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2017

Document Version:
Publisher's PDF, also known as Version of record

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LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Belfragesalen, BMC, Lund. 16th of November at 1pm.

Faculty opponent
Professor Andrew Maas

Organization	Document name
LUND UNIVERSITY	PhD Thesis
	Date of issue 25th of october 2017
Author Olga Calcagnile	Sponsoring organization

Title Clinical validation of S100B and the Scandinavian Guidelines for management of traumatic brain injury in adults

Abstract

Background: Mild traumatic brain injury (mTBI) is one of the most common reasons for patients to seek the emergency department and is associated with a significant health economic burden. CT scans have become the general method to identify patients with intracranial complications. Several clinical guidelines have been designed in order to attempt to identify patients that should undergo a CT scan. In 2013, the SNC13 were published; they are the first guidelines that combine clinical risk factors with a biomarker, S100B.

The aim of this thesis was to investigate and validate the SNC13 guidelines and the clinical use of S100B in adults with TBI.

Methods: Study 1 was a clinical report on implementation of S100B into the SNC guidelines. Study 2 analyzed how S100B performed in intoxicated patients and on elderly population. Study 3 was an external validation of the SNC13 in an independent cohort. Study 4 was a cost analysis of the implementation of S100B into guidelines. Paper 5 is a multicenter study protocol, designed to internally validate the SNC13.

Results: 1144 with mTBI patients were prospectively registered at Halland Hospital Halmstad from 2007-2017. 30% of patients had negative S100B results and could be safely discharged with no CT scan needed. Patients younger than 65 years had a negative S100B result in 40% of cases. Moreover, 662 patients from an independent cohort were analyzed; the SNC13 showed high sensitivity and 34% specificity for intracranial complications on CT findings.

Conclusion: S100B can be safely implemented into guidelines and is useful for reduction of CT scans particularly in intoxicated patients and patients under 65 years of age. The SNC13 stratifies patients into adequate risk groups and can lead to a further reduction in CT scans without missing important intracranial complications.

Key words Traumatic brain injury, S100B, clinical guidelines

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language
ISSN 1652-8220		ISBN 978-91-7619-545-1
Recipient's notes	Number of pages 59	Price
	Security classification	

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Coverphoto by Roberta Giamminelli

ISBN 978-91-7619-545-1
ISSN 1652-8220
Lund University, Faculty of Medicine Doctoral Dissertation Series 2017:163

Printed in Sweden by Media-Tryck, Lund University
Lund 2016

Brevis a natura vita vobis data est, at memoria bene redditae vitae sempiterna

Marcus Tullius Cicero (106 B.C.- 43 B.C.)

Content

Abbreviations

(TBI) traumatic brain injury,

(ED) emergency department,

(mTBI) mild traumatic brain injury,

(GCS) Glasgow Coma Scale,

(LOC) loss of consciousness,

(RLS) Reaction Level Scale,

(FOUR) Full Outline of UnResponsiveness,

(CT) computed tomography,

(MR) magnetic resonance,

(SAH) subarachnoid hemorrhages,

(PCS) post-concussive syndrome,

(BBB) blood brain barrier,

(CDRs) Clinical Decision Rules,

(CCHR) Canadian CT Head Rule,

(NOC) New Orleans criteria,

(NICE) National Institute of Clinical Excellence,

(CHIP) CT in head injury patients Prediction Rule,

(NCWFNS) Neurotraumatology Committee of the World Federation of Neurosurgical Society,

(NEXUS-II) National Emergency X-Radiography Utilization Study II,

(SNC13) Scandinavian Neurotrauma Committee 2013

Introduction

Background

Traumatic brain injuries (TBI) are one of the most common reasons for patients to seek emergency department (ED) care (Cassidy JD et al., 2004). TBI has received increasing attention during the past decades due to the considerable health economic impact involved with this patient group; not only concerning management in the acute post-injury phase but also due to substantial morbidity and mortality.

A meta-analysis from 2015 showed an overall European incidence of 262 per 100,000 population for admitted TBI, although a large national and regional variation was reported (Peeters W et al., 2015). Swedish data showed an incidence of 546/100,000 (Andersson EH et al., 2003). Moreover all published data refer to patients that seek the ED; many patients with these injuries do not seek medical help and the true incidence is likely to be much higher (Cassidy JD et al., 2004).

Patients seeking the ED because of TBI constitute a very heterogeneous group, both regarding epidemiology and severity of injuries. The highest rate of TBI is registered among the elderly population, aged over 75 years, and the infant and young children population, 0 to 4 years old. The primary causes of injury are falls, but in younger adults (15-24 years old) motor-vehicle accidents are the most common cause. The majority of TBI patients are men, except in the elderly population where women have higher incidence (Taylor CA et al., 2017). 30-40% of patients hospitalized after a TBI are alcohol intoxicated. Alcohol use is associated with many types of injuries but its association with TBI is disproportionately high (Faul M et al., 2015). TBI is one of the leading causes of death for the younger population (1-44 years) and is associated with life-long disabilities; in severe TBI more than a third of the patients die and more than 60% of surviving patients will have moderate to severe disabilities (Faul M et al., 2015).

There are two specific categories of TBI patients that deserve to be mentioned separately due to the large attention they have received from national governments and private associations. The first one is sport-related TBI and has an estimated incidence of 3.8 million per year in the USA. Even in this case, the true incidence rate may be higher as many patients with mild injuries do not seek medical help (Harmon KG et al., 2013). Sports related TBI are often defined as "a traumatically induced transient disturbance of brain function", and are considered a subgroup of mild TBI. Concussions may resolve by themselves, but lifelong disabilities may occur, especially in repetitive TBI, leading to efforts trying to prevent or predict those unfavorable outcomes (Harmon KG et al., 2013). The second group is war-related TBI, with an increasing incidence of combat-related mild TBI. In this specific case, TBI is associated with a specific injury mechanism due to explosions, also called blast injuries. Even here, recovery should take place within 3 months but as many as 25% of individuals with war-related mild TBI may suffer chronical disabilities (Ware JB et al., 2016).

Definition and classification

The definition of TBI has long been a debated matter. A recent definition is: "an alteration in brain function, or other evidence of brain pathology, caused by external force" (Menon DK et al., 2010). The "alteration in brain function" has been defined by mean of clinical signs: any period of loss of consciousness (LOC), any loss of memory before or after the injury, any neurological deficit including sensory loss or weakness or any alteration of mental state including disorientation or confusion. It is important to consider that alteration of mental state can be induced by different causes than TBI, such as alcohol, drugs or simply pain and fear: nevertheless, these elements should not prevent clinicians in recognizing TBI. The inclusion of "other evidence of brain pathology" in the definition has opened the door, not only for the diagnostic tools such as neuroimaging and biomarkers, but also for disabilities diagnosed later in the injury process. The "external force" causing a TBI has been defined as the head being struck or striking an object, acceleration/deceleration movement of the brain without external trauma, penetrating trauma to the brain, forces generated by an explosion or blast or other forces that lack definition (Menon DK et al., 2010).

TBI is usually sub-classified into mild, moderate and severe TBI depending on severity indicators and level of consciousness on presentation to the ED. Glasgow Coma Scale (GCS) is the most accredited method to evaluate level of consciousness. The score is determined by a combined evaluation of eye, verbal and motor response with a range from 3, representing deep coma, to 15, representing fully awake patients. The GCS score, however, was never designed to be used for these injuries and is by no means perfect (Haukoos JS et al., 2007)

(Green SM et al., 2011). In the past decades, efforts have been made to design scales that are easier to use in everyday clinical practice and that truly reflect the patient's degree of awareness. One example is the Reaction Level Scale (RLS), an 8-step scale which aims to assess reaction level of patients in order to determine their awareness degree (Starmark JE et al., 1988) somewhat similar to an extended GCS motor score. This scale is simpler to use than GCS and can also be used in intubated patients or patients with facial injuries. RLS is widely used in Sweden. A more modern attempt at a new scale is instead the FOUR (Full Outline of UnResponsiveness) score that has shown promising results as a successor of GCS (Wijdicks EF et al., 2005), but is still somewhat complicated to use. However, the GCS is still the only scale universally used and reported; alternatives have only recently been validated; hence, the general worldwide consensus still lies with the GCS system (Teasdale G et al., 2014).

Mild TBI (mTBI) has previously been defined as patients with a GCS of 13 to15, loss of consciousness for less than 30 minutes and/or posttraumatic amnesia no greater than 24 hours (ACRM, 1993). In the past years, alteration of consciousness (AOC), for example confusion or disorientation, was included as additional criteria (Veterans Affair, Department of Defense, 2009). Moreover, the same researchers have suggested that patients with GCS 13 should be redefined as moderate TBI due to the high frequency of serious CT findings in this patient group (Uchino Y et al., 2001). 75-90% of patients with TBI fulfill criteria for mTBI (Cassidy JD et al., 2004) (Bazarian J et al., 2005).

Approximately 5% of patients with mTBI have pathological Computed Tomography (CT) findings and even fewer, less than 1%, require a specific intervention, such as neurosurgical procedures (Borg J et al., 2004). Coupled with the fact that these patients are so common, this presents a challenging management problem.

Neuroimaging

CT is the most common method to screen for acute intracranial complications. It is relatively cheap, fast and accessible in virtually all ED's (Lee H et al., 2008). CT is able to detect focal lesions, bleeding, midline shift, ventricular and basal cistern compressions. As previously mentioned, only a small minority of mTBI patients will have positive CT findings, sometimes defined as "complicated mTBI" (Jeter CB et al., 2013). The most common findings are skull fractures and cerebral contusions (Lee H et al., 2008). Nevertheless, other methods have shown to be even more accurate in detecting pathological findings and have a higher sensitivity to predict outcome. 30% of mTBI patients with normal CT have been reported to have abnormal Magnetic Resonance Imaging (MRI) results (Ingebrigtsen T et al.,

1999) and an even higher sensitivity has been shown with Diffusion Tensor Imaging (DTI) (Kulbe JR et al., 2016).

Most of the pathological findings missed by CT, but detected by other modalities, are related to white matter tract damage. On the other hand, CT may instead show small subarachnoid hemorrhages (SAH) that were not found a few days later on MRI, but these may have resolved spontaneously and MRI may have been just "too late" to detect them (Lee H et al., 2008). However, MRI is considerably more resource dependent than CT and the imaging process takes significantly longer (Yuh EL et al., 2013).

In conclusion, MRI is not cost effective as a screening tool in the acute phase management, but may be considered for TBI patients without full recovery.

Injury mechanism and outcome

Defining mTBI as "mild" has misled general opinion in believing that there are no consequences after such injuries. Unfortunately, besides rare cases where neurosurgical intervention is needed, approximately 15% of patients classified as having mTBI suffer from persistent cognitive and behavioral disabilities (McAllister TW et al., 2006) (Levin HS et al., 2015). Post-concussive syndrome (PCS) is a combination of physical symptoms, such as headache, dizziness and fatigue, and cognitive symptoms, such as working memory disabilities and concentration deficit, that may even lead to an increase risk in developing psychiatric diagnoses such as depression or anxiety. There is today no predictive tool to accurately identify which patients that will develop PCS. Multiple TBI´s over time, often seen for instance in sports such as fotboll and ice hockey, may be associated with long term sequel. Repetitive mTBI has been shown to increase the risk of developing chronic encephalopathy and young onset dementia (Nordström P et al., 2014) (Kulbe JR et al., 2016).

The pathophysiological events that occur into the brain after an injury are still largely unknown. Many researchers have stressed the fundamental concept that TBI is not just an isolated event caused by a primary injury (Kulbe JR et al., 2015) (Maas AIR et al., 2008). Long after the primary incident, several biochemical events will affect the brain tissues and generate a secondary injury. The first reaction will be an inflammatory response with a massive neurotransmitter releases. It develops within hours and continues several days after TBI with intensified ion-leakage that negatively affects all cell lines. Glutamate overproduction, for instance, generates an excessive accumulation of intracellular calcium that leads to mitochondrial damage with subsequent energy crisis. Calcium accumulation and ion leakage cause astrocyte swelling and disruption of the blood brain barrier (BBB). Free radical generation contributes into inducing

neuronal death or axonal damage. (Wilberger J et al., 2006) (Maas AIR et al., 2008) (Choe MC, 2016). However, such a massive response is not only negative: the inflammatory response is needed in order to aid the damaged tissue in promoting cell migration and regeneration (Maas A et al., 2008).

Clinical Guidelines

Although most patients with mTBI seek medical attention, only a minority will need urgent neurosurgical intervention. In order to identify those rare, but nevertheless feared, intracranial complications patients generally undergo a CT examination. Several research groups have investigated the possibility of optimizing the number of CT in mTBI patients by designing guidelines or Clinical Decision Rules (CDRs) that would help clinicians in identifying patients that have a higher risk for intracranial complications.

In the past 15 years, several guidelines have been published, validated both internally and externally and most are clinically implemented. These guidelines have many similarities in their clinical approach, evaluating self-reported patient history, clinical signs and examination elements, but differ in the choice and weight of risk factors within the respective guidelines. Their well-established application has been proven to be safe with no or few missed complications. However, their main goal, the reduction of CT scanning, has not been successfully achieved with a CT rate after mTBI still being high (Boyle A et al., 2004).

Example of these guidelines are: the Canadian CT Head Rule (CCHR) (Stiell IG et al., 2001), the New Orleans criteria (NOC) (Haydel MJ et al., 2000), the National Institute of Clinical Excellence (NICE) (in 2000), the CT in head injury patients Prediction Rule (CHIP) (Smits M et al., 2007), the Neurotraumatology Committee of the World Federation of Neurosurgical Society (NCWFNS) (Servadei F et al., 2001), the National Emergency X-Radiography Utilization Study II (NEXUS-II) (Mower WR et al., 2002) and the Scandinavian Neurotrauma Committee guidelines (SNC13) (Undén J et al., 2013).

The CCHR is a decision rule specific to the mTBI population, stratifying patients with GCS 13-15 that have suffered LOC, have amnesia for the trauma or are disoriented. High risk patients are those who suffer a decrease in GCS at 2 hours after injury, have signs of skull fracture or basal skull fracture, have more than 2 episodes of vomiting or are 65 years or older; in this group CT is mandatory because of elevated risk for neurosurgical intervention. In the CCHR medium risk group CT is only recommended. These patients report amnesia of more than 30 minutes before impact and a dangerous mechanism of injury (pedestrian struck by

motor vehicle, occupant ejected from motor vehicle, fall from height higher than 5 stairs). The CCHR primary outcome measure is the need for neurological intervention and the secondary outcome measure is clinically relevant brain injury on CT, defined as contusions larger than 5mm in diameter, subarachnoid bleeding thicker than 1mm, subdural hematoma thicker than 4mm, pneumocephaly that will need intervention and depressed skull fracture through the inner table (Stiell IG et al., 2001).

The NOC includes only patients with LOC and a GCS of 15, implying that those patients with GCS 14 or less should undergo a CT. According to the NOC posttraumatic headache, any vomiting, post-traumatic seizures, intoxication, persistent anterograde amnesia, contusion of the skull as well as any injury above clavicles, signs of skull fracture or facial fracture, and age over 60 years old are conditions that require a CT investigation. The NOC outcome measure is any acute traumatic intracranial lesion on CT (Haydel MJ et al., 2000).

The NICE guidelines are based upon the CCHR and divide patients that are eligible for CT into two groups. Firstly patients that suffer a decrease in GCS at 2 hours after injury, have signs of skull fracture or basal skull fracture, have post-traumatic seizures, focal neurological deficit and/or more than 1 episode of vomiting should perform a CT within 1 hour. Patients that are 65 years old or older, have a history of clotting or bleeding disease, reported a dangerous mechanism of injury and/or have more than 30 minutes retrograde amnesia for events before head injury should do a CT within 8 hours (National Institute of Clinical Excellence, 2000).

The CHIP prediction rule does not have strict inclusion criteria except for GCS 13-15, and recommends CT if patients are affected by a major risk factor (GCS less than 15 or GCS deterioration 1 hour after presentation at the ED, age 60 years or older, posttraumatic seizures, anticoagulant therapy, vomiting, posttraumatic amnesia longer than 4 hours, signs of skull fracture, serious injury mechanism (pedestrian or cyclist versus vehicle, ejected from vehicle) or at least 2 minor factors (fall from any elevation, anterograde amnesia or posttraumatic amnesia between 2 and 4 hours, contusion of the skull, neurological deficit or age 40 to 60 years). The CHIP primary outcome measure is any intracranial traumatic finding on CT, and the secondary outcome is all neurosurgical intervention after the initial CT (Smits M et al., 2007).

The NCWFNS guidelines identify three levels of risk for intracranial complications. Low risk patients with GCS 15, no clinical findings, no neurological deficit and no risk factors (coagulopathy, drug or alcohol consumption, previous neurosurgical procedures, epilepsy and age over 60 years old) can be dismissed without any further investigation. Patients with medium (clinical findings of LOC, amnesia, vomiting and/or headache) and with high risk

(GCS 14, neurological deficits, skull fracture), in both case CT should be obtained (Servadei F et al., 2001).

NEXUS-II recommend a CT in all patients with abnormal alertness or behavior, age of 65 years old or older, recurrent vomiting, coagulopathy, any sign of neurological deficit or suspected skull fracture or scalp hematoma. The NEXUS II outcome measure is any intracranial injury on CT (Mower WR et al., 2002).

The SNC13 were published in 2013. They are the first guidelines that use a biomarker, S100B, as a diagnostic tool for reducing CT scanning in low risk patients following mTBI. S100B has a low specificity for intracranial complications; nevertheless, its high sensitivity in this patient group makes it suitable as a negative predictor following mTBI.

The SNC13 include all patients with minimal, mild and moderate head injury within 24 hours after injury. Patients are stratified into high risk (posttraumatic seizures, focal neurological deficits, clinical signs of depressed or basal skull fracture, shunt-treatment, coagulopathy or anticoagulation), medium risk (age 65 years or older with anti-platelet medication) or low risk patients (GCS 14, or GCS 15 with LOC or repeated vomiting). Low risk patients with S100B levels below 0.10 µg/L can be discharged directly without a CT scan. The SNC13 primary outcome is the need for any neurosurgical intervention; the secondary outcome measures are identification of non-neurosurgical intracranial traumatic complications (Undén J et al., 2013). Our preliminary data have shown that in this particular patient group, S100B has reduced CT scans by 50%.

All guidelines share similar theoretical limitations; they are based upon self-reported history or symptoms as risk factors which may be associated with considerable bias; even GCS evaluation has been proven to be dependent on clinician's experience and judging ability (Green SM et al., 2011). From this point of view, the integration of a biomarker into clinical guidelines is not only a practical tool for reducing CT scans, but also adds an objective variable that is not affected by clinical judgement.

Biomarkers

Biomarkers have become powerful objective tools to support decision making in a variety of clinical areas. Technical progress has enabled the possibility to analyze and quantify proteins and chemical elements that have changed clinical management routines in almost every medical field. Some areas have been easier to implement than others; the diagnosis of myocardial ischemic injuries, for instance, has been assisted by the use of biomarkers since the 1960s and can now

rely on the effectiveness of Troponin-T. Biomarkers have a central, often essential, role in the diagnosis and prognosis of diverse medical conditions such as infectious disease, kidney disease, liver disease, cancer and pulmonary embolism. Similar efforts were launched for neurological disease, including TBI, with most effort being focused on the outcome prognosis of moderate to severe TBI (Raabe A et al., 1998) (Vos PE, 2011) (Thelin EP et al., 2017). In the past decades, more focus has been put on mTBI; as previously mentioned, this is a highly heterogeneous group where diagnosis is historically based on patients history and clinical examination. These aspects may be unreliable, especially in TBI and/or intoxicated patients. Many CDRs contain very specific risk factors such as exact speed of vehicles, number of step fallen down, centimeters fallen and minutes of amnesia. The potential diagnostic bias involved with these risk factors in this patient group in is obvious.

Different working groups have suggested a definition of the "perfect" brain biomarker (Strimbu K et al., 2010) (Papa L et al., 2008) (Kulbe JR et al., 2016); it should be specific to the brain, easily measured, have high sensitivity and specificity to brain injuries, have a well-defined release curve, help to identify risk-patients, monitor progress of the disease or response to treatment, and predict outcome. However appealing, it is utopian to believe that a single biomarker would possess all these characteristics. Nevertheless, a panel of biomarkers will allow for different aspects in TBI to be evaluated. Prognostic, diagnostic and monitoring aspects are all important parts of TBI management.

How brain biomarkers reach the bloodstream

One important aspect that must be discussed when dealing with brain biomarkers is the mechanisms that allow them to be measurable in peripheral blood. These are today still largely unknown. There are two major theories, one implying a rupture of the blood-brain-barrier (BBB) and the other focusing on glymphaticsystem activity (Iliff JJ et al., 2012) (Kawata K et al., 2016).

The BBB is considered a unique anatomical structure due to the fact that several cell types cooperate in order to create a selective diffusion barrier between the blood circulation and the central nervous system. The brain microvascular endothelial cells are connected by mean of tight junctions and create the capillary lumen, characterized by the lack of fenestration. Outside the endothelial cells we find the pericytes, cells rich in contractile proteins that influence BBB permeability. The basal lamina enfolds the endothelial cells and pericytes and is in contact with the end feet of astrocytes that envelope the capillaries (Hawkins BT et al., 2005). The main function of the BBB is to allow free passage, by passive diffusion, of water and lipid-soluble molecules along with the passage of glucose

and amino acids fundamental to neural metabolism and functioning. At the same time, it prevents other substances to freely pass in order to protect the brain from potentially neurotoxic substances.

During a TBI, not only neurons may be damaged but even astrocytes, oligodendrocytes, microglia, and brain microvascular endothelial cells: the entire neurovascular unit (NVU) is affected by both the primary and the secondary injury (Kawata K et al., 2016). The primary injury is caused by the mechanical rupture of the BBB structure (Wright RM el al., 2012) and compromising the tight junction stability. Neural and gliafactors are released into blood circulation resulting in a BBB compromise and/or increased permeability (Chodobski A et al., 2011).

The secondary injury mechanism varies depending on the pathophysiological mechanism of the primary injury. As previously mentioned, secondary damages develop after hours or days following the primary incident and compromise all cell-functions, including BBB integrity. The secondary injury starts with an inflammatory response with the expression of pro-inflammatory cytokines, mitochondrial dysfunction and free radical generation, the activation of the coagulation pathway and the exposure of matrix metalloproteases. All these events affect the tight junction function, generating a leakage of brain-specific proteins into the bloodstream (Maas A et al., 2008) (Kawata K et al., 2016).

Beside the BBB disruption theory, researchers have recently investigated a glymphatic pathway that drains the interstitial fluid of the brain independently to the BBB integrity (Iliff JJ et al., 2012). The cerebrospinal fluid flows into the space that surrounds neurons and astrocytes to the interstitial space between brain microvascular endothelial cells and basal lamina (Jessen NA et al., 2015). Neurospecific proteins can then be transported into the paravenous space by astrocyte water channel aquaporine-4. In an animal model, it was demonstrated that when the glymphatic system was compromised brain biomarkers such as S100B, neuron-specific enolase (NSE) and glial fibrillary acid protein (GFAP) were not detectable in serum after induced TBI (Plog BA et al., 2015).

The glymphatic pathway theory has opened up for an alternate explanation for the presence of biomarkers in peripheral blood. Nevertheless, it is still impossible to monitor the glymphatic system in humans leaving much to be studied in this field.

Biomarkers in mTBI

In the past decades several biomarkers have been studied in order to find the perfect brain specific biomarker that would aid in the clinical management of patients with TBI. Most studies have been conducted in the moderate to severe

TBI group but recently more attention has been focused on mTBI, due to its unpredictable outcome and the high health economic costs involved in its management.

The pathophysiological mechanism initiated after mTBI are still poorly understood and, so far, researchers have primarily focused their attention towards a biomarker that would identify astrocytic and neuronal damage. The first studies on possible biomarker candidates reported on lactate dehydrogenase (LDH) and creatine kinase BB (CK-BB) but their results were so poor both for sensitivity and specificity that they were quickly discarded as potentially useful tests (Kulbe JR et al., 2016).

The biomarker that has been most investigated, generating several thousands of publications, is S100B. S100B is the most important calcium-binding protein present in astrocytes and is considered a marker of astrocyte injury or death (Papa L et al., 2008). Another marker for astrocyte damage is the glial fibrillary acidic protein (GFAP), the primary protein of the cytoskeleton of the astrocytes. Neuron specific enolase (NSE), present in the cytoplasm of neuron, ubiquitin C-terminal hydrolase-L1 (UCH-L1) and heart-type fatty acid-binding proteins (H-FABP) are more neuron specific biomarkers. Other proteins that have been studied are myelin basic protein (MBP), spectrin break down products (SBDP), tau and neurofilaments (NF) primarily released after axonal damage (Kulbe JR et al., 2016) (Di Battista AP et al., 2013).

S100B

S100B is a Ca2+ binding protein, belonging to the S100 protein family, a group of small proteins of 9-14 kDa. All proteins share a homodimeric structure, consisting of two identical polypeptides held together by non-covalent bonds, forming a helix-loop-helix ("EF-hand type") conformation, expressing two calcium-binding sites. Each molecular structure is unique for each S100 protein (Donato R, 2001) (Strynadka NC et al., 1989). The name, S100, has been given for their characteristic of being 100% soluble in ammonium sulfate at neutral pH.

25 members of the S100 family have been identified so far (Santamaria-Kisiel L et al., 2006). They are involved in several important functions such as cell proliferation, differentiation, a Ca2+ homeostasis, inflammatory response and apoptosis. S100 proteins are only present in vertebrates (Schäfer BW et al., 1996) and each S100 protein has a cell-specific expression pattern so that each member has specific functions. S100 proteins can act both intracellularly as regulators and extracellularly as signaling proteins, they can be secreted or released after cell injury and regulate cell activities as transmitters.

S100B is a dimeric molecule consisting of two β- subunits, with an atomic weight of 21kDa. S100B can be found in different type of cells both inside the central nervous system (CNS) as outside; it is mostly present in mature perivascular astrocytes (Thelin EP et al., 2017) but even in maturing olygodendrocytes, neural progenitor cells, dendritic cells, Schwann cells, melanocytes, chondrocytes, adipocytes, skeletal myofibers and lymphocytes (Donato R et al., 2009) (Donato R et al., 2013). S100B has multiple functions and works both as an intracellular regulator and an extracellular signal molecule (Donato R et al., 2009). Intracelluraly, S100B is localized in the centrosomes, cytoplasmic microtubules and type III intermediat filaments, thus effecting cytoskeleton interaction. S100B is involved in cell proliferation, it interacts for example with the tumor suppressor p53 inhibiting its phosphorylation. S100B protein is also an inhibitor of cell differentiation and it is responsible for maintenance of Ca2+ homeostasis in astrocytes. The extra-cellular value of S100B is also quite fascinating: both in vitro and in vivo studies have shown that S100B has a neurotrophic effect: it enhances growth and regeneration, stimulates neuronal survival after injury and protects neurons from toxic stimuli both by upregulating anti-apoptotic factors and indirectly by promoting uptake of glutamate by astrocytes. It has been observed that low concentrations of S100B, at nanomolar doses in the extracellular space, have clear neurotrophic effects yet doses higher than 500nM have a toxic effect upregulating apoptotic mechanism (Donato R et al., 2009).

S100B is eliminated from the blood via kidney filtration. The half-life in serum seems to be approximately 25-30 minutes but may be longer in TBI with possible ongoing S100B release (Jönsson H et al., 2000) (Ghanem G et al., 2001) (Thelin EP et al., 2017). The mean half-life of S-100B in the circulation after minor head trauma has been reported approximately 97 minutes (Townend W et al., 2006).

S100B is considered a stable protein; it is not affected by hemolysis or freezing (Raabe A et al., 2003) but does not tolerate long term storing (Müller K et al., 2006). On the market, there are several available analysis kits (Smit LH et al., 2005). In laboratories, the most used method is ELISA, produced by several manufacturers; this type of analysis requires a 4-6 hours procedure, which makes it unsuitable for clinical practice. The most used devices in the clinical settings are the LIASON-mat S100 system and Elecsys S100B system. LIASON system is a quantitative automated luminometric immunoassay and was firstly designed as to detect S100B in malignant melanoma. The Elecsys S100B system instead is an electrochemiluminecsence immunoassay which, due to its quick analysis time, 20 minutes after centrifuging of the blood sampling, is the most suitable method for clinical scenarios where speedy results are desired (Smit LH et al., 2005).

S100B and TBI

S100B is by far the most studied biomarker in TBI with the first publications dated in the early 1990s (Ingebrigtsen T et al., 1995). Moreover is the only biomarker in clinical use as a screening tool following TBI (Undén et al., 2013). Nevertheless S100B has a low specificity due to extra cranial sources. Notwithstanding its very high sensitivity and negative predictive value has made it a reliable tool for ruling out intracranial complications after TBI.

The clinical cut off used in TBI is 0.10 μg/l (Undén J et al., 2010); such a cut off has been chosen in order to reach a 100% sensitivity for complications. This approach renders the specificity low, at approximately 30% (Undén J et al., 2010).

The low S100B specificity in TBI patients is due mainly to the extra cranial sources of S100B. Adipocytes, for instance, express only marginally less mRNA of S100B compared with cerebral cortex, in comparison with other biomarker, such as GFAP, which show a 500 times higher expression in cortex compared to other extra cranial tissues (Sjöstedt E et al., 2015). Multitrauma patients with no TBI show high S100B levels in serum (Undén J et al., 2005)but clearance from the bloodstream seems faster compared to TBI (Thelin EP et al., 2017).

S100B's high sensitivity has been tested even in relation to neuroimaging and S100B has been proven to be more sensitive than CT in detecting findings that were detected on MRI (Ingebrigtsen T et al., 1996). The "false positive" results may be in fact partially explained by the detecting of lesions not visible on CT scans but visible on MRI. This fact has recently been investigated with similar results (Linsenmaier et al., 2016). Nevertheless, clinical relevance of these findings may be questionable as many such MRI findings have little clinical relevance, especially in acute mangement.

GFAP

Glial fibrillary acid protein (GFAP) is an intermediate filament protein component of the astrocyte cytoskeleton. It is specific highly for the CNS and breakdown products (GFAP-BD) may be found in peripheral blood after astrocyte damage (McMahon PJ et al., 2015). GFAP-BD is detectable in serum and increases after TBI; high levels sampled within 24h after injury are associated with poor outcome at 3 months in moderate and severe TBI and it has shown diagnostic potential even in mTBI patients with a reduction of CT scans (Okonkwo DO et al., 2013) (Kulbe JR et al., 2016). Despite this, the body of evidence concerning GFAP and mTBI is small and the biomarker cannot be recommended for clinical use.

NSE

The Neuron specific enolase (NSE) is a cytosolic protein involved in axonal transport; during axonal damage NSE levels increase in order to keep cellular homeostasis and regulate energy needs. High levels of NSE have been observed after different neurological insults, including mTBI and its transport outside the brain it is supposedly dependent on the glymphatic pathway more than on BBB impairment (Kawata K et al., 2016).

Nevertheless, NSE is unfortunately not brain-specific and can be found also in thrombocytes and erythrocytes, being very sensitive to hemolysis. Some studies have investigated the prognostic value of NSE, while other studies have shown that, despite a very high specificity, NSE showed low sensitivity to the magnitude of injury in the acute evaluation (Ingebrigtsen T et al., 2003). Although the clinical value of NSE in TBI seems doubtful, its use for prognostication in anoxic brain damage patients following cardiac arrest is recommended (Stammet P et al., 2015).

UCH-**L1**

Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is present in the cytoplasm of neurons, both in the central nervous system and in the peripheral nervous system and muscular junctions. It has been found in minor concentration in aortic endothelial and smooth muscles. UCH-L1 is an enzyme responsible for cleaning up oxidized or misfolded proteins (Kulbe JR et al., 2016).

UCH-L1 measures neuronal dysfunction instead for astrocyte damage (S100B, GFAP) or axonal damage (NSE, MBP, Tau, NF, etc) and would therefore be an interesting complement to other biomarkers (Diaz-Arrastia R et al., 2014). Few studies have been conducted trying to establish UCH-L1 role as a biomarker for TBI. Promising results have shown that UCH-L1 may have a higher performance compared to S100B and GFAP in mTBI patients (Welch RD et al., 2016). In a study with severe TBI patients elevated UCH-L1 was specific for diffuse injury opposed to focal injury. However, another study on moderate and mTBI did not show the same results leaving the future of UCH-L1 still to be determined (Kulbe JR et al., 2016).

H-FABP

Fatty acid-binding proteins (FABP) are a family of non-enzymatic proteins, named after the tissue where they were fist found. B-FABP, the brain type, is found only

in astrocytes of the white matter while H-FABP, the heart type, besides being a biomarker for cardiac injuries is also to be found in the neuronal cell bodies of the grey matter (Pelsers MM et al., 2004).

Only a few studies have been published that have considered H-FABP as a biomarker for TBI, with special attention to mTBI in comparison to S100B. Those studies have showed that H-FABP is more specific that S100B (Lagerstedt L et al., 2017). Nevertheless S100B results were much lower than those reported in other, larger studies. Further investigations are therefore necessary.

MBP

Myelin basic protein (MBP) is a major protein component of myelin in axons. MBP can be found in the PNS but is primarily found in the CNS. High levels of MBP can be measured after axonal injury or demyelinating disease and for this reason has been thought to be a possible biomarker for TBI (Kulbe JR et al., 2016).

The evidence available on MBP is poor and has so far only focused on the pediatric population with severe TBI with interesting, although limited, results (Su E et al., 2012).

SBDP

αII-spectrin is a cytoskeletal protein present in axons and pre-synaptic terminals of neurons. During cell death or cell damage, it is divided into spectrin break down products (SBDP). αII-spectrin N-terminal fragment (SNTF) increases after TBI and cerebral ischemia (Kulbe JR et al., 2016).

Few studies have been conducted with SBDP in mTBI cohorts. These show a potential prognostic value of the protein for detection of diffuse axonal injury and impaired cognitive functions (Siman R et al., 2013).

Tau

Tau is a microtubule binding protein, primarily expressed within axons. The main function is to allow the brain to stretch and retract against small mechanical forces. However, if these forces should be greater than threshold, the microtubule network will break causing diffuse axonal injury (DAI). Tau has been studied as a biomarker for axonal injury and more specifically for DAI (Kawata K et al.,

2016). Several studies have focused on the prognostic value of high TAU levels in severe TBI but unfortunately data have not been consistent (Kulbe JR et al., 2016).

Besides axonal mechanical damage, Tau is also affected by pathological mechanism as in Alzheimer's disease where high level of phosphorylated Tau can be measured. The phosphorylated tau does not function as normal tau promoting microtubule assembly, but instead, it has a neurotoxic effect by inhibiting assembly and disrupting microtubules (Iqbal K et al., 2010).

NF

Neurofilament (NF) is a protein found in axons and in dendrites of neurons. Increased levels of NF can be measured in plasma and CSF is several neurodegenerative disorders, such as MS or Alzheimer's disease. Only few studies have investigated NF levels in mTBI. The most interesting findings to date may be that an increase in NF after TBI can be measured 24-72 hours after injury and has shown very high sensitivity and specificity for intracranial complications (Kulbe JR et al., 2016). This time frame, however, is not clinically useful in acute mTBI management as most patients seek care within the first hours following injury. As a biomarker for long-term outcome after mTBI, however, this biomarker may allow better prognostication of post-concussion symptoms

	Biomarkers	CNS	Outside CNS	In mTBI
Astrocyte damage	**S100B**	Perivascular astrocytes, maturing olygodendrocytes, neural progenitor cells, dendritic cells, Schwann cells,	Melanocytes, chondrocytes, adipocytes, skeletal myofibers and lymphocytes	Well studied High sensitivity Low specificity
	GFAP	Astrocyte cytoskeleton		Few studies God performances
Axonal damage	**NSE**	Axonal cytosolic protein	Thrombocytes and erythrocytes	Doubtful results
	MBP	Myelin in axons	Myelin in axons	Poor evidence
	NF	Axons and dendrites of neurons		High sensitivity and specificity but slow release
	SBDP	Axons and pre-synaptic terminals of neurons		Few studies
	Tau	Microtubule binding protein within axons		No consistent results
Neuronal damage	**UCH-L1**	Neurons	Neurons PNS and muscular junctions	Opposite study results
	H-FABP	Neuronal cell bodies of the grey matter	Heart muscle cells	Few studies God specificity

Aim of the thesis

This thesis has been structured around the clinical aspects of the Scandinavian guidelines for management of minimal, mild and moderate head injury in adults with special focus on the role of S100B in this patient group.

The primary aims are to:

- Investigate the clinical implementation of S100B into the existing SNC guidelines.
- Investigate the relationship of older age and alcohol intoxication to S100B serum levels in mTBI patients.
- Externally validate the performance of the new SNC guidelines (SNC13) for important intracranial outcomes in a mTBI cohort.
- Investigate the health economic benefit of S100B implementation into clinical guidelines.
- To design and initiate a pragmatic, prospective, multicenter validation study for the SNC13 in Sweden.

Study design and Methods

All the studies included in this thesis present S100B from a clinical perspective.

In total 1806 patients have been included in these studies; 1144 patients from Hallands Hospital Halmstad with mTBI from November 2007 until May 2017 and 662 from the multicenter study from New York and Pennsylvania between 2008 and 2010.

Paper 1

A prospective, observational-study was performed in Hallands Hospital Halmstad, Sweden, from November 2007 to May 2011. S100B was introduced into the existing SNC guidelines 6 months prior to commencing the study, creating new local management routine, based upon the available evidence at that time. These guidelines were therefore used clinically to manage head injury patients in the ED from May 2007.

All adult patients with mTBI and S100B sampling were enrolled for the study. Inclusion criteria were the same as for mTBI patients in the SNC guidelines with the addition of the S100B sample; specifically, adult patients with acute trauma to the head, presenting at the ED within 24 hours from trauma, with GCS 14-15 during examination, loss of consciousness < 5 minutes and/or amnesia. Exclusion criteria were presence of neurological deficits and/or additional risk factors (therapeutic anticoagulation or haemophilia, radiographically demonstrated skull fracture, clinical signs of depressed skull fracture or skull base fracture, posttraumatic seizures, shunt-treated hydrocephalus and multiple injuries), age less than 18 years, no Swedish personal identification number (difficult to follow up) and patients where serum sampling for S100B was done more than 3 hours post-injury.

Details of how patients were managed, including patient characteristics, type of injury, patient history, medications, clinical examination results, S100B levels, CT results, admission type and duration were documented. Patients were asked to answer a questionnaire sent by mail 3 months after the injury. Included in this questionnaire were questions that would identify a significant intracranial lesion (i.e. symptoms from such an injury, cases of new neuroimaging, new health care

contacts and other complaints). In cases where patients could not be reached by mail or telephone, medical records and national mortality databases were consulted for evidence of complications and/or death. Medical records and mortality data for the whole country are available to the Swedish health care providers for all persons with a Swedish personal identification number. Patients who suffered significant (enough to seek medical care) intracranial complications after discharge could therefore be identified, thus this was chosen as the major outcome variable for the study.

Data was registered on an Excel® file. Sensitivity, specificity, positive and negative predictive values were calculated from cross tabulation between S100B and intracranial complications.

Paper 2

A prospective, observational-study was performed in Hallands Hospital Halmstad, Sweden, from June 2008 to December 2012. The same inclusion and exclusion criteria for paper 1 were applied.

Patient age at the time of injury and alcohol intoxication was documented: the age limit of ≤ 65 was chosen as a pre-determined cut off for analysis accordingly to previously published clinical guidelines (Stiell IG et al., 2001) (Haydel MJ et al., 2000) (NICE, 2000) (Mower WR et al., 2002) (Smits M et al., 2007). Alcohol levels were measured only upon the discretion of treating physicians.

The difference in S100B levels between age groups and intoxicated/sober patients were calculated.

Cost analysis (paper 4)

The aim of this paper was to examine the impact in a real-life clinical setting and safety of S100B in management of mTBI patients.

The study setting was Halland Hospital Halmstad, Sweden. From November 2007 to December 2013 we prospectively enrolled consecutive adult patients with mTBI and S100B sampling, according to former SNC guidelines. S100B sampling was done within 3 hours post-injury.

Details of how patients were managed as well as the follow up questionnaire were similar to those applied in paper 1 and 2.

A comparison of number of sick days between the two groups of patients was performed. Cost analysis was based upon standard costs according to our hospital

accounts or (where data is missing) national reports. We did not calculate a monetary value regarding the opportunity costs related to time spent by patients in the ED (difficult to assess) and we did not consider socioeconomic costs associated with increased cancer risks from CT scans at all (theoretically based and difficult to estimate). Not considering these aspects would lead to an under-estimation of the cost-saving potential of S100B implementation.

Clinical validation (papers 3 and 5)

Papers 3 and 5 were designed with the ambition to clinically validate the SNC13. Paper 3 had the advantage of being able to use a foreign database in order to apply an "external" validation of the guidelines. Paper 5 is a protocol design for an ongoing prospective, pragmatic, multicenter study to validate the performance of SNC13 in Sweden.

Paper 3

We performed a nested cohort study of adults with mTBI where S100B was sampled within 6 hours form injury in 6 hospitals in New York and Pennsylvania between 2008 and 2010. Accordingly to the existing local guidelines, all patients underwent a CT scan. Follow up telephone interview one month after ED visit was performed to assess missed intracranial complications and recovery after TBI.

Registered clinical variables and S100B levels were used to classify patients according to the SNC13 into moderate TBI, mTBI-high risk, mTBI-medium risk, mTBI-low risk and minimal TBI. The prevalence of traumatic CT findings was calculated and compared for each category. As the prevalence of positive CT findings were only $\leq$5% in two groups (moderate and mTBI-high risk), Fisher exact test was used.

The need for CT scans as predicted by the SNC13 was compared to the CT results to determine sensitivity and specificity of SNC13.

Paper 5

This paper outlines the design of an ongoing study to validate the performance of the SNC13 in predicting intracranial complications in adult patients presenting with traumatic head injury in Swedish hospitals. A secondary aim is to compare the performance of SNC 13 with 6 other clinical guidelines, with respect to important outcomes. Moreover, we want to explore the performances of different

biomarkers in predicting intracranial complications in predefined subgroups of TBI. Finally, we want to evaluate the possibility of further improvement of the SNC13 guidelines.

In September 2017, we will perform a prospective, multicenter, pragmatic, observational study of adults with a GCS 9-15 presenting with traumatic head injury at the ED within 24h after TBI. The study will be set in Halmstad, Malmö, Lund, Örebro and Linköping, Sweden. All data necessary for analysis including predictor variables and outcome data for all the seven guidelines included in the study will be registered. Patients will be managed clinically accordingly to the judgment of the responsible physician and/or local guidelines. A follow up questionnaire will be sent 3 months after TBI in order to detect missed intracranial complications. In cases where patients could not be reached by mail or telephone, medical records and national mortality databases were consulted for evidence of complications and/or death. The Swedish health care systems gives medical professionals full access to medical records and mortality data for the whole country, for residents with a Swedish identification number. Therefore no patient suffering late complications will be missed.

Sensitivity, specificity, predictive values and likelihood ratios will be calculated for the SNC13 for identifying; a) traumatic intracranial complications, b) patients needing neurosurgery or neurointensive care for the TBI, and c) new, acute, traumatic intracranial pathology on CT including intracranial hematomas of any size, cerebral contusions and depressed skull fractures, as well as clinically relevant CT findings, defined as contusions larger than 5mm in diameter, subarachnoid bleeding thicker than 1mm, subdural hematoma thicker than 4mm, pneumocephaly that will need intervention, depressed skull fracture through the inner table.

Moreover, we will calculate sensitivity, specificity, predictive values and likelihood ratios of each guideline in identifying traumatic intracranial CT finding when applied to the same TBI population (comparison cohort). This cohort will include only patients with a GCS of 13-15. We will also measure frequency of CT scans. We will investigate the performance of the SNC13 in comparison to other guidelines in reducing CT frequency without missing complications. We hypothesize that the implementation of a biomarker as S100B into clinical guidelines will achieve a further reduction in CT scans. Accuracy variables will be statistically compared with Chi-squared test.

In a explorative analysis we will compare S100B with GFAP, SBP-50 and Tau on the same selected mTBI population in order to determine the potential value of a panel of biomarkers for identifying high and low risk patients. We will calculate sensitivity, specificity, predictive values and likelihood ratios of each biomarker in identifying traumatic intracranial CT finding when applied to the same TBI

population. ROC curves will be calculated in order to compare cut off values. We will also use the net reclassification index to see if each biomarker improves the accuracy of the classification.

Finally, we would like to study is the derivation of a new improved guideline. Binary logistic regressions analysis of all the variables taken into account and registered during the study will be performed. We will a priori divide the population into a derivation cohort, obtain ROC curves, and use these cutoffs after the bootstrapping process and other clinical and biochemical variables to construct a mode losing multivariable analysis

S100B sampling

S100B analysis has been conducted in 3 of 4 studies by the same laboratory at the Clinical Chemistry Department of Halland Hospital Halmstad, Sweden. 5ml of blood was drawn from patients' cubital vein in the ED and was analyzed with the fully automated Elecsys® S100 (Roche AB). Roche AB reports a range between 0.005µg/L and 39µg/L and a within-series coefficient of variance of <2.1%. Based on the available evidence at the time of the studies, we chose a cut-off level for normal levels of less than 0.10µg/L and a window of sampling of 3 hours from the time of the accident in paper 1,2 and 4, and a window of sampling of 6 hours from injury in paper 3 and 5.

Ethical approval

Papers 1, 2 and 4 were approved by the Regional Ethical Review Board of Lund (approval number 19/2007). For paper 3, ethical approval was granted from the Institutional Review Boards for each of the participating centre in the USA where the parent study was conducted.

For paper 5, ethical approval was granted from the Regional Ethical Board of Lund (approval number 2012/574). In this study informed verbal consent is sufficient for patients to be included in data registration, while written consent is necessary from all patients where the extra blood sampling is collected for biomarker analysis (other than that needed for S100B analysis for clinical management).

Results

From 2007 until 2017, a total of 1144 patients with mTBI with S100B sampling have been included. Initially, patients were defined as mTBI according to the old SNC guidelines and S100B was sampled only if patients seek ED within 3 hours form injury. Since 2013 the sampling interval has been extended to 6 hours with the updated SNC13 guidelines.

57 patients (5% of the cohort) showed intracranial CT findings and all of them had pathological S100B levels. A neurosurgical consult was done for 14 patients but none needed neurosurgical intervention. One patient died as a consequence of TBI; as reported in previous article, a 83-year-old man with an S100B level of 0.23µg/L who was deemed to frail for neurosurgery and received palliative care. 30% of patients had negative S100B levels and could be discharged. None of these had any intracranial complications at follow up.

Descriptive statistics for the entire population shows 100% sensitivity and 34% specificity (95% CI, 31% to 37%). Importantly, the NPV was 100% with narrow confidence intervals (lower 95% CI of 99%). AUC is 0.81 for the entire population for a cut off level of 0.10µg/L.

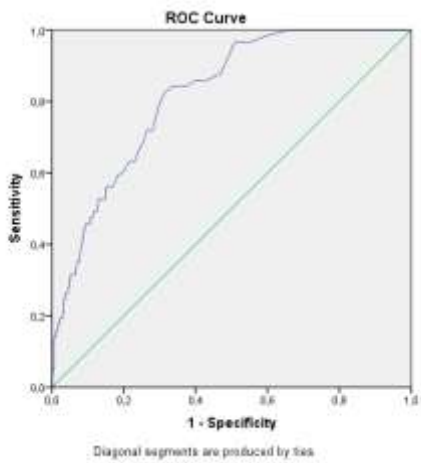

Figure 1 ROC S100B results for the whole population

Diagonal segments are produced by ties.

Table 1: Descriptive statistics for the whole population

Sensitivity	100.00%	93.73% to 100.00% (95%CI)
Specificity	34.13 %	31.31% to 37.04%
Positive Likelihood Ratio	1.52	1.45 to 1.58
Negative Likelihood Ratio	0.00	
CT findings prevalence	4.98%	3.80% to 6.41%
Positive Predictive Value	7.37%	7.09% to 7.67%
Negative Predictive Value	100.00 %	98.72% to 100%

When we stratified our population according to age, we saw a statistically significant difference in S100B levels and an increase specificity of S100B to almost 40% in the patients younger than 65 years. AUC was 0.84, while in elderly patients the AUC was 0.69.

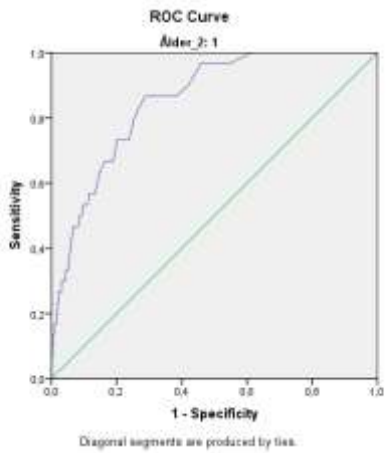

Figure 2 ROC S100B in patients < 65 years old

Table 2: Descriptive statistics for patients < 65 years old

Sensitivity	100%	88.43% ot 100% (95% CI)
Specificity	38.66%	35.40% to 42%
Positive Likelihood Ratio	1.63	1.55 to 1.72
Negative Likelihood Ratio	0.00	
CT findings prevalence	3.36%	2.28% to 4.76%
Positive Predictive Value	5.36%	5.10% to 5.63%
Negative Predictive Value	100%	98.58% to 100%

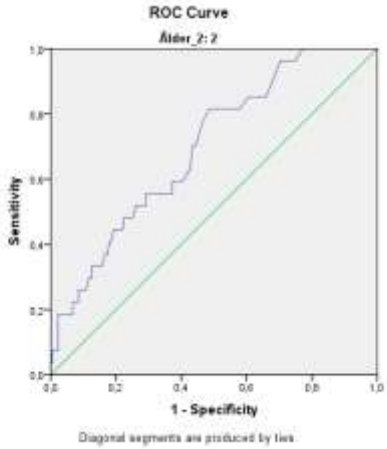

Figure 3 ROC S100B in patients ≥ 65 years old

Table 3: Descriptive statistics for patients ≥ 65 years old

Sensitivity	100%	87.23% to 100.00% (95% CI)
Specificity	14.80%	10.64% to 19.82%
Positive Likelihood Ratio	1.17	1.11 to 1.24
Negative Likelihood Ratio	0.00	
CT findings prevalence	9.75%	6.52% to 13.86%
Positive Predictive Value	11.25%	10.74% to 11.78%
Negative Predictive Value	100%	88.28% to 100%

Paper 1

512 patients with MHI who were managed with the aid of S100B levels were enrolled between November 2007 and May 2011. 26 patients had cranial CT pathology but only 24 (4.7%) showed traumatic CT abnormalities (isolated skull fracture n=3, cerebral contusions n=7, acute subdural hematoma n=3, intracranial air n=1, combinations of traumatic intracranial findings n=10). No patients needed neurosurgical intervention.

138 patients (27%) had a S100B level less than 0.10μg/L and 374 patients (73%) showed a S100B level higher or equal to 0.10μg/L. The follow up questionnaire was completed for 414 patients (81%). Medical records and the mortality database

were successfully checked for all remaining patients. No patients with a normal S100B level showed significant intracranial complication. 215 patients (42%) were alcohol intoxicated.

S100B had sensitivity and NPV of 100% for significant intracranial complications, a specificity of 28% and a positive predictive value (PPV) of 6%.

Table 4: Population Paper 1

	S100B < 0.10µg/L	S100B ≥ 0.10µg/L	All
Male	85 (61,6%)	229 (61,3%)	314 (61,5%)
Female	53 (38,4%)	145 (38,7%)	198 (38,5%)
Age (mean)	32,6	46,6	42,2
Total	138	374	512

Table 5: Descriptive statistics Paper 1

Sensitivity	100%	85.75% to 100.00%(95% CI)
Specificity	28.28 %	24.32% to 32.50%
Positive Likelihood Ratio	1.39	1.32 to 1.47
Negative Likelihood Ratio	0.00	
CT findings prevalence	4.69%	3.03% to 6.89%
Positive Predictive Value	6.42%	6.09% to 6.76%
Negative Predictive Value	100.00 %	96.62 % to 100%

Paper 2

621 patients with MHI who were managed with the aid of S100B levels were enrolled for this study.

29 patients had cranial CT pathology but only 26 (4.7%) showed traumatic abnormalities (isolated skull fracture n=3, cerebral contusions n=9, acute subdural hematoma n=3, intracranial air n=1, combinations of traumatic intracranial findings n=10). 18/26 patients (69%) with CT findings were younger than 65 years old. No patients needed neurosurgical intervention.

280 patients (45%) were intoxicated by ethanol; in 197 patients an increased blood alcohol level was detected. No correlation was seen between S100B levels and alcohol intoxication or S100B levels and ethanol levels

Table 6: Population Paper 2

	S100B < 0.10µg/L	Median S100B µg/L	S100B ≥ 0.10µg/L	Median S100B µg/L
<65 years	171 patients	0.07 (0.03-0.09)	335 patients	0.19 (0.10-4.51)
≥65 years	9 patients	0.07 (0.04-0.09)	106 patients	0.23 (0.10-6.75)
No Alcohol intoxication	106 patients	0.07 (0.03-0.09)	235 patients	0.20 (0.10-6.75)
Alcohol intoxication	74 patients	0.07 (0.03-0.09)	206 patients	0.20 (0.10-4.51)

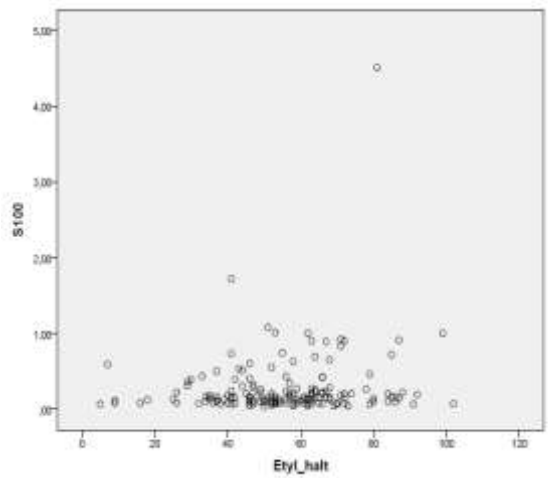

Figure4: correlation S100B and blood ethanol concentration

180 patients (28.9%) had a S100B level lower than 0.10µg/L. 171 of these patients (95%) were younger than 65 years of age and the mean S100b levels was 0.06µg/L. 9 patients (5%) were older than 65 years of age and even in this group the mean S100b levels was 0.06µg/L.

441 patients (71%) showed a S100B level higher or equal to 0.10µg/L. 315 of these patients (71.5%) were younger than 65 years old and had a mean S100B level of 0.32µg/L while the 126 patients older than 65 years old had a mean S100B level of 0.55µg/L (t-test p=0.000). Medical records and the mortality database were successfully checked for all patients. No patients with a normal S100B level showed significant intracranial complication

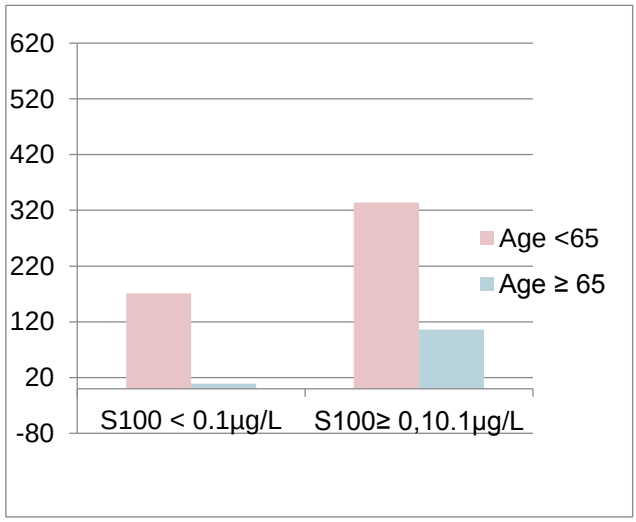

Figure 5: S100B and age

S100B had a sensitivity and NPV of 100% for significant intracranial complications and a specificity of 30 % for the entire cohort. The specificity increased to 35 % if only the patients younger than 65 years were considered. The likelihood ratio that a S100B level higher or equal to 0.10µg/L was predicting a pathological CT was 1.44 for the entire cohort while it increased to 1.54 if only patients younger than 65 years of age were considered.

Table 7: Descriptive statistics Paper 2

Sensitivity	100%	86.77% to 100.00%(95% CI)
Specificity	30.25 %	26.58% to 34.12%
Positive Likelihood Ratio	1.44	1.36 to 1.51
Negative Likelihood Ratio	0.00	
CT findings prevalence	4.19%	2.75% to 6.07%
Positive Predictive Value	5.90%	5.61% to 6.20%
Negative Predictive Value	100.00 %	97.39% to 100%

Table 8: Descriptive statistics patients < 65 years old Paper 2

Sensitivity	100%	81.47% to 100.00% (95% CI)
Specificity	35.04 %	30.81% to 39.46%
Positive Likelihood Ratio	1.54	1.44 to 1.64
Negative Likelihood Ratio	0.00	
CT findings prevalence	3.56%	2.12% to 5.56%
Positive Predictive Value	5.37%	5.61% to 6.20%
Negative Predictive Value	100.00 %	97.26% to 100%

Table 9: Statistics patients ≥ 65 years old, Paper 2

Sensitivity	100%	63.06% to 100.00% (95% CI)
Specificity	8.41 %	3.92% to 15.37%
Positive Likelihood Ratio	1.09	1.03 to 1.16
Negative Likelihood Ratio	0.00	
CT findings prevalence	6.96%	3.05% to 13.25%
Positive Predictive Value	7.55%	7.16% to 7.96%
Negative Predictive Value	100.00 %	62.88% to 100%

Cost analysis (Paper 4)

We enrolled 795 patients with mTBI and S100B levels. 69 patients were excluded according to exclusion criteria and the final population was therefore 726 patients. Compliance to guidelines was reasonable with more than 67% of patients managed correctly according to the guidelines. 229 patients had a S100B level lower than 0.10μg/L (29% of the population).

32 patients had pathology on CT, but only 29 of these (4.7%) were classed as traumatic abnormalities. No patients needed neurosurgical intervention. One patient with a small cerebral contusion was discharged without hospitalization. One patient died as reported earlier as a result of the head injury. The follow up questionnaire was completed for 589 patients (81%) and the medical records were checked for the remainder; no patient with negative S100B levels sought the emergency room for missed complications.

The actual cost was calculated for the 726 patients strictly taking into account only S100B analysis, CT and hospitalization cost, for an average of 242 € per patient per admission. To calculate the potential reduction in cost, we calculated several potential costs given different assumptions; 1) potential cost if S100B is not used in the guidelines and assuming the same practices regarding CT and hospitalization for all patients as for the 570 patients that had high S100B levels in the actual cohort (281 € per patient), and 2) potential cost if the guidelines with S100B are followed strictly and assuming that only CT is used, as recommended in the guidelines, for the 497 patients with S100B levels higher than 0.10 ug/L (110 €). If the guidelines were followed strictly and CT only was used as the management option, the potential savings per patient was 71 € for this cohort. Given the actual use of S100B and CT/ hospitalization for our cohort (i.e. compliance to the guidelines was not perfect), actual savings were limited to 39 € per patient.

Table 10: Descriptive statistics for population Paper4

	S100B < 0.10µg/L	S100B ≥ 0.10µg/L	All
Male	140 (61.1%)	305 (61.3%)	445 (61.3 %)
Female	89 (38.9%)	192 (38.7%)	281 (38.7 %)
Age (mean)	31,8 years (Range 18-89y)	46,6 years (Range 18-92y)	42,2 years
Alcohol intoxication	94 (41%)	231 (46.4%)	325 (44.7%)
Total	229	497	726

Table 11: cost analysis

		S100= 21 €	CT= 130 €	Hospitalization= 366 €	Tot
ACTUAL COST in follow-up (cost per patient)		726 x 21 €= 15 246 €	398 x 130 €= 51 740 €	297 x 366 €= 108 702 €	175 688 € (242 €)
POTENTIAL COST given different assumptions	S100B not in guidelines and assuming same use of CT and hospitalization as for cohort		0,7x726 x130 € = 66 066 €	0,52x726x366 € = 138 172 €	204 238 € (281€)
	Strict compliance based on guidelines for S100 + CT only	726 x 21 €= 15 246 €	CT(S100B+) 497 x 130€= 64 610 €		79 856 € (110 €)

Validation (papers 3 and 5)

Paper 3

784 patients were enrolled accordingly to the parent study, 93 were children and 29 patient had missing data; 662 patients were considered eligible for our validation study. CT findings were judged as positive in 36 patients (5%) and no patients needed neurosurgical intervention.

The prevalence of CT findings was highest in the moderate TBI group (5%) and lowest in the minimal TBI group (0%).

The SNC13 defined 451 patients in need of CT scan and 211 as not needing one; the SNC guidelines showed a 97% sensitivity (95% CI, 84-100%) and 34% specificity (95% CI, 30-37%) for predicting traumatic CT findings. One patient, a 20 years old male presenting to the ED after a motor vehicle accident with a GCS 14 and LOC, had a borderline normal S100B levels (0.09µg/L). He had a small cerebral contusion on CT that had disappeared after a repeat CT 1 week later. This patient did not have any neurological sequel on follow up and did not require any treatment for his TBI.

Table 11: Statistics Paper 3

Sensitivity	97.22%	83.79% to 99.85% (95% CI)
Specificity	33.46 %	29.88% to 37.41%
Positive Likelihood Ratio	1.46	1.35 to 1.58
Negative Likelihood Ratio	0.08	0.01 to 0.57
CT findings prevalence	5.44%	3.84% to 7.45%
Positive Predictive Value	7.55%	7.16% to 7.96%
Negative Predictive Value	99.52%	96.97% to 99.97%

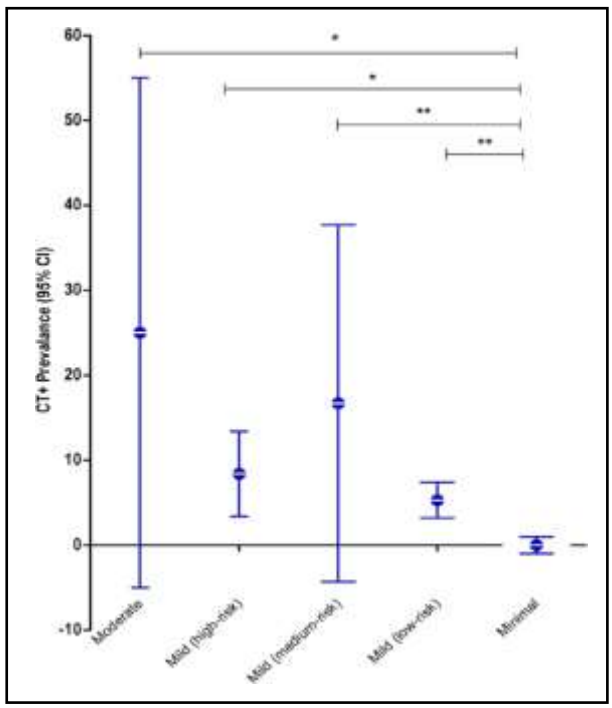

Figure 6: Prevalence of traumatic CT abnormalities by SNC guideline severity categories. *P<0.01, **P= 0.01-0.05,

Paper 5

Paper 5 is a study protocol; no results were available when this thesis was written.

Discussion

Mild TBI patients have been extensively studied during the past decades and the clinical management of this specific group of patients has evolved accordingly. For the first time, a brain biomarker, S100B, was allowed to influence clinical management of patients at ED. Some aspects of the first published articles may be considered anachronistic, as for instance the sampling window for S100B or the inclusion/exclusion criteria of the former SNC guidelines. Nevertheless, the general conclusion, that S100B can be safely used in mTBI patients as a screening tool for safely reducing the number of CT scans, is still valid today. The implementation of S100B into SNC13 was the logical evolution of a process that started in Halmstad in 2007. Among all the risk factor data included in the design of the SNC13, S100B was the only negative predictor and displayed the most consistent data (Undén et al., 2013).

Clinical observation studies

The main aim of the first studies was to show that S100B worked clinically and was a helpful tool in reducing number of CT scans in a safe and objective way. Our observational studies confirmed a reduction in CT frequency by approximately 30%, similar to previous studies where S100B was not used clinically (Biberthaler P et al., 2006). Results are even more impressive when the study population is stratified by age; when tested on patients younger than 65 years, S100B specificity reached almost 40%.The ROC curve analysis confirms and strengthens the results shown in the second paper and support the use of age as a risk factor and also the risk stratification chosen for the SNC13.

A difficult aspect to deal with in an ED scenario is intoxicated patients. In mTBI, almost 45% of patients are affected by alcohol (Calcagnile O et al., 2013). Considering that clinical guidelines evaluate patient history and symptoms, to determine if a patient should or should not undergo a CT scan, this particular patient group offers obvious management challenges. Our results clearly show no correlation between S100B levels and alcohol in mTBI patients, with sustained clinical performance in intoxicated patients. Biomarkers should be as independent as possible from other variables in order to ensure objective accuracy; alcohol

intoxication is so common in this patient group that only biomarkers not affected by ethanol levels can be considered useful.

Cost analysis

We live in a society with limited resources and mTBI is a particular group where the economic impact has become considerable owing to a high incidence of patients seeking ED. Nevertheless, to measure benefits after changes in guidelines is complicated.

In our cost analysis study, we quantify how clinical implementation of S100B has helped the health care system to save resources. These calculations were very reductive considering that many aspects were not taken into account. These may include the potential cost savings from an easier discharge procedure for S100B negative patients, or the limitation of ionizing radiation. These potential cost savings are very complex to quantify and investigate.

Both the clinical observational studies and the cost analysis have highlighted another very important topic of discussion; in all these studies compliance to guidelines was not perfect. Nevertheless, guidelines are only tools available for clinicians in order to rationalize difficult medical situations but should never overrule the individual physician judgment.

Validation

Clinical validation is necessary before widespread implementation of guidelines can be initiated. Thanks to the cooperation with other research groups, we were able to validate the SNC13 results in a well-designed cohort. When the guidelines were introduced, we could dispose of an independent patient database and apply the SNC algorithm to identify intracranial complications. This was an elegant expedient that allowed us to draw important results shortly after the publications of the guidelines. Other than showing a high sensitivity for intracranial complications, the SNC13 would have reduced CT scans is this pre-selected cohort. Also, analysis showed that the risk stratification used in the guidelines seemed valid. Despite this, an internal validation in the health care system for which the guidelines were designed for was needed.

The most complex study that is included in this thesis is the planned validation study where multiple aspects of SNC13 and biomarkers analysis will be taken into account. The central core of the study will be the validation of SNC13 in Sweden in respect to the primary endpoints. Unpublished analysis of the first 100 patients with low-risk mTBI shows that S100B is negative in approximately 50% of

patients. Part of the validation will be also to compare the SNC13 to the other 6 guidelines in the same Swedish cohort in order to evaluate which guideline is more suitable in the Swedish health care system. The addition of S100B results into these guidelines will also be investigated.

Another important aspect will be focused on biomarker analysis. S100B is not the perfect biomarker for mTBI considering its low specificity, so it is important to evaluate the performance of other biomarkers. We now have a well-established tradition of clinical using a biomarker as a screening tool in this patient group; for this reason Scandinavia could be an adequate setting to investigate other biomarkers with potentially better performances, in particular specificity, than S100B.

Finally, it is fundamental to look forward and explore the possibility of improving the SNC13 by means of analysis of all data that will be collected during the multicenter study.

Strengths and Limitations

The main strength of this thesis is its consistency concerning the theme of moving towards clinical use with the SNC13 and S100B. The clinical impact of the first study has been important in the evidence process that has generated the SNC13. These reports reflect more accurately the actual clinical situations rather than a controlled study. In these reports, S100B was used as a clinical tool and hence the results reflect a clinical reality. Following this, internal and external validation adds to the essential information needed for widespread clinical implementation.

Nevertheless, all studies have limitations. The studies overlap in time and part of the same population has been included in more than one study. However, different aspects were studied and endpoints were decided *a priori*.

As inclusion criteria for studies 1-4 included the measurement of S100B, possible selection bias exists. We did not register all mTBI patients, i.e. patients with mTBI that did not, for some reason, have sampling for S100B analysis. This, however, reflects the clinical reality. Guideline compliance was not perfect and some patients are managed without using the approved management routines. As the final study, the prospective validation of the SNC13 in Sweden, is a general validation of the entire guideline, not just S100B, this study will include all patients with TBI and GCS scores of 9-15.

One may argue that S100B negative patients should be exempted from intracranial CT findings because CT was not performed (verification bias). Nevertheless, we tried to reduce the possibility of missed complications with the structured follow up questionnaire and access to medical records and/or mortality database. This

combination is judged as adequately robust to detect missed complications of any real clinical consequence. There is, however, a possibility that some of these patients had subclinical findings that would be evident on CT. None of these, however, needed any intervention and all patients had a good outcome. The alternative would be a CT scan on all patients, similar to prior studies. Considering that S100B was already proven to be safe in clinical scenario by multiple studies at the time of initiation, to submit healthy individuals to unnecessary radiations was deemed ethically questionable. At some point in the evolution of new clinical routines, implementation must take place and adequately reported, as in this thesis.

Finally, in our entire cohort, the prevalence of CT findings is approximately 5%. Prevalence is low but consistent with other studies (Bazarian J et al., 2005) (Biberthaler P et al., 2006). A low prevalence will push towards a high NPV and a low PPV (Altman et al., 1994). As with sensitivity and specificity, likelihood ratios are not influenced by the prevalence of the condition in question, and may therefore be more robust measurements of diagnostic accuracy (Altman DG et al., 1994). However, as long as the prevalence in the target population for a test is similar to the prevalence in the study population, bias should be limited. Considering predictive values are possibly the most clinically relevant parameter (i.e. a test result exists and the chance of disease or no disease is desired), and that the target population is similar to populations from studies (as in this case), predictive values are still of importance. Nevertheless, we report all values for the current work, in order to allow readers to choose their own parameters depending on the intended use.

Conclusions

The following main conclusions were reached in this thesis:

- S100B can be safely integrated into existing clinical guidelines.
- Patients with low S100B levels after mTBI can safely be discharged without a CT scan.
- S100B is not affected by alcohol intoxication in mTBI patients.
- S100B levels are higher in elderly patients with mTBI.
- The performance of S100B improves if applied to patients under 65 years of age.
- SNC13 can further reduce the number of CT scans in mTBI patients already selected for CT scanning.
- The risk stratification in the SNC13 guidelines is justified.
- The implementation of S100B into clinical guidelines was cost saving.

Acknowledgements

First and foremost I want to express my enormous gratitude towards Johan Undén my main supervisor. You have been able to inspire and encourage me from my very first weeks as an intern at HSH. My S100B-adventure started as a small project but, back then, you already had a clear vision of how we could develop it into this wonderful thesis! Without your guidance, support, encouragement and inspiration I would have never reached this point.

I would also like to thank my co-supervisor, Michelle Chew. It was a real honor to have you on board this fantastic trip. Your guidance and supervision was a fundamental complement to my work, you are a true source of inspiration for your accuracy, knowledge and your ambition.

My sincere thanks also go to the entire FoU team, especially Anders Holmén, Amir Baigi, Hanna Svensson, Marit Petrius, Yvonne Johnelius, Ola Andersson. You were my statistical "lab"; you were always there when I needed help and even made statistics almost funny! Without your precious support it would not have been possible to conduct this research.

I want to thank all my fellow colleagues at the Pediatric Department; thank you for encouraging me, covering up for me when I was unfocused, believing that I would make it. To all of you who were so patient with me after my sleepless nights in front of a computer. In particular, I am grateful to Attila and Dörte, my desk neighbors, for all your patience and support!

Last but not the least, I would like to thank my family: my husband and daughters that have seen me so little lately, in particular Roberta that was so proud that mummy was writing a book and helped with the front page. You three are the best of my life! To my parents, you made me who I am today, you always believed in me even when I took questionable decisions. To all my friends, in particular Valeria and Catherine, all of you showed so much support and warmth.

Thank you all for being there for me!

References

ACRM The American Congress of Rehabilitation Medicine. Definition of mild traumatic brain injury. J Head Trauma Rehabil 1993;8(3):86-87.

Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ. 1994 Jul 9;309(6947):102.

Andersson EH, Björklund R, Emanuelson I, Stålhammar D. Epidemiology of traumatic brain injury: a population based study in western Sweden. Acta Neurol Scand. 2003 Apr;107(4):256-9.

Bazarian J, McClung J, Cheng Y, Flesher W, and Schneider S. Emergency department management of mild traumatic brain injury in the USA. Emerg Med J. 2005 Jul; 22(7): 473–477.

Biberthaler P, Linsenmeier U, Pfeifer KJ, Kroetz M, Mussack T, Kanz KG, Hoecherl EF, Jonas F, Marzi I, Leucht P, Jochum M, Mutschler W. Serum S-100B concentration provides additional information for the indication of computed tomography in patients after minor head injury: a prospective multicenter study. Shock. 2006 May;25(5):446-53.

Borg J, Holm L, Cassidy JD, Peloso PM, Carroll LJ, von Holst H, Ericson K; WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. Diagnostic procedures in mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain InjuryJ Rehabil Med. 2004 Feb;(43 Suppl):61-75.

Boyle A, Santarius L, Maimaris C. Evaluation of the impact of the Canadian CT head rule on British practice. Emerg Med J. 2004 Jul;21(4):426-8.

Calcagnile O, Holmén A, Chew M, Undén J. S100B levels are affected by older age but not by alcohol intoxication following mild traumatic brain injury. Scand J Trauma Resusc Emerg Med. 2013; 21: 52. Published online 2013 Jul 6.

Cassidy JD, Carroll LJ, Peloso PM, Borg J, von Holst H, Holm L, Kraus J, Coronado VG; WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med. 2004 Feb;(43 Suppl):28-60.

Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood-brain barrier pathophysiology in traumatic brain injury. Transl Stroke Res. 2011 Dec;2(4):492-516.

Choe MC. The Pathophysiology of Concussion. Curr Pain Headache Rep. 2016 Jun;20(6):42.

Diaz-Arrastia R, Wang KK, Papa L, Sorani MD, Yue JK, Puccio AM, McMahon PJ, Inoue T, Yuh EL, Lingsma HF, Maas AI, Valadka AB, Okonkwo DO, Manley GT;

TRACK-TBI Investigators. Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. J Neurotrauma. 2014 Jan 1;31(1):19-25.

Di Battista AP, Rhind SG, Baker AJ. Application of blood-based biomarkers in human mild traumatic brain injury. Front Neurol. 2013 May 1;4:44.

Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol. 2001 Jul;33(7):637-68.

Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, Giambanco I. S100B's double life: intracellular regulator and extracellular signal. Biochim Biophys Acta. 2009 Jun;1793(6):1008-22.

Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, Geczy CL. Functions of S100 proteins. Curr Mol Med. 2013 Jan;13(1):24-57.

Faul M, Coronado V. Epidemiology of traumatic brain injury. Handb Clin Neurol. 2015;127:3-13.

Ghanem G, Loir B, Morandini R, Sales F, Lienard D, Eggermont A, Lejeune F; EORTC Melanoma Group.On the release and half-life of S100B protein in the peripheral blood of melanoma patients. Int J Cancer. 2001 Nov;94(4):586-90

Green SM. Cheerio, laddie! Bidding farewell to the Glasgow Coma Scale. Ann Emerg Med. 2011 Nov;58(5):427-30.

Harmon KG, Drezner JA, Gammons M, Guskiewicz KM, Halstead M, Herring SA, Kutcher JS, Pana A, Putukian M, Roberts WO. American Medical Society for Sports Medicine position statement: concussion in sport. Br J Sports Med. 2013 Jan;47(1):15-26.

Haukoos JS, Gill MR, Rabon RE, Gravitz CS, Green SM. Validation of the Simplified Motor Score for the prediction of brain injury outcomes after trauma. Ann Emerg Med. 2007 Jul;50(1):18-24. Epub 2006 Nov 16.

Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev. 2005 Jun;57(2):173-85.

Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PM. Indications for computed tomography in patients with minor head injury. N Engl J Med. 2000 Jul 13;343(2):100-5.

Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med. 2012 Aug 15;4(147):147ra111.

Ingebrigtsen T, Romner B. Serial S-100 protein serum measurements related to early magnetic resonance imaging after minor head injury. Case report. J Neurosurg. 1996 Nov;85(5):945-8.

Ingebrigtsen T, Romner B, Kongstad P, Langbakk B. Increased serum concentrations of protein S-100 after minor head injury: a biochemical serum marker with prognostic value? J Neurol Neurosurg Psychiatry. 1995 Jul;59(1):103-4

Ingebrigtsen T, Waterloo K, Jacobsen EA, Langbakk B, Romner B. Traumatic brain damage in minor head injury: relation of serum S-100 protein measurements to magnetic resonance imaging and neurobehavioral outcome. Neurosurgery. 1999 Sep;45(3):468-75; discussion 475-6.

Ingebrigtsen T, Romner B. Biochemical serum markers for brain damage: a short review with emphasis on clinical utility in mild head injury. Restor Neurol Neurosci. 2003;21(3-4):171-6.

Iqbal K, Liu F, Gong CX, Grundke-Iqbal I. Tau in Alzheimer disease and related tauopathies. Curr Alzheimer Res. 2010 Dec;7(8):656-64.

Jessen NA, Munk AS, Lundgaard I, Nedergaard M. The Glymphatic System: A Beginner's Guide Neurochem Res. 2015 Dec;40(12):2583-99.

Jeter CB, Hergenroeder GW, Hylin MJ, Redell JB, Moore AN, Dash PK. Biomarkers for the diagnosis and prognosis of mild traumatic brain injury/concussion. J Neurotrauma. 2013 Apr 15;30(8):657-70.

Jönsson H, Johnsson P, Höglund P, Alling C, Blomquist S. Elimination of S100B and renal function after cardiac surgery J Cardiothorac Vasc Anesth. 2000 Dec;14(6):698-701.

Kawata K, Liu CY,Merkel SF, Ramirez SH, Tierney RT, Langford D Blood biomarkers for brain injury: What are we measuring? Neurosci Biobehav Rev. 2016 Sep; 68: 460–473.

Kulbe JR, Geddes JW.Current status of fluid biomarkers in mild traumatic brain injury. Exp Neurol. 2016 Jan;275 Pt 3:334-352.

Lagerstedt L, Egea-Guerrero JJ, Bustamante A, Montaner J, Rodríguez-Rodríguez A, El Rahal A, Turck N, Quintana M, García-Armengol R, Prica CM, Andereggen E, Rinaldi L, Sarrafzadeh A, Schaller K, Sanchez JC. H-FABP: A new biomarker to differentiate between CT-positive and CT-negative patients with mild traumatic brain injury. PLoS One. 2017 Apr 18;12(4):e0175572.

Lee H, Wintermark M, Gean AD, Ghajar J, Manley GT, Mukherjee P. Focal lesions in acute mild traumatic brain injury and neurocognitive outcome: CT versus 3T MRI. J Neurotrauma. 2008 Sep;25(9):1049-56.

Levin HS, Diaz-Arrastia RR. Diagnosis, prognosis, and clinical management of mild traumatic brain injury.

Lancet Neurol. 2015 May;14(5):506-17.

Linsenmaier U, Wirth S, Kanz KG, Geyer LL. Imaging minor head injury (MHI) in emergency radiology: MRI highlights additional intracranial findings after measurement of trauma biomarker S-100B in patients with normal CCT. Br J Radiol. May 2016; 89(1061): 20150827. Published online 2016 Mar 4.

Maas AI, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. Lancet Neurol. 2008 Aug;7(8):728-41.

McAllister TW, Flashman LA, McDonald BC, Saykin AJ. Mechanisms of working memory dysfunction after mild and moderate TBI: evidence from functional MRI and neurogenetics. J Neurotrauma. 2006 Oct;23(10):1450-67.

McMahon PJ, Panczykowski DM, Yue JK, Puccio AM, Inoue T, Sorani MD, Lingsma HF, Maas AI, Valadka AB, Yuh EL, Mukherjee P, Manley GT, Okonkwo DO; TRACK-TBI Investigators. Measurement of the glial fibrillary acidic protein and its breakdown products GFAP-BDP biomarker for the detection of traumatic brain injury compared to computed tomography and magnetic resonance imaging. J Neurotrauma. 2015 Apr 15;32(8):527-33.

Menon DK, Schwab K, Wright DW, Maas AI; Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward Common Data Elements for Research on Traumatic Brain Injury and Psychological Health. Position statement: definition of traumatic brain injury. Arch Phys Med Rehabil. 2010 Nov;91(11):1637-40.

Mower WR, Hoffman JR, Herbert M, Wolfson AB, Pollack CV Jr, Zucker MI; NEXUS II Investigators. National Emergency X-Radiography Utilization Study. Developing a clinical decision instrument to rule out intracranial injuries in patients with minor head trauma: methodology of the NEXUS II investigation. Ann Emerg Med. 2002 Nov;40(5):505-14.

Mrak RE, Griffin WS. Trisomy 21 and the brain. J Neuropathol Exp Neurol. 2004 Jul;63(7):679-85.

Müller K, Elverland A, Romner B, Waterloo K, Langbakk B, Undén J, Ingebrigtsen T. Analysis of protein S-100B in serum: a methodological study. Clin Chem Lab Med. 2006;44(9):1111-4.

National Clinical Guideline Centre (UK). Head Injury: Triage, Assessment, Investigation and Early Management of Head Injury in Children, Young People and Adults. London: National Institute for Health and Care Excellence (UK); 2014 Jan.

Nordström P, Michaëlsson K, Gustafson Y, Nordström A. Traumatic brain injury and young onset dementia: a nationwide cohort study. Ann Neurol. 2014 Mar;75(3):374-81.

Okonkwo DO, Yue JK, Puccio AM, Panczykowski DM, Inoue T, McMahon PJ, Sorani MD, Yuh EL, Lingsma HF, Maas AI, Valadka AB, Manley GT; Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Investigators. GFAP-BDP as an acute diagnostic marker in traumatic brain injury: results from the prospective transforming research and clinical knowledge in traumatic brain injury study. J Neurotrauma. 2013 Sep 1;30(17):1490-7.

Papa L, Robinson G, Oli M, Pineda J, Demery J, Brophy G, Robicsek SA, Gabrielli A, Robertson CS, Wang KK, Hayes RL. Use of biomarkers for diagnosis and management of traumatic brain injury patients. Expert Opin Med Diagn. 2008 Aug;2(8):937-45.

Peeters W, van den Brande R, Polinder S, Brazinova A, Steyerberg EW, Lingsma HF, Maas AI. Epidemiology of traumatic brain injury in Europe. Acta Neurochir (Wien). 2015 Oct;157(10):1683-96.

Pelsers MM, Hanhoff T, Van der Voort D, Arts B, Peters M, Ponds R, Honig A, Rudzinski W, Spener F, de Kruijk JR, Twijnstra A, Hermens WT, Menheere PP, Glatz JF. Brain- and heart-type fatty acid-binding proteins in the brain: tissue distribution and clinical utility. Clin Chem. 2004 Sep;50(9):1568-75.

Plog BA, Dashnaw ML, Hitomi E, Peng W, Liao Y, Lou N, Deane R, Nedergaard M. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J Neurosci. 2015 Jan 14;35(2):518-26.

Raabe A, Grolms C, Keller M, Döhnert J, Sorge O, Seifert V. Correlation of computed tomography findings and serum brain damage markers following severe head injury. Acta Neurochir (Wien). 1998;140(8):787-91; discussion 791-2.

Raabe A, Kopetsch O, Gross U, Zimmermann M, Gebhart P. Measurements of serum S-100B protein: effects of storage time and temperature on pre-analytical stability. Clin Chem Lab Med. 2003 May;41(5):700-3.

Santamaria-Kisiel L, Rintala-Dempsey AC, Shaw GS. Calcium-dependent and -independent interactions of the S100 protein family. Biochem. J. (2006) 396, 201–214.

Schäfer BW, Heizmann CW. The S100 family of EF-hand calcium-binding proteins: functions and pathology. Trends Biochem Sci. 1996 Apr;21(4):134-40.

Servadei F, Teasdale G, Merry G; Neurotraumatology Committee of the World Federation of Neurosurgical Societies. Defining acute mild head injury in adults: a proposal based on prognostic factors, diagnosis, and management. J Neurotrauma. 2001 Jul;18(7):657-64.

Siman R, Giovannone N, Hanten G, Wilde EA, McCauley SR, Hunter JV, Li X, Levin HS, Smith DH. Evidence That the Blood Biomarker SNTF Predicts Brain Imaging Changes and Persistent Cognitive Dysfunction in Mild TBI Patients. Front Neurol. 2013 Nov 18;4:190.

Sjöstedt E, Fagerberg L, Hallström BM, Häggmark A, Mitsios N, Nilsson P, Pontén F, Hökfelt T, Uhlén M, Mulder J. Defining the Human Brain Proteome Using Transcriptomics and Antibody-Based Profiling with a Focus on the Cerebral Cortex. PLoS One. 2015 Jun 15;10(6):e0130028.

Smit LH, Korse CM, Bonfrer JM. Comparison of four different assays for determination of serum S-100B. Int J Biol Markers. 2005 Jan-Mar;20(1):34-42.

Smits M, Dippel DW, Steyerberg EW, de Haan GG, Dekker HM, Vos PE, Kool DR, Nederkoorn PJ, Hofman PA, Twijnstra A, Tanghe HL, Hunink MG. Predicting intracranial traumatic findings on computed tomography in patients with minor head injury: the CHIP prediction rule. Ann Intern Med. 2007 Mar 20;146(6):397-405.

Stammet P, Collignon O, Hassager C, Wise MP, Hovdenes J, Åneman A, Horn J, Devaux Y, Erlinge D, Kjaergaard J, Gasche Y, Wanscher M, Cronberg T, Friberg H, Wetterslev J, Pellis T, Kuiper M, Gilson G, Nielsen N; TTM-Trial Investigators. Neuron-Specific Enolase as a Predictor of Death or Poor Neurological Outcome After Out-of-Hospital Cardiac Arrest and Targeted Temperature Management at 33°C and 36°C. J Am Coll Cardiol. 2015 May 19;65(19):2104-14. doi: 10.1016/j.jacc.2015.03.538. Erratum in: J Am Coll Cardiol. 2015 Aug 25;66(8):983.

Starmark JE, Stålhammar D, Holmgren E. The Reaction Level Scale (RLS85). Manual and guidelines. Acta Neurochir (Wien). 1988;91(1-2):12-20.

Stein SC, Fabbri A, Servadei F, Glick HA. A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann Emerg Med. 2009 Feb;53(2):180-8.

Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, McKnight RD, Verbeek R, Brison R, Cass D, Eisenhauer ME, Greenberg G, Worthington J. The Canadian CT Head Rule for patients with minor head injury. Lancet. 2001 May 5;357(9266):1391-6.

Strimbu K, Tavel JA What are Biomarkers? Curr Opin HIV AIDS. 2010 Nov; 5(6): 463–466.

Strynadka NC, James MN. Crystal structures of the helix-loop-helix calcium-binding proteins. Annu Rev Biochem. 1989;58:951-98.

Su E, Bell MJ, Kochanek PM, Wisniewski SR, Bayir H, Clark RS, Adelson PD, Tyler-Kabara EC, Janesko-Feldman KL, Berger RP. Increased CSF concentrations of myelin basic protein after TBI in infants and children: absence of significant effect of therapeutic hypothermia. Neurocrit Care. 2012 Dec;17(3):401-7.

Teasdale G, Maas A, Lecky F, Manley G, Stocchetti N, Murray G. The Glasgow Coma Scale at 40 years: standing the test of time. Lancet Neurol. 2014 Aug;13(8):844-54.

Thelin EP, Zeiler FA, Ercole A, Mondello S, Büki A, Bellander BM, Helmy A, Menon DK, Nelson D. Serial Sampling of Serum Protein Biomarkers for Monitoring Human Traumatic Brain Injury Dynamics: A Systematic Review. Front Neurol. 2017 Jul 3;8:300.

Thelin EP, Nelson DW, Bellander BM. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. Acta Neurochir (Wien). 2017 Feb;159(2):209-225.

Taylor CA, Bell JM, Breiding MJ, Xu L. Traumatic Brain Injury-Related Emergency Department Visits, Hospitalizations, and Deaths - United States, 2007 and 2013. MMWR Surveill Summ. 2017 Mar 17;66(9):1-16.

Townend W, Dibble C, Abid K, Vail A, Sherwood R, Lecky F. Rapid elimination of protein S-100B from serum after minor head trauma. J Neurotrauma. 2006 Feb;23(2):149-55.

Uchino Y, Okimura Y, Tanaka M, Saeki N, Yamaura A. Computed tomography and magnetic resonance imaging of mild head injury--is it appropriate to classify patients with Glasgow Coma Scale score of 13 to 15 as "mild injury"? Acta Neurochir (Wien). 2001 Oct;143(10):1031-7.

Undén J, Bellner J, Eneroth M, Alling C, Ingebrigtsen T, Romner B. Raised serum S100B levels after acute bone fractures without cerebral injury. J Trauma. 2005 Jan;58(1):59-61.

Undén J, Romner B. Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: an evidence-based review and meta-analysis. J Head Trauma Rehabil. 2010 Jul-Aug;25(4):228-40.

Undén J, Ingebrigtsen T, Romner B; Scandinavian Neurotrauma Committee (SNC). Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. BMC Med. 2013 Feb 25;11:50.

Vos PE. Biomarkers of focal and diffuse traumatic brain injury. Crit Care. 2011 Aug 18;15(4):183.

Veterans Affair, Department of Defense. VA/DoD Clinical Practice Guideline for Management of Concussion/Mild Traumatic Brain Injury. J Rehabil Res Dev. 2009;46(6):CP1-68.

Ware JB, Biester RC, Whipple E, Robinson KM, Ross RJ, Nucifora PG. Combat-related Mild Traumatic Brain Injury: Association between Baseline Diffusion-Tensor Imaging Findings and Long-term Outcomes. Radiology. 2016 Jul;280(1):212-9.

Welch RD, Ayaz SI, Lewis LM, Unden J, Chen JY, Mika VH, Saville B, Tyndall JA, Nash M, Buki A, Barzo P, Hack D, Tortella FC, Schmid K, Hayes RL, Vossough A, Sweriduk ST, Bazarian JJ. Ability of Serum Glial Fibrillary Acidic Protein, Ubiquitin C-Terminal Hydrolase-L1, and S100B To Differentiate Normal and Abnormal Head Computed Tomography Findings in Patients with Suspected Mild or Moderate Traumatic Brain Injury.J Neurotrauma. 2016 Jan 15;33(2):203-14. doi: 10.1089/neu.2015.4149. Epub 2015 Dec 18.

Wijdicks EF, Bamlet WR, Maramattom BV, et al. Validation of a new coma scale: The FOUR score. Ann Neurol. 2005;58:585–93.

Wilberger J, Ortega J, Slobounov S. Concussion Mechanisms and Pathophysiology. Foundations of Sport-Related Brain Injuries pp 45-63 Editors: Slobounov S, Sebastianelli W. 2006

Wright RM, Ramesh KT. An axonal strain injury criterion for traumatic brain injury. Biomech Model Mechanobiol. 2012 Jan;11(1-2):245-60.

Yuh EL, Mukherjee P, Lingsma HF, Yue JK, Ferguson AR, Gordon WA, Valadka AB, Schnyer DM, Okonkwo DO, Maas AI, Manley GT; TRACK-TBI Investigators.Magnetic resonance imaging improves 3-month outcome prediction in mild traumatic brain injury Ann Neurol. 2013 Feb;73(2):224-35.

Paper I

Calcagnile et al. BMC Emergency Medicine 2012, 12:13
http://www.biomedcentral.com/1471-227X/12/13

BMC Emergency Medicine

RESEARCH ARTICLE Open Access

Clinical validation of S100B use in management of mild head injury

Olga Calcagnile[1,2], Linda Undén[3] and Johan Undén[2,4*]

Abstract

Background: Despite validated guidelines, management of mild head injury (MHI) is still associated with excessive computed tomography (CT) scanning. Reports concerning serum levels of S100B have shown promise concerning safe reduction in CT scanning but clinical validation and actual impact on patient management is unclear. In 2007, S100B was introduced into emergency department (ED) clinical management routines in Halmstad, Sweden. MHI patients with low (<0.10 mikrogram/L) levels of S100B could be discharged without CT. Our aim was to examine the clinical impact and performance of S100B in clinical use for MHI patients.

Methods: Adult ([≥]18 years) patients with MHI (GCS 14–15, loss of consciousness and/or amnesia and no additional risk factors) and S100B sampling within 3 hours were prospectively included in this validation study. Patients were managed according to the adapted guidelines and management was documented. Outcome was determined with a questionnaire 3 months post-trauma and medical records to identify significant intracranial complications such as new neuroimaging, neurosurgery and/or death related to the trauma.

Results: 512 patients were included. 24 (4.7%) showed traumatic abnormalities on CT and 1 patient died (0.2%). 138 patients (27%) had normal S100B levels and 374 patients (73%) showed elevated S100B levels. No patients with a normal S100B level showed significant intracranial complication. 44 patients (32%) were managed with CT despite the guidelines recommending discharge (all these CT scans were normal) and 28 patients (7%) were discharged despite a CT recommendation (follow-up was normal in all these patients). S100B had a sensitivity of 100% (95% CI 83-100%) and a specificity of 28% (95% CI 24-33%) for significant intracranial complications.

Conclusion: The clinical use of S100B within our existing guidelines for management of MHI is safe and effective. Adult MHI patients, without additional risk factors and with normal S100B levels within 3 hours of injury, can safely be discharged from the hospital.

Background

Traumatic brain injuries (TBI) result in almost 17 000 emergency department (ED) visits per year in Sweden and account for more than 1 million ED visits each year in both the United States of America and the United Kingdom [1-3]. Most of them (up to 95%) are classified as mild head injuries (MHI) [4], commonly defined as a head trauma with short loss of consciousness (LOC) or amnesia for the accident, Glasgow Coma Scale (GCS) 14–15 and no neurological deficits at the time of medical inspection. These patients have been notoriously difficult to manage since they have a low, but not negligible, risk of an intracranial complication, which may be life threatening [5]. Pathological computed tomography (CT) results after MHI are found in 0.5-20% of patients (0-8% for significant complications) and the need for neurosurgical intervention is between 0-1% [6].

Scandinavian guidelines for management of minimal, mild and moderate head injuries were presented by the Scandinavian Neurotrauma Committee (SNC) in the year 2000 [1]. For patients with GCS 14–15 and LOC and/or amnesia, these guidelines recommend head CT or, as a secondary option, hospital admission with clinical observation. Similar guidelines have been published from other groups [7-9] and all have the same goal; to stratify patients with MHI into risk groups for intracranial complications. In order to ensure that guidelines

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do not miss patients with intracranial complications, substantial over-triage to CT has historically been accepted (between 80–99,5% of CT's after MHI are normal [6,10]). In recent years, however, focus has been put on reducing unnecessary CT scans due to limitations in health care resources along with reports of increased cancer risks associated with exposure to medical radiation [11,12]. External comparisons of different clinical decision rules have shown favourable results for the SNC guidelines [10,13].

During the last fifteen years, protein S100B has received increasing attention as a possible biomarker for neurological disease [14,15]. Low serum levels of the protein are found in healthy individuals while patients with head trauma have a level of S100B proportionate with the severity of their brain injury [16]. S100B has a very high sensitivity for brain injuries, possibly even higher than CT [17], which would result in a high negative predictive value (NPV) in the MHI setting. Based on several studies from separate research groups and a meta-analysis [18-22], S100B has shown a NPV of over 99% for intracranial complications and close to 100% for neurosurgical lesions after MHI. Considering the theoretical CT reduction of 30%, S100B seems useful in the management of this patient group.

Despite these promising studies, S100B has not been validated in clinical practice and the impact on decision-making in a real-life setting is unclear. The aim of this study was therefore to examine the clinical impact and diagnostic performance of serum S100B levels in actual management of MHI patients.

Methods

Study setting and population

In early 2007, S100B was introduced into clinical practice within the existing SNC guidelines to create new local management routines (Figure 1). The addition of S100B was applied to a group of patients, typically considered as intermediate risk for intracranial complication, where CT is normally recommended. We set the time interval for S100B sampling at 3 hours post injury, reflecting the evidence available in 2007 [23]. Also, evidence for S100B use in children at this time was relatively weak and the new guidelines were therefore used only in adults.

After a 6 months adjustment period, we undertook a prospective cohort validation study in Halmstad Regional hospital, Sweden, from November 2007 to May 2011, to evaluate the adapted guidelines explained above. Our hospital is a level II trauma centre with 24-hour emergency care, anaesthesiology, radiology, surgery and intensive care.

We consecutively enrolled all adult patients with MHI and S100B sampling. Initial inclusion criteria were therefore analogous to the MHI group in the SNC guidelines; adult patients with acute trauma to the head with GCS 14–15 during examination and loss of consciousness < 5 minutes and/or amnesia, with the addition of the S100B sample. Patients with anti-platelet agents (such as aspirin or clopidogrel) were included. Exclusion criteria were age less than 18 years, non-Swedish citizens (difficult to follow up), neurological deficits, additional risk factors from the SNC guidelines (therapeutic anticoagulation or haemophilia, clinical signs of depressed skull fracture or skull base fracture, posttraumatic seizures, shunt-treated hydrocephalus and multiple injuries) and patients where serum sampling for S100B was done more than 3 hours post-injury.

Our goal was to include 500 patients in the study, based upon consensus in the study group when considering the aim of the study. A sample size calculation was not performed.

The study was conducted in accordance to the Helsinki Declaration and approved by the Lund regional ethical committee, Lund, Sweden (reference number 19/2007). Since the study did not involve any change in patient management and based upon clinical practice, informed consent was not necessary and the ethics committee concurred with this decision.

Blood sampling and biochemical analysis

A 5ml blood sample was drawn from each patient's cubital vein in the ED. Samples were analysed with the fully automated Elecsys® S100 (Roche AB) at the Clinical Chemistry Department of Halmstad Regional hospital, Sweden. Roche AB report a range between 0.005 μg/L and 39 μg/L and a within-series coefficient of variance of <2.1%. Based on the available evidence at this time, we chose a cut-off level for normal levels of less than 0.10μg/L and a window of sampling of 3 hours from the time of the accident [19,23]. Lab results were available to treating physicians within 1 hour after sampling.

CT examinations

CT scans were performed with a GE VCT Ligthspeed 64 multislice detector with a 0,625/0,625mm, 0,5 seconds rotation time and pitch of 0,531:1. 10mm thick slices were used as part of the standard CT protocol for these patients. CT scans are always analysed by a board certified radiologist and confirmed by a consultant radiologist. Since S100B was used clinically, radiologists were not blinded to S100B results. A CT scan was considered positive if any signs of cranial (skull fracture) or intracranial pathology (hematoma, air or contusion) were present.

Standardized assessment of patients

Supervised interns and surgical residents from the ED of the Halmstad Regional Hospital assessed patients. These physicians underwent several educational sessions on evaluating patients with MHI using the new guidelines.

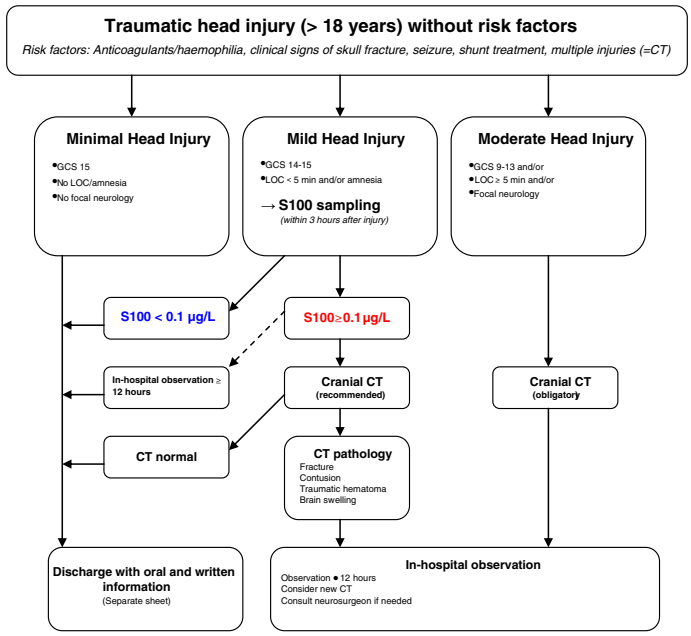

Figure 1 Modified Scandinavian Neurotrauma Committee (SNC) guidelines including S100B sampling. Dotted line indicates secondary management option. GCS = Glasgow Come Scale, CT = Computed Tomography.

Physicians were instructed to follow the new guidelines for all non-severe head injury patients even though deferral from these due to clinical judgement was allowed.

Data registration and follow-up

Details of how patients were managed, including patient characteristics, type of injury, patient history, medications, clinical examination results, CT results, admission type and duration were documented in an Excel spreadsheet.

Patients were asked to answer a questionnaire sent by mail 3 months after the injury, which was repeated if no answer was received. For patients who did not return the questionnaire after these attempts, a blinded assessor conducted the questionnaire via telephone. Included in this questionnaire were questions that would identify a significant intracranial complication [7]. In cases where patients could not be reached by mail or telephone, medical records and national mortality databases were consulted for evidence of complications and/or death. Considering the rigid and transparent organisation of the health care system in Sweden, these methods would identify all patients with significant (enough to result in new neuroimaging, neurosurgery or death) intracranial complications.

Our outcome endpoint for the study was significant intracranial complication, which was defined as either a traumatic complication on emergency CT or, via follow-up, new neuroimaging showing traumatic intracranial complication or neurosurgery and/or death due to an intracranial complication.

Sensitivity, specificity, positive and negative predictive values were estimated from cross tabulation between S100B and significant intracranial complications and reported with corresponding 95% confidence intervals. Values are reported to two significant figures.

Results

Between November 2007 and May 2011, we enrolled 512 patients (see Figure 2 for inclusion process and Table 1 for descriptive statistics). 26 patients had cranial CT pathology but only 24 (4.7%) showed traumatic abnormalities (isolated skull fracture n=3, cerebral contusions n=7, acute subdural hematoma n=3, intracranial air n=1, combinations of traumatic intracranial findings n=10). 2 patients showed CT pathology not related to trauma (cerebral tumour n=1 and pathological intracranial calcification n=1). No patients needed neurosurgical

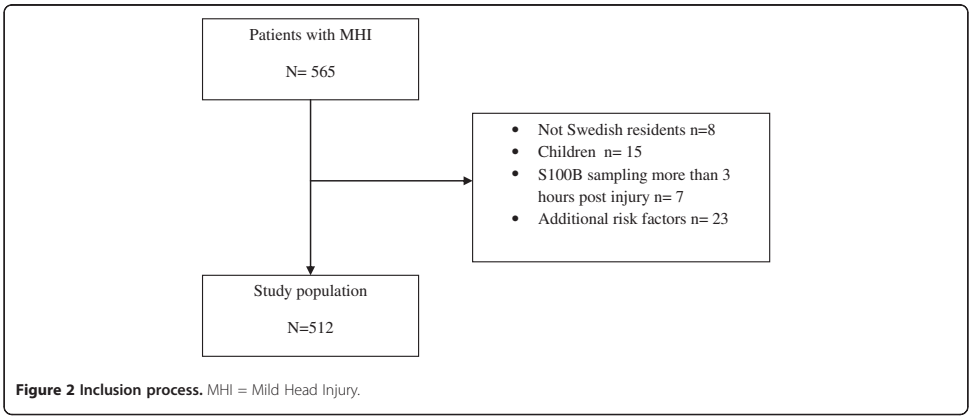

Figure 2 Inclusion process. MHI = Mild Head Injury.

intervention. One patient died as a result of a head injury; an 83-year-old man with an S100B level of 0.23μg/L and a CT showing expansive cerebral contusions who died from increased intracranial pressure. Neurosurgical care was denied due to advanced age.

138 patients (27%) had a S100B level less than 0.10μg/L and 374 patients (73%) showed a S100B level higher or equal to 0.10μg/L. Details of how patients were managed are presented in Figure 3. The follow up questionnaire was completed for 414 patients (81%). Medical records and the mortality database were successfully checked for all remaining patients. No patients with a normal S100B level showed significant intracranial complication, either on CT or on follow-up, see Figure 3.

Over-triage (CT or admission performed when the guidelines recommended discharge) occurred in 44 patients (32%) with normal S100B levels. 15 of these had a CT scan, 20 were admitted and 9 patients had both a CT and admission. All of these patients had normal CT findings and/or normal follow-up. Under-triage (not performing a CT when recommended) occurred in 28 patients (7%) with elevated S100B levels. None of these patients had any significant intracranial complications on follow-up.

S100B displayed a sensitivity and NPV of 100% for significant intracranial complications, a specificity of 28% and a positive predictive value (PPV) of 6%, see Table 2.

Table 1 Descriptive statistics

	S100B < 0.10μg/L	S100B ≥ 0.10μg/L	All
Male	85 (61.6%)	229 (61.3%)	314 (61.5%)
Female	53 (38.4%)	145 (38.7%)	198 (38.5%)
Age (mean)	32.6	46.6	42.2
Total	138	374	512

Discussion

The first report concerning serum S100B as a possible biomarker in MHI was published in 1995 [15]. Since then, numerous reports and a meta-analysis, documenting the potential of S100B to safely reduce CT scans following MHI, have increased the evidence for clinical use [20-24]. However, actual clinical validation has never been reported despite the biomarker being used clinically in several European countries. In 2007, S100B was introduced as a clinical tool in the management of MHI in our hospital, in an attempt to reduce CT scans after these injuries. This study shows that this implementation has been successful and that S100B, using a cut-off of less than 0.10 μg/L for normal values and a time window of 3 hours from injury, shows similar predictive values to the derivation studies.

Low compliance to guidelines is a common problem [5]. 32% of patients with normal S100B levels were over-triaged with CT, admission or both. None of these had any intracranial complications. It is natural to expect caution when using new routines, especially concerning an injury where biomarkers have never been used before. Also, physicians must always be free to exert clinical judgement since management guidelines are merely an aid in the clinical process. Some patients cannot be sent home from the ED irrespective of S100B and/or CT findings (for example; elderly patients without support in their home environment, serious intoxication and patients with other injuries).

Our adapted guidelines are based upon the evidence-based SNC management guidelines from the year 2000 [1]. Since this publication, considerable new evidence has emerged in this field, including validated guidelines based upon patient history and clinical examination [7-9]. The impact of including S100B in other guidelines

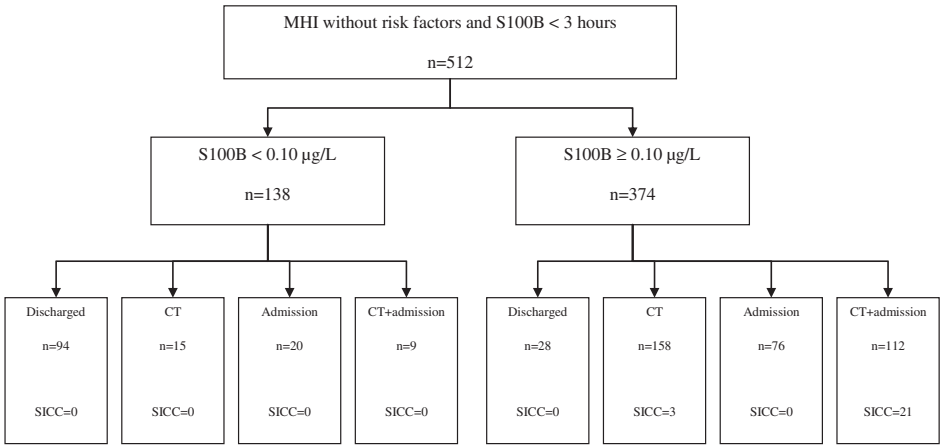

Figure 3 Patient management in the study cohort including number of intracranial injuries. CT= computed tomography, MHI= mild head injury, SICC=Significant Intracranial Complication.

is unknown. However, the SNC guidelines have proved accurate in comparison studies [8,10] so the implementation of S100B into these is justifiable. Despite this, the examination of S100B within other guidelines is naturally warranted.

Owing to the predictive properties of S100B, the biomarker is best adapted into an intermediate risk group of patients, such as in this study. The prevalence of traumatic intracranial injury in this group was 4.7%, similar to other cohorts. These patients would normally receive a CT recommendation according to the SNC guidelines, which is justifiable considering the prevalence level. However, interpreting S100B levels in minimal head injury would lead to substantial over-triage (false positives) and using levels in more severe head injuries could lead to under-triage and may risk missing important complications (false negatives) [12].

This study has several limitations. Firstly, one may argue that our method of determining the outcome measure, significant intracranial complications, may miss patients that may in fact have CT abnormalities. However, if these exist, these abnormalities would not have resulted in any change in management and/or outcome for these patients. The organisation of the state-owned Swedish health care system, with personal identification numbers connected with all medical journals, allows us to accurately identify new neuroimaging, neurosurgery and/or death in all patients who were not followed up with the questionnaire and therefore identifies any cases of important intracranial injury. This also allows us to minimise recall bias arising from the questionnaire. Secondly, none of our patients needed neurosurgery. If this was the endpoint, one could suggest that all our patients could have been discharged without S100B or CT. This management, however, would not be accepted in Sweden and the results must be considered in relation to the existing guidelines, which recommend CT in all these patients, similar to guidelines in other countries. Thirdly, the timing of S100B sampling after injury may be of importance. We used a time window of 3 hours based upon

Table 2 Cross tabulation showing statistical values for S100B and significant intracranial complications

	SICC + Total = 24	**SICC - Total = 488**	
S100B ≥ 0.10μg/L	24	350	**PPV**: 6,4%
Total = 374			(95% CI 4.2-10%)
S100B < 0.10μg/L	0	138	**NPV**: 100%
Total = 138			(95% CI 97-100%)
	Sensitivity: 100% (95% CI 83-100%)	**Specificity**: 28% (95% CI 24-33%)	

SICC = Significant intracranial complications, NPV= Negative predictive value, PPV= Positive predictive value.

the evidence at this time [23] and worries concerning the short half-life of S100B in blood [25]. Recently, a large prospective study has utilised a time window of 6 hours [20] with maintained predictive ability of S100B. It seems reasonable that a time window of 6 hours may be more applicable to this population and should be considered in future studies and/or clinical practice. Finally, deviation from the guidelines was seen. Although this was allowed in the study protocol, reasons for the deviation were not explored in depth and would have been an interesting point to examine. Future studies should include a comparison of clinical rules with unstructured physician assessment, in order to fully explore this aspect, including reasons for deviation from a guideline.

Conclusion

Incorporation of S100B into existing guidelines for management of MHI in adults is safe and effective. Adult MHI patients without additional risk factors and with normal S100B levels within 3 hours of injury can safely be discharged from the hospital.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JU conceived and designed the study, with input from LU and OC. JU, OC and LU acquired data. OC and JU did the analysis and interpretation of data. OC drafted the manuscript, JU and LU did critical revision and all authors finally approved the manuscript.

Acknowledgements

We would like to thank Anders and Linnea Holmén for help with follow-up forms. We would like to thank the FoU department at Halmstad Regional Hospital for support during this work.
This study was funded with non-commercial (Swedish State) funds via the Scientific Committee (Vetenskapsrådet) at the Halmstad Regional Hospital and Region Skåne, Sweden. The funding bodies had no input on any aspects of the final study.

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Received: 10 July 2012 Accepted: 24 October 2012
Published: 27 October 2012

References

1. Ingebrigtsen T, Romner B, Kock-Jensen C: **Scandinavian guidelines for initial management of minimal, mild, and moderate head injuries. The Scandinavian Neurotrauma Committee.** *J Trauma* 2000, **48**(4):760–766.
2. Saab M, Gray A, Hodgkinson D, Irfan M: **Warfarin and the apparent minor head injury.** *J Accid Emerg Med* 1996, **13**(3):208–209.
3. Rutland-Brown W, Langlois JA, Thomas KE, Xi YL: **Incidence of traumatic brain injury in the United States, 2003.** *J Head Trauma Rehabil* 2006, **21**(6):544–548.
4. Cassidy JD, Carroll LJ, Peloso PM, Borg J, von Holst H, Holm L, *et al*: **Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury.** *J Rehabil Med: Official J UEMS European Board of Physical Rehabil Med* 2004, **43**(Suppl):28–60.
5. Morton MJ, Korley FK: **Head computed tomography use in the emergency department for mild traumatic brain injury: integrating evidence into practice for the resident physician.** *Ann Emerg Med* 2012, **60**(3):361–367.
6. Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PM: **Indications for computed tomography in patients with minor head injury.** *N Engl J Med* 2000, **343**(2):100–105.
7. Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, *et al*: **The Canadian CT Head Rule for patients with minor head injury.** *Lancet* 2001, **357**(9266):1391–1396.
8. Smits M, Dippel DW, Steyerberg EW, de Haan GG, Dekker HM, Vos PE, *et al*: **Predicting intracranial traumatic findings on computed tomography in patients with minor head injury: the CHIP prediction rule.** *Ann Intern Med* 2007, **146**(6):397–405.
9. Yates DW: **The NICE head injury guidelines.** *Emergency Med J: EMJ* 2003, **20**(2):117.
10. Stein SC, Fabbri A, Servadei F, Glick HA: **A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults.** *Ann Emerg Med* 2009, **53**(2):180–188.
11. Hall P, Adami HO, Trichopoulos D, Pedersen NL, Lagiou P, Ekbom A, *et al*: **Effect of low doses of ionising radiation in infancy on cognitive function in adulthood: Swedish population based cohort study.** *BMJ* 2004, **328**(7430):19.
12. Ruan S, Noyes K, Bazarian JJ: **The economic impact of S-100B as a pre-head CT screening test on emergency department management of adult patients with mild traumatic brain injury.** *J Neurotrauma* 2009, **26**(10):1655–1664.
13. Smits M, Dippel DW, de Haan GG, Dekker HM, Vos PE, Kool DR, *et al*: **Minor head injury: guidelines for the use of CT–a multicenter validation study.** *Radiology* 2007, **245**(3):831–838.
14. Raabe A, Wissing H, Zwissler B: **Brain cell damage and S-100B increase after acute lung injury.** *Anesthesiology* 2005, **102**(4):713–714.
15. Ingebrigtsen T, Romner B, Kongstad P, Langbakk B: **Increased serum concentrations of protein S-100 after minor head injury: a biochemical serum marker with prognostic value?** *J Neurol Neurosurg Psychiatry* 1995, **59**(1):103–104.
16. Pelinka LE, Kroepfl A, Leixnering M, Buchinger W, Raabe A, Redl H: **GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome.** *J Neurotrauma* 2004, **21**(11):1553–1561.
17. Romner B, Ingebrigtsen T, Kongstad P, Borgesen SE: **Traumatic brain damage: serum S-100 protein measurements related to neuroradiological findings.** *J Neurotrauma* 2000, **17**(8):641–647.
18. Biberthaler P, Mussack T, Wiedemann E, Gilg T, Soyka M, Koller G, *et al*: **Elevated serum levels of S-100B reflect the extent of brain injury in alcohol intoxicated patients after mild head trauma.** *Shock* 2001, **16**(2):97–101.
19. Muller K, Townend W, Biasca N, Unden J, Waterloo K, Romner B, *et al*: **S100B serum level predicts computed tomography findings after minor head injury.** *J Trauma* 2007, **62**(6):1452–1456.
20. Zongo D, Ribereau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, *et al*: **S100-B protein as a screening tool for the early assessment of minor head injury.** *Ann Emerg Med* 2012, **59**(3):209–218.
21. Bouvier D, Oddoze C, Ben Haim D, Moustafa F, Legrand A, Alazia M, *et al*: **Interest of S100B protein blood level determination for the management of patients with minor head trauma.** *Ann Biol Clin (Paris)* 2009, **67**(4):425–431.
22. Unden J, Romner B: **Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: an evidence-based review and meta-analysis.** *J Head Trauma Rehabil* 2010, **25**(4):228–240.
23. Biberthaler P, Linsenmeier U, Pfeifer KJ, Kroetz M, Mussack T, Kanz KG, *et al*: **Serum S-100B concentration provides additional information fot the indication of computed tomography in patients after minor head injury: a prospective multicenter study.** *Shock* 2006, **25**(5):446–453.
24. Muller K, Elverland A, Romner B, Waterloo K, Langbakk B, Unden J, *et al*: **Analysis of protein S-100B in serum: a methodological study.** *Clin Chem Laboratory Med: CCLM / FESCC* 2006, **44**(9):1111–1114.
25. Jonsson H, Johnsson P, Hoglund P, Alling C, Blomquist S: **Elimination of S100B and renal function after cardiac surgery.** *J Cardiothorac Vasc Anesth* 2000, **14**(6):698–701.

doi:10.1186/1471-227X-12-13
Cite this article as: Calcagnile *et al.*: **Clinical validation of S100B use in management of mild head injury.** *BMC Emergency Medicine* 2012 **12**:13.

Paper II

Calcagnile et al. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine 2013, 21:52
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ORIGINAL RESEARCH Open Access

S100B levels are affected by older age but not by alcohol intoxication following mild traumatic brain injury

Olga Calcagnile[1], Anders Holmén[2], Michelle Chew[3] and Johan Undén[4*]

Abstract

Introduction: Biomarkers of brain damage and head injury are potentially useful tools in the management of afflicted patients. Particularly S100B has received much attention and has been adapted into clinical guidelines. Alcohol intoxication and higher age (65 years and over) have been used as risk factors for serious complications following head injury. The effect of these factors on S100B levels has not been fully established in a relevant patient cohort.

Methods: We prospectively included 621 adult patients with mild traumatic brain injury (TBI) and S100B sampling. Mild TBI was defined as Glasgow Come Scale 14–15 with loss of consciousness and/or amnesia, but without high-risk factors for intracranial complications. These patients would normally require CT scanning according to local and most international guidelines. S100B was sampled within 3 hours following trauma.

Results: 280 patients (45%) were intoxicated by alcohol. Alcohol intoxication had no effect on S100B levels ($p = 0.65$) and the performance of S100B remained unchanged in these patients. 115 patients (22%) were 65 years or older with elevated S100B levels being more common in this group compared to patients under 65 ($p = 0.029$). Although the sensitivity of S100B was unchanged in older patients, the specificity was poorer.

Conclusion: S100B can be used reliably in mild TBI patients with alcohol intoxication. The clinically utility of S100B in older patients may be limited by very poor specificity leading to only a small decrease in CT scanning.

Introduction

Biochemical markers are used as screening and diagnostic tools in many clinical scenarios. Recently, biomarkers for diagnosis and prognosis of brain injury have developed [1]. These may allow faster and more accurate management of brain disease, similarly to biomarkers used in other organ systems. Traumatic brain injury (TBI) is a leading cause of death, especially in younger individuals [2]. Management usually involves computed tomography of the brain to detect lesions that may need neurosurgical or medical intervention. However, most of there injuries are not serious in nature, often called mild TBI or minor head injury. Serious complications following mild TBI are rare, with approximately 5% displaying traumatic CT pathology and less than 1% needing specific intervention [3,4]. Despite this, CT is recommended in these patients due to the seriousness of the complications [5-7]. Attempts to reduce CT use have been based upon aspects of patient history and clinical examination. However, these may be inaccurate in any patients, especially in those with head trauma or brain injury. An objective biomarker would therefore we welcomed in the management of these patients.

S100B is a small calcium-binding protein weighing approximately 21 kDa, predominantly expressed by glia cells. When brain tissues and/or the blood brain barrier (BBB) are damaged, S100B is released and can be detected in the peripheral blood. Many studies have shown the potential of S100B as a biomarker in brain injuries [8-10]. In particular, the potential of S100B to reduce unnecessary CT scans following mild TBI has received considerable attention. Recently, international guidelines have been published including S100B as a management option [11].

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Many patients with mild TBI are intoxicated by ethanol [12]. Although some studies have shown little effect of alcohol on S100B levels [12], others have shown conflicting results [13]. This aspect is important if the biomarker is to function effectively in this population. False high S100B due to alcohol intoxication would limit the CT-reducing ability of the biomarker.

Children have higher levels of S100B than adults [14]. However, levels of S100B in elderly patients following mild TBI have not been investigated. Since older age (most often defined as over 65 years of age) is often included as a risk factor for complications after TBI [15,16], this aspect is also of importance.

The aim of this study is to investigate the relationship of older age and alcohol intoxication to serum S100B levels following mild TBI in a large prospective cohort.

Methods

Study setting and cohort population

We undertook a prospective study in Halmstad Regional hospital, Sweden, from June 2008 to December 2012. Our hospital is a level II trauma centre with 24-hour emergency care, anesthesiology, radiology, surgery and intensive care. Approximately 6 months prior to the study, local guidelines for management of mild TBI, including S100B sampling, were introduced into clinical practice.

We consecutively enrolled all adult patients with mild TBI and subsequent S100B sampling. Inclusion criteria were; adult patients with trauma to the head with GCS 14–15 during examination and loss of consciousness < 5 minutes or amnesia. Exclusion criteria were; age less than 18 years, focal neurological deficit, therapeutic anticoagulation or haemophilia, radiographically demonstrated skull fracture, clinical signs of depressed skull fracture or skull base fracture, posttraumatic seizure, shunt-treated hydrocephalus, multiple organ trauma and patients where serum sampling for S100B was taken more than 3 hours post-injury.

Patient age at time of trauma and alcohol intoxication (yes/no based upon patient history and examination) was prospectively documented. Determination of blood alcohol levels was determined based upon the discretion of the treating physician. The age limit of 65 years or older was the pre-determined cut-off for analysis based upon published management rules [11,16].

The study was approved by the regional ethical board (approval number 19/2007).

Blood sampling and biochemical analysis

A 5 ml blood sample was drawn from patient's cubital vein in the ED. Samples were analyzed with the fully automated Elecsys® S100 (Roche AB) at the Clinical Chemistry Department of Halmstad Regional hospital, Sweden. Roche AB report a range between 0.005 μg/L and 39 μg/L and a within-series coefficient of variance of <2.1%. Based on the available evidence at this time, we chose a cut-off level for normal levels of less than 0.10 μg/L and a window of sampling of 3 hours from the time of the accident [17,18]. Lab results were available to treating physicians within 1 hour after sampling.

CT examinations

Cranial CT scans were performed with a GE VCT Ligthspeed 64 multislice detector with a 0,625/0,625 mm, 0,5 seconds rotation time and pitch of 0,531:1. 10 mm thick slices were used as part of the standard CT protocol for these patients. CT scans are always analysed by a board certified radiologist and confirmed by a consultant radiologist. Since S100B was used clinically, radiologists were not blinded to S100B results. A CT scan was considered positive if any sings of cranial (skull fracture) or intracranial pathology (hematoma, air or contusion) were present.

Follow-up

Patients were followed up after 3 months post-trauma by questionnaire. This contained information regarding clinical symptoms suggestive of intracranial complications and included additional (new) CT scans and/or exposure to the health care system. Patients who were lost to follow-up were checked by examination of medical records and national mortality databases for signs of intracranial complications or death.

Statistic analysis

Data was registered on an Excel® file. The difference in S100B levels between age groups and intoxicated/sober patients were calculated with a Mann–Whitney test. A non-parametric test was chosen due to a skewed distribution of data.

Results

Between June 2008 and December 2012, we enrolled 621 patients with mild TBI and S100B levels. 351 patients had CT scans as part of their management (322 with S100B levels equal to or higher than 0.10 μg/L and 29 patients with S100 levels lower than 0.10 μg/L). 513 (83%) of patients had successful and complete follow-up including 242 (90%) of the 270 patients not receiving an initial CT. No patients showed any new signs of intracranial complications. A total of 29 patients had cranial CT pathology but only 26 (4.7%) of these showed traumatic abnormalities (isolated skull fracture n = 3, cerebral contusions n = 9, acute subdural hematoma n = 3, intracranial air n = 1, combinations of traumatic intracranial findings n = 10). The remaining 3 patients had non-traumatic findings unrelated to the injury.

280 patients (45%) were intoxicated by alcohol and a blood alcohol level was determined in 197 patients. All

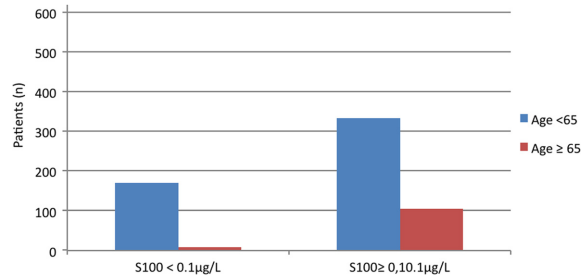

Figure 1 Bar graph showing the relationship between age and S100B levels.

patients who had blood drawn for alcohol analysis were clinically suspected of intoxication and all had measureable levels. 115 patients (19%) of the total cohort were 65 years or older.

180 patients (29%) had a S100B level lower than 0.10 μg/L: 171 of these (95%) were younger than 65 years and only 9 patients (5%) were older or equal than 65 years of age. Figure 1 shows a bar graph of age and S100B levels in the study population. 441 patients (71%) showed a S100B level higher or equal to 0.10 μg/L: 335 (76%) of these were younger than 65 years old and 106 patients (24%) were older or equal than 65 years old. The difference in S100B levels between the age groups was significant (p = 0.029), see Table 1. A scatter plot of age verses S100B levels is shown in Figure 2.

Table shows the number of patients (and interquartile range in brackets) with S100B levels above and below 0.10 μg/L and the number of patients above or below 65 years of age and with or without alcohol intoxication. The difference in S100B levels between patients 65 and over with patients under 65 years was significant (p = 0.029) and the difference between intoxication and no intoxication was not significant (p = 0.65).

206 of the 280 patients (74%) who were intoxicated by alcohol had a S100B level higher or equal to 0.10 μg/L. 235 of the 338 (70%) patients without alcohol intoxication had elevated S100B levels. There was no statistical difference in S100B levels between those patients with and without intoxication (p = 0.65), see Table 1. For the 197 patients where serum ethanol levels were determined, a scatterplot was created, see Figure 3. 10 patients were both 65 years or older and intoxicated by alcohol.

S100B had a sensitivity of 100% and a specificity of 30% for CT findings in the entire population. The specificity increased to 35% if only patients younger than 65 years were considered. The positive likelihood ratio that a S100B level higher or equal to 0.10 μg/L would predict a pathological CT was 1.44 for the entire population while it increased to 1.54 if we considered only patients younger than 65 years of age.

Discussion

The initial management of mild TBI is still under debate. Several guidelines and decision rules, derived from different cohorts from different countries, have been published and are currently used clinically [11,15,16,19]. The introduction of S100B into clinical practice has been shown to improve the management of mild TBI [20] with a reduction of CT scans following these injuries. This has the potential to reduce costs

Table 1 S100B levels in 621 patients with mild TBI

	S100b < 0.10 μg/L	Median S100b μg/L	S100b ≥ 0.10 μg/L	Median S100b μg/L
<65 years	171 patients	0.07 (0.03-0.09)	335 patients	0.19 (0.10-4.51)
≥65 years	9 patients	0.07 (0.04-0.09)	106 patients	0.23 (0.10-6.75)
No Alcohol intoxication	106 patients	0.07 (0.03-0.09)	235 patients	0.20 (0.10-6.75)
Alcohol intoxication	74 patients	0.07 (0.03-0.09)	206 patients	0.20 (0.10-4.51)

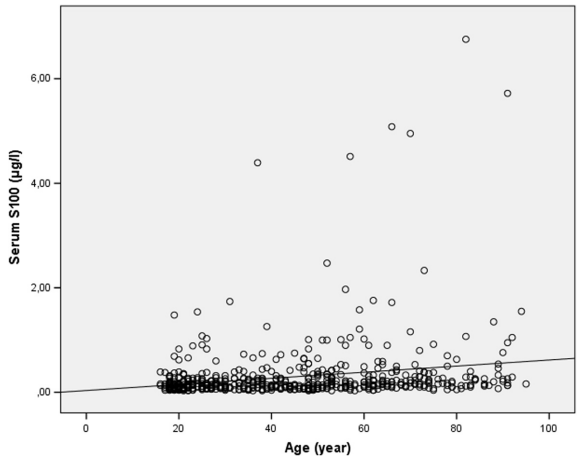

Figure 2 Scatter plot showing S100B levels verses age in patients following mild TBI. Spearman's rho 0,295, p = 0,000.

and potential radiation dosages to patients with head injury.

New, updated international guidelines, including blood sampling for S100B, are presently being implemented in Scandinavia [11]. These guidelines recommend S100B sampling in adult patients with loss of consciousness and/or repeated (more than one episode) vomiting if other risk factors are absent. One such risk factor is older age (65 years or older) in combination with anti-thrombocyte medication. As many elderly patients take these medications, the majority of these patients will have this risk factor and not be eligible for S100B sampling. Based upon the results of this study, this approach seems reasonable. Even if the sensitivity of S100B for CT findings was still 100% in elderly patients, the specificity was worse. In practice, this results in a smaller potential reduction in CT scanning after mild

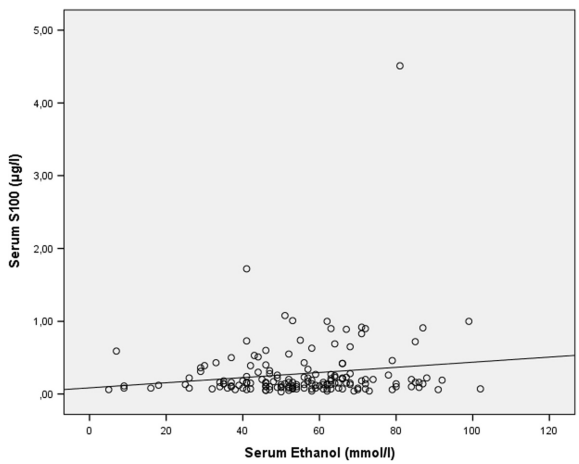

Figure 3 Scatter plot showing S100B levels verses serum ethanol levels in patients following mild TBI. Spearman's rho 0,092, p = 0,249.

TBI and hence a weaker clinical indication for the test. The reason for this observation is unclear. One may speculate that the higher S100B levels observed are merely a reflection of the increased risk of brain injury these patients have following brain trauma. If this is the case, S100B levels may still correctly classify these patients as high risk mild TBI indicating the necessity of a CT scan. Also, older patients often have concurrent chronic disease and may also have neurological disease such as Alzheimer's disease or Parkinson's disease. In the present study, non-neurological disease was not registered and the prevalence of neurodegenerative disease was too low to perform any meaningful analysis. It may also be argued that the cut-off for S100B should be higher in older patients. This was, however, not an endpoint of this study. Higher cut-off levels have been shown to be more specific in previous studies [21]. However, the large body of evidence and current clinical practice is focused on the 0.10 μg/L level and is seems reasonable to primarily consider this cut-off although to ensure maximal sensitivity in clinical practice. These issues should be confirmed in future studies.

We found no affect of alcohol on S100B levels, irrespective of whether alcohol intoxication was derived from patient history and clinical examination or from objective blood ethanol levels. This confirms previous observations from another cohort [12] but is somewhat in contrast to other reports [13]. Different methods of S100B analysis may have influenced these conflicting results [22]. The results from this study are based upon a much larger cohort than the previous studies and consider a pragmatic and clinically relevant patient material. This observation is important considering the frequency of alcohol intoxication in these patients. Indeed, in this study, 45% of patients were intoxicated by alcohol.

Considering these results, S100B can be used freely in mild TBI patients with alcohol intoxication. This is naturally welcomed, due to the difficulties of assessing patient history, performing adequate and reliable clinical examination and obtaining a cranial CT scan of intoxicated patients. Although S100B shows a 100% sensitivity for CT findings after mild TBI in all age groups, the performance of the biomarker, specifically the ability of S100B to decrease unnecessary CT scans, will likely be reduced in elderly (65 years or older) patients. Although this is in accordance to recent guidelines [11], this should also be considered in other scenarios. The health economic implications of S100B use in this patient group remains to be shown.

Conclusion

In patients with mild TBI, S100B is unaffected by alcohol intoxication and may be used effectively in this patient group. Patients aged 65 years and older had higher S100B levels and the overall ability of S100B to reduce CT scans in the elderly may be impaired.

Competing interests

JU has in previous studies (unconnected with the current study) received S100B analysis kits from Roche AB, Sweden and Diasorin AB, Sweden. JU has previously lectured for Roche AB, Sweden. AH, OC and MC did not have any competing interests.

Authors' contributions

JU and OC conceived the study. JU and OC acquired data. JU, AH, MC and JU did data analysis. JU and OC drafted the paper with input from AH and MC. All authors read and approved the final manuscript.

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Received: 15 May 2013 Accepted: 3 July 2013
Published: 6 July 2013

References

1. Ingebrigtsen T, Romner B, Kongstad P, Langbakk B: **Increased serum concentrations of protein S-100 after minor head injury: a biochemical serum marker with prognostic value?** *J Neurol Neurosurg Psychiatry* 1995, **59**(1):103–104.
2. Soreide K, Kruger AJ, Vardal AL, Ellingsen CL, Soreide E, Lossius HM: **Epidemiology and contemporary patterns of trauma deaths: changing place, similar pace, older face.** *World J Surg* 2007, **31**(11):2092–2103.
3. Morton MJ, Korley FK: **Head computed tomography use in the emergency department for mild traumatic brain injury: integrating evidence into practice for the resident physician.** *Ann Emerg Med* 2012, **60**(3):361–367.
4. Af Geijerstam JL, Britton M: **Mild head injury - mortality and complication rate: meta-analysis of findings in a systematic literature review.** *Acta Neurochir (Wien)* 2003, **145**(10):843–850. discussion 850.
5. Stein SC: **Indications for computed tomography after minor head injury.** *N Engl J Med* 2000, **343**(21):1570. author reply 1571.
6. Stein SC: **Mild head injury.** *J Neurosurg* 2005, **102**(1):183–184. author reply 185.
7. Ingebrigtsen T, Romner B, Kock-Jensen C: **Scandinavian guidelines for initial management of minimal, mild, and moderate head injuries. The Scandinavian Neurotrauma Committee.** *J Trauma* 2000, **48**(4):760–766.
8. Pelinka LE, Kroepfl A, Leixnering M, Buchinger W, Raabe A, Redl H: **GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome.** *J Neurotrauma* 2004, **21**(11):1553–1561.
9. Foerch C, Wunderlich MT, Dvorak F, Humpich M, Kahles T, Goertler M, Alvarez-Sabin J, Wallesch CW, Molina CA, Steinmetz H, *et al*: **Elevated serum S100B levels indicate a higher risk of hemorrhagic transformation after thrombolytic therapy in acute stroke.** *Stroke* 2007, **38**(9):2491–2495.
10. Mussack T, Biberthaler P, Wiedemann E, Kanz KG, Englert A, Gippner-Steppert C, Jochum M: **S-100b as a screening marker of the severity of minor head trauma (MHT)–a pilot study.** *Acta Neurochir Suppl* 2000, **76**:393–396.
11. Unden J, Ingebrigtsen T, Romner B: **Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update.** *BMC Med* 2013, **11**:50.
12. Mussack T, Biberthaler P, Kanz KG, Heckl U, Gruber R, Linsenmaier U, Mutschler W, Jochum M: **Immediate S-100B and neuron-specific enolase plasma measurements for rapid evaluation of primary brain damage in alcohol-intoxicated, minor head-injured patients.** *Shock* 2002, **18**(5):395–400.
13. Lange RT, Iverson GL, Brubacher JR: **Clinical utility of the protein S100B to evaluate traumatic brain injury in the presence of acute alcohol intoxication.** *J Head Trauma Rehabil* 2012, **27**(2):123–134.
14. Astrand R, Romner B, Lanke J, Unden J: **Reference values for venous and capillary S100B in children.** *Clin Chim Acta* 2011, **412**(23–24):2190–2193.

15. Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PM: **Indications for computed tomography in patients with minor head injury.** *N Engl J Med* 2000, **343**(2):100–105.
16. Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, McKnight RD, Verbeek R, Brison R, Cass D, *et al*: **The Canadian CT head rule for patients with minor head injury.** *Lancet* 2001, **357**(9266):1391–1396.
17. Biberthaler P, Linsenmeier U, Pfeifer KJ, Kroetz M, Mussack T, Kanz KG, Hoecherl EF, Jonas F, Marzi I, Leucht P, *et al*: **Serum S-100B concentration provides additional information fot the indication of computed tomography in patients after minor head injury: a prospective multicenter study.** *Shock* 2006, **25**(5):446–453.
18. Muller K, Townend W, Biasca N, Unden J, Waterloo K, Romner B, Ingebrigtsen T: **S100B serum level predicts computed tomography findings after minor head injury.** *J Trauma* 2007, **62**(6):1452–1456.
19. Smits M, Dippel DW, Steyerberg EW, de Haan GG, Dekker HM, Vos PE, Kool DR, Nederkoorn PJ, Hofman PA, Twijnstra A, *et al*: **Predicting intracranial traumatic findings on computed tomography in patients with minor head injury: the CHIP prediction rule.** *Ann Intern Med* 2007, **146**(6):397–405.
20. Calcagnile O, Unden L, Unden J: **Clinical validation of S100B use in management of mild head injury.** *BMC Emerg Med* 2012, **12**:13.
21. Cervellin G, Benatti M, Carbucicchio A, Mattei L, Cerasti D, Aloe R, Lippi G: **Serum levels of protein S100B predict intracranial lesions in mild head injury.** *Clin Biochem* 2012, **45**(6):408–411.
22. Muller K, Elverland A, Romner B, Waterloo K, Langbakk B, Unden J, Ingebrigtsen T: **Analysis of protein S-100B in serum: a methodological study.** *Clin Chem Lab Med* 2006, **44**(9):1111–1114.

doi:10.1186/1757-7241-21-52
Cite this article as: Calcagnile *et al.*: S100B levels are affected by older age but not by alcohol intoxication following mild traumatic brain injury. *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine* 2013 **21**:52.

Paper III

Undén et al. BMC Medicine (2015) 13:292
DOI 10.1186/s12916-015-0533-y

BMC Medicine

RESEARCH ARTICLE Open Access

Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults

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Abstract

Background: Acute management of traumatic brain injury (TBI), in particular mild TBI, focuses on the detection of the 5–7 % who may be harboring potentially life-threatening intracranial hemorrhage (IH) using CT scanning. Guidelines intending to reduce unnecessary head CT scans using available clinical variables to detect those at high IH risk have shown varying results. Recently, the Scandinavian Neurotrauma Committee (SNC) derived a new set of high-IH risk variables for adults with TBI using an evidence-based literature review. Unlike previous guidelines, the SNC guideline incorporates serum values of the brain protein S100B with clinical variables.

Methods: We performed a nested cohort study of adults with mild TBI presenting to six emergency departments in New York and Pennsylvania within 6 h of injury. Patients were managed according to existing guidelines for CT selection. All patients underwent head CT scanning and serum S100B measurement, as well as prospective collection of clinical variables, as a requirement of the parent study. Using the SNC guidelines, S100B values and clinical variables were applied to these subjects, classifying each into one of five pre-defined severity categories, as well as predicting the need for head CT scanning to identify IH. This classification was then compared to actual head CT results to determine guideline sensitivity and specificity.

Results: In total, 662 adults (mean age 42 years, range 18–96; 258 females, 549 Caucasians) were available for analysis; 36 (5 %) had IH on head CT scan. The SNC guidelines had a sensitivity of 97 % (95 % CI, 84–100 %) and a specificity of 34 % (95 % CI, 30–37 %) for the detection of IH on head CT. Application of the SNC guidelines would have resulted in a CT reduction of 32 % (211/662 patients). One patient with low-risk mild TBI and a S100B level under 0.10 μg/L had a traumatic CT abnormality and would have been discharged with strict adherence to the guidelines. However, this patient did not need any intervention for the injury and had a good outcome.

Conclusion: Using the SNC guideline could save approximately one third of CT scans in a pre-selected cohort of mild TBI patients with little or no impact on patient outcome.

Keywords: Biomarkers, Brain injury, Computed tomography, Decision rule, Guidelines, Head injury, Management, Mild traumatic brain injury, S100B/S100/S100BB, Traumatic brain injury

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Background

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity [1], and one of the most common reasons to seek emergency department (ED) care [2, 3]. The vast majority of patients with acute (<24 h after injury) TBI are conscious on ED arrival with a Glasgow Come Scale (GCS) of 13–15. These patients are typically defined as mild TBI (mTBI) and constitute approximately 95 % of all TBIs [4]. Although conscious on arrival, a small portion of these patients will have traumatic intracranial findings on computed tomography (CT) and some will require neurosurgical intervention [5]. Many of these are therefore subjected to CT scanning, hospital admission or both. Considering the economic implications of CT scanning and hospital admission, coupled with escalating concerns for radiation risks from CT scans [6, 7], several guidelines and decision rules have been published aiming to guide ED physicians to minimize unnecessary CT scans and/or admission while ensuring a safe triage for mTBI patients [8, 5]. Some of these have been externally validated with varying results [9–11]. Unfortunately, these guidelines are generally not applicable to all mTBI patients presenting in a typical ED. Further, there are concerns that introduction of new guidelines may actually lead to an increase in CT scans [12].

Recently, attention has been focused on efforts using brain-specific biomarkers, mainly protein S100B, in an attempt to reduce unnecessary CT scanning following mTBI [13, 14]. S100B is a dimeric astroglial protein of approximately 21 kD. Although the specific function of the protein has not been established, it seems to have both intracellular and extracellular effects [15]. The half-life of S100B is short, with recent data suggesting a half-life of less than 30 min [16]. Although first thought to be brain specific, studies have shown that low levels of S100B exist in extracerebral tissues and may limit the clinical specificity of S100B in TBI management [17]. Despite this, the high sensitivity and clinical negative predictive value of S100B justifies the use of the protein in TBI management. However, since much of the clinical evidence concerning S100B is relatively recent, it has not been included in clinical guidelines but is nevertheless used clinically in many European countries [18].

In 2013, the Scandinavian Neurotrauma Committee (SNC) published evidence-based guidelines for initial management of TBI for adults [19] (Fig. 1). These guidelines are designed for patients with acute (<24 h from injury) TBI and for detection of important intracranial injuries, such as those needing neurosurgical intervention and/or intensive care support. The classification of mTBI has further been divided into high, medium and low risk depending on the presence of certain risk factors. The guidelines also include biomarker S100B as a clinical tool for reducing CT scans in a subset of mTBI patients. Although these guidelines were designed for the Scandinavian healthcare systems, validation in an external cohort would be of interest.

Methods

We performed a retrospective nested cohort study of adults with mTBI presenting to the ED within 6 h of injury. The parent study was a prospective, multicenter, cohort study designed to determine the classification accuracy of serum S100B, serum apolipoprotein A1, and clinical variables for identifying patients with mild TBI and for identifying patients with traumatic abnormalities on head CT [14]. Given the similarities between the variables collected and the variables contained in the SNC, these data permitted an assessment of the performance of the SNC.

Participants were enrolled in the parent study at five hospitals in Upstate New York and one hospital in Pennsylvania between 2008 and 2010. Subjects were eligible for inclusion in the parent study if they were aged 1 year or older, had mTBI as defined by the Centers for Disease Control and Prevention's National Center for Injury Prevention and Control (a blow to the head or rapid acceleration/deceleration resulting in at least one of the following: a loss of consciousness (LOC) ≤30 min, post-traumatic amnesia ≤24 h, neuropsychological abnormality [any transient period of confusion, disorientation, or impaired consciousness; in children ≤2 years old: irritability, lethargy, or vomiting post-injury], or neurological abnormality [seizure acutely following injury, hemiplegia, or diplopia]) [1]. An additional inclusion criteria was the availability of head CT scanning as part of their clinical care. The Institutional Review Boards for each of the six participating centers approved this study and the process of informed consent. All participants (or guardians of participants) gave informed consent.

Participants

Subjects were selected from the parent study into this nested cohort if they were adults ≥18 years of age (the SNC guidelines are designed and intended for adults) and had sufficient data present in order to classify patients according to the guidelines.

Clinically-relevant variables

Subjects participating in the parent cohort were interviewed in the ED by trained research assistants for injury mechanism, initial symptoms, demographics, and medical history. The emergency provider was also interviewed and the emergency chart was reviewed to determine physical exam signs, associated injuries, and GCS score. The decision to collect specific clinical variables was based on their inclusion in two head CT clinical decision rules that were in use at the time the parent study was conducted, namely the New Orleans Criteria (NOC) [8] and the Canadian CT

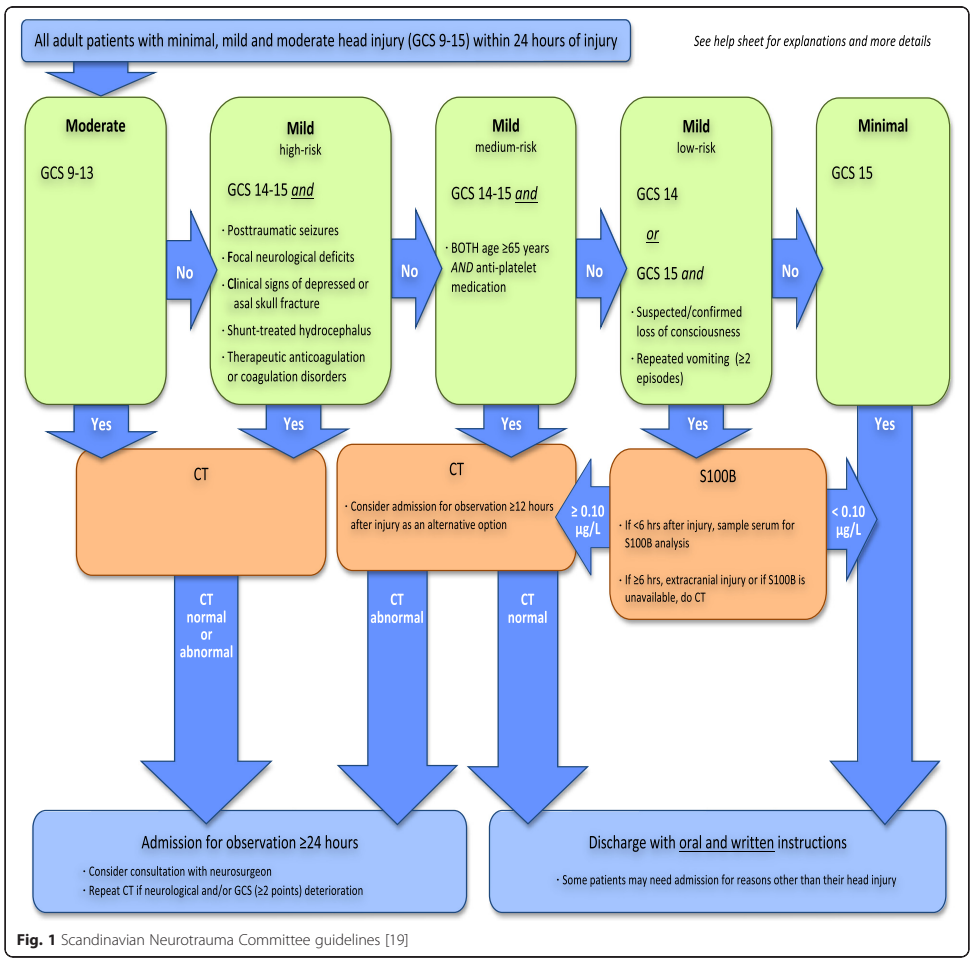

Fig. 1 Scandinavian Neurotrauma Committee guidelines [19]

Head Rule [5]. The SNC Head CT guideline, which was published after the parent study was completed, recommended a slightly different set of clinical variables [19]. The subset of clinical variables collected in the parent study that were identical or similar to the variables in the SNC head CT guideline were analyzed in the nested cohort. The extent to which this subset of clinical variables overlap with the variables recommended by the SNC head CT guideline is shown in Table 1.

As the variables collected in the parent study were not chosen with the SNC guidelines in mind, certain assumptions were made a priori. As double vision and paralysis were the only neurologically specific symptoms recorded, these were composited to the variable of focal neurological deficit. Further, suspected/confirmed LOC from the guidelines was equated with unsure/confirmed LOC from the cohort data. Significant extracerebral injury was met if internal organ injury, fractures and blast/burn/electrocution injuries were noted. Minor injuries, such as lacerations and bruises, were not classified as significant extracerebral injuries.

Head CT scans

At each study site, head CT scans were interpreted by board-certified radiologists who were blinded to the laboratory results. The final reading entered into the radiology

Table 1 Comparison of clinical variables collected and those included in SNC guideline

Clinical variables collected	SNC head CT guideline [19]
Post-traumatic seizure	Post-traumatic seizure
Age	Age ≥65 years
Vomiting, number of times	Vomiting ≥2 times
Glasgow Coma Scale score	Glasgow Coma Scale score
Suspected open skull fracture	Clinical signs of depressed skull fracture
Signs of basilar skull fracture	Clinical signs of basilar skull fracture
Diplopia, paralysis	Focal neurologic deficit
All current neurologic conditions, including hydrocephalus	Shunt-treated hydrocephalus
prothrombin ratio and international normalized ratio, not collected	Coagulation disorders
All current medications including antiplatelet and anticoagulants	Antiplatelet medication
	Therapeutic anticoagulation
Loss of consciousness	Suspected or confirmed loss of consciousness
All extracranial injuries	Significant extracerebral injury
S100B levels	S100B ≤0.10 μg/L

image database at each institution was used to determine the presence or absence of intracranial abnormalities. Traumatic CT abnormalities were defined as subdural hematomas, epidural hematomas, subarachnoid hemorrhage, edema, skull fracture, and cerebral contusions.

Blood draw and sample handling

Blood for S100B sampling was drawn from mTBI subjects within 6 h of the time of injury. Four milliliters of whole blood was drawn into a serum separator tube and immediately placed on ice. Within 60 min, the blood was centrifuged at 3000 rpms for 10 min and the serum was aliquoted into 500 μL tubes frozen at −80 °C.

S100B assay

Serum S100B concentrations were determined by a fully automatic electrochemoluminometric immunoassay (Elecsys S100; Roche Diagnostics, Penzberg, Germany) with a detection limit of 0.005–39 lg/L. The analyte was sandwiched between two monoclonal antibodies directed against the beta-chain of the S100 dimer. Then, streptavidin-coated microparticles were added and the immunocomplex was bound to the solid phase. In the measurement cell, unbound components were removed and a defined voltage used to initiate the electrochemiluminescent reaction. The resultant light emission was then measured using a photomultiplier. S100B assays were performed from November to December 2010. Resulting S100B values were not available to the emergency physicians caring for the subjects involved in this study, nor where they available to interviewers and trained research assistants. Thus, providers and research personnel were blinded to S100B results.

Outcome

One month after the initial ED visit, outcome was determined by telephone interview using the Rivermead Post Concussion Questionnaire [20, 21]. Subjects were asked to rate the severity of 16 post-concussive symptoms (such as headache), compared to pre-injury, on a Likert scale ranging from "0" (absent) to "4" (severe). Total scores thus ranged from 0–64. The interviewer was blinded to the details of the ED visit.

Analysis

Using the SNC guidelines, S100B values and clinical variables were used to classify each subject into one of the five SNC-defined head injury severity categories (moderate TBI, mTBI/high risk, mTBI/medium risk, mTBI/low risk, and minimal TBI). In order to estimate the ability of the SNC to determine head injury severity, the prevalence of traumatic CT abnormalities in each severity category was calculated and compared. Because the number of CT+ subjects was ≤5 in two severity groups (moderate-risk and medium-risk mTBI), the Fishers exact test, rather than the χ^2 test, was used to make these comparisons. Given the fixed samples sizes of each SNC severity group and the number of CT+ subjects in each group, the power to detect the observed differences in CT+ prevalence between groups – assuming a Type 1 error rate of 0.05 – ranged from 0.132 to 0.717. The need for head CT scanning as predicted by the SNC was then compared to actual head CT results to determine guideline sensitivity and specificity.

Results

During the study period, 784 subjects with mTBI were enrolled into the parent study; 93 were children and therefore not considered for the guidelines. In 29 patients, vital data was missing (mainly GCS scores), which made it impossible to accurately classify the patients and they were therefore excluded. Thus, 662 patients were eligible for analysis (Fig. 2). Most subjects in the nested cohort were Caucasian and male (Table 2).

SNC guidelines and head injury severity

CT scans were positive (CT+) for traumatic abnormalities in 36/662 patients (5 %). Eight patients showed cerebral contusions, six had traumatic subarachnoid hemorrhage, four had subdural hematomas, two had petechial hemorrhage/shear injury, one had cerebral edema, one had a linear skull fracture, and one had an epidural hematoma. The remaining 13 patients had a combination of intracranial traumatic abnormalities. No

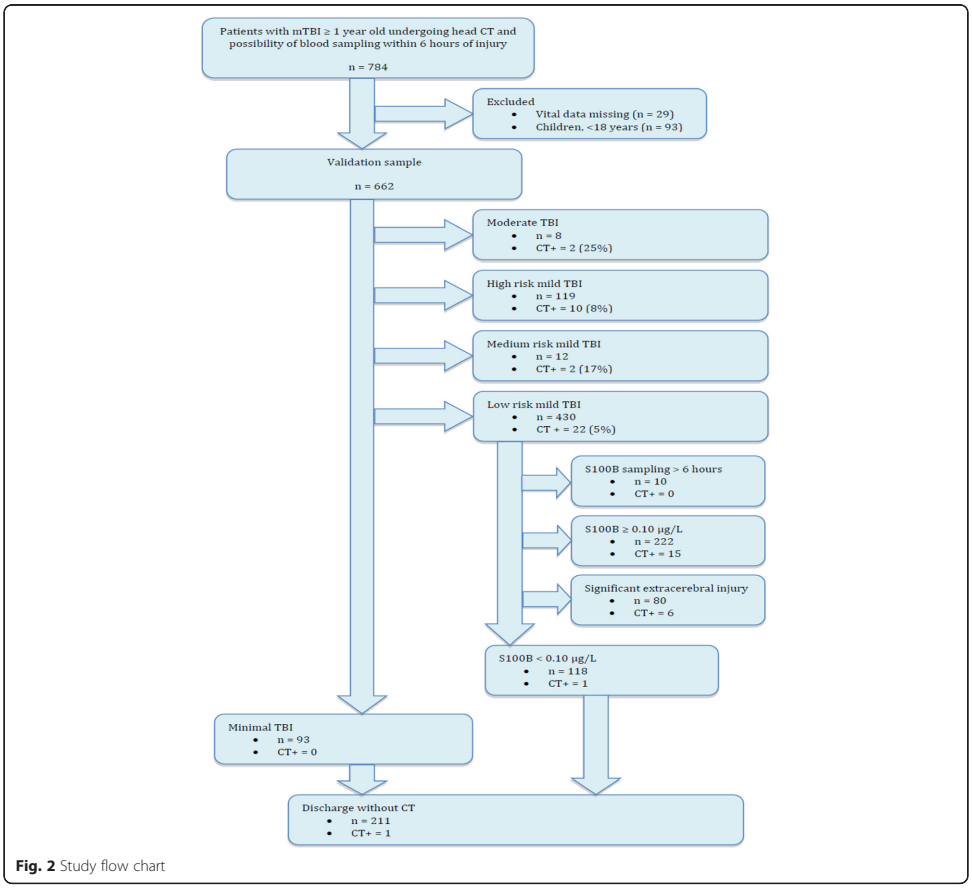

Fig. 2 Study flow chart

patients in the cohort needed neurosurgical intervention and none died as a result of the TBI.

Eight subjects were classified by SNC as moderate TBI, 119 as high-risk mTBI, 12 as medium-risk mTBI, 430 as low-risk mTBI, and 93 as minimal TBI (Table 3). The prevalence of CT+ was highest in the moderate TBI group (25 %) and lowest in the minimal TBI group (0 %). Compared to the minimal TBI group, the CT+ prevalence was significantly higher in the moderate TBI ($P = 0.006$), in the high-risk mTBI group ($P = 0.003$), in the medium-risk mTBI group ($P = 0.012$), and in low-risk mTBI group ($P = 0.021$; Fig. 3). The CT+ prevalence in the moderate TBI group (25 %) was higher than the low-risk mTBI group (5 %), but this difference did not reach statistical significance. The CT+ prevalence in the medium-risk mTBI (17 %) was higher than that of the high-risk mTBI (8 %), but this difference did not reach statistical significance.

Table 2 Characteristics of the nested cohort

Variable	
Age, mean (range)	42 (18–96) years
Sex, percentage female	39 %
Race	83 % Caucasian
Head CT results	37 CT+ (6 %)

SNC guidelines and prediction of traumatic abnormalities on head CT

The SNC guidelines classified 451 subjects as needing a head CT scan, and 211 as not needing one. The SNC guidelines had a sensitivity of 97 % (95 % CI, 84–100 %)

Table 3 Performance of SNC guideline in validation cohort

		CT results		
		+	–	Total
SNC guideline	CT	35	416	451
	No CT	1	210	211
	Total	36	626	662

Overall, a 32 % reduction in CT scanning was observed if SNC guidelines were used; 1 missed patient (low-risk mild with S100B ≤0.10 μg/L), see text for details; Prevalence of CT findings: 5 %; Sensitivity: 97 % (95 % CI, 84–100 %); Specificity: 34 % (95 % CI, 30–37 %); Negative predictive value, 100 % (95 % CI, 97–100 %); Positive predictive value, 8 % (95 % CI, 6–11 %)

and a specificity of 34 % (95 % CI, 30–37 %) for predicting traumatic CT abnormalities (Table 3).

In patients with high-risk mTBI, 84 patients exhibited double vision as a risk factor and four of these had CT+ findings. Of 19 patients with paralysis, none had a CT+ lesion. Of 18 patients with seizures and 12 patients with clinical suspicion of open/depressed skull fracture, two cases in each showed CT+ findings. Finally, eight patients with anticoagulant use and six patients with clinical signs of basal skull fractures each showed one case of CT+. Overall, 430 patients were classed as low-risk mTBI (65 % of the total sample). Of these, 340 were eligible for S100B sampling according to the SNC guidelines (10 underwent sampling more than 6 h after injury and 80 patients had significant extracerebral injuries). Of these, 118 had levels <0.10 μg/L and 222 had levels ≥0.10 μg/L (35 % below cut-off). In patients with extracerebral injury, six had CT+ and all of these had elevated S100B levels. None of the 10 patients with sampling done after 6 h from injury had CT+ results.

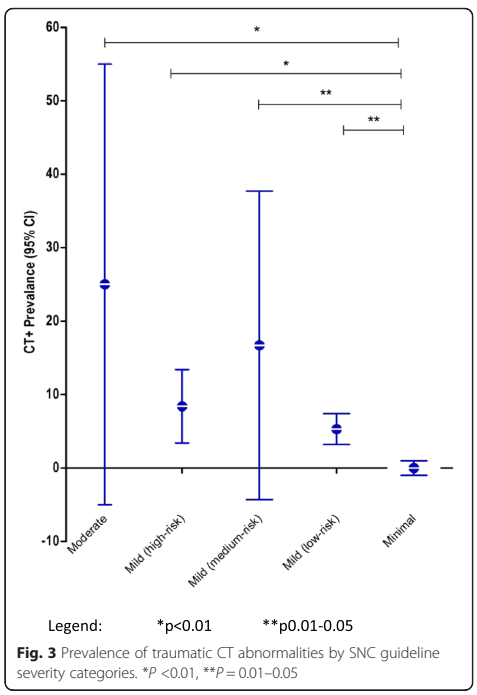

Fig. 3 Prevalence of traumatic CT abnormalities by SNC guideline severity categories. *P <0.01, **P = 0.01–0.05

In total, application of the SNC guidelines to this validation sample would have resulted in a CT reduction of 32 % (211/662 patients); one patient with a low-risk mild TBI and a S100B level under 0.10 μg/L had a traumatic CT abnormality. This patient was a 20-year-old male presenting at the ED after a motor vehicle accident (without ejection) with a GCS of 14 and LOC (unclear time period). CT showed a small cerebral contusion which subsided on follow-up CT scans (Fig. 4). He was discharged home from the inpatient unit without needing medical or surgical intervention for his injury and had a good neurological outcome on follow-up. His total Rivermead Post Concussion Questionnaire score was low (11 out of 64) and he had new symptoms of moderate fatigue and mild issues of frustration and poor memory.

Discussion

In response to escalating healthcare costs, care providers have a responsibility to manage patients within health-economic considerations [22]. TBI, in particular mTBI, represents a significant burden for hospitals and ED facilities in developed countries. Existing guidelines for management of such patients differ in sensitivity and specificity with respect to detection of CT findings, traumatic CT findings, clinically important CT findings and need for neurosurgical/intensive care intervention [9, 11]. The SNC guideline offers a comprehensive aid to management of all adults with TBI and includes CT management options.

The results indicate that the SNC guidelines seem to predict TBI severity within a cohort of patients with GCS 13–15. The CT+ prevalence in the minimal TBI group was significantly lower than each of the other four severity groups. In addition, the CT+ prevalence in the moderate TBI group was five times higher than that of the low-risk mTBI group, but this difference was not statistically significant, likely due to the small number of subjects (n = 8) in the moderate risk group. Counterintuitively, the CT+ prevalence in the medium-risk mTBI group was over twice that of the high-risk mTBI group, but this difference was also not statistically significant, likely due to the small number of subjects (n = 12) in the medium-risk mTBI group.

Adherence to the SNC guidelines would have resulted in a 32 % reduction in CT scans in the present population – a population clinically judged to need a CT scan according to

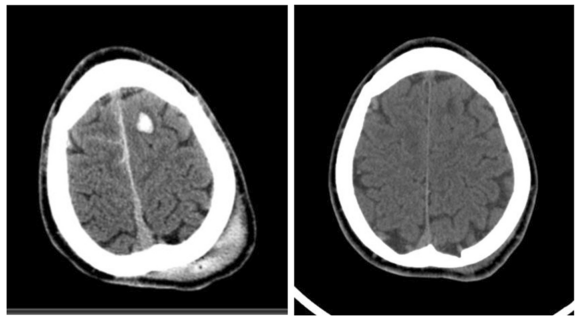

Fig. 4 False negative subject. A 20-year-old with GCS 14 and unclear episode of loss of consciousness. The patient would be classed as low-risk mild traumatic brain injury and had a S100B of 0.09 μg/L. CT shows a small contusion and extracranial soft tissue swelling. The contusion subsided on follow-up CT after 25 days. The patient did not need any intervention or treatment and was discharged from the inpatient unit with a good neurological outcome

local routines. Since many of these patients were also admitted to hospital, the cost saving potential is substantial. However, one patient, who was classified by the SNC guideline as low-risk mTBI and had a low S100B level (0.09 μg/L, just below cut-off), showed a traumatic abnormality on CT scanning. The finding was relatively minor (small contusion) and did not need any specific treatment. Missing this CT abnormality would therefore not have had an impact on the care and outcome of this patient. S100B is not 100 % sensitive and less significant, non-neurosurgical lesions, such as the lesion in this study, may be missed using the present cut-off [23, 24]. Additionally, the SNC guidelines were designed to primarily detect patients needing neurosurgical or other specific intervention, with traumatic CT abnormalities being a secondary, yet important, goal [19]. It is likely that the difference in medico-legal attitudes between countries may influence the view on this matter. In Scandinavia, missing uncomplicated intracranial complications that do not need specific intervention in patients with good outcome is acceptable, especially if this implies resource saving. However, this may not be true for other countries such as the United States and Canada.

Since S100B is currently unavailable in the US, the management according to the SNC guidelines would have differed in that patients with low-risk mTBI would have had a CT scan. In this scenario, no patients would have been missed but the CT use would have been reduced to 14 % (93/662).

S100B is included as an option in the guidelines for those centers with the ability to perform 24/7 real-time analysis. As most Scandinavian centers have this possibility, S100B is now widely used in this setting. Published reports of S100B in clinical use [18] and unpublished summaries of current use with the new guidelines have shown very promising results. However, these recent observations need to be scientifically examined, a process which is currently underway via a validation study in Scandinavia. Further, the practicality of using the SNC guidelines would be of interest but could not be examined in the present study. A guideline would have to be practically viable for the treating of professionals in order for such a tool to be clinically useful.

The sensitivity and specificity figures reported here are not reflective of an unselected cohort of TBI patients, but rather a selection of patients where current guidelines advocate a CT scan. In a more unselected cohort, the sensitivity and negative predictive ability would reasonably be higher as the present study had already selected a population with a higher risk for CT abnormalities (higher pre-test probability). Therefore, it is also difficult to compare the performance of different guidelines as the cohort is pre-selected. The NOC criteria, for example, would reasonably advocate CT scans on many patients not considered for inclusion into this cohort and can only be used on the subset of patients with GCS 15. Additionally, both the NOC and Canadian CT Head Rule criteria can only be applied to patients with specific symptoms (LOC and LOC, amnesia or confusion, respectively), unlike the SNC guidelines, which are designed for all adult patients following a non-severe TBI. The only correct method of comparing these guidelines would be in an unselected series of all TBI patients presenting at the ED.

Limitations

The S100B cutoff used in the SNC guidelines was derived from studies involving mostly Caucasian populations. This cutoff might not perform the same in subjects of color, such as some of those in the current study. However, this would rather affect the specificity of S100B (i.e. more false

positives) and therefore theoretically reduce the CT saving ability of S100B in non-Caucasian populations. S100B as a single test had a 35 % CT reduction ability in this study, which is similar to other cohorts [25].

Although the reported cohort is relatively large, the absolute number of CT+ patients was small and no patients needed neurosurgical or specific medical intervention. A much larger cohort would be necessary to fully examine these aspects. Further, although the original patient inclusion was prospective, the validation of the SNC guidelines was retrospective. A purpose-designed prospective study is recommended and currently underway.

Conclusion

The updated SNC guidelines can accurately classify injury severity and may further reduce CT scans in a selected population of patients with TBI requiring CT scanning. The one patient missed by the guidelines did not require any intervention and had a good outcome.

Availability of supporting data

Raw data is currently not available to publicly share as further analysis of the parent cohort is planned. However, the authors (specifically JB) will consider sharing data upon personal request.

Abbreviations

CT: Computed tomography; ED: Emergency department; GCS: Glasgow Come Scale; LOC: Loss of consciousness; mTBI: Mild TBI; NOC: New Orleans Criteria; SNC: Scandinavian Neurotrauma Committee; TBI: Traumatic brain injury.

Competing interests

JB states that he has a patent "Method of Diagnosing Mild Traumatic Brain Injury", PCT International Application Number PCT/US2012/069002, US Serial No. 14/365,550. This patent involves the use the peripheral protein Apolipoprotein A1 to aid in the diagnosis of concussion. As it does not involve S100B, there are no competing interests with the content of the current manuscript. JB is a consultant for Banyan Biomarkers. This consulting involved efforts to design a multicenter study to test the serum markers GFAP and UCHL1, not S100B. Thus, there are no competing interests with the content of the current manuscript. The other authors have no competing interests.

Authors' contributions

LU, OC and JU conceived the study. All authors were involved in specific study design. JB was responsible for data acquisition for the parent study. LU and OC performed data analysis with assistance from JU and PR. The manuscript was drafted by LU with input from all authors. LU addressed reviewer comments with input from JB, PR, OC and JU. All authors approved the final manuscript.

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Received: 9 July 2015 Accepted: 23 November 2015
Published online: 09 December 2015

References

1. Centers for Disease Control and Prevention. National Center for Injury Prevention and Control. Report to Congress. Mild traumatic brain injury in the United States: steps to prevent a serious public health problem. Atlanta, GA: CDC; 2003.
2. Faul MXL, Wald MM, Coronado VG. Traumatic brain injury in the United States: emergency department visits, hospitalizations and deaths 2002–2006. Atlanta, GA: CDC, National Center for Injury Prevention and Control; 2010.
3. Tagliaferri F, Compagnone C, Korsic M, Servadei F, Kraus J. A systematic review of brain injury epidemiology in Europe. Acta Neurochir. 2006;148:255–68. discussion 68.
4. Corrigan JD, Selassie AW, Orman JA. The epidemiology of traumatic brain injury. J Head Trauma Rehabil. 2010;25:72–80.
5. Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, et al. The Canadian CT Head Rule for patients with minor head injury. Lancet. 2001;357:1391–6.
6. Brenner DJ, Hall EJ. Computed tomography – an increasing source of radiation exposure. N Engl J Med. 2007;357(22):2277–84.
7. Choi SJ, Kim EY, Kim HS, Choi HY, Cho J, Yang HJ, et al. Cumulative effective dose associated with computed tomography examinations in adolescent trauma patients. Pediatr Emerg Care. 2014;30:479–82.
8. Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PM. Indications for computed tomography in patients with minor head injury. N Engl J Med. 2000;343:100–5.
9. Stein SC, Fabbri A, Servadei F, Glick HA. A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann Emerg Med. 2009;53:180–8.
10. Stiell IG, Clement CM, Rowe BH, Schull MJ, Brison R, Cass D, et al. Comparison of the Canadian CT Head Rule and the New Orleans Criteria in patients with minor head injury. JAMA. 2005;294:1511–8.
11. Smits M, Dippel DW, de Haan GG, Dekker HM, Vos PE, Kool DR, et al. External validation of the Canadian CT Head Rule and the New Orleans Criteria for CT scanning in patients with minor head injury. JAMA. 2005;294:1519–25.
12. Kelly JC, O'Callaghan A, McMullin L, Clinton O, Binchy J. Management of traumatic head injuries in a rural Irish hospital: implications of the NICE guidelines. Ir J Med Sci. 2010;179:557–60.
13. Unden J, Romner B. Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: an evidence-based review and meta-analysis. J Head Trauma Rehabil. 2010;25:228–40.
14. Bazarian JJ, Blyth BJ, He H, Mookerjee S, Jones C, Kiechle K, et al. Classification accuracy of serum ApoA-I and S100B for the diagnosis of mild TBI and prediction of abnormal initial head CT. J Neurotrauma. 2013;30(20):1747–54. doi:10.1089/neu.2013.2853.
15. Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, et al. Functions of S100 proteins. Curr Mol Med. 2013;13:24–57.
16. Jonsson H, Johnsson P, Hoglund P, Alling C, Blomquist S. Elimination of S100B and renal function after cardiac surgery. J Cardiothorac Vasc Anesth. 2000;14:698–701.
17. Pelinka LE, Szalay L, Jafarmadar M, Schmidhammer R, Redl H, Bahrami S. Circulating S100B is increased after bilateral femur fracture without brain injury in the rat. Br J Anaesth. 2003;91:595–7.
18. Calcagnile O, Unden L, Unden J. Clinical validation of S100B use in management of mild head injury. BMC Emerg Med. 2012;12:13.
19. Unden J, Ingebrigtsen T, Romner B, Scandinavian NC. Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. BMC Med. 2013;11:50.
20. Crawford S, Wenden FJ, Wade DT. The Rivermead head injury follow up questionnaire: a study of a new rating scale and other measures to evaluate outcome after head injury. J Neurol Neurosurg Psychiatry. 1996;60:510–4.
21. King NS, Crawford S, Wenden FJ, Moss NE, Wade DT. The Rivermead Post Concussion Symptoms Questionnaire: a measure of symptoms commonly experienced after head injury and its reliability. J Neurol. 1995;242:587–92.
22. Ruan S, Noyes K, Bazarian JJ. The economic impact of S-100B as a pre-head CT screening test on emergency department management of adult patients with mild traumatic brain injury. J Neurotrauma. 2009;26:1655–64.

23. Biberthaler P, Linsenmeier U, Pfeifer KJ, Kroetz M, Mussack T, Kanz KG, et al. Serum S-100B concentration provides additional information for the indication of computed tomography in patients after minor head injury: a prospective multicenter study. Shock. 2006;25:446–53.
24. Muller K, Townend W, Biasca N, Undén J, Waterloo K, Romner B, et al. S100B serum level predicts computed tomography findings after minor head injury. J Trauma. 2007;62:1452–6.
25. Unden J, Romner B. A new objective method for CT triage after minor head injury–serum S100B. Scand J Clin Lab Invest. 2009;69:13–7.

Paper IV

Calcagnile et al. BMC Neurology (2016) 16:200
DOI 10.1186/s12883-016-0723-z

BMC Neurology

RESEARCH ARTICLE Open Access

The addition of S100B to guidelines for management of mild head injury is potentially cost saving

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Abstract

Background: Mild traumatic brain injury (TBI) is associated with substantial costs due to over-triage of patients to computed tomography (CT) scanning, despite validated decision rules. Serum biomarker S100B has shown promise for safely omitting CT scans but the economic impact from clinical use has never been reported. In 2007, S100B was adapted into the existing Scandinavian management guidelines in Halmstad, Sweden, in an attempt to reduce CT scans and save costs.

Methods: Consecutive adult patients with mild TBI (GCS 14-15, loss of consciousness and/or amnesia), managed with the aid of S100B, were prospectively included in this study. Patients were followed up after 3 months with a standardized questionnaire. Theoretical and actual cost differences were calculated.

Results: Seven hundred twenty-six patients were included and 29 (4.7 %) showed traumatic abnormalities on CT. No further significant intracranial complications were discovered on follow-up. Two hundred twenty-nine patients (27 %) had normal S100B levels and 497 patients (73 %) showed elevated S100B levels. Over-triage occurred in 73 patients (32 %) and under-triage occurred in 39 patients (7 %). No significant intracranial complications were missed. The introduction of S100B could save 71 € per patient if guidelines were strictly followed. As compliance to the guidelines was not perfect, the actual cost saving was 39 € per patient.

Conclusion: Adding S100B to existing guidelines for mild TBI seems to reduce CT usage and costs, especially if guideline compliance could be increased.

Background

Head injury is a serious health problem in developed countries and associated with a substantial economic burden [1]. Most (up to 95 %) of head injuries are classified as mild head injury (MHI), commonly defined as Glasgow Coma Scale (GCS) 13-15 with the presence of certain risk factors such as loss of consciousness (LOC) and/or amnesia [2, 3].

Typical management of MHI involves computed tomography (CT) of the brain to identify complications such as intracranial haemorrhage and cerebral contusions [4]. These complications are rare but may occasionally need neurosurgical intervention [5]. Guidelines have therefore recommended liberal CT examinations in this patient group. Patients with GCS 15 and no risk factors have a very low risk of intracranial complication [6] and can be discharged from the emergency department (ED) without a CT scan [7].

Due to the considerable resource use and high number of unnecessary CT scans, recent efforts have been concentrated on optimizing CT use after MHI [7–12]. These decision rules are based upon risk factors from patient history and clinical examination. However, due to the high socioeconomic cost of missing cases of intracranial complication, CT rates remain high [12].

Another aspect to be taken into account is the logistics of patients waiting in the ED to have a CT scan. Some departments may obtain a CT result within minutes but in smaller facilities patients may need to wait several hours before a CT can be carried out, stocking the work flow at the ED [12].

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Several groups have considered the use of brain biomarker S100B in this clinical setting. Studies show that serum levels of the protein may reduce CT scans in the MHI subgroup of patients by 30 % without missing intracranial complications [13–15]. Serum levels of S100B are also not affected by ethanol intoxication [16] and represent an objective addition to the more subjective risk factors included in existing guidelines. Despite theoretical reports of the potential of S100B to reduce costs in this patient group, no reports of clinical S100B use, and hence actual cost and time reduction, exist.

In the year 2000, the Scandinavian Neurotrauma Committee (SNC) published guidelines for management of non-severe head injuries [7]. In 2007, serum S100B measurements were introduced into these guidelines at Halmstad Regional Hospital, Sweden, based upon the evidence available at the time, in an attempt to reduce CT scans, costs and waiting time in the ED. The aim of the present study was to establish if this change in management routines resulted in a decrease in health care costs and waiting time for patients.

Methods

Study setting and population

The study setting is the Halmstad Regional hospital, Sweden; a level II trauma centre with 24-h emergency care, anaesthesiology, radiology, surgery and intensive care. From November 2007 (6 months following the introduction of S100B into clinical care, see Fig. 1 for management routines) to December 2013 we prospectively

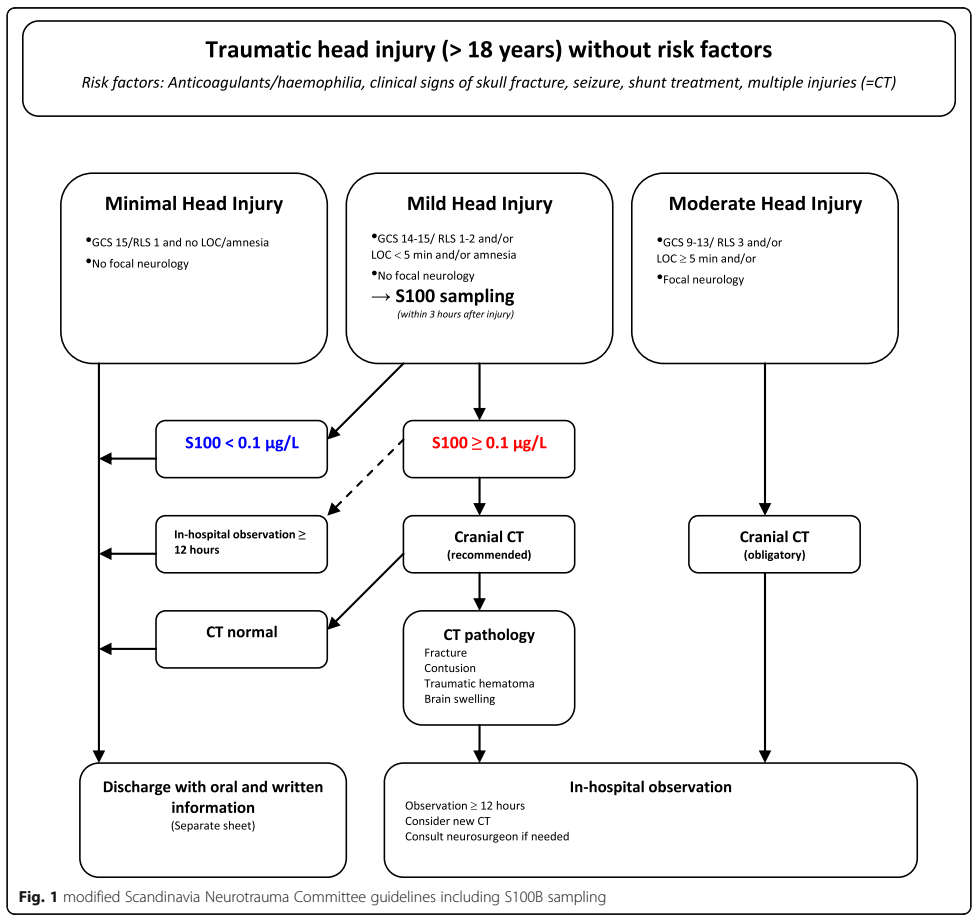

Fig. 1 modified Scandinavia Neurotrauma Committee guidelines including S100B sampling

enrolled consecutive adult patients with MHI and S100B sampling, according to these clinical guidelines. In September 2013, an update to the Scandinavian Guidelines was introduced. This study did not take into account the new guidelines and age or antiplatelet medications were not considered as risk factors. The inclusion criteria in this study were: adult patients with acute trauma to the head with GCS 14-15 during examination and/or loss of consciousness for less than 5 min with no neurological deficits nor additional risk factors (therapeutic anticoagulation or haemophilia, clinical signs of depressed skull fracture or skull base fracture, posttraumatic seizures, shunt-treated hydrocephalus and multiple injuries). According to SNC guidelines, trauma history was not considered as a risk factor.

Exclusion criteria were: age less than 18 years, non-Swedish citizens (difficult to follow up) and patients where serum sampling for S100B was done more than 3 h post-injury.

Ethical approval was granted from the regional ethics board (approval number 19/2007).

S100B analysis

A 5 ml blood sample was drawn from patient's cubital vein in the ED. Samples were analysed with the fully automated Elecsys® S100 (Roche AB) at the Clinical Chemistry Department of Halmstad Regional hospital, Sweden, with results being available to treating physicians within 1 h. Based on previous studies [13, 14], we set a cut-off level for normal levels of S100B at less than 0.10 μg/L and a window of sampling of within 3 h from the time of the accident.

CT examinations

CT scans were performed with a GE VCT Ligthspeed 64 multislice CT scanner including 10 mm thick slices. CT scans are always analysed by a board certified radiologist.

Data registration and follow-up

Details of how patients were managed, including patient characteristics, injury type, patient history, clinical examination results, current medications, CT details including time needed from the writing of the request to the radiologist result, admission type and duration were documented in a pre-determined database.

Compliance to the guidelines was calculated by examining the actual patient management compared to the suggested management from the guidelines. All patients were asked to answer a questionnaire sent by mail 3 months after the injury. The questionnaire was repeated if no answer was received. If no answer was received from these attempts, patients were contacted via telephone. Included in this questionnaire were questions that would identify a significant intracranial lesion [9], occupation, data concerning sick-days, new contacts with medical professionals and information concerning functionality and quality of life. In cases where patients could not be reached by mail or telephone, medical records and national mortality databases were consulted for evidence of complications and/or death. Patients who would suffer significant (enough to seek medical care) intracranial complications after discharge would therefore be identified.

Data was registered on an Excel® file. Descriptive statistics was analysed using IBM SPSS® Statistics Version 20 software. Comparison of number of sick days between the two groups of patients was performed with the non-parametric Mann–Whitney U-test.

Cost analysis

The Swedish health care is state-owned; it is partially difficult to determine the costs on an individual basis considering that state refund of hospital expenses are based on hospital annual budget more than refund per service. Our cost analysis is therefore based upon standard costs according to our hospital accounts or (where data is missing) national reports. The average cost for S100B analysis during the study period was 21€ and the average cost for a non-contrast cranial CT was 130€. The cost of one day in the surgery ward (the typical admission ward for MHI patients) was 600€. Using data from the OCTOPUS study [4], the costs for a patient that is admitted only for MHI observation was calculated to be 61 % of the total costs, i.e. 366€ a day. We decided not to calculate a monetary value regarding the opportunity costs related to time spent by patients in the ED (difficult to assess) and we did not consider socioeconomic costs associated with increased cancer risks from CT scans at all (theoretically based). Not considering these aspects would lead to an under-estimation of the cost-saving potential of S100B implementation.

Results

We enrolled 795 patients with MHI and S100B levels. Sixty-nine patients were excluded according to exclusion criteria: 15 patients were younger than 18 years of age, 45 patients did not live in Sweden, 9 patients had their S100B blood sampling more than 3 h post-injury. The final population was therefore 726 patients. Descriptive statistics are presented in Table 1.

Compliance to guidelines was reasonable; more than 67 % of patients were managed according to guidelines. Two hundred twenty-nine patients had a S100B lower than 0.10 μg/L and among them 156 patients (68 %) were directly discharged without a CT or being admitted for in-hospital observation (Fig. 2). Even among patients

Table 1 Descriptive statistics

	S100B < 0.10 μg/L	S100B ≥ 0.10 μg/L	All
Male	140 (61.1 %)	305 (61.3 %)	445 (61.3 %)
Female	89 (38.9 %)	192 (38.7 %)	281 (38.7 %)
Age (mean)	31, 8 years (Range 18-89y)	46, 6 years (Range 18-92y)	42, 2 years
Alcohol intoxication	94 (41 %)	231 (46.4 %)	325 (44.7 %)
Total	229	497	726

with elevated S100B levels, we registered cases of poor compliance to the guidelines where patients with normal CT were admitted to hospital or patients with normal 12-24 h in hospital observation still underwent a CT scan (121 patients) (Fig. 2).

Thirty- two patients had pathology on CT but only 29 of these (4.7 %) were classed as traumatic abnormalities (isolated skull fracture $n = 4$, cerebral contusions $n = 9$, acute subdural hematoma n = 3, intracranial air $n = 2$, combinations of traumatic intracranial findings $n = 11$). No patients needed neurosurgical intervention. One patient with a small cerebral contusion was dismissed without hospitalization. One patient died as a result of the head injury; an 83-year-old male with expansive cerebral contusions that later resulted in a fatal intracranial pressure increase. He had an admission S100B level of 0.23 μg/L. Details of how patients were managed are presented in Fig. 2.

The follow up questionnaire was completed for 589 patients (81 %), consisting of 190 patients with normal S100B levels (83 % of population with normal S100B levels) and 399 patients with elevated S100B levels (80 % of population with elevated S100B levels). No patient with negative S100B levels sought the emergency room for missed complications. In the questionnaire, patients reported number of sick-days; there was no significant difference in number of sick-days between patients with normal S100B levels and those with elevated levels ($p = 0.352$).

Average waiting time to CT was 4 h and 14 min, calculated from the 398 patients that underwent a CT examination, with a waiting time range from 1 h and 35 min to 8 h and 35 min (Fig. 3).

The actual cost were calculated for the 726 patients strictly taking into account only S100B analysis, CT and hospitalisation cost, for an average of 242 € per patient.

To calculate the potential reduction in cost, we calculated several potential costs given different assumptions (Table 2): 1) potential cost if S100B is not used in the guidelines and assuming the same practices regarding CT and hospitalization for all patients as for the 570 patients that had high S100B levels in the actual cohort (281 € per patient), and 2) potential cost if the guidelines with S100B are followed strictly and assuming that only CT is used, as recommended in the guidelines, for the 497 patients with S100B levels higher than 0.10 ug/L (110 €). If the guidelines were followed strictly and CT

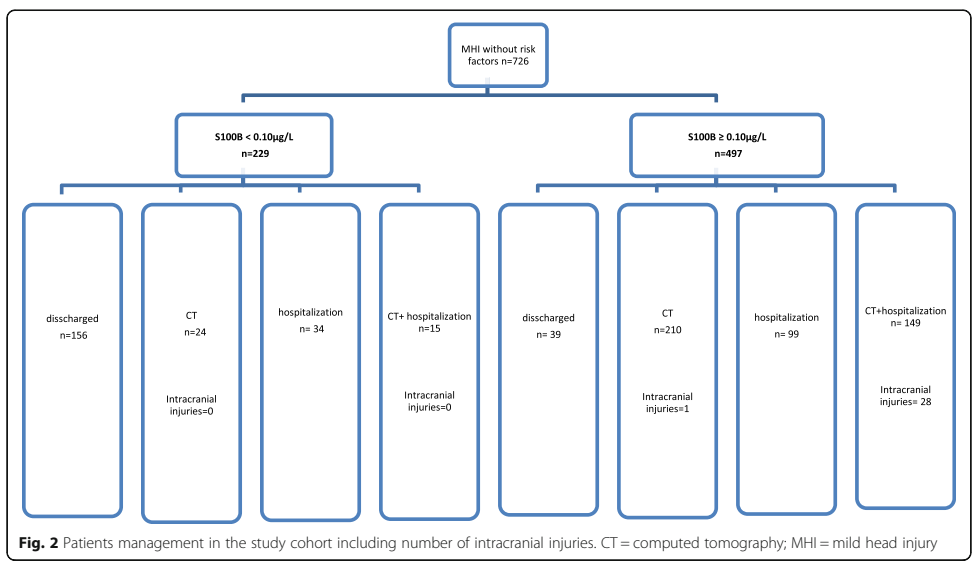

Fig. 2 Patients management in the study cohort including number of intracranial injuries. CT = computed tomography; MHI = mild head injury

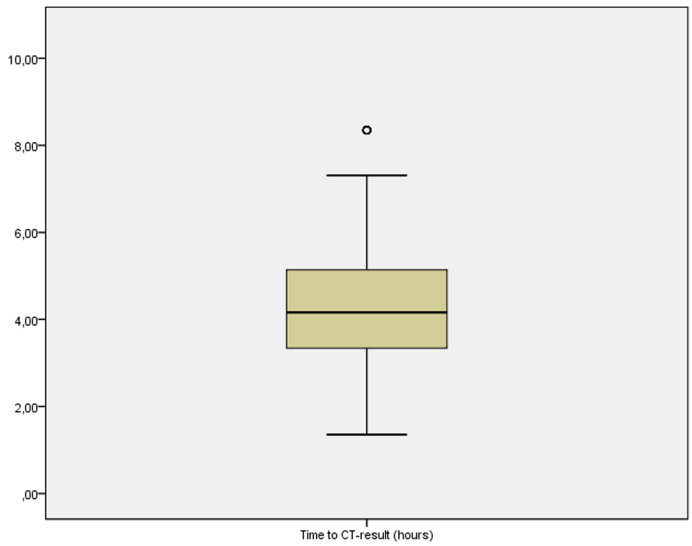

Fig. 3 Time to CT-result (hours)

only was used as the management option, the potential savings per patient was 71 € for this cohort. Given the actual use of S100B and CT/ hospitalization for our cohort, the calculated savings was limited to 39 € per patient.

Discussion

Considering the scarcity of health care resources, socioeconomic aspects of patient management should be fundamental [12, 17]. MHI is a common reason for ED contact and is associated with considerable use of health care resources [17]. These are partly due to the ineffective triage of patients to either discharge or further examinations/admission. However, these routines have been warranted due to the significant consequences of missing a significant brain injury after MHI for both patients and health care providers [18].

Although several rules have been suggested in MHI management, they are only based upon positive predictors, i.e. risk factors that should lead to a CT scan if present. The decision to incorporate S100B into the existing SNC guidelines in our hospital in 2007 was based upon the negative predictive ability of this biomarker, i.e. an aspect that could potentially reduce resource use. Since 2007, additional studies and a meta-analysis have confirmed findings showing the potential of S100B to safely reduce CT scans in this patient group [19–21].

Table 2 Actual cost for 726 patients = cost for S100B + cost for all the CT taken + cost for all the patients hospitalized

		S100 = 21 €	CT = 130 €	Hospitalization = 366 €	Tot
ACTUAL COST in follow-up (cost per patient)		726 × 21 € = 15 246 €	398 × 130 € = 51 740 €	297 × 366 € = 108 702 €	175 688 € (242 €)
POTENTIAL COST given different assumptions	S100B not in guidelines and assuming same use of CT and hospitalization as for cohort		0,7 x 726 x 130 € = 66 066 €	0,52 x 726 x 366 € = 138 172 €	204 238 € (281€)
	Strict compliance based on guidelines for S100 + CT only	726 × 21 € = 15 246 €	CT (S100B+) 497 × 130€ = 64 610 €		79 856 € (110 €)

Potential cost for 726 patients given different assumptions:
-if S100B is not included in guidelines= 156 patients with S100B negative were directly dismissed, we calculated an hypothetical cost if they underwent a CT or were hospitalized
-strict compliance based on guidelines= we considered that all the patients with a negative S100B were dismissed (138 patients) and took into account only the cost for S100 B positive patients

Our findings show a reduction in costs after S100B implementation in a typical ED setting. However, compliance to the new guidelines regarding S100 and use of CT and hospitalization was not perfect and both over- and under-triage was observed. Since the routines were relatively new (we allowed 6 months before initiating the study) it is understandable that physicians over-triaged patients with normal S100B levels. None of these showed any intracranial complications.

It is important that guidelines in this setting show a very high sensitivity (high negative predictive value) for significant intracranial injuries. The cost of missing a patient with such a complication is substantial [18]. Even though we have included over 700 patients in our study, a much larger cohort would be needed to include enough patients with significant complications to clearly examine this aspect. However, it may be unreasonable to expect 100 % sensitivity in a guideline and clinical advice and/or follow-up should be included in order to identify and treat patients missed from the initial triage [11].

Adapting our results into other cohorts may be difficult. Firstly, adapting S100B into guidelines other than the SNC proposal will naturally show different results. However, independent economic and clinical comparisons of the most prominent decision rules have shown the SNC guidelines to be similar, if not superior, in performance [8, 12, 22]. Despite this, validation and cost analysis of clinical S100B use in other guidelines using other cohorts are naturally warranted. Also, costs for the different aspects of the management routines will differ between sites. Caregivers should, however, be able to adapt their costs into our results to give an estimation of the economic impact our management routines in other health care systems. Finally, our results are based upon some assumptions regarding the use of CT and/or hospitalization. The guidelines recommend CT as the primary management option. However, our results show that many patients were hospitalized, sometimes in addition to CT scanning. Our assumptions therefore also included a model including the use of CT and hospitalisation that was observed in the present cohort.

Conclusion

Adding S100B to existing guidelines as a negative predictor for normal CT scans is potentially cost saving, although actual savings will ultimately be determined by compliance to guidelines and local costs for CT and hospitalisation.

The biomarker should be considered as a clinical tool, especially when CT rates of MHI patients are high.

Abbreviations

CT: Computed tomography; ED: Emergency department; GCS: Glasgow Coma Scale; LOC: Loss of consciousness; MHI: Mild head injury; SNC: Scandinavian Neurotrauma Committee

Acknowledgements

We thank Anders Holmén who provided statistical support.

Funding

Our founding source is FoUU Halmstad regional Hospital, the recipient of the award is OC.

Availability of data and material

The datasets during and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

OC designed the study, acquired the data that was later analyzed and interpreted, and wrote the article. AA critically reviewed the manuscript for the health economic data. JU was the study supervisor and critically reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Ethical approval was granted from our regional ethics board (Regionala Etikprövningsnämnden Lund, Sweden) (approval number 19/2007). Consent to participate was not applicable.

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Received: 11 December 2015 Accepted: 15 October 2016
Published online: 20 October 2016

References

1. Marshall LF. Head injury: recent past, present, and future. Neurosurgery. 2000;47(3):546–61.
2. Servadei F, Teasdale G, Merry G. Defining acute mild head injury in adults: a proposal based on prognostic factors, diagnosis, and management. J Neurotrauma. 2001;18(7):657–64.
3. Cassidy JD, Carroll LJ, Peloso PM, Borg J, von Holst H, Holm L, et al. Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med. 2004;(43 Suppl): 28-60.
4. Norlund A, Marke LA, af Geijerstam JL, Geijerstam JL, Oredsson S, Britton M. Immediate computed tomography or admission for observation after mild head injury: cost comparison in randomised controlled trial. BMJ. 2006;333(7566):469.
5. Morton MJ, Korley FK. Head computed tomography use in the emergency department for mild traumatic brain injury: integrating evidence into practice for the resident physician. Ann Emerg Med. 2012.
6. Stein SC, Spettell C. The Head Injury Severity Scale (HISS): a practical classification of closed-head injury. Brain injury : [BI]. 1995;9(5):437–44.
7. Ingebrigtsen T, Romner B, Kock-Jensen C. Scandinavian guidelines for initial management of minimal, mild, and moderate head injuries. The Scandinavian Neurotrauma Committee. J Trauma. 2000;48(4):760–6.
8. Smits M, Dippel DW, Steyerberg EW, de Haan GG, Dekker HM, Vos PE, et al. Predicting intracranial traumatic findings on computed tomography in patients with minor head injury: the CHIP prediction rule. Ann Intern Med. 2007;146(6):397–405.
9. Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, et al. The Canadian CT Head Rule for patients with minor head injury. Lancet. 2001;357(9266):1391–6.
10. Yates DW. The NICE head injury guidelines. Emerg Med J. 2003;20(2):117.

11. Mower WR, Hoffman JR, Herbert M, Wolfson AB, Pollack Jr CV, Zucker MI. Developing a decision instrument to guide computed tomographic imaging of blunt head injury patients. J Trauma. 2005;59(4):954–9.
12. Ruan S, Noyes K, Bazarian JJ. The economic impact of S-100B as a pre-head CT screening test on emergency department management of adult patients with mild traumatic brain injury. J Neurotrauma. 2009;26(10):1655–64.
13. Muller K, Townend W, Biasca N, Unden J, Waterloo K, Romner B, et al. S100B serum level predicts computed tomography findings after minor head injury. J Trauma. 2007;62(6):1452–6.
14. Biberthaler P, Mussack T, Wiedemann E, Kanz KG, Koelsch M, Gippner-Steppert C, et al. Evaluation of S-100b as a specific marker for neuronal damage due to minor head trauma. World J Surg. 2001;25(1):93–7.
15. Biberthaler P, Linsenmeier U, Pfeifer KJ, Kroetz M, Mussack T, Kanz KG, et al. Serum S-100B concentration provides additional information fot the indication of computed tomography in patients after minor head injury: a prospective multicenter study. Shock. 2006;25(5):446–53.
16. Biberthaler P, Mussack T, Wiedemann E, Gilg T, Soyka M, Koller G, et al. Elevated serum levels of S-100B reflect the extent of brain injury in alcohol intoxicated patients after mild head trauma. Shock. 2001;16(2):97–101.
17. Pandor A, Goodacre S, Harnan S, Holmes M, Pickering A, Fitzgerald P, et al. Diagnostic management strategies for adults and children with minor head injury: a systematic review and an economic evaluation. Health Technol Assess. 2011;15(27):1–202.
18. Smits M, Dippel DW, Nederkoorn PJ, Dekker HM, Vos PE, Kool DR, et al. Minor head injury: CT-based strategies for management–a cost-effectiveness analysis. Radiology. 2010;254(2):532–40.
19. Zongo D, Ribereau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, et al. S100-B protein as a screening tool for the early assessment of minor head injury. Ann Emerg Med. 2012;59(3):209–18.
20. Bouvier D, Oddoze C, Ben Haim D, Moustafa F, Legrand A, Alazia M, et al. Interest of S100B protein blood level determination for the management of patients with minor head trauma. Ann Biol Clin. 2009;67(4):425–31.
21. Unden J, Romner B. Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: an evidence-based review and meta-analysis. J Head Trauma Rehabil. 2010;25(4):228–40.
22. Stein SC, Fabbri A, Servadei F, Glick HA. A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann Emerg Med. 2009;53(2):180–8.

Paper V

Multicenter validation of the Scandinavian Guidelines for management of minimal, mild and moderate head injury in adults: a study protocol.

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Multicenter validation of the Scandinavian Guidelines for management of minimal, mild and moderate head injury in adults: a study protocol.

Introduction

Traumatic brain injuries (TBI) are one of the most common reasons for patients to attend the emergency department (ED) with an estimated incidence in Europe of 260 per 100 000 for admitted TBI[1],[2],[3]. Approximately 90% of patients with TBI are defined as mild TBI (mTBI)[4]. These patients have a normal or minimally altered level of consciousness and Glasgow Coma Scale (GCS) 13 to 15 when attending the ED.
A small minority of patients with mTBI would show pathological results, such as intracranial hemorrhages or cerebral contusions on a computed tomography (CT), and even fewer need neurosurgical intervention[5]. Nevertheless, complications would be so severe, if neurosurgical intervention is delayed, that it has become common practice to subject all patients with mTBI to CT[6]. The high number of CT scans has an impact on health care resources but may also involve risk by subjecting patients through potentially harmful ionizing radiations[7].

In the past years, several independent research groups have attempted to optimize CT use in mTBI patients by forming guidelines that aim to identify patients at high risk for intracranial complications[8]. Most guidelines have been published in the past 15 years and have been validated both prospectively internally and externally; all guidelines have been shown to be safe when implemented in clinical use with few missed complications. However the number of CT scans has not been reduced dramatically, in some cases it has even increased[9].

In 2013, the new Scandinavian guidelines (SNC13) were published. They are the first guidelines that use a biomarker, S100B, as a tool for managing patients with mTBI. Although S100B has a low specificity for intracranial complications, a high sensitivity makes it suitable to be implemented into clinical practice as a tool for CT reduction.
Previous SNC guidelines have been compared to other prominent guidelines with impressive results[10]. The SNC13 have been externally validated in a retrospective multicenter center study from the USA that was underpowered for important outcomes [11]. Nevertheless, SNC13 have already been partially implemented in clinical practice in Scandinavia. However, a strict

multicenter validation has not been performed yet nor a systematic comparison to other available guidelines.

Aims

Our primary aim is to validate the performance of the SNC13 in predicting intracranial complications in adult patients presenting with traumatic head injury in Swedish hospitals. A secondary aim is to compare the performance of SNC 13 with 6 other clinical guidelines, with respect to important outcomes. Moreover, we want to explore the performances of different biomarkers in predicting intracranial complications in predefined subgroups of TBI. Finally, we want to evaluate the possibility of further improvement of the SNC13 guidelines.

Methods

Design

We will perform a prospective, multicenter, pragmatic, observational study of adults presenting with traumatic head injury at the ED.

All data necessary for analysis including predictor variables and outcome data for all the seven guidelines included in the study will be registered (table 1). Patients will be managed clinically accordingly to the judgment of the responsible physician and/or local guidelines.

Study setting and population

The study will be set in Halmstad, Malmö, Lund, Örebro and Linköping, Sweden. Hallands Hospital Halmstad (HS) is a level II trauma centre, Skåne University Hospital in Malmö and Lund (SUS)**,** Örebro University Hospital, Linköping University Hospital are level I trauma centers.

The coordinating site for the study will be HS where the statistical and the comparative biomarker analysis will be performed.

Inclusion criteria

From September 2017 we will prospectively enroll all adult patients with a GCS 9-15 that seek the ED within 24h after TBI.

Exclusion criteria

We will exclude:

- patients younger than 18 years of age;
- patients without a Swedish personal identification number due to difficulties in performing the follow up phase;

- all patients that refuse to participate.

Primary endpoint

The primary endpoint for this study will be the sensitivity, specificity, predictive values and likelihood ratios for the SNC13 for identifying traumatic intracranial complications.

Definition

Traumatic intracranial complications are defined as a composite variable of death as consequence of the TBI, need for neurosurgical intervention or marked abnormality on CT. CT abnormalities are defined as any new, acute, traumatic intracranial pathology including intracranial hematomas of any size, cerebral contusions and depressed skull fractures.

Secondary endpoints

- Measure the sensitivity, specificity, predictive values and likelihood ratios for the SNC13 for identifying patients needing neurosurgery or neurointensive care for the TBI within the first week following trauma.
- Measure the sensitivity, specificity, predictive values and likelihood ratios for the SNC13 for identifying patients with new, acute, traumatic intracranial pathology on CT including intracranial hematomas of any size, cerebral contusions and depressed skull fractures.
- Measure the sensitivity, specificity, predictive values and likelihood ratios for the SNC13 for identifying patients with clinically relevant CT findings (according to the CCHR) [12], defined as contusions larger than 5mm in diameter, subarachnoid bleeding thicker than 1mm, subdural hematoma thicker than 4mm, pneumocephaly that will need intervention, depressed skull fracture through the inner table.
- We will calculate sensitivity, specificity, predictive values and likelihood ratios of each guideline in identifying traumatic intracranial CT finding when applied to the same TBI population. We will also measure frequency of CT scans. We will investigate the performance of the SNC13 in comparison to other guidelines in reducing CT frequency without missing complications. We hypothesize that the implementation of a biomarker as S100B into clinical guidelines will achieve a further reduction in CT scans. Accuracy variables will be statistically compared with Chi-squared test.
- Measure performances of novel biomarkers such as GFAP, SBP-50 and TAU. S100B is the most studied brain biomarker and is the only one that is clinically used as a screening tool. However, S100B is not the perfect brain biomarker for TBI and new

biomarkers appear promising. In a explorative analysis we will compare S100B with GFAP, SBP-50 and TAU on the same selected mTBI population in order to determine the potential value of a panel of biomarkers for identifying high and low risk patients. We will calculate sensitivity, specificity, predictive values and likelihood ratios of each biomarker in identifying traumatic intracranial CT finding when applied to the same TBI population. ROC curves will be calculated in order to compare cut off values. We will also use the net reclassification index to see if each biomarker improves the accuracy of the classification.

- The final aspect that we would like to study is the derivation of a new improved guideline: binary logistic regressions analysis of all the variables taken into account and registered during the study will be performed. We will a priori divide the population into a derivation cohort, obtain ROC curves, and use these cutoffs after the bootstrapping process and other clinical and biochemical variables to construct a mode losing multivariable analysis.

Guidelines

A secondary goal of the study is to compare how the same mTBI population would be managed according to 7 guidelines that are clinically used in this particular patient group; we included the Canadian CT Head Rule[13] (CCHR), the New Orleans criteria[14] (NOC), the National Institute of Clinical Excellence[15] (NICE), the CT in head injury patients Prediction Rule[16] (CHIP) , the Neurotraumatology Committee of the World Federation of Neurosurgical Society[17] (NCWFNS), the National Emergency X-Radiography Utilization Study II[18] (NEXUS-II) and the SNC 2013[19].

Each guideline has specific inclusion and exclusion criteria, and outcome measures, see table 2.

For each guideline patients will be divided into different groups: those who should be dismissed without a CT, those who should do a CT and those who do not fit the inclusion criteria for the guideline. A comparison of the 7 guidelines is shown in table 3.

The CCHR includes only patients with GCS 13-15 that have suffered LOC, have amnesia for trauma or are disoriented. Patients are divided into a high risk group, where CT is mandatory because of elevated risk for neurosurgical intervention, and medium risk for CT complications, in which case CT is only recommended. Both groups are analyzed independently but reported together for the group where CT scan should be performed. The CCHR primary outcome measure is the need for neurological intervention and the secondary

outcome is clinically relevant brain injury on CT. Clinically relevant CT findings are defined as contusions larger than 5mm in diameter, subarachnoid bleeding thicker than 1mm, subdural hematoma thicker than 4mm, pneumocephaly that will need intervention, depressed skull fracture through the inner table.

The NOC includes only patients with GCS of 15, thus according to these guidelines patients with GCS 14 or less were considered to have an indication for CT[20]. The NOC outcome measure is any acute traumatic intracranial lesion on CT.

The NICE guidelines stratify patients that are eligible for CT into two groups, those who should undergo a CT within 1 hour and those within 8 hours. Both groups are analyzed independently but reported together for the group where CT scan should be performed.

The CHIP prediction rule does not have strict inclusion criteria, and recommends CT in the presence of one major or at least 2 minor risk factors. Both groups are analyzed independently but reported together for the group where CT scan should be performed. The CHIP primary outcome measure is any intracranial traumatic finding on CT, secondary outcome is all neurosurgical intervention after the initial CT.

The NCWFNS guidelines identify three levels of risk for intracranial complications. Low risk patients can be dismissed without any further investigation while patients with medium and high risk should have a CT scan and therefore are analyzed together.

NEXUS II does not stratify patients or take into account injury mechanism, it focuses mostly on symptoms at presentation at ED. The NEXUS II outcome measure is any intracranial injury on CT.

The SNC13 guidelines include all patients with head injury within 24h and a GCS 9-13. Patients with mild head injury are divided into high risk, medium risk or low risk for intracranial complications. Low risk patients (GCS 14 or GCS 15 and LOC or repeated vomiting) with normal S100B can be dismissed directly. The SNC13 primary outcome is the need for any neurosurgical intervention. The secondary outcome measures are identification of non-neurosurgical intracranial traumatic complications.

Data registration and follow-up

Details of how patients are managed, including patient characteristics, injury type, patient history, clinical examination results, current medications and CT findings will be documented in a pre-determined case-report form by the triage nurse and/or physician on call.

All patients will be asked to answer a questionnaire sent by mail 3 months after the injury. The questionnaire will be re-sent if no answer is received. If no answer is received from these

attempts, patients will be contacted via telephone. The questionnaire includes questions that would identify a significant intracranial lesion, data concerning sick-days, new contacts with medical professionals and information concerning quality of life. In cases where patients can not be reached by mail or telephone, medical records and national mortality databases will be consulted for evidence of complications and/or death. The Swedish health care system allows full visibility of data for persons with a Swedish personal identification number for medical records and mortality database over the whole country. Patients who suffer significant (enough to seek medical care) intracranial complications after discharge would therefore be identified.

Details on study period are specified on figure 1 with an algorithm for patient eligibility and data analysis.

Data will be registered in an Excel® file. Descriptive statistics will be analysed using IBM SPSS® Statistics Version 20 software.

S100B analysis

A 5ml blood sample is drawn from patient's cubital vein in the ED. Samples are analysed with the fully automated Elecsys® S100 (Roche AB) at the Clinical Chemistry Department of HS, SUS, Örebro University Hospital and Linköping University Hospital, Sweden. Cut-off level for normal levels of S100B according to the SNC guidelines is less than 0.10µg/L and a window of sampling of within 6 hours from the time of the injury.

From all patients seeking care within 24h form injury with medium and low risk TBI, according to SNC13 (including multitrauma patients), a 5ml blood sample will be drawn, centrifuged and frozen at -70 degrees Celsius. Samplings will be coded and registered for analysis of GFAP, SBP-50 and TAU.

CT examinations

CT scans are always analysed by a board certified radiologist.

Sample size

We assume that the Scandinavian guidelines will recommend discharge (i.e. neither CT nor admission) in approximately 50% of patients and a prevalence of our primary outcome of 5% (from our own observations and from data derived from a pre-selected cohort)[21]. Allowing for one missed case, a sensitivity of >99% with a lower 95% confidence interval, a sample size of 2490 patients is required to detect traumatic intracranial complications according to the SNC13. Allowing for a 10% lost to follow-up, our desired sample size is 2767 patients.

Interim analysis

After 1000 patients we will measure prevalence for the primary outcome in order to be able to reevaluate sample size.

Ethics

Ethical approval was granted from the Regional Ethical Review Board of Lund (approval number 2012/574).

Informed verbal consent will be obtained and registered by nurses responsible for triage at ED.

Patients' data and social security number will be stored and handled accordingly to Swedish Personal Data Act, (PUL 1998: 204).

Written consent will be obtained from all patients from whom the extra blood sampling for biomarker analysis will be requested. Sampling will be coded and patients will be able at any time to refuse to be part of the study.

Discussion

The main purpose of this study is to perform a prospective validation of the new SNC13 guidelines. The external validation already performed has the limitation of being applied to a preselected population; nevertheless it showed that the SNC13 have a potential impact for reducing frequency of CT scans[22]. In order to complement the previous study we designed this multicenter prospective study that will collect enough data to support the safety and efficacy of the SNC13. The study is pragmatic in nature, including all adult patients with GCS 9-13 within 24h of head injury, with very few exclusion criteria.

The second important aim was to compare the SNC13 to the other 6 guidelines. Different guidelines applied to the same population will perform with very different results, as previous studies have already shown[23],[24]. The first aspect to be discussed in this comparative work is how many patients of this TBI population could be managed according to different guidelines. Both NOC, NICE and CCRH guidelines have strict inclusion criteria that may exclude a substantial portion of the TBI patients leaving physicians with no other choice than CT. Nevertheless, more flexible guidelines with no exclusion criteria like CHIP prediction rule or the NCWFNS guidelines have been proven to only marginally reduce the number of CTs. Nevertheless, beside restricted inclusion criteria, the NICE guidelines have shown to be one of the better performing guidelines for reducing CT frequancy[23].

In the SNC13 guidelines, all patients could be managed with no exclusion criteria pre-defined, except for the time frame of 24h. According to previous studies, we would have expected a S100B negative rate for about 30% of sampled patients but, considering the new grading of patients, we expect better performances.

S100B is not the perfect biomarker for mTBI considering its low specificity. The perfect biomarker should be able to be brain specific, easily detectable, have a high sensitivity for intracranial complications and adverse outcome, with 100% negative predictive value and high clinical specificity. In recent years researchers agree on the possibility of defining a brain biomarker-panel; it therefore is fundamental to compare a well-studied biomarker as S100B with other new biomarkers. . The present study includes the most promising of these.

Strength and limitations

The main strength of this study is its design being an adequately powered multicenter study. Another important aspect is that it tests the SCN13 in the health care system for which it was intended for.

However every study has its limitation: a proper validation should be performed with a randomized study design; however this method would be ethically questionable.

The SNC13 were designed primarily for the Scandinavian health care system and its validity outside Scandinavia cannot be assumed.

Biomarker analysis will only be performed in a pre-defined subgroup of patients. This could lead to selection bias; however, the remaining patients do not have any theoretical advantage in management with biomarker results.

Table 1. Variables studied

Variable	Data Format
Demografic/anamnestic variables	
Age	Years (continuous)
Gender	Male/female
Cause of injury	Patient report
Pedestrian/cyclist versus vehicle	Yes/no
Ejected from vehicle	Yes/no
Fall	Yes/no
Fall >1m	Yes/no
Signs of skull fracture	Yes/no
Contusion of the skull	Yes/no
Fracture above clavicles	Yes/no
Alcohol/drug intoxication	Yes/no
Ethanol levels	mmol/l (continuous)
LOC*	Yes/no
Duration LOC	Minutes (continuous)
Amnesia	Yes/no
Duration Amnesia	Minutes (continuous)
Persistent anterograd amnesia	Yes/no
Headache	Yes/no
Worsening headache	Yes/no
Vomiting episodes	Number (continuous)
Neurological deficit	Yes/no
Pretraumatic seizure	Yes/no
Posttraumatic seizure	Yes/no
GCS	Number (continuous)
GCS deterioration**	Number (continuous)
Antiplatlet medication	Yes/no
Anticoagulation therapy	Yes/no
Drug registration	Drug name
Bleeding disorder	Yes/no
Shunt-treatment	Yes/no
Dismissed***	Yes/no
S100B	µg/L (continuous)
CT	Yes/no
Admitted	Yes/no
Other causes to admission than TBI	Yes/no
Length of admission	Days (continuous)
Complications during admission	Yes/no
*** LOC= loss of conciousness** **** deterioration of GCS 2 after injury** ***** dismissed with no intervention (CT or admission)**	

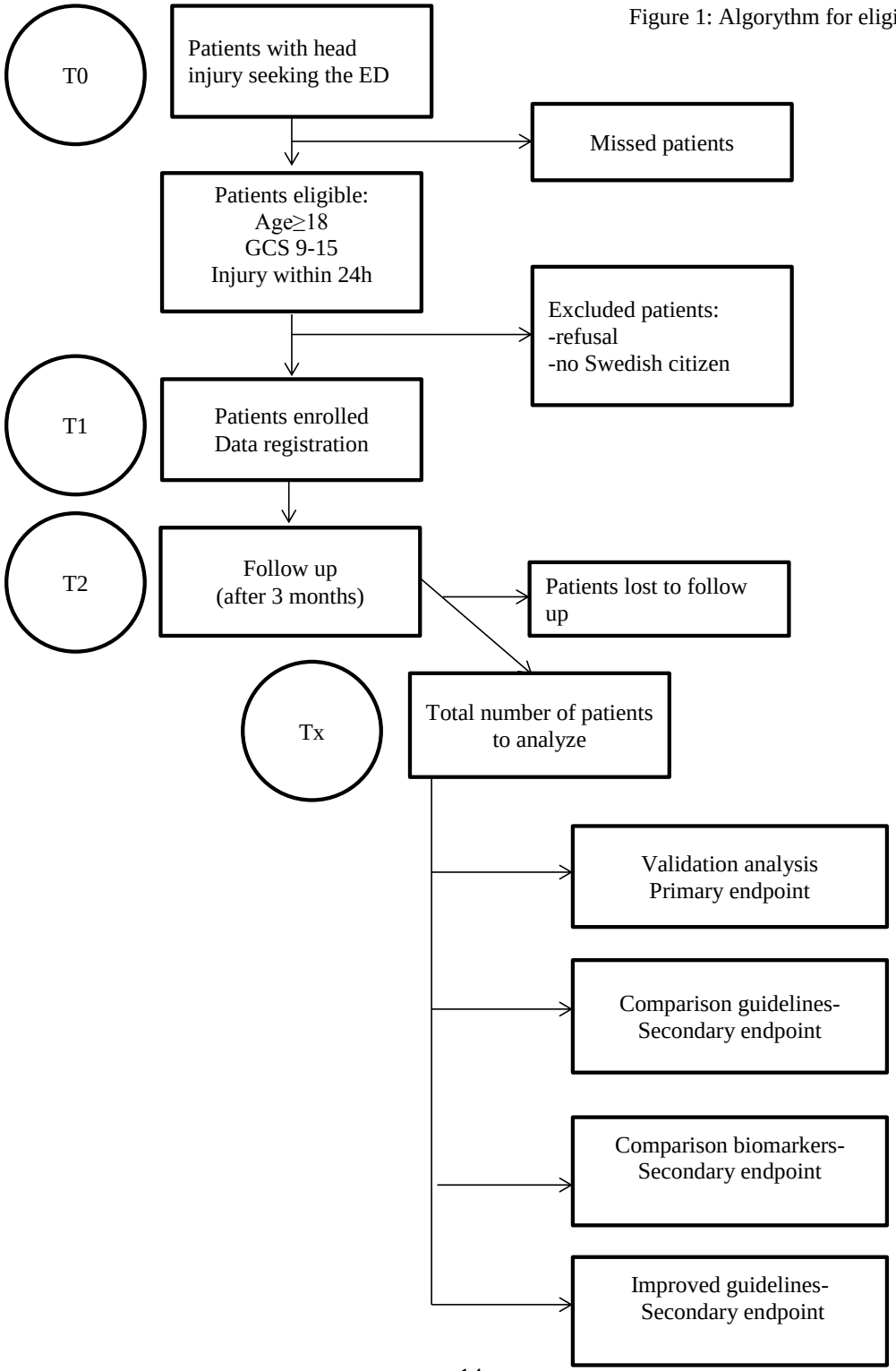
Table 2. Inclusion criteria, exclusion criteria, primary outcomes, secondary outcomes

	CCHR	NOC	NICE	CHIP	NCWFNS	NEXUS II	SNC13
Inclusion criteria	Age ≥ 16 Presenting within 24h of head injury	Age ≥ 18 Presenting within 24h of head injury GCS 15	Age ≥ 18	Age ≥ 16 Presenting within 24h of head injury GCS 13-14 or GCS 15 and LOC or amnesia or pst-traumatic seizure or vomiting or severe headache or alcohol/drug intoxication or coagulopathy/ oral anticoagulants or injury above the clavicle or neurological deficit	Age ≥ 18 Presenting within 12h of head injury GCS 14-15	Age ≥ 18 GCS 15	Age ≥ 18 Presenting within 24h of head injury GCS 9-15
Exclusion criteria	-No clear history of trauma as primary event -Penetrating skull injury or depressed fracture -bleeding disorder or oral anticoagulants - neurological deficit	GCS ≤14					
Primary outcomes	Any need for neurological intervention	Acute traumatic intracranial lesion on CT		Any intracranial traumatic finding on CT		Any intracranial injury on CT	Any neurosurgical intervention
Secondary outcomes	Clinically relevant brain injury on CT			All neurosurgical intervention after initial CT			Any non-neurosurgical intracranial traumatic complications

Table 3. Comparison of the 7 guidelines used in clinical practice for initial screening of TBI.

Clinical findings	CCHR	NOC	NICE	CHIP	NCWFNS	NEXUS II	SNC 2013
Age	≥65	>60	≥65	≥60 major 40-60 minor	>60	≥65	≥65 and anti-platelet treatment
Pedestrian/cyclist versus vehicle	Minor		Minor	Major			
Ejected from vehicle	Minor		Minor	Major			
Fall >1m	Minor		Minor	Minor			
Signs of skull fracture	Any	Any	Any	Any	Any	Any	Any
Contusion of the skull						Any	
Fracture above clavicles		Any					
Alcohol/drug intoxication	Any				Any		
LOC*	Inclusion	Inclusion	Inclusion	Minor	Any		Low risk
Amnesia	Retrograde > 30 min	Antegrade	Retrograde > 30 min	≥4h major 2-<4h minor	Any	-	-
Headache		Severe			Any		
Vomiting episodes	>2	Any	>2	Major	Any	>2	>2 and GCS 14 low-risk
Neurological deficit	Excluded	Excluded	Any	Minor	Any	Any	Any
Pretraumatic seizure					Yes		
Posttraumatic seizure	Excluded	Any	Any	major	Any		Any
GCS <15	After 2h	Exclusion	After 2h	≥2 points deterioration Major 1point=minor	Always	Always	14 and no other risk-factor= low risk
Antiplatlet medication							And ≥65y
Anticoagulation therapy	Exclusion	-	Any	Major	Any	Any	Any
Bleeding disorder	Exclusion			Major			Any
Shunt-treatment					Any previous neurosurgical intervention		Yes

Figure 1: Algorythm for eligibility



REFERENCES

[1] Cassidy JD, Carroll LJ, Peloso PM, Borg J, von Holst H, Holm L, Kraus J, Coronado VG; WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med. 2004 Feb;(43 Suppl):28-60.

[2] Peeters W, van den Brande R, Polinder S, Brazinova A, Steyerberg EW, Lingsma HF, Maas AI. Epidemiology of traumatic brain injury in Europe. Acta Neurochir (Wien). 2015 Oct;157(10):1683-96

[3] McKinlay A, Grace RC, Horwood LJ, Fergusson DM, Ridder EM, MacFarlane MR. Prevalence of traumatic brain injury among children, adolescents and young adults: prospective evidence from a birth cohort. Brain Inj. 2008 Feb;22(2):175-81

[4] Servadei F, Teasdale G, Merry G; Neurotraumatology Committee of the World Federation of Neurosurgical Societies. Defining acute mild head injury in adults: a proposal based on prognostic factors, diagnosis, and management. J Neurotrauma. 2001 Jul;18(7):657-64.

[5] Bazarian J, McClung J, Cheng Y, Flesher W, and Schneider S. Emergency department management of mild traumatic brain injury in the USA. Emerg Med J. 2005 Jul; 22(7): 473–477.

[6] Geijerstam JL, Britton M. Mild head injury - mortality and complication rate: meta-analysis of findings in a systematic literature review. Acta Neurochir (Wien). 2003 Oct;145(10):843-50; discussion 850.

[7] Smith-Bindman R, Lipson J, Marcus R, Kim KP, Mahesh M, Gould R, Berrington de González A, Miglioretti DL. Radiation dose associated with common computed tomography examinations and the associated lifetime attributable risk of cancer. Arch Intern Med. 2009 Dec 14;169(22):2078-86.

[8] Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PM. Indications for computed tomography in patients with minor head injury. N Engl J Med. 2000 Jul 13;343(2):100-5.

[9] Boyle A, Santarius L, Maimaris C. Evaluation of the impact of the Canadian CT head rule on British practice. Emerg Med J. 2004 Jul;21(4):426-8.

[10] Stein SC, Fabbri A, Servadei F, Glick HA. A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann Emerg Med. 2009 Feb;53(2):180-8.

[11] Undén L, Calcagnile O, Undén J, Reinstrup P, Bazarian J. Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults. BMC Med. 2015 Dec 9;13:292.

[12] Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, McKnight RD, Verbeek R, Brison R, Cass D, Eisenhauer ME, Greenberg G, Worthington J. The Canadian CT Head Rule for patients with minor head injury. Lancet. 2001 May 5;357(9266):1391-6.

[13] Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, McKnight RD, Verbeek R, Brison R, Cass D, Eisenhauer ME, Greenberg G, Worthington J. The Canadian CT Head Rule for patients with minor head injury. Lancet. 2001 May 5;357(9266):1391-6.

[14] Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PM. Indications for computed tomography in patients with minor head injury. N Engl J Med. 2000 Jul 13;343(2):100-5.

[15] National Clinical Guideline Centre (UK). Head Injury: Triage, Assessment, Investigation and Early Management of Head Injury in Children, Young People and Adults. London: National Institute for Health and Care Excellence (UK); 2014 Jan.

[16] Smits M, Dippel DW, Steyerberg EW, de Haan GG, Dekker HM, Vos PE, Kool DR, Nederkoorn PJ, Hofman PA, Twijnstra A, Tanghe HL, Hunink MG. Predicting intracranial traumatic findings on computed tomography in patients with minor head injury: the CHIP prediction rule. Ann Intern Med. 2007 Mar 20;146(6):397-405.

[17] Servadei F, Teasdale G, Merry G; Neurotraumatology Committee of the World Federation of Neurosurgical Societies. Defining acute mild head injury in adults: a proposal based on prognostic factors, diagnosis, and management. J Neurotrauma. 2001 Jul;18(7):657-64.

[18] Mower WR, Hoffman JR, Herbert M, Wolfson AB, Pollack CV Jr, Zucker MI; NEXUS II Investigators. National Emergency X-Radiography Utilization Study. Developing a clinical decision instrument to rule out intracranial injuries in patients with minor head trauma: methodology of the NEXUS II investigation. Ann Emerg Med. 2002 Nov;40(5):505-14.

[19] Undén J, Ingebrigtsen T, Romner B; Scandinavian Neurotrauma Committee (SNC). Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. BMC Med. 2013 Feb 25;11:50.

[20] Stein SC, Fabbri A, Servadei F, Glick HA. A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann Emerg Med. 2009 Feb;53(2):180-8.

[21] Undén L, Calcagnile O, Undén J, Reinstrup P, Bazarian J.

Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults. BMC Med. 2015 Dec 9;13:292.

[22] Undén L, Calcagnile O, Undén J, Reinstrup P, Bazarian J. Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults. BMC Med. 2015 Dec 9;13:292.

[23] Smits M, Dippel DW, Nederkoorn PJ, Dekker HM, Vos PE, Kool DR, van Rijssel DA, Hofman PA, Twijnstra A, Tanghe HL, Hunink MG. Minor head injury: CT-based strategies for management--a cost-effectiveness analysis. Radiology. 2010 Feb;254(2):532-40.

[24] Stein SC, Fabbri A, Servadei F, Glick HA. A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann Emerg Med. 2009 Feb;53(2):180-8.