



Predictive value of S100-B and copeptin for outcomes following seizure: the BISTRO International Cohort Study.

Freund, Y; Bloom, B; Bokobza, J; Baair, N; Laribi, S; Harris, T; Navarro, V; Bernard, M; Pearse, R; Riou, B; Hausfater, P; BISTRO Investigators,

© 2015 Freund et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

For additional information about this publication click this link.

<http://qmro.qmul.ac.uk/xmlui/handle/123456789/10968>

Information about this research object was correct at the time of download; we occasionally make corrections to records, please therefore check the published record when citing. For more information contact scholarlycommunications@qmul.ac.uk

RESEARCH ARTICLE

Predictive Value of S100-B and Copeptin for Outcomes following Seizure: The BISTRO International Cohort Study

Yonathan Freund^{1,2*}, Benjamin Bloom^{3,10}, Jerome Bokobza², Nacera Baair⁴, Said Laribi⁵, Tim Harris^{3,10}, Vincent Navarro⁶, Maguy Bernard^{7,9}, Rupert Pearce^{8,10}, Bruno Riou^{1,2}, Pierre Hausfater^{1,2}, the BISTRO investigators[¶]

1 Sorbonne universités, UPMC Univ Paris 06, UMRS INSERM 1166, IHU ICAN, Paris, France, **2** Emergency Department, Hôpital Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris (APHP), Paris, France, **3** Emergency Department, Royal London Hospital, London, United Kingdom, **4** Emergency Department, Hôpital Tenon, APHP, Paris, France, **5** Emergency Department, Hôpital Lariboisière, APHP, Paris, France, **6** Neurology Department, Hôpital Pitié-Salpêtrière, APHP, Paris, France, **7** Biochemistry Department, Hôpital Pitié-Salpêtrière, APHP, Paris, France, **8** Adult critical care unit, Royal London Hospital, London, United Kingdom, **9** Université Paris 5 Paris Cité, Paris, France, **10** Queen Mary University of London, London, United Kingdom

¶ Membership of the BISTRO investigators is listed in the Acknowledgments.

* yonathanfreund@gmail.com



OPEN ACCESS

Citation: Freund Y, Bloom B, Bokobza J, Baair N, Laribi S, Harris T, et al. (2015) Predictive Value of S100-B and Copeptin for Outcomes following Seizure: The BISTRO International Cohort Study. PLoS ONE 10(4): e0122405. doi:10.1371/journal.pone.0122405

Academic Editor: Johan Pallud, Sainte-Anne Hospital Center, FRANCE

Received: October 22, 2014

Accepted: February 20, 2015

Published: April 7, 2015

Copyright: © 2015 Freund et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Because the study involved human subjects, the authors do not have authorization from the Ethics Committees to house data in a public repository. Anonymised data are fully available upon request to any of the authors. Individuals can contact the following to request data: Dr Yonathan Freund, yonathan.freund@psl.aphp.fr (corresponding author) Dr Benjamin Bloom, ben.bloom@nhs.uk (Local investigator UK), Pr Bruno Riou, bruno.riou@psl.aphp.fr (Primary investigator), Dr Anne-Laure Feral Pierrssens, anne-laure.feral-pierrssens@egp.aphp.fr (third party).

Abstract

Objective

To evaluate the performance of S100-B protein and copeptin, in addition to clinical variables, in predicting outcomes of patients attending the emergency department (ED) following a seizure.

Methods

We prospectively included adult patients presented with an acute seizure, in four EDs in France and the United Kingdom. Participants were followed up for 28 days. The primary endpoint was a composite of seizure recurrence, all-cause mortality, hospitalization or rehospitalisation, or return visit in the ED within seven days.

Results

Among the 389 participants included in the analysis, 156 (40%) experienced the primary endpoint within seven days and 195 (54%) at 28 days. Mean levels of both S100-B (0.11 µg/l [95% CI 0.07–0.20] vs 0.09 µg/l [0.07–0.14]) and copeptin (23 pmol/l [9–104] vs 17 pmol/l [8–43]) were higher in participants meeting the primary endpoint. However, both biomarkers were poorly predictive of the primary outcome with a respective area under the receiving operator characteristic curve of 0.57 [0.51–0.64] and 0.59 [0.54–0.64]. Multivariable logistic regression analysis identified higher age (odds ratio [OR] 1.3 per decade [1.1–1.5]), provoked seizure (OR 4.93 [2.5–9.8]), complex partial seizure (OR 4.09 [1.8–9.1]) and first seizure (OR 1.83 [1.1–3.0]) as independent predictors of the primary outcome. A second regression

Funding: Roche Diagnostics France (Meylan, France) provided free reagents and kits for S100B assays, and Thermo Fisher scientific B.R.A.H.M.S (Aktiengesellschaft, Hennigsdorf, Germany) provided free reagents and kits for copeptin assays.

ThermoFisher BRAHMS provided the copeptin assays free of charge, and funded our biochemistry technicians' time of work in order to process all the analysis. Roche diagnostic provided the S100B assays free of charge. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication. Yonathan Freund received a grant from the French society of emergency medicine (SFMU) and from the Assistance Publique—Hôpitaux de Paris for this study. The funders had no role in the study design, data collection, analysis, and interpretation, or the writing of the report.

Competing Interests: Roche Diagnostics France (Meylan, France) provided free reagents and kits for S100B assays, and Thermo Fisher scientific B.R.A.H.M.S (Aktiengesellschaft, Hennigsdorf, Germany) provided free reagents and kits for copeptin assays. Roche diagnostics and ThermoFisher scientific did not participate in the analysis of the data and did not see the manuscript before submission. ThermoFisher BRAHMS provided the copeptin assays free of charge, and funded our biochemistry technicians' time of work in order to process all the analysis. Roche diagnostic provided the S100B assays free of charge. YF, BB, JB, NB, SL, TH, VN and MB had no other conflict of interest with this study. PH received lecture fees and clinical studies fees from ThermoFisher scientific and honorarium from ROCHE diagnostic. BR received lecture fees from ThermoFisher scientific. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

analysis including the biomarkers showed no additional predictive benefit (S100-B OR 3.89 [0.80–18.9] copeptin OR 1 [1.00–1.00]).

Conclusion

The plasma biomarkers S100-B and copeptin did not improve prediction of poor outcome following seizure. Higher age, a first seizure, a provoked seizure and a partial complex seizure are independently associated with adverse outcomes.

Introduction

Patients attending the emergency department (ED) with seizure account for 0.5 to 7% of all ED visits, and approximately one million visits per year in the United States [1–5]. The impact of one or more seizures on an individual includes the potential for physical trauma, time off work, degeneration into status epilepticus and the risk of a life threatening acute anoxic event [6–8]. Therefore the ability to risk assess for recurrence is of critical importance.

The rate of long term recurrence is high, with a three year risk of 30% after acute symptomatic seizures and 50 to 70% after an unprovoked seizure [9–12]. The rate of early seizure recurrence (ESR) is less well established. ESR rates have been reported to be 19% in the first 24 hours, and up to 30% in cases of alcohol related seizure [4,13]. One prospective study has evaluated predictors of ESR, and found that alcoholism, low plasma glucose, and a Glasgow coma scale (GCS) less than 15 were independently associated with a higher risk of ESR [13]. As the risk of other adverse events, such as hospitalisation or death, following a seizure have not been studied, there may be further variables in addition to the three identified that can assist in the risk stratification of patients presenting to the ED with seizure.

The astroglial S100-B protein is a specific marker of cerebral injury. Raised S100-B has value in predicting adverse neurological outcomes in cardiac arrest and traumatic brain injury [14–16]. S100-B concentration is normal following febrile seizure in children. That febrile seizures are considered to be relatively harmless contributes to the hypothesis that elevated S100-B might predict adverse neurological outcomes [17,18]. Copeptin, the c-terminal part of the vasopressin molecule, is a biomarker of endogenous stress. Recently, it has been described as a good prognostic marker in neurological disorders, such as traumatic brain injury [19], intracerebral hemorrhage, and stroke [20,21].

We hypothesised that these two biomarkers may have an incremental added prognostic value to routine clinical data to predict adverse events following seizure related ED visits.

Methods

Study design, setting and participants

The Biomarkers In Seizure To predict Recurrences and severe Outcomes (BISTRO) is a prospective international cohort study (NCT01774500), conducted from January 2013 to December 2013. The primary objective is to establish the incremental value of combining S100-B and copeptin levels with standard clinical variables to identify patients most at risk of complications following presentation in the ED with seizure.

We enrolled patients from four centres: one in London, UK and three in Paris, France. Participants' informed signed consent was sought prior to enrolment, and institutional review boards from both countries approved the study (Comité de protection des personnes—Paris

Ile de France 6, Paris, France; and NHS Health Research Authority, National Research Ethics Service Camberwell St Giles, United Kingdom). In cases in which informed consent could not be obtained from the patient due to a decreased level of consciousness, a next-of-kin signed informed consent was mandatory prior to enrolment. After the patient returned to a normal level of consciousness, their signed informed consent was then sought. When this was not obtained, the patient was excluded from the study.

The study design and report is in accordance with the STROBE statement [22]. Patients were eligible to become study participants if they were 18 years or older and had had one or more convulsive seizures within 24 hours. Patients were excluded if they were less than 18 years; pregnant; prisoners; and those for whom seven or 28 day follow up was deemed impossible. Patients were screened in real time in the EDs of the participating centres.

Outcomes

The primary endpoint was a composite endpoint of seizure recurrence, or all cause death, hospitalisation, or rehospitalisation or return visit in the ED within seven days.

Secondary endpoints included seizure recurrence at seven and 28 days; ICU admission; death within seven and 28 days; and length of hospitalization within seven and 28 days. The decision to hospitalise a patient depends on individual physicians and as such may be considered subjective. To reduce the effect of this subjectivity, a sensitivity analysis was run with a modified primary endpoint that excluded those patients that were hospitalised for less than 24 hours. Finally, as predicting adverse events in discharged patients is of great importance, we ran a sub-analysis focusing only on patients that were not admitted after their first ED visit.

Variables

Clinical and physiological data were recorded; white cell count, sodium, calcium, glucose, and lactate were routinely measured within the participating centres. Venous blood samples were taken in heparinised tubes to measure S100-B and copeptin. The sample for S100-B and copeptin was frozen at -80°C and all samples were measured in a single batch at the end of the study to avoid bias from assay discrepancy. The assay for copeptin measurement was performed on a KRYPTOR analyzer using the commercial sandwich immunoluminometric assay (B.R.A.H.M. S Aktiengesellschaft, Hennigsdorf, Germany). The lower detection limit is 4.8 pmol/L, and the functional assay sensitivity is < 12 pmol/L. The limit of quantification (10% coefficient of variation [CV]) is 14.1 pmol/L. In our laboratory, the CV were found to be <5% (4.4% at 28.86 pmol/L and 4.6% at 95.84 pmol/L). S100-B measurement was performed on an Elecsys (Roche Diagnostics, Mannheim, Germany). The lower detection limit is 0.005 µg/L and the functional assay sensitivity is 39 µg/L. In our laboratory, the CV was found to be <5%. Copeptin and S100-B determinations were performed blinded to the clinical assessment of the emergency physicians. Follow up was performed either by telephone or hospital visit.

Since the definition of “epilepsy” is controversial, and has varied in recent years [23–25], a patient was considered epileptic if a neurologist had ever diagnosed the condition, if the patient had an unprovoked seizure and evidence of remote CNS lesion or if the patient was currently on antiepileptic drug. A remote lesion is a CNS lesion that is stable and is not acute (for instance a stroke sequellum). This approach is in accordance with recommendations from the International League Against Epilepsy (ILAE) for a pragmatic definition of epilepsy [25]. A seizure was classified, according to ILAE guidelines, as provoked if it could have been related to an acute systemic insult or acute CNS lesion (there are many causes for a provoked seizure, for instance alcohol intoxication, alcohol withdrawal, hypoglycemia) occurring within the previous seven days, or unprovoked if not. Unprovoked seizures were classified as idiopathic, or

remote symptomatic in the presence of a known CNS lesion. Seizures in the setting of sleep deprivation were not considered provoked [26].

Patients were followed up for 28 days, and were called (or visited if still in the hospital) at day seven and 28 to assess endpoints. Participants with missing data regarding the two biomarkers, and participants lost to follow up were excluded.

Study size

On the basis of pre-existing literature, we estimated the rate of the primary endpoint at day seven to be 20%. To avoid overfitting and in order to be able to include at least 10 variables in the logistic regression model, there needed to be at least 100 events in our sample [27]. Furthermore, this minimal number of 100 events is warranted for external validation [28]. Therefore a total sample size of 500 participants was required for this study. An interim analysis of outcome showed a higher rate of the endpoint than expected (35%), which reduced the required sample size to 350 participants.

Statistical analysis

Data are expressed as mean \pm standard deviation (SD) for Gaussian variables; median and 25 to 75% interquartile range for non-Gaussian variables; and number and percentage for categorical variables with 95% confidence interval. Normality was assessed using the Kolmogorov-Smirnov test. Measures of diagnostic accuracy were calculated with their 95% confidence intervals (CI) for S100-B and copeptin. Receiving operator characteristics (ROC) curves were constructed and their area under the curve was calculated. Thresholds were determined using the Youden's method. Comparison of the two groups was performed using the Student t test, the Mann-Whitney U test, and Fisher's exact method when appropriate.

A multiple logistic regression was performed to assess independent variables associated with the primary endpoint, and odds ratios (ORs) with their 95% CI were calculated. To avoid overestimation, a conservative approach was used and all clinically relevant variables were included [29]. These variables were determined a priori upon previous literature and clinical relevance (namely age, first seizure, history of epilepsy, neuromuscular impairment, chronic alcohol intake, focal neurological deficit, complex partial seizure, provoked seizure, GCS < 15, body temperature > 37.5°C) and the two studied biomarkers, S-100 and copeptin. Correlation between all variables were calculated, and in case of a coefficient of correlation $R^2 > 0.6$, only the most clinically significant variable was entered in the model. Calibration of the model was estimated with Hosmer-Lemeshow test, and discrimination with the c-index. Internal validation was assessed using the bootstrap resampling method (n = 500, without replacement) [30]. To present the internal validation, the difference (optimism) between the c-statistics observed in the population and in the bootstrapped sample was calculated [30].

All analyses were performed using SPSS software (IBM, Armonk, NY), all comparisons were two-tailed and a p value of 0.05 was required to reject the null hypothesis. The statistical plan was decided before the onset of the study.

Results

In the period of inclusion, 443 participants were enrolled. Twenty two participants had no S100-B and copeptin measurements, and 32 were lost to follow up (Fig 1). Therefore 389 participants were included in the analysis, of which 87 (22%) were from the United Kingdom and 302 (78%) from France. The mean age of the studied population was 44 years (SD 18), and 58% were male. One hundred and thirty (33%) presented to the ED with a first seizure and 259

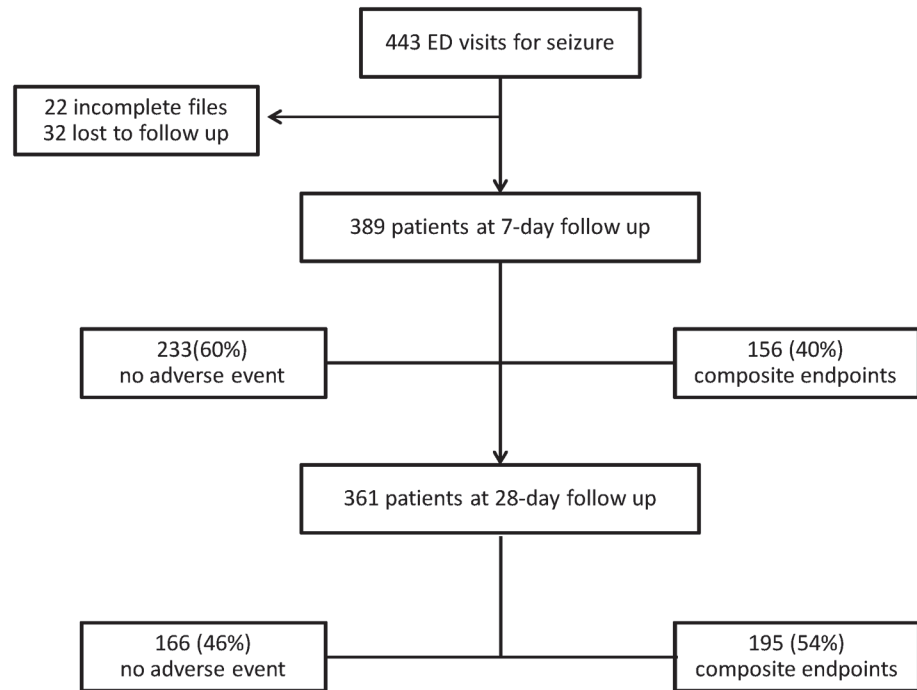


Fig 1. Flow chart ED: emergency department. Composite endpoint of recurrence, hospitalization or death at day seven.

doi:10.1371/journal.pone.0122405.g001

(67%) were considered epileptic according to the definition above. Main baseline characteristics are summarized in [Table 1](#).

One hundred and fifty six participants (40%) experienced the primary endpoint of death, hospitalization, seizure recurrence, rehospitalisation or return visit to the ED within seven days and 195 (54%) at 28 days. The primary endpoint occurred in 56%, 40%, 31% and 26% in participants from Royal London Hospital, Pitié-Salpêtrière, Tenon and Lariboisière hospitals respectively ($p = 0.003$ for UK vs France). Sixty patients (15%) had a seizure recurrence within seven days. Main outcomes are summarized in [Table 2](#).

Copeptin and S100-B were significantly higher in participants that experienced the primary combined endpoint than in the others: $0.11 [0.07-0.20]$ vs $0.09 [0.07-0.14]$ $\mu\text{g/l}$ ($p = 0.02$) for S100-B and $23 [9-104]$ vs $17 [8-43]$ pmol/l ($p < 0.001$) for copeptin ([Fig 2](#)).

ROC curves for S100-B and copeptin are reported in [Fig 3](#), with a respective area under the curve of $0.57 [95\% \text{ CI } 0.51-0.64]$ and $0.59 [95\% \text{ CI } 0.54-0.64]$ ($p < 0.05$ for both). Using Youden’s method, a threshold value of $0.1 \mu\text{g/l}$ for S100-B and 100 pmol/l for copeptin was found, which corresponded to a sensitivity and specificity of $57\% [49-65\%]$ and $53\% [46-59\%]$ respectively for S100-B, and $24\% [18-31\%]$ and $92\% [88-95\%]$ respectively for copeptin. Complete diagnostic performances are reported in [Table 3](#) with different thresholds. Of note, we studied “positive S100 AND positive copeptin”, as well as “Positive S100 OR positive copeptin”, with different thresholds and we found that no combination resulted in satisfactory diagnostic performances (data not shown). When considering more homogenous populations, for example epileptic patients, or patients with provoked seizure, neither of these two biomarkers showed good diagnostic performances (data not shown).

A multivariable logistic regression was performed with pre-specified variables. “Epilepsy” as a variable was not included because it was correlated with the variable “first seizure” ($R^2 = 0.66$). We kept “first seizure” instead of “epilepsy” in the model, because the diagnosis of

Table 1. Characteristics of study cohort.

Characteristic		Total	All patients	No event in 7 days		Recurrence or severe outcome at day 7	
		389	389	233 (60%)	156 (40%)		
Characteristic	Age, mean (SD), y		44 (18)	40 (16)	51 (20)		
	Sex Male, No. (%)	229	(58%)	143 (61%)	86 (55%)		
	Sex Female, No. (%)	160	(42%)	90 (39%)	70 (45%)		
	Seizure in the ED, No. (%)	73	(19%)	21 (9%)	52 (33%)		
	Seizure	259	(67%)	170 (0.72)	89 (57%)		
Past Medical History, No. (%)	Epilepsy	217	(56%)	147 (63%)	70 (45%)		
	Stroke	32	(8%)	16 (7%)	16 (10%)		
	Meningitis	14	(4%)	7 (3%)	7 (4.5%)		
	Neuromuscular impairment	22	(6%)	7 (3%)	15 (10%)		
	Chronic alcohol intake	50	(13%)	20 (9%)	30 (20%)		
	Drug	13	(3%)	8 (3%)	5 (3%)		
Current medication, No. (%)	Benzodiazepin	56	(14%)	37 (16%)	19 (12%)		
	Anti epileptic drug	172	(44%)	114 (49%)	58 (37%)		
	Headache	100	(26%)	62 (27%)	38 (24%)		
On Examination, No. (%)	Photophobia	13	(3%)	9 (4%)	4 (3%)		
	Confusion	43	(11%)	14 (6%)	29 (19%)		
	Neurological deficit	13	(3%)	1 (0.5%)	12 (8%)		
	Partial simple	24	(6%)	13 (6%)	11 (7%)		
Type of seizure, No. (%)	Complex partial	41	(10%)	13 (6%)	28 (18%)		
	Generalised tonic clonic	290	(75%)	179 (77%)	111 (71%)		
	Absence	31	(8%)	22 (9%)	9 (6%)		
	Acute Symptomatic	67	(17%)	17 (7%)	50 (32%)		
	Remote symptomatic	49	(13%)	25 (11%)	24 (15%)		
	Idiopathic	273	(70%)	191 (82%)	82 (53%)		
	Witnessed	280	(72%)	160 (69%)	120 (77%)		
	Time from Seizure to ED visit, median [IQR], hours	1.5	[1–2]	1.5 [1–2]	0.7 [0–2]		
	Heart rate, mean (SD)	378	89 (19)	89 (19)	90 (17)		
	Physiological parameters on admission	Systolic BP, mean (SD)	380	129 (21)	129 (19)	129 (24)	
Diastolic BP, mean (SD)		380	77 (15)	77 (13)	79 (17)		
Temperature, mean (SD)		376	36.6 (0.6)	36.6 (0.5)	36.8 (0.7)		
GCS, median [IQR]		379	15 [15–15]	15 [15–15]	15 [15–15]		
GCS<15, No (%)		389	45 (12%)	25 (11%)	20 (13%)		
Pulse oxymetry, median [IQR]		380	97 [96–99]	98% [96–99]	97% [95–99]		
WBC (Giga/l), median [IQR]		325	9.8 [7.0–13]	9.5 [6.5–12.7]	10.4 [7.4–13]		
Glucose (mmol/l), median [IQR]		270	6.1 [5.2–7.3]	5.8 [5.1–6.8]	6.4 [5.4–8]		
Sodium (mmol/l), mean (SD)		365	137 (12)	137 (13)	137 (11)		
Calcium (mmol/l), median [IQR]		289	2.3 [1.3–2.4]	2.4 [2.3–2.5]	2.3 [1.2–2.4]		
Laboratory results	Lactate (mmol/l), median [IQR]	176	1.9 [1.2–3.6]	1.65 [1.2–3.3]	2.1 [1.3–3.7]		
	S100B (µg/l), median [IQR]	389	0.10 [0.07–0.16]	0.09 [0.07–0.14]	0.11 [0.07–0.2]		
	Copeptin (pmol/l), median [IQR]	389	19 [8–54]	17 [8–43]	23 [9–104]		

SD, standard deviation; IQR, 25–75% interquartile range; ED, emergency department; GCS, Glasgow coma scale; WBC, white blood cells. All laboratory results were obtained from venous blood.

doi:10.1371/journal.pone.0122405.t001

Table 2. Outcomes and follow up of the study cohort.

		Total	All patients	
Disposition from ED	Home	389	243	(63%)
	Observation unit		95	(29%)
	Hospitalisation		126	(32%)
	Admission in ICU		11	(3%)
	Admission in neurosurgery		15	(4%)
	Death		2	(1%)
	Follow up day 7	Seven days free of hospital	389	224
Recurrence			60	(15%)
Re hospitalisation			16	(4%)
Number of hospital free days, median [IQR]			7	[4–7]
ICU admission			14	(4%)
Death			5	(1%)
Follow up day 28	28 days free of hospital	361	185	(51%)
	Recurrence		97	(27%)
	Rehospitalisation		29	(8%)
	Hospital free days, median [IQR]		28	[25–28]
	ICU admission		16	(4%)
	Death		10	(2%)

ED, emergency department, ICU, intensive care unit; IQR, 25–75% interquartile range.

doi:10.1371/journal.pone.0122405.t002

epilepsy can be more subject to diagnostic disagreement than a “first seizure”. Two models are presented; one not including and the other including the biomarkers. In the first model independent risk factors for the primary outcome were found to be higher age; complex partial seizure; provoked seizure; and first seizure (Table 4). Discrimination of the model was good with a c-statistic of 0.77 [95% CI 0.72 to 0.81] and Hosmer-Lemeshow goodness-of-fit test had a $p = 0.51$. Bootstrap sampling confirmed the internal validity of the model, with an optimism of

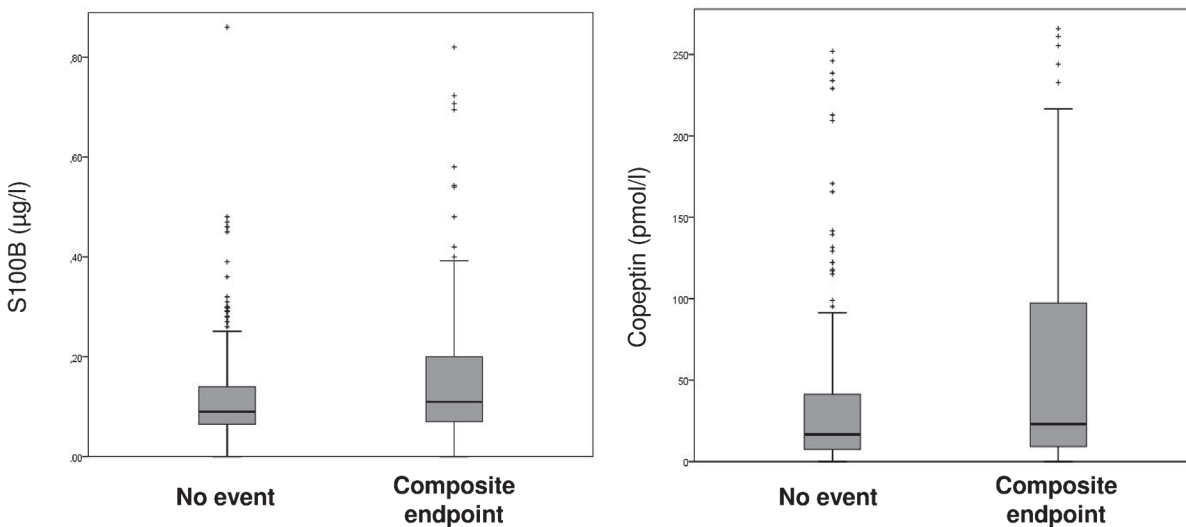


Fig 2. S100B and copeptin values in the two groups. Box plot with median, interquartile range, and 5th and 95th centile. Composite endpoint of recurrence, hospitalization or death at day seven.

doi:10.1371/journal.pone.0122405.g002

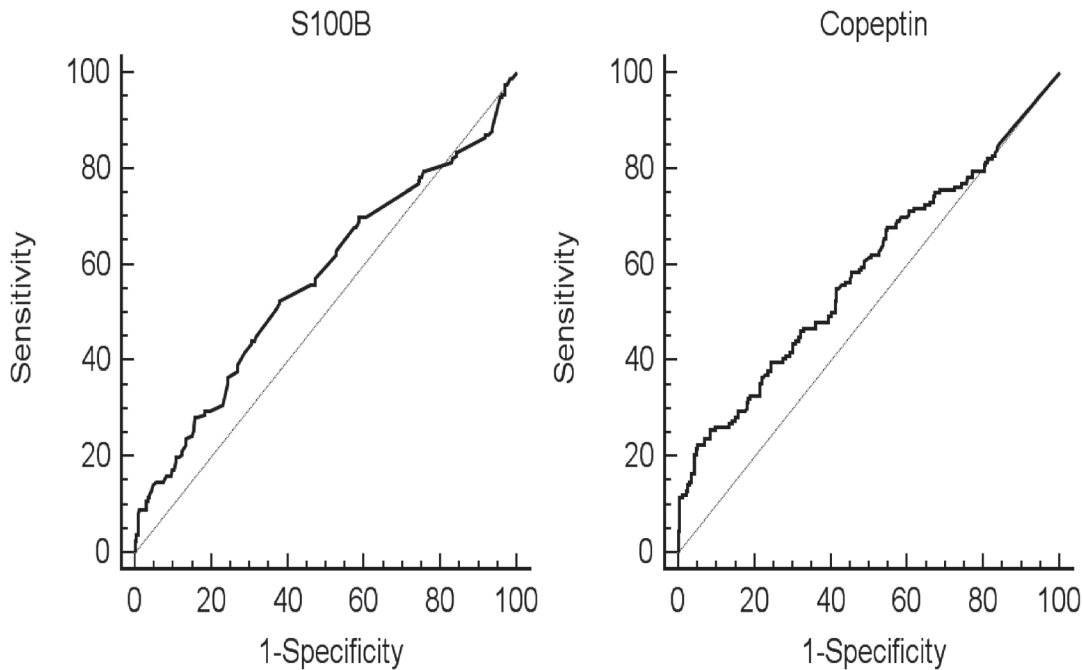


Fig 3. Receiving operator characteristics curve for Copeptin and S100B. Area under the curve 0.57 [95% CI 0.51–0.64] for S100B, $p = 0.01$, and 0.59 [95% CI 0.54–0.64] for copeptin, $p = 0.02$.

doi:10.1371/journal.pone.0122405.g003

0.01, and a corrected c-stat of 0.76. When adding S100-B and copeptin, the model was left unchanged, and neither of the two biomarkers was independently associated with the primary endpoint (c-stat 0.78, optimism 0.02, corrected c-stat 0.76).

With a modified primary endpoint that excluded those with hospitalisation for less than 24 hours, there was no improvement in terms of diagnostic performances for either of the two biomarkers. The clinical model of logistic regression showed one supplemental variable independently associated with the endpoint: pre-existing neuromuscular impairment (OR 11.9 [95% CI 1.44–98.60]).

Finally, the subgroup of participants that were not admitted following their ED visit was analysed. There were 263 participants (69%) that were discharged home from the ED. Amongst them, 30 (11%) met the primary endpoint within seven days. Values of S100-B and copeptin were similar in the two groups, with a median of respectively 0.09 $\mu\text{g/l}$ and 17 pmol/l . Complex partial seizures was the only significant predictor of increased risk of recurrence (OR 5.7

Table 3. Diagnostic performances of S100-B and Copeptin, and 95% confidence interval.

Biomarker	Threshold	Sensitivity	Specificity	PPV	NPV	LR+	LR-
S100-B ($\mu\text{g/l}$)	0.1	57% [49%- 65%]	53% [46%- 59%]	45% [38% -52%]	65% [57%- 71%]	1.21 [0.99–1.46]	0.81 [0.65–1.01]
	0.2	24% [18%- 32%]	85% [80%- 89%]	52% [40%- 64%]	63% [57%- 68%]	1.62 [1.07–2.46]	0.89 [0.80–0.98]
	0.5	8% [5%- 14%]	99% [97%- 100%]	87% [59%- 98%]	62% [57%- 67%]	9.71 [2.6–58]	0.92 [0.87–0.96]
Copeptin (pmol/l)	14	67% [59%- 74%]	45% [39%- 52%]	45% [39%- 52%]	67% [59%- 74%]	1.22 [1.04–1.44]	0.73 [0.56–0.95]
	50	33% [26%- 40%]	79% [73%- 83%]	51% [40%- 61%]	64% [58%- 69%]	1.52 [1.09–2.13]	0.86 [0.75–0.97]
	100	24% [18%- 31%]	92% [88%-95%]	66% [52%- 78%]	64% [58%- 69%]	2.91 [1.76–4.96]	0.83 [0.75–0.91]

PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative LR.

doi:10.1371/journal.pone.0122405.t003

Table 4. Adjusted odds ratios of independent predictors for composite endpoint.

Variables	Adjusted ORs	95% CI	Variables	Adjusted ORs	95% CI
Provoked seizure	4.93	2.47–9.84	Provoked seizure	4.71	2.32–9.56
Complex partial	4.09	1.84–9.08	Complex partial	4.26	1.90–9.52
First seizure	1.83	1.10–3.02	First seizure	1.73	1.03–2.89
Age (per 10 year older)	1.27	1.11–1.45	Age (per 10 year older)	1.26	1.11–1.45
			S100B	3.89	0.80–18.9
			Copeptin	1.00	1.00–1.00

OR, odds ratio; CI, confident interval.

a) clinical model, Hosmer-Lemeshow goodness-of-fit statistics p value 0.5, c-stat 0.77.

b) model with S100-B and copeptin, Hosmer Lemeshow goodness-of-fit statistics p value 0.04, c-stat 0.78.

doi:10.1371/journal.pone.0122405.t004

[95% CI 1.96–16.7]). No association was found between the level of S100-B or copeptin and the rate of secondary endpoints—only copeptin was associated with ICU admission at day seven and 28 (Table 5).

Discussion

With this study, we aimed to determine whether S100-B and copeptin are of added prognostic value to usual assessment following seizure. The first result from our study is a negative result: measurement of S100-B and copeptin has no significant added value to predict the risk of seizure recurrence or severe outcome. We found that the primary endpoint was more frequent than we expected with a rate of 40%. Finally, we present four independent clinical factors that are associated with a significant increased risk of adverse events after a seizure: higher age; acute symptomatic seizure; complex partial seizures; and a first seizure.

Although the long term rate of recurrence is well known, there is scarce data on the risk of early seizure recurrence. In its last clinical policy on evaluation of adults presenting with seizures, the American College of Emergency Physicians [31] tried to identify patient that do not need to be admitted to prevent adverse events. In contrast with literature regarding long term outcome, their level C recommendations lack studies focusing on early recurrence. As stated by Huff et al., the immediate need for admission and observation after ED evaluation has not been specifically addressed [31]. We chose a composite endpoint of early complications after ED visit that included seizure recurrence; hospital admission; death within seven days; or return visit to hospital within seven days. We consider these endpoints to be sufficiently severe that they merited being addressed collectively. The timeframe of seven days is consistent with previous literature [32,33].

Table 5. Median of S100B and copeptin, with their 25%-75% interquartile range.

		Day seven		Day seven		Day 28		Day 28	
		No recurrence	Recurrence	No ICU admission	ICU admission	No recurrence	Recurrence	No ICU admission	ICU admission
S100B	(µg/l)	0.1 [0.07–0.16]	0.09 [0.06–0.17]	0.1 [0.07–0.16]	0.1 [0.08–0.20]	0.1 [0.07–0.18]	0.09 [0.06–0.15]	0.09 [0.06–0.16]	0.11 [0.08–0.20]
Copeptin	(pmol/l)	19 [8.3–54]	18 [5.2–48.5]	19 [8–53]	33 [8.2–296]	23 [9.9–66.2]	17 [0–47]	20 [8.6–54]	74 [11.1–311]

ICU: Intensive Care Unit.

doi:10.1371/journal.pone.0122405.t005

In recent years S100-B has been reported to have a very high specificity for death (95% to 98%) and unfavourable neurological outcomes (85 to 98%) [34], and a very high sensitivity for the diagnosis of brain lesions (99 to 100%) [16,35] in traumatic brain injury. In the context of seizure, we report very low diagnostic performances of S100-B, with failure to obtain thresholds that would allow greater sensitivity with acceptable specificity, or vice versa. There was a very high rate of S100-B false positive (47% and 15% for a respective threshold of 0.1 and 0.2 µg/l), i.e. S100-B was raised in many cases that did not meet the primary endpoint. This suggests that there is a pathophysiological increase in blood concentration of S100-B after a seizure, regardless of whether that patient will go on to develop the primary endpoint or not. Similarly, we report no added value of copeptin in the setting of convulsive seizure. We failed to determine a threshold of S100-B or copeptin value that can help the clinician either to rule in or exclude the occurrence of adverse events.

The high frequency of the primary endpoint is in contrast to previously published work. This could be explained by the fact that our endpoint is a composite whereas previous studies report singular primary endpoints such as seizure recurrence. In their study in France, Choquet et al. found an early seizure recurrence rate of 19% (within 24 hours) [13], and Breen et al. suggested that a rate of at least 28% patients that were not initially admitted experienced the endpoint in the next six weeks [36]. In our study, more than a tenth of patients who were initially not admitted had an early seizure recurrence or re hospitalization within seven days.

The four independent factors we found to be associated with a significantly increased risk of adverse events after a seizure were higher age; provoked seizure; complex partial seizures; and first seizure. Besides higher age, those three conditions can contribute to the overall risk assessment a physician makes when encountering a patient that has just had a seizure. Other factors reported in the literature as carrying an increased risk of recurrence are a higher blood glucose level, a decreased GCS, and a context of alcoholism. We confirmed the influence of blood glucose level although only in the univariate analysis. However, we did not find that a decreased GCS was associated with the occurrence of adverse events—probably due to a lack of power. We also found that provoked seizure (therefore including those in the context of alcohol) is an independent risk factor of recurrence and severe outcome. This is a very valuable result as most previous studies focused on the risk of long term recurrences, and reported that provoked seizures have a lower rate of recurrence at three years (30 vs 50–70% [9–12,26]).

Limitations

Our study has some limitations. There is a significant difference in the rate of the endpoint between France and UK. There may be inclusion bias as the ED systems of the two countries are markedly different: in the UK centre, less severe patients were managed in a different part of the ED (out-of-hours general practitioners' clinic, or minors unit) where recruitment did not take place. Another limitation was the choice of our composite endpoint that included subjective data such as "hospitalization". However, we determined that inclusion of hospitalization was not a serious shortcoming by running a sensitivity analysis with modified composite endpoints (with the exclusion of patients hospitalized less than 24 hours for example, and focusing only on critically ill patients), and the conclusions remained the same. Finally, there may be an element of inclusion bias because the diagnosis of seizure may be uncertain in the ED, and consequently we may have included some patients that did not have a true epileptic seizure, and may have had a pseudo-epileptic seizure or convulsive syncope. This limitation is inherent to the design and reflects the day to day work of an emergency physician, in which it is sometimes impossible to fully confirm than an epileptic seizure has occurred. In the same way, the collected data on the type of seizure were made upon patient and witness interrogation, and are

consequently subject to bias. This again mirrors the real life information to which a clinician has access. A third of patients had no witness account of their seizure. To avoid inconsistencies in classification of seizure type, we classified any seizure with loss of consciousness as generalised although some of them could have been absence or focal seizure with lost of consciousness.

Conclusion

In summary, S100-B and copeptin have very low added value to predict adverse events after an ED visit for seizure. We report four independent clinical predictors of early seizure recurrence and severe outcome: higher age; provoked seizure; complex partial seizure; and first seizure. Since the rate of adverse events is high (40%) we suggest that these conditions should alert emergency physicians to increased risk and lower the threshold for admission to the hospital.

Acknowledgments

BISTRO Investigator study Group:

Lead author: Patrick Ray, MD, PhD, Emergency Department, Hopital Tenon, Paris, France.
Email: Patrick.ray@tnn.aphp.fr

Jason Pott, RN, Geoffrey Bellhouse, RN, (Emergency Department, Royal London Hospital, London, United Kingdom)

Amanda Smith, RN, Kirsty Everingham, RN, (Adult Critical Care Unit, Royal London Hospital, London, United Kingdom)

Andrew Kelso, MD (Neurology Department, Royal London Hospital, London, United Kingdom)

Julie Pernet, MD, (Emergency Department, Hôpital Tenon, Paris, France)

Guillaume Lefevre, MD, (biochemistry department, Hôpital Tenon, Paris, France)

Jérôme Denis, MD, PhD, (biochemistry department, Hôpital Lariboisière, Paris France)

Christine Brochet, MD, Francoise Imbert-Bismuth, MD, PhD, (biochemistry department, Hopital Pitie-Salpetriere, Paris, France)

Nicolas Segal, MD, PhD (Emergency Department, Hôpital Lariboisière, Paris, France)

Anne-Laure Philippon, MD (Emergency Department, Hôpital Pitié-Salpêtrière, Paris, France)

Hinda Maa, Djalila Bensetti-Houari (from the Emergency Department, Hôpital Pitié-Salpêtrière, Paris, France).

Author Contributions

Conceived and designed the experiments: YF BB RP BR PH. Performed the experiments: YF BB JB NB SL TH. Analyzed the data: YF VN BR. Contributed reagents/materials/analysis tools: MB. Wrote the paper: YF BB RP BR PH.

References

1. Huff JS, Morris DL, Kothari RU, Gibbs MA, Emergency Medicine Seizure Study Group (2001) Emergency department management of patients with seizures: a multicenter study. *Acad Emerg Med* 8: 622–628. PMID: [11388937](https://pubmed.ncbi.nlm.nih.gov/11388937/)
2. Krumholz A, Grufferman S, Orr ST, Stern BJ (1989) Seizures and seizure care in an emergency department. *Epilepsia* 30: 175–181. PMID: [2924743](https://pubmed.ncbi.nlm.nih.gov/2924743/)
3. Rathlev NK, D'Onofrio G, Fish SS, Harrison PM, Bernstein E, Hossack RW, et al. (1994) The lack of efficacy of phenytoin in the prevention of recurrent alcohol-related seizures. *Ann Emerg Med* 23: 513–518. PMID: [8135426](https://pubmed.ncbi.nlm.nih.gov/8135426/)
4. Tardy B, Lafond P, Convers P, Page Y, Zeni F, Viallon A, et al. (1995) Adult first generalized seizure: etiology, biological tests, EEG, CT scan, in an ED. *Am J Emerg Med* 13: 1–5. PMID: [7832926](https://pubmed.ncbi.nlm.nih.gov/7832926/)

5. Pallin DJ, Goldstein JN, Moussally JS, Pelletier AJ, Green AR, Camargo A. (2008) Seizure visits in US emergency departments: epidemiology and potential disparities in care. *Int J Emerg Med* 1: 97–105. doi: [10.1007/s12245-008-0024-4](https://doi.org/10.1007/s12245-008-0024-4) PMID: [19384659](https://pubmed.ncbi.nlm.nih.gov/19384659/)
6. Marinas A, Elices E, Gil-Nagel A, Salas-Puig J, Sánchez JC, Carreno M, et al. (2011) Socio-occupational and employment profile of patients with epilepsy. *Epilepsy Behav* 21: 223–227. doi: [10.1016/j.yebeh.2011.01.025](https://doi.org/10.1016/j.yebeh.2011.01.025) PMID: [21620775](https://pubmed.ncbi.nlm.nih.gov/21620775/)
7. Holland P, Lane S, Whitehead M, Marson AG, Jacoby A (2009) Labor market participation following onset of seizures and early epilepsy: Findings from a UK cohort. *Epilepsia* 50: 1030–1039. doi: [10.1111/j.1528-1167.2008.01819.x](https://doi.org/10.1111/j.1528-1167.2008.01819.x) PMID: [19178562](https://pubmed.ncbi.nlm.nih.gov/19178562/)
8. DeLorenzo RJ, Hauser WA, Towne AR, Boggs JG, Pellock JM, Penberthy L, et al. (1996) A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia. *Neurology* 46: 1029–1035. PMID: [8780085](https://pubmed.ncbi.nlm.nih.gov/8780085/)
9. Berg AT, Shinnar S (1991) The risk of seizure recurrence following a first unprovoked seizure: a quantitative review. *Neurology* 41: 965–972. PMID: [2067659](https://pubmed.ncbi.nlm.nih.gov/2067659/)
10. Marson A, Jacoby A, Johnson A, Kim L, Gamble C, Chadwick D. (2005) Immediate versus deferred antiepileptic drug treatment for early epilepsy and single seizures: a randomised controlled trial. *Lancet* 365: 2007–2013. PMID: [15950714](https://pubmed.ncbi.nlm.nih.gov/15950714/)
11. Kim LG, Johnson TL, Marson AG, Chadwick DW, MRC MESS Study group (2006) Prediction of risk of seizure recurrence after a single seizure and early epilepsy: further results from the MESS trial. *Lancet Neurol* 5: 317–322. PMID: [16545748](https://pubmed.ncbi.nlm.nih.gov/16545748/)
12. Brown JW, Lawn ND, Lee J, Dunne JW (2014) When is it safe to return to driving following first-ever seizure? *J Neurol Neurosurg Psychiatry*: 2013–307529.
13. Choquet C, Depret-Vassal J, Doumenc B, Sarnel S, Casalino E (2008) Predictors of early seizure recurrence in patients admitted for seizures in the Emergency Department. *Eur J Emerg Med* 15: 261–267. doi: [10.1097/MEJ.0b013e3282f63d](https://doi.org/10.1097/MEJ.0b013e3282f63d) PMID: [18784504](https://pubmed.ncbi.nlm.nih.gov/18784504/)
14. Böttiger BW, Möbes S, Glätzer R, Bauer H, Gries A, Bärtsch P, et al. (2001) Astroglial protein S-100 is an early and sensitive marker of hypoxic brain damage and outcome after cardiac arrest in humans. *Circulation* 103: 2694–2698. PMID: [11390339](https://pubmed.ncbi.nlm.nih.gov/11390339/)
15. Song KJ, Shin SD, Ong MEH, Jeong JS (2010) Can early serum levels of S100B protein predict the prognosis of patients with out-of-hospital cardiac arrest? *Resuscitation* 81: 337–342. doi: [10.1016/j.resuscitation.2009.10.012](https://doi.org/10.1016/j.resuscitation.2009.10.012) PMID: [20047785](https://pubmed.ncbi.nlm.nih.gov/20047785/)
16. Zongo D, Ribéreau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, et al. (2012) S100-B protein as a screening tool for the early assessment of minor head injury. *Ann Emerg Med* 59: 209–218. doi: [10.1016/j.annemergmed.2011.07.027](https://doi.org/10.1016/j.annemergmed.2011.07.027) PMID: [21944878](https://pubmed.ncbi.nlm.nih.gov/21944878/)
17. Atici Y, Alehan F, Sezer T, Tuygun N, Haberal A, Yazici AC, et al. (2012) Serum S100B levels in children with simple febrile seizures. *Seizure J Br Epilepsy Assoc* 21: 175–177. doi: [10.1016/j.seizure.2011.11.003](https://doi.org/10.1016/j.seizure.2011.11.003) PMID: [22221921](https://pubmed.ncbi.nlm.nih.gov/22221921/)
18. Mikkonen K, Pekkala N, Pokka T, Romner B, Uhari M, Rantala H (2012) S100B proteins in febrile seizures. *Seizure J Br Epilepsy Assoc* 21: 144–146. doi: [10.1016/j.seizure.2011.10.006](https://doi.org/10.1016/j.seizure.2011.10.006) PMID: [22130006](https://pubmed.ncbi.nlm.nih.gov/22130006/)
19. Dong X-Q, Huang M, Yang S-B, Yu W-H, Zhang Z-Y (2011) Copeptin is associated with mortality in patients with traumatic brain injury. *J Trauma* 71: 1194–1198. doi: [10.1097/TA.0b013e31821283f2](https://doi.org/10.1097/TA.0b013e31821283f2) PMID: [21502880](https://pubmed.ncbi.nlm.nih.gov/21502880/)
20. Katan M, Fluri F, Morgenthaler NG, Schuetz P, Zweifel C, Bingisser R, et al. (2009) Copeptin: a novel, independent prognostic marker in patients with ischemic stroke. *Ann Neurol* 66: 799–808 doi: [10.1002/ana.21783](https://doi.org/10.1002/ana.21783) PMID: [20035506](https://pubmed.ncbi.nlm.nih.gov/20035506/)
21. Zweifel C, Katan M, Schuetz P, Siegemund M, Morgenthaler NG, Merlo A, et al. (2010) Copeptin is associated with mortality and outcome in patients with acute intracerebral hemorrhage. *BMC Neurol* 10: 34. doi: [10.1186/1471-2377-10-34](https://doi.org/10.1186/1471-2377-10-34) PMID: [20504314](https://pubmed.ncbi.nlm.nih.gov/20504314/)
22. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP (2007) The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 370: 1453–1457. PMID: [18064739](https://pubmed.ncbi.nlm.nih.gov/18064739/)
23. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, et al. (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 46: 470–472. PMID: [15816939](https://pubmed.ncbi.nlm.nih.gov/15816939/)
24. Panayiotopoulos CP (2012) The new ILAE report on terminology and concepts for the organization of epilepsies: critical review and contribution. *Epilepsia* 53: 399–404. doi: [10.1111/j.1528-1167.2011.03381.x](https://doi.org/10.1111/j.1528-1167.2011.03381.x) PMID: [22242702](https://pubmed.ncbi.nlm.nih.gov/22242702/)

25. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. (2014) ILAE Official Report: A practical clinical definition of epilepsy. *Epilepsia* 55: 475–482. doi: [10.1111/epi.12550](https://doi.org/10.1111/epi.12550) PMID: [24730690](https://pubmed.ncbi.nlm.nih.gov/24730690/)
26. Lawn N, Lieblich S, Lee J, Dunne J (2014) Are seizures in the setting of sleep deprivation provoked? *Epilepsy Behav EB* 33: 122–125. doi: [10.1016/j.yebeh.2014.02.008](https://doi.org/10.1016/j.yebeh.2014.02.008) PMID: [24657503](https://pubmed.ncbi.nlm.nih.gov/24657503/)
27. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR (1996) A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol* 49: 1373–1379. PMID: [8970487](https://pubmed.ncbi.nlm.nih.gov/8970487/)
28. Vergouwe Y, Steyerberg EW, Eijkemans MJC, Habbema JDF (2005) Substantial effective sample sizes were required for external validation studies of predictive logistic regression models. *J Clin Epidemiol* 58: 475–483. PMID: [15845334](https://pubmed.ncbi.nlm.nih.gov/15845334/)
29. Sun GW, Shook TL, Kay GL (1996) Inappropriate use of bivariable analysis to screen risk factors for use in multivariable analysis. *J Clin Epidemiol* 49: 907–916. PMID: [8699212](https://pubmed.ncbi.nlm.nih.gov/8699212/)
30. Molinaro AM, Simon R, Pfeiffer RM (2005) Prediction error estimation: a comparison of resampling methods. *Bioinforma* 21: 3301–3307.
31. Huff JS, Melnick ER, Tomaszewski CA, Thiessen MEW, Jagoda AS, Fesmire FM (2014) Clinical policy: Critical issues in the evaluation and management of adult patients presenting to the emergency department with seizures. *Ann Emerg Med* 63: 437–447 doi: [10.1016/j.annemergmed.2014.01.018](https://doi.org/10.1016/j.annemergmed.2014.01.018) PMID: [24655445](https://pubmed.ncbi.nlm.nih.gov/24655445/)
32. Sauvin G, Freund Y, Saïdi K, Riou B, Hausfater P (2013) Unscheduled return visits to the emergency department: Consequences for triage. *Acad Emerg Med* 20: 33–39 doi: [10.1111/acem.12052](https://doi.org/10.1111/acem.12052) PMID: [23570476](https://pubmed.ncbi.nlm.nih.gov/23570476/)
33. Gabayan GZ, Asch SM, Hsia RY, Zingmond D, Liang L-J, Han W, et al. (2013) Factors associated with short-term bounce-back admissions after emergency department discharge. *Ann Emerg Med* 62: 136–144.e1. doi: [10.1016/j.annemergmed.2013.01.017](https://doi.org/10.1016/j.annemergmed.2013.01.017) PMID: [23465554](https://pubmed.ncbi.nlm.nih.gov/23465554/)
34. Mercier E, Boutin A, Lauzier F, Fergusson DA, Simard J-F, Zarychanski R, et al. (2013) Predictive value of S-100 protein for prognosis in patients with moderate and severe traumatic brain injury: systematic review and meta-analysis. *BMJ* 346: f1757–f1757. doi: [10.1136/bmj.f1757](https://doi.org/10.1136/bmj.f1757) PMID: [23558282](https://pubmed.ncbi.nlm.nih.gov/23558282/)
35. Egea-Guerrero JJ, Revuelto-Rey J, Murillo-Cabezas F, Muñoz-Sánchez MA, Vilches-Arenas A, Sánchez-Linares P, et al. (2012) Accuracy of the S100 β protein as a marker of brain damage in traumatic brain injury. *Brain Inj* 26: 76–82. doi: [10.3109/02699052.2011.635360](https://doi.org/10.3109/02699052.2011.635360) PMID: [22149446](https://pubmed.ncbi.nlm.nih.gov/22149446/)
36. Breen DP, Dunn MJG, Davenport RJ, Gray AJ (2005) Epidemiology, clinical characteristics, and management of adults referred to a teaching hospital first seizure clinic. *Postgrad Med J* 81: 715–718. PMID: [16272236](https://pubmed.ncbi.nlm.nih.gov/16272236/)