WORSHEET for Evidence-Based Review of Science for Emergency Cardiac Care

Worksheet author(s)

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Clinical question.

"In adult patients who are comatose after cardiac arrest (pre-hospital or in-hospital) (P), does the use of biochemical markers (I) as opposed to standard care (C), allow accurate prediction of outcome (O) (e.g. survival)?"

Is this question addressing an intervention/therapy, prognosis or diagnosis? Prognosis
State if this is a proposed new topic or revision of existing worksheet: Revision

Conflict of interest specific to this question

Do any of the authors listed above have conflict of interest disclosures relevant to this worksheet? No

Search strategy (including electronic databases searched).

- Cochrane Library (Databases: Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects)
Database search strategy according to PICO format:
Query 1   "cardiac arrest" OR "heart arrest"
Query 2   "biological marker*"
Query 3   1 AND 2 ⇨ 44 hits (July 28, 2008)

- PubMed
Database search strategy according to PICO format:
Query 1   "Heart Arrest"[Mesh] OR "Death, Sudden, Cardiac"[Mesh]
Query 2   "Biological Markers"[Mesh]
Query 3   1 AND 2 ⇨ 249 hits (July 28, 2008)

The results of the Query 3 have been filtered by the "Clinical Queries" tool   (category: prognosis; scope: broad, sensitive) and restricted to ⇨ 66 hits (July 28, 2008)

- Embase (1988-2008)
Database search strategy according to PICO format:
Query 1   "biomarker.mp. or exp Biological Marker/"
Query 2   "cardiac arrest.mp. or exp Heart Arrest/"
Query 3   1 AND 2 ⇨ 41 hits (July 28, 2008)

- ECC Endnote Master library
Search strategy
Query 1   "biomarker" [any field] OR "biological marker" [any field] ⇨ 28 hits (July 28, 2008)

• State inclusion and exclusion criteria

 Only human studies on cases of non-traumatic cardiac arrest were included for analysis.
 Animal studies, traumatic cardiac arrest, circulatory arrest during surgery with extracorporeal circulation, pediatric cardiac arrest, single-case
   reports were excluded. Non English language articles, abstract only studies and not peer-reviewed articles were not considered for further review.

• Number of articles/sources meeting criteria for further review:

 After publication list consolidation, elimination of duplicates, and title analysis, 119 references were considered for further review. During the following steps of the review process, several references were added after manual cross-check of the bibliography of selected publications. The studies analyzed in the pivotal systematic reviews and meta-analyses by Zandbergen et al. 2001 1661-1667 and Wijdicks et al. 2006 203-210 were appropriately flagged with a symbol (* and †, respectively). After analysis of the abstracts and of the publications *in extenso*, comprehensive analysis was restricted to 32 publications exactly matching the worksheet topic, as stated in the PICO format ("*In adult patients who are comatose after cardiac arrest (pre-hospital or in-hospital) (P), does the use of biochemical markers (I) as opposed to standard care (C), allow accurate prediction of outcome (O) (e.g. survival)?"* , were eventually included in the worksheet. Of these, 21 were LOE P1 (*Inception [prospective] cohort studies [or meta-analyses of inception cohort studies], or validation of Clinical Decision Rule [CDR]*), 1 was LOE P2 (*Follow up of untreated control groups in RCTs [or meta-analyses of follow-up studies], or derivation of CDR, or validated on split-sample only*), 10 were LOE P3 (*Retrospective cohort studies*), none were LOE P4 (*Case series*) or LOE P5 (*Studies not directly related to the specific patient/population [(eg. different patient/population, animal models, mechanical models etc*.]))

Summary of evidence

Evidence Supporting Clinical Question

Good	*Martens 1996 126-31 s-NSE [D] Meynaar et al. 2003 189-95 s-NSE [D E] Mussack et al. 2002 2669-2674 s-S100 [D] Schoerkhuber et al. 1999 1598-1603 s-NSE [D] Wijdicks et al. 2006 203-10 s-NSE [D] s-S-100 [D] CSF-CKBB [D] Zandbergen et al. 2001 1661-1667 s-NSE [D] s-S100 [D] CSF-NSE [D] CSF-CKBB [D]				
Fair	†Bottiger et al. 2001 2694-2698 s-NSE [A B D] s-S-100 [A B D] †*Fogel et al. 1997 1133-1138 s-NSE [D] Hachimi-Idrissi et al. 2002 251-7 s-S-100 [D] Karkela et al. 1992 378-86 CSF-CK [D] CSF-CK-BB [D] CSF-LDH tot [D] CSF-LDH iso 1, 2 and 3 CSF-Acid Phosphatase [D] CSF-lactate [D] * Longstreth et al. 1984 834-7 CSF-CK [D] *Martens 1996 126-31 CSF-CPK-BB [D] CSF-LDH [D] *†Martens et al. 1998 2363-2366 s-NSE s-S100 CSF-NSE CSF-S100 †Pfeifer et al. 2005 49-55 s-NSE [B D] s-S-100 [B D]	Prohl et al. 2007 1230-7 S-NSE [C D E] (multivariate) S-S100 [C D E] (univariate)	Adib-Conquy et al. 2007 406-410 s-TREM and PCT [E] †*Dauberschmidt et al. 1991 237-45 s-NSE [D] Geppert et al. 2003 805-11 vWF [D] Rosen et al. 2001 183-91 s-NSE [D] s-S100 [D] *Rosen et al. 2004 19-24 CSF-NFL [D] *Sherman et al. 2000 889-94 CSF-CKBB [D] Sodeck et al. 2007 439-445 BNP [B E]		

	1	2	3	4	5
	†*Roine et al. 1989 753-6 s-NSE CSF-NSE CSF-CKBB

*†Rosen et al. 1998 473-477 s-S-100 [E]

†Tiainen et al. 2003 2881-2886 s-NSE [D] s-S100[D] *(Therapeutic hypothermia)*

†Zandbergen et al. 2006 62-8 s-NSE [C D] s-S100 [C D]

Zingler et al. 2003 79-84 s-NSE[E] | | | | |
| **Poor** | *Martens 1996 126-31 CSF-GOT [D]

Mussack et al. 2001 539-543; discussion 544 s-S100 [E]

Rothstein et al. 1991 101-7 CSF-CKBB [C,D] | | †*Longstreth et al. 1981 455-8 CSF-CKBB [D]

*Tirschwell et al. 1997 352-7 CSF-CKBB | | |
| | **1** | **2** | **3** | **4** | **5** |
| **Level of evidence** | | | | | |

A = Return of spontaneous circulation   C = Survival to hospital discharge   E = Other endpoint
B = Survival of event   D = Intact neurological survival   *Italics = Animal studies*

* Included in Zandbergen 2001 1661-67
† Included in Wijdicks 2006 203-10

*

Evidence Neutral to Clinical question

	1	2	3	4	5
Good					
Fair			Adrie et al. 2002 562-8: sTNFRII, IL-6, IL-8, IL-10 Geppert et al. 2003 805-11 ICAM [D]		
Poor					
	1	**2**	**3**	**4**	**5**
			Level of evidence		

A = Return of spontaneous circulation   C = Survival to hospital discharge   E = Other endpoint
B = Survival of event   D = Intact neurological survival   *Italics = Animal studies*

* Included in Zandbergen 2001 1661-67
† Included in Wijdicks 2006 203-10

Evidence Opposing Clinical Question

	1	2	3	4	5
Good	Mussack et al. 2002 2669-2674 IL8 [D]				
Fair	Karkela et al. 1992 378-86 CSF-beta DN acetylglucosaminidase [D] CSF-pyruvate [D]		Karkela et al. 1992 378-86 CSF-beta DN acetylglucosaminidase [D] CSF-pyruvate [D] Adrie et al. 2002 562-8[B] IL1-ra RANTES		
Poor	*Clemmensen et al. 1987 235-6 CSF-CKBB [C D]				
	1	**2**	**3**	**4**	**5**
			Level of evidence		

A = Return of spontaneous circulation   C = Survival to hospital discharge   E = Other endpoint
B = Survival of event   D = Intact neurological survival   *Italics = Animal studies*

* Included in Zandbergen 2001 1661-67
† Included in Wijdicks 2006 203-10

REVIEWER'S FINAL COMMENTS AND ASSESSMENT OF BENEFIT / RISK:

Several biochemical markers (designed as "biomarkers" throughout the worksheet) have been evaluated to assess their ability to predict the outcome of comatose survivors of cardiac arrest. Among the studies which explored the predictive performance of different biomarkers to assess the prognosis of comatose survivors of cardiac arrest, 32 were considered eligible for the present worksheet and were related to serum biomarkers (NSE, S-100, BNP, IL8, vWF, ICAM-1, Plasma endotoxin, sTNFRII, IL-1ra, IL-6, IL-8, IL-10, RANTES, sTREM-1 and PCT) or cerebrospinal fluid biomarkers (CK, CK-BB, NSE, S-100, LDH, GOT and CSF-neurofilament). The results of the systematic review upon which the worksheet's conclusions are based are presented hereafter.

Serum biomarkers

Serum NSE

Sixteen eligible studies investigated the predictive performance of s-NSE to assess the prognosis of comatose survivors of cardiac arrest. A role of s-NSE to assess the prognosis of comatose survivors of cardiac arrest was supported by:

13 LOE P1 studies ((*LOE P1: Inception [prospective] cohort studies [or meta-analyses of inception cohort studies], or validation of Clinical Decision Rule[CDR]);* of these, 5 were QOE good [Martens 1996 126-31, Meynaar 2003 189-95, Schoerkhuber 1999 1598-603; Wijdicks 2006 203-10   Zandbergen et al. 2001 1661-1667] and 8 QOE fair [Bottiger 2001 2694-98, Fogel 1997 1133-38, Martens 1998 2363-66, Tiainen 2003 2881-86, Pfeifer 2005 49-55; Roine 1989 753-6; Zingler 2003 79-84; Zandbergen 2006 62-8]

1 LOE P2 study *(LOE P2: Follow up of untreated control groups in RCTs (or meta-analyses of follow-up studies), or derivation of CDR, or validated on split-sample only)* [Prohl 2007 1230-7] whose QOE was fair

2 LOE P3 studies *(LOE P3: retrospective cohort studies)* [Rosen 2001 183-91, Dauberschmidt 1991 237-45] whose QOE was fair

The predictive performance of s-NSE has been extensively analyzed in the following "*Citation list"* section and is here summarized as follows (for abbreviations see the *Abbreviations list* at the end of the worksheet):

*Martens 1996 126-31

Study design: prospective inception cohort study (LOE P1).
Study population: Fifty-two consecutive patients who remained comatose after successful resuscitation from OHCA.
Biomarker under evaluation: Serum NSE levels assessed on blood samples collected 24 hours after cardiac arrest; in 16 pts who remained alive but comatose a lumbar puncture was performed to assess CSF CPK-BB, CSF-AST and CSF-LDH also. Biomarkers levels were not used to take treatment decisions
Outcome assessed: neurological outcome, defined as return of consciousness *vs.* death due to CNS failure; pts regaining consciousness but eventually dying from non-neurologic causes as well as pts with neurologic impairment were considered as pts with successful outcome.
Results: S-NSE, GCS on admission, serum glucose on admission and total epinephrine dose before ROSC were significantly higher in pts with unfavorable outcome. A correlation between S-NSE on admission and outcome was confirmed by logistic regression analysis after adjustment for the possible confounding role of best GCS on admission, blood glucose on admission and total epinephrine dose before ROSC (adjusted OR 5.8; p=0.034). The predictive performance of different levels of s-NSE was not reported by the authors and could not be assessed by the reviewer, as individual data were not presented.
In conclusion, S-NSE appeared to be correlated to outcome both at univariate and multivariate analysis; unfortunately, the predictive performance of abnormal s-NSE concentrations was not assessed, with limited clinical applicability of the results of logistic regression analysis. Therefore, the study has been ranked as supportive of a prognostic role of S-NSE (LOE P1 QOE good) to assess neurological outcome of comatose survivors of cardiac arrest.

Meynaar 2003 189-95

Study design: prospective inception cohort study (LOE P1)
Study population: 110 comatose pts admitted to the ICU immediately following CPR after circulatory arrest for any cause. Patients received full intensive treatment until recovery or until absence of cortical response to somato-sensory evoked potentials (defined as bilateral absence of N20 on median nerve stimulation); moreover If GCS was still below 8 on the 6th day after CPR, treatment was withdrawn.
Biomarkers under evaluation: s-NSE as determined on blood samples collected on admission and daily for 5 days.

Outcome assessed: survival and neurological outcome, defined as the regain of consciousness (obviously awake or able to obey simple commands at least once, independent of successive survival or death)

Results: In the patients who regained consciousness, s-NSE was lower than in the patients remaining comatose. This difference was significant at 24 h (P <0.001) and 48 h (P =0.001) after admission. In addition, in the patients who remained comatose, s-NSE concentrations increased after admission with highest levels measured at 48 h after ICU admission. In contrast, in the patients who regained consciousness, s-NSE concentrations remained low. Finally, no s-NSE concentration higher than 25.0 μg/l was measured at any time in a patient eventually regaining consciousness. There were, however, several patients with s-NSE levels below 25.0 μg/l who remained comatose and died. Sensitivity, specificity, PPV and NPV have been computed for a NSE cut-off level of 25 μg/l at 48 hours (59%, 100% [FPR 0%], 100% and 10% respectively); Sensitivity, specificity, PPV and NPV have also been computed for the absence of N20 at SSEP (64%, 100% (FPR 0%), 100% and 4% respectively); of great interest, the prognostic performance of the above mentioned criteria was significantly improved when they were combined so that in the presence of s-NSE levels >25 μg/l OR bilateral absence of N20 at SSEP sensitivity, specificity, PPV and NPV were improved (78%, 100% (FPR 0%), 100%, 18%). The ability of s-NSE levels was confirmed at multivariate analysis (logistic regression analysis).

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. The biomarker under evaluation appeared to be predictive of outcome both at univariate and multivariate analysis. In addition, the authors did compare the predictive performance of biomarkers under investigations to SSEP. Therefore, the study has been ranked as supportive of a prognostic role of s-NSE levels at 24 and 48 hours to predict survival and neurological outcome with an additional value over SSEPs alone. (LOE P1, QOE good)

Schoerkhuber 1999 1598-603

Study design: prospective inception cohort trial (LOE P1)
Study population: 56 comatose pts resuscitated from OHCA.
Biomarker under evaluation: Serum NSE levels as determined on blood samples collected at 12, 24, 48 and 72 hours after ROSC.
Outcome assessed: neurological outcome as assessed by the best CPC achieved within 6 months from ROSC (CPC 1-2 good outcome *vs.* CPC 3-4 bad outcome).
Results: Levels of NSE were lower in pts with favorable outcome compared to those with unfavorable outcome at 12, 24, 48 and 72 hours. The predictive performance of NSE levels at different sampling times and of the highest NSE level over the first 72 hours after ROSC has been evaluated comparing the respective AUCs. The best predictive performance was observed for NSE at 72 hours (AUC 0.92±0.04). In a multivariate analysis (logistic regression) exploring the independent predictive role of maximum NSE level measured within 72 hours after cardiac arrest, the no-flow time and low-flow time, the cumulative dose of epinephrine, basic life support (yes/no), the location of cardiac arrest, age, and gender, maximum level of NSE achieved during the 72 hours after ROSC was predictive of a poor neurological outcome (OR 1.09 95% CI 1.01- 1.19, *p*=0.04). Also of interest, a different time course of NSE levels was observed between the 2 outcome groups; while NSE levels tended to increase in patients with a bad neurological outcome, they tended to decrease in those with a good neurological outcome. The following table was adapted from the original source to show the predictive performance of s-NSE in this study:

	NSE LEVEL cut-point	Sensitivity	Specificity (FPR)
	μg/l	%	%
12 h	38.5	17.4	100 (0)
24h	40.0	8.0	100 (0)
48h	25.1	48.0	100 (0)
72 h	16.4	70.0	100 (0)
Highest concentration within 72h	27.3	28.6	100 (0)

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. The levels of biomarker under evaluation actually were not available for all pts at all times. The biomarker under evaluation appeared to be predictive of outcome, which was confirmed at a multivariate analysis considering also the no-flow time and low-flow time, the cumulative dose of epinephrine, basic life support (yes/no), the location of cardiac arrest, age, and gender . Therefore, the study has been ranked as supportive of a predictive role of NSE at 72 hours to estimate the probability of unfavorable neurological outcome (LOE P1, QOE good)

Wijdicks 2006 203-10

Study design: Meta-analysis (LOE P1)
Study population: see individual studies (marked with † throughout the worksheet)
Biomarkers evaluated: serum NSE, serum S100, cerebrospinal fluid CKBB, cerebrospinal fluid neurofilament
Results: *serum NSE:* One class I study [Zandbergen 2006 62-8], four class III studies [Pfeifer 2005 49-55, Tiainen 2003 2881-86, Fogel 1997 1133-38, Martens 1998 2363-66], and one class IV study [Dauberschmidt 1991 237-45] have investigated the usefulness of increased serum NSE as a marker of poor outcome. In the class I study [Zandbergen 2006 62-8], 60% of 231 patients had NSE >33 µg/L at day 1 to 3 after CPR. All these patients had a poor outcome (FPR 0; 95% CI: 0 to 3). Most other studies also found an increase in serum NSE at day 3. However, the cutoff points for a 0 FPR value vary greatly (20 to 65 µg/L). An FPR could not be obtained in two studies, and it ranged from 0 to 11% in class III studies. Of interest, group analyses have shown that serum levels of NSE, but not those of S100, are significantly lower in patients treated with induced hypothermia compared with those of untreated patients.
Conclusions (as reported by the authors of the meta-analysis). "Serum NSE, S100, and CSF CKBB have been investigated as a predictor for outcome with studies using variable cutoff points. For serum NSE levels > 33 µg/L at days 1 to 3, one class I study [Zandbergen 2006 62-8] demonstrates a 0 FPR with narrow 95% CIs. Recommendations. Serum NSE levels >33 µg/L at days 1 to 3 post-CPR accurately predict poor outcome (recommendation level B). There are inadequate data to support or refute the prognostic value of other serum and CSF biochemical markers in comatose patients after CPR (recommendation level U)"
The author of the worksheet endorsed this conclusions: this systematic review supports a role of s-NSE to assess the prognosis of comatose survivors of cardiac arrest (LOE P1 QOE good)

Zandbergen 2001 1661-67

Study design: Meta-analysis of prognostic studies (LOE P1). The specific objectives of the review were stated clearly. The study was carefully designed and criteria for inclusion were described. Characteristics and methodological quality of each trial were detailed and a log of excluded studies with reasons for exclusion was reported. The studies cited in the present meta-analysis and included in the worksheet are marked with an asterisk throughout the worksheet. A methodological limitation of the present meta-analysis is that data were pooled irrespective of the nature of the study (either prospective or retrospective) and of blinding of physician to the results of the biomarker level.
In conclusion, the study is a valuable systematic review of the literature, but the studies included in the meta-analysis were both prospective and retrospective and blinded and unblinded. However, the present study is supportive of a predictive role s-NSE to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their clinical application is limited by the wide confidence intervals of the pooled estimates of false positive rates as detailed in the following table. (LOE P1 QOE good)

	95% CI of the pooled false positive rates
Serum	
NSE>17 ng/ml	10.8-42.5
NSE>20 ng/ml	4.2-26.8
NSE>33 ng/ml	0.1-22.8

†Bottiger 2001 2694-98

Study design: prospective inception cohort study (LOE P1)
Study population: 66 patients undergoing cardiopulmonary resuscitation after non-traumatic cardiac arrest.
Biomarkers under investigation: Levels of S-100 (as determined on blood samples collected on admission and at 15, 30, 45, and 60 minutes; 2, 8, 24, 48, and 72 hours; and 7 days after initiation of cardiopulmonary resuscitation) and of NSE (as determined on blood samples collected between 2 hours and 7 days
Other prognostic markers under investigation: If patients survived for >48 hours, brain damage was assessed by a combination of neurological, cranial CT, and electrophysiological examinations.
Outcome evaluated: ROSC, survival and neurological outcome (defined as: group 1: no brain damage [patients discharged from the hospital fully oriented and without any communication defects (CPC1)]; group 2: documented brain damage (i.e., according to neurological, cranial CT, and electrophysiological evaluations systematically performed between 48 hours and 96 hours after cardiac arrest; to focus on all patients, the data of surviving and non-surviving patients were combined here; group 3: no ROSC; group 4: patients who died soon after ROSC before assessment of brain damage.
Results: The study was mainly centered on the diagnostic performance of s-S-100 (which is presented separately in the appropriate

relative section). With regard to s-NSE, significant differences between patients with documented brain damage and those with no brain damage were found at 24, 48, and 72 hours and 7 days but a more detailed assessment of the predictive performance of NSE was not performed.
 Quality of evidence: The present study supports a role of s-NSE to assess the prognosis of comatose survivors of cardiac arrest, but is inconclusive (QOE fair)

†* Fogel 1997 1133-38

Study design: prospective inception cohort study (LOE P1).
Study population: 50 pts resuscitated from cardiac arrest
Biomarker under evaluation: NSE as determined on blood samples collected within 12 hours from admission and daily for 7 days.
Outcomes assessed: Survival and neurological outcome at 3 months as determined by the Barthel index.
Results: seven pts died before neurological status could be evaluated and were excluded from analysis. Of the remaining 43 pts, 25 patients remained comatose and subsequently died while 18 patients survived the first 3 months and had no relevant functional deficit at 3-month follow-up. NSE was significantly lower in pts who survived and had a favorable neurological outcome. None of the pts with NSE levels > 33 ng/ml at any sampling time regained consciousness; however, 25 pts who remained comatose had NSE levels persistently ≤ 33 ng/ml; none of the pts who survived had NSE levels >33 ng/ml at any sampling time. The authors reported the sensitivity of s-NSE levels > 33ng/ml from day 1 to day 7 after cardiac arrest; at this cut-off the specificity of s-NSE levels to predict a poor outcome in this study was uniformly 100% (FPR 0%). The predictive performance of s-NSE levels > 33ng/ml is presented hereafter in a table modified from the original source

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Sensitivity (%)	25	60	63	65	65	38	30
Specificity (%)	100	100	100	100	100	100	100

In conclusion, the study is a prospective inception cohort trial (LOE P1). Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. However, even though at univariate analysis the biomarker under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive (QOE fair) of a predictive role of s-NSE to assess the prognosis of comatose survivors of cardiac arrest

†*Martens 1998 2363-66

Study design: prospective inception cohort trial (LOE P1)
Study population: 64 pts who remained comatose > 24 hours after resuscitation from cardiac arrest.
Biomarkers under evaluation: NSE and S100 levels as determined on blood samples collected at 24 hours after admission and NSE and S100 levels as determined on cerebrospinal fluid collected by lumbar puncture at 48 hours after admission.
Outcome assessed: outcome was dichotomized into 2 groups: group 1 consisted of patients who died or remained in vegetative state, and group 2 consisted of patients who regained consciousness (i.e., obeyed simple verbal commands). Regaining consciousness was considered an end point for follow-up, regardless of outcome at discharge or at 6 months. Patients who eventually died of multiorgan failure but who had clearly regained consciousness after cardiac arrest were classified in group 2.
Results: Serum S-100, serum NSE, S-100 CSF and NSE CSF were significantly higher in patients who never regained consciousness compared with those who regained consciousness. S-100 and NSE correlated well with each other in the CSF; both CSF concentrations also correlated with their serum concentrations; S-100 and NSE did not correlate at all with each other in serum. In detail, the predictive performance of S-NSE (cut-off 20 µg/L) was: Sensitivity 51%, Specificity 89%, FPR 11%
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a prognostic role of s-NSE (and of s-S100, CSF-NSE, CSF-S100 as discussed separately in the relative appropriate sections) to assess the probability of a favorable neurological outcome. (LOE P1, QOE fair).

†Pfeifer 2005 49-55

Study design: Prospective (inception) cohort study (LOE P1).
Study population: 97 pts arrived at ICU within 12 hours of ROSC after non-traumatic cardiac arrest (both in-hospital and out-of-

hospital) and survived for a minimum of 48 hours.

Prognostic biomarker under evaluation: Serum NSE and S-100 sampled for 5 days (once to three times a day).

GCS was also assessed on a daily basis.

Outcome evaluated: survival and neurological outcome at 28 days (bad outcome defined as Glasgow Outcome Scale (GOS) 1-2 vs. good outcome defined as GOS 3-5).

Results: s-NSE and s-S100 levels were significantly higher in pts with unfavorable outcome at 24 and 48 hours after ROSC, respectively.

Interestingly, while s-NSE and s-S-100 levels reached their peak and declined within 48 hours in pts with favorable outcome, NSE and S-100 levels reached their peak at 4 and 5 days respectively in pts with unfavorable outcome. NSE at day 3 $\geq$ 65 ng/ml had a sensitivity of 50%, a specificity of 96% (FPR 4%) and a positive predictive value of 97% to predict a poor neurological outcome.

Conclusions: The authors did not attempt multivariate analyses, as the study population was not large enough to allow control for all known confounders and give conclusive results. Therefore, the study has been ranked as supportive of a predictive role of levels of s-NSE (and of s-S-100 as discussed separately in the relative appropriate section) and their combination to assess the neurological outcome of comatose survivors of CA (LOE P1, QOE fair)

†*Roine 1989 753-6

Study design: prospective inception cohort study LOE P1

Study population: 75 consecutive victims of out of-hospital cardiac arrest, but it was not overtly stated whether they were comatose on admission. GCS was assessed at 1 h, 24 h and 7 days after admission but was not reported. The study was included in both the seminal systematic reviews on the topic by Wijdicks 2006 203-10 and Zandbergen 2001 1661-67 and therefore the ILCOR reviewer decided to report it

Biomarkers under evaluation: NSE and CKBB as measured in samples of cerebrospinal fluid (CSF) and serum (CSF-NSE, s-NSE, CSF-CKBB, s-CKBB)

Outcome assessed: The recovery of consciousness was defined as the ability to follow verbal commands. All patients were followed up for 3 months or until death. The 3-month outcome was classified according to the Glasgow Outcome Scale.

Results: The serum level of NSE was assessed in 65 patients and the serum level of CKBB in 71 patients. In pts with poor neurological outcome, the serum levels of NSE were significantly higher than in pts with a favorable neurological outcome (P<.001). If an arbitrary cut-off value of 17 ng/mL was chosen for serum NSE, the test had a sensitivity of 40 %, a specificity of 98% (FPR 2%) , a positive predictive value of 89%, and negative predictive value of 79% in detecting patients who did not recover consciousness (P<.001).

In conclusion the study has been ranked as supportive of a prognostic role on s-NSE to assess the prognosis of comatose survivors of cardiac arrest (QOE fair)

†Tiainen 2003 2881-86

Study design: sub-study of a prospective inception cohort study (Hypothermia after Cardiac Arrest Trial Study) (LOE P1) Study population: 70 adult pts arrived at ED after a cardiac arrest with several restrictive inclusion criteria: witnessed CA, cardiac rhythm with VF or pulseless VT as the initial rhythm, a presumed cardiac origin of the arrest, an estimated interval of 5 to 15 minutes from collapse to EMS intervention and an interval from collapse to ROSC < 60 min. Patients were randomized to therapeutic hypothermia (TH) or standard treatment (ST) .

Prognostic biomarker under evaluation: Serum NSE and S-100 sampled at 24, 36, 48 hours after ROSC.

Outcome evaluated: survival and neurological outcome at 6 months (bad outcome defined as CPC score $\geq$ 3 v.s. good outcome defined as CPC score 1-2).

Results: 36 out of the 70 pts were randomized to TH and 34 to ST. NSE and S-100 were available for 35/36 HT pts and 33/34 ST pts. After 6 months, a favorable outcome was observed in 69% of HT pts *vs*. 47 % of ST pts. A decrease of NSE levels between 24 and 48 hours was observed in 88% of HT pts as compared to 50% of ST pts; a decrease of NSE but not of S-100 between 24 and 48 hours was associated with a favorable outcome at 6 months. Cut-off levels associated with at least 95% specificity for predicting unfavorable outcome were calculated by ROC analysis for both NSE and S-100 at 24, 36 and 48 hours in HT and ST as separate groups. Cut-off levels associated with at least 95% specificity for predicting unfavorable outcome were higher in HT as compared to ST group, and sensitivity values associated to these cut-offs were remarkably lower in HT as compared to ST pts. The following table, extracted from the manuscript, details cut-off values and their performance

	Hypothermia				Normothermia			
	Cut-off	Specificity	FPR	Sensitivity	Cut-off	Specificity	FPR	Sensitivity
	µg/L	(%)	(%)	(%)	µg/L	(%)	(%)	(%)
NSE								
24 h	31.2	96	4	22	13.3	100	0	59

| 36 h | 26.0 | 96 | 4 | 30 | 12.6 | 100 | 0 | 59 |
| 48 h | 25.0 | 96 | 4 | 25 | 8.8 | 100 | 0 | 76 |

Of interest, the AUCs at all study times were lower in the hypothermia group as compared to the normothermia group, again suggesting a less favorable predictive performance of the biomarkers under investigation in pts treated with therapeutic hypothermia.

	Hypothermia (AUC)	Normothermia (AUC)
NSE		
24 h	0.72	0.89
36 h	0.85	0.88
48 h	0.80	0.89

AUC: Area under the curve at ROC analysis

In conclusion, the study has been ranked as supportive of a predictive role of NSE as determined on blood samples at 24, 36 and 48 hours to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their predictive performance and cut-off levels at comparable levels of specificity are lower in pts treated with therapeutic hypothermia (LOE P1, QOE fair)

†Zandbergen 2006 62-8

Study design: Prospective (inception) cohort study (LOE P1).
Study population: 407 adult comatose survivors of CA.
 Prognostic biomarker under evaluation: Serum NSE and S-100 sampled at 24, 48 and 72 hours after CPR.
Other prognostic markers evaluated: clinical characteristics, median nerve SSEP at 24, 48 and 72 hours, and EEG at 72 h. Outcome evaluated: neurological outcome (Glasgow outcome scale at 1 month and at 1 year in survivors
Results: All patients unconscious a 72 h with NSE>33 µg/ml (but a blood sample was available only in 231/305) at any time had a poor outcome (95% CI of false positive rate 0-3%); pts with bilateral absence of N20 or NSE>33 µg/ml overlapped only partially; all pts with bilateral absence of N20 or NSE>33 µg/ml had a poor outcome (95% CI 0-2%). In pts without absent N20 and NSE not > 33 µg/ml, a small number of pts with poor outcome could be identified with EEG (burst suppression or no voltage > 20 µV). In conclusion, 252 out of 356 pts with poor outcome could be predicted with these 3 variables in the first 3 days after CPR. False positive rate (95% CI) of NSE levels >33 µg/ml were 0 (0-3), 0 (0-3), 0 (0-4), 0 (0-3) respectively at 24, 48, 72 and 24-72 hours;
Quality of evidence: Comparison groups were clearly defined. Outcome was assessed in the same way. Blood samples were not available in many enrolled pts, so that a *detection bias* cannot be excluded. Treatment could be modified according to the results of neurological examination, SSEP at 72 h and EEG with possible *performance bias.* Therapeutic hypothermia was introduced during the study and used in a limited number of pts, and a confounding effect cannot be entirely excluded. Conclusions: The study therefore has been ranked as supportive of a predictive role of NSE to assess neurological outcome in comatose survivors of cardiac arrest (LOE P1, fair).

Zingler 2003 79-84

Study design: Prospective inception cohort study (LOE P1).
Study population: 27 consecutive comatose survivors of either in or out-of-hospital cardiac arrest.
Prognostic biomarker under evaluation: NSE and S100 levels as assessed on blood samples collected on days 1, 2, 3 and 7 after cardiac arrest. Other prognostic factors under investigation: SSEP as assessed on days 2 and 7, GCS, EEG and standardized neurological examination.
Outcome evaluated: neurological outcome at 2, 4 and 12 weeks after cardiac arrest (bad outcome defined as best CPC score during f.u. ≥ 3 vs. good outcome defined as best CPC score <3 during 6 months f.u).
 Results: NSE levels were higher in pts with unfavorable outcome on days 2, 3 and 7 but not on day 1. NSE release pattern was characterized by a progressive increase over days 1-3 in pts with unfavorable outcome but not in pts with favorable outcome. NSE levels ≥ 43 µg/l had a 100% specificity and a 90% sensitivity to identify pts with unfavorable outcome. The presence of both NSE levels ≥ 43 µg/l and S100 levels ≥ 0.5 µg/l was not associated with an improvement of predictive performance.
Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (presumably blinded) and follow up of pts was complete. Even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of its independent predictive role was not performed. The authors also assessed the correlation between SSEP and neurological outcome without a formal comparison with the biomarkers under investigation.
Conclusions. The study has been ranked as supportive of a predictive role of NSE (and of S100 as discussed separately in the relative

appropriate sections) to assess survival and neurological outcome in comatose survivors of cardiac arrest. (LOE P1, QOE fair)

Prohl 2007 1230-7

Study design: prospective study with derivation of a clinical decision rule (LOE P2).
Study population: 80 consecutive comatose pts admitted after either a IHCA or OHCA.
Prognostic biomarkers under evaluation: NSE and S-100B at days 2, 3 and 4 after CA. Prognostic biomarkers were investigated together with standardized clinical examinations (clinical examination score *CES* assessed on days 2 and 4), sensory-evoked potentials (N10, N20, N70 on day 4), and neuropsychological assessments (≤1 and 6 months) in a multidimensional prognostic assessment model.
Outcome evaluated: neurological outcome (bad outcome defined as CPC score ≥ 4 vs. good outcome defined as CPC score <4) and scores at neuropsychological testing.
Results: NSE and S-100B levels at all sampling times (days 2,3 and 4) were higher in pts with bad neurological outcome. Interestingly, NSE and S-100B peaked on day 2 and then decreased in pts with good outcome, while kept rising to reach a peak in day 3 (S-100B) or day 4 (NSE) in pts with unfavorable outcome. At univariate analysis by ROC, NSE at day 2, 3 and 4 were predictive of neurological outcome (cut-off, sensitivity and specificity respectively: day 2, 29.1 ng/ml, 33, 100 (FPR 0); day 3, 31.65 ng/ml, 33, 100 (FPR 0); day 4 27.85 ng/ml, 67,100 (FPR 0)). An attempt for multivariate analysis of several predictors (age, NSE at day 4, S-100B at days 2-4, CES at day 4, SEPs N10, N20 and N70) was made on 41 out of 66 pts who survived the first 4 days. This multivariate logistic-regression analysis resulted in a model in which 85% of the variance in the dichotomized CPC was explained by s-NSE at day 4, clinical examination score at day 4, and age. This predictor index had a sensitivity of 92% and a specificity of 93% (FPR 7%). ROC analysis was also performed to assess the diagnostic performance of predictors and to assess the sensitivity associated to the cut-off levels which were selected to give a 100 % specificity, or in other words to identify the most sensitive predictor at a pre-set specificity of 100% (NSE>27.85 at day 4 had a 100% specificity (FPR 0) and a 67 sensitivity to identify pts with unfavorable outcome).
Conclusions: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete for CPC assessment at 1 and 6 months while NPS assessment was available in 26 out of 33 survivors; NSE and S-100B on days 2, 3 and 4 were available in 69 pts; CES was available in 63pts; SEPs were available in 51 pts. The authors performed a multivariate analyses, which however was limited to 41 out of 66 survivors at day 4, thus hindering the relevance of the observations. Therefore, the study has been ranked as supportive of an independent predictive role of NSE at day 4 to assess the neurological outcome of comatose survivors of cardiac arrest (LOE P2 QOE fair), as the multivariate analysis presented a possible *selection bias*)

†*Dauberschmidt 1991 237-45

Study design: not unequivocally stated that the study was prospective, and therefore conservatively rated as retrospective LOE P3
Study population: 18 patients admitted to the intensive care unit after cardiac arrest and resuscitation
Prognostic biomarkers under evaluation: Serum NSE measured daily from admission
Outcome assessed: Survival and neurological outcome (defined as regaining consciousness)
Results: the NSE concentration was significantly higher after cardiac arrest in survivors as well as in non-survivors in comparison to the reference values in controls with slipped disc. In addition, the NSE concentration of the non-survivors was significantly higher as compared to survivors. In seven non-survivors the NSE concentration was in the pathological range (defined as a concentration above 7 µg//L) from first day, in one non-survivor it increased to pathological values within 4 days from admission. However, in six of the non-survivors (43%) NSE concentration never raised above 7.0 µg//L. In one survivor, NSE was above the threshold level of 7.0 µg/L. From these data the ILCOR reviewer has calculated the following parameters of predictive performance (death) of a s-NSE above 7 µg/L obtained within 4 days from admission from comatose survivors of cardiac arrest: Sensitivity: 57% (95% CI: 33-79%) Specificity: 75% (95% CI: 30-95); FPR 25% (95% CI 5-70).
Conclusions: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. This LOE P3 study offers fair evidence of a role of s-NSE in assessing prognosis of comatose survivors of cardiac arrest.

Rosen 2001 183-91

Study design: the study design has not been explicitly characterized as prospective and therefore it has been considered retrospective (LOE P3)
Study population: 66 consecutive comatose pts resuscitated from OHCA
Biomarker under evaluation: Serum S100 and NSE levels as determined on blood samples collected on days 1, 2 and 3; the timing of determination of blood levels has not been specified (determination short after sampling or retrospectively determined on stored frozen samples).
Other prognostic factors evaluated: brain stem reflexes, anoxia time and coma level on admission.
Outcome assessed: neurological outcome defined as favorable outcome (GOS 3-5) *vs.* unfavorable outcome (GOS 1-2).

Results: levels of S-100 and NSE on days 1–3 were higher among patients with a poor outcome compared with those with a good outcome. Positive predictive values and negative predictive values were computed ad different cut-offs separately on days 1, 2 and 3 for both S100 and NSE and are presented *in extenso* in the original manuscript. Of clinical interest: At day 1, NSE levels ≥25 µg/l had a PPV of 100% with a corresponding NPV of 39%; At day 2, NSE levels ≥25 µg/l had a PPV of 100% with a corresponding NPV of 47%; At day 3, NSE levels ≥15 µg/l had a PPV of 100% with a corresponding NPV of 53%.

In conclusion, the study has been ranked as a retrospective cohort study. Of note, it is unclear whether blood samples on days 2 and 3 were collected from still comatose pts. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded); follow up of pts was incomplete as a few pts were lost to follow-up, but for 2 out of 3 the GOS could be estimated. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a predictive role of s-NSE (and s-S100, as discussed separately in the appropriate section) from days 1 to 3 to assess neurological outcome of comatose survivors of OHCA (LOE P3, QOE fair)

Serum S-100

Thirteen eligible studies investigated the predictive performance of s-S100 to assess the prognosis of comatose survivors of cardiac arrest. A role of s-S100 to assess the prognosis of comatose survivors of cardiac arrest was supported by:

11 LOE P1 studies ((*LOE P1: Inception [prospective] cohort studies [or meta-analyses of inception cohort studies], or validation of Clinical Decision Rule[CDR]);* of these, 3 were QOE good [Mussack 2002 2669-74 Wijdicks 2006 203-10; Zandbergen et al. 2001 1661-1667], 7 were QOE fair [Bottiger 2001 2694-8; Hachimi-Idrissi 2002 251-7; Martens 1998 2363-66; Pfeifer 2005 49-55; Rosen 1998 473-77; Tiainen 2003 2881-86; Zandbergen 2006 62-8], 1 was QOE poor [Mussack 2001 539-43; discussion 44]

1 LOE P2 study *(LOE P2: Follow up of untreated control groups in RCTs (or meta-analyses of follow-up studies), or derivation of CDR, or validated on split-sample only)*, whose QOE was fair [Prohl 2007 1230-7]

1 LOE P3 studies *(LOE P3: retrospective cohort studies)* whose QOE was fair [Rosen 2001 183-91]

The predictive performance of s-S100 has been extensively analyzed in the following "*Citation list*" section and is here summarized as follows (for abbreviations see the *Abbreviations list* at the end of the worksheet):

Mussack 2002 2669-74

Study design: prospective inception cohort study LOE P1
Study population: 20 consecutive pts resuscitated from OHCA and surviving >12 h. The pts were not explicitly comatose at both sampling times but were presumably so as the authors state that "*Due to unconsciousness of the patients, informed consent was obtained for the first blood sampling retrospectively and for the second sampling prospectively in all cases within 6 hrs after study entry by the patients' relatives who were instructed about the purpose of blood*".
Biomarkers under evaluation: S100 and IL-8 as measured on blood samples collected on admission and 12 hours thereafter.
Outcome assessed: neurological outcome (GOS 1-3 unfavorable outcome; GOS 4-5 favorable outcome).
Results: S100 and IL 8 levels on admission after CA are similar to those observed after traumatic brain injury, and higher compared to healthy control. 12 hours after CA, median values of S100 decreased and IL8 increased compared to basal values. At multivariate logistic regression analysis, only age and S100 levels proved to be independent predictors of neurologic outcome. ROC analysis allowed to identify an optimal cut-off level of 0.76 ng/ml. At a cut-off of 0.76 ng/ml the positive predictive value was 100% (95% CI 56–100%), the specificity 100% (95% CI 31-100, i.e. FPR 0% (0-69%)), the negative predictive value 33 (95% CI 9-69%) and the sensitivity 54% (95% CI 26-80%).
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. S-100 levels appeared to be predictive of outcome both at univariate and multivariate analysis. Therefore, the study has been ranked as supportive of a predictive role of S-100 levels at 12 hours to assess neurological outcome. (LOE P1, QOE good)

Wijdicks 2006 203-10

Study design: Meta-analysis (LOE P1)
Study population: see individual studies (marked with † throughout the worksheet)
Biomarkers evaluated: serum NSE, serum S100, cerebrospinal fluid CKBB, cerebrospinal fluid neurofilament
Results: *Serum astroglial S100* has been investigated in one class I study [Zandbergen 2006 62-8], four class III studies [Pfeifer 2005 49-55, Tiainen 2003 2881-86, Martens 1998 2363-66, Rosen 1998 473-77] and one class IV study [Bottiger 2001 2694-98]. The median FPR was 2% (range 0 to 54%) in the four studies that allowed this calculation, and it was 5% in the class I study. Predictions were based on values measured within the

first 2 days after cardiac arrest.

Conclusions (as reported by the authors of the meta-analysis). "Serum NSE, S100, and CSF CKBB have been investigated as a predictor for outcome with studies using variable cutoff points. For serum NSE levels > 33 μg/L at days 1 to 3, one class I study Zandbergen 2006 [62-8] demonstrates a 0 FPR with narrow 95% CIs. Recommendations. Serum NSE levels >33 μg/L at days 1 to 3 post-CPR accurately predict poor outcome (recommendation level B). There are inadequate data to support or refute the prognostic value of other serum and CSF biochemical markers in comatose patients after CPR (recommendation level U)". Actually, the meta-analysis suggests that s-S-100 levels are associated to the prognosis of comatose survivors of cardiac arrest, but we agree that the predictive performance is unsatisfactory for clinical use.

†Zandbergen 2001 1661-67

Study design: Meta-analysis of prognostic studies (LOE P1). The specific objectives of the review were stated clearly. The study was carefully designed and criteria for inclusion were described. Characteristics and methodological quality of each trial were detailed and a log of excluded studies with reasons for exclusion was reported. The studies cited in the present meta-analysis and included in the worksheet are marked with an asterisk in the Tables and in the citation list. A methodological limitation of the present meta-analysis is that data were pooled irrespective of the nature of the study (either prospective or retrospective) and of blinding of physician to the results of the biomarker level. In conclusion, the study is a valuable systematic review of the literature, but the studies included in the meta-analysis were both prospective and retrospective and blinded and unblinded. However, the present study is supportive of a predictive role of S100 as assessed on blood samples to assess survival and neurological outcome in comatose survivors of cardiac arrest

	95% CI of the pooled false positive rates
Serum	
S100 > 0.7 μg/l	0.1-22.8

†Bottiger 2001 2694-98

Study design: prospective inception cohort study (LOE P1)
Study population: 66 patients undergoing cardiopulmonary resuscitation after non-traumatic cardiac arrest.
Biomarkers under investigation: Levels of S-100 (as determined on blood samples collected on admission and at 15, 30, 45, and 60 minutes; 2, 8, 24, 48, and 72 hours; and 7 days after initiation of cardiopulmonary resuscitation) and of NSE (as determined on blood samples collected between 2 hours and 7 days
Other prognostic markers under investigation: If patients survived for >48 hours, brain damage was assessed by a combination of neurological, cranial CT, and electrophysiological examinations.
Outcome evaluated: ROSC, survival and neurological outcome (defined as: group 1: no brain damage (patients discharged from the hospital fully oriented and without any communication defects (CPC1); group 2: documented brain damage (ie, according to neurological, cranial CT, and electrophysiological evaluations systematically performed between 48 hours and 96 hours after cardiac arrest; to focus on all patients, the data of surviving and non-surviving patients were combined here; group 3: no ROSC; group 4: patients who died soon after ROSC before assessment of brain damage.
Results: Maximum S-100 levels within 2 hours after cardiac arrest were significantly higher in patients with documented brain damage; significant differences between these 2 groups were observed from 30 minutes until 7 days after cardiac arrest. At 48 hours after cardiac arrest, an S-100 serum level of > 1.10 μg/L revealed a specificity of 100% (FPR = 0%) for the diagnosis of brain damage. The positive predictive value of the S-100 test at 2 hours for fatal outcome within 14 days was 79%, and the negative predictive value was 100% (P < 0.01). At 24 hours, the corresponding figures were 87% and 100% (P<0.001), and at 48 hours, they were 75% and 100% (P < 0.01), respectively.
Conclusions: groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis s-S-100 appeared to be predictive of outcome, a multivariate analysis of its independent predictive role was not performed. Therefore, the study has been ranked as supportive of a role of serum S-100 in predicting prognosis of comatose survivors of cardiac arrest (QOE fair)

Hachimi-Idrissi 2002 251-7

Study design: prospective inception cohort study (LOE P1)
Study population: Fifty-eight comatose patients resuscitated from out-of-hospital CA.
Biomarker under evaluation: S-100 levels on blood samples collected on admission and at 24 hours
Outcome assessed: neurological outcome defined as follows: Group 1 consisting of patients who died or remained in vegetative state;

Group 2 consisting of patients who regained consciousness. Of note, regaining consciousness was considered as a clinical end point regardless of the definitive outcome on discharge, therefore patients who eventually died later but who regained consciousness at any time during their stay were included in Group 2.

Results: Serum S-100 was significantly higher at admission and 24 h later in-group 1 compared with group 2. The highest specificity value for a poor outcome on admission was 85% (FPR 15%), obtained with a cut-off of 0.7 µg/l; at this cut-off, the sensitivity was 66.6%, the PPV was 84%, the NPV was 78% and the accuracy was 77.6%. All these values increased 24 h later up to 88.2 (FPR 11.8%), 100%, 86%, 100% and 93% respectively for specificity, sensitivity, PPV, NPV and accuracy.

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not formally performed. Therefore, the study has been ranked as supportive of a prognostic role of s-S-100 to assess neurological outcome. (LOE P1, QOE fair)

†*Martens 1998 2363-66

Study design: prospective inception cohort trial (LOE P1)
Study population: 64 pts who remained comatose > 24 hours after resuscitation from cardiac arrest.
Biomarkers under evaluation: NSE and S100 levels as determined on blood samples collected at 24 hours after admission and NSE and S100 levels as determined on cerebrospinal fluid collected by lumbar puncture at 48 hours after admission.
Outcome assessed: outcome was dichotomized into 2 groups: group 1 consisted of patients who died or remained in vegetative state, and group 2 consisted of patients who regained consciousness (ie, obeyed simple verbal commands). Regaining consciousness was considered an end point for follow-up, regardless of outcome at discharge or at 6 months. Patients who eventually died of multiorgan failure but who had clearly regained consciousness after cardiac arrest were classified in group 2.
Results: Serum S-100, serum NSE, S-100 CSF and NSE CSF were significantly higher in patients who never regained consciousness compared with those who regained consciousness. S-100 and NSE correlated well with each other in the CSF; both CSF concentrations also correlated with their serum concentrations; S-100 and NSE did not correlate at all with each other in serum. In detail, the predictive performance of the s-S-100 levels were: (cut-off 0.7 µg/L): Sensitivity 55%, Specificity 96%, FPR 4%;
 In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a prognostic role of s-S-100 (and of s-NSE, CSF-NSE, CSF-S-100, as presented in the appropriate relative sections),to assess the probability of a favorable neurological outcome. (LOE P1, QOE fair).

†Pfeifer 2005 49-55

Study design: Prospective (inception) cohort study (LOE P1).
Study population: 97 pts arrived at ICU within 12 hours of ROSC after non-traumatic cardiac arrest (both in-hospital and out-of-hospital) and survived for a minimum of 48 hours.
Prognostic biomarker under evaluation: Serum NSE and S-100 sampled for 5 days (once to three times a day).
GCS was also assessed on a daily basis. Outcome evaluated: survival and neurological outcome at 28 days (bad outcome defined as Glasgow Outcome Scale (GOS) 1-2 vs. good outcome defined as GOS 3-5).
Results: NSE and S-100 levels were significantly higher in pts with unfavorable outcome at 24 and 48 hours after ROSC, respectively.
Interestingly, while NSE and S-100 levels reached their peak and declined within 48 hours in pts with favorable outcome, NSE and S-100 levels reached their peak at 4 and 5 days respectively in pts with unfavorable outcome. S-100 levels ≥ 1.5 µg/l (not explicitly defined at which time, probably at any time) had a sensitivity of 34%, a specificity of 96% (FPR 4%) and a positive predictive value of 96% to predict a poor neurological outcome.
The authors did not attempt multivariate analyses, as the study population was not large enough to allow control for all known confounders and give conclusive results. Therefore, the study has been ranked as supportive of a predictive role of levels S-100 (and of NSE, as discussed separately in the relative section), and their combination to assess the neurological outcome of comatose survivors of CA (LOE P1, QOE fair)

†*Rosen 1998 473-77

Study design: prospective inception cohort trial (LOE P1).
Study population: 41 comatose pts resuscitated from OHCA.
Biomarker under evaluation: S100 levels as determined on blood samples collected on days 1, 2 and 3.
Outcome assessed: survival at 14 days.

Results: Levels of S100 were lower in survivors as compared to non-survivors at all sampling times. A cut-off of S100 levels ≤0.2 µg/L at day 1 had a PPV of 71% and a NPV of 85%. A cut-off of S100 levels ≤0.2 µg/L at day 2 had a PPV of 100% and a NPV of 89%. Sensitivity and specificity have been computed from the data presented on the manuscript. Sensitivity and specificity at day 1 were 77% and 81%, respectively (FPR 19%); Sensitivity and specificity at day 2 were 78% and 100% (FPR 0%), respectively.
 In conclusion, the study was a prospective inception cohort study. Of note, 7 pts entered the study on day 2 and it is unclear whether blood samples on days 2 and 3 were collected from still comatose pts. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a predictive role of S100 at days 1 and 2 to assess the probability of survival at 14 days (LOE P1, QOE fair)

†Tiainen 2003 2881-86

Study design: sub-study of a prospective inception cohort study (Hypothermia after Cardiac Arrest Trial) (LOE P1).
Study population: 70 adult pts arrived at ED after a cardiac arrest with several restrictive inclusion criteria: witnessed CA, cardiac rhythm with VF or pulseless VT as the initial rhythm, a presumed cardiac origin of the arrest, an estimated interval of 5 to 15 minutes from collapse to EMS intervention and an interval from collapse to ROSC < 60 min. Patients were randomized to therapeutic hypothermia (TH) or standard treatment (ST) .
Prognostic biomarker under evaluation: Serum NSE and S-100 sampled at 24, 36, 48 hours after ROSC.
Outcome evaluated: survival and neurological outcome at 6 months (bad outcome defined as CPC score ≥ 3 v.s. good outcome defined as CPC score 1-2).
Results: 36 out of the 70 pts were randomized to TH and 34 to ST. NSE and S-100 were available for 35/36 HT pts and 33/34 ST pts. After 6 months, a favorable outcome was observed in 69% of HT pts *vs*. 47 % of ST pts. A decrease of NSE levels between 24 and 48 hours was observed in 88% of HT pts as compared to 50% of ST pts; a decrease of S-100 levels between 24 and 48 hours was observed in 50% of HT pts as compared to 45% of ST pts. A decrease of NSE but not of S-100 between 24 and 48 hours was associated with a favorable outcome at 6 months. Cut-off levels associated with at least 95% specificity for predicting unfavorable outcome were calculated by ROC analysis for both NSE and S-100 at 24, 36 and 48 hours in HT and ST as separate groups. Cut-off levels associated with at least 95% specificity for predicting unfavorable outcome were higher in HT as compared to ST group, and sensitivity values associated to these cut-offs were remarkably lower in HT as compared to ST pts. The following table, extracted from the manuscript, details cut-off values and their performance

	Hypothermia				Normothermia			
	Cut-off µg/L	Specificity (%)	FPR (%)	Sensitivity (%)	Cut-off µg/L	Specificity (%)	FPR (%)	Sensitivity (%)
S-100								
24 h	0.21	100	0	30	0.19	100	0	59
36 h	0.21	96	4	20	0.12	100	0	88
48 h	0.23	96	4	22	0.12	100	0	88

Of interest, the AUCs at all study times were lower in the hypothermia group as compared to the normothermia group, again suggesting a less favorable predictive performance of the biomarkers under investigation in pts treated with therapeutic hypothermia.

	Hypothermia	Normothermia
	(AUC)	(AUC)
S-100		
24 h	0.65	0.90
36 h	0.69	0.85
48 h	0.63	0.91

AUC: Area under the curve at ROC analysis

Conclusions: The study population was a sample of 70 pts admitted to ED after CA with very selective inclusion criteria and randomized to TH or ST. Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was adequate. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a predictive role of NSE (data presented separately in the relative section) and S100 levels as determined on blood samples at 24, 36 and 48 hours to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their predictive performance and cut-off levels at comparable levels of specificity are different in pts treated with therapeutic hypothermia (LOE P1, QOE fair)

†Zandbergen 2006 62-8

Study design: Prospective (inception) cohort study (LOE P1).
Study population: 407 adult comatose survivors of CA.
 Prognostic biomarker under evaluation: Serum NSE and S-100 sampled at 24, 48 and 72 hours after CPR.
Other prognostic markers evaluated: clinical characteristics, median nerve SSEP at 24, 48 and 72 hours, and EEG at 72 h. Outcome
evaluated: neurological outcome (Glasgow outcome scale at 1 month and at 1 year in survivors
Results: Predictive performance of S-100 levels > 0.7 µg/ml was less satisfactory than NSE levels >33 µg/ml. False positive rate
(95% CI) of S-100 levels > 0.7 µg/ml were 3 (1-8), 2 (0-7), 0 (0-5), 2 (1-7) respectively at 24, 48, 72 and 24-72 hours;
 Conclusions: comparison groups were clearly defined. Outcome was assessed in the same way. Blood samples were not available in
many enrolled pts, so that a *detection bias* cannot be excluded. Treatment could be modified according to the results of neurological
examination, SSEP at 72 h and EEG with possible *performance bias.* Therapeutic hypothermia was introduced during the study and
used in a limited number of pts, and a confounding effect cannot be entirely excluded. The univariate predictive performance of
clinical variables, SSEP, NSE and S100-B was confirmed
Conclusions: The study therefore has been ranked as supportive of a predictive role of s-S100 levels (and of s-NSE, as discusses
separately in the appropriate section) to assess neurological outcome in comatose survivors of cardiac arrest (LOE P1, fair).

Mussack 2001 539-43; discussion 44

Study design: prospective inception cohort study (LOEP1)
Study population: 16 pts resuscitated after OHCA, unconscious on admission; it is unclear whether they were comatose at 12 hours.
Biomarkers under evaluation: S100, sP-selectin and sE-selectin as determined on blood samples collected shortly after ROSC and 12
hours later.
Outcome assessed: survival at 24 hours, 30 days and to hospital discharge.
Results: 11 pts survived longer than 24 hours, 3 pts longer than 30 days, 2 pts were discharged from hospital with no neurologic
impairment. According to the authors "Survival analyses revealed consistently high S-100b serum levels on scene (3.11 ng/ml;
2.23–3.98 ng/ml) and at 12 hours after ROSC (1.82 ng/ml; 1.59 –2.04 ng/ml) for the two patients dying within the first 24 hours,
whereas serum levels significantly decreased in patients surviving longer than 24 hours or 30 days", but the significance levels
of these differences as assessed by formal statistical testing were not provided.
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were
measured in the same way and follow up of pts was complete. The study has been ranked as supportive of a possible predictive role
of the course of S100 levels over the first 12 hours after resuscitation from a cardiac arrest, but is not conclusive as the pts population
is small, cut-offs have not been proposed, a multivariate analysis has not been performed and it is unclear whether pts were still
comatose at the time of the second blood sample (LOE P1, QOE poor)

Prohl 2007 1230-7

Study design: prospective study with derivation of a clinical decision rule (LOE P2).
Study population: 80 consecutive comatose pts admitted after either a IHCA or OHCA.
Prognostic biomarkers under evaluation: NSE and S-100B at days 2, 3 and 4 after CA. Prognostic biomarkers were investigated
together with standardized clinical examinations (clinical examination score *CES* assessed on days 2 and 4), sensory-evoked
potentials (N10, N20, N70 on day 4), and neuropsychological assessments (≤1 and 6 months) in a multidimensional prognostic
assessment model.
Outcome evaluated: neurological outcome (bad outcome defined as CPC score ≥ 4 v.s. good outcome defined as CPC score <4) and
scores at neuropsychological testing.
Results: NSE and S-100B levels at all sampling times (days 2,3 and 4) were higher in pts with bad neurological outcome.
Interestingly, NSE and S-100B peaked on day 2 and then decreased in pts with good outcome, while kept rising to reach a peak in
day 3 (S-100B) or day 4 (NSE) in pts with unfavorable outcome. At univariate analysis by ROC, S100 at day 2, 3 and 4 were
predictive of neurological outcome (cut-off, sensitivity and specificity respectively: day 2, 2.14 ng/ml, 17, 100 (FPR 0); day 3, 2.76
ng/ml, 17, 100 (FPR 0); day 4 1.16 ng/ml, 33,100 (FPR 0)).   An attempt for multivariate analysis of several predictors (age, NSE at
day 4, S-100B at days 2-4, CES at day 4, SEPs N10, N20 and N70) was made on 41 out of 66 pts who survived the first 4 days. This
multivariate logistic-regression analysis resulted in a model in which 85% of the variance in the dichotomized CPC was explained by
s-NSE at day 4, clinical examination score at day 4, and age. This predictor index had a sensitivity of 92% and a specificity of 93%
(FPR 7%).
Conclusions: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow
up of pts was complete for CPC assessment at 1 and 6 months while NPS assessment was available in 26 out of 33 survivors; NSE
and S-100B on days 2, 3 and 4 were available in 69 pts; CES was available in 63pts; SEPs were available in 51 pts. The authors
performed a multivariate analyses, which however was limited to 41 out of the 66 survivors at day 4, thus hindering the relevance of
the observations. Therefore, the study has been ranked as supportive of an independent predictive role of NSE at day 4 to assess the
neurological outcome of comatose survivors of CA, while S100, which was predictive at univariate analysis, failed to demonstrate a
similar predictive role at a multivariate analysis which however was limited to 41 out of the 66 survivors at day 4 with a possible

selection bias (LOE P2 Quality: fair)

Rosen 2001 183-91

Study design: the study design has not been explicitly characterized as prospective and therefore it has been considered retrospective (LOE P3)

Study population: 66 consecutive comatose pts resuscitated from OHCA; it has not been specified whether part of these pts were enrolled in a previous study from the same group and published in 1998.

Biomarker under evaluation: Serum S100 and NSE levels as determined on blood samples collected on days 1, 2 and 3; the timing of determination of blood levels has not been specified (determination short after sampling or retrospectively determined on stored frozen samples).

Other prognostic factors evaluated: brain stem reflexes, anoxia time and coma level on admission.

Outcome assessed: neurological outcome defined as favorable outcome (GOS 3-5) *vs.* unfavorable outcome (GOS 1-2).

Results: levels of S-100 and NSE on days 1–3 were higher among patients with a poor outcome compared with those with a good outcome. Positive predictive values and negative predictive values were computed ad different cut-offs separately on days 1, 2 and 3 for both S100 and NSE and are presented *in extenso* in the original manuscript. Of clinical interest: at day 1, S100 levels ≥0.4 µg/l had a PPV of 88% with a corresponding NPV of 55%; at day 2, S100 levels ≥0.25 µg/l had a PPV of 100% with a corresponding NPV of 58%; at day 3, S100 levels ≥0.20µg/l had a PPV of 100% with a corresponding NPV of 54%.

In conclusion, the study has been ranked as a retrospective cohort study. Of note, it is unclear whether blood samples on days 2 and 3 were collected from still comatose pts. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded); follow up of pts was incomplete as a few pts were lost to follow-up, but for 2 out of 3 the GOS could be estimated. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a predictive role of NSE (data presented separately in the appropriate relative section) and S100 from days 1 to 3 to assess neurological outcome of comatose survivors of OHCA (LOE P3, QOE fair)

Serum BNP

A single eligible study investigated the predictive performance of Serum BNP to assess the prognosis of comatose survivors of cardiac arrest

1 LOE P3 study *(LOE P3: retrospective cohort studies)* [Sodeck 2007 439-45], whose QOE was fair supports an association between serum BNP and the prognosis of comatose survivors of cardiac arrest.

The predictive performance of s-BNP has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet):

Sodeck 2007 439-45

Study design: Retrospective cohort study (observational case series with convenience sampling, LOE P3).

Study population: 155 non-consecutive comatose cardiac arrest survivors.

Prognostic biomarker under evaluation: BNP on admission to emergency department from arterial line.

Outcome evaluated: survival and neurological outcome at 6months (bad outcome defined as best CPC score during 6 months f.u. ≥ 3 vs. good outcome defined as best CPC score <3 during 6 months f.u).

Results: BNP on admission was significantly higher in pts with unfavorable outcome. At univariate analysis, BNP levels on admission in the upper quartile (> 230 pg/ml) were predictive of the combined end-point of non-survival or unfavorable outcome. The authors attempted a multivariate analysis to adjust the results for the confounding factors: age, diabetes, chronic heart failure, initial rhythm, cumulative epinephrine, lactate levels on admission; at multivariate analysis, BNP on admission in the 4th quartile (> 230 pg/ml) was independently predictive of survival and unfavorable neurologic outcome.

Quality of evidence: The study population was a convenience sample of 155 out of 697 comatose CA pts admitted to ED after ROSC. Comparisons groups were clearly defined, outcomes were measured in the same way (presumably blinded) and follow up of pts was complete. However, even though the authors attempted multivariate analyses, the study population was not large enough to allow control for all known confounders and give conclusive results. Therefore, the study has been ranked as supportive of a predictive role of BNP to assess survival and neurological outcome in comatose survivors of cardiac arrest. (LOE P3, QOE fair)

Serum IL8

Two eligible studies investigated the predictive performance of Serum IL8 to assess the prognosis of comatose survivors of cardiac arrest

1 LOE P3 study *(LOE P3: retrospective cohort studies)* [Adrie 2002 562-8], whose QOE was fair, offered a neutral evidence for a role of IL-8 to assess prognosis of comatose survivors of cardiac arrest

1 LOE P1 study(*(LOE P1: Inception [prospective] cohort studies [or meta-analyses of inception cohort studies], or validation of Clinical Decision Rule[CDR])* [Mussack 2002 2669-74], whose QOE was good opposed a role of IL-8 to assess prognosis of comatose survivors of cardiac arrest

The predictive performance of s-IL8 has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet):

Adrie 2002 562-8

Study design: Not overtly characterized as prospective and therefore ranked as LOE P3
Study population: 61 comatose survivors aged>16 resuscitated after OHCA and a Glasgow coma score of 3 (range 3 to 4) on admission
Prognostic biomarkers under evaluation: Plasma endotoxin, sTNFRII, IL-1ra, IL-6, IL-8, IL-10, RANTES, Lactate, bicarbonate and ex-vivo cytokine production.
Other prognostic factors under evaluation: clinical characteristics of pts on admission.
Outcome evaluated: survival.
Results. Levels of lactate, bicarbonate, sTNFRII, IL-6, IL-8, and IL-10 on admission were significantly higher in non-survivors than in survivors. Among the above cited cytokines or soluble receptors, there was no independent variable predictive of death compared with the interval from collapse to basic life support and duration of cardiopulmonary resuscitation..
Conclusions. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis several biomarkers under evaluation appeared to be significantly different in survivors compared to non-survivors, at multivariate analysis none of them was independently predictive of outcome after correction for the confounding effect of time from collapse to BLS and duration of resuscitation maneuvers; the possibility that the study was underpowered to detect an independent predictive role of the prognostic variables under investigation should be considered. In conclusion, the study has been ranked as of neutral [favoring a role at univariate analysis but opposing a role at underpowered multivariate analysis) evidence of a role of s-IL-8, to the clinical question (QOE fair).

Mussack 2002 2669-74

Study design: prospective inception cohort study LOE P1
Study population: 20 consecutive pts resuscitated from OHCA and surviving >12 h. The pts were not explicitly comatose at both sampling times but were presumably so
Biomarkers under evaluation: S100 and IL-8 as measured on blood samples collected on admission and 12 hours thereafter.
Outcome assessed: neurological outcome (GOS 1-3 unfavorable outcome; GOS 4-5 favorable outcome).
Results: S100 and IL 8 levels on admission after CA are similar to those observed after traumatic brain injury, and higher compared to healthy control. 12 hours after CA, median values of S100 decreased and IL8 increased compared to basal values. At multivariate logistic regression analysis, only age and S100 levels proved to be independent predictors of neurologic outcome. In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. S-S-100 levels but not of s-IL-8 appeared to be predictive of outcome both at univariate and multivariate analysis. Therefore, the study has been ranked as opposing a predictive role of s-IL-8 at 12 hours to assess neurological outcome. (LOE P1, QOE good)

Serum vWF and ICAM-1

One eligible study investigated the predictive performance of *Serum vWF and ICAM-1 t*o assess the prognosis of comatose survivors of cardiac arrest

One LOE P3*(LOE P3: retrospective cohort studies)* [Geppert 2003 805-11] study, whose QOE was fair, supports an independent role of serum vWF to assess prognosis of comatose survivors of cardiac arrest.

One LOE P3*(LOE P3: retrospective cohort studies)* [Geppert 2003 805-11] study, whose QOE was fair, supports a role of serum ICAM1 at univariate but not at possibly underpowered multivariate analysis to assess prognosis of comatose survivors of cardiac arrest and therefore has been considered of neutral evidence

The predictive performance of s-vWF and s-ICAM1 has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet):

Geppert 2003 805-11

Study design: retrospective study on stored plasma samples (LOE P3).
Study population: 35 non-consecutive pts who survived > 24 h after either a IHCA or OHCA not awake after 24 hours after cardiac arrest (not excluded because they were awake and transferred to the ward within the first 24 hours after CA.)
Prognostic biomarkers under evaluation: vWF and s-ICAM on stored plasma samples collected 2 days after CA.
Outcome evaluated: neurological outcome (bad outcome defined as CPC score $\geq$ 3 vs. good outcome defined as CPC score <3).
Results: vWF concentration was significantly higher in pts with bad neurological outcome ($p<0.001$). ICAM-1 concentration was non-significantly higher in pts with bad neurological outcome ($p<0.097$). vWF >166% and ICAM-1 > 500 ng/ml were 100% specific (FPR=0, with a sensitivity of 80% and 70% respectively) to identify pts with unfavorable outcome after OHCA, but clinically useful cut-offs could not be found for IHCA. A cardiac arrest score was developed on the basis of factors resulted to be predictive of adverse outcome at univariate analysis (renal dysfunction/failure, severe cardiovascular failure, systemic inflammatory syndrome and CPR duration > 15 minutes) whose predictive accuracy was improved by vWF and ICAM concentrations. At multivariate analysis, vWF concentration and CPR duration were the most significant predictors of outcome.
 Conclusions: Comparisons groups were clearly defined, outcomes were measured in the same way (presumably blinded) and follow up of pts was complete. At univariate analysis the biomarkers under evaluation appeared to be predictive of outcome; the authors also attempted a multivariate analyses to confirm an independent prognostic role of vWF and ICAM. At multivariate analysis, vWF concentration and CPR duration were the most significant predictors of outcome. Therefore, the study has been ranked as supportive, (LOE P3, QOE fair) of a role of vWF (neurological outcome) to assess the prognosis of comatose patients after OHCA; evidence on a possible prognostic role of ICAM appeared to be weaker and conflicting (predictive at univariate but not at multivariate analysis on a small sized sample) and has been ranked as neutral (neurological outcome) LOE P3 QOE fair.

Plasma endotoxin, sTNFRII, IL-1ra, IL-6, IL-10, RANTES

One study investigated the predictive performance of *Plasma endotoxin, sTNFRII, IL-1ra, IL-6, IL-10, RANTES* in comatose survivors of cardiac arrest.

One LOE P3 *(LOE P3: retrospective cohort studies)* study[Adrie 2002 562-8], whose QOE was fair, showed that levels of sTNFRII, IL-6, IL-8, and IL-10, lactate and bicarbonate on admission were significantly higher in non-survivors than in survivors. However, among the cytokines or soluble receptors, there was no independent variable predictive of death compared with the interval from collapse to basic life support and duration of cardiopulmonary resuscitation. An independent predictive role of lactate and bicarbonate was not explicitly stated. In conclusion, the study has been ranked as of neutral [favoring a role at univariate analysis but opposing a role at underpowered multivariate analysis) evidence of a role of plasma sTNFRII, IL-6, IL-8, IL-10 to assess the prognosis of comatose survivors of cardiac arrest.

One LOE P3 study*(LOE P3: retrospective cohort studies)* [Adrie 2002 562-8], whose QOE was fair, showed that levels of IL1-ra and RANTES were not significantly different in non-survivors as compared to survivors and that there was not any correlation between plasma endotoxin and mortality.

The predictive performance of *Plasma endotoxin, sTNFRII, IL-1ra, IL-6, IL-10, RANTES* has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

Adrie 2002 562-8

Study design: Not explicitly characterized as prospective and therefore ranked as LOE P3
Study population: 61 comatose survivors aged>16 resuscitated after OHCA and a Glasgow coma score of 3 (range 3 to 4) on admission (Table 1).
Prognostic biomarkers under evaluation: Plasma endotoxin, sTNFRII, IL-1ra, IL-6, IL-8, IL-10, RANTES, Lactate, bicarbonate and ex-vivo cytokine production.
Other prognostic factors under evaluation: clinical characteristics of pts on admission.
Outcome evaluated: survival.
Results. levels of lactate, bicarbonate, sTNFRII, IL-6, IL-8, and IL-10 on admission were significantly higher in non-survivors than in survivors. No significant differences in the concentration of IL1-ra and RANTES could be found between survivors and non-survivors. However, among the above cited cytokines or soluble receptors, there was no independent variable predictive of death compared with the interval from collapse to basic life support and duration of cardiopulmonary resuscitation. A significant

independent prognostic role of lactate and bicarbonate at multivariate analysis was not explicitly evidenced. Moreover, there was no correlation between the presence of endotoxin and mortality. Production of cytokines in whole-blood assays on in vitro stimulation and in vitro plasma exchange experiments showed several differences between survivors of cardiac arrest and controls, but their ability to assess prognosis of comatose survivors of cardiac arrest was not explored.

Conclusions: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis several biomarkers under evaluation appeared to be significantly different in survivors compared to non-survivors, at multivariate analysis none of them was independently predictive of outcome after correction for the confounding effect of time from collapse to BLS and duration of resuscitation maneuvers; the possibility that the study was underpowered to detect an independent predictive role of the prognostic variables under investigation should be considered.

In conclusion, the study has been ranked as of neutral [favoring a role at univariate analysis but opposing a role at underpowered multivariate analysis) evidence of a role of plasma sTNFRII, IL-6, IL-10 (and IL-8, as discussed separately in the appropriate section) to the clinical question (QOE fair). Moreover, the study has been considered as opposing a possible role of IL1-ra and RANTES to assess the prognosis of comatose survivors of cardiac arrest (QOE fair)

Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) and procalcitonin (PCT)

One eligible study investigated the predictive performance of *Serum sTREM-1* and *PCT* to assess the prognosis of comatose survivors of cardiac arrest

One LOE P3 study *(LOE P3: retrospective cohort studies)* [Adib-Conquy 2007 406-10], whose QOE was fair, offered evidence in favor of a possible role of serum sTREM-1 and PCT to assess the prognosis of comatose survivors of cardiac arrest

The predictive performance of sTREM-1 and PCT has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

Adib-Conquy 2007 406-10

Study design: retrospective cohort study (LOE P3).

Study population: 54 pts with successfully resuscitated OHCA, retrospectively retrieved from a database of previous studies (GCS on admission not specified in the present study, but extracted from previous studies GCS=3 [3-4]) and compared with: (1) a group of pts undergoing cardiac surgery (2) a group of healthy controls and (3) a group of pts with severe sepsis.

Prognostic biomarkers under evaluation: Levels of soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) and procalcitonin (PCT), as determined on stored plasma samples collected on admission, at day 2 and day 3

Outcome evaluated: survival and neurological outcome categorized in 3 groups: (Group 1) survival and CPC 1-2; (Group 2) death from neurological failure; (Group 3) death from shock.

Results. Both sTREM-1 and PCT were significantly increased in cardiac arrest patients who died of refractory shock as compared to those who died of neurological failure or survived without major neurological damage, but no clinically useful cut-off were proposed In conclusion there is fair evidence that sTREM-1 and PCT are predictive of death from refractory shock at univariate analysis the authors do not propose indicators of predictive performance at different cut-offs (i.e. sensitivity, specificity, positive and negative predictive values, false positive rate). For these reasons the study has been ranked as supporting an association between the biomarkers under evaluation and death from refractory shock, but is of limited clinical applicability (QOE fair)

Cerebrospinal fluid (CSF) biomarkers

CSF-CK

Two eligible studies investigated the predictive performance of CSF-CK to assess the prognosis of comatose survivors of cardiac arrest

2LOE P1 study (LOE P1: Inception (prospective) cohort studies (or meta-analyses of inception cohort studies), or validation of Clinical Decision Rule (CDR)) [Longstreth 1984 834-7, Karkela 1992 378-86], whose QOE was fair, support an association between CSF-CK and the prognosis of comatose survivors of cardiac arrest

The predictive performance of CSF-CK has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

*Longstreth 1984 834-7

Study design: prospective inception cohort study (LOE P1).

Study population: Thirty patients resuscitated from OHCA
Biomarker under evaluation: Total CK levels on cerebrospinal fluid collected at a mean time of 18 hours (range 5-53).
Outcome assessed: neurological outcome defined as awakening (following commands or having comprehensible speech) *vs.* not awakening. Results: One pt was excluded from analysis before knowing CSF-CK levels or outcome as soon as the authors became aware that he had a head trauma. CSF-CK was significantly higher in non-awakening as compared to awakening pts (120U/l *vs.* 10 U/L respectively, *p<0.007*). Using a cut-off level of 25U/L "only five patients were misclassified"; from the data presented in the paper, a sensitivity of 64%, a specificity of 100% (FPR 0%), a PPV of 100% and a NPV of 75% were calculated for the proposed cut-off; CSF-CK was still significantly higher in non-awakening *vs.* awakening pts even after excluding from the analysis 2 pts with brain death on physical examination and isotopic flow study (120U/l *vs.* 10 U/L respectively, *p<0.03*).
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way; follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not formally performed. Therefore, the study has been ranked as supportive of a prognostic role of CSF-CK levels to assess neurological outcome. (LOE P1, QOE fair)

Karkela 1992 378-86

Study design: Prospective inception cohort study (LOE P1).
Study population: 20 comatose survivors of cardiac arrest.
Prognostic biomarkers under evaluation: Concentrations in the CSF of CK, CK-BB, LDH and its isoenzymes (1-5), acid phosphatase, beta-D-N-acetylglucosaminidase, lactate, pyruvate, sodium, potassium and calcium as determined on samples collected at 4, 28, 76 and 172 hours after the initiation of resuscitation.
Outcome evaluated: neurological outcome at 1 year
Results: 8 pts recovered while 12 pts remained comatose or neurologically disabled until death. 6 out of the 8 pts who presented neurological recovery were discharged without intellectual or neurological deficit but with short term memory disturbances. CSF-CK was higher in pts with unfavorable outcome at 28 and 76 hours (p<0.001 and p=0.018 respectively); CSF-CKBB was higher in pts with unfavorable outcome at 28 and 76 hours (p=0.014 and p=0.010 respectively).
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. The results of the dosages of the biomarkers under evaluation do not appear to have influenced the treatment. Even though several biomarkers levels appeared to be significantly different at univariate analysis in pts with unfavorable outcome, the authors have not performed a multivariate analysis to allow control for several confounders. No cut-off for the biomarkers have been suggested. Therefore, the study has been ranked as supportive of a prognostic role of CSF-CK at 28 and 76 hours, but is not conclusive. (LOE P1, QOE fair).

CSF CK-BB

Nine eligible studies investigated the predictive performance of CSF-CKBB to assess the prognosis of comatose survivors of cardiac arrest

A role of CSF-CKBB to assess the prognosis of comatose survivors of cardiac arrest was supported by:

6 LOE P1 studies ((LOE P1: Inception [prospective] cohort studies [or meta-analyses of inception cohort studies], or validation of Clinical Decision Rule[CDR]); of these 2 were QOE good [Wijdicks 2006 203-10; Zandbergen 2001 1661-67], 2 were QOE fair [Karkela 1992 378-86 Martens 1996 126-31, Roine 1989 753-6], and 1 was QOE poor, [Rothstein 1991 101-7]
2 LOE P3 studies (LOE P3: retrospective cohort studies); of these 1 was QOE fair [Sherman 2000 889-94] and 1 was QOE poor [Longstreth 1981 455-8 Tirschwell 1997 352-7]

A role of CSF-CKBB to assess the prognosis of comatose survivors of cardiac arrest was opposed by:

1 LOE P1 (LOE P1: Inception [prospective] cohort studies [or meta-analyses of inception cohort studies], or validation of Clinical Decision Rule[CDR]) eligible study [Clemmensen 1987 235-6,] whose QOE was poor

The predictive performance of CSF-BB has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

Wijdicks 2006 203-10

Study design: Meta-analysis (LOE P1)
Study population: see individual studies (marked with † throughout the worksheet)

Biomarkers evaluated: serum NSE, serum S100, cerebrospinal fluid CKBB, cerebrospinal fluid neurofilament

Six class III studies [Longstreth 1981 455-8, Clemmensen 1987 235-6, Roine 1989 753-6, Rothstein 1991 101-7, Tirschwell 1997 352-7, Sherman 2000 889-94] investigated the usefulness of CSF CKBB as an indicator of poor outcome. Values used to identify those with poor outcome varied widely. The median FPR was 15% (range 0 to 33%) in six studies allowing the calculations, indicating a poor prognostic ability. Furthermore, the availability of this test result could have influenced the decision to withdraw life support.

Conclusions (as reported by the authors of the meta-analysis). "Serum NSE, S100, and CSF CKBB have been investigated as a predictor for outcome with studies using variable cutoff points. For serum NSE levels > 33 μg/L at days 1 to 3, one class I study [Zandbergen 2006 62-8] demonstrates a 0 FPR with narrow 95% CIs. Recommendations. Serum NSE levels >33 μg/L at days 1 to 3 post-CPR accurately predict poor outcome (recommendation level B). There are inadequate data to support or refute the prognostic value of other serum and CSF biochemical markers in comatose patients after CPR (recommendation level U)". Actually, the meta-analysis provides evidence in favor of an association between CSF-CKBB and the prognosis of comatose survivors of cardiac arrest, but the predictive performance is unsatisfactory for clinical use.

Zandbergen 2001 1661-67

Study design: Meta-analysis of prognostic studies (LOE P1). The specific objectives of the review were stated clearly. The study was carefully designed and criteria for inclusion were described. Characteristics and methodological quality of each trial were detailed and a log of excluded studies with reasons for exclusion was reported. The studies cited in the present meta-analysis and included in the worksheet are marked with an asterisk in the Tables and in the citation list. A methodological limitation of the present meta-analysis is that data were pooled irrespective of the nature of the study (either prospective or retrospective) and of blinding of physician to the results of the biomarker level. In conclusion, the study is a valuable systematic review of the literature, but the studies included in the meta-analysis were both prospective and retrospective and blinded and unblinded. However, the present study is supportive of a predictive role of CKBB, NSE, S100, LDH and GOT levels as determined on the CSF and of CKBB, NSE and S100 as assessed on blood samples to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their clinical application for non-treatment decisions is limited mainly by the wide confidence limits of the pooled estimates of false positive rates and of positive-likelihood ratio as detailed in the following table.

	95% CI of the pooled false positive rates
CSF	
CKBB>20 UI/L	3.0-7.9
CKBB>20ng/ml	2.7-32.4
CKBB>204U/l	0-2.3

Karkela 1992 378-86

Study design: Prospective inception cohort study (LOE P1).
Study population: 20 comatose survivors of cardiac arrest.
Prognostic biomarkers under evaluation: Concentrations in the CSF of CK, CK-BB, LDH and its isoenzymes (1-5), acid phosphatase, beta-D-N-acetylglucosaminidase, lactate, pyruvate, sodium, potassium and calcium as determined on samples collected at 4, 28, 76 and 172 hours after the initiation of resuscitation.
Outcome evaluated: neurological outcome at 1 year
Results: 8 pts recovered while 12 pts remained comatose or neurologically disabled until death. 6 out of the 8 pts who presented neurological recovery were discharged without intellectual or neurological deficit but with short term memory disturbances. CSF-CK was higher in pts with unfavorable outcome at 28 and 76 hours (p<0.001 and p=0.018 respectively); CSF-CKBB was higher in pts with unfavorable outcome at 28 and 76 hours (p=0.014 and p=0.010 respectively);
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. The results of the dosages of the biomarkers under evaluation do not appear to have influenced the treatment. Even though several biomarkers levels appeared to be significantly different at univariate analysis in pts with unfavorable outcome, the authors have not performed a multivariate analysis to allow control for several confounders. No cut-off for the biomarkers have been suggested. Therefore, the study has been ranked as supportive of a prognostic role of CSF-CK at 28 and 76 hours, CSF-CKBB at 28 and 76 hours to assess neurological outcome, but is not conclusive and of limited applicability for clinical use. (LOE P1, QOE fair).

*Martens 1996 126-31

Study design: prospective inception cohort study (LOE P1).
Study population: Fifty-two consecutive patients who remained comatose after successful resuscitation from OHCA.
Biomarker under evaluation: Serum NSE levels assessed on blood samples collected 24 hours after cardiac arrest; in 16 pts who

remained alive but comatose a lumbar puncture was performed to assess CSF CPK-BB, CSF-AST and CSF-LDH.

Outcome assessed: neurological outcome, defined as return of consciousness vs. death due to CNS failure; pts regaining consciousness but eventually dying from non-neurologic causes as well as pts with neurologic impairment were considered as pts with successful outcome.

Results: The predictive performance of CSF-CK-BB and CSF-LDH was calculated by the ILCOR reviewer in 15 of 16 pts in whom the lumbar puncture was performed (one pts had an undetermined outcome and was excluded from the analysis; CSF-GOT was not determined in 4 pts and therefore calculations were not attempted). Sensitivity, specificity, and FPR for CSF-CPK-BB were 83% (95% CI 55-95), 100% (95% CI 44-100) and 0 % (95%CI 0-66). Biomarkers levels were not used to take treatment decisions.

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and in a blinded fashion; follow up of pts was complete. CSF-CPK-BB (and CSF-GOT and CSF-LDH as discussed separately in the appropriate sections) were predictive of outcome at some extent at univariate analysis, but were assessed only in a subgroup of pts. Therefore, the study has been ranked as supportive of a prognostic role CSF-CPK-BB to assess neurological outcome of comatose survivors of cardiac arrest (QOE fair)

*Rothstein 1991 101-7

Study design: Prospective inception cohort study (LOE P1)

Study population: 16 out of 40 patients comatose for at least 6 hours after cardiac arrest

Prognostic biomarker under evaluation: CK-BB obtained on CSF by lumbar puncture 18-26 hours after cardiac arrest (cut-off arbitrarily set at 20 ng/ml)

Other prognostic factors under evaluation: EEG and SSEP

Outcome evaluated: poor outcome (defined as death without awakening plus survival with motor/cognitive impairment)

Results: CSF-CKBB was obtained only in 16 out of 40 pts enrolled in the study (primary endpoint of the study was evaluation of the predictive performance of SSEP and EEG). CSF-CKBB was below the arbitrary cut-off of 20 ng/ml (normal) in 6 pts, 2 of which died without awakening, 1 survived with neurological deficit and 3 recovered without deficits. CSF-CKBB was equal or above the arbitrary cut-off of 20 ng/ml (abnormal) in 10 pts, 8 of which died without awakening and 2 survived with neurological deficit. The following predictive performance indicators of an abnormal value of CSF-CKBB to predict death without awakening were reported by the authors: sensitivity 80%, specificity 67%, NPV 67%, PPV 80%. The ILCOR reviewer calculated, on the basis of the published data, the following further indicators of predictive performance: sensitivity 80 (95%CI 49-94), specificity 67 % (95% CI 30-90), FPR 33% (95% CI 10-70).

Quality of evidence: The prognostic biomarkers under evaluation was assessed only in 16 out of 40 pts (it was not the primary objective of the study) and reasons for not assessing are not reported, resulting in a significant selection bias. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded). Even though at univariate analysis the biomarker under evaluation appeared to be predictive of outcome, a multivariate analysis of its independent predictive role was not performed. Therefore, the study has been ranked as supportive of abnormal CK-BB levels as determined on CSF samples collected at days 18-26 h after CA to predict death without awakening, but with significant methodological limitations and with an unsatisfactory predictive performance (LOE P1, QOE poor)

*Sherman 2000 889-94

Study design: retrospective cohort study (LOE P3).

Study population: 52 pts out of 72 comatose pts after cardiac arrest.

Prognostic biomarker under evaluation: Levels of CKBB as determined on cerebrospinal fluid sampled by lumbar puncture (17/52 between 48-72 hours after CA, 31/52 between 2-3 days after CA, no details about timing of lumbar puncture in the remaining pts). Prognostic biomarker was investigated together with somato-sensory evoked potentials (SEP), GCS, early pupillary reactivity, EEG results, CT results, presence of myoclonus and seizures all as abstracted from medical records.

Outcome evaluated: neurological outcome defined as awakening (ability to follow command, to use comprehensible speech or both).

Results: CSF-CKBB ≥ 205 U/L predicted non-awakening with a sensitivity of 49% and a specificity of 100% (FPR 0).

Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded). Results of the test under investigation may have affected treatment decision, with a possible performance bias. The univariate predictive performance of the markers under investigation and their combination has been retrospectively assessed, but multivariate analysis has not been attempted. Therefore, the study has been ranked as supportive of a predictive role of CK-BB levels as determined on CSF to assess neurological outcome of comatose survivors of cardiac arrest. (LOE P3, QOE fair).

†*Longstreth 1981 455-8

Study design: retrospective study (LOE P3)

Study population: 52 patients retrospectively extracted from a population of 55 pts admitted after resuscitation from OHCA

Prognostic biomarkers under evaluation: Serum CKBB levels (as determined on 2 serial samples collected <6 h after cardiac arrest

and > 6h after cardiac arrest) and, in a subgroup of 20 pts in which they were available, CSF CKBB levels.
Outcome evaluated: survival and neurological outcome, defined as: group 1, no neurologic recovery and brief survival; group 2, full neurologic recovery; group 3, incomplete neurological recovery (ranging from dementia to persistent vegetative state); group 4, cardiac death.
 Results: CKBB levels in CSF and serum were not available in all patients (see Table), thus limiting the value of the following analyses.

	CSF	Serum < 6 h	Serum > 6 h
Group 1 (n=10)	5/10	9/10	10/10
Group 2 (n=26)	8/26	26/26	26/26
Group 3 (n=11)	7/11	10/11	11/11
Group 4 (n=8)	0/8	5/8	n.a.

Sampled pts in Group 1 had higher CSF-CKBB levels compared to Group 2 (p<0.001) and Group 3 (p<0.003); sampled pts in Group 3 had higher CSF-CKBB levels compared to Group 2 (p<0.001); The authors concluded that both the levels of CSF-CKBB may be a useful predictor of neurologic outcome after cardiac arrest.
 Quality of evidence: the study was retrospective in nature and assessment of biomarker's levels was not available in all pts, with a potential for a detection bias. Comparisons groups were clearly defined, outcomes were measured in the same way (not stated whether in a blinded fashion, presumably not) and follow up of pts was complete. At univariate analysis the biomarkers under evaluation (positive vs. negative) appeared to be predictive of outcome; the authors did not attempt a multivariate analyses to confirm an independent prognostic role of the biomarkers under evaluation. Therefore, the study has been ranked as supportive, of a prognostic role of CSF-CKBB to assess survival and neurological outcome (LOE P3, QOE poor).

*Tirschwell 1997 352-7

Study design: Retrospective cohort study (LOE P3).
Study population: 351 comatose cardiac arrest survivors extracted from a database of 474 pts admitted to an ICU who had their CK-BB levels assessed on CSF for prognostic stratification.
Prognostic biomarker under evaluation: CK-BB levels assessed on CSF (given the retrospective nature of the study, sampling was not performed according to a standardized protocol at specific times, but "usually…48-72 hours after cardiac arrest") .
Outcome evaluated: neurological outcome defined as awakening vs. non-awakening on the basis of information extracted from medical charts (awakening was defined as pt having comprehensible speech, following commands or both).
Results: patients. CSF CKBB was significantly higher for those who never awakened compared with those who ever awakened. The predictive performance of CSF CKBB was analyzed at different cut-offs, as detailed in the following table.

CSF-CKBB cut-off (U/l)	Sensitivity	Specificity	FPR	PPV
10	90	62	38	92
20	87	72	28	94
30	84	79	21	95
50	82	85	15	96
100	69	92	8	98
150	59	97	3	99
205	48	100	0	100
250	41	100	0	100

Quality of evidence: The study population was retrospectively assembled by extracting the medical charts of 474 pts in whom a determination of CKBB CSF levels was ordered by the treating physician to assess prognosis. 351 of out of these 474 were survivors of cardiac arrest. Timing of CSF sampling by lumbar puncture could not be assessed precisely but is reported by the authors to be "usually" 48-72 hours after cardiac arrest. Of methodological relevance, the results of CSF CKBB determination might have affected treatment in some pts, with a performance bias and a possible so-called self-fulfilling prophecy . Comparisons groups were clearly defined, outcomes were measured in the same way (not blinded). All patients were followed until they awakened or died, except for three patients who were unconscious at the time of the last contact at 63, 107, and 109 months their arrests. Even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of its independent

predictive role was not performed. Therefore, the study has been ranked as supportive of a predictive role of CSF CKBB levels as determined in comatose survivors of cardiac arrest 48-72 hours after the event (LOE P3, QOE poor)

*Clemmensen 1987 235-6

Study design: prospective inception cohort trial LOE P1
Study population: 21 consecutive pts who remained comatose after successful resuscitation from OHCA.
Prognostic biomarkers under evaluation: CK-BB levels as assessed form cerebrospinal fluid sampled by lumbar puncture between 6 and 72 hours after resuscitation.
Outcome assessed: Survival, defined as survival to hospital discharge, and neurological outcome, without a clear definition.
Results: The median values of CSF-CKBB were 5 U/l in non-survivors as compared to values below the detection limit of the assay in survivors (p= n.s). The positive predictive value of a positive test was 66.6%; the negative predictive value of a positive test was 25%. The authors reported that "no correlation between cerebral recovery and CSF-CKBB was found" but no other individual or aggregate data are presented.
Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. Even though at univariate analysis the biomarkers under evaluation did not result to be predictive of outcome, the power of the study was rather limited due to the small size of the patient population.
Therefore, the study has been ranked as of opposing evidence to the clinical question (QOE poor)

CSF-NSE

Three eligible studies investigated the predictive performance of CSF-NSE to assess the prognosis of comatose survivors of cardiac arrest.

3 LOE P1 studies (LOE P1: Inception (prospective) cohort studies (or meta-analyses of inception cohort studies), or validation of Clinical Decision Rule (CDR)) supported a role of CSF NSE to assess prognosis of comatose survivors of cardiac arrest. One was QOE good [Zandbergen 2001 1661-67]   and 2 were QOE fair [Martens 1998 2363-66; Roine 1989 753-6]

The predictive performance of CSF-NSE has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

Zandbergen 2001 1661-67

Study design: Meta-analysis of prognostic studies (LOE P1). The specific objectives of the review were stated clearly. The study was carefully designed and criteria for inclusion were described. Characteristics and methodological quality of each trial were detailed and a log of excluded studies with reasons for exclusion was reported. The studies cited in the present meta-analysis and included in the worksheet are marked with an asterisk in the Tables and in the citation list. A methodological limitation of the present meta-analysis is that data were pooled irrespective of the nature of the study (either prospective or retrospective) and of blinding of physician to the results of the biomarker level. In conclusion, the study is a valuable systematic review of the literature, but the studies included in the meta-analysis were both prospective and retrospective and blinded and unblinded. However, the present study is supportive of a predictive role of CKBB, NSE, S100, LDH and GOT levels as determined on the CSF and of CKBB, NSE and S100 as assessed on blood samples to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their clinical application for non-treatment decisions is limited mainly by the wide confidence limits of the pooled estimates of false positive rates as detailed in the following table.

	95% CI of the pooled false positive rates
CSF	
NSE>24 ng/l	0-23.2
NSE>50 ng/l	0.1-19.0

*Martens 1998 2363-66

Study design: prospective inception cohort trial (LOE P1)
Study population: 64 pts who remained comatose > 24 hours after resuscitation from cardiac arrest.
Biomarkers under evaluation: NSE and S100 levels as determined on blood samples collected at 24 hours after admission and NSE and S100 levels as determined on cerebrospinal fluid collected by lumbar puncture at 48 hours after admission.

Outcome assessed: outcome was dichotomized into 2 groups: group 1 consisted of patients who died or remained in vegetative state, and group 2 consisted of patients who regained consciousness (i.e., obeyed simple verbal commands). Regaining consciousness was considered an end point for follow-up, regardless of outcome at discharge or at 6 months. Patients who eventually died of multiorgan failure but who had clearly regained consciousness after cardiac arrest were classified in group 2.

Results: Serum S-100, serum NSE, S-100 CSF and NSE CSF were significantly higher in patients who never regained consciousness compared with those who regained consciousness. S-100 and NSE correlated well with each other in the CSF; both CSF concentrations also correlated with their serum concentrations; In detail, the predictive performance of CSF-NSE (cut-off 50 µg/L) was: Sensitivity 89%, Specificity 83%, FPR 17%

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed.). Therefore, the study has been ranked as supportive of a prognostic role of CSF-NSE (and of s-NSE, s-S100, CSF-S100, as discussed in the appropriate relative sections) to assess the probability of a favorable neurological outcome, but the predictive performance is unsatisfactory. (LOE P1, QOE fair).

*Roine 1989 753-6

Study design: prospective inception cohort study LOE P1

Study population: 75 consecutive victims of out of-hospital cardiac arrest, but it was not explicitly stated whether they were comatose on admission. GCS was assessed at 1 h, 24 h and 7 days after admission but was not reported. As the lower bound of cardiac arrest duration and resuscitation duration was 1 min, it is not entirely clear whether all patients were comatose on admission, but it is likely that most were so.

Biomarkers under evaluation: NSE and CK-BB as measured in samples of cerebrospinal fluid (CSF) and serum (CSF-NSE, s-NSE, CSF CK-BB, s-CK-BB)

Outcome assessed: The recovery of consciousness was defined as the ability to follow verbal commands. All patients were followed up for 3 months or until death. The 3-month outcome was classified according to the Glasgow Outcome Scale.

Results: Cerebrospinal fluid was obtained by lumbar puncture approximately 24 hours (20 - 26 hours) after cardiac arrest in 68 patients. Lumbar puncture was unsuccessful or contraindicated in seven patients. CSF-NSE was determined in 59 patients and the level of CSF-CK-BB was determined in 67 patients. All patients with CSF-NSE concentrations higher than 24 ng/mL remained unconscious and died. When this level is taken as a cut-off value, the test had a sensitivity of 74%, specificity and positive predictive value of 100% (FPR 0%), and negative predictive value of 89% in detecting patients who did not recover.

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. Therefore, the study has been ranked as supportive of a predictive role of CSF-NSE at 24 hours to assess neurological outcome. (LOE P1, QOE fair)

CSF-S100

1 eligible study investigated the predictive performance of CSF-S100 to assess the prognosis of comatose survivors of cardiac arrest.

One LOE P1 study (LOE P1: Inception (prospective) cohort studies (or meta-analyses of inception cohort studies), or validation of Clinical Decision Rule (CDR)) [Martens 1998 2363-66], whose QOE was fair, supported a role of CSF S100 to assess prognosis of comatose survivors of cardiac arrest. This study is also cited in the systematic review by Zandbergen 2001 1661-67 as the sole eligible study to investigate this topic. As the conclusions reported in the cited meta-analysis are based only on the study from Martens 1998 2363-66, the meta-analysis from Zandbergen 2001 1661-67 (whose abstract is however reported hereafter for the readers' convenience) is not considered as an additional evidence.

The predictive performance of CSF-S100 has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

*Martens 1998 2363-66

Study design: prospective inception cohort trial (LOE P1)

Study population: 64 pts who remained comatose > 24 hours after resuscitation from cardiac arrest.

Biomarkers under evaluation: NSE and S100 levels as determined on blood samples collected at 24 hours after admission and NSE and S100 levels as determined on cerebrospinal fluid collected by lumbar puncture at 48 hours after admission.

Outcome assessed: outcome was dichotomized into 2 groups: group 1 consisted of patients who died or remained in vegetative state, and group 2 consisted of patients who regained consciousness (ie, obeyed simple verbal commands). Regaining consciousness was

considered an end point for follow-up, regardless of outcome at discharge or at 6 months. Patients who eventually died of multiorgan failure but who had clearly regained consciousness after cardiac arrest were classified in group 2.

Results: Serum S-100, serum NSE, CSF S-100 and CSF NSE were significantly higher in patients who never regained consciousness compared with those who regained consciousness. Of note, only some of the pts assessed by serum NSE and S100 received a lumbar puncture to assess CSF-NSE s-S100. S-100 and NSE correlated well with each other in the CSF; both CSF concentrations also correlated with their serum concentrations; S-100 and NSE did not correlate at all with each other in serum. In detail, the predictive performance of the biomarkers were: CSF-S100 (cut-off 6 µg/L): Sensitivity 93%, Specificity 60%, FPR 40%;

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a prognostic role CSF-S100 (and of s-NSE, s-S100, CSF-NSE,as discussed separately in the appropriate relative sections), to assess the probability of a favorable neurological outcome, , but with an unsatisfactory predictive performance (CSF-S100 [cut-off 6 µg/L]: Sensitivity 93%, Specificity 60%) (LOE P1, QOE fair).

Zandbergen 2001 1661-67

Study design: Meta-analysis of prognostic studies (LOE P1). The specific objectives of the review were stated clearly. The study was carefully designed and criteria for inclusion were described. Characteristics and methodological quality of each trial were detailed and a log of excluded studies with reasons for exclusion was reported. The studies cited in the present meta-analysis and included in the worksheet are marked with an asterisk in the Tables and in the citation list. A methodological limitation of the present meta-analysis is that data were pooled irrespective of the nature of the study (either prospective or retrospective) and of blinding of physician to the results of the biomarker level. In conclusion, the study is a valuable systematic review of the literature, but the studies included in the meta-analysis were both prospective and retrospective and blinded and unblinded. However, the present study is supportive of a predictive role of CSF-S100 (and of CKBB, NSE, LDH and GOT levels on CSF samples, as discussed separately in the relative sections) to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their clinical application for non-treatment decisions is limited mainly by the wide confidence limits of the pooled estimates of false positive rates and of positive-likelihood ratio as detailed in the following table.

	95% CI of the (pooled) false positive rates
CSF	
S100>6 µg/l	2.2-27.3

CSF LDH

Two eligible studies investigated the predictive performance of CSF-LDH to assess the prognosis of comatose survivors of cardiac arrest

Two LOE P1 studies (LOE P1: Inception (prospective) cohort studies (or meta-analyses of inception cohort studies), or validation of Clinical Decision Rule (CDR)) [Martens 1996 126-31; Karkela 1992 378-86], whose QOE were fair, supported a role of CSF LDH to assess prognosis of comatose survivors of cardiac arrest arrest. One of these studies [Martens 1996 126-31] is also cited in the systematic review by Zandbergen 2001 1661-67 as the sole eligible study to investigate this topic. As the conclusions reported in the cited meta-analysis are based only on the study from Martens 1996 126-31, the meta-analysis from Zandbergen 2001 1661-67 (whose abstract is however reported hereafter for the readers' convenience) is not considered as an additional evidence.

The predictive performance of CSF-LDH has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

Karkela 1992 378-86

Study design: Prospective inception cohort study (LOE P1).
Study population: 20 comatose survivors of cardiac arrest.
Prognostic biomarkers under evaluation: Concentrations in the CSF of CK, CK-BB, LDH and its isoenzymes (1-5), acid phosphatase, beta-D-N-acetylglucosaminidase, lactate, pyruvate, sodium, potassium and calcium as determined on samples collected at 4, 28, 76 and 172 hours after the initiation of resuscitation.
Outcome evaluated: neurological outcome at 1 year
Results: 8 pts recovered while 12 pts remained comatose or neurologically disabled until death. 6 out of the 8 pts who presented neurological recovery were discharged without intellectual or neurological deficit but with short term memory disturbances. Total LDH activity and LDH isoenzymes 1 and 2 activity was higher in pts with unfavorable outcome at 76 hours (*p=0.003* for all

comparisons); LDH isoenzyme 3 activity was higher in pts with unfavorable outcome at 28 and 76 hours (*p<0.021* and *p=0.003* respectively); No other significant differences between groups were found for all the other prognostic biomarkers under evaluation. In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. The results of the dosages of the biomarkers under evaluation do not appear to have influenced the treatment. Even though several biomarkers dosages appeared to be significantly different at univariate analysis in pts with unfavorable outcome, the authors have not performed a multivariate analysis to allow control for several confounders. No cut-off for the biomarkers have been suggested. Therefore, the study has been ranked as supportive of a prognostic role of total LDH activity and LDH isoenzymes 1 and 2 activity at 76 hours, LDH isoenzyme 3 activity at 28 and 76 hours to assess neurological outcome, but is not conclusive. (LOE P1, QOE fair).

*Martens 1996 126-31

Study design: prospective inception cohort study (LOE P1).
Study population: Fifty-two consecutive patients who remained comatose after successful resuscitation from OHCA.
Biomarker under evaluation: Serum NSE levels assessed on blood samples collected 24 hours after cardiac arrest; in 16 pts who remained alive but comatose a lumbar puncture was performed to assess CSF CPK-BB, CSF-AST and CSF-LDH.
Outcome assessed: neurological outcome, defined as return of consciousness vs. death due to CNS failure; pts regaining consciousness but eventually dying from non-neurologic causes as well as pts with neurologic impairment were considered as pts with successful outcome.
Results: The predictive performance of CSF-CK-BB and CSF-LDH was also calculated by the ILCOR reviewer in 15 of 16 pts in whom the lumbar puncture was performed (one pts had an undetermined outcome and was excluded from the analysis; CSF-GOT was not determined in 4 pts and therefore calculations were not attempted). Sensitivity, specificity and FPR for CSF-LDH were 75 % (95% CI 47-91), 100 % (95% CI 44-100) and 0% (95% CI 0-66) respectively (%). The Biomarkers levels were not used to take treatment decisions.
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and in a blinded fashion; follow up of pts was complete. S-NSE appeared to be correlated to outcome both at univariate and multivariate analysis; unfortunately, the predictive performance of abnormal s-NSE concentrations was not assessed, with limited clinical applicability of the results of logistic regression analysis. CSF-CPK-BB, CSF-GOT and CSF-LDH were predictive of outcome at some extent at univariate analysis, but were assessed only in a subgroup of pts. Therefore, the study has been ranked as supportive of a prognostic role of CSF-LDH (LOE P1 QOE fair) to assess neurological outcome of comatose survivors of cardiac arrest, but with an unsatisfactory predictive performance

Zandbergen 2001 1661-67

Study design: Meta-analysis of prognostic studies (LOE P1). The specific objectives of the review were stated clearly. The study was carefully designed and criteria for inclusion were described. Characteristics and methodological quality of each trial were detailed and a log of excluded studies with reasons for exclusion was reported. The studies cited in the present meta-analysis and included in the worksheet are marked with an asterisk in the Tables and in the citation list. A methodological limitation of the present meta-analysis is that data were pooled irrespective of the nature of the study (either prospective or retrospective) and of blinding of physician to the results of the biomarker level. In conclusion, the study is a valuable systematic review of the literature, but the studies included in the meta-analysis were both prospective and retrospective and blinded and unblinded. However, the present study is supportive of a predictive role of CKBB, NSE, S100, LDH and GOT levels as determined on the CSF and of CKBB, NSE and S100 as assessed on blood samples to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their clinical application for non-treatment decisions is limited mainly by the wide confidence limits of the pooled estimates of false positive rates as detailed in the following table.

	95% CI of the pooled false positive rates
CSF	
LDH>82 U/l	0-33.6

CSF-GOT

1 eligible study investigated the predictive performance of CSF-GOT to assess the prognosis of comatose survivors of cardiac arrest.
One LOE P1 study (LOE P1: Inception (prospective) cohort studies (or meta-analyses of inception cohort studies), or validation of

Clinical Decision Rule (CDR)) [Martens 1996 126-31],whose QOE was poor, supported a role of CSF GOT to assess prognosis of comatose survivors of cardiac arrest arrest . This study is also cited in the systematic review by Zandbergen 2001 1661-67 as the sole eligible study to investigate this topic. As the conclusions reported in the cited meta-analysis are based only on the study from Martens 1996 126-31 the meta-analysis from Zandbergen 2001 1661-67 (whose abstract is however reported hereafter for the readers' convenience) is not reported as an additional evidence.

The predictive performance of CSF-GOT has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

*Martens 1996 126-31
Study design: prospective inception cohort study (LOE P1).
Study population: Fifty-two consecutive patients who remained comatose after successful resuscitation from OHCA.
Biomarker under evaluation: Serum NSE levels assessed on blood samples collected 24 hours after cardiac arrest; in 16 pts who remained alive but comatose a lumbar puncture was performed to assess CSF CPK-BB, CSF-AST and CSF-LDH.
Outcome assessed: neurological outcome, defined as return of consciousness vs. death due to CNS failure; pts regaining consciousness but eventually dying from non-neurologic causes as well as pts with neurologic impairment were considered as pts with successful outcome.
Results: S-NSE, GCS on admission, serum glucose on admission and total epinephrine dose before ROSC were significantly higher in pts with unfavorable outcome. A correlation between S-NSE on admission and outcome was confirmed by logistic regression analysis after adjustment for the possible confounding role of best GCS on admission, blood glucose on admission and total epinephrine dose before ROSC (adjusted OR 5.8; p=0.034). The predictive performance of CSF-CK-BB and CSF-LDH was also calculated by the ILCOR reviewer in 15 of 16 pts in whom the lumbar puncture was performed (one pts had an undetermined outcome and was excluded from the analysis; CSF-GOT was not determined in 4 pts and therefore calculations were not attempted). Sensitivity, specificity, and FPR for CSF-CPK-BB were 83% (95% CI 55-95), 100% (95% CI 44-100) and 0 % (95%CI 0-66). Sensitivity, specificity and FPR for CSF-LDH were   75 % (95% CI 47-91), 100 % (95% CI 44-100) and 0% (95% CI 0-66) respectively (%). The predictive performance of s-NSE could not be assessed because individual data were not available. Biomarkers levels were not used to take treatment decisions. In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and in a blinded fashion; follow up of pts was complete. S-NSE appeared to be correlated to outcome both at univariate and multivariate analysis; unfortunately, the predictive performance of abnormal s-NSE concentrations was not assessed, with limited clinical applicability of the results of logistic regression analysis. CSF-CPK-BB, CSF-GOT and CSF-LDH were predictive of outcome at some extent at univariate analysis, but were assessed only in a subgroup of pts. Therefore, the study has been ranked as supportive of a prognostic role CSF-GOT (LOE P1 QOE poor) (and of of S-NSE, CSF-CPK-BB and CSF-LDH as discussed separately in the relative sections) and , to assess neurological outcome of comatose survivors of cardiac arrest.

Zandbergen 2001 1661-67

Study design: Meta-analysis of prognostic studies (LOE P1). The specific objectives of the review were stated clearly. The study was carefully designed and criteria for inclusion were described. Characteristics and methodological quality of each trial were detailed and a log of excluded studies with reasons for exclusion was reported. The studies cited in the present meta-analysis and included in the worksheet are marked with an asterisk in the Tables and in the citation list. A methodological limitation of the present meta-analysis is that data were pooled irrespective of the nature of the study (either prospective or retrospective) and of blinding of physician to the results of the biomarker level. In conclusion, the study is a valuable systematic review of the literature, but the studies included in the meta-analysis were both prospective and retrospective and blinded and unblinded. However, the present study is supportive of a predictive role of CKBB, NSE, S100, LDH and GOT levels as determined on the CSF and of CKBB, NSE and S100 as assessed on blood samples to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their clinical application for non-treatment decisions is limited mainly by the wide confidence limits of the pooled estimates of false positive rates as detailed in the following table.

	95% CI of the (pooled) false positive rates
CSF	
GOT>62 U/l	0-36.9

CSF neurofilament

1 eligible study investigated the predictive performance of CSF-neurofilament to assess the prognosis of comatose survivors of cardiac arrest. This study has also been cited in the systematic review by Wijdicks 2006 203-10 as the sole eligible study to address this topic. As the conclusions by Wijdicks 2006 203-10 on CSF neurofilament (whose abstract is however reported hereafter for readers' convenience) are based only on the following study [Rosen 2004 19-24], the systematic review itself has not been considered as additional evidence to investigate the topic.

One LOE P3 study (LOE P3: Retrospective cohort study) [Rosen 2004 19-24], whose QOE was fair, supported a role of CSF neurofilament to assess prognosis of comatose survivors of cardiac arrest arrest

The predictive performance of CSF-neurofilament has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet

Rosen 2004 19-24

Study design: the study has not been unambiguously characterized as prospective and has been considered retrospective cohort study (LOE P3)

Study population: 22 pts admitted after non traumatic OHCA with ROSC of ≥12 days.

Prognostic biomarker under evaluation: Levels of neurofilament protein (NFL) as determined on cerebrospinal fluid sampled by lumbar puncture between days 12-14. Prognostic biomarker was investigated together with standardized neurologic examination (coma level assessed by RLS 85 score; neurological status assessed by NIH stroke scale) on admission (t0), at days 2-4 (t1), days 12-14 (t1), day 45 (t3), 3 months (t4), 1 year (t5) .

 Outcome evaluated: neurological outcome defined as the best Glasgow outcome scale score assessed at t2, t3, t4 and t5 (favorable outcome defined as GOS 1-2, unfavorable outcome defined as GOS 3-5); cognitive function defined as the best mini-mental state examination (MMSE) score at t3, t4, t5; dependence/independence in activities of daily living defined as the best Katz score (assessed at t3, t4, t5).

Results: NFL as determined on a CSF sample at day 17.5±1: (1) is significantly increased in pts with unfavorable as compared to those with favorable neurological outcome; (2) is significantly increased in pts with abnormal as compared to those with normal cognitive function; (3) is significantly increased in dependent pts as compared to those independent in ADL. A significant correlation has been shown between NFL and GOS, MMSE and dependence in ADL. Sensitivity and specificity of several cut-off levels are analyzed for all the outcome measures. Of special interest, for the purposes of the present worksheet, a NFL level in the CSF at 12-14 days > 12800 µg/l had a 100% sensitivity, 75% specificity (FPR 25 %), 100% PPV and 77% NPV; NFL level in the CSF at 12-14 days > 9622 µg/l had a 90% sensitivity, 92% specificity (FPR 8 %), 92% PPV and 90% NPV; NFL level in the CSF at 12-14 days > 5888 µg/l had a 80% sensitivity, 92% specificity (FPR 8%), 92% PPV and 90% NPV.

Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded). The study sample was extracted from a population of 105 pts after exclusion of 43 pts who died before day 12, 10 pts who refused, 17 pts who had contraindication to lumbar puncture, 5 pts who were lost to follow-up. Even though at univariate analysis the biomarker under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed.

In conclusion, this study supports a role of CSF Neurofilament concentration as assessed on average at day 15 after cardiac arrest to assess prognosis of comatose survivors of cardiac arrest (predictive performance for a poor outcome); NFL level in the CSF at 12-14 days > 9622 µg/l had a 90% sensitivity, 92% specificity (FPR 8%); NFL level in the CSF at 12-14 days > 5888 µg/l had a 80% sensitivity, 92% specificity (FPR 8%) (LOE P3, QOE fair).

CSF- acid phosphatase, beta-D-N-acetylglucosaminidase, lactate, pyruvate

One eligible study investigated the predictive performance of CSF- acid phosphatase, beta-D-N-acetylglucosaminidase, lactate, pyruvate to assess the prognosis of comatose survivors of cardiac arrest [Karkela 1992 378-86].

One LOE P1 study [Karkela 1992 378-86] ,whose QOE was fair, supported a role of CSF- acid phosphatase and CSF-lactate to assess the prognosis of comatose survivors of cardiac arrest

One LOE P1 study [Karkela 1992 378-86] ,whose QOE was fair, opposed a role of beta-D-N-acetylglucosaminidase, and pyruvate to assess the prognosis of comatose survivors of cardiac arrest

The predictive performance of CSF- acid phosphatase, beta-D-N-acetylglucosaminidase, lactate, pyruvate has been extensively analyzed in the following "Citation list" section and is here summarized as follows (for abbreviations see the Abbreviations list at the end of the worksheet)

Karkela et al. 1992 378-86

Study design: Prospective inception cohort study (LOE P1).
Study population: 20 comatose survivors of cardiac arrest.
Prognostic biomarkers under evaluation: Concentrations in the CSF of CK, CK-BB, LDH and its isoenzymes (1-5), acid phosphatase, beta-D-N-acetylglucosaminidase, lactate, pyruvate as determined on samples collected at 4, 28, 76 and 172 hours after the initiation of resuscitation.
Outcome evaluated: neurological outcome at 1 year
Results: 8 pts recovered while 12 pts remained comatose or neurologically disabled until death. 6 out of the 8 pts who presented neurological recovery were discharged without intellectual or neurological deficit but with short term memory disturbances. Of interest for the topic under evaluation, CSF-Acid Phosphatase was higher in pts with unfavorable outcome at 76 hours (p=0.010); CSF-lactate was higher in pts with unfavorable outcome at 4, 28 and 76 hours (p=0.039, p=0.002 and p=0.010 respectively). No other significant differences between groups were found for all the other prognostic biomarkers under evaluation.
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. The results of the dosages of the biomarkers under evaluation do not appear to have influenced the treatment. Even though several biomarkers dosages appeared to be significantly different at univariate analysis in pts with unfavorable outcome, the authors have not performed a multivariate analysis to allow control for several confounders. No cut-off for the biomarkers have been suggested. Therefore, the study has documented an association between CSF-Acid Phosphatase at 76 hours, CSF-lactate at 4, 28 and 76 hours and neurological outcome (and of CSF-CK, CSF-CKBB, total LDH activity and LDH isoenzymes as detailed in the relative appropriate sections), and has been ranked as supportive. (LOE P1, QOE fair) but is not conclusive. Moreover, the study opposed a role of CSF-pyruvate and beta-D-N-acetylglucosaminidase to assess   the prognosis of comatose survivors of cardiac arrest (LOE P1, QOE fair)

Citation List

Adib-Conquy 2007 406-10
"Increased plasma levels of soluble triggering receptor expressed on myeloid cells 1 and procalcitonin after cardiac surgery and cardiac arrest without infection." Shock 28(4): 406-410.

Reviewer's analysis
Study design: retrospective study (LOE P3).
Study population: 54 pts with successfully resuscitated OHCA, retrospectively retrieved from a database of previous studies (GCS on admission not specified in the present study, but extracted from previous studies GCS=3 [3-4]) and compared with: (1) a group of pts undergoing cardiac surgery (2) a group of healthy controls and (3) a group of pts with severe sepsis.
Prognostic biomarkers under evaluation: Levels of *soluble triggering receptor expressed on myeloid cells 1* (sTREM-1) and procalcitonin (PCT), as determined on stored plasma samples collected on admission, at day 2 and day 3
Outcome evaluated: survival and neurological outcome categorized in 3 groups: (Group 1) survival and CPC 1-2; (Group 2) death from neurological failure; (Group 3) death from shock.
Results. Both sTREM-1 and PCT were significantly increased in cardiac arrest patients who died of refractory shock as compared to
those who died of neurological failure or survived without major neurological damage, but no clinically useful cut-off were proposed
In conclusion there is fair evidence that sTREM-1 and PCT are predictive of death from refractory shock at univariate analysis the
authors do not propose indicators of predictive performance at different cut-offs (i.e. sensitivity, specificity, positive and negative
predictive values, false positive rate). For these reasons the study has been ranked as supporting an association between the biomarkers under evaluation and death from refractory shock, but is of limited clinical applicability (QOE fair)

Adrie 2002 562-8
"Successful cardiopulmonary resuscitation after cardiac arrest as a "sepsis-like" syndrome." Circulation 106(5): 562-8

Reviewer's analysis

Study design: Not overtly characterized as prospective and therefore ranked as LOE P3

Study population: 61 comatose survivors aged>16 resuscitated after OHCA and a Glasgow coma score of 3 (range 3 to 4) on admission (Table 1).

Prognostic biomarkers under evaluation: Plasma endotoxin, sTNFRII, IL-1ra, IL-6, IL-8, IL-10, RANTES, Lactate, bicarbonate and ex-vivo cytokine production.

Other prognostic factors under evaluation: clinical characteristics of pts on admission.

Outcome evaluated: survival.

Results. levels of lactate, bicarbonate, sTNFRII, IL-6, IL-8, and IL-10 on admission were significantly higher in non-survivors than in survivors. No significant differences in the concentration of IL1-ra and RANTES could be found between survivors and non-survivors. However, among the above cited cytokines or soluble receptors, there was no independent variable predictive of death compared with the interval from collapse to basic life support and duration of cardiopulmonary resuscitation. A significant independent prognostic role of lactate and bicarbonate at multivariate analysis was not explicitly evidenced. Moreover, there was no correlation between the presence of endotoxin and mortality. Production of cytokines in whole-blood assays on in vitro stimulation and in vitro plasma exchange experiments showed several differences between survivors of cardiac arrest and controls, but their ability to assess prognosis of comatose survivors of cardiac arrest was not explored.

Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis several biomarkers under evaluation appeared to be significantly different in survivors compared to non-survivors, at multivariate analysis none of them was independently predictive of outcome after correction for the confounding effect of time from collapse to BLS and duration of resuscitation maneuvers; the possibility that the study was underpowered to detect an independent predictive role of the prognostic variables under investigation should be considered.

In conclusion, the study has been ranked as of neutral (favoring a role at univariate analysis but opposing a role at underpowered multivariate analysis) evidence of a role of plasma sTNFRII, IL-6, IL-10 and IL-8, respect to the clinical question (QOE fair). Moreover, the study has been considered as opposing a possible role of IL1-ra and RANTES to assess the prognosis of comatose survivors of cardiac arrest (QOE fair)

†**Bottiger 2001 2694-98**

"Astroglial protein S-100 is an early and sensitive marker of hypoxic brain damage and outcome after cardiac arrest in humans." Circulation **103(22): 2694-2698.**

Reviewer's analysis

Study design: prospective inception cohort study (LOE P1)

Study population: 66 patients undergoing cardiopulmonary resuscitation after non-traumatic cardiac arrest.

Biomarkers under investigation: Levels of S-100 (as determined on blood samples collected on admission and at 15, 30, 45, and 60 minutes; 2, 8, 24, 48, and 72 hours; and 7 days after initiation of cardiopulmonary resuscitation) and of NSE (as determined on blood samples collected between 2 hours and 7 days

Other prognostic markers under investigation: If patients survived for >48 hours, brain damage was assessed by a combination of neurological, cranial CT, and electrophysiological examinations.

Outcome evaluated: ROSC, survival and neurological outcome (defined as: group 1: no brain damage (patients discharged from the hospital fully oriented and without any communication defects (CPC1); group 2: documented brain damage (ie, according to neurological, cranial CT, and electrophysiological evaluations systematically performed between 48 hours and 96 hours after cardiac arrest; to focus on all patients, the data of surviving and non-surviving patients were combined here; group 3: no ROSC; group 4: patients who died soon after ROSC before assessment of brain damage.

Results: Maximum S-100 levels within 2 hours after cardiac arrest were significantly higher in patients with documented brain damage; significant differences between these 2 groups were observed from 30 minutes until 7 days after cardiac arrest. At 48 hours after cardiac arrest, an S-100 serum level of > 1.10 μg/L revealed a specificity of 100% (FPR = 0%) for the diagnosis of brain damage. The positive predictive value of the S-100 test at 2 hours for fatal outcome within 14 days was 79%, and the negative predictive value was 100% (P < 0.01). At 24 hours, the corresponding figures were 87% and 100% (P<0.001), and at 48 hours, they were 75% and 100% (P < 0.01), respectively. With regard to neuron-specific enolase, significant differences between patients with documented brain damage and those with no brain damage were found at 24, 48, and 72 hours and 7 days but a more detailed assessment of the predictive performance of NSE was not performed.

Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a role of serum S-100 and NSE in predicting prognosis of comatose survivors of cardiac arrest (univariate). QOE fair

*Clemmensen 1987 235-6

"Cerebrospinal fluid creatine kinase isoenzyme BB levels do not predict the clinical outcome in patients unconscious following cardiac resuscitation." <u>Clin Cardiol</u> 10(4): 235-6.

Reviewer's analysis
Study design: prospective inception cohort trial LOE P1
Study population: 21 consecutive pts who remained comatose after successful resuscitation from OHCA.
Prognostic biomarkers under evaluation: CK-BB levels as assessed form cerebrospinal fluid sampled by lumbar puncture between 6 and 72 hours after resuscitation.
Outcome assessed: Survival, defined as survival to hospital discharge, and neurological outcome, without a clear definition.
Results: The median values of CSF-CKBB were 5 U/l in non-survivors as compared to values below the detection limit of the assay in survivors (*p=n.s*). The positive predictive value of a positive test was 66.6%; the negative predictive value of a positive test was 25%. The authors reported that "no correlation between cerebral recovery and CSF-CKBB was found" but no other individual or aggregate data are presented.
Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. Even though at univariate analysis the biomarkers under evaluation did not result to be predictive of outcome, the power of the study was rather limited due to the small size of the patient population. Therefore, the study has been ranked as of opposing evidence to the clinical question (poor).

†*Dauberschmidt 1991 237-45
"Changes of neuron-specific enolase concentration in plasma after cardiac arrest and resuscitation." <u>Mol Chem Neuropathol</u> 14(3): 237-45.

Reviewer's analysis
Study design: not unequivocally stated that the study was prospective, and therefore conservatively rated as retrospective LOE P3
Study population: 18 patients admitted to the intensive care unit after cardiac arrest and resuscitation
Prognostic biomarkers under evaluation: Serum NSE measured daily from admission
Outcome assessed: Survival and neurological outcome (defined as regaining consciousness)
Results: the NSE concentration was significantly higher after cardiac arrest in survivors as well as in non-survivors in comparison to the reference values in controls with slipped disc. In addition, the NSE concentration of the non-survivors was significantly higher as compared to survivors. In seven non-survivors the NSE concentration was in the pathological range (defined as a concentration above 7 µg//L) from first day, in one non-survivor it increased to pathological values within 4 days from admission. However, in six of the non-survivors (43%) NSE concentration never raised above 7.0 µg//L. In one survivor, NSE was above the threshold level of 7.0 µg/L.
From these data the ILCOR reviewer has calculated the following parameters of predictive performance (death) of a s-NSE above 7 µg/L obtained within 4 days from admission from comatose survivors of cardiac arrest: Sensitivity: 57% (95% CI: 33-79%) Specificity: 75% (95% CI: 30-95); FPR 25% (95% CI 5-70).
Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed
Conclusions: This LOE P3 study offers evidence of a role of s-NSE in assessing prognosis of comatose survivors of cardiac arrest. (QOE fair)

†* Fogel 1997 1133-38
"Serum neuron-specific enolase as early predictor of outcome after cardiac arrest." <u>Crit Care Med</u> 25(7): 1133-1138.

Reviewer's analysis
Study design: prospective inception cohort study (LOE P1).
Study population: 50 pts resuscitated from cardiac arrest (not explicitly stated whether INHCA or OHCA or both; not explicitly stated whether all pts were comatose on admission but presumably so
Biomarker under evaluation: NSE as determined on blood samples collected within 12 hours from admission and daily for 7 days. Outcomes assessed: Survival and neurological outcome at 3 months as determined by the Barthel index.
Results: seven pts died before neurological status could be evaluated and were excluded from analysis. Of the remaining 43 pts, 25 patients remained comatose and subsequently died while 18 patients survived the first 3 months and had no relevant functional deficit at 3-month follow-up. NSE was significantly lower in pts who survived and had a favorable neurological outcome. None of the pts with NSE levels > 33 ng/ml at any sampling time regained consciousness; however, 25 pts who remained comatose had NSE levels persistently ≤ 33 ng/ml; none of the pts who survived had NSE levels >33 ng/ml at any

sampling time. The authors reported the sensitivity of s-NSE levels > 33ng/ml from day 1 to day 7 after cardiac arrest; at this cut-off the specificity of s-NSE levels to predict a poor outcome in this study was uniformly 100% (FPR 0%). The predictive performance of s-NSE levels > 33ng/ml is presented hereafter in a table modified from the original source

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Sensitivity (%)	25	60	63	65	65	38	30
Specificity (%)	100	100	100	100	100	100	100

Quality of evidence. The study is a prospective inception cohort trial (LOE P1). Comparisons groups were clearly defined, outcomes were measured in the same way and follow up of pts was complete. However, even though at univariate analysis the biomarker under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive (LOE fair) of s-NSE levels to assess the prognosis of comatose survivors of cardiac arrest

Geppert 2003 805-11

"Plasma concentrations of von Willebrand factor and intracellular adhesion molecule-1 for prediction of outcome after successful cardiopulmonary resuscitation." <u>Crit Care Med</u> 31(3): 805-11.

Reviewer's analysis
Study design: retrospective study on stored plasma samples (LOE P3).
Study population: 35 non-consecutive pts who survived > 24 h after either a IHCA or OHCA not awake after 24 hours after cardiac arrest (not excluded because they were awake and transferred to the ward within the first 24 hours after CA.)
Prognostic biomarkers under evaluation: vWF and sICAM at 2 days after CA.
Outcome evaluated: neurological outcome (bad outcome defined as CPC score ≥ 3 v.s. good outcome defined as CPC score <3).
Results: vWF concentration was significantly higher in pts with bad neurological outcome ($p<0.001$). ICAM-1 concentration was non-significantly higher in pts with bad neurological outcome ($p<0.097$). vWF >166% and ICAM-1 > 500 ng/ml were 100% specific (FPR=0, with a sensitivity of 80% and 70% respectively) to identify pts with unfavorable outcome after OHCA, but clinically useful cut-offs could not be found for IHCA. A cardiac arrest score was developed on the basis of factors resulted to be predictive of adverse outcome at univariate analysis (renal dysfunction/failure, severe cardiovascular failure, systemic inflammatory syndrome and CPR duration > 15 minutes) whose predictive accuracy was improved by vWF and ICAM concentrations. At multivariate analysis, vWF concentration and CPR duration were the most significant predictors of outcome.
 Conclusions: Comparisons groups were clearly defined, outcomes were measured in the same way (presumably blinded) and follow up of pts was complete. At univariate analysis the biomarkers under evaluation appeared to be predictive of outcome; the authors also
attempted a multivariate analyses to confirm an independent prognostic role of vWF and ICAM. At multivariate analysis, vWF concentration and CPR duration were the most significant predictors of outcome. Therefore, the study has been ranked as supportive, (LOE P3, QOE fair) of a role of vWF (neurological outcome) to assess the prognosis of comatose patients after OHCA; evidence on a possible prognostic role of ICAM appeared to be weaker and conflicting (predictive at univariate but not at multivariate analysis on a small sized sample) and has been ranked as neutral (neurological outcome) LOE P3 QOE fair.

Hachimi-Idrissi 2002 251-7
"S-100 protein as early predictor of regaining consciousness after out of hospital cardiac arrest." <u>Resuscitation</u> 53(3): 251-7.

Reviewer's analysis
Study design: prospective inception cohort study (LOE P1)
Study population: Fifty-eight comatose patients resuscitated from out-of-hospital CA.
Biomarker under evaluation: S-100 levels on blood samples collected on admission and at 24 hours
Outcome assessed: neurological outcome defined as follows: Group 1 consisting of patients who died or remained in vegetative state; Group 2 consisting of patients who regained consciousness. Of note, regaining consciousness was considered as a clinical end point regardless of the definitive outcome on discharge, therefore patients who eventually died later but who regained consciousness at any time during their stay were included in Group 2.
Results: Serum S-100 was significantly higher at admission and 24 h later in-group 1 compared with group 2. The   highest specificity value for a poor outcome on admission was 85% (FPR 15%), obtained with a cut-off of 0.7 μg/l; at this cut-off, the sensitivity was 66.6%, the PPV was 84%, the NPV was 78% and the accuracy was 77.6%. All these values increased 24 h later up to 88.2 (FPR 11.8%), 100%, 86%, 100% and 93% respectively for specificity, sensitivity, PPV, NPV and accuracy.

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not formally performed. Therefore, the study has been ranked as supportive of a prognostic role of s-S-100 to assess neurological outcome of comatose survivors of cardiac arrest. (LOE P1, QOE fair)

Karkela 1992 378-86

"Evaluation of hypoxic brain injury with spinal fluid enzymes, lactate, and pyruvate." <u>Crit Care Med</u> 20(3): 378-86

Reviewer's analysis

Study design: Prospective inception cohort study (LOE P1).

Study population: 20 comatose survivors of cardiac arrest.

Prognostic biomarkers under evaluation: Concentrations in the CSF of CK, CK-BB, LDH and its isoenzymes (1-5), acid phosphatase, beta-D-N-acetylglucosaminidase, lactate, pyruvate, sodium, potassium and calcium as determined on samples collected at 4, 28, 76 and 172 hours after the initiation of resuscitation.

Outcome evaluated: neurological outcome at 1 year

Results: 8 pts recovered while 12 pts remained comatose or neurologically disabled until death. 6 out of the 8 pts who presented neurological recovery were discharged without intellectual or neurological deficit but with short term memory disturbances. CSF-CK was higher in pts with unfavorable outcome at 28 and 76 hours (p<0.001 and p=0.018 respectively); CSF-CKBB was higher in pts with unfavorable outcome at 28 and 76 hours (p=0.014 and p=0.010 respectively); total LDH activity and LDH isoenzymes 1 and 2 activity was higher in pts with unfavorable outcome at 76 hours (p=0.003 for all comparisons); LDH isoenzyme 3 activity was higher in pts with unfavourable outcome at 28 and 76 hours (p<0.021 and p=0.003 respectively); CSF-Acid Phosphatase was higher in pts with unfavorable outcome at 76 hours (p=0.010); CSF-lactate was higher in pts with unfavorable outcome at 4, 28 and 76 hours (p=0.039, p=0.002 and p=0.010 respectively). No other significant differences between groups were found for all the other prognostic biomarkers under evaluation.

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. The results of the dosages of the biomarkers under evaluation do not appear to have influenced the treatment. Even though several biomarkers levels appeared to be significantly different at univariate analysis in pts with unfavourable outcome, the authors have not performed a multivariate analysis to allow control for several confounders. No cut-off for the biomarkers have been suggested. Therefore, the study has been ranked as supportive of a prognostic role of CSF-CK at 28 and 76 hours, CSF-CKBB at 28 and 76 hours, total LDH activity and LDH isoenzymes 1 and 2 activity at 76 hours, LDH isoenzyme 3 activity at 28 and 76 hours, CSF-Acid Phosphatase at 76 hours, CSF-lactate at 4, 28 and 76 hours to assess neurological outcome, but is not conclusive. Moreover, the opposed a role of beta-D-N-acetylglucosaminidase, and pyruvate to assess the prognosis of comatose survivors of cardiac arrest. (LOE P1, QOE fair).

†*Longstreth 1981 455-8

"Cerebrospinal fluid and serum creatine kinase BB activity after out-of-hospital cardiac arrest." <u>Neurology</u> 31(4): 455-8.

Reviewer's analysis

Study design: retrospective study (LOE P3).

Study population: 52 patients retrospectively extracted from a population of 55 pts admitted after resuscitation from OHCA

Prognostic biomarkers under evaluation: Serum CKBB levels (as determined on 2 serial samples collected <6 h after cardiac arrest and > 6h after cardiac arrest) and, in a subgroup of 20 pts in which they were available, CSF CKBB levels.

Outcome evaluated: survival and neurological outcome, defined as: group 1, no neurologic recovery and brief survival; group 2, full neurologic recovery; group 3, incomplete neurological recovery (ranging from dementia to persistent vegetative state); group 4, cardiac death.

Results: CKBB levels in CSF and serum were not available in all patients (see Table), thus limiting the value of the following analyses.

	CSF	Serum < 6 h	Serum > 6 h
Group 1 (n=10)	5/10	9/10	10/10
Group 2 (n=26)	8/26	26/26	26/26
Group 3 (n=11)	7/11	10/11	11/11

Group 4 (n=8)	0/8	5/8	n.a.

Sampled pts in Group 1 had higher CSF-CKBB levels compared to Group 2 ($p<0.001$) and Group 3 ($p<0.003$); sampled pts in Group 3 had higher CSF-CKBB levels compared to Group 2 ($p<0.001$); serum CKBB was detectable in Group 1 in all sampled pts both <6h and >6h; serum CKBB was detectable in Group 2 in 20/26 sampled pts at <6h and in 1/26 at>6h; serum CKBB was detectable in Group 3 in 9/10 sampled pts at <6h and in 5/11 at>6h; serum CKBB was detectable in Group 4 in all sampled pts at <6h. The authors conclude that both the levels of CSF-CKBB and the delayed presence of serum CKBB may be useful predictors of neurologic outcome after cardiac arrest.

 Quality of evidence: the study was retrospective in nature and assessment of biomarker's levels was not available in all pts, with a potential for a *detection bias*. Comparisons groups were clearly defined, outcomes were measured in the same way (not stated whether in a blinded fashion, presumably not) and follow up of pts was complete. At univariate analysis the biomarkers under evaluation (positive vs. negative) appeared to be predictive of outcome; the authors did not attempt a multivariate analyses to confirm an independent prognostic role of the biomarkers under evaluation. The authors did not compare the predictive performance of biomarkers under investigations to standard clinical or electrophysiological predictors of prognosis (as defined on 2005 guidelines). Therefore, the study has been ranked as supportive, of a prognostic role of CSF-CKBB and serum CKBB to assess survival and neurological outcome (LOE P3, QOE poor).

*Longstreth 1984 834-7

"Cerebrospinal fluid creatine kinase activity and neurologic recovery after cardiac arrest." <u>Neurology</u> 34(6): 834-7.

Reviewer's analysis
Study design: prospective inception cohort study (LOE P1).
Study population: Thirty patients resuscitated from OHCA
Biomarker under evaluation: Total CK levels on cerebrospinal fluid collected at a mean time of 18 hours (range 5-53). Outcome assessed: neurological outcome defined as awakening (following commands or having comprehensible speech) *vs.* not awakening. Results: One pt was excluded from analysis before knowing CSF-CK levels or outcome as soon as the authors became aware that he had a head trauma. CSF-CK was significantly higher in non-awakening as compared to awakening pts (120U/l *vs.* 10 U/L respectively, *p<0.007*). Using a cut-off level of 25U/L "only five patients were misclassified"; from the data presented in the paper, a sensitivity of 64%, a specificity of 100%, a PPV of 100% and a NPV of 75% were calculated for the proposed cut-off; CSF-CK was still significantly higher in non-awakening *vs.* awakening pts even after excluding from the analysis 2 pts with brain death on physical examination and isotopic flow study (120U/l *vs.* 10 U/L respectively, *p<0.03*).
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and in a blinded fashion; follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not formally performed. Therefore, the study has been ranked as supportive of a prognostic role of CSF-CK levels to assess neurological outcome. (LOE P1, QOE fair)

*Martens 1996 126-31

"Serum neuron-specific enolase as a prognostic marker for irreversible brain damage in comatose cardiac arrest survivors." <u>Acad Emerg Med</u> 3(2): 126-31.

Reviewer's analysis
Study design: prospective inception cohort study (LOE P1).
Study population: Fifty-two consecutive patients who remained comatose after successful resuscitation from OHCA.
Biomarker under evaluation: Serum NSE levels assessed on blood samples collected 24 hours after cardiac arrest; in 16 pts who remained alive but comatose a lumbar puncture was performed to assess CSF CPK-BB, CSF-AST and CSF-LDH.
Outcome assessed: neurological outcome, defined as return of consciousness *vs.* death due to CNS failure; pts regaining consciousness but eventually dying from non-neurologic causes as well as pts with neurologic impairment were considered as pts with successful outcome.
Results: S-NSE, GCS on admission, serum glucose on admission and total epinephrine dose before ROSC were significantly higher in pts with unfavorable outcome. A correlation between S-NSE on admission and outcome was confirmed by logistic regression analysis after adjustment for the possible confounding role of best GCS on admission, blood glucose on admission and total epinephrine dose before ROSC (adjusted OR 5.8; p=0.034). The predictive performance of CSF-CK-BB and CSF-LDH was also calculated by the ILCOR reviewer in 15 of 16 pts in whom the lumbar puncture was performed (one pts had an undetermined outcome and was excluded from the analysis; CSF-GOT was not determined in 4 pts and therefore calculations

were not attempted). Sensitivity, specificity, and FPR for CSF-CPK-BB were 83% (95% CI 55-95), 100% (95% CI 44-100) and 0 % (95%CI 0-66). Sensitivity, specificity and FPR for CSF-LDH were 75 % (95% CI 47-91), 100 % (95% CI 44-100) and 0% (95% CI 0-66) respectively (%). The predictive performance of s-NSE could not be assessed because individual data were not available. Biomarkers levels were not used to take treatment decisions. In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way and in a blinded fashion; follow up of pts was complete. S-NSE appeared to be correlated to outcome both at univariate and multivariate analysis; unfortunately, the predictive performance of abnormal s-NSE concentrations was not assessed, with limited clinical applicability of the results of logistic regression analysis. CSF-CPK-BB, CSF-GOT and CSF-LDH were predictive of outcome at some extent at univariate analysis, but were assessed only in a subgroup of pts. Therefore, the study has been ranked as supportive of a prognostic role of S-NSE (LOE P1 QOE good) and CSF-CPK-BB and CSF-LDH (LOE P1 QOE fair) and , CSF-GOT (LOE P1 QOE poor) to assess neurological outcome of comatose survivors of cardiac arrest.

†*Martens 1998 2363-66
"Serum S-100 and neuron-specific enolase for prediction of regaining consciousness after global cerebral ischemia." Stroke 29(11): 2363-2366.

Reviewer's analysis
Study design: prospective inception cohort trial (LOE P1)
Study population: 64 pts who remained comatose > 24 hours after resuscitation from cardiac arrest.
Biomarkers under evaluation: NSE and S100 levels as determined on blood samples collected at 24 hours after admission and NSE and S100 levels as determined on cerebrospinal fluid collected by lumbar puncture at 48 hours after admission. Of note, only some of the pts assessed by serum NSE and S100 received a lumbar puncture to assess CSF-NSE s-S100.
Outcome assessed: outcome was dichotomized into 2 groups: group 1 consisted of patients who died or remained in vegetative state, and group 2 consisted of patients who regained consciousness (i.e., obeyed simple verbal commands). Regaining consciousness was considered an end point for follow-up, regardless of outcome at discharge or at 6 months. Patients who eventually died of multiorgan failure but who had clearly regained consciousness after cardiac arrest were classified in group 2.
Results: Serum S-100, serum NSE, S-100 CSF and NSE CSF were significantly higher in patients who never regained consciousness compared with those who regained consciousness. S-100 and NSE correlated well with each other in the CSF; both CSF concentrations also correlated with their serum concentrations; S-100 and NSE did not correlate at all with each other in serum. The highest positive predictive values for predicting poor outcome were 95% for serum S-100 (cutoff value, 0.7 µg/L) and 96% for CSF-NSE (cutoff value, 50 µg/L). Highest sensitivity values were 93% for CSF-S100 and 89% for CSF-NSE. Specificity was highest with serum S-100 (96%,i.e. FPR 4) and serum NSE (89%, i.e. FPR 11)). In detail, the predictive performance of the biomarkers were: CSF-NSE (cut-off 50 µg/L) : Sensitivity 89%, Specificity 83%, FPR 17%; CSF-S100 (cut-off 6 µg/L): Sensitivity 93%, Specificity 60%, FPR 40%; S-NSE (cut-off 20 µg/L): Sensitivity 51%, Specificity 89%, FPR 11%; S-S100 (cut-off 0.7 µg/L): Sensitivity 55%, Specificity 96%, FPR 4%;
In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a prognostic role of s-NSE, s-S100, CSF-NSE, CSF-S100 to assess the probability of a favorable neurological outcome. (LOE P1, QOE fair).

Meynaar 2003 189-95
"Serum neuron-specific enolase predicts outcome in post-anoxic coma: a prospective cohort study." Intensive Care Med 29(2): 189-95.

Reviewer's analysis
Study design: prospective inception cohort study (LOE P1)
Study population: 110 comatose pts admitted to the ICU immediately following CPR after circulatory arrest for any cause. Patients received full intensive treatment until recovery or until absence of cortical response to somato-sensory evoked potentials (defined as bilateral absence of N20 on median nerve stimulation); moreover If GCS was still below 8 on the 6th day after CPR, treatment was withdrawn.
Biomarkers under evaluation: serum NSE as determined on blood samples collected on admission and daily for 5 days.
Outcome assessed: survival and neurological outcome, defined as the regain of consciousness (obviously awake or able to obey simple commands at least once, independent of successive survival or death).
Results: In the patients who regained consciousness, NSE was lower than in the patients remaining comatose. This difference was significant at 24 h (P <0.001) and 48 h (P =0.001) after admission. In addition, in the patients who remained comatose, NSE concentrations increased after admission with highest levels measured at 48 h after ICU admission. In contrast, in the patients who regained consciousness, NSE concentrations remained low. Finally, no NSE

concentration higher than 25.0 µg/l was measured at any time in a patient eventually regaining consciousness. There were, however, several patients with NSE levels below 25.0 µg/l who remained comatose and died. Sensitivity, specificity, PPV and NPV have been computed for a NSE cut-off level of 25 µg/l at 48 hours (59, 100 (FPR 0), 100 and 10 respectively); Sensitivity, specificity, PPV and NPV have also been computed for the absence of N20 at SSEP (64, 100 (FPR 0), 100 and 4 respectively); of great interest, the prognostic performance of the above mentioned criteria was significantly improved when they were combined so that [NSE levels >25 OR bilateral absence of N20 at SSEP] sensitivity, specificity, PPV and NPV were improved (78, 100 (FPR 0), 100, 18).

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. The biomarker under evaluation appeared to be predictive of outcome both at univariate and multivariate analysis. In addition, the authors did compare the predictive performance of biomarkers under investigations to SSEP. Therefore, the study has been ranked as supportive of a prognostic role of s-NSE levels at 24 and 48 hours to predict survival and neurological outcome with an additional value over SSEPs alone. (LOE P1, QOE good)

Mussack 2001 539-43; discussion 44

"S-100b, sE-selectin, and sP-selectin for evaluation of hypoxic brain damage in patients after cardiopulmonary resuscitation: pilot study." World J Surg 25(5): 539-543; discussion 544.

Reviewer's analysis
Study design: prospective inception cohort study (LOEP1)
Study population: 16 pts resuscitated after OHCA, unconscious on admission; it is unclear whether they were still comatose at 12 hours.
Biomarkers under evaluation: S100, sP-selectin and sE-selectin as determined on blood samples collected shortly after ROSC and 12 hours later.
Outcome assessed: survival at 24 hours, 30 days and to hospital discharge.
Results: 11 pts survived longer than 24 hours, 3 pts longer than 30 days, 2 pts were discharged from hospital with no neurologic impairment. According to the authors "Survival analyses revealed consistently high S-100b serum levels on scene (3.11 ng/ml; 2.23–3.98 ng/ml) and at 12 hours after ROSC (1.82 ng/ml; 1.59 –2.04 ng/ml) for the two patients dying within the first 24 hours, whereas serum levels significantly decreased in patients surviving longer than 24 hours or 30 days", but the significance levels of these differences as assessed by formal statistical testing were not provided. Similarly, the authors state that "P-selectin serum levels on admission increased slightly but not significantly above normal levels in patients with a survival of less than 24 hours and in those with longer survival", but the significance levels of these differences as assessed by formal statistical testing were not provided. Increasing E-selectin serum levels did not show a significant relation to survival time.
 In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. The authors did not compare the predictive performance of biomarkers under investigations to standard clinical or electrophysiological predictors of prognosis (as defined on 2005 guidelines). Therefore, the study has been ranked as supportive of a possible predictive role of the course of S100 during the first 12 hours after resuscitation from a cardiac arrest, but is not conclusive as the pts population is small, cut-offs have not been proposed, a multivariate analysis has not been performed and it is unclear whether pts were still comatose at the time of the second blood sample(LOE P1, QOE poor).

Mussack 2002 2669-74
"Serum S-100B and interleukin-8 as predictive markers for comparative neurologic outcome analysis of patients after cardiac arrest and severe traumatic brain injury." Crit Care Med 30(12): 2669-2674.

Reviewer's analysis
Study design: prospective inception cohort study LOE P1
Study population: 20 consecutive pts resuscitated from OHCA and surviving >12 h. The pts were not explicitly comatose at both sampling times but were presumably so as the authors state that "*Due to unconsciousness of the patients, informed consent was obtained for the first blood sampling retrospectively and for the second sampling prospectively in all cases within 6 hrs after study entry by the patients' relatives who were instructed about the purpose of blood*".
Biomarkers under evaluation: S100 and IL-8 as measured on blood samples collected on admission and 12 hours thereafter.
Outcome assessed: neurological outcome (GOS 1-3 unfavourable outcome; GOS 4-5 favorable outcome).
Results: S100 and IL 8 levels on admission after CA are similar to those observed after traumatic brain injury, and higher compared to healthy control. 12 hours after CA, median values of S100 decreased and IL8 increased compared to basal values. At multivariate logistic regression analysis, only age and S100 levels proved to be independent predictors of neurologic outcome. ROC analysis allowed to identify an optimal cut-off level of 0.76 ng/ml. At a cut-off of 0.76 ng/ml the positive

predictive value was 1.00 (95% CI 0.56–1.00), the specificity 1.00 (95% CI 0.31–1.00, i.e. FPR 0 (0-69)), the negative predictive value 0.33 (95% CI 0.09–0.69) and the sensitivity 0.54 (95% CI 0.26–0.80).

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. S-100 levels appeared to be predictive of outcome both at univariate and multivariate analysis. . Therefore, the study has been ranked as supportive of a predictive role of S-100 levels at 12 hours and opposing a role of IL-8 to assess neurological outcome in pts after cardiac arrest. (LOE P1, QOE good)

†Pfeifer 2005 49-55
"Outcome after cardiac arrest: predictive values and limitations of the neuroproteins neuron-specific enolase and protein S-100 and the Glasgow Coma Scale." <u>Resuscitation</u> 65(1): 49-55.

Reviewer's analysis
Study design: Prospective (inception) cohort study (LOE P1).

Study population: 97 pts arrived at ICU within 12 hours of ROSC after non-traumatic cardiac arrest (both in-hospital and out-of-hospital) and survived for a minimum of 48 hours.

Prognostic biomarker under evaluation: Serum NSE and S-100 sampled for 5 days (once to three times a day).

GCS was also assessed on a daily basis. Outcome evaluated: survival and neurological outcome at 28 days (bad outcome defined as Glasgow Outcome Scale (GOS) 1-2 v.s. good outcome defined as GOS 3-5).

Results: NSE and S-100 levels were significantly higher in pts with unfavorable outcome at 24 and 48 hours after ROSC, respectively.

Interestingly, while NSE and S-100 levels reached their peak and declined within 48 hours in pts with favorable outcome, NSE and S-100 levels reached their peak at 4 and 5 days respectively in pts with unfavorable outcome. NSE at day $3 \geq 65$ ng/ml had a sensitivity of 50%, a specificity of 96% (FPR 4%) and a positive predictive value of 97% to predict a poor neurological outcome. Similarly, S-100 levels ≥ 1.5 μg/l (not explicitly defined at which time, probably at any time) had a sensitivity of 34%, a specificity of 96% (FPR 4%) and a positive predictive value of 96% to predict a poor neurological outcome. Of note, in 10 pts with poor neurological outcome NSE levels were above the while S-100 was below the respective proposed cut-off values and in 4 pts with poor neurological outcome S-100 levels were above while the NSE was below the respective proposed cut-off values. However, a GCS<6 at day 3 plus both NSE and S-100 above the proposed cut-offs had a sensitivity of 20%, a specificity of 100% (FPR 0 %) and a positive predictive value of 100% to predict a poor neurological outcome; a GCS <6 at day 3 and NSE or S-100 above the respective proposed cut-offs had a sensitivity of 52%, a specificity of 96% (FPR 4%) and a positive predictive value of 96% to predict a poor neurological outcome; in pts in whom GCS assessment at day 3 was not possible (pts on muscle relaxants for mechanical ventilation), NSE or S-100 were above the respective proposed cut-off levels in 74% of pts but complete data to compute sensitivity, specificity and positive predictive value in this subset of pts were not available.

Quality of evidence: The study population was a sample of 97 pts comatose CA pts admitted to ICU within 12 hours after ROSC and surviving for a minimum of 48 hours. It is not explicitly stated whether these pts were consecutive. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was adequate. Of interest, the authors state that "during the period of the survey the results of NSE and S-100 had not influence on the treatment the first 7 days after ROSC"; it is therefore unclear whether NSE and S-100 had any influence on the treatment after the first 7 days, with a potential for *performance bias*. The authors did not attempt multivariate analyses, as the study population was not large enough to allow control for all known confounders and give conclusive results. Therefore, the study has been ranked as supportive of a predictive role of levels of NSE, S-100 and their combination to assess the neurological outcome of comatose survivors of CA (LOE P1, QOE fair)

Prohl 2007 1230-7
"Prediction of short-term and long-term outcomes after cardiac arrest: a prospective multivariate approach combining biochemical, clinical, electrophysiological, and neuropsychological investigations." <u>Crit Care Med</u> 35(5): 1230-7.

Reviewer's analysis
Study design: prospective study with derivation of a clinical decision rule (LOE P2).

Study population: 80 consecutive comatose pts admitted after either a IHCA or OHCA.

Prognostic biomarkers under evaluation: NSE and S-100B at days 2, 3 and 4 after CA. Prognostic biomarkers were investigated together with standardized clinical examinations (clinical examination score *CES* assessed on days 2 and 4), sensory-evoked potentials (N10, N20, N70 on day 4), and neuropsychological assessments ($\leq$1 and 6 months) in a multidimensional prognostic assessment model.

Outcome evaluated: neurological outcome (bad outcome defined as CPC score ≥ 4 vs. good outcome defined as CPC score <4)

and scores at neuropsychological testing.

Results: NSE and S-100B levels at all sampling times (days 2,3 and 4) were higher in pts with bad neurological outcome. Interestingly, NSE and S-100B peaked on day 2 and then decreased in pts with good outcome, while kept rising to reach a peak in day 3 (S-100B) or day 4 (NSE) in pts with unfavorable outcome. CES was significantly lower in pts with unfavorable outcome. The presence of SEP N70 had a significant correlation with a favorable outcome, while the correlation was weaker for N20 or absent for N10. At univariate analysis by ROC, NSE at day 2, 3 and 4 were predictive of neurological outcome (cut-off, sensitivity and specificity respectively: day 2, 29.1 ng/ml, 33, 100 (FPR 0); day 3, 31.65 ng/ml, 33, 100 (FPR 0); day 4 27.85 ng/ml, 67,100 (FPR 0)). At univariate analysis by ROC, S100 at day 2, 3 and 4 were predictive of neurological outcome (cut-off, sensitivity and specificity respectively: day 2, 2.14 ng/ml, 17, 100 (FPR 0); day 3, 2.76 ng/ml, 17, 100 (FPR 0); day 4 1.16 ng/ml, 33,100 (FPR 0)).   At univariate analysis by ROC, CES at day 2 and 4 were predictive of neurological outcome (cut-off, sensitivity and specificity respectively: day 2, 11, 93, 8; day 4 15, 42, 100). An attempt for multivariate analysis of several predictors (age, NSE at day 4, S-100B at days 2-4, CES at day 4, SEPs N10, N20 and N70) was made on 41 out of 66 pts who survived the first 4 days. This multivariate logistic-regression analysis resulted in a model in which 85% of the variance in the dichotomized CPC was explained by neuron-specific enolase at day 4, clinical examination score at day 4, and age. This predictor index had a sensitivity of 92% and a specificity of 93% (FPR 0.07). ROC analysis was also performed to assess the diagnostic performance of predictors and to assess the sensitivity associated to the cut-off levels which were selected to give a 100 % specificity, or in other words to identify the most sensitive predictor at a pre-specified specificity of 100% (NSE>27.85 at day 4 had a 100% specificity (FPR 0) and a 67 sensitivity to identify pts with unfavorable outcome). In addition, 26 patients (out of 33 survivors) underwent neuropsychological testing at 6 months. Significant correlations were found with selected cognitive variables and S-100B at day 3, long-latency sensory-evoked potential at day 4, and neuropsychological bedside screening.

Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete for CPC assessment at 1 and 6 months while NPS assessment was available in 26 out of 33 survivors; NSE and S-100B on days 2, 3 and 4 were available in 69 pts; CES was available in 63pts; SEPs were available in 51 pts. The authors performed a multivariate analyses, which however was limited to 41 out of 66 survivors at day 4, thus hindering the relevance of the observations. Therefore, the study has been ranked as supportive of an independent predictive role of NSE at day 4 to assess the neurological outcome of comatose survivors of cardiac arrest (LOE P2 QOE fair), as the multivariate analysis presented a possible *selection bias*)

†*Roine 1989 753-6
"Neurological outcome after out-of-hospital cardiac arrest. Prediction by cerebrospinal fluid enzyme analysis." <u>Arch Neurol</u> 46(7): 753-6.

Reviewer's analysis

Study design: prospective inception cohort study LOE P1

Study population: 75 consecutive victims of out of-hospital cardiac arrest, but it was not overtly stated whether they were comatose on admission. GCS was assessed at 1 h, 24 h and 7 days after admission but was not reported. The study was included in both the seminal systematic reviews on the topic by Wijdicks 2006 203-10 and Zandbergen 2001 1661-67 and therefore the ILCOR reviewer decided to report it

Biomarkers under evaluation: NSE and CK-BB as measured in samples of cerebrospinal fluid (CSF) and serum (CSF-NSE, s-NSE, CSF CK-BB, s-CK-BB)

Outcome assessed: The recovery of consciousness was defined as the ability to follow verbal commands. All patients were followed up for 3 months or until death. The 3-month outcome was classified according to the Glasgow Outcome Scale.

Results: Cerebrospinal fluid was obtained by lumbar puncture approximately 24 hours (20 - 26 hours) after cardiac arrest in 68 patients. Lumbar puncture was unsuccessful or contraindicated in seven patients. CSF-NSE was determined in 59 patients and the level of CSF-CK-BB was determined in 67 patients. All patients with CSF-NSE concentrations higher than 24 ng/mL remained unconscious and died. When this level is taken as a cut-off value, the test had a sensitivity of 74%, specificity and positive predictive value of 100% (FPR 0%), and negative predictive value of 89% in detecting patients who did not recover. Most of the patients who never recovered consciousness (group 2) had a CSF CK-BB concentration more than 10 times higher than those in group 1. With an arbitrary cut-off value of 17 ng/mL, the CSF CK-BB measurement had a sensitivity of 52%, specificity of 98% (FPR 2%), positive predictive value of 93%, and negative predictive value of 77% in detecting patients who never regained consciousness.

The serum level of NSE was determined in 65 patients and the level of CK-BB was determined in 71 patients. In group 2, the serum
level of NSE was significantly higher than in group 1(P<.001). If an arbitrary cut-off value of 17 ng/mL was chosen for serum NSE, the test had a sensitivity of 40 %, a specificity of 98% (FPR 2%) , a positive predictive value of 89%, and negative predictive value of 79% in detecting patients who did not recover consciousness (P<.001).. Although there was a borderline difference (P<.05) in the serum levels of CK-BB between groups 1and 2, the overlap was considerable, no cut-off value could be recognized, and the predictive indexes were not calculated.

Conclusions: The authors did not attempt multivariate analyses, as the study population was not large enough to allow control for

all known confounders and give conclusive results. Therefore, the study has been ranked as supportive of a predictive role of levels of s-NSE, CSF-NSE, CSF-CKBB at 24 hours to assess the neurological outcome of comatose survivors of CA (LOE P1, QOE fair). The present study also opposed a role of s-CKBB to assess the prognosis of comatose survivors of cardiac arrest.

†*Rosen 1998 473-77

"Increased serum levels of the S-100 protein are associated with hypoxic brain damage after cardiac arrest." Stroke 29(2): 473-477.

Reviewer's analysis
Study design: prospective inception cohort trial (LOE P1)
Study population: 41 comatose pts resuscitated from OHCA.
Biomarker under evaluation: S100 levels as determined on blood samples collected on days 1, 2 and 3.
Outcome assessed: survival at 14 days. Results: Levels of S100 were lower in survivors as compared to non-survivors at all sampling times. A cut-off of S100 levels ≤0.2 µg/L at day 1 had a PPV of 71% and a NPV of 85%. A cut-off of S100 levels ≤0.2 µg/L at day 2 had a PPV of 100% and a NPV of 89%. Sensitivity and specificity have been computed from data presented on the manuscript. Sensitivity and specificity at day 1 were 77% and 81%, respectively (FPR 19); Sensitivity and specificity at day 2 were 78% and 100% (FPR 0), respectively. In conclusion, the study was a prospective inception cohort study. Of note, 7 pts entered the study on day 2 and it is unclear whether blood samples on days 2 and 3 were collected from still comatose pts. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a predictive role of S100 at days 1 and 2 to assess the probability of survival at 14 days (LOE P1, QOE fair)

Rosen 2001 183-91

"Serum levels of the brain-derived proteins S-100 and NSE predict long-term outcome after cardiac arrest." Resuscitation 49(2): 183-91.

Reviewer's analysis
Study design: the study design has not been explicitly characterized as prospective and therefore it has been considered retrospective (LOE P3)
Study population: 66 consecutive comatose pts resuscitated from OHCA; it has not been specified whether part of these pts were enrolled in a previous study from the same group and published in 1998.
Biomarker under evaluation: Serum S100 and NSE levels as determined on blood samples collected on days 1, 2 and 3; the timing of determination of blood levels has not been specified (determination short after sampling or retrospectively determined on stored frozen samples).
Other prognostic factors evaluated: brain stem reflexes, anoxia time and coma level on admission.
Outcome assessed: neurological outcome defined as favorable outcome (GOS 3-5) *v.s.* unfavorable outcome (GOS 1-2).
Results: levels of S-100 and NSE on days 1–3 were higher among patients with a poor outcome compared with those with a good outcome. Positive predictive values and negative predictive values were computed ad different cut-offs separately on days 1, 2 and 3 for both S100 and NSE and are presented *in extenso* in the original manuscript. Of clinical interest: at day 1, S100 levels ≥0.4 µg/l had a PPV of 88% with a corresponding NPV of 55%; at day 2, S100 levels ≥0.25 µg/l had a PPV of 100% with a corresponding NPV of 58%; at day 3, S100 levels ≥0.20µg/l had a PPV of 100% with a corresponding NPV of 54%. At day 1, NSE levels ≥25 µg/l had a PPV of 100% with a corresponding NPV of 39%; At day 2, NSE levels ≥25 µg/l had a PPV of 100% with a corresponding NPV of 47%; At day 3, NSE levels ≥15 µg/l had a PPV of 100% with a corresponding NPV of 53%.
In conclusion, the study has been ranked as a retrospective cohort study. Of note, it is unclear whether blood samples on days 2 and 3 were collected from still comatose pts. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded); follow up of pts was incomplete as a few pts were lost to follow-up, but for 2 out of 3 the GOS could be estimated. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. However, the authors investigated also other prognostic factors in the same cohort (brainstem reflexes, anoxia time, neurological status on admission).. Therefore, the study has been ranked as supportive of a predictive role of NSE and S100 from days 1 to 3 to assess neurological outcome of comatose survivors of OHCA (LOE P3, QOE fair)

Rosen 2004 19-24

"CSF levels of neurofilament is a valuable predictor of long-term outcome after cardiac arrest." J Neurol Sci 221(1-2): 19-24.

Reviewer's analysis

Study design: the study has not been unambiguously characterized as prospective and has been considered retrospective cohort study (LOE P3)

Study population: 22 pts admitted after non traumatic OHCA with ROSC of ≥12 days.

Prognostic biomarker under evaluation: Levels of *neurofilament protein* (NFL) as determined on cerebrospinal fluid sampled by lumbar puncture between days 12-14. Prognostic biomarker was investigated together with standardized neurologic examination (coma level assessed by RLS 85 score; neurological status assessed by NIH stroke scale) on admission (t0), at days 2-4 (t1), days 12-14 (t1), day 45 (t3), 3 months (t4), 1 year (t5) .

Outcome evaluated: neurological outcome defined as the best Glasgow outcome scale score assessed at t2, t3, t4 and t5 (favorable outcome defined as GOS 1-2, unfavorable outcome defined as GOS 3-5); cognitive function defined as the best mini-mental state examination (MMSE) score at t3, t4, t5; dependence/independence in activities of daily living defined as the best Katz score (assessed at t3, t4, t5).

Results: NFL as determined on a CSF sample at day 17.5±1: (1) is significantly increased in pts with unfavorable as compared to those with favorable neurological outcome; (2) is significantly increased in pts with abnormal as compared to those with normal cognitive function; (3) is significantly increased in dependent pts as compared to those independent in ADL. A significant correlation has been shown between NFL and GOS, MMSE and dependence in ADL. Sensitivity and specificity of several cut-off levels are analyzed for all the outcome measures. Of special interest, for the purposes of the present worksheet, a NFL level in the CSF at 12-14 days > 12800 µg/l had a 100% sensitivity, 75% specificity (FPR 25), 100% PPV and 77% NPV; NFL level in the CSF at 12-14 days > 9622 µg/l had a 90% sensitivity, 92% specificity (FPR 8), 92% PPV and 90% NPV; NFL level in the CSF at 12-14 days > 5888 µg/l had a 80% sensitivity, 92% specificity (FPR 8), 92% PPV and 90% NPV. The performance of NSE levels in the CSF to predict dependency in activities of daily living and neurocognitive function was also assessed. The prognostic value of the clinical characteristics of the pts (standardized neurological evaluation, as previously detailed) was also assessed.

Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded). The study sample was extracted from a population of 105 pts after exclusion of 43 pts who died before day 12, 10 pts who refused, 17 pts who had contraindication to lumbar puncture, 5 pts who were lost to follow-up. Even though at univariate analysis the biomarker under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. In conclusion, this study supports a role of CSF Neurofilament concentration as assessed on average at day 15 after cardiac arrest to assess prognosis of comatose survivors of cardiac arrest (predictive performance for a poor outcome); NFL level in the CSF at 12-14 days > 9622 µg/l had a 90% sensitivity, 92% specificity (FPR 8%); NFL level in the CSF at 12-14 days > 5888 µg/l had a 80% sensitivity, 92% specificity (FPR 8%) (LOE P3, QOE fair)

*Rothstein 1991 101-7

"Predicting outcome in hypoxic-ischemic coma. A prospective clinical and electrophysiologic study." <u>Electroencephalogr Clin Neurophysiol</u> **79(2): 101-7.**

Reviewer's analysis

Study design: Prospective inception cohort study (LOE P1)

Study population: 16 out of 40 patients comatose for at least 6 hours after cardiac arrest

Prognostic biomarker under evaluation: CK-BB obtained on CSF by lumbar puncture 18-26 hours after cardiac arrest (cut-off arbitrarily set at 20 ng/ml)

Other prognostic factors under evaluation: EEG and SSEP

Outcome evaluated: poor outcome (defined as death without awakening plus survival with motor/cognitive impairment)

Results: CSF-CKBB was obtained only in 16 out of 40 pts enrolled in the study (primary objective of the study was evaluation of the predictive performance of SSEP and EEG). CSF-CKBB was below the arbitrary cut-off of 20 ng/ml (normal) in 6 pts, 2 of which died without awakening, 1 survived with neurological deficit and 3 recovered without deficits. CSF-CKBB was equal or above the arbitrary cut-off of 20 ng/ml (abnormal) in 10 pts, 8 of which died without awakening and 2survived with neurological deficit). The following predictive performance indicators of an abnormal value of CSF-CKBB to predict death without awakening were reported by the authors: sensitivity 80%, specificity 67%, NPV 67%, PPV 80%. The ILCOR reviewer calculated, on the basis of the published data, the following further indicators of predictive performance: sensitivity 80 (95%CI 49-94), specificity 67 % (95% CI 30-90), FPR 33% (95% CI 10-70).

Quality of evidence: The prognostic biomarkers under evaluation was assessed only in 16 out of 40 pts (it was not the primary objective of the study) and reasons for not assessing are not reported, resulting in a significant *selection bias.* Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded). Even though at univariate analysis the biomarker under evaluation appeared to be predictive of outcome, a multivariate analysis of its independent predictive role was not performed. Therefore, the study has been ranked as supportive of abnormal CK-BB levels as determined on CSF samples collected at days 18-26 h after CA to predict death without awakening, but with significant methodological limitations and with an unsatisfactory predictive performance (LOE P1, QOE poor)

Schoerkhuber 1999 1598-603
"Time course of serum neuron-specific enolase. A predictor of neurological outcome in patients resuscitated from cardiac arrest." Stroke 30(8): 1598-1603.

Reviewer's analysis
Study design: prospective inception cohort trial (LOE P1)
 Study population: 56 comatose pts resuscitated from OHCA.
Biomarker under evaluation: Serum NSE levels as determined on blood samples collected at 12, 24, 48 and 72 hours after ROSC.
Outcome assessed: neurolgical outcome as assessed by the best CPC achieved within 6 months from ROSC (CPC 1-2 good outcome *vs.* CPC 3-4 bad outcome).
Results: Levels of NSE were lower in pts with favorable outcome compared to those with unfavorable outcome at 12, 24, 48 and 72 hours. The predictive performance of NSE levels at different sampling times and of the highest NSE level over the first 72 hours after ROSC has been evaluated comparing the respective AUC. The best predictive performance was observed for NSE at 72 hours (AUC 0.92 ± 0.04). In a multivariate analysis (logistic regression) exploring the independent predictive role of maximum NSE level measured within 72 hours after cardiac arrest, the no-flow time and low-flow time, the cumulative dose of epinephrine, basic life support (yes/no), the location of cardiac arrest, age, and gender, the maximum level of NSE achieved during the 72 hours after ROSC was predictive of a poor neurological outcome (OR 1.09 95% CI 1.01- 1.19, p=0.04). Also of interest, a different time course of NSE levels was observed between the 2 outcome groups; while NSE levels tended to increase in patients with a bad neurological outcome, they tended to decrease in those with a good neurological outcome; If NSE concentrations increased by >15 µg/L between 12 and 72 hours, prognosis seems to be unfavorable.

	NSE LEVEL cut-point	Sensitivity	Specificity (FPR)
	µg/l	%	%
12 h	38.5	17.4	100 (0)
24h	40.0	8.0	100 (0)
48h	25.1	48.0	100 (0)
72 h	16.4	70.0	100 (0)
Highest concentration within 72h	27.3	28.6	100 (0)

In conclusion, the study was a prospective inception cohort study. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded) and follow up of pts was complete. The levels of biomarker under evaluation actually were not available for all pts at all times. The biomarker under evaluation appeared to be predictive of outcome, which was confirmed at a multivariate analysis considering also the no-flow time and low-flow time, the cumulative dose of epinephrine, basic life support (yes/no), the location of cardiac arrest, age, and gender . Therefore, the study has been ranked as supportive of a predictive role of NSE at 72 hours to estimate the probability of unfavorable neurological outcome (LOE P1, QOE good)

*Sherman 2000 889-94

"Somatosensory potentials, CSF creatine kinase BB activity, and awakening after cardiac arrest." Neurology 54(4): 889-94.

Reviewer's analysis
Study design: retrospective cohort study (LOE P3).
Study population: 52 pts out of 72 comatose pts after cardiac arrest.
Prognostic biomarker under evaluation: Levels of CKBB as determined on cerebrospinal fluid sampled by lumbar puncture (17/52 between 48-72 hours after CA, 31/52 between 2-3 days after CA (unclear difference), no details about timing of lumbar puncture in the remaining pts). Prognostic biomarker was investigated together with somatosensory evoked potentials (SEP), GCS, early pupillary reactivity, EEG results, CT results, presence of myoclonus and seizures all as abstracted from medical records.
Outcome evaluated: neurological outcome defined as awakening (ability to follow command, to use comprehensible speech or both). Results: CKBB ≥ 205 U/L predicted nonawakening with a sensitivity of 49% and a specificity of 100% (FPR 0). Bilateral absence of the N1 peak predicted nonawakening with a sensitivity of 53% and a specificity of 100% (FPR 0). CKBB ≥ 205 U/L

or bilaterally absent SEP N1 peaks predicted nonawakening with a sensitivity of 69% and a specificity of 100% (FPR 0). CKBB ≥ 205 U/L, bilaterally absent N1 peaks, bilateral N3 ≥ 176 msec or absent, or some combination predicted non-awakening with a sensitivity of 78% and a specificity of 100% (FPR 0).

Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitly blinded). Results of the test under investigation may have affected treatment decision, with a possible *performance bias.* The univariate predictive performance of the markers under investigation and their combination has been retrospectively assessed, but multivariate analysis has not been attempted. The authors also assessed the predictive performance of several clinical findings and electrophysiological predictors of prognosis on the same cohort (GCS, early pupillary reactivity, EEG results, CT results, presence of myoclonus and seizures). Therefore, the study has been ranked as supportive of a predictive role of CK-BB levels as determined on CSF to assess neurological outcome of comatose survivors of cardiac arrest. (LOE P3, QOE fair).

Sodeck 2007 439-45
"Can brain natriuretic peptide predict outcome after cardiac arrest? An observational study." <u>Resuscitation</u> 74(3): 439-445.

Reviewer's analysis
Study design: Retrospective cohort study (observational case series with convenience sampling) (LOE P3).
Study population: 155 non-consecutive comatose cardiac arrest survivors.
Prognostic biomarker under evaluation: BNP on admission to emergency department from arterial line.
 Outcome evaluated: survival and neurological outcome at 6months (bad outcome defined as best CPC score during 6 months f.u. ≥ 3 v.s. good outcome defined as best CPC score <3 during 6 months f.u).
 Results: BNP on admission was significantly higher in pts with unfavorable outcome. At univariate analysis, BNP levels on admission in the upper quartile (> 230 pg/ml) were predictive of the combined end-point of non-survival or unfavorable outcome. The authors attempted a multivariate analysis to adjust the results for the confounding factors: age, diabetes, chronic heart failure, initial rhythm, cumulative epinephrine, lactate levels on admission; at multivariate analysis, BNP on admission in the 4th quartile (> 230 pg/ml) was independently predictive of survival and unfavorable neurologic outcome.
Quality of evidence: The study population was a convenience sample of 155 out of 697 comatose CA pts admitted to ED after ROSC. Comparisons groups were clearly defined, outcomes were measured in the same way (presumably blinded) and follow up of pts was complete. However, even though the authors attempted multivariate analyses, the study population was not large enough to allow control for all known confounders and give conclusive results. Therefore, the study has been ranked as supportive of a predictive role of BNP to assess survival and neurological outcome in comatose survivors of cardiac arreste. (LOE P3, QOE fair)

†Tiainen 2003 2881-86
"Serum neuron-specific enolase and S-100B protein in cardiac arrest patients treated with hypothermia." <u>Stroke</u> 34(12): 2881-2886.

Reviewer's analysis
Study design: Prospective (inception) cohort study (LOE P1) as a sub-study of the Hypothermia after Cardiac Arrest Trial (HACA) . Study population: 70 adult pts arrived at ED after a cardiac arrest with several restrictive inclusion criteria: witnessed CA, cardiac rhythm with VF or pulseless VT as the initial rhythm, a presumed cardiac origin of the arrest, an estimated interval of 5 to 15 minutes from collapse to EMS intervention and an interval from collapse to ROSC < 60 min. Patients were randomized to therapeutic hypothermia (TH) or standard treatment (ST) .
Prognostic biomarker under evaluation: Serum NSE and S-100 sampled at 24, 36, 48 hours after ROSC.
Outcome evaluated: survival and neurological outcome at 6 months (bad outcome defined as CPC score ≥ 3 v.s. good outcome defined as CPC score 1-2). Results: 36 out of the 70 pts were randomized to TH and 34 to ST. NSE and S-100 were available for 35/36 HT pts and 33/34 ST pts. After 6 months, a favorable outcome was observed in 69% of HT pts *v.s.* 47 % of ST pts. A decrease of NSE levels between 24 and 48 hours was observed in 88% of HT pts as compared to 50% of ST pts; a decrease of S-100 levels between 24 and 48 hours was observed in 50% of HT pts as compared to 45% of ST pts. A decrease of NSE but not of S-100 between 24 and 48 hours was associated with a favorable outcome at 6 months. Cut-off levels associated with at least 95% specificity for predicting unfavorable outcome were calculated by ROC analysis for both NSE and S-100 at 24, 36 and 48 hours in HT and ST as separate groups. Cut-off levels associated with at least 95% specificity for predicting unfavorable outcome were higher in HT as compared to ST group, and sensitivity values associated to these cut-offs were remarkably lower in HT as compared to ST pts. The following table, extracted from the manuscript, details cut-off values and their performance

	Hypothermia				Normothermia			
	Cut-off	Specificity	FPR	Sensitivity	Cut-off	Specificity	FPR	Sensitivity
	μg/L	(%)	(%)	(%)	μg/L	(%)	(%)	(%)
NSE								

24 h	31.2	96	4	22	13.3	100	0	59
36 h	26.0	96	4	30	12.6	100	0	59
48 h	25.0	96	4	25	8.8	100	0	76
S-100								
24 h	0.21	100	0	30	0.19	100	0	59
36 h	0.21	96	4	20	0.12	100	0	88
48 h	0.23	96	4	22	0.12	100	0	88

Of interest, the AUCs at all study times were lower in the hypothermia group as compared to the normothermia group, again suggesting a less favorable predictive performance of the biomarkers under investigation in pts treated with therapeutic hypothermia.

	Hypothermia	Normothermia
	(AUC)	(AUC)
NSE		
24 h	0.72	0.89
36 h	0.85	0.88
48 h	0.80	0.89
S-100		
24 h	0.65	0.90
36 h	0.69	0.85
48 h	0.63	0.91

AUC: Area under the curve at ROC analysis

Quality of evidence: The study population was a sample of 70 pts admitted to ED after CA with very selective inclusion criteria and randomized to TH or ST. Comparisons groups were clearly defined, outcomes were measured in the same way (not explicitely blinded) and follow up of pts was adequate. However, even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of their independent predictive role was not performed. Therefore, the study has been ranked as supportive of a predictive role of NSE and S100 levels as determined on blood samples at 24, 36 and 48 hours to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their predictive performance and cut-off levels at comparable levels of specificity are different in pts treated with therapeutic hypothermia (LOE P1, QOE fair)

*Tirschwell 1997 352-7
"Cerebrospinal fluid creatine kinase BB isoenzyme activity and neurologic prognosis after cardiac arrest." <u>Neurology</u> 48(2): 352-7

Reviewer's analysis
Study design: Retrospective cohort study (LOE P3).
Study population: 351 comatose cardiac arrest survivors extracted from a database of 474 pts admitted to an ICU who had their CK-BB levels assessed on CSF for prognostic stratification.
Prognostic biomarker under evaluation: CK-BB levels assessed on CSF (given the retrospective nature of the study, sampling was not performed according to a standardized protocol at specific times, but "usually…48-72 hours after cardiac arrest") .
Outcome evaluated: neurological outcome defined as awakening *vs.* non-awakening on the basis of information extracted from medical charts (awakening was defined as pt having comprehensible speech, following commands or both).
Results: patients. CSF CKBB was significantly higher for those who never awakened compared with those who ever awakened. The predictive performance of CSF CKBB was analyzed at different cut-offs, as detailed in the following table.

CSF-CKBB cut-off (U/l)	Sensitivity	Specificity	FPR	PPV
10	90	62	38	92
20	87	72	28	94
30	84	79	21	95
50	82	85	15	96
100	69	92	8	98

150	59	97	3	99
205	48	100	0	100
250	41	100	0	100

Quality of evidence: The study population was retrospectively assembled by extracting the medical charts of 474 pts in whom a determination of CKBB CSF levels was ordered by the treating physician to assess prognosis. 351 of out of these 474 were survivors of cardiac arrest. Timing of CSF sampling by lumbar puncture could not be assessed precisely but is reported by the authors to be "usually" 48-72 hours after cardiac arrest. Of methodological relevance, the results of CSF CKBB determination might have affected treatment in some pts, with a *performance bias* and a possible so-called *self-fulfilling prophecy* . Comparisons groups were clearly defined, outcomes were measured in the same way (not blinded). All patients were followed until they awakened or died, except for three patients who were unconscious at the time of the last contact at 63, 107, and 109 months their arrests. Even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of its independent predictive role was not performed. Therefore, the study has been ranked as supportive of a predictive role of CSF CKBB levels as determined in comatose survivors of cardiac arrest 48-72 hours after the event (LOE P3, QOE poor)

Wijdicks 2006 203-10
"Practice parameter: prediction of outcome in comatose survivors after cardiopulmonary resuscitation (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology." Neurology 67(2): 203-10.

Reviewer's analysis
Study design: Meta-analysis (LOE P1)
Study population: see individual studies (marked with † throughout the worksheet)
Biomarkers evaluated: serum NSE, serum S100, cerebrospinal fluid CKBB, cerebrospinal fluid neurofilament
Results: *serum NSE:* One class I study [Zandbergen 2006 62-8], four class III studies [Pfeifer 2005 49-55, Tiainen 2003 2881-86, Fogel 1997 1133-38, Martens 1998 2363-66], and one class IV study [Dauberschmidt 1991 237-45] have investigated the usefulness of increased serum NSE as a marker of poor outcome. In the class I study [Zandbergen 2006 62-8], 60% of 231patients had NSE >33 μg/L at day 1 to 3 after CPR. All these patients had a poor outcome (FPR 0; 95% CI: 0 to 3). Most other studies also found an increase in serum NSE at day 3. However, the cutoff points for a 0 FPR value vary greatly (20 to 65 μg/L). An FPR could not be obtained in two studies, and it ranged from 0 to 11% in class III studies. Of interest, group analyses have shown that serum levels of NSE, but not those of S100, are significantly lower in patients treated with induced hypothermia compared with those of untreated patients.
Serum astroglial S100 has been investigated in one class I study [Zandbergen 2006 62-8], four class III studies [Pfeifer 2005 49-55, Tiainen 2003 2881-86, Martens 1998 2363-66, Rosen 1998 473-77] and one class IV study [Bottiger 2001 2694-98]. The median FPR was 2% (range 0 to 54%) in the four studies that allowed this calculation, and it was 5% in the class I study. Predictions were based on values measured within the first 2 days after cardiac arrest. Six class III studies [Longstreth 1981 455-8, Clemmensen 1987 235-6, Roine 1989 753-6, Rothstein 1991 101-7, Tirschwell 1997 352-7, Sherman 2000 889-94] investigated the usefulness of *CSF CKBB* as an indicator of poor outcome. Values used to identify those with poor outcome varied widely. The median FPR was 15% (range 0 to 33%) in six studies allowing the calculations, indicating a poor prognostic ability. Furthermore, the availability of this test result could have influenced the decision to withdraw life support. *Neurofilament in CSF* was measured in one class IV study [Rosen 2004 19-24] 2 to 3 weeks after resuscitation in a series of 22 patients and yielded an FPR of 10%.
Conclusions (as reported by the authors of the meta-anaysis): "Serum NSE, S100, and CSF CKBB have been investigated as a predictor for outcome with studies using variable cutoff points. For serum NSE levels > 33 μg/L at days 1 to 3, one class I study [Zandbergen 2006 62-8] demonstrates a 0 FPR with narrow 95% CIs. Recommendations. Serum NSE levels >33 μg/L at days 1 to 3 post-CPR accurately predict poor outcome (recommendation level B). There are inadequate data to support or refute the prognostic value of other serum and CSF biochemical markers in comatose patients after CPR (recommendation level U)". The author of the worksheet concluded that this systematic review supports a role of serum NSE, serum S100, cerebrospinal fluid CKBB, cerebrospinal fluid neurofilament to assess the prognosis of comatose survivors of cardiac arrest, but s-NSE showed the best predictive performance (LOE P1 QOE good)

Zandbergen 2001 1661-67
"Systematic review of prediction of poor outcome in anoxic-ischaemic coma with biochemical markers of brain damage." Intensive Care Med 27(10): 1661-1667.

Reviewer's analysis
Study design: Meta-analysis of prognostic studies (LOE P1). The specific objectives of the review were stated clearly. The study was carefully designed and criteria for inclusion were described. Characteristics and methodological quality of each trial were detailed and a log of excluded studies with reasons for exclusion was reported. The studies cited in the present meta-analysis and included in the worksheet are marked with an asterisk throughout the worksheet A methodological limitation of the present meta-analysis is that data were pooled irrespective of the nature of the study (either prospective or retrospective) and of blinding of physician to the results of the biomarker level. In conclusion, the study is a valuable systematic review of the literature, but

the studies included in the meta-analysis were both prospective and retrospective and blinded and unblinded. However, the present study is supportive of a predictive role of CKBB, NSE, S100, LDH and GOT levels as determined on the CSF and of CKBB, NSE and S100 as assessed on blood samples to assess survival and neurological outcome in comatose survivors of cardiac arrest, but their clinical application for non-treatment decisions is limited mainly by the wide confidence limits of the pooled estimates of false positive rates

	95% CI of the pooled false positive rates
CSF	
CKBB>20 UI/L	3.0-7.9
CKBB>20ng/ml	2.7-32.4
CKBB>204U/l	0-2.3
NSE>24 ng/l	0-23.2
NSE>50 ng/l	0.1-19.0
S100>6 µg/l	2.2-27.3
LDH>82 U/l	0-33.6
GOT>62 U/l	0-36.9
Serum	
CKBB present	4.1-45.6
CKBB>7.5 U/l	29.9-92.5
NSE>17 ng/ml	10.8-42.5
NSE>20 ng/ml	4.2-26.8
NSE>33 ng/ml	0.1-22.8
S100 > 0.7 µg/l	0.1-22.8

†Zandbergen 2006 62-8

"Prediction of poor outcome within the first 3 days of postanoxic coma." <u>Neurology</u> **66(1): 62-8.**

Reviewer's analysis
Study design: Prospective (inception) cohort study (LOE P1).
Study population: 407 adult comatose survivors of CA.
Prognostic biomarker under evaluation: Serum NSE and S-100 sampled at 24, 48 and 72 hours after CPR.
Other prognostic markers evaluated: clinical characteristics, median nerve SSEP at 24, 48 and 72 hours, and EEG at 72 h.
Outcome evaluated: neurological outcome (Glasgow outcome scale at 1 month and at 1 year in survivors
Results: All patients unconscious at 72 h with bilateral absence of N20 at any time had a poor outcome (95%CI of false positive rate 0-3%); All patients unconscious a 72 h with NSE>33 µg/ml (but a blood sample was available only in 231/305) at any time had a poor outcome (95% CI of false positive rate 0-3%); pts with bilateral absence of N20 or NSE>33 µg/ml overlapped only partially; all pts with bilateral absence of N20 or NSE>33 µg/ml had a poor outcome (05% CI 0-2%). In pts without absent N20 and NSE not > 33 µg/ml, a small number of pts with poor outcome could be identified with EEG (burst suppression or no voltage > 20 µV). In conclusion, 252 out of 356 pts with poor outcome could be predicted with these 3 variables in the first 3 days after CPR. Predictive performance of S-100 levels > 0.7 µg/ml was less satisfactory than NSE levels >33 µg/ml. False positive rate (95% CI) of S-100 levels > 0.7 µg/ml were 3 (1-8), 2 (0-7), 0 (0-5), 2 (1-7) respectively at 24-48-72 and 24-72 hours; False positive rate (95% CI) of NSE levels >33 µg/ml were 0 (0-3), 0 (0-3), 0 (0-4), 0 (0-3) respectively at 24-48-72 and 24-72 hours;
Quality of evidence: Comparison groups were clearly defined. Outcome was assessed in the same way. Blood samples were not available in many enrolled pts, so that a *detection bias* cannot be excluded. Treatment could be modified according to the results of neurological examination, SSEP at 72 h and EEG with possible *performace bias*. Therapeutic hypothermia was introduced during the study and used in a limited number of pts, and a confounding effect cannot be entirely excluded. The univariate predicitive performance of clinical variables, SSEP, NSE and S100-B was assessed with derivation of a clinical decision rule, which however deserve a prospective validation.

Conclusions: The study therefore has been ranked as supportive of a predictive role of NSE and S100 levels to assess neurological outcome in comatose survivors of cardiac arrest (LOE P1, fair).

Zingler 2003 79-84

"Early prediction of neurological outcome after cardiopulmonary resuscitation: a multimodal approach combining neurobiochemical and electrophysiological investigations may provide high prognostic certainty in patients after cardiac arrest." Eur Neurol **49**(2): 79-84.

Reviewer's analysis
Study design: Prospective inception cohort study (LOE P1).
Study population: 27 consecutive comatose survivors of either in or out-of-hospital cardiac arrest.
Prognostic biomarker under evaluation: NSE and S100 levels as assessed on blood samples collected on days 1, 2, 3 and 7 after cardiac arrest. Other prognostic factors under investigation: SSEP as assessed on days 2 and 7, GCS, EEG and standardized neurological examination.
Outcome evaluated: neurological outcome at 2, 4 and 12 weeks after cardiac arrest (bad outcome defined as best CPC score during f.u. ≥ 3 v.s. good outcome defined as best CPC score <3 during 6 months f.u).
Results: NSE levels were higher in pts with unfavorable outcome on days 2, 3 and 7 but not on day 1. NSE release pattern was characterized by a progressive increase over days 1-3 in pts with unfavorable outcome but not in pts with favorable outcome. NSE levels ≥ 43 µg/l on day 2 had a 100% specificity and a 90% sensitivity to identify pts with unfavorable outcome. S100 levels were higher in pts with unfavorable outcome on days 1, 2, 3 (not specified on day 7). S100 levels ≥ 0.5 µg/l on day 3 had a 100% specificity (FPR 0) and a 75% sensitivity to identify pts with unfavorable outcome. The presence of both NSE levels ≥ 43 µg/l and S100 levels ≥ 0.5 µg/l was not associated with an improvement of predictive performance.
Quality of evidence: Comparisons groups were clearly defined, outcomes were measured in the same way (presumably blinded) and follow up of pts was complete. Even though at univariate analysis the biomarkers under evaluation appeared to be predictive of outcome, a multivariate analysis of its independent predictive role was not performed. The authors also assessed the correlation between SSEP and neurological outcome without a formal comparison with the biomarkers under investigation.
Conclusions Therefore, the study has been ranked as supportive of a predictive role of NSE and S100 to assess survival and neurological outcome in comatose survivors of cardiac arrest. (LOE P1, QOE fair)

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ABBREVIATION LIST

BNP	Brain natriuretic peptide
CA	Cardiac arrest
ICAM-1	intracellular adhesion molecule-1
IHCA	In-hospital cardiac arrest
IL-10	Interleukin 10
IL-1ra	Interleukin 1 receptor antagonist
IL-6	Interleukin 6
IL-8	Interleukin 8
NSE	Neuron specific enolase
OHCA	Out-of-hospital cardiac arrest
PCT	Procalcitonin
s-IL-8	Serum Interleukin 8
s-NSE	Serum neuron specific enolase
s-S-100	Serum astroglial protein S-100
sTNFRII	soluble TNF receptor type II
sTREM-1	Soluble triggering receptor expressed on myeloid cells 1
TH	Therapeutic hypothermia
vWF	von Willebrand factor