological deficits.

1.3 Data collection.

On the basis of a study by Tapia et al.[38], a questionnaire for the standardized capture of relevant research data was developed (see Appendix). Demographics, clinic parameters, CCT, MRI, surgery, complications and outcomes were assessed. Initially, it was suggested that statins may induce an anti-inflammatory effect and may promote recovery after TBI, and thus a question on statin treatment was included on the questionnaire. However, the intake of statins stated on the original questionnaire used for data collection was excluded because of a potential treatment bias. The clinical parameters on admission, such as median arterial pressure, heart rate, respiratory rate and laboratory measurements at days 0, 3 and 7 were used from this questionnaire [38].

2. Methods.

2.1 Patient management and treatment.

Patients were admitted to the emergency room and handled according to Advanced Trauma Life Support (ATLS) and Brain Trauma Guidelines. The treatment team of the regional trauma center at the Evangelical Hospital in Oldenburg, based on these guidelines, consists of: one anesthesiologist, two traumatologists, one neurosurgeon and two specialized emergency room nurses. The primary survey occurs starting from the arrival of the patient to the emergency room for a maximum of 5 minutes. In this period of time, the ABCD protocol is applied:

a. **Airway maintenance with cervical spine protection**.

   This is the first stage of the primary survey to ensure patency of the airway. A cervical immobilization collar is worn all the time until a fracture of the cervical spine can be ruled out.

b. **Breathing and ventilation**.

   The chest is examined by inspection, palpation, percussion and auscultation.

c. **Circulation with hemorrhage control.**

   Two large-bore intravenous lines are established and crystalloid solution may be given. In this phase, the traumatologist performs a focused assessment sonography in trauma (FAST) in order to rule out internal bleeding while the second traumatologist performs a body check in order to rule out any lesions that should be immediately treated, such as exposed fractures or severe bleeding from any wound (Fig. 6).

d. **Disability/neurological assessment**.

That is the point in where the neurosurgeon takes part in patient assessment, by actively performing a basic neurological assessment, with the patient being classified as alert, responsive to verbal stimuli, responsive to painful stimuli or unresponsive. This establishes the patient's level of consciousness (this is when the important Glasgow Coma Scale is performed by the neurosurgeon), pupil size and reaction, lateralizing signs and spinal cord injury level.

In the next 6-15 minutes (secondary survey), once the patient is stabilized, blood samples for hematic biometry, blood chemistry and serum electrolytes (sodium, potassium, calcium, ionized calcium, S100B protein, IL-6, etc.) and arterial blood gases are taken. After this period, the pertinent radiological studies such as cranial CT, cervical spine CT and in some cases when the patient was classified as suffering a polytrauma (see later) full scans (CT thorax-abdomen) are done and the patient is admitted according to the severity of the injuries in the Intermediate Care Unit or the Intensive Care Unit.

Clinical variables upon hospital admission consist of: age, gender, associated lesions, seizures, pupillary reaction assessment; the Glasgow Coma Scale upon hospital admission is applied as described (Table 1) for the primary survey. Respiratory and cardiac frequency, arterial systolic and diastolic pressure as well as mean arterial pressure are monitored upon arrival of the patient to the emergency room. The Glasgow Outcome Score (Table 2) is applied at the time of discharge or death of the patient.

Fig. 6. Trauma management in the emergency room at the Regional Trauma Center at Evangelical Hospital in Oldenburg following the primary survey guidelines from the Advance Trauma Life Support, in this case a FAST (see text).

2.2 Statistical analysis.

The statistical program JMP-7 [43] was used in order to perform the analysis of descriptive statistics, providing values of central tendency and dispersion such as mean and standard deviation (SD) of all the variables (Bartlett's test for homoscedasticity is automatically performed by the JMP-7 program). For the comparative analysis, Student's t-test for normally distributed continuous variables or the Wilcoxon/Kruskal-Wallis test for non-normally distributed continuous variables were used. For categorical variables, the Chi-square test was applied, and for tables with cells less than 5 the Fisher's exact test was utilized. Statistical significance was defined as $p<0.05$. We calculated the odds ratio (OR) and relative risk (RR) with 95% confidence intervals (CI). Using these data, we were able to determine the risk of moderate/severe disability and

death for patients with hypocalcemia. We performed a logistic regression analysis with the variables that showed a significant difference ($p<0.05$) in the bivariate analysis and with the variable pupillary reactivity. This latter variable was significant in the pilot study performed on Mexican patients [11, 12]. As pupillary reactivity is of crucial clinical importance as a prognostic factor for mortality in TBI, it was analyzed in the final regression model independently of its significance in the comparative analysis [44-46]. Therefore, we did not use stepwise selection of covariates [46, 47] in the abovementioned analysis.

In the final model, the results were expressed in the retrospective data with OR (CI 95%) and in the prospective part of the study with RR (CI 95%).

RESULTS

1. Demographic and clinical variables.

After evaluating the clinical files in the **first part** of our study, data were compiled retrospectively from 99 patients with moderate and severe TBI fulfilling the inclusion criteria. These patients had a median age of 42 years; the youngest was 16 years old and the oldest was 87 years old. Of these, two thirds of the patients (67.67%) were male and 32 (32.32%) female. Almost half of the patients (52.52%) had a Glasgow Outcome Score ≤3 and 47 (47.47%) had a Glasgow Outcome Score >3. A total of 17 patients were excluded from the study as they did not fulfill the inclusion criteria according to the records in the clinical files.

In general, clinical variables did not show a significant difference, with the exception of the GCS upon admission ($p=0.041$), the GCS ($p<0.001$) at discharge and the mean arterial pressure upon admission ($p=0.018$) between group one (death/disability) and group two (survivors) depict in Table 3.

One patient had to be excluded from the evaluation of isocoria due to a stabbing injury to the right eye.

The duration of stay in the ICU, the respiratory and cardiac rate as well as the acid-base state (pH value) upon admission and at day 3 did not differ between the groups (Table 3).

In **the second part** of our study, we prospectively recruited 61 patients with moderate and severe TBI who fulfilled the inclusion criteria with a median age of 42 years (range: 17 to 86). Of the studied patients, 43 (70.50%) were male and 18 (29.50%) were female. Twenty-one (34.42%) patients had a GOS ≤3 and 40 (65.58%) had a GOS >3 (Table 4). A total of 14

patients were excluded from the study as they did not fulfill the inclusion criteria. The demographic and clinical variables, baseline pH levels, and duration of stay in the ICU of the included patients are shown in (Table 4).

After evaluation of the hospitalization variables, it could be seen that most patients did not show a significant difference between groups, with the exception of age (<0.001), Glasgow Coma Scale at discharge (0.041), duration of ICU stay (0.002), mean arterial pressure (0.015), pH on day 3 (0.022) and pupillary reactivity (0.003). Age is a well-known factor for mortality and morbidity in TBI and was particularly taken into account in the statistical analysis in order to avoid potential selection bias (Table 4 and Fig. 7).

In the collective of patients in both studies (160 patients in total) the cause of TBI was a fall in 79 (49%); of these patients, 69 (88%) had a fall in the home environment and 10 (12%) had a fall at work. Seventy-one (44%) cases of TBI were due to a traffic accident (motorized vehicle, bicycle); of these patients, the accident occurred as part of a private trip in 54 (76%) and to or from work in 17 (34%). Violence was the cause of TBI in 6 (3.7%) cases and an accident at work (not fall or a vehicular accident) was the cause of TBI in 4 (3.3%) patients. There were a total of 17 (10.6%) fatal events in this collective of 160 patients.

The mean time until admission to hospital care for the total collective of patients was 2 hours (range: 30 minutes-24 hours) after TBI. 12.5% (20 patients) patients suffered from polytrauma in the collective of both studies.

Tab. 3. Demographic and clinical variables from the 99 retrospective studied patients.

	Group 1 (Death/disability=52)	Group 2 (Survivors=47)	p-Value
Gender (M/ F)	32/20	35/12	0.167 §
Age (years) †	36.5 (16-80)	45.2 (16-87)	0.079 ‡
Glasgow Coma Scale at admittance *	8 ± 3	7 ± 3	0.041 ‡
Glasgow Coma Scale at discharge *	10 ± 1	14 ± 1	<0.001‡
ICU days *	18 ± 11	20 ± 12	0.314 ‖
Mean Arterial Pressure (mmHg) *	104 ± 17	111 ± 14	0.018 ‖
Heart Rate * (beats/minute)	92 ± 26	91 ± 26	0.845 ‖
Respiratory frequency * (respirations/minute)	15 ± 2	15 ± 2	0.186 ‖
pH *	7.36 ± 0.08	7.39 ±0.83	0.193 ‖
pH day 3 *	7.39 ± 0.06	7.41 ± 0.05	0.159 ‖
Isocoria			
Yes	47/52 (90.3%)	35/**46**** (76.1%)	
No	5/52 (9.6%)	11/**46**** (26.9%)	0.054 ¶
Pupillary reactivity			
Yes	40/52 (76.9%)	30/47 (63.8%)	
No	12/52 (23.1%)	17/47 (46.2%)	0.152 ¶

* Mean ± Standard Deviation (SD)

† Median (ranges).

‡Wilcoxon ranges.

§ Fisher's exact test.

‖ Student's t-test.

¶ Chi-squared test.

** Due to a stabbing wound at the eye n=1 patient was not taken into account for isocoria.

Tab. 4. Demographic and clinical variables from the 61 prospective studied patients.

	Group 1 (Death/disability=21)	Group 2. (Survivors=40)	p-Value
Gender (M/ F)	15/6	28/12	0.907§
Age (years) †	65.5 ± (27-86)	47.5 ± (17-80)	0.001‡
Glasgow Coma Scale at admittance *	9 ± 3	9 ± 4	0.445 ‡
Glasgow Coma Scale at discharge *	9 ± 4	14 ± 1	<0.001‡
ICU days *	17 ± 13	9 ± 7	0.002‖
Mean Arterial Pressure (mmHg) *	110 ± 13	118 ± 11	0.015‖
Heart rate * (beats/minute)	100 ± 22	97 ± 19	0.640‖
Respiratory frequency * (respirations/minute)	18 ± 1	14 ± 2	0.120‖
pH day 0*	7.40 ± 0.09	7.38 ± 0.07	0.402‖
pH day 3 *	7.45 ± 0.06	7.42 ± 0.04	0.022‖
pH day 7 * ††	7.42 ± 0.08	7.42 ± 0.05	0.921‖
Isocoria			
Yes	15/21(71.4%)	33/**39**** (84.6%)	
No	6/21(28.6%)	6/**39**** (15.4%)	0.231¶
Pupillary reactivity			
Yes	11/21 (52.3%)	34/40 (77.5%)	
No	10/21 (47.7%)	5/40 (32.5%)	0.003¶

* Mean ± Standard Deviation (SD).

† Median (ranges).

‡Wilcoxon ranges.

§ Fisher's exact test.

‖ Student's t-test.

¶ Chi-squared test.

†† In 4 patients because of early death (< 7 days after admission) this variable could not be assessed.

** Due to a stabbing wound at the eye n=1 patient was not taken into account for isocoria.

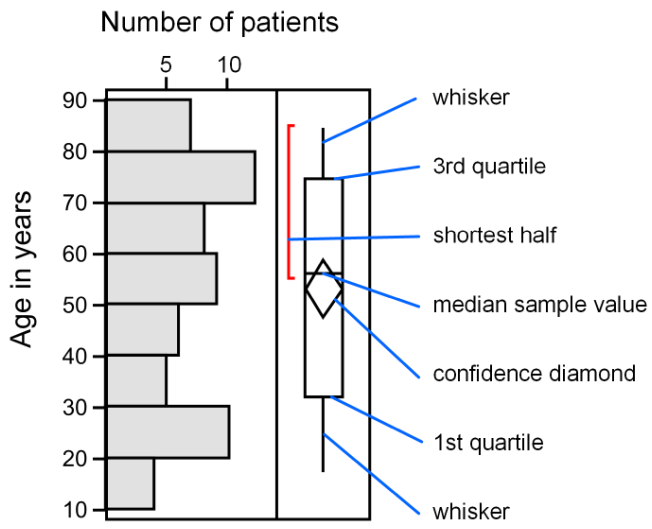

Fig. 7. Graphical representation of the age of the 61 enrolled patients in years at the time of TBI. The lines extending vertically from the box plot on the right side of the graphic indicates variability outside the upper and lower quartiles (whisker). The spacing between the different parts of the box indicates the degree of dispersion (spread) and skewness in the data (notice the non-parametric distribution for patients older than 65 years of age). The band inside the box represents the second quartile (the median) and the diamond, called a "means diamond" in the statistical progam JMP, is centered around the sample mean, with endpoints spanning the 95% normal confidence interval for the sample mean.

2. Chemistry variables and blood cellularity at days 0, 3 and 7.

In the retrospectively analyzed group of 99 patients, we found a significant difference in ionized serum calcium levels on day 3 after admission (Table 5 and Fig. 8) between patients with GOS ≤3 and >3 (0.008). According to this result, the lower calcium levels were correlated with the group with worse outcomes (GOS ≤3). Mean serum sodium levels on day 7 were also found to be significantly different ($p<0.047$) between these groups. Patients with a worse outcome (GOS ≤3) showed a lower level of sodium in serum, compared to patients with a better outcome (GOS >3).

Mean hemoglobin levels showed significant differences on day 3 ($p<0.047$), but this value did not correlate with the proposed final logistic regression model (discussed later). This parameter was considered to be a possible bias, as seen in the next paragraphs.

In the collective of 61 prospectively analyzed patients, we found a significant statistical difference (0.009) in ionized serum calcium levels, S100B protein (0.002), IL-6 (0.007) and hemoglobin (0.011) on day 3 after admission between the groups with GOS ≤3 and >3 (disability/death). Regarding hemoglobin levels on days 3 and 7, we also found a statistically significant difference in hemoglobin on day three (0.011) (as seen in the 99 retrospectively evaluated patients, $p=0.020$), as well as for potassium on day 7 (0.039) between groups (Table 6).

Surprisingly, the calcium level on day 3, in this collective from the German population, was not significantly different between the studied groups. The mean value of hemoglobin on day 3 after trauma seems to be a confusing factor and was discarded to have a prognostic value in the logistic regression model together with potassium on day 7, as explained below.

Other chemistry and blood cellularity variables considered important in the pathophysiology of TBI such as glucose, hematocrit, magnesium, phosphate and total leukocytes were not significantly different between groups (Table 6).

Tab. 5. Chemistry variables and blood cellularity at 0, 3rd and 7th day from the 99 retrospective studied patients.

	Group 1 (Death/disability=52)	Group 2 (Survivors=47)	p-Value
Day 0			
Total leukocytes (X 10^3/µL) *	13.5 ± 5.9	11.6 ± 5.5	0.118†
Hematocrit (%) *	34.2 ± 7.1	37.9 ± 8.4	0.550†
Hemoglobin (g/dL) *	11.8 ± 1.9	13.4 ± 7.0	0.596†
Sodium (mmol/ L) *	139.5 ± 5.3	140.3 ± 4.3	0.399†
Potassium (mmol/L) *	3.7 ± 0.4	3.7 ± 0.4	0.900†
Calcium (mmol/L) *	2.0 ± 0.5	2.0 ± 0.3	0.823†
Ca^{++} ion (mmol/L) *	1.1 ± 0.2	1.1 ± 0.1	0.453†
Glucose (mg/dL) *	136.0 ± 32.9	138.0 ± 43.3	0.880†
Day 3			
Total leukocytes (X 10^3/ µL) *	10.6 ± 5.4	10.3 ± 4.2	0.773†
Hematocrit (%) *	31.1 ± 5.6	28.9 ± 6.4	0.065†
Hemoglobin (g/dL) *	10.4 ± 1.9	9.6 ± 2.2	0.047†
Sodium (mmol/L) *	142.1 ± 6.6	143.8 ± 6.9	0.189†
Potassium (mmol/L) *	3.9 ± 0.4	3.9± 0.6	0.943†
Calcium (mmol/L) *	2.0 ± 0.2	2.0 ± 0.1	0.069†
Ca^{++} ion (mmol/L) *	**1.09 ± 0.1**	**1.15 ± 0.1**	**0.008**†
Glucose (mg/dL) *	130.9 ± 35.6	141.9 ± 42.5	0.213†
Day 7 ‡			
Total leukocytes (X 10^3/µL) *	11.0 ± 4.2	9.8 ± 3.1	0.151†
Hematocrit (%) *	30.7 ± 5.7	28.2 ± 6.8	0.077†

Hemoglobin (g/dL) *	10.5 ± 2.4	10.1 ± 2.7	0.494†
Sodium (mmol/ L) *	139.8 ± 7.6	142.9 ± 5.8	0.047†
Potassium (mmol/L) *	3.0 ± 0.5	4.1 ± 0.4	0.355†
Calcium (mmol/L) *	1.8 ± 0.7	2.0 ± 0.2	0.263†
Ca^{++} ion (mmol/L) *	1.13 ± 0.1	1.17 ± 0.2	0.428†
Glucose (mg/dL) *	143.7 ± 49.3	140.6 ± 41.8	0.790†

* Mean ± Standard Deviation (SD).

† Student's t-test.

‡ In 6 patients because of early death (< 7 days after admission) this variable could not be assessed.

Tab. 6. Chemistry variables and blood cellularity at 0, 3rd and 7th day from the 61 prospective studied patients.

	Group 1 (Death/disability=21)	Group 2 (Survivors=40)	p-Value
Day 0			
Total leukocytes (X 10^3/µL) *	10.1 ± 3.6	10.7 ± 4.1	0.599†
Hematocrit (%) *	35.0 ± 6.2	36.0 ± 7.4	0.615†
Hemoglobin (g/dL) *	12.3 ± 2.5	12.4 ± 1.9	0.877†
Sodium (mmol/ L) *	140.0 ± 3.4	140.3 ± 3.7	0.439†
Potassium (mmol/L) *	3.9 ± 0.5	3.9 ± 0.6	0.689†
Calcium (mmol/L) *	2.2 ± 0.2	2.2 ± 0.1	0.394†
Ca^{++} ion (mmol/L) *	1.2 ± 0.2	1.1 ± 0.1	0.337†
Glucose (mg/dL) *	160.5 ± 66.5	139.3 ± 47.6	0.157†
Protein S-100B (ng/dL)*	0.4 ± 0.5	0.3 ± 0.2	0.169†
IL6 (ng/dL) *	185.0 ± 242.0	96.1 ± 183.3	0.117†

Magnesium (mmol/L) *	0.8 ± 0.1	0.8 ± 0.1	0.479†
Phosphate (mmol/L) *	0.8 ± 0.3	0.9 ± 0.2	0.098†
Day 3			
Total leukocytes (X 10^3/ µL) *	9.7 ± 3.6	10 ± 4.7	0.825†
Hematocrit (%) *	27.2 ± 7.9	30.3 ± 9.3	0.196†
Hemoglobin (g/dL) *	9.4 ± 1.9	10.8 ± 2.1	0.011†
Sodium (mmol/L) *	135.0 ± 30.3	141.0 ± 4	0.223†
Potassium (mmol/L) *	4.1 ± 0.6	4.0 ± 0.4	0.488†
Calcium (mmol/L) *	2.1 ± 0.2	2.1 ± 0.1	0.264†
Ca^{++} ion (mmol/L) *	**1.1** ± 0.1	**1.2 ± 0.1**	**0.009**†
Glucose (mg/dL) *	146.2 ± 36.7	135.8 ± 31	0.250†
Protein S-100B (ng/dL) *	0.2± 0.1	0.1±0.1	0.002†
IL6 (ng/dL) *	131.5 ± 202.5	39 ± 44.2	0.007†
Magnesium (mmol/L) *	0.8 ± 0.1	0.8 ± 0.1	0.188†
Phosphate (mmol/L)*	0.9 ± 0.3	0.8 ± 0.3	0.405†
Day 7 ‡			
Total leukocytes (X 10^3/µL) *	10 ± 3.6	9.6 ± 4.9	0.800†
Hematocrit (%) *	27.8 ± 7.2	31.9 ± 8	0.075†
Hemoglobin (g/dL) *	9.8 ± 1.7	11.2 ± 2.7	0.020†
Sodium (mmol/ L) *	139 ± 6.2	135.7 ± 22.3	0.548†
Potassium (mmol/L) *	4.2 ± 0.4	3.9 ± 0.6	0.039†
Calcium (mmol/L) *	2.1 ± 0.2	2.2 ± 0.1	0.058†
Ca^{++} ion (mmol/L) *	1.18 ± 0.2	1.17 ± 0.1	0.694†
Glucose (mg/dL) *	152.6 ± 30.0	130.5 ± 44.3	0.067†
Protein S-100B (ng/dL)	0.1 ± 0.1	0.1 ± 0.1	0.095†
IL6 (ng/dL) *	36.6 ± 24.1	25.3 ± 21.4	0.082†

Magnesium (mmol/L) *	0.9 ± 0.1	0.8 ± 0.1	0.739†
Phosphate (mmol/L) *	0.9 ± 0.2	1.0 ± 0.3	0.163†

* Mean ± Standard Deviation (SD).

† Student's t-test.

‡ In 4 patients because of early death (< 7 days after admission) this variable could not be assessed.

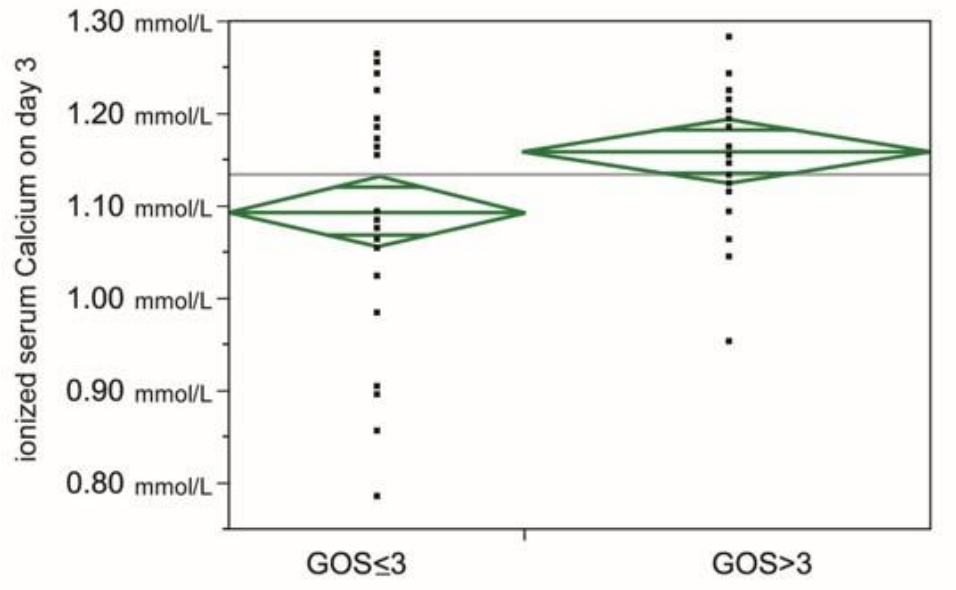

Fig. 8. Diamond-shaped boxplot for ionized calcium levels on day 3 of the *first part (retrospective)* collective. The means diamonds presents the sample averages as point estimates (center lines) and as confidence intervals (top and bottom points of the diamonds). The width of the diamond is proportional to the size of the group. It includes overlapping lines. If the interval between these lines for one group does not overlap the interval between these lines for the other group, then the group means are significantly different (note the gray line).

3. Logistic regression model: Glasgow Outcome Score ≤3.

In the final logistic regression model of the 99 retrospectively analyzed patients, we included absent pupillary reactivity, hypocalcemia of ionized serum calcium (<1.10 mmol/L) on day 3 and low serum sodium on day 7 (Table 7 and Fig. 9). These variables were substantiated in conjunction with poor Glasgow Outcome Scores (p-value 0.002, not shown in the table), explaining the poor outcomes in patients with severe disability.

Pupillary reactivity, hypocalcemia of ionized serum calcium on day 3 and serum sodium deregulation on day 7 following TBI were significant predictive factors for a poor outcome in our study. However, in comparison to our initial results, hemoglobin on day 3, MAP upon admission and GCS upon admission were not significant in the logistic regression model and therefore were considered as potential bias in the explanation of poor outcomes in patients with severe disability (Table 7).

Regarding our collective of 61 prospectively analyzed patients, the best logistic regression model included age, absent pupillary reactivity, hypocalcemia of ionized serum calcium (<1.10 mmol/L), S100B protein and IL-6 on day 3 (Table 8). These variables were substantiated in conjunction with poor Glasgow Outcome Scores with a p-value <0.001(not shown in the table).

In this final logistic regression model, as with the collective of 99 retrospectively analyzed patients, we attempted to analyze all the significant variables plus pupillary reactivity (as seen in the statistical analysis section) in order to establish which variables influenced the dependent variable, i.e. the Glasgow Outcome Score, in the prospectively studied patients. However, in comparison with our initial results (Table 4 and 6), the significant variables regarding poor outcome, i.e. pH on day 3, duration of ICU stay, hemoglobin on days 3 and 7 and potassium deregulation on day 7, according to the this proposed logistic regression model, were no more

significant regarding patients with severe disability/death (GOS ≤3) and therefore we considered these variables as potential bias in our study.

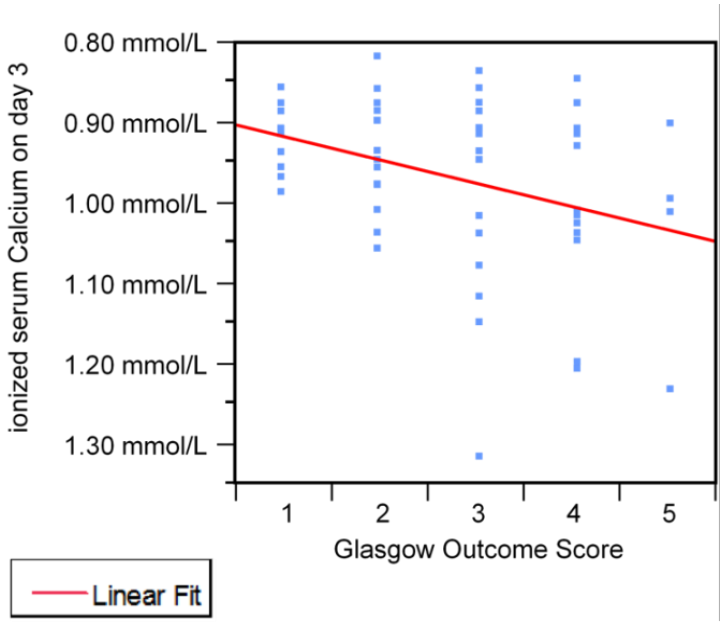

Fig. 9. Graphical representation explaining the Glasgow Outcome Score (GOS) in the collective of 99 patients with ionized serum calcium on day 3 using the logistic regression model. The straight red line is the fitted normal distribution.

Tab. 7. Logistic regression model in the 99 prospective studied patients for Glasgow Outcome Score ≤3 (death/disability).

Parameter	OR	Lower 95 %	Upper 95%	p value
Pupillary reactivity	7.1	1.34	51.4	0.01
Hypocalcaemia of serum ionized calcium (<1.10 mmol/L) on day 3	3.03	1.32	9.14	0.004
Seventh day serum sodium	1.18	1.05	1.35	0.002

Tab. 8. Logistic regression model in the 61 prospective studied patients for Glasgow Outcome Score ≤3 (death/disability).

Parameter	RR	Lower 95 %	Upper 95%	p value
Pupillary reactivity	12.4	9.42	15.38	0.004
Hypocalcaemia of serum ionized calcium (<1.10 mmol/L) on day 3	3.14	2.49	3.78	0.05
Age in years	8.45	6.48	10.42	0.003
Protein S-100B	5.00	3.88	6.12	0.025
IL-6	4.60	3.65	5.67	0.032

3.1 Hypocalcemia on day 3.

In the **first part (retrospective)**, we found a statistically significant difference ($p<0.008$) in ionized serum calcium levels on day 3 after admission between the groups with GOS >3 and ≤3 (disability/death).

In the **second part (prospective),** a statistically significant difference ($p<0.009$) between ionized serum calcium levels on day 3 after admission between group 1 (GOS >3) and group 2 (GOS ≤3) was observed.

Surprisingly, serum calcium on day 3 was not found to be significant in both retrospective and prospective parts with p-values of 0.069 and 0.264, respectively. These findings indicate the pathophysiological hypothetical limitation of non-ionized calcium in TBI to serve as an active electrolytic exchanger. This phenomenon was likely related to an overwhelming pro-inflammatory reaction concomitant with death[11].

3.2 OR and RR ranges with prognostic value.

In the retrospective **first part** of the study, we calculated an odds ratio (OR) regarding death/disability of 3.03 (with 1.32 as the lowest value and 9.14 as the highest value; 95% CI: 1.32-9.14; p-value=0.004) for the association between hypocalcemia of ionized serum calcium as a single marker (<1.10 mmol/L) on day 3 after TBI and a poor outcome. Therefore, the odds of a poor outcome, entailing significant disability or death, was 3.03 (lowest value 1.32 and highest value 9.14) times greater than the odds of a good outcome in patients with an ionized serum calcium level of <1.10 mmol/L on day 3 (Table 7).

In the **second prospective part**, we calculated a relative risk (RR) of 3.14 (with 2.49 as the lowest value and as 3.78 the highest value; 95% CI: 2.49-3.78; p-value=0.05) for the association between hypocalcemia of ionized serum calcium (<1.10 mmol/L) as single marker on day 3 after TBI in the disability/death group. Therefore, the probability of a poor outcome, entailing significant disability or death, was 3.14 (lowest value 2.49 and highest value 3.78) times greater than the relative risk of a good outcome in patients with an ionized serum calcium level of <1.10 mmol/L on day 3 (Table 8).

Furthermore, an association with the included pro-inflammatory proteins/molecules namely protein S-100B and IL-6 could be found with a RR of 5.00 (lowest value 3.88 and 6.12 the highest value; 95% CI: 3.88-6.12; p-value=0.025) for protein S-100B and a RR of 4.60 for IL-6 (lowest value 3.65 and 5.67 the highest value; 95% CI: 3.65-5.67; p-value=0.032) (Table 8).

DISCUSSION

At the beginning of this study, the expectation that non-ionized serum calcium (serum calcium), as seen in the collective of 122 Mexican patients, to be a significant predictive factor regarding TBI. Surprisingly, this was not the case; rather, in both retrospective and prospective parts of this study in a German patient collective, this factor was non-significant. Instead, a more specific calcium measure, namely ionized serum calcium, was significant as a predictor regarding mortality/morbidity in patients with TBI.

The overall results between the two parts (retrospective and prospective) were strikingly similar, confirming each other. The clinically relevant factors in these studies in patients with a poor outcome were monovalent cations such as sodium and potassium on day 7 after TBI and pH on day 3; the latter supports the theory of metabolic acidosis due to mitochondrial dysfunction and subsequent lactate accumulation, disrupting the fine balance of intracellular calcium activating programmed cellular death (apoptosis) [24, 26].

This was significant only in the second prospective part maybe because of the more sophisticated data collection.

However, two of these variables (potassium on day 3 after TBI and pH on day 3 after TBI) were dismissed as prognostic variables after failing significance in logistic regression models.

Critical appraisal of data collection.

GCS values appeared to be decisive in terms of the impact on outcome following TBI. However, this value is prone to considerable variability between observers. This variability is mainly seen

in unexperienced health care workers that have little contact with patients suffering from neurological disorders.

The difference found in GCS on admission in patients with TBI in the first phase of the study could be explained because of this variability. Although there is always a neurosurgeon on duty according to the trauma protocols of the regional trauma center at the Evangelical Hospital in Oldenburg, as part of the trauma team in the emergency room, inconsistences in the documentation of this variable (GCS by admittance) could accentuate the errors inherent in this assessment.

Another aspect that could have incurred errors is that the mean time until arrival at the shock room was two hours. In this time period, the value of the Glasgow Coma Scale in critical patients could change very quickly, coupled with unexperienced health care workers assessing patients with neurological disorders in pre-admission care. In the second part of this work, we tried to avoid inconsistences in data documentation by corroborating the data obtained in less than 24 hours by an experienced health care (neurosurgeon on duty). However, when a patient arrived at the emergency room intubated and deep sedated, the achieved GCS was 3 points (Table 1). 6 patients fulfilled this characteristic being prone to fall into selection bias and therefore incurring again in errors or inconsistences in data documentation.

In the same manner, the recent reconstruction (in the last two years) of the emergency department at the Evangelical Hospital in Oldenburg, as part of the regional trauma network, permitted the neurosurgeon on duty to estimate the pre-admission GCS by staying in contact via telephone with health care workers until the arrival of the patient at the shock room. In the first retrospective part of our study, data collection consisted of patient files. For this reason, in cases

of high inter-observer variance and inconsistences upon patient admission, these data could contribute to a selection bias.

In the second prospective part, the GCS was assessed by the neurosurgeon on duty on admission of the patient in the emergency room and within <24 hours was confirmed/corrected by me in order to avoid inter-observer bias.

The Glasgow Outcome Score, a scale similar to the GCS, is also prone to considerable inter-observer variance. This scale was assessed in the same manner and performed by a health care worker experienced in assessing patients with neurological disorders upon discharge from the hospital. However, in patients requiring rehabilitation, the GOS could not be assessed after completing the rehabilitation phase; these quite important data are missing. These valuable data could not be documented because of the lack of a unique reference center in the city of Oldenburg. Most of the studied patients, depending of their rehabilitation needs, had to be transferred to another city to carry on with their rehabilitation program. Therefore, morbidity and mortality were assessed only in the early phase following TBI.

As a discussion of these results, several answers to the questions enquired at the beginning of this thesis, need to be addressed.

1. Are lower serum levels of calcium and ionized calcium on day 3 reliable predictors of mortality/morbidity in moderate/severe TBI?

We found ionized calcium values in the serum on the third post-traumatic day to be a prognostic factor for mortality and morbidity in moderate/severe TBI, with p-values of 0.008 and 0.009 for the retrospective and prospective study, respectively. This similarity between studies, far from

contradicting each other, supports the significant difference on day 3 after TBI between dead/disabled patients and those patients considered to be survivors.

However, in this collective of 160 patients, non-ionized serum calcium alone was not significant regarding mortality/morbidity, as seen in the study on 122 patients from the Mexican population. Back then, only mortality was evaluated (and not morbidity) and ionized calcium was not assessed [11].

Furthermore, the patient collective from the German population in comparison with the Mexican collective was treated with different health standards and treatment quality in a completely different context, i.e. in a hospital and a health care system in a first world country. It is quite likely that German patients who have a severe disability after surviving a critical health condition that could cost their lives (such as moderate/severe TBI) has not concluded in a fatal outcome thanks to complex treatment in the ICU. If these patients had been treated in the Mexican health care system, i.e. in a developing country with deficiencies and financial shortcomings associated with the treatment of critical patients, such as those with moderate/severe TBI, the trauma could have been fatal.

Nevertheless, despite our expectations, in the German collective of 160 patients, 17 deaths (10.6%) occurred while in the collective of 122 Mexican patients, there were only 9 (7.3%) deaths [11]. Outcomes regarding morbidity (severe disability) between these two collectives were not analyzed. Nonetheless, the overall death rates provide a rough estimation of outcomes, and were comparable in the two patient collectives.

In support of these results and in order to clarify this rough approximation, a more advanced and sensitive method of calcium measurement (ionized serum calcium) was brought about. This

measurement is quicker and easier to assess and plays a more important role regarding the intracellular biomolecular signaling processes in programmed cell death. The correlation with hypocalcemia in ionized serum calcium in the collective of 160 German patients was striking: over 95% (70/73) of the patients with GOS scores ≤3 and with low ionized calcium died or had a poor outcome with severe disability, while in the collective of Mexican patients, 8 of the 9 patients (88.8%) who presented hypocalcemia in non-ionized serum calcium on day 3 after trauma died. Findings like this should alert physicians treating patients suffering from moderate/severe TBI.

2. Does hypocalcemia correlate with the severity of mortality/morbidity in moderate/severe TBI?

A higher rate of hypocalcemia has already been reported in other critically ill patients, although the underlying pathologies are variable [53]. Most commonly, this phenomenon has been observed in sepsis syndrome [54]. However, the severity and incidence of hypocalcemia in non-septic but critically ill patients has not been well-examined. Some authors have studied this phenomenon and found it to be frequent in critically ill adults and associated with mortality and severe organ dysfunction in children. They described a correlation with the severity of illness associated with sepsis and burns, but not with a specific illness per se [55].

Regarding these observations, the exact role of hypocalcemia after moderate/severe TBI still remains unclear. However, based on the proposed pathophysiological mechanisms, there is a tendency that hypocalcemia is likely to influence cerebral edema by activating neuronal cell death with the release of pro-inflammatory cytokines. This phenomenon could be assessed with minimally-invasive sampling techniques that are used for the continuous measurement of free, unbound analyte (neurotransmitters, hormones, glucose, proteins, etc.) concentrations in the extracellular fluid of the brain using microdialysis with a molecular weight cut-off range of approximately 6-100 kD. However, ions are very small particles that cannot be measured with

this technique. Despite this limitation, the variety of mechanisms that have been postulated to be involved in the decline in clinical condition after TBI, such as neuro-inflammation, neuronal hypoxia, loss of cerebral vessel autoregulation and brain edema, correlate with our proposed hypothesis regarding calcium depletion. As part of these mechanisms other molecules that are easy to assess with this technique (microdialysis), especially S100B protein and IL-6, have turned out to be reliable prognostic markers[54-59], being indirect indicators in calcium depletion. For this reason, the culmination of this section will be dedicated to the role of these molecules regarding hypocalcemia in the pathological mechanism associated with TBI.

It should to be taken into account that not only intracranial lesions are responsible for hypocalcemia [9], but this might also be a result of a whole-body inflammatory reaction [55]. This inflammatory reaction could be seen in patients who had been subjected to multiple traumatic injuries, i.e. polytrauma, defined by an Injury Severity Score (ISS) equal to or greater than 16 points[40]. The referred inflammatory reaction together with direct neuronal damage and consequent hypo-oxygenation, with subsequent secondary neuro-inflammation, leads to the liberation of IL-6 and promotes the release of S100B protein into the extracellular space from surrounding damaged neurons. These molecules bind extracellular calcium ions and cause a transmembrane concentration imbalance with consequent disturbances in the intracellular equilibrium, thereby activating signaling pathways related to programmed cellular death [25,27, 28]. However, in our study, only 20 (12.5%) of our patients were in such a severe condition with the associated whole-body inflammatory reaction. The epidemiological data from our study support the theory that hypocalcemia following TBI is not an isolated finding in patients suffering from severe injuries, but rather an epiphenomenon that is tied to brain injury itself.

Moreover, we have a determinant influence on our results regarding outcomes in patients with TBI, the results obtained in the collective of 160 patients are hard to deny. It is possible that

hypocalcemia is just an epiphenomenon that occurs apart from TBI as a host defense response, as seen with cortisone levels after trauma [48]. However, hypocalcemia does not seem to be an isolated finding following moderate/severe TBI, as it also correlated with mortality/morbidity in the acute/subacute phase (less than 15 days) of the complex treatment/recovery process after TBI, despite the inherent bias and inconsistences in data documentation characteristic of an observational study.

In our study hypocalcemia correlated and was useful in predicting mortality/morbidity in the early phase following TBI.

3. Can any other clinical, chemistry or blood cellularity variables be identified as prognostic factors regarding moderate/severe TBI?

Hyponatremia is a frequently observed electrolyte abnormality in patients after TBI [49]. Several mechanisms, such as syndrome of inappropriate antidiuretic hormone (SIADH), hypopituitarism and cerebral salt wasting syndrome (CSWS), have been observed with variable incidence [48]. This phenomenon has been described to appear within the first week after TBI with a mean duration of 1.78 days [48, 49]. In the first phase of our study, similar findings were observed: hyponatremia peaked on day 7 after trauma, possibly because of a disturbed hypothalamic-pituitary axis, as part of the loss of regulation in neuronal tissue after TBI, and correlated with trauma severity and poor outcomes.

Changes in the epidemiological patterns of TBI show that the median age of individuals who experience TBI is increasing, and falls have now surpassed road traffic incidents as the leading cause of this injury in developed countries [50]. Age is a well-known factor predisposing to unfavorable outcomes in patients with TBI [51]. Elderly TBI patients, in general, have a deteriorated medical condition before injury, compared to younger adults. Other aging-related

changes include cerebrovascular atherosclerosis and decreased free radical clearance [52]. This deficiency in free radical clearance could increase the risk of injury or cause a secondary insult, thereby activating neuronal programmed death. Based on our results and according to global trends, most of the patients included in this work were older than 65 years of age at the time of TBI, particularly in the prospective phase (Fig. 7). These patients more often had a severe disability or even died (GOS ≤3). Falls were the main cause of admission in the shock room following TBI. This finding could lead to a selection bias as elderly people tend to have worse outcomes overall, mainly due to a deteriorated medical condition and deficient free radical clearance with the accumulation of pro-inflammatory proteins/cytokines that activate cellular death following TBI.

Nevertheless, the logistic regression confirmed that hypocalcemia in serum ionized calcium on day 3 is an independent finding regarding age and therefore this studied variable (age) was considered a potential selection bias in our study.

Radiological findings like supratentorial compressive lesions and diffuse axonal injury (DAI), as prognostic factors for hypocalcemia, play an important role in mediating direct damage to neuronal tissue. As a consequence of supratentorial compressive lesions, impaired pupillary reactivity (anisocoria) can occur, coupled to a rise in intracranial pressure to critical levels. The latter is mainly caused by three types of herniation, i.e. diencephalic, subfalcine and temporal lobe (uncus) herniation through the foramen magnum, with compression of the parasympathetic mesencephalic nuclei in the brainstem being an indirect sign of secondary neuronal oxygen delivery disturbances and therefore a correlating prognostic factor regarding outcomes in our proposed pathophysiological model. A compressive mass effect prevents the adequate delivery of oxygen to already insulted neuronal cells, resulting in neuro-inflammation with the release of

pro-inflammatory proteins and molecules that bind calcium, such as S100B protein and IL-6, which thereby activate cellular death processes such as caspase activation.

Furthermore, impaired pupillary reactivity and hyponatremia on day 7 after TBI, according to the logistic regression model, were concomitant factors associated with ionized serum hypocalcemia and higher mortality and disability rates in patients with moderate and severe TBI. Regarding the importance of S100B protein and IL-6 after TBI, a special section in this discussion was reserved for the description of these two mediators, as they are highly important in the pathological mechanism after TBI (see below).

4. Could pro-inflammatory proteins/molecules such as S100B protein and IL-6 be related to the pathogenesis of hypocalcemia on day 3 driving mortality/morbidity after moderate/severe TBI?

Based on the hypothetical pathophysiological mechanisms of the present study and correlating with results in the literature regarding the function and utility of S100B protein[56-59], the link between this protein and the decrease in calcium in its active form (ionized calcium) in this pathological status (neuronal oxygen deficit after TBI), the following statements are addressed:

1. S100B protein is liberated into the extracellular space by injured neurons.
2. Subsequently, calcium binding by this protein is increased.
3. This causes hypocalcemia and consequently activates apoptotic intraneuronal processes.
4. Apoptosis leads to neuronal loss manifested as disability and even death (Fig. 4).

In addition, prompt and adequate treatment regimens, such as maintaining adequate cerebral blood perfusion, avoiding large disturbances in serum glucose levels and neurosurgical procedures (Fig.3) taking into account the host defense reaction, may improve oxygen delivery at the neuronal level and influence S100B and pro-inflammatory cytokine (e.g. IL-6) release,

thereby preventing inflammation and disturbances in the aerobic mitochondrial phase, which would decrease Ca^{2+} concentrations, activate caspases and consequently lead to cellular death[56-59].

Another marker that indicates the grade of neuro-inflammation and regulates the liberation of S100B protein with consequent calcium binding is the cytokine IL-6 [60]. This interleukin acts as a pro-inflammatory cytokine. It is secreted by T cells and macrophages and stimulates the immune response during infection and after trauma, especially with tissue damage, and thereby promotes inflammation. It is capable of crossing the blood-brain barrier and stimulating the synthesis of acute phase proteins such as tumor necrosis factor alpha (TNF-α) [61]. IL-6 can be used as an indirect indicator of the liberation of S100B and therefore Ca^{2+} chelation, and was significant in our final logistic regression model.

In conclusion, it can be stated that hypocalcemia is a marker of the degree of brain damage as a result of a cascade of various pathological mechanisms such as direct mechanical trauma, neuro-inflammation, altered vessel autoregulation and hypoxia. Hypocalcemia seems to be an epiphenomenon driven by several factors. It seems to play a role as a prognostic marker, although not as a target for therapy. Ca^{2+} administration does not have the potential to influence neuronal death[10-12, 33, 36, 56, 59 ,61].

Further studies should assess the role of calcium in TBI regarding the on-going pathophysiological process. Regarding this, the next step will be to initiate a prospective study with a larger population and a longer follow-up period in order to improve the statistical significance of the obtained data and avoid sources of bias such as selection bias or discrepancies in patient data, with the aim of assessing the long-term effects of hypocalcemia after moderate/severe TBI.

ZUSAMENFASSUNG

Das Schädel-Hirn-Trauma (SHT) ist eine schwerwiegende Verletzung, deren individuelle und sozio-ökonomische Auswirkungen verheerend sein können. Trotz des wissenschaftlichen Fortschrittes und verbesserter Diagnose- und Behandlungsmethoden bleiben die Möglichkeiten einer frühzeitigen Prognoseabschätzung nach einem schweren Schädel-Hirn-Trauma zurzeit noch begrenzt.

Ein Hauptproblem besteht darin, dass einfach zu bestimmende Marker fehlen, mit deren Hilfe bereits zu einem frühen Behandlungszeitpunkt das Ausmaß der Schädigung erkannt und somit der klinische Verlauf besser prognostiziert werden kann.

Die Hypothese der vorliegenden Studie lautet, dass eine Hypokalzämie im Serum (definiert als: <2,1 mmol/l {8,5 mg/dl}) und ein Mangel von ionisiertem Kalzium im Serum (definiert als: <1,10 mmol/l {4,5 mg/dl}) in der Frühphase eines mittelschweren oder schweren Schädel-Hirn-Traumas als prognostische Faktoren für die Mortalität und Morbidität (definiert über den Glasgow Outcome Score ≤3) dienen.

Basierend auf einer früheren Studie, in der gezeigt wurde, dass Kalzium bezüglich einer frühen Mortalität nach moderater/schwerer Schädel-Hirn-Verletzung eine Rolle spielt, wurde als **ersten Teil der Studie** retrospektiv in einem Fall-Kontroll-Studiendesign, Kalzium in seiner aktiven Kationen-Form analysiert. Diese aktive Form interagiert in einer intrazellulären Signalkaskade, die zum Zelltod führt und spielt daher eine wichtige Rolle als prognostischer Faktor. Es wurden die Daten von teilnehmenden Patienten gesammelt, die zwischen Januar 2004 und Dezember 2012 ein Schädel-Hirn-Trauma erlitten haben und in der Universitätsklinik für Neurochirurgie des

Evangelischen Krankenhauses Oldenburg behandelt wurden. Parameter unserer Studie entnahmen wir Aufzeichnungen des klinischen Status bei Aufnahme, bildgebender Befunde (CCT) bei Aufnahme, nach 12 und nach 36 Stunden sowie den Laborergebnissen am Aufnahmetag sowie 3 und 7 Tage nach der Aufnahme im Krankenhaus.

In gleicher Weise wurde als **zweiten Teil der Studie** eine prospektive Kohortenstudie von Januar 2014 bis Dezember 2015 bezüglich der Mortalität und Morbidität nach moderatem/schwerem Schädel-Hirn-Trauma durchgeführt, um den Serumspiegel ionisierten Kalziums als möglichen prognostischen Faktor zu bewerten. In Ergänzung der prospektiven Studie wurde es, der Hypothese folgend, dass proinflammatorische Proteine/Moleküle die Hauptursache für eine Hypokalzämie nach Schädel-Hirn-Trauma sind, zudem Protein S-100b (Marker neuronaler Schädigung) und IL-6 (proinflammatorisches Cytosin) am Aufnahmetag, sowie am dritten und siebten Tag nach dem Schädel-Hirn-Trauma bestimmt.

Die Patientenrekrutierung erfolgte in der Universitätsklinik Evangelisches Krankenhaus Oldenburg in der Abteilung für Neurochirurgie unter Genehmigung der Ethikkommission der Carl von Ossietzky Universität mit der Nummer: Drs.21/4/2014.

Im **ersten retrospektiven Studienteil** wurde eine Gruppe von 99 Patienten analysiert. Es fand sich ein signifikanter Unterschied des ionisierten Serum-Kalzium-Spiegels am dritten Tag nach Aufnahme zwischen Patienten mit einem Glasgow Outcome Score ≤3 Punkte (Gruppe 1) und >3 Punkte (Gruppe 2) (p=0,008).

Die endgültigen logistischen Regressionsmodelle enthalten für diesen Studienteil fehlende Pupillenreaktion, Serumspiegel ionisierten Kalziums (<1,10 mmol/l {4,5 mg/dl}) am dritten Tag und Hyponatriämie am siebten Tag nach Schädel-Hirn-Trauma.

In der Phase 1 berechneten wir eine Odds Ratio (OR) von 3,03 (95% CI: 1,32-9,14) (p = 0,004) bezüglich der Assoziation eines verminderten Serumspiegels von ionisiertem Kalzium (<1,10 mmol/l {4,5 mg/dl}) am dritten Tag nach Schädel-Hirn-Trauma und von Invalidität oder Tod.

Im **zweiten prospektiven Studienteil** an Hand von 61 Patienten (Phase 2) ergab sich ein statistisch signifikanter Unterschied des Serumspiegels von ionisiertem Kalzium (p=0,009), Protein S-100b (p=0,002), IL 6 (p=0,007) und Hämoglobin (p=0,011) am dritten Tag nach Aufnahme zwischen den Patienten von Gruppe 1 und Gruppe 2.

Für diese Phase enthalten die endgültigen logistischen Regressionsmodelle Alter, fehlende Pupillenreaktion und die Serumspiegel des ionisierten Kalziums, des Protein S-100b und von IL-6 am dritten Tag nach Schädel-Hirn-Trauma. Diese Variablen untermauerten einen schlechten Glasgow Outcome Score bei Entlassung.

Das relative Risiko (RR) von 3,14 (95% CI: 2,49-3,78) (p=0,05) bezüglich der Assoziation eines verminderten Serumspiegels ionisierten Kalziums (<1,10 mmol/l {4,5 mg/dl}) am dritten Tag nach Schädel-Hirn-Trauma und Behinderung oder Tod wurde kalkuliert.

Es wurde erwartet, dass die Bedeutung des Serumspiegels des nicht-ionisierten Kalziums, wie in einer früheren Studie, als prädiktiven Faktor bezüglich des Outcomes nach mittelschwerem oder schwerem Schädel-Hirn-Trauma nachweisen würde. Überraschenderweise fand sich diese Korrelation nicht, sondern blieb sowohl im retrospektiven als auch im prospektiven Studienteil nicht signifikant. Stattdessen fand sich eine deutliche Signifikanz des Serumspiegels des ionisierten Kalziums als Prädiktor hinsichtlich der Mortalität und Morbidität von Patienten, die ein Schädel-Hirn-Trauma erlitten haben. Die Gesamtergebnisse der retrospektiven und der prospektiven Kollektive waren sehr ähnlich. Beide Studien unterstützen sich daher gegenseitig.

Pathophysiologisch erklärt sich die Entstehung der Hypokalzämie aus dem Zusammenspiel verschiedener, gleichzeitig ablaufender Kaskaden im Rahmen des Schädel-Hirn-Traumas, nämlich einem erhöhten pathologischen zellulären Kalzium-Einstrom mit vermehrter Bindung von ionisiertem, freien Kalzium an proinflammatorische Proteine wie Protein S-100b und IL-6.

In Abhängigkeit von der Schwere der Verletzung wird eine komplexe Abfolge von Prozessen aktiviert, die weitere endogene Veränderungen bedingen sowie auch Störungen der zellulären Homöostase nach sich ziehen.

Somit ist die Aussagekraft des Wertes von Kalzium im Serum im Sinne einer Hypokalzämie als Marker für das Ausmaß der Schädigung im Rahmen eines Schädel-Hirn-Traumas nachgewiesen und zwar spätestens für den dritten Tag nach Schädel-Hirn-Trauma. Dieser Wert könnte damit als entscheidender, bislang fehlender diagnostischer Marker und somit auch als Prädiktor für den weiteren klinischen Verlauf verwandt werden.

Weitere größer angelegte Studien sind hierfür erforderlich.

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APPENDIX

Questionnaire.

University Department of Neurosurgery Evangelic Hospital Oldenburg, Germany.

Researcher **patient's pseudonym**

Date of traumatic brain injury:

Sex: Male Age (years): pre-admittance care Yes/No
Female Date of birth:

Time accident to admittance (hours):

1. ≤30 minutes 2. > 30 minutes ≤2 hours 3. >2 hours-24 hours

Mechanism of injury:

1. Fall 2. Vehicle 3. Violence 4. Other

Comorbidities:

Diabetes Mellitus: Yes/No Hypertension: Yes/No other: Yes/No (specify)

Medication:

Alcoholic intoxication: Yes/No Intoxication by other substances: Yes/No (specify)
Nausea: Yes/No vomiting: Yes/No Cephalea: Yes/No

Loss of consciousness: Yes/No (time)
GCS admission: Motor verbal ocular Total
GCS discharge: Motor verbal ocular Total

Arterial pressure: Systolic/Diastolic
Mean Arterial pressure:
Respiratory rate: Cardiac Rate:

Seizures:	Yes/No	
pupils:	1. Isocoria	2. Anisocoria
pupillary Reactivity:	Left: Yes/No	Right: Yes/No

Other injuries:

Initial CCT: Time to acquisition: Admission after 12 hours after 36 hours

(Specify) 1. Subdural Hematoma
2. Epidural Hematoma
3. Contusions
4. Edema
5. Subarachnoid Haemorrhage

Labs/days.	**0**	**3**	**7**		**0**	**3**	**7**
Hemoglobin				Na			
Hematocrit				K			
Leucocytes				**Ca**			
Glucose				**Ionized Ca**			
Protein S-100b				IL-6			
Magnesium				Phosphat			

Blood gas analysis:	**0**	**3**	**7**
pO2			
pCO2			
pH			
Saturation 02			

Management: Intubation: Yes/No

Solutions: 1. NaCl 0.9%
2. NaCl 3%
3. Dextrose-NaCl
4. Ringer
5. Glucose 5%

Anticonvulsant:

Protectors of gastric mucosa:

1. Inhibitors Protons 2. H2 3. Sucralfat 4. Other

Anti-edema therapies: 1. Acetazolamide or another
2. Furosemide
3. Mannitol
4. Barbiturate

Monitoring intracerebral pressure: Yes/No

Intensive care unit days:

Surgery: Intracranial: 1. Subdural hematoma evacuation.
2. Epidural hematoma evacuation.
3. Intraventricular drainage.
4. Decompression.
5. Intraparenchymatous hematoma evacuation.
6. Intracerebral pressure probe.

Extracranial:

Days in hospital:

Glasgow Outcome Score at discharge:

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LEBENSLAUF

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Ausbildung

1992-1998	Grundschule Zertifikat von " Escuela Primaria Justa Ledesma"
1998-2001	Mittelschule Zertifikat von "Escuela Secundaria Ing. Camilo Arriaga"
2001-2003	Oberschule Zertifikat von "Instituto Salesiano Carlos Gomez"

Studium

2003-2010	Medizin Studium an der Autonoma Universität in San Luis Potosi, Mexiko. (UASLP)

Praktisches Jahr

2009-2010	Tätigkeit in der Neurochirurgie , Hospital Central ,Chefarzt Dr. Torres Corzo.

Weiterbildung

Seit März/2011	Tätig im Evangelischen Krankenhaus, Oldenburg als Assistenzarzt in der Universitätsklinik für Neurochirurgie. Direktor: Prof. Dr. Thomas Kretschmer

03.03.2014	**“ Approbation als Arzt”** Niedersachsen Ärzte Kammer(AEKN)
	Fellowships
05/2008-06/2008	Langen-Debstedt in Bremerhaven, Deutschland Klinik für Wirbelsäulechirurgie/Orthopädie Chef: PhD. M.D. Ulrich Wagner
	Observership
06/15-16/2015	Queen´s Medical Center Nottingham Center for Spinal Studies and Surgery(CSSS)
	Zertifikaten
	Bestätigungszertifikat der Teil 1 der EANS Prüfung. September 2014 Nikosia, Zypern.
	Veröffentlichungen:
	„ Transventricular neuroendoscopic exploration and biopsy of the basal cisterns in patients with Basal meningitis and hydrocephalus. “ Torres-Corzo J, Viñas-Rios JM, Sanchez-Aguilar M, Vecchia RR, Chalita-Williams JC, Rangel-Castilla L. *World of Neurosurgery 2012*
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	„ Effect of rosuvastatin on cytokines after traumatic head injury. “Sánchez-Aguilar M, Tapia-Pérez JH, Sánchez-Rodríguez JJ, Viñas-Ríos JM, Martínez-Pérez P, de la Cruz-Mendoza E, Sánchez-Reyna M, Torres-Corzo JG, Gordillo-Moscoso A. *Journal of Neurosurgery 2013*

„ Flexible neuroendoscopy biopsy of brainstem gliomas”
Torres-Corzo J, Sanchez-Rodriguez JJ, Lucino Castillo J, Viñas-Rios JM, Falcon-Escobedo R,Cervantes D, Vecchia RR. Central European Neurosurgery 2015.

“ Extension of Right Renal Vein in Renal Transplant from Deceased Donors: Cohort Study”. Miguel Angel Jaramillo Gante, Jesus Martín Sánchez-Aguilar, J. Humberto Tapia-Perez,Yadiralia Torres Medina, Viñas-Rios Juan Manuel Daniel González Aguirre, Jorge Luis Montes de Oca Arce. Experimental and Clinical Transplantation 2015

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Manuel VR, Martin SA, Juan SR, Fernando MA, Frerk M, Thomas K, Christian H. *Asian Journal of Neurosurgery 2015*

Endoscopic Transventricular Exploration with Biopsy of the Basal Cisterns and the Role of Endoscopic Third Ventriculostomy in Patients suffering Basal Cistern Meningitis and consecutive Hydrocephalus
Jaime Torres-Corzo, Juan Manuel Vinas-Rios, Jesus Antonio Viana Rojas , Dominic Cervantes, Martin Sánchez- Aguilar, Juan Carlos Chalita-Williams, Roberto Rodriguez-Dellavecchia, Jose Juan Sanchez-Rodriguez. Neurological Research 2016.

“Influence of the state of the subarachnoid space of the cranial base in the neuroendoscopic resolution of hydrocephalus.Subarachnoid space and endoscopic success in hydrocephalus”.
Sánchez-Rodríguez JJ ,Torres-Corzo JG,Cervantes DS ,Rodríguez-DellaVecchia R,Gordillo-Moscoso AA ,Vinas-Rios JM ,Sánchez-Aguilar M. Central European Neurosurgery ACCEPTED

Vorträge in Kongressen:

“Hypocalcaemia as a prognostic factor of early mortality in moderate and severe traumatic brain injury” 64. Annual Meeting der Deutsche Gesellschaft für Neurochirurgie (DGNC) Düsseldorf, Deutschland

Poster Vorträge in Kongressen:

Hypocalcemia as prognostic factor for mortality- morbidity in moderate/severe Traumatic Brain Injury:

EANS Annual Meeting: Prag, Tschechien, Oktober 2014
ANIM: Annual Neurointensiv Meeting: Berlin, Deutschland, Januar 2015.

Predictors of shunt-dependency after non-traumatic subarachnoid hemorrhage:

EANS Annual Meeting: Madrid, Spanien, Oktober 2015

The Role of High-Frequency Ultrasound and MRI in the Management of Schwanommas affecting Major Nerves

World Congress of Brachial Plexus and Peripheral Nerve Surgery: New Delhi, Indien, Februar 2016.

Hypocalcemia as prognostic factor for mortality- morbidity in moderate/severe Traumatic Brain Injury and its role with protein S-100b

EANS Annual Meeting: Athen, Griechenland, September 2016

Mitgliedschaft in ärztlichen Gesellschaften .

Mitglied der Deutsche Wirbelsäule Gesellschaft (DWG) seit 2012.

Mitglied der Deutsche Gesellschaft für Neurochirurgie (DGNC) seit Mai 2014.

Mitglied der European Association of Neurological Societies (EANS) seit June 2014.

Mitglied der Deutsche Gesellschaft für Neuro-Intensiv Medizin (DGNI) seit Januar 2015.

Mitglied der AOSpine seit Januar 2016

Sprachen

Deutsch	(B2 von Goethe Institut Mexiko)
Englisch	(TOEFL ITP 557 Punkte in Februar 2016)
Spanisch	(Muttersprache)

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