ORIGINAL ARTICLE

Usefulness of determining serum S-100β protein expression in screening patients with minor head injury in a hospital emergency department

Fernando RICHARD ESPIGA[1], Juan Tomás VICENTE CARRERO[1], María Victoria PONCELA GARCÍA[2], Evelia MALLA PÉREZ[2], María Jesús RUBIO SANZ[3], Esther RIÑONES MENA[3]

[1]Servicio de Urgencias. [2]Servicio de Análisis Clínicos. [3]Servicio de Radiodiagnóstico. Complejo Asistencial Universitario de Burgos. Burgos, Spain.

Correspondence:
Fernando Richard Espiga
Servicio de Urgencias
Complejo Asistencial
Universitario de Burgos
Avenida del Cid, 96
09005 Burgos, Spain
E-mail: frichardespiga@gmail.com

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Objectives: To validate testing for S-100β protein expression in minor head injury in comparison with computed tomography (CT) brain scans as the gold standard. To evaluate the role this screening approach might play in a hospital emergency department and set a cutoff point that could identify patients without CT evidence of brain injury.
Methods: Data for 100 patients over the age of 14 years who came to the emergency department with minor head injury were included. Some patients had additional symptoms. All agreed to participate in the study. Serum S-100β levels were determined and a CT scan performed in all enrolled patients. The findings, as well as symptoms and the findings of physical examination, were recorded. Statistical analysis included the study of sensitivity, specificity, predictive values, likelihood ratios, contingency tables, and the area under the receiver operating characteristic curve.
Results: CT scans were normal in patients with a serum S-100β level below 0.1375 µg/L. Concentrations below that cutoff were found in 44% of the sample. CT scans were positive in 7% and these patients (mean age, 42 years) all had S-100β levels above the cutoff. The sensitivity of the test was 100%; specificity was 47%. The negative predictive value was 100%, but the positive predictive value was 12%. The overall predictive value was 51%. The positive and negative likelihood ratios were 1.9 and 0, respectively. The area under the receiver operating characteristic curve of S-100β levels was 0.653.
Conclusions: Early determination of S-100β blood levels could reduce the number of CT scans required for patients with minor head injury by nearly half. The number of patients admitted to the observation unit could also be reduced. [Emergencias 2011;23:15-21]

Key words: Head injury. S-100β protein Computed tomography. Glasgow Coma Scale.

Introduction

Traumatic brain injury (TBI) is a serious public health problem in developed countries; it is a major cause of death in young people under 45 years of age, carries high morbidity and mortality and a large number disabilities, and involves substantial health, social and economic costs[1-3].

TBI is classified according to level of consciousness, measured by the Glasgow Coma Scale (GCS)[4] which evaluates three patient responses to voice call or pain: eye opening (4 points), best verbal response (5 points) and best motor response (6 points). GCS score is the sum of points for each parameter, with a maximum of 15 points and a minimum of 3 points. Based on this GCS score, TBI is classified as: severe (≤ 8 points), moderate (9-13 points) and mild (14 or 15 points).

Mild TBI, accounting for 70-80% of all TBI, is one of the most frequent reasons for visiting the hospital emergency department (ED). The con-

dition generates great uncertainty, despite being generally benign (only 5-7% are associated intracranial injury), and causes considerable resource consumption cost [virtually routine computerized tomography (CT), admission to the observation unit, neurosurgery appointments, etc.]. The aim of the emergency physician is to direct all their efforts towards early diagnosis of brain injury for early management, and reduce morbidity and mortality.

Protein S-100β[5-7] is a small dimeric molecule (10.5 kDa) which belongs to the multi-gene family of calcium binders. It is produced in the cytoplasm of astroglial, Swann and melanoma cells, and is released into the bloodstream after blood-brain barrier breakdown caused by TBI. It can be measured in blood (venous or arterial) and cerebrospinal fluid (CSF) by ELISA. Its values are not altered by hemolysis and remain stable in serum for hours (which allows non-immediate analysis), but has the limitation of a short half-life[7,8]. S-100β can also be determined in urine, where it remains detectable for a longer period of time. It also offers the advantage of being rapidly determined (18 minutes) at little cost, and its values are unaltered by blood alcohol levels, a common situation in this pathology which also hinders the clinical assessment of these patients.

We set out to perform a prospective, observational study of a significant number of consecutive TBI patients, using computerized tomography (CT) scan as the gold standard. The objective was to validate the role of protein S-100β in the screening of patients with mild head injury in the ED, and to determine whether a particular cut-off value of protein S-100β can be used to discriminate patients who will not present intracranial CT lesions. If confirmed, S-100β levels could be included in the protocol for management of mild TBI patients attending the ED, which would avoid a large number of costly CT scans and admissions to the observation unit.

Method

The study was conducted in accordance with the protocol and standards of good clinical practice, as described in the Harmonized Tripartite Norms of ICH E6 for Good Clinical Practice 1996, Directive 2001/20 EC, R.D. 223/2004, and the Helsinki declaration and amendments relating to medical research involving human beings. The study was approved by the Clinical Research Ethics Committee of the hospital.

We carried out the study prospectively during the months of June to November 2009, with 100 patients attending our ED after suffering mild head injury.

The inclusion criteria are listed in Table 1. All patients included were informed about the nature of the study and voluntarily signed the informed consent form.

Blood samples were obtained from all patients to determine protein S-100β levels, and CT scan was performed within 6 hours after head injury (but not before the first hour). The determination of S-100β was carried out by immunoassay with Elecsys® 1010 using electrochemiluminescence.

The CT study was performed by the radiologist on duty, and subsequently validated by the neuro-radiologist.

In the first patients we also collected urine samples for S-100β determination , but on observing the lack of correlation between these values and CT scan results this collection was abandoned.

A database was used to prospectively record clinical and physical examination data of each patient, the corresponding cranial CT findings and laboratory data. We recorded age and sex of the patient, time of trauma, time of ED attention and blood sampling, type of injury, TBI-associated injuries, clinical signs and symptoms, GCS score, medical history, previous medication, serum S-100 protein values, cranial CT findings and degree of injury according to the traumatic coma data base (TCDB)[9] (Table 2).

From the results obtained, we performed statistical analysis of the different variables to calculate the sensitivity (Se), specificity (Sp), predictive value, likelihood ratio, contingency table and receiver operating characteristic (ROC) curve. For the analysis of qualitative variables we used frequency distribution, and for quantitative variables we calculated the mean and standard deviation.

Table 1. Study inclusion criteria

Mild TBI (GCS: 14-15).
Age >14 years.
Less than 3 hours after the trauma.
Any accompanying symptom:
– Transient loss of consciousness.
– Amnesia (peri-traumatic).
– Headache.
– Dizziness / vertigo.
– Vomiting.
– Seizure.
Informed consent.

TBI: traumatic brain injury; GCS: Glasgow Coma Scale.

Table 2. Computed tomography (CT) findings, according to the traumatic coma database (TCDB)

TCDB grade	Category	Definition	Total (100)	With sub-arachnoid bleeding
Intra-cranial injury (ICS)				
I	Type I diffuse injury	No intracranial pathology visible on CT	93	
II	Type II diffuse injury	Cisterns with midline shift 0-5 mm		
		No hyperdense or mixed injuries > 25 ml	5	2
III	Type III diffuse injury	Cisterns compressed or absent with 0-5 mm midline shift		
		No hyperdense or mixed injuries > 25 ml	0	
IV	Type IV diffuse injury	Midline shift > 5 mm		
		No hyperdense or mixed injuries > 25 ml	0	
V	Removed mass injury	Any surgically removed mass injury	1	
VI	Mass injury not removed	No surgically removed mass injury	1	1
Cranial injury				
		Fractures		3
		Suture diastasis		1
		Depression ≥ 1/2 diploid diameter		0

We used Pearson's chi-square test to analyze the relationship between qualitative variables, and Student's t test for the comparison of means. The S-100β cut-off point was established by fitting the data to a ROC curve.

Differences with a p value of 0.05 were considered statistically significant. The program used for statistical analysis was SPSS version 14.02.

Results

The mean age of patients was 49 years, with 63% men and 37% women. Trauma was closed in 76% and open in 24% of cases. Regarding type of trauma, the main cause was accidental falls (54%), followed by traffic accidents (18%), occupational accidents (13%) and sports accidents (11%); 32% of cases were associated with other injuries, mainly of the limbs (18%) and chest trauma and multiple contusion (15%) (Table 3). Regarding symptoms accompanying clinical TBI, the most common was headache (73%), followed by transient loss of consciousness (55%) and amnesia (40%). GCS score was 15 in 95% of cases. Regarding medical history, dementia and heart failure was found in 7% of patients; and regarding medication being taken at the time of the accident, 13% were treated with acetylsalicylic acid and 7% with oral anticoagulation (Table 3).

The mean level of S-100β in blood was 0.58 (SD: 1.05). The cut-off value below which no alterations were observed in cranial CT was 0.1375 µg/L mg/L; 44% of cases showed values below this point.

Pathologic CT scan secondary to trauma was observed in 7% of patients, with a mean age of 42 years. The levels of serum S-100β in these patients were always higher than the cutoff, with a mean value of 0.403 µg/L (0.137 to 0.857). Intracranial CT findings are presented in Table 2. In 4 of these patients we also detected cranial injury (3 craniofacial fractures and 1 suture diastasis fracture). Mean time between trauma and blood collection was 81.6 min, and levels of S-100β above the cut-off point were more frequently observed with earlier blood collection (Table 4).

Sensitivity (Se) of the test was 100% and specificity 47.3%. Negative predictive value (NPV) was 100% but positive predictive value (PPV) was 12.5%, with an overall predictive value (OPG) of 51% and a positive likelihood ratio (LR) of 1.9 and a negative LR of 0 (Table 5). ROC curve values of protein S-100β presented an area under the curve of 0.653 (Figure 1).

Although there seemed to be a statistical relationship between the presence of TBI-associated trauma and high levels of S-100β, we did not find any significant variable that predicted CT alteration in these patients, nor with any other variables included in the study (type of injury, associated trauma, age, symptoms, history, medication, etc.).

Of the 100 patients, 9 were hospitalized, with good outcome and subsequent discharge. There were no deaths, and none required neurosurgical intervention or presented neurological deterioration. One patient who was discharged on the day of ED attention required admission 72 hours later for trauma-associated injury (splenic contusion), but did not require intervention and was then discharged. Subsequent monitoring of patients (6-month follow up) showed no complications secondary to TBI.

Table 3. Type and clinical features of head injury

	Plasma S-100β < 0.1375 µg/L N (%)	Plasma S-100β > 0.1375 µg/L N (%)	Total N (%)	p
Sex				0.10
Men	20 (45.5)	43 (76.8)	63 (63)	
Women	24 (54.5)	13 (23.2)	37 (37)	
Type of injury				1.00
Open	11 (25)	13 (23.2)	24 (24)	
Closed	33 (75)	43 (76.8)	76 (76)	
Cause of trauma				0.19
Fall	29 (65.9)	25 (44.6)	54 (54)	
Traffic accident	6 (13.6)	12 (21.4)	18 (18)	
Occupational accident	5 (11.3)	8 (14.3)	13 (13)	
Sports accident	2 (4.5)	9 (16.1)	11 (11)	
Assault	1 (2.3)	2 (3.6)	3 (3)	
Other	1 (2.3)	0 (0.0)	1 (1)	
Associated injuries				< 0.05
Yes	6 (13.6)	26 (46.4)	32 (32)	
No	38 (86.4)	30 (53.6)	68 (68)	
Trauma				
Chest	7 (15.9)	8 (14.3)	15 (15)	1.00
Abdominal	1 (2.3)	0 (0.0)	1 (1)	0.44
Limbs	3 (6.8)	15 (26.8)	18 (18)	0.08
Contusion	3 (6.8)	12 (21.4)	15 (15)	0.05
GCS (points)				0.22
15	44 (100)	51 (91.1)	95 (95)	
14	0 (0.0)	5 (8.9)	5 (5)	
Symptoms				
Headache	33 (75.0)	40 (71.4)	73 (73)	0.821
Nausea	11 (25.0)	11 (19.6)	22 (22)	0.628
Vomiting	5 (11.4)	6 (10.7)	11 (11)	1.000
PLoss of consciousness	21 (47.7)	34 (60.7)	55 (55)	0.228
Amnesia	12 (27.3)	28 (50.0)	40 (40)	0.025
Seizure	1 (2.3)	2 (3.6)	3 (3)	1.000
> 2 symptoms	21 (47.7)	45 (80.4)	66 (66)	0.282
Medical history				
Cancer	0 (0.0)	0 (0.0)	0 (0)	Not calculable
Epilepsy	3 (6.8)	1 (1.8)	4 (4)	1.00
CVA	2 (4.5)	1 (1.8)	3 (3)	0.58
Dementia	4 (9.1)	2 (3.6)	6 (6)	0.40
Heart failure	3 (6.8)	4 (7.1)	7 (7)	0.69
Renal failure	1 (2.3)	0 (0.0)	1 (1)	0.44
Premedication				
Aspirin	9 (20.5)	4 (7.1)	13 (13)	0.12
Clopidogrel	1 (2.3)	1 (1.8)	2 (2)	1.00
Ticlopidine	0 (0.0)	0 (0.0)	0 (0)	Not calculable
Anticoagulation	2 (4.5)	5 (8.9)	7 (7)	1.00

GCS: Glasgow Coma Score: CVA: Cardiovascular accident.

Discussion

Mild head injury is a frequent complaint in the ED, and generates a great deal of uncertainty regarding patient management since there are no previous risk factors, clinical signs or symptoms that allow the clinician to assume with certainty the existence or absence of intracranial injury[2]. Ct scan is considered the most sensitive tool for diagnosis of TBI[10-12], and even economically beneficial compared with the cost of admission to ED observation units[13]. However, different availability of ED resources (even within the same center, based on availability at the time), together with the important and sometimes forgotten ethical issue posed by exposure to radiation, make it difficult to establish a uniform protocol of management of these patients.

This means that we emergency physicians are often uncertain about the best way to manage these patients and on many occasions unnecessarily solicit cranial CT scans or refer patients to observation or short-stay units to be able to then discharge them with greater guarantee of safety. The result is we compound the demand, already high, for limited resources available to the ED. It would therefore be helpful to have a biomarker that discriminates those patients without intracranial damage after a mild head injury. In this regard, a number of biological markers have been

Table 4. S-100β levels and time lapse between traumatic brain injury (TBI) and blood extraction

Time from TBI to extraction (P < 0.001)		
S-100β < 0.1375 µg/L	**S-100β > 0.1375 µg/L**	**Total (Mean time)**
92.4 min (mín: 25; max: 180)	73.2 min (mín: 5; max: 180)	81.6 min

proposed for the management of head trauma, such as neurospecific enolase (NSE), microtubule protein (TAU) or glial fiber acidic protein (GFAP)[14], among others, but the results (mostly obtained in severe TBI) have so far been inconclusive, with low sensitivity and specificity.

Currently, the main clinical utility of determining S-100β levels is in the diagnosis of melanoma, as well as the detection of metastasis and monitoring its progression. It is also known that S-100β levels increase in other situations and conditions, such as cerebral hypoxia, stroke, neurodegenerative diseases, psychiatric disorders, etc.[15].

Previous studies have shown that protein S-100β is a good marker of brain injury, able to discriminate intracranial injury in TBI patients. Most studies have been carried out in severe TBI[16-18], but some studies in mild TBI appear to show that protein S-100β is a good negative predictive marker of intracranial lesions[19-21]. Many studies also highlight the value of determining S-100β to assess future brain damage[22-26], neuropsychological dysfunction[27] or worse prognosis after suffering a severe head injury.

The determination of S-100β in peripheral blood in patients attending the ED after mild TBI is a simple, fast and low-cost procedure, with a sensitivity of 100% when using a cutoff point of less than 0.1375 µg/L, although specificity in our study was only 47.31%. It has the added advantage that S-100β values are not altered by hemolysis or alcohol levels (common in these patients); however, it has a short half-life, which limits its utility for patients who have suffered head trauma more than 3 hours before assessment (although there is evidence showing that serum levels remain elevated for up to 72 hours after trauma).

Table 5. Contingency table (value of S-100β / CT findings) and statistical analysis

	Normal TC N	**Pathological CT N**	**Total**
S-100β Negative (< 0.1375 µg/L)	44	0	44
S-100β Positive (> 0.1375 µg/L)	49	7	56
Total	**93 (93.0)**	**7 (7.0)**	**100**
Prevalence			7/100: 7%
Confidence interval			95%
Sensitivity (S): TP / (TP + FN)			100% (92.86-100)
Specificity (Sp): TN / (TN + FP)			47.31% (36.63-58)
Positive predictive value: TP / TP + FP			12.5% (2.95-22.1)
Negative predictive value: TN / TN + FN			100% (92.86-100)
Overall value (OV): TP + TN / TP + TN + FP + FN			51% (40.7-61.3)
Positive LR (Likelihood Ratio)			1.9 (1.57-2.3)
Negative LR (Likelihood Ratio)			0

CT: computed tomography. TP: true positive. TN: true negative. FP: false positives. FN: false negative.

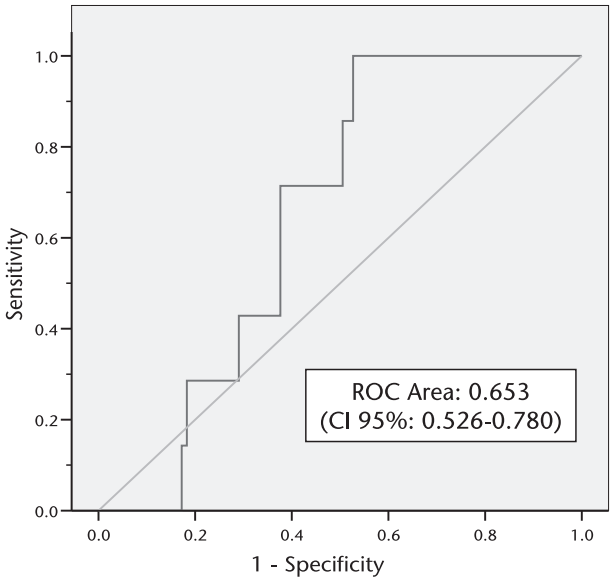

Figure 1. ROC curve for discriminative values of protein S-100β for computed tomography lesions.

Attempting to circumvent this limitation of short half-life, we initially considered performing a comparative study of S-100β levels in blood versus urine. We were aware that S-100β determination involved a urine sample with previous bladder drain and subsequent monitoring of diuresis, which entailed significant discomfort for the patient and made it difficult to carry out the study as initially designed. On observing the lack of correlation between blood and urine values obtained, the latter were suspended. However, we believe that this comparison should be carried out in future studies because, if good correlation between blood and urinary S-100β levels could be established, it would simplify clinical practice with ED determination, and obviate the disadvantage of short half-life in blood.

We were struck by the lack of proportionality between extremely high values of S-100β and CT scan findings. In our series, none of the 14 patients with values of S-100β > 1 µg/L showed CT

alterations. Nor were we able to determine a relationship with any of the variables studied that would explain this fact.

It should be pointed out that a large number of patients with mild TBI also present TBI-associated extra-cranial lesions that may be responsible for elevated levels of S-100β, especially when adipose tissue and cartilage (contusion, chest fractures, burns etc.) are affected[28-30]. On considering only those patients with mild TBI, without extracranial injuries, the specificity of S-100β levels in blood significantly improved, but a great number of these patients did in fact have other injuries (in our series 46.4% of patients with high levels of S-100β).

We believe that the great advantage of this test is its high sensitivity or negative predictive value which allows ruling out intracranial injury.

According to the results of the study, a negative test (S-100β < 0.1375 µg/L) rules out intracranial injury in mild head injury, while a positive test (S-100β > 0.1375 µg/L) does not confirm the diagnosis, so cranial CT scan is mandatory.

These results may be applied in other studies, in large series, together with other studies confirming the absence of medium-term brain injury in mild TBI when S-100β levels are below the proposed cutoff point. We should continue assessing all the evidence, with larger samples and more diverse variables, such as time from trauma, the cutoff point, use in pediatric mild head injury, etc, which involve improved performance of the test.

In summary, we believe that being able to safely rule out intracranial damage in a significant number of patients with this simple test would constitute a great contribution to clinical practice in the ED, as a crucial and distinctive element in decision-making, and as a reliable test to greatly reduce the number of CT scans performed. All this suggests that S-100β testing could be introduced as part of the protocol of care for patients with mild head injury in the ED.

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Utilidad de la determinación de la proteína S-100β en el manejo del traumatismo craneoencefálico leve en un servicio de urgencias hospitalario

Richard Espiga F, Vicente Carrero JT, Poncela García MV, Malla Pérez E, Rubio Sanz MJ, Riñones Mena E

Objetivos: Validar una técnica de cribaje para la valoración del traumatismo craneoencefálico (TCE) leve con respecto a un patrón-oro de neuroimagen, para confirmar el papel de la proteína S-100β en los servicios de urgencias hospitalarios (SUH), y si un determinado punto de corte es capaz de discriminar a los pacientes que no presentarán lesión intracraneal en la tomografía computarizada (TC).
Método: Se incluyeron 100 pacientes mayores de 14 años que acudieron al SUH tras TCE leve en las 3 horas previas, con algún síntoma acompañante y que aceptaron participar en el estudio. A todos se les determinó los niveles de S-100β, y se realizó TC craneal. Se recogieron las variables referentes a la clínica y exploración física, hallazgos de la TC craneal y los niveles de S-100β. Se calculó sensibilidad, especificidad, valor predictivo, razón de verosimilitud, tabla de contingencia y curva ROC.
Resultados: El nivel de S-100β en sangre, por debajo del cual la TC fue normal, fue de 0,1375 µg/L. Valores inferiores se encontraron en el 44%. El TC fue patológico en el 7%, con una edad media de 42 años y con niveles de S-100β siempre superiores al punto de corte. La sensibilidad de la prueba ha sido del 100%, con una especificidad del 47%. El valor predictivo negativo fue del 100% con un valor predictivo positivo del 12%; un valor global del 51%, y una razón de verosimilitud positiva del 1,9 y negativa de 0. La tabla ROC para los valores de proteína S-100β, presenta un área bajo la curva de 0,653.
Conclusiones: La determinación de S-100β tras un TCE leve, siempre que se determine precozmente, podría reducir en casi la mitad el número de TC realizadas y el ingreso en unidades de observación. [Emergencias 2011;23:15-21]

Palabras clave: Traumatismo craneoencefálico (TCE). Proteína S-100β. Tomografía axial computarizada (TC). Escala de Coma de Glasgow (GCS).