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Serum Biomarkers of Brain Injury after Pediatric Cardiac Arrest

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Abstract

Objective—Morbidity and mortality in children with cardiac arrest (CA) largely result from neurologic injury. Serum biomarkers of brain injury can potentially measure injury to neurons (neuron specific enolase [NSE]), astrocytes (S100b), and axons (myelin basic protein [MBP]). We hypothesized that serum biomarkers can be used to predict outcome from pediatric CA.

Design—Prospective observational study.

Setting—Single tertiary pediatric hospital.

Patients—Forty-three children with cardiac arrest.

Interventions—None.

Measurements and Main Results—We measured serum NSE, S100b, and MBP on days 1–4 and 7 after CA. We recorded demographics, details of the CA and resuscitation, and Pediatric Cerebral Performance Category (PCPC) at hospital discharge and 6 months. We analyzed the association of biomarker levels at 24, 48, and 72 hours with good (PCPC 1–3) or poor (PCPC 4–6) outcome and mortality. Forty-three children (49% female; mean age of 5.9 ± 6.3 were enrolled and 17 (40%) died. Serum concentration at 24 hours for all 3 biomarkers predicted mortality (all $p<0.05$). Additionally, serum NSE and S100b concentrations were increased in the poor outcome vs. good outcome group and in subjects who died at all time points (all $p<0.05$). Receiver operator curves for serum S100b and NSE to predict good vs. poor outcome at 6 months was superior to

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clinical predictors. There was no association between serum biomarker concentrations and subject temperature.

Conclusions—Preliminary data show that serum S100b, NSE, and MBP may aid in outcome prediction of children surviving CA.

Keywords

Biomarker; heart arrest; hypoxia-ischemia, brain; child; outcome assessment (health care); resuscitation

INTRODUCTION

Children with cardiac arrest (CA) have mortality rates of about 50% for in-hospital CA and 80% for out-of-hospital CA, with many survivors having neurological disability[1–8]. These poor outcomes are partly attributable to the fact that unlike in adults, most pediatric CAs begin with respiratory arrest or shock, followed by hypoxemia, bradycardia, and finally, global ischemia. Furthermore, unlike adult CA or neonatal asphyxia, there are no novel treatment strategies proven to be effective in improving outcomes in pediatric CA.

Current clinical and laboratory tests performed early in the hospital course in pediatric CA are not robustly associated with long term outcomes. Uncertainty of prognosis can be excruciating for families faced with making medical decisions. Early recognition of brain injury on physical examination can be obscured by medications and developmental stage, and under-recognized brain injury may prevent implementation of neuroprotective therapies and monitoring. In addition, the use of broad entry criteria without stratification for severity of injury in RCTs runs the risk of a heterogeneous sample which can obscure subgroups that may derive benefit from the intervention being evaluated despite an overall negative study[9].

Serum brain-specific biomarkers may help predict outcome after various pediatric brain insults. Serum biomarkers of brain injury such as neuron specific enolase (NSE), S100b, and myelin basic protein (MBP), from neurons, astrocytes, and axons respectively, have the potential to assist in the early detection and quantification of the severity of brain injury, response to therapeutic interventions, and prediction of outcome after CA[10–13]. Brain biomarkers have different patterns of release and limitations to their use[14,15]. As a result, clinical applicability may be limited by type of brain injury (i.e., trauma, stroke, hypoxia-ischemia, cardiopulmonary bypass)[16–20]. A prospective pilot study involving pediatric subjects who received 24 hours of therapeutic hypothermia found that serum NSE and S100b had excellent specificity and fair sensitivity at 48 hours or later to predict outcome at hospital discharge[10]. White matter injury seen after hypoxia-ischemia suggests a potential role for MBP but clinical studies have been limited[21,22].

In this single center prospective study, we examined the time course of serum NSE, S100b, and MBP levels in children during the first week after CA and tested the accuracy of serum and clinical biomarkers to predict good vs. poor outcome at 6 months. We hypothesized that serum biomarkers of brain injury targeting various cell types (neurons, astrocytes, axons) would predict subject outcome at clinically relevant time points (24 hours).

MATERIALS AND METHODS

Design and Setting

Between November 2009 and September 2011, 43 subjects with CA were enrolled in one of 2 studies at the Children's Hospital of Pittsburgh (Figure 1). Twenty-five subjects were enrolled in an RCT (NCT00797680) and were randomized to either 24 or 72 hours of therapeutic hypothermia (target temperature 33 ± 1°C). Eighteen subjects who did not meet criteria for the RCT were enrolled in an observational study, and the pediatric ICU physician made decisions about post-resuscitation temperature management. Duration of hypothermia is not disclosed in this manuscript because both studies remain open to enrollment. Both studies were approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from the subject's parent or guardian.

Inclusion and Exclusion Criteria

We studied children between the ages 1 week and 17 years who were admitted to the ICU with ROSC after in- or out-of-hospital CA. CA was defined as receipt of chest compressions for pulselessness by a healthcare worker. Subjects were included if they had an indwelling arterial or venous catheter for blood draws. Subjects were excluded if they had a do not resuscitate status, were pregnant, had any contraindication for MRI, had another simultaneous acute brain disease, were undergoing brain death evaluation, or had a metabolic disease affecting the brain. Subjects were also excluded from the RCT if they had active hemorrhage or a pre-existing coagulation defect or if they had a long bone fracture (because of the potential for false positive S100b concentrations). Additionally, specifically for the RCT, subjects were included if Glasgow Coma Scale (GCS) score was ≤ 8 after ROSC and if they had therapeutic hypothermia initiated by their ICU attending.

Post-Resuscitation Care

Post-resuscitation care in the PICU consisted of prevention of secondary neurologic insults, treatment of organ dysfunction, and investigation of cause of CA if unknown[23]. Standards of care for most children post-CA included intubation and mechanical ventilation and placement of central venous and arterial catheters. Targets for PaO_2 and $PaCO_2$ were ~100 mmHg and 40 mmHg, respectively, as well as maintenance of normal mean arterial blood pressure for age. Pain and sedation medications were used at the discretion of the attending physician and were often withheld initially to obtain an accurate neurological examination. Electroencephalography (EEG) and brain CT were commonly obtained in the first 24–48 hours after ROSC, but continuous EEG was not routinely used. Prophylactic anti-epileptic medications were not used as standard of care. Rectal and/or esophageal temperature probes were used for continuous temperature reading. Fever (>38 °C) was treated aggressively in all subjects with methods similar to those used for cooling induction as well as with anti-pyretics[24].

Data Collection

Data were collected from medical charts using the Utstein template for CA, including subject demographics, details about the CA and resuscitation, post-resuscitation care, and

outcomes[25]. Inotrope score was calculated using the highest concentrations within the first 24 hours post-arrest[26]. Initial and subsequent GCS score, GCS motor score, and pupillary reactivity were recorded from nurse charting. EEG result was designated as 'good' if it was read by the attending neurologist as a continuous background without diffuse slowing or 'poor' if the background was discontinuous, demonstrated diffuse slowing, burst suppression, or was markedly attenuated[27]. Brain CT scan was recorded as 'acute brain lesion' if the attending neuroradiologist identified brain edema, loss of gray-white differentiation, or herniation was present or 'no acute lesion' in the absence of those findings.

Serum Biomarkers

Three ml of blood were collected twice daily (days 1–4) and once on day 7 after ROSC. For the first 4 subjects, the initial blood sample was obtained only after informed consent was acquired. Subsequently, the IRB granted permission for the initial sample to be drawn prior to consent. If consent was not obtained, the sample was discarded. Samples were centrifuged, aliquoted, frozen at −70°C, and analyzed in batches. Serum NSE, S100b, and MBP were measured in duplicate using commercially available ELISAs (International Point of Care, Toronto, Ontario, Canada). NSE concentration was corrected for hemolysis[14]. The sensitivity of the assays was 0.1 ng/mL for NSE and MBP and 0.01 ng/mL for S100b. The coefficient of variation for each assay was < 10%. An experienced technician blinded to subject treatment and outcome performed all biomarker measurements. Clinical team members were unaware of the biomarker results. Serum biomarker time points were measured from time of ROSC as documented in the chart. Samples closest to but not greater than 24, 48, 72, 96, and 120 hours after ROSC were used in the analysis.

Outcome Measures

Subjects were followed until 6 months post-CA. The primary outcome was the accuracy of serum brain biomarker concentrations to predict good (Pediatric Cerebral Performance Category (PCPC) score 1–3) or poor (PCPC 4–6) outcome[28]. The PI, who assigned the pre-arrest, hospital discharge, and 6 month PCPC scores, was blinded to biomarker results but not to clinical course. Six month outcomes were performed in surviving children either over the telephone or during in-person interview with the parent or guardian during a scheduled outpatient visit. Secondary outcomes included mortality at hospital discharge and 6 months. Additional analyses included effect of temperature on serum biomarker concentration and clinical variable assessment for prediction of primary and secondary outcomes.

Data analysis

Biomarker data are presented as median (interquartile range [IQR]) since data were skewed. Other continuous variables are presented as mean and standard deviation (SD). The data were analyzed for outcome group differences with Fisher's exact tests for categorical variables. Median serum biomarkers were represented graphically by outcome group. The Wilcoxon rank sum was used to compare serum biomarker concentration and outcome. Spearman's rank correlation was used to test correlation between serum biomarker concentration and temperature and age. Kruskal-Wallis test was used to detect if there were any differences between categorical temperature groups (< 34°C, 34–36°C, > 36°C). Mann-

Whitney U test was then used to determine where differences existed between groups. Receiver operating characteristic (ROC) curves were used to evaluate the probability that a serum or clinical biomarker would correctly classify an outcome. Clinical biomarkers chosen for ROC curves were continuous variables that predicted outcome with $p < 0.05$ in univariate analysis. ROC curves were generated using raw data from all time points. Sensitivity analysis was performed for serum biomarkers at clinically relevant time points. All *p*-values were two-sided. Data analysis was performed using SPSS.

RESULTS

Forty-three subjects were enrolled and ranged in age from infancy to adolescence (Table 1). The sample was evenly split by sex and a strong majority of the participants had asphyxia or shock as the etiology of CA. Seventeen (40%) subjects died and 19 (60%) overall had poor outcome at 6 months post-CA (Table 2). Subjects with poor outcome were more likely to have had an unwitnessed CA and asystole as the first documented rhythm. Motor and pupillary examination findings, first lactate and blood pH, and EEG early post-CA were associated with good vs. poor outcome. Thirty-five (81%) subjects received therapeutic hypothermia for ≤ 72 hours while the remaining 8 subjects were maintained normothermic.

Serum biomarker patterns after pediatric cardiac arrest

Serum S100b concentration peaked the earliest after ROSC at median of 19 hours followed by NSE at 37.5 hours, and lastly MBP at 57.5 hours (Figures 2 a–b). NSE peaked earlier in subjects with good outcome vs. poor outcome (30 vs. 47.3 hours, p=.043) but there were no differences in time to peak by mortality.

Serum biomarker concentrations measured between 0–120 hours post-ROSC [median (± 95% CI)] were plotted by both good vs. poor outcome and mortality (Figures 3 a–f). Subjects with good outcome had values within the normal range for all 3 biomarkers when compared to historical controls[12]. Serum S100b increased and decreased rapidly in subjects with poor outcome or who died. Serum NSE remained increased at 120 hours in most subjects with poor outcome or who died. Serum MBP showed a delayed, sustained increase in subjects with poor outcome or who died.

Serum NSE, S100b, and MBP concentrations and outcome

Participants who died had significantly higher 24 hour NSE, S100b, and MBP levels when compared to survivors (Table 3). Serum NSE and S100b concentrations were increased in the poor outcome vs. good outcome group and in subjects who died vs. survived at 48, and 72 hours post-ROSC, all $p<0.05$. Mean, median, and peak serum MBP concentrations were increased in subjects with poor vs. good outcome. Additionally, serum MBP significantly predicted good vs. poor outcome and mortality at 72 hours.

Serum biomarker correlation with temperature and age

Core temperatures were noted at the time of biomarker sampling. MBP had a positive correlation with subject temperature ($r=0.241$, $p < .001$) while NSE and S100b concentrations were not correlated with temperature. Subject temperature was categorized as

< 34°C, 34–36°C, and > 36°C. There were no differences between temperature groups for NSE. S100b was increased in the 34–36°C group vs. <34°C and > 36 °C and MBP concentrations were increased in the > 36°C group compared to both <34°C and 34–36°C groups (all $p<0.05$).

Age was inversely correlated with initial S100b ($r=-0.40$, $p=0.008$), peak S100b ($r=-0.31$, $p=0.042$) and at the 24 hour S100b ($r=-0.30$, $p=0.05$). Initial NSE was inversely correlated with age ($r=-0.41$, $p=0.006$) while MBP concentration was not correlated with age.

Serum biomarker receiver operating characteristics (ROC) and sensitivity analysis

The area under the curve (AUC) [95% CI] for serum S100b to predict poor 6 month outcome and mortality were 0.955 [0.922–0.987] and 0.908 [0.866–0.950], respectively (Figures 4 a–f). Similarly, serum NSE AUC was 0.859 [0.796–0.922] and 0.787 [0.721–0.852] and serum MBP was 0.732 [0.647–0.817] and 0.727 [0.654–0.799] (all $p<0.05$). AUC for CPR-ROSC time, first lactate and blood pH are shown in Figures 4 g–l. AUC for S100b and NSE for both outcomes were superior to all clinical variables tested (Table 4).

Tables 5a–b feature biomarker concentration thresholds for best sensitivity and specificity and corresponding positive and negative predictive value at 24 and 48 hour time points. For example, a cut point of 0.008 ng/ml for 24 hour S100b yielded an extremely high probability of correctly classifying good vs. poor outcome using just one biomarker score. Similar results were obtained for mortality. A cut point of 53.10 ng/ml for 24 hour NSE resulted in a very high probability of correctly classifying mortality coupled with the best specificity on that variable.

DISCUSSION

In summary, we found that serum biomarkers of brain injury, S100b, NSE, and MBP, have unique patterns of release after pediatric CA. Furthermore, despite a relatively small sample size, biomarker concentrations at multiple time points predicted good or poor outcome and mortality at 6 months with remarkable accuracy. Importantly, the initial and 24 hour biomarker time points have potential value to clinicians in helping to decide whether to initiate a neuroprotective therapy and assisting in counseling families.

Serum S100b can detect early brain injury,[11,29] change in response to secondary brain insults[30], and predict outcome after various adult and pediatric CNS injuries[18,29,31–34]. Berger et al found that initial and peak serum S100b concentrations were increased in children with hypoxic-ischemic injury and traumatic brain injury (TBI) but children with TBI peaked earlier (6 vs. 9 hours)[20]. In our study, S100b was the most robust biomarker to predict outcome at all time points. S100b was also found to be superior to NSE to predict outcome in a study in adults with CA[35]. In children with CA, Topjian et al found that serum S100b concentrations predicted survival but not good vs. poor outcome when measured at 48 and 72 hours while our study found all time points to be associated with neurological outcome and survival[10]. S100b consistently peaked prior to the neuronal and myelin biomarkers but the reasons for this are unclear. However, inflammatory mediators released

acutely after ischemia-reperfusion by the blood-brain-barrier's epithelial layer may provoke astrocytes to release cytokines and S100b[36,37].

Peak serum NSE concentrations occurred between days 1–2 after CA and tended to remain increased for up to a week or longer in children who fared poorly. The later appearance and often prolonged release of NSE in serum may reflect neuronal cell death from the instigating event as well as ongoing secondary cell death from apoptosis[38]. This finding of delayed neuronal death is predicted from classic studies of global brain ischemia[39]. Serum NSE has been evaluated in neonates, children, and adults with hypoxic-ischemic injury and correlates with severity of injury and predicts outcome[40,41, 20,42–45]. Notably, serum NSE was endorsed by the American Academy of Neurology as an early clinical marker to predict outcome. However, this recommendation was prior to hypothermia becoming standard of care in adults with CA, inviting re-evaluation[46]. Additionally, in adults surviving CA, NSE concentrations were decreased in subjects randomized to hypothermia compared to subjects in the normothermic group. NSE concentrations correlated with improved gross outcome at 6 months in the hypothermia group, and trended towards improved cognitive and neurophysiological scores in the authors' follow-up study[13,47].

MBP, accounting for 30% of protein in the myelin sheath, peaks late in serum after TBI and can be increased for up to 2 weeks in subjects with poor outcome[12,48]. MBP has been previously documented in the cerebrospinal fluid in children after severe hypoxic insult[49]. MBP's relatively late peak after pediatric CA is consistent with white matter being more resistant to ischemia as compared with gray matter[50,51]. The pathophysiology of myelin injury after CA is unknown, but presence of white matter injury in the splenium of the corpus callosum suggests a role for Wallerian degeneration after adult CA[52]. Focal and diffuse white matter injury also occurs after neonatal asphyxia[53].

Our analysis suggests that subject temperature did not affect biomarker trajectories. These results could be validated in a larger study or an RCT where subject temperature is controlled.

Initial serum S100b and NSE concentrations were inversely correlated with age in this study. Notably, similar to previous reports, S100b concentrations at later time points also displayed this trend, but NSE did not[54]. However, population norms have been established for S100b that may allow for its use in all age groups[55]. Unlike pediatric TBI, younger age was not inversely correlated with MBP concentrations at any time point despite having less brain myelination developmentally[56].

Remarkably, serum NSE and S100b performed better than clinical variables in discriminating subject outcome. Clinical predictors of outcome after pediatric CA that have been assessed include duration of pulselessness, first blood gas pH upon ROSC, motor and pupillary examination at 24 hours after ROSC, and EEG[57–61].

Evidence suggests that each brain disease requires separate studies to determine their accuracy and cut-off values with and without treatments such as hypothermia to optimize their use. Our findings, in particular the robust ROC results, strongly suggest the need for validation in a larger sample of subjects and consideration for a biomarker panel to

maximize accuracy of outcome prediction. The ultimate objective would be development of a point of care test which can be used for rapid results at the bedside for clinical and research purposes.

Study limitations

There were several limitations to the design of this study. Laboratory, imaging, and EEG studies were not mandated in this study. Not all subjects survived to the 7 day study time point, therefore not contributing a full complement of serum biomarkers. However, this is intrinsic to studying a disease with high mortality. Missing data was not imputed. The relatively limited sample size precluded multivariate analysis to see if the highly accurate prediction rate could be improved on by modeling a panel of serum and clinical biomarkers. PCPC was assigned in a non-blinded fashion by the study PI. It was only necessary to contact the families of children who survived to 6 months and the PI was sometimes also part of the clinical team treating study subjects. Finally, the outcome measure we used was the PCPC which is a gross measure of function and may not accurately depict outcome in infants and young children[62].

CONCLUSIONS

Our preliminary data show that serum biomarkers S100b, NSE, and MBP have potential to aid in guiding treatment decisions and outcome prediction of children surviving pediatric CA. Modeling and validation of serum and clinical biomarkers may strengthen early prognostication and assist with risk stratification in future clinical studies.

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Conflicts of Interest and Source of Funding: Drs. Berger and Kochanek are provisional co-patent holders on a biomarker panel for abusive head trauma in infants. Dr. Callaway has patents and royalties with Medtronic ERS, Inc. related to timing of defibrillation, licensed to a manufacturer of defibrillators.

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REFERENCES

1. Kuisma M, Suominen P, Korpela R. Paediatric out-of-hospital cardiac arrests--epidemiology and outcome. Resuscitation. 1995 Oct; 30(2):141–150. [PubMed: 8560103]
2. Reis AG, Nadkarni V, Perondi MB, Grisi S, Berg RA. A prospective investigation into the epidemiology of in-hospital pediatric cardiopulmonary resuscitation using the international Utstein reporting style. Pediatrics. 2002; 109(2):200–209. [PubMed: 11826196]
3. Young KD, Gausche-Hill M, McClung CD, Lewis RJ. A prospective, population-based study of the epidemiology and outcome of out-of-hospital pediatric cardiopulmonary arrest. Pediatrics. 2004 Jul; 114(1):157–164. [PubMed: 15231922]

4. Nadkarni VM, Larkin GL, Peberdy MA, et al. First documented rhythm and clinical outcome from in-hospital cardiac arrest among children and adults. JAMA. 2006 Jan 4; 295(1):50–57. [PubMed: 16391216]
5. de Mos N, van Litsenburg RR, McCrindle B, Bohn DJ, Parshuram CS. Pediatric in-intensive-care-unit cardiac arrest: incidence, survival, and predictive factors. Crit Care Med. 2006 Apr; 34(4): 1209–1215. [PubMed: 16484906]
6. Hickey RW, Painter MJ. Brain injury from cardiac arrest in children. Neurol Clin. 2006 Feb; 24(1): 147–158. [PubMed: 16443136]
7. Donoghue AJ, Nadkarni V, Berg RA, et al. Out-of-hospital pediatric cardiac arrest: an epidemiologic review and assessment of current knowledge. Ann Emerg Med. 2005 Dec; 46(6): 512–522. [PubMed: 16308066]
8. O'Rourke PP. Outcome of children who are apneic and pulseless in the emergency room. Crit Care Med. 1986 May; 14(5):466–468. [PubMed: 3698611]
9. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. Lancet. 2005 Feb 19–25; 365(9460):663–670. [PubMed: 15721471]
10. Topjian AA, Lin R, Morris MC, et al. Neuron-specific enolase and S-100B are associated with neurologic outcome after pediatric cardiac arrest. Pediatr Crit Care Med. 2009 Jul; 10(4):479–490. [PubMed: 19307814]
11. Steffen IG, Hasper D, Ploner CJ, et al. Mild therapeutic hypothermia alters neuron specific enolase as an outcome predictor after resuscitation: 97 prospective hypothermia patients compared to 133 historical non-hypothermia patients. Crit Care. 2010; 14(2):R69. [PubMed: 20403168]
12. Berger RP, Adelson PD, Pierce MC, Dulani T, Cassidy LD, Kochanek PM. Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. J Neurosurg. 2005 Jul; 103 Suppl(1):61–68. [PubMed: 16122007]
13. Tiainen M, Roine RO, Pettila V, Takkunen O. Serum neuron-specific enolase and S-100B protein in cardiac arrest patients treated with hypothermia. Stroke. 2003 Dec; 34(12):2881–2886. [PubMed: 14631087]
14. Berger R, Richichi R. Derivation and validation of an equation for adjustment of neuron-specific enolase concentrations in hemolyzed serum. Pediatr Crit Care Med. 2009 Mar; 10(2):260–263. [PubMed: 19188872]
15. Anderson RE, Hansson LO, Nilsson O, Dijlai-Merzoug R, Settergren G. High serum S100B levels for trauma patients without head injuries. Neurosurgery. 2001 Jun; 48(6):1255–1258. discussion 1258–1260. [PubMed: 11383727]
16. Gonzalez-Garcia S, Gonzalez-Quevedo A, Fernandez-Concepcion O, et al. Short-term prognostic value of serum neuron specific enolase and S100B in acute stroke patients. Clin Biochem. 2012 Nov; 45(16–17):1302–1307. [PubMed: 22820433]
17. Fassbender K, Schmidt R, Schreiner A, et al. Leakage of brain-originated proteins in peripheral blood: temporal profile and diagnostic value in early ischemic stroke. J Neurol Sci. 1997 May 1; 148(1):101–105. [PubMed: 9125396]
18. Lardner D, Davidson A, McKenzie I, Cochrane A. Delayed rises in serum S100B levels and adverse neurological outcome in infants and children undergoing cardiopulmonary bypass. Paediatr Anaesth. 2004 Jun; 14(6):495–500. [PubMed: 15153214]
19. Bechtel K, Frasure S, Marshall C, Dziura J, Simpson C. Relationship of serum S100B levels and intracranial injury in children with closed head trauma. Pediatrics. 2009 Oct; 124(4):e697–e704. [PubMed: 19786430]
20. Berger RP, Adelson PD, Richichi R, Kochanek PM. Serum biomarkers after traumatic and hypoxemic brain injuries: insight into the biochemical response of the pediatric brain to inflicted brain injury. Dev Neurosci. 2006; 28(4–5):327–335. [PubMed: 16943655]
21. Liu Y, Silverstein FS, Skoff R, Barks JD. Hypoxic-ischemic oligodendroglial injury in neonatal rat brain. Pediatric Research. 2002; 51(1):25–33. [PubMed: 11756636]
22. Garcia-Alix A, Cabanas F, Pellicer A, Hernanz A, Stiris TA, Quero J. Neuron-specific enolase and myelin basic protein: relationship of cerebrospinal fluid concentrations to the neurologic condition of asphyxiated full-term infants. Pediatrics. 1994; 93(2):234–240. [PubMed: 7510064]

23. Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication: a scientific statement from the International Liaison Committee on Resuscitation; the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; the Council on Stroke (Part II). Int Emerg Nurs. 2010 Jan; 18(1):8–28. [PubMed: 20129438]
24. Fink EL, Kochanek PM, Clark RS, Bell MJ. Fever control and application of hypothermia using intravenous cold saline. Pediatr Crit Care Med. 2012 Jan; 13(1):80–84. [PubMed: 21037507]
25. Langhelle A, Nolan J, Herlitz J, et al. Recommended guidelines for reviewing, reporting, and conducting research on post-resuscitation care: the Utstein style. Resuscitation. 2005 Sep; 66(3): 271–283. [PubMed: 16129543]
26. Wernovsky G, Wypij D, Jonas RA, et al. Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants. A comparison of low-flow cardiopulmonary bypass and circulatory arrest. Circulation. 1995 Oct 15; 92(8):2226–2235. [PubMed: 7554206]
27. Nishisaki A, Sullivan J 3rd, Steger B, et al. Retrospective analysis of the prognostic value of electroencephalography patterns obtained in pediatric in-hospital cardiac arrest survivors during three years. Pediatr Crit Care Med. 2007 Jan; 8(1):10–17. [PubMed: 17251876]
28. Fiser DH. Assessing the outcome of pediatric intensive care. J Pediatr. 1992 Jul; 121(1):68–74. [PubMed: 1625096]
29. Berger RP, Pierce MC, Wisniewski SR, Adelson PD, Kochanek PM. Serum S100B concentrations are increased after closed head injury in children: a preliminary study. J Neurotrauma. 2002 Nov; 19(11):1405–1409. [PubMed: 12490005]
30. Unden J, Astrand R, Waterloo K, et al. Clinical significance of serum S100B levels in neurointensive care. Neurocrit Care. 2007; 6(2):94–99. [PubMed: 17522791]
31. Jauch EC, Lindsell C, Broderick J, Fagan SC, Tilley BC, Levine SR. Association of serial biochemical markers with acute ischemic stroke: the National Institute of Neurological Disorders and Stroke recombinant tissue plasminogen activator Stroke Study. Stroke. 2006 Oct; 37(10): 2508–2513. [PubMed: 16960091]
32. Petzold A, Green AJ, Keir G, et al. Role of serum S100B as an early predictor of high intracranial pressure and mortality in brain injury: a pilot study. Crit Care Med. 2002 Dec; 30(12):2705–2710. [PubMed: 12483062]
33. Foerch C, Otto B, Singer OC, et al. Serum S100B predicts a malignant course of infarction in patients with acute middle cerebral artery occlusion. Stroke. 2004 Sep; 35(9):2160–2164. [PubMed: 15297628]
34. James ML, Blessing R, Phillips-Bute BG, Bennett E, Laskowitz DT. S100B and brain natriuretic peptide predict functional neurological outcome after intracerebral haemorrhage. Biomarkers. 2009 Sep; 14(6):388–394. [PubMed: 19505208]
35. Shinozaki K, Oda S, Sadahiro T, et al. Serum S-100B is superior to neuron-specific enolase as an early prognostic biomarker for neurological outcome following cardiopulmonary resuscitation. Resuscitation. 2009 Aug; 80(8):870–875. [PubMed: 19535196]
36. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006 Jan; 7(1):41–53. [PubMed: 16371949]
37. Abbott NJ. Inflammatory mediators and modulation of blood-brain barrier permeability. Cell Mol Neurobiol. 2000 Apr; 20(2):131–147. [PubMed: 10696506]
38. Beilharz EJ, Williams CE, Dragunow M, Sirimanne ES, Gluckman PD. Mechanisms of delayed cell death following hypoxic-ischemic injury in the immature rat: evidence for apoptosis during selective neuronal loss. Brain Res Mol Brain Res. 1995 Mar; 29(1):1–14. [PubMed: 7769986]
39. Kirino T. Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res. 1982 May 6; 239(1):57–69. [PubMed: 7093691]
40. Meynaar IA, Straaten HM, van der Wetering J, et al. Serum neuron-specific enolase predicts outcome in post-anoxic coma: a prospective cohort study. Intensive Care Medicine. 2003; 29(2): 189–195. [PubMed: 12594583]
41. Tekgul H, Yalaz M, Kutukculer N, et al. Value of biochemical markers for outcome in term infants with asphyxia. Pediatr Neurol. 2004 Nov; 31(5):326–332. [PubMed: 15519113]

42. Berger RP. The use of serum biomarkers to predict outcome after traumatic brain injury in adults and children. J Head Trauma Rehabil. 2006; 21(4):315–333. [PubMed: 16915008]
43. Berger RP, Pierce MC, Wisniewski SR, et al. Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatrics. 2002 Feb.109(2):E31. [PubMed: 11826241]
44. Satchell MA, Lai Y, Kochanek PM, et al. Cytochrome c, a biomarker of apoptosis, is increased in cerebrospinal fluid from infants with inflicted brain injury from child abuse. J Cereb Blood Flow Metab. 2005 Jul; 25(7):919–927. [PubMed: 15744250]
45. Schoerkhuber W, Kittler H, Sterz F, et al. Time course of serum neuron-specific enolase. A predictor of neurological outcome in patients resuscitated from cardiac arrest. Stroke. 1999 Aug; 30(8):1598–1603. [PubMed: 10436107]
46. Wijdicks EF, Hijdra A, Young GB, Bassetti CL, Wiebe S. 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. 2006 Jul 25; 67(2):203–210. [PubMed: 16864809]
47. Tiainen M, Poutiainen E, Kovala T, Takkunen O, Happola O, Roine RO. Cognitive and neurophysiological outcome of cardiac arrest survivors treated with therapeutic hypothermia. Stroke. 2007 Aug; 38(8):2303–2308. [PubMed: 17585081]
48. Thomas DG, Palfreyman JW, Ratcliffe JG. Serum-myelin-basic-protein assay in diagnosis and prognosis of patients with head injury. Lancet. 1978 Jan 21; 1(8056):113–115. [PubMed: 87549]
49. Kohlschutter A. Myelin basic protein in cerebrospinal fluid from children. Eur J Pediatr. 1978 Mar 13; 127(3):155–161. [PubMed: 648537]
50. Falcao AL, Reutens DC, Markus R, et al. The resistance to ischemia of white and gray matter after stroke. Ann Neurol. 2004 Nov; 56(5):695–701. [PubMed: 15505775]
51. Bristow MS, Simon JE, Brown RA, et al. MR perfusion and diffusion in acute ischemic stroke: human gray and white matter have different thresholds for infarction. J Cereb Blood Flow Metab. 2005 Oct; 25(10):1280–1287. [PubMed: 15889043]
52. Bianchi MT, Sims JR. Restricted diffusion in the splenium of the corpus callosum after cardiac arrest. The open neuroimaging journal. 2008; 2:1–4. [PubMed: 19018311]
53. Sugiura H, Kouwaki M, Kato T, et al. Magnetic resonance imaging in neonates with total asphyxia. Brain Dev. 2012 May 12.
54. Shore PM, Berger RP, Varma S, et al. Cerebrospinal fluid biomarkers versus glasgow coma scale and glasgow outcome scale in pediatric traumatic brain injury: the role of young age and inflicted injury. J Neurotrauma. 2007 Jan; 24(1):75–86. [PubMed: 17263671]
55. Bouvier D, Castellani C, Fournier M, et al. Reference ranges for serum S100B protein during the first three years of life. Clin Biochem. 2011 Jul; 44(10–11):927–929. [PubMed: 21601568]
56. Su E, Bell MJ, Kochanek PM, et al. Increased CSF Concentrations of Myelin Basic Protein After TBI in Infants and Children: Absence of Significant Effect of Therapeutic Hypothermia. Neurocrit Care. 2012 Dec; 17(3):401–407. [PubMed: 22890910]
57. Abend NS, Topjian A, Ichord R, et al. Electroencephalographic monitoring during hypothermia after pediatric cardiac arrest. Neurology. 2009 Jun 2; 72(22):1931–1940. [PubMed: 19487651]
58. Abend NS, Topjian AA, Kessler S, et al. Outcome prediction by motor and pupillary responses in children treated with therapeutic hypothermia after cardiac arrest. Pediatr Crit Care Med. 2012 Apr 14.:32–38. [PubMed: 21499174]
59. Moler FW, Donaldson AE, Meert K, et al. Multicenter cohort study of out-of-hospital pediatric cardiac arrest. Crit Care Med. 2011 Jan; 39(1):141–149. [PubMed: 20935561]
60. Meert KL, Donaldson A, Nadkarni V, et al. Multicenter cohort study of in-hospital pediatric cardiac arrest. Pediatr Crit Care Med. 2009 Sep; 10(5):544–553. [PubMed: 19451846]
61. Fink EL, Clark RS, Kochanek PM, Bell MJ, Watson RS. A tertiary care center's experience with therapeutic hypothermia after pediatric cardiac arrest. Pediatr Crit Care Med. 2010 Jan; 11(1):66–74. [PubMed: 19935440]
62. Fiser DH, Long N, Roberson PK, Hefley G, Zolten K, Brodie-Fowler M. Relationship of pediatric overall performance category and pediatric cerebral performance category scores at pediatric

intensive care unit discharge with outcome measures collected at hospital discharge and 1- and 6-month follow-up assessments. Crit Care Med. 2000 Jul; 28(7):2616–2620. [PubMed: 10921604]

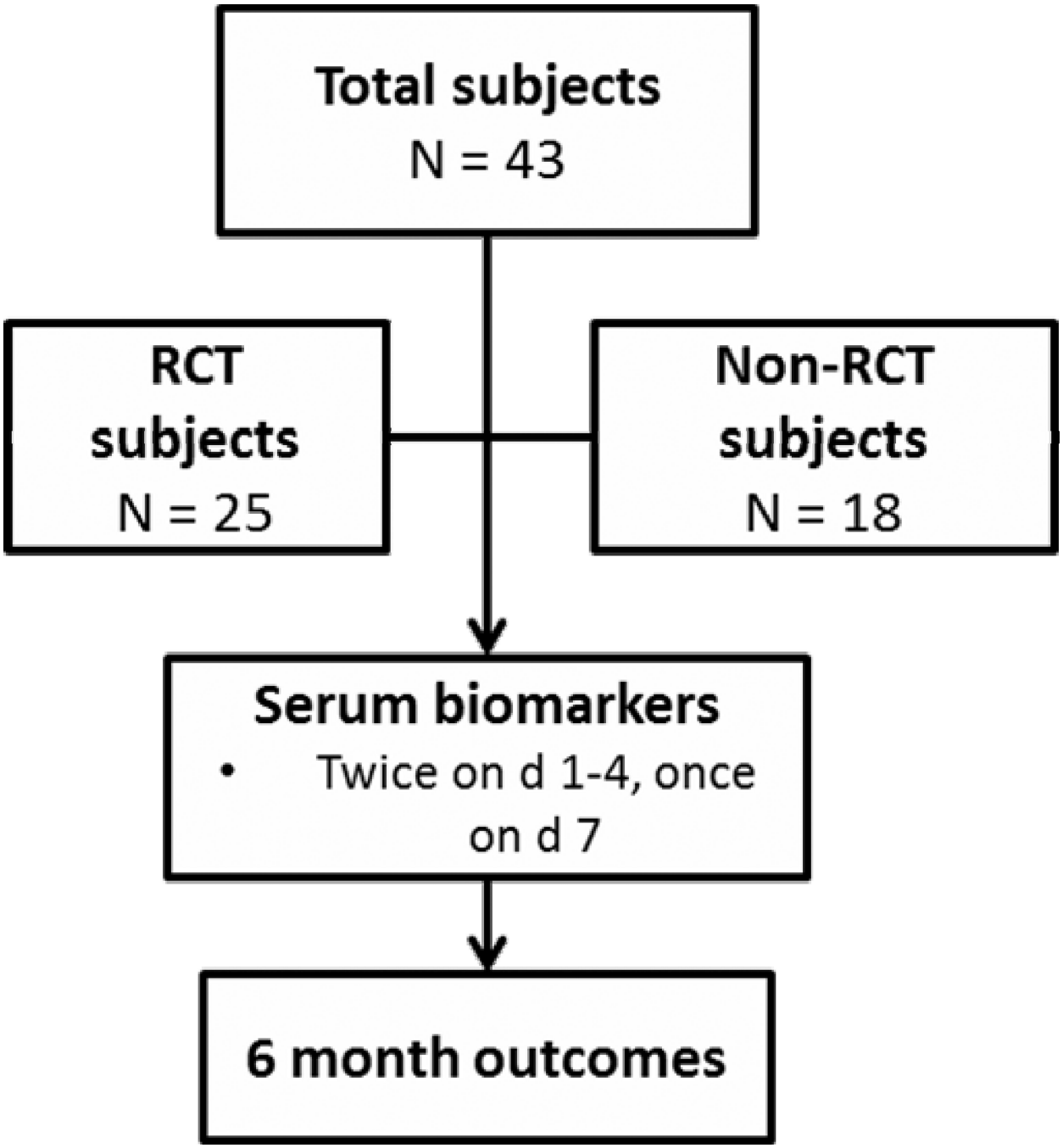
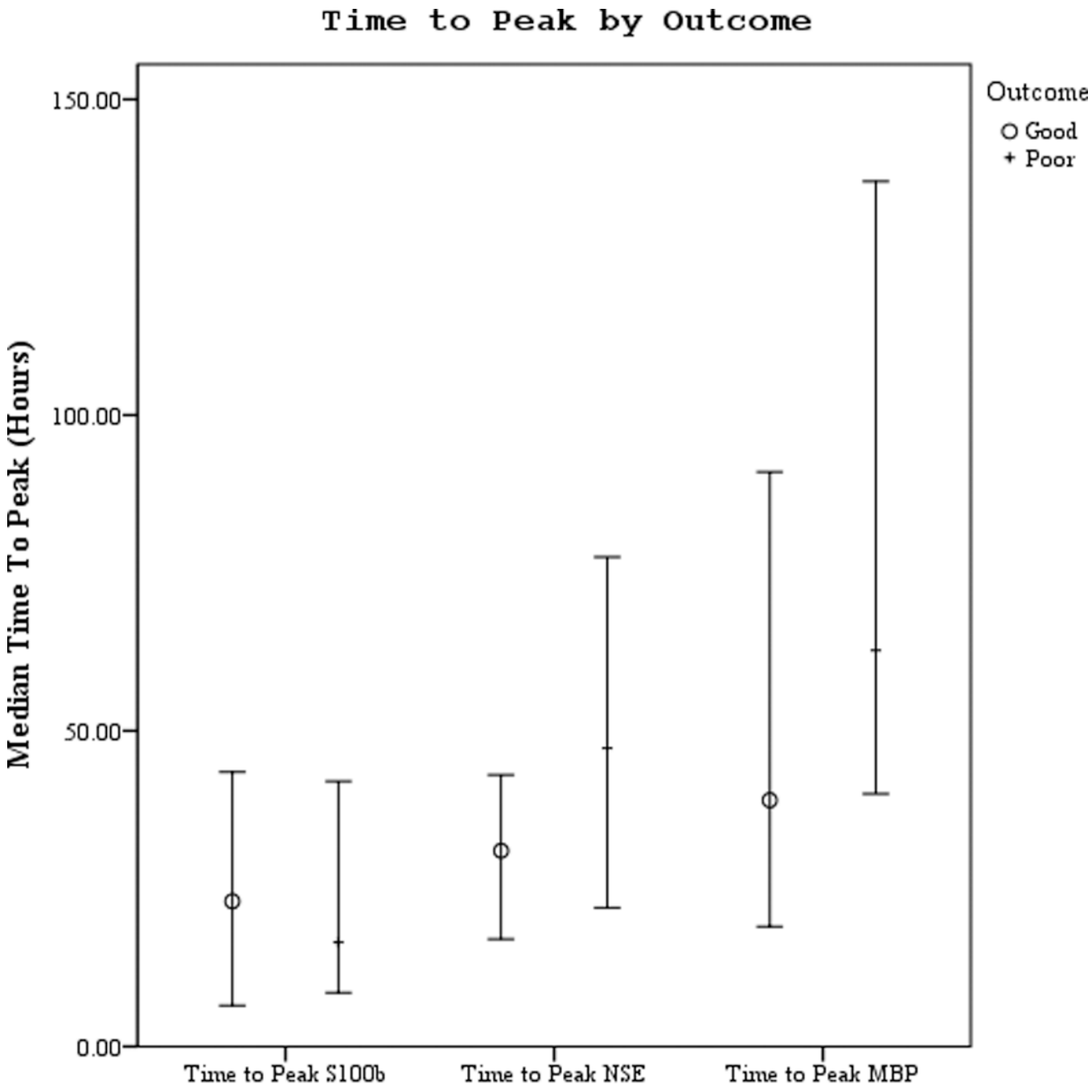
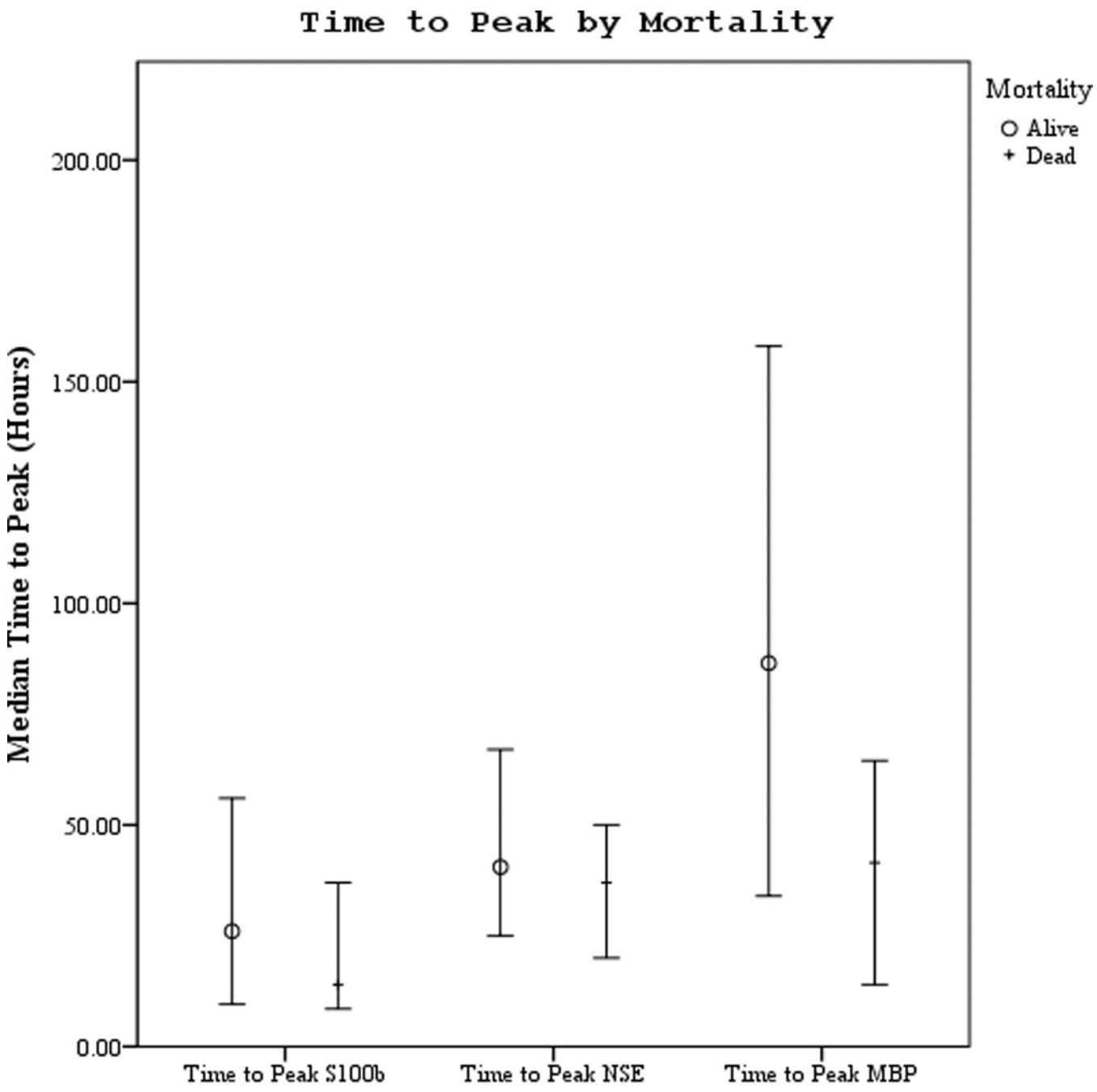


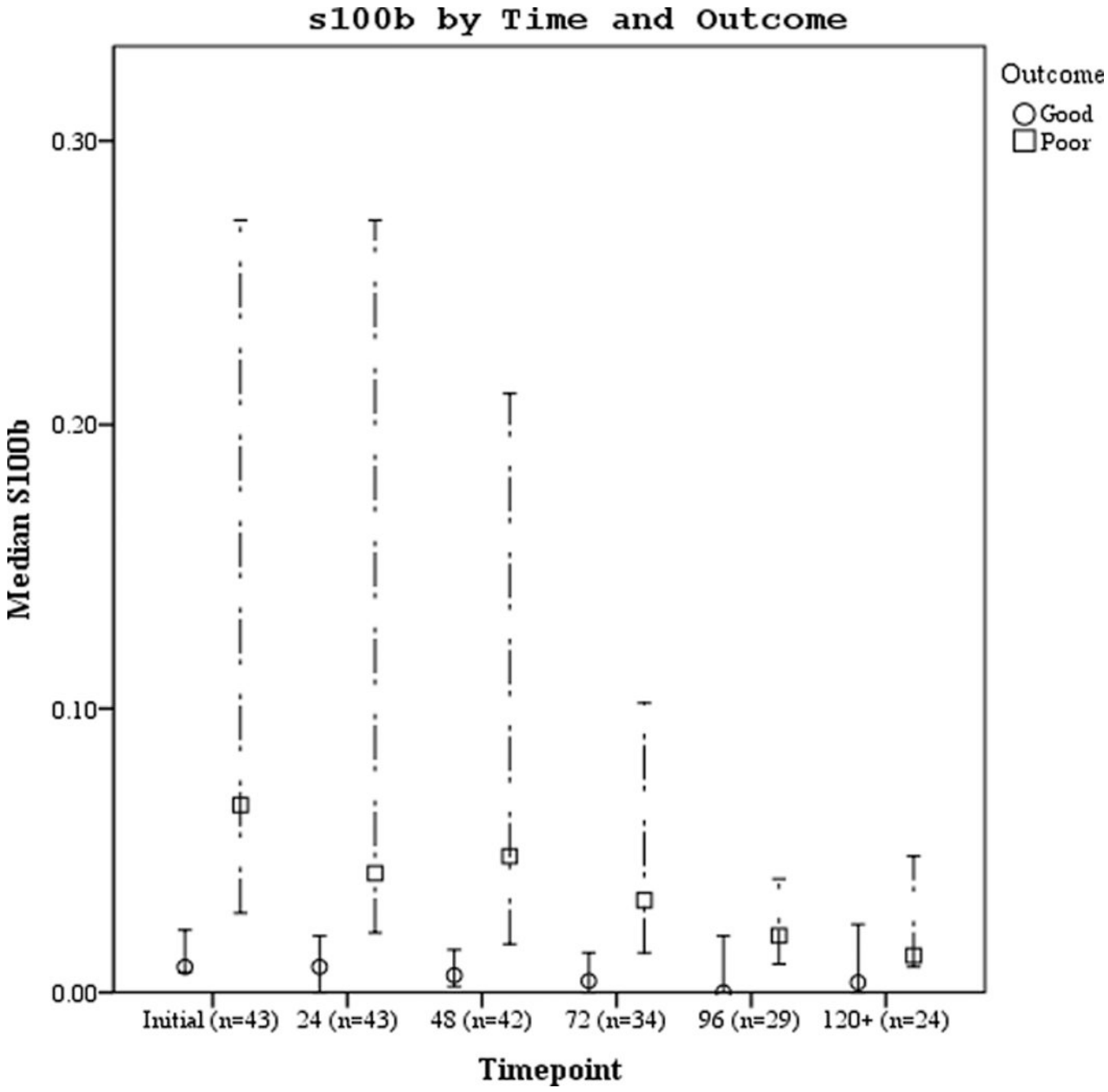
Figure 1.
Study flowchart.

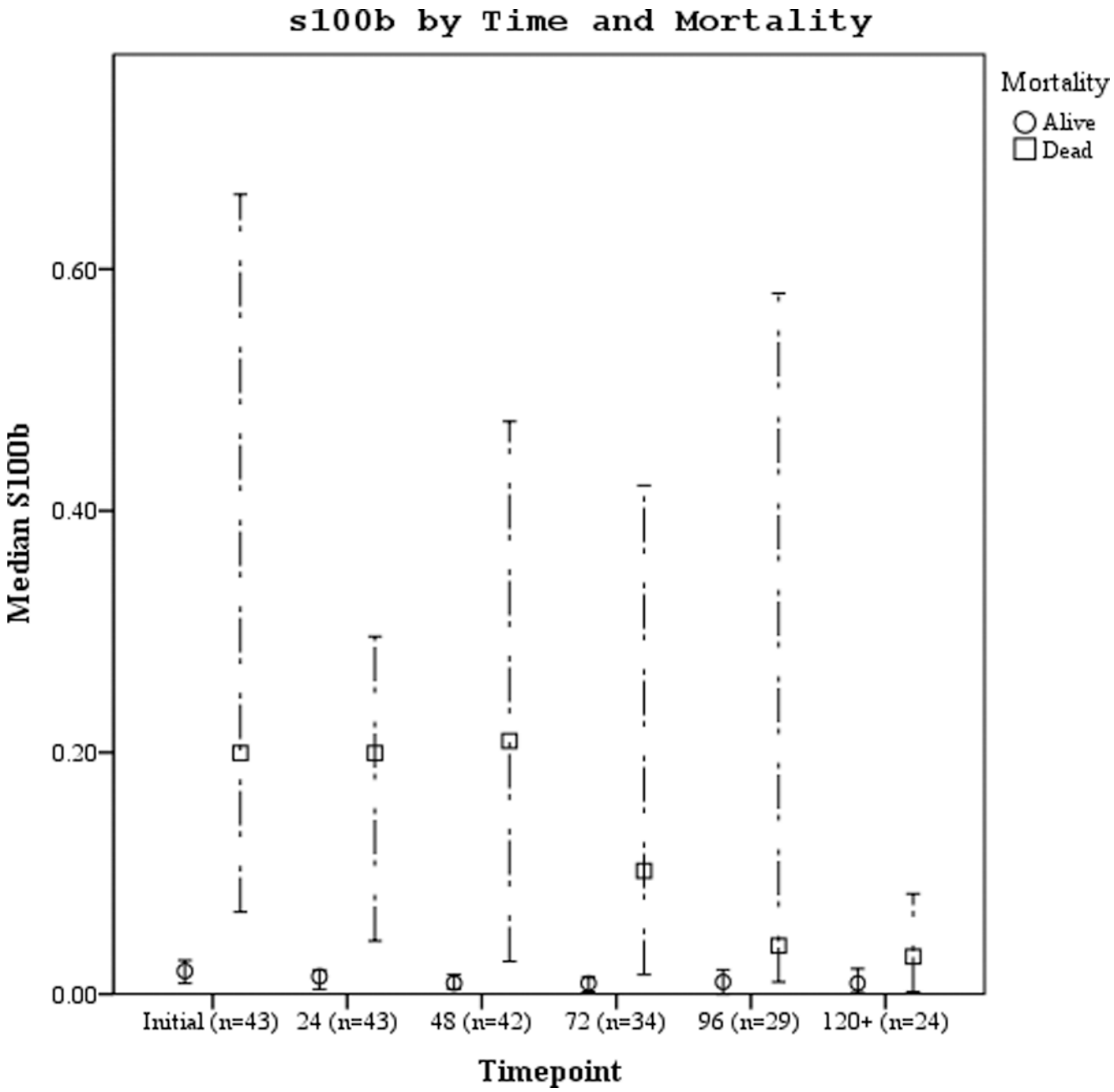


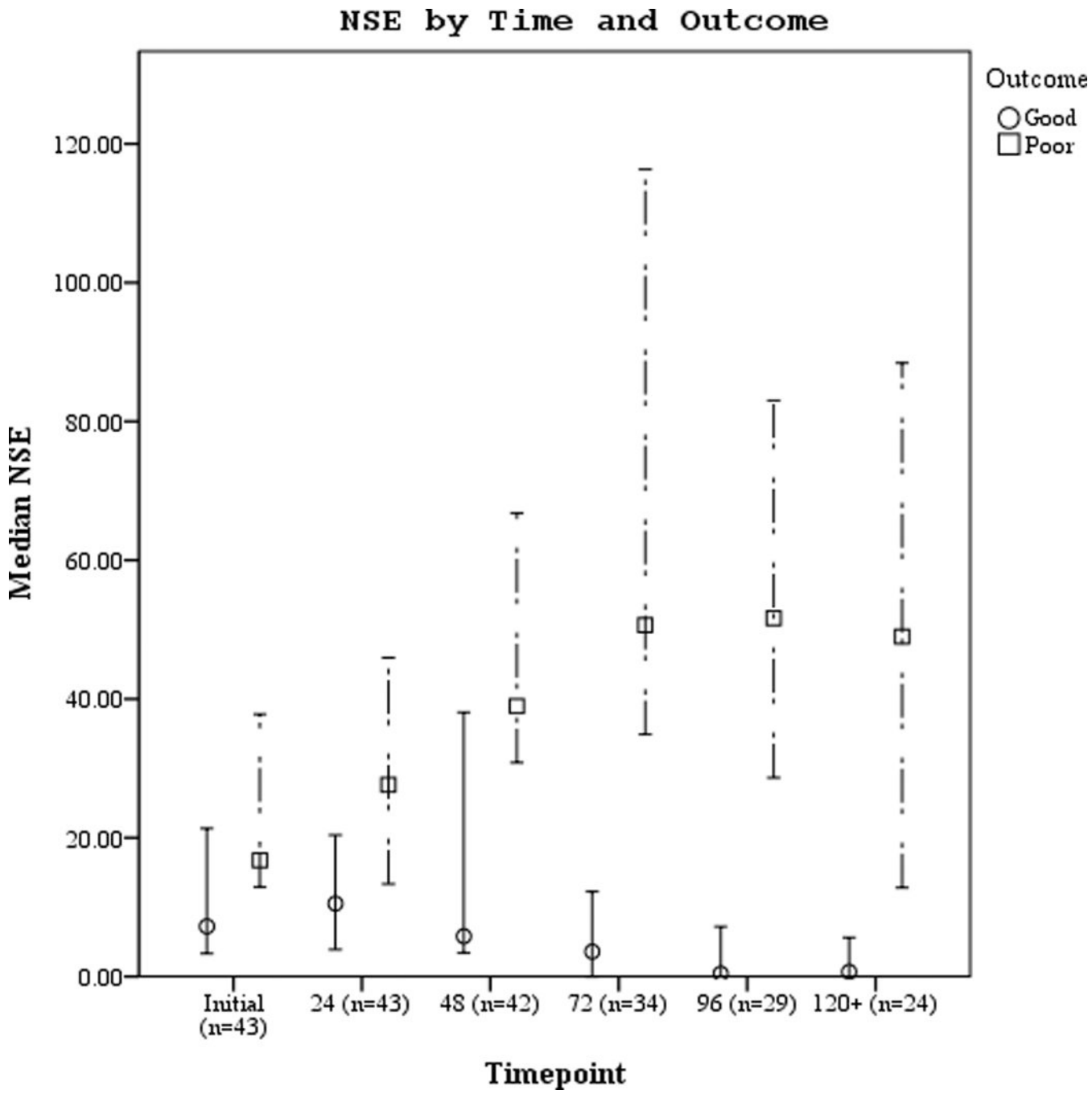


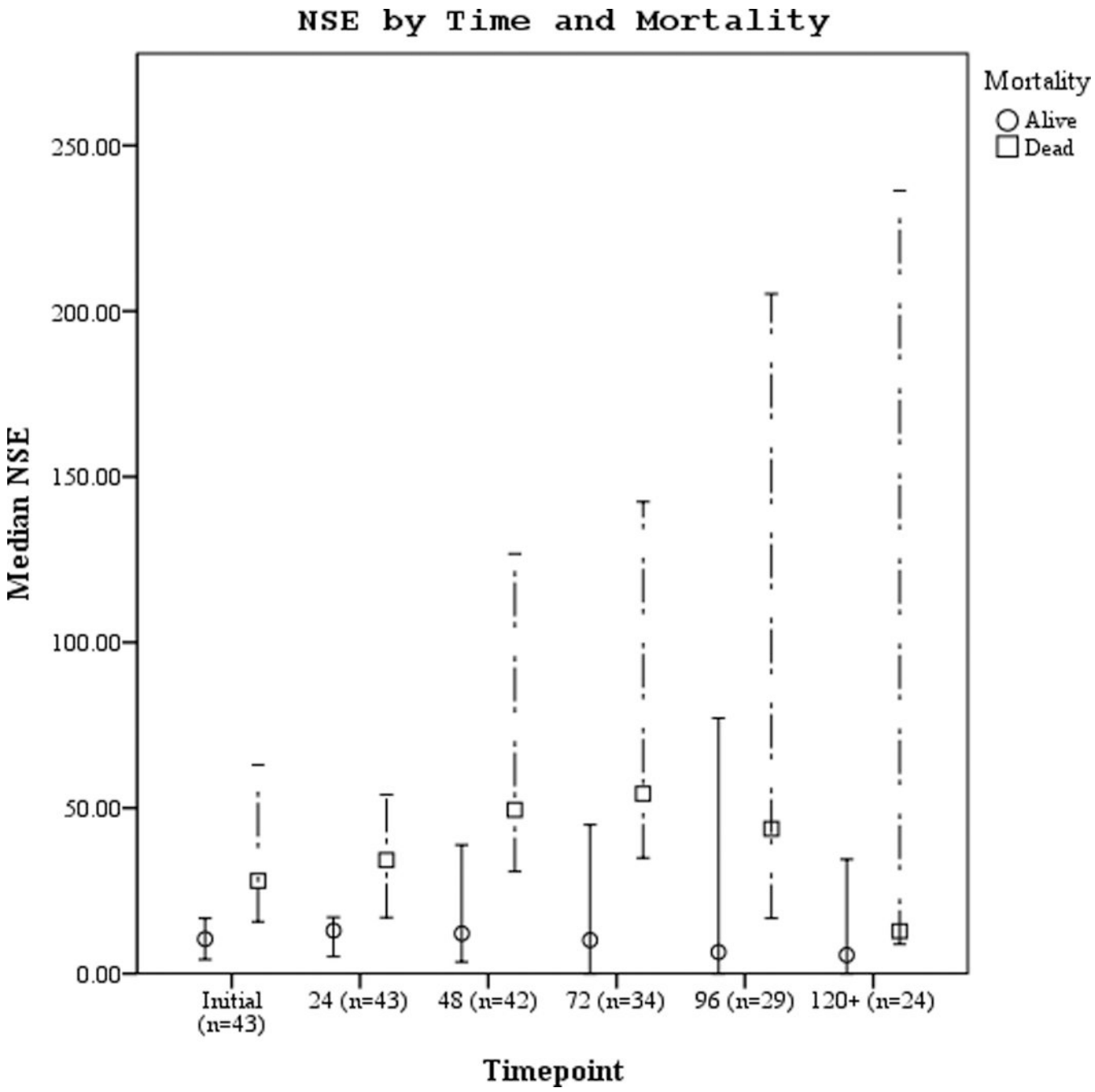
Figures 2.

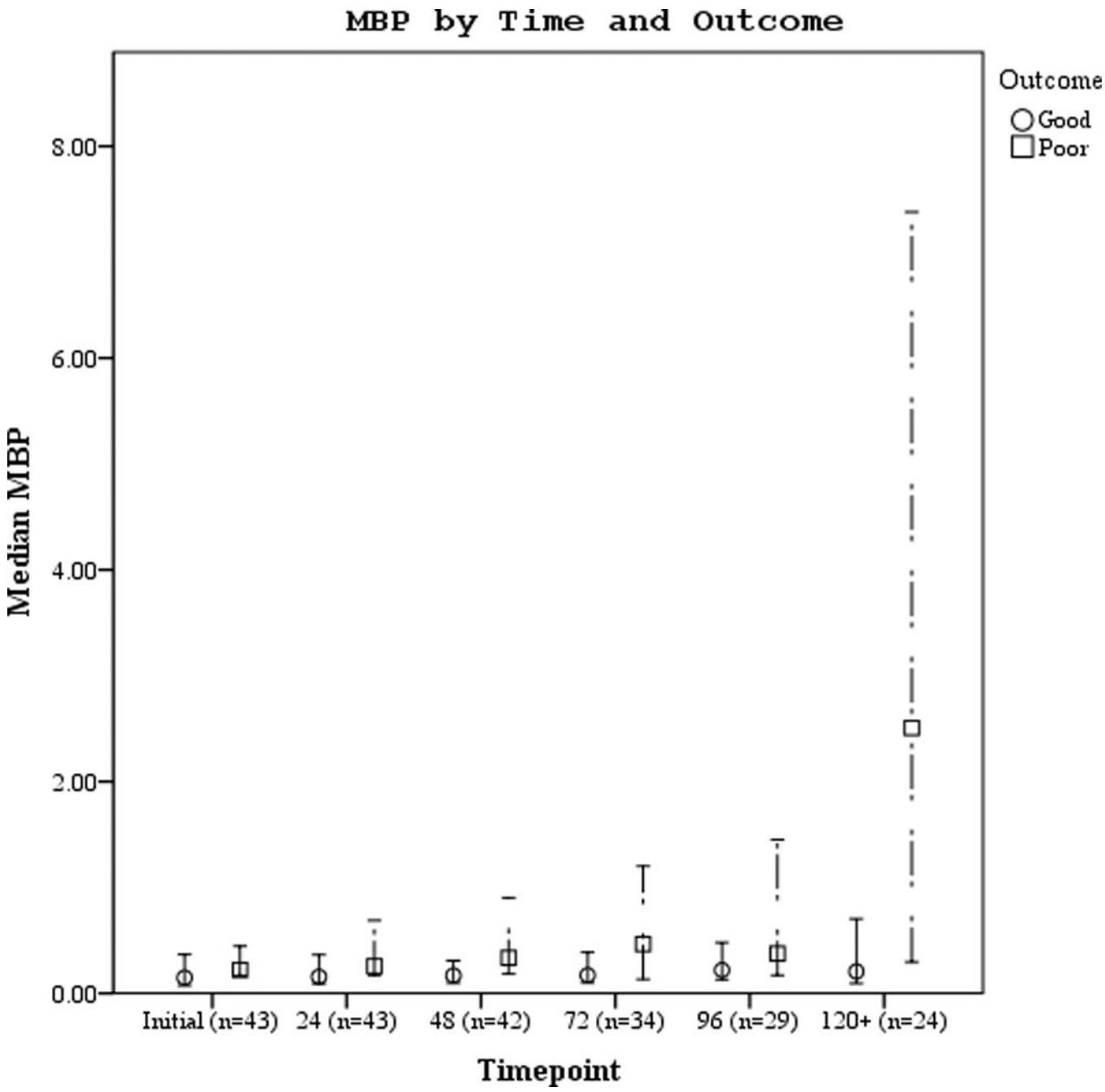
a–b. Time to peak serum NSE, S100, and MBP concentration by good vs. poor outcome and mortality at 6 months.

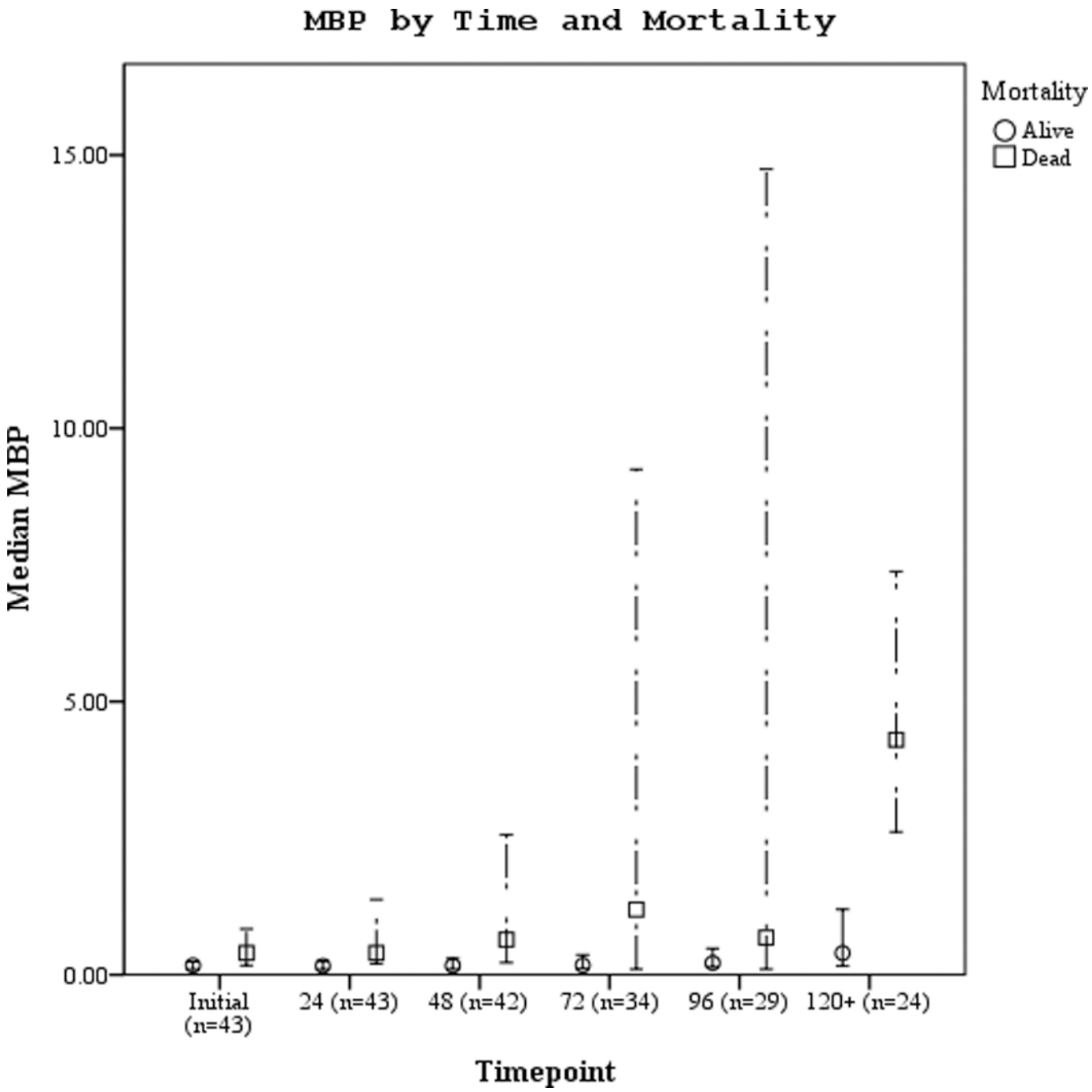






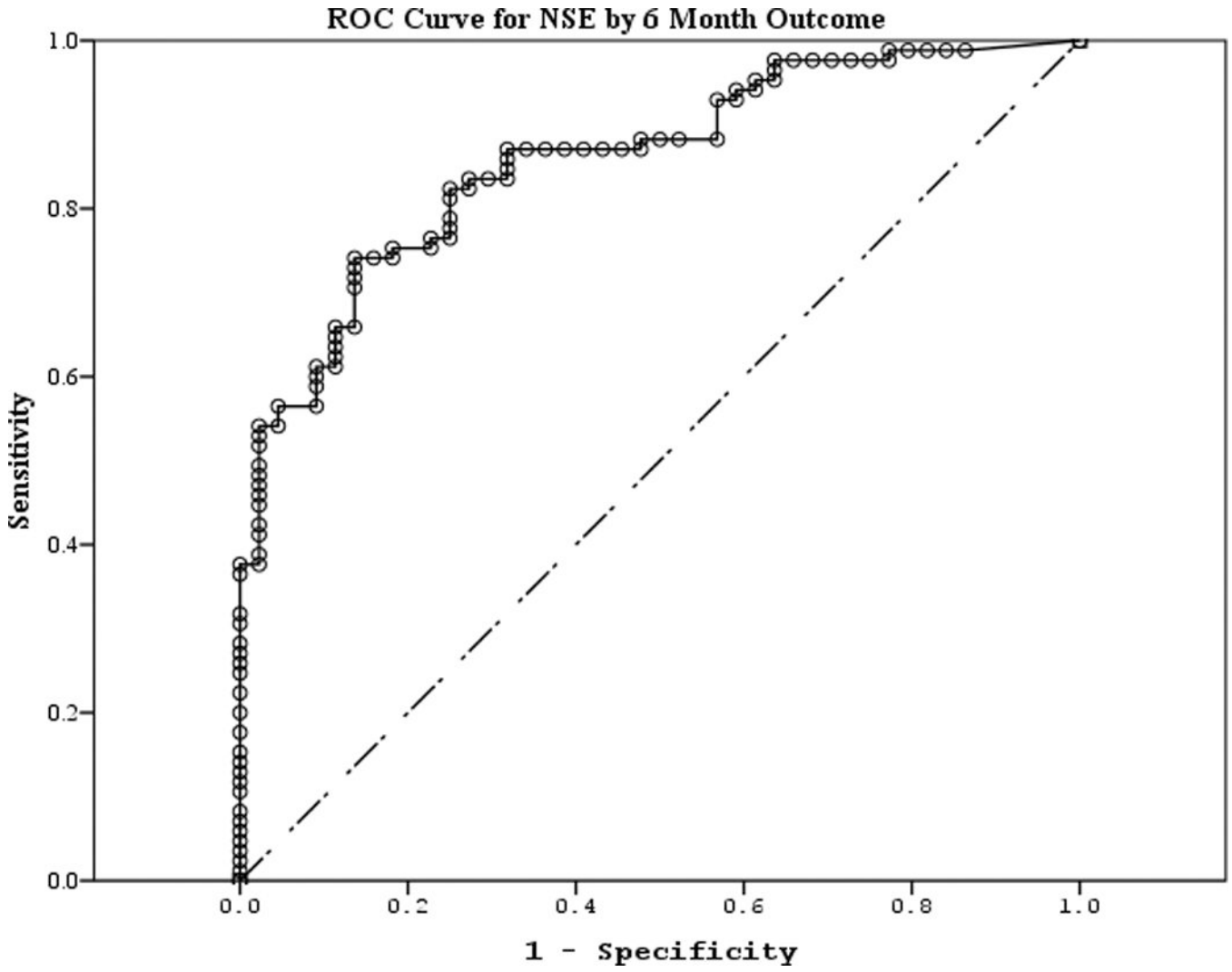


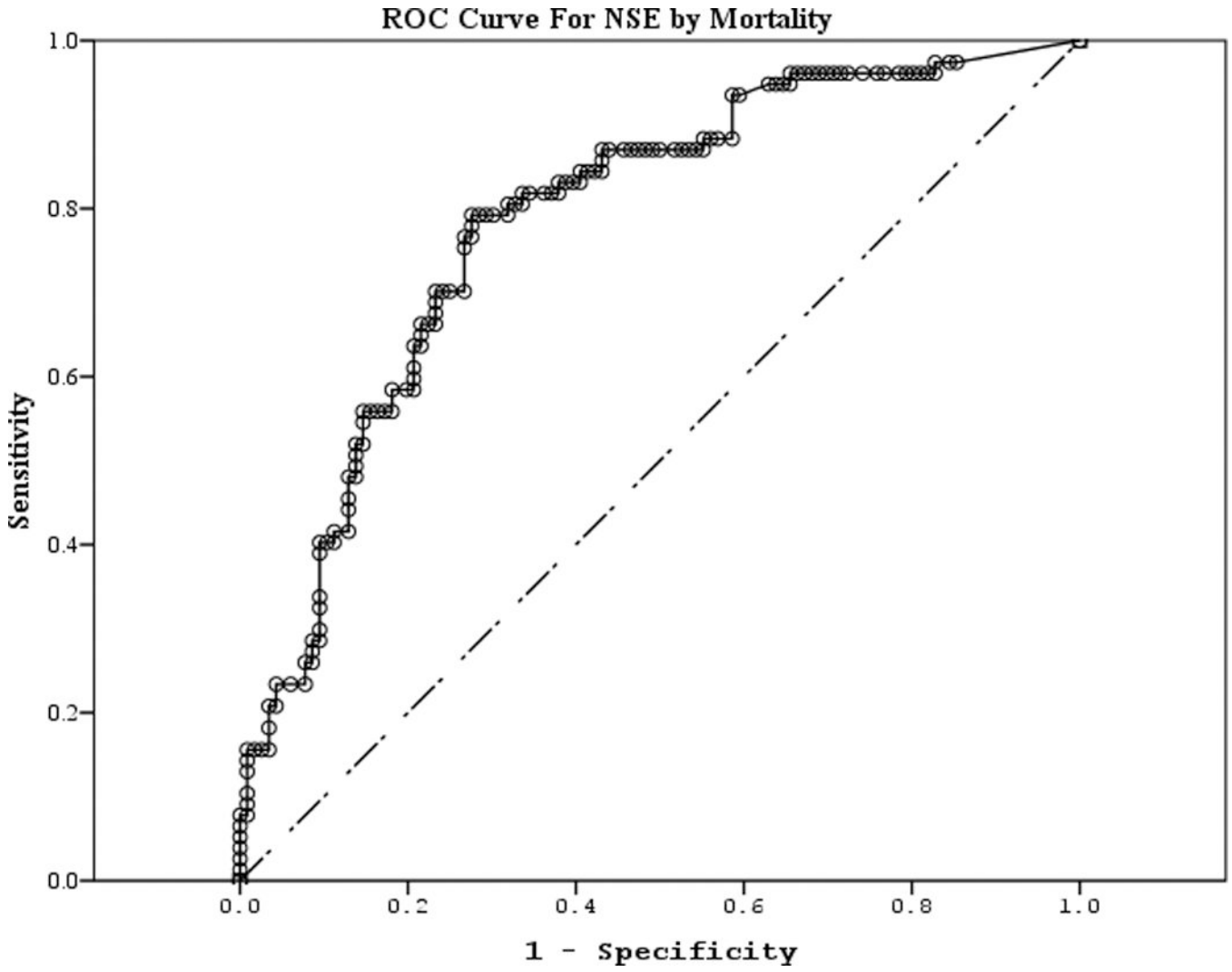


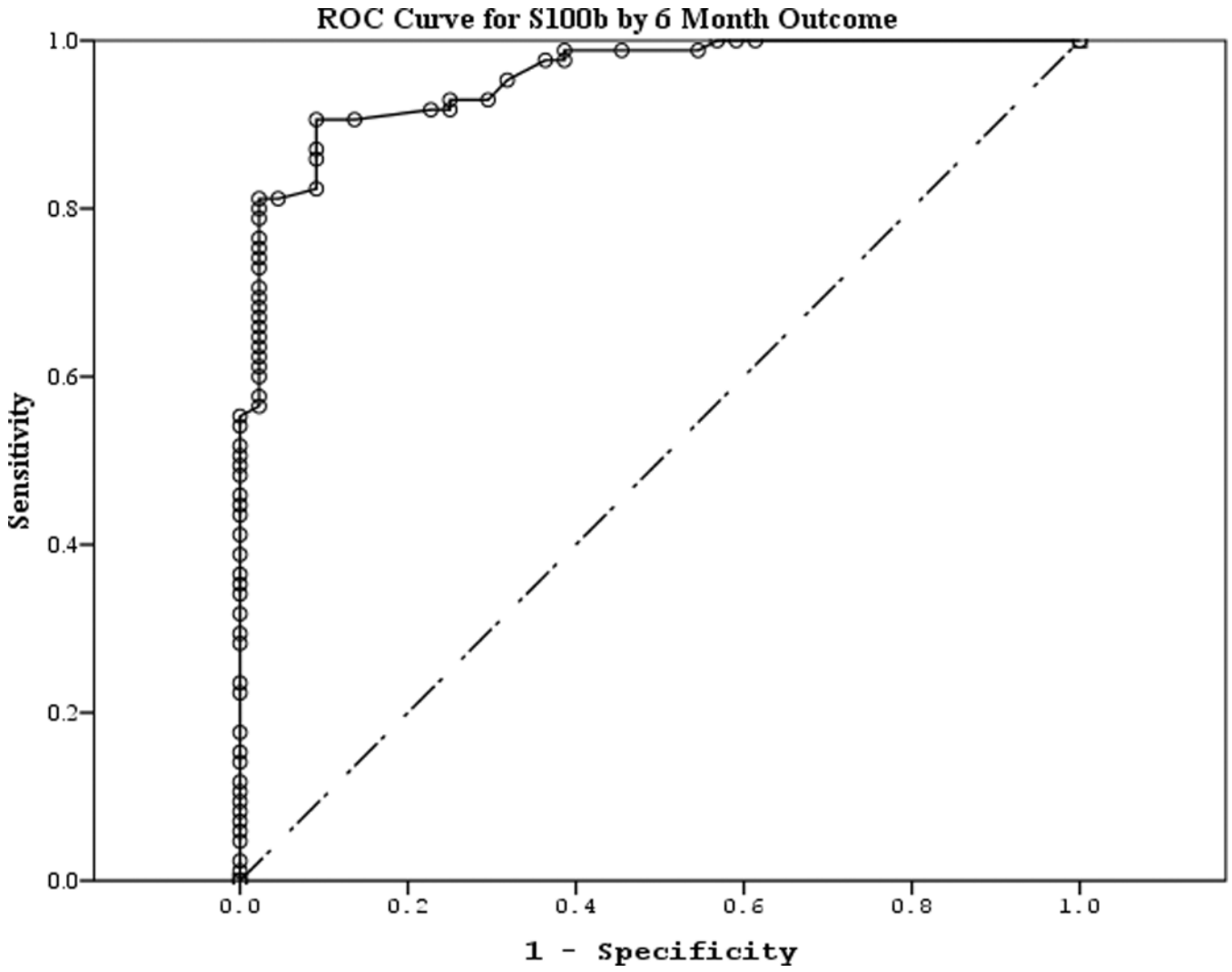


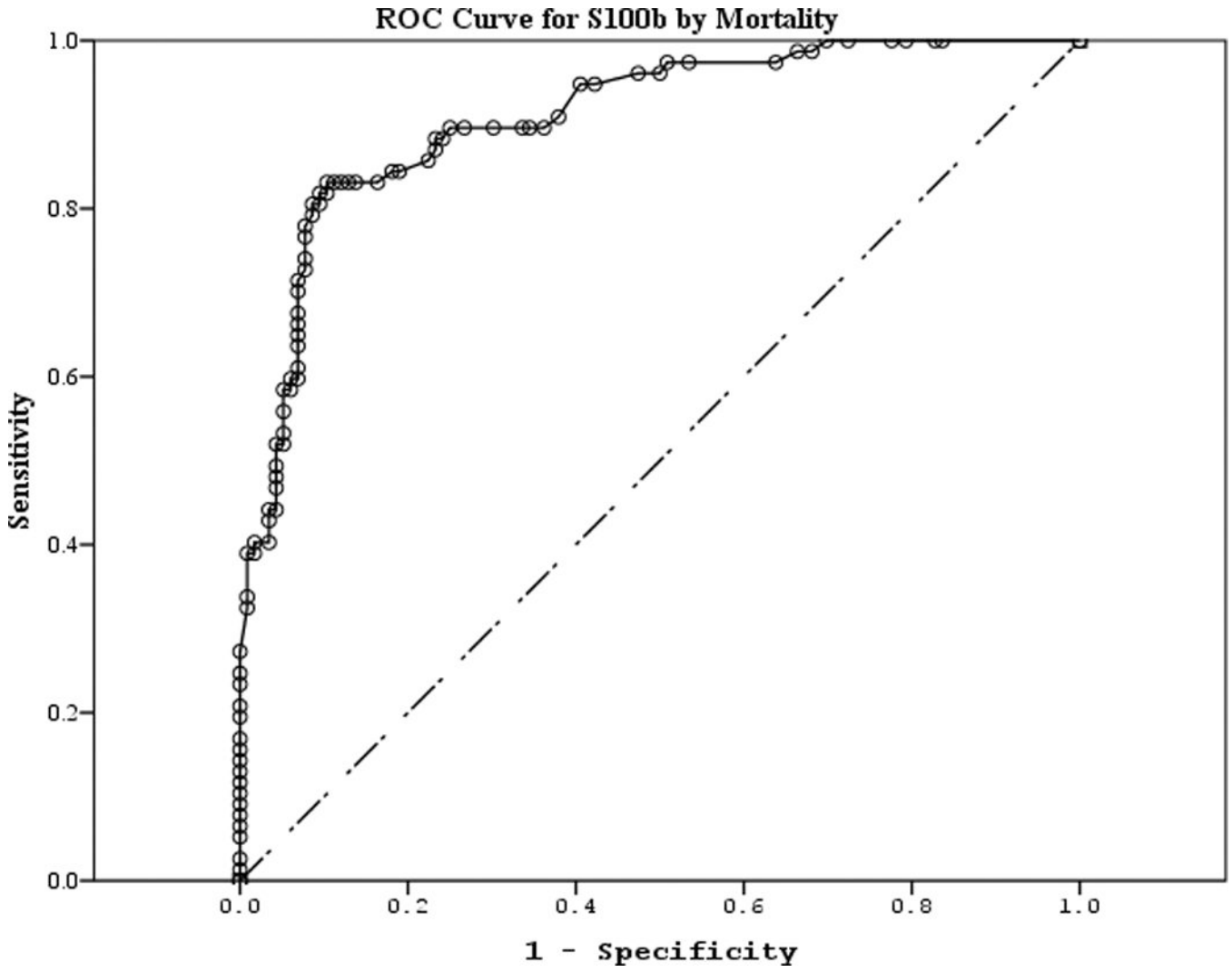
Figures 3.

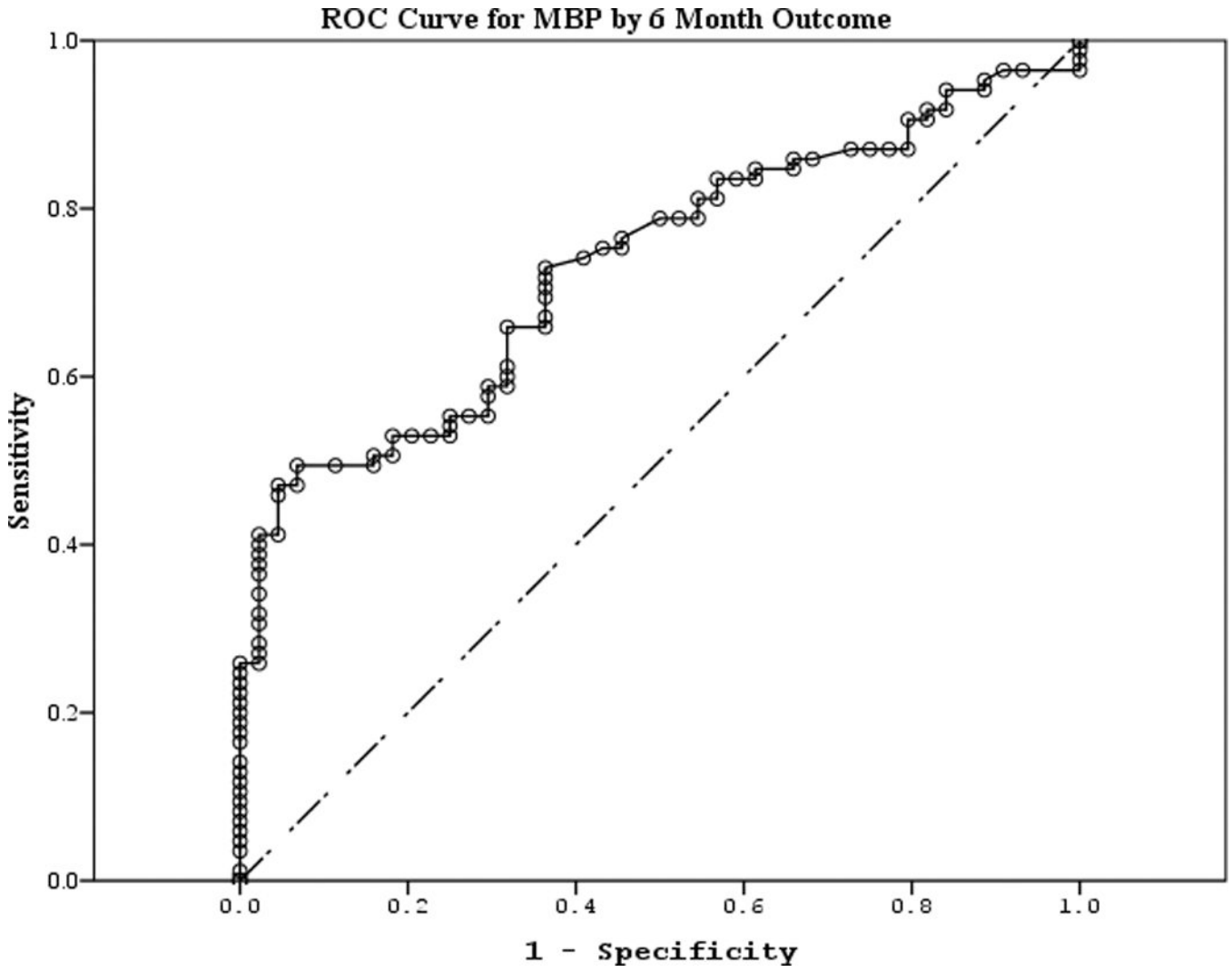
a–f. Serum NSE, S100, and MBP concentrations over the study period by good vs. poor outcome and mortality at 6 months.

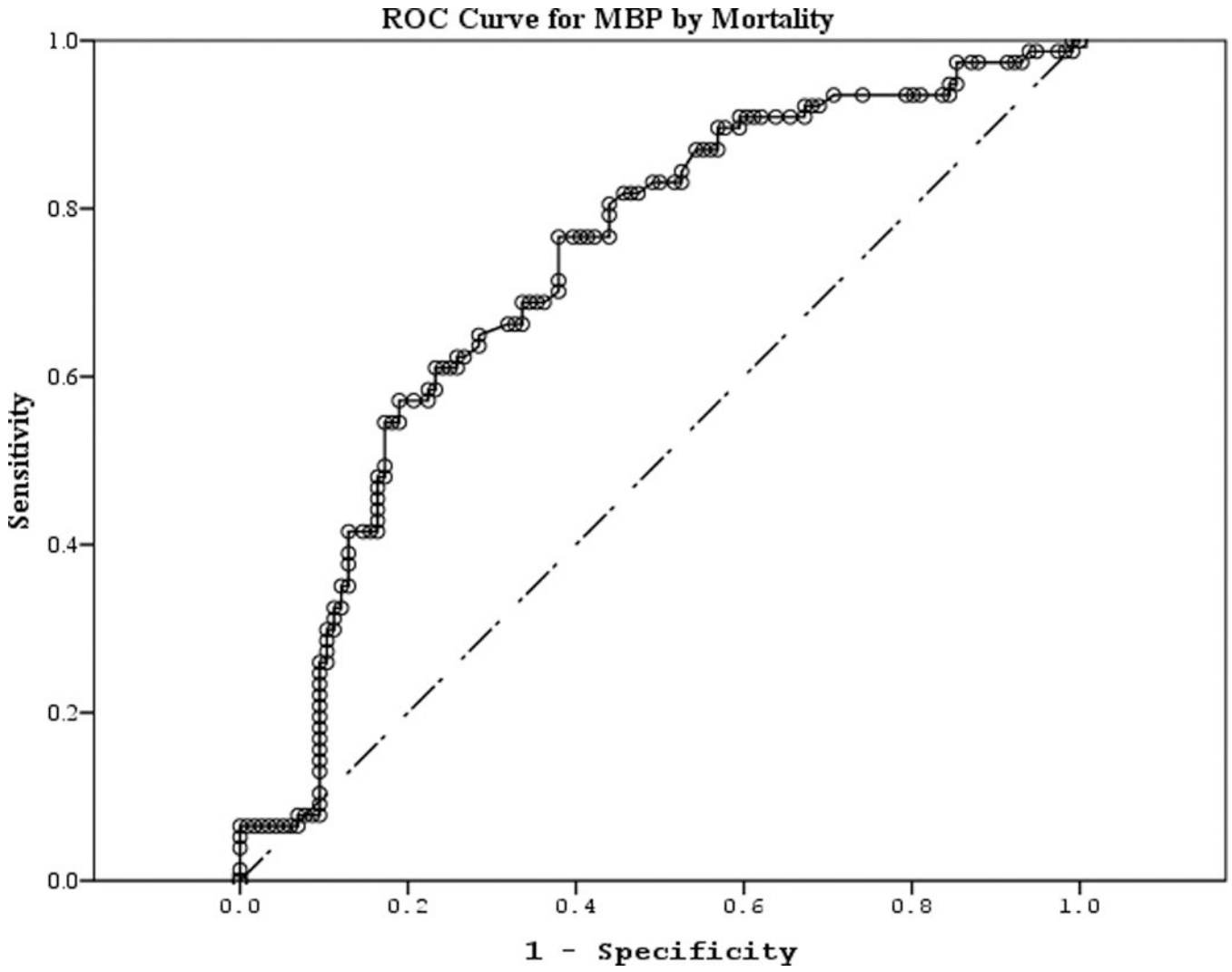


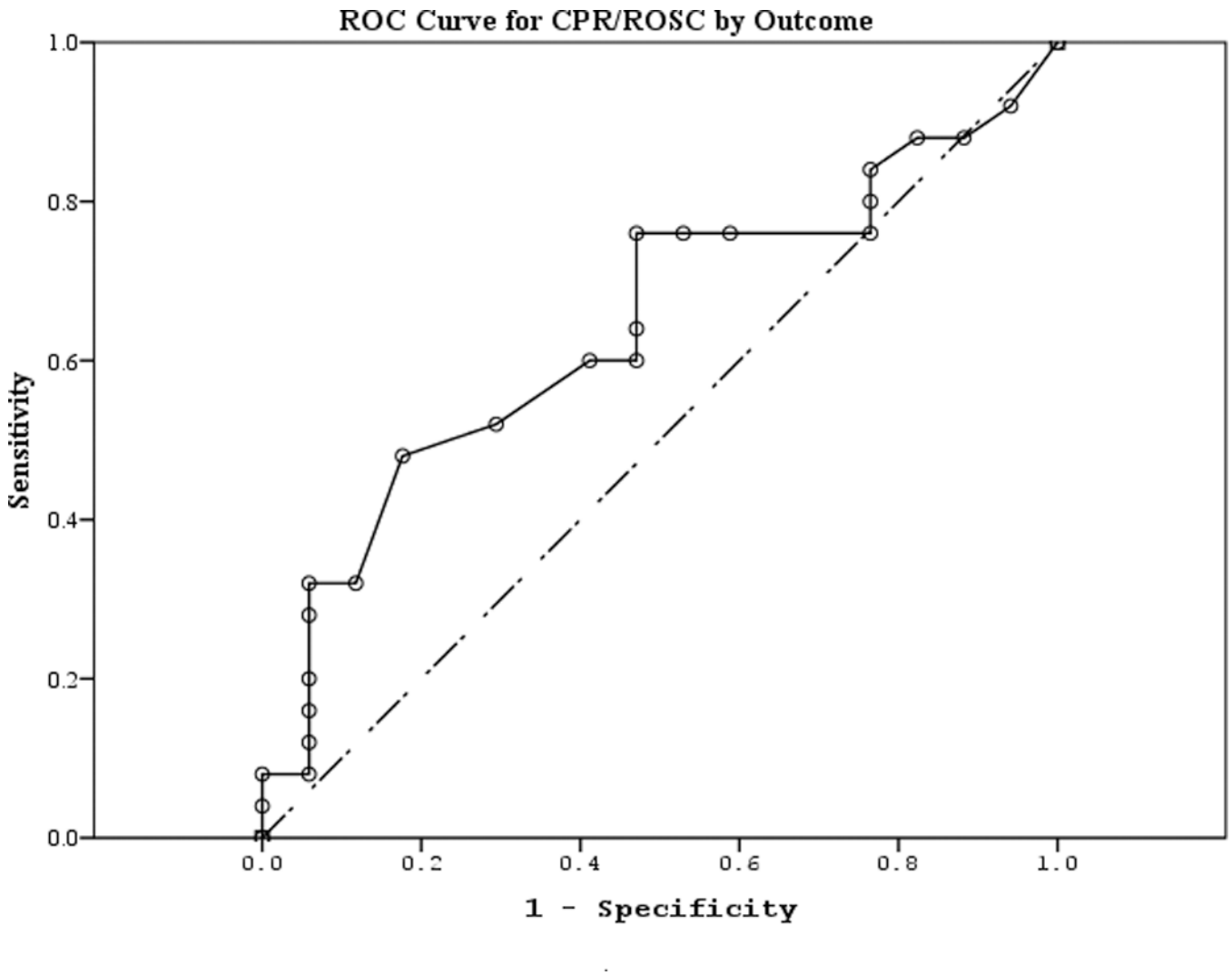


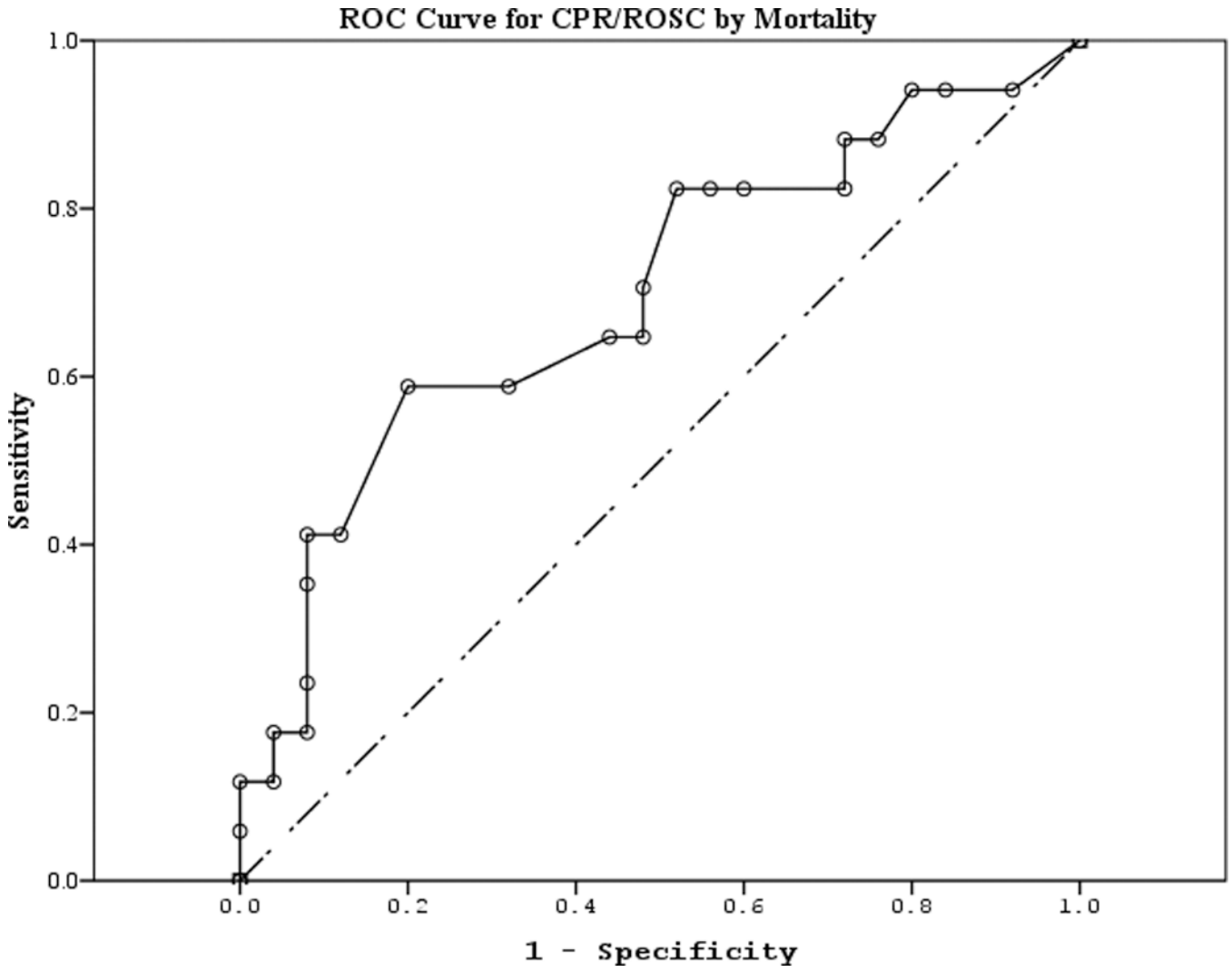


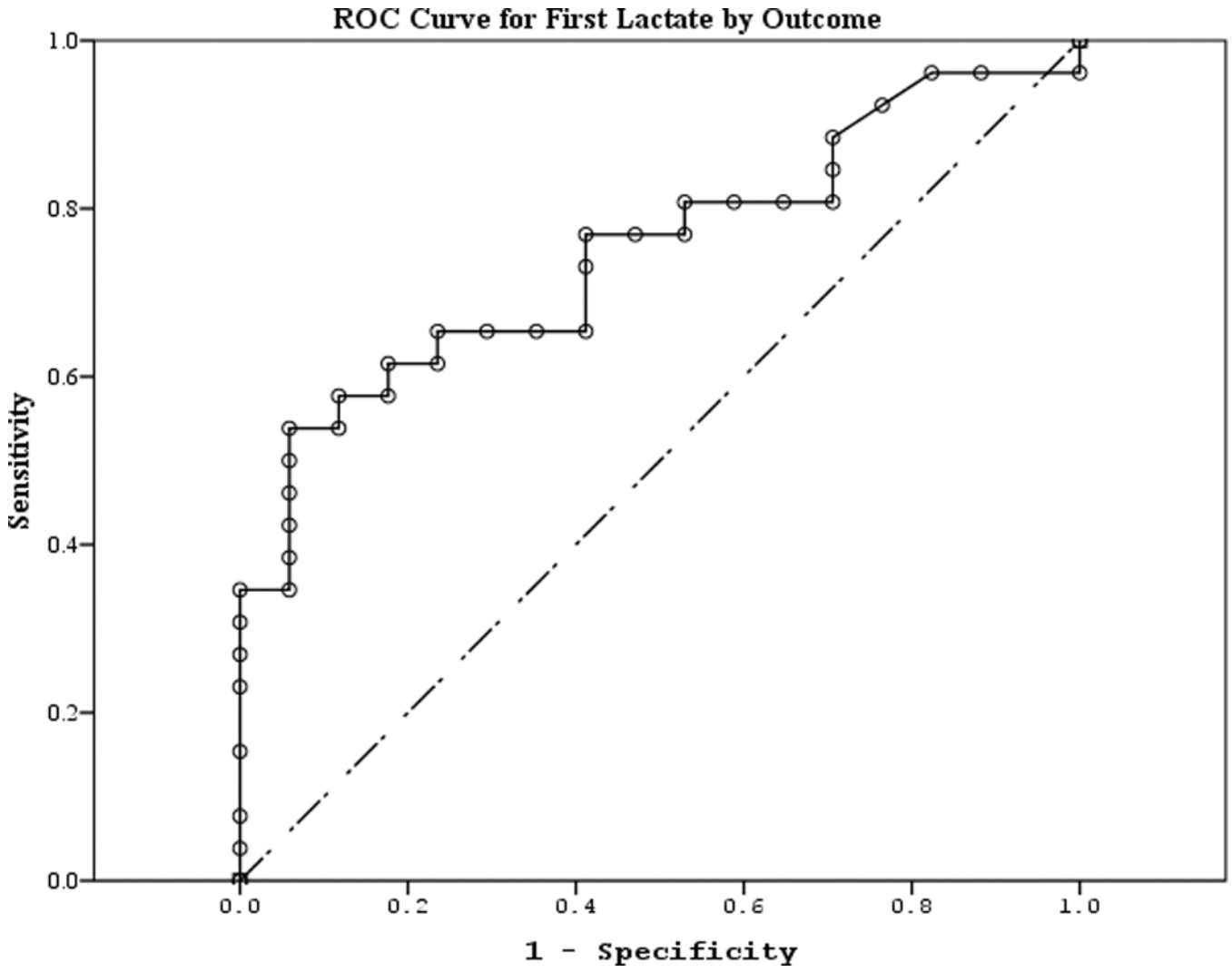


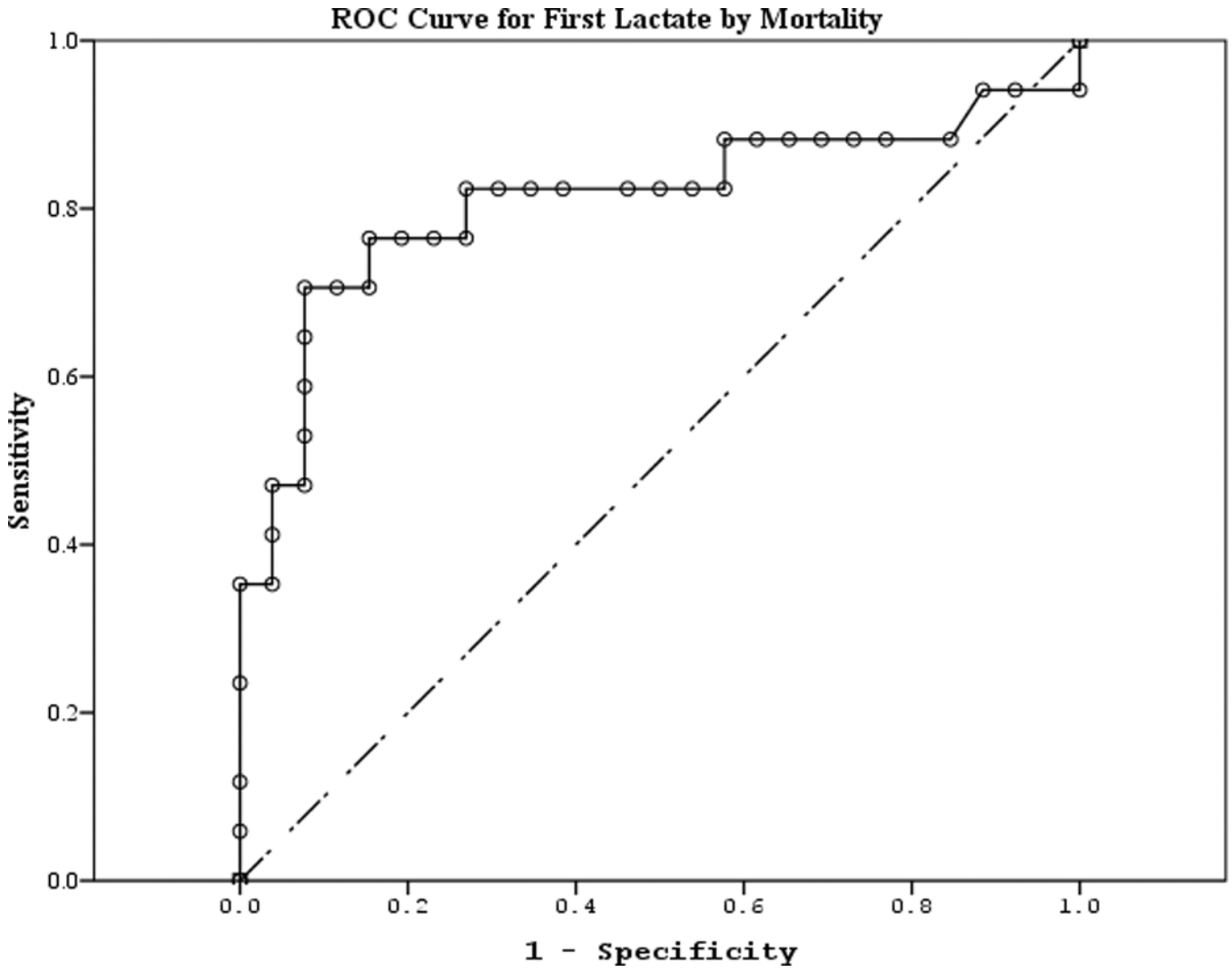


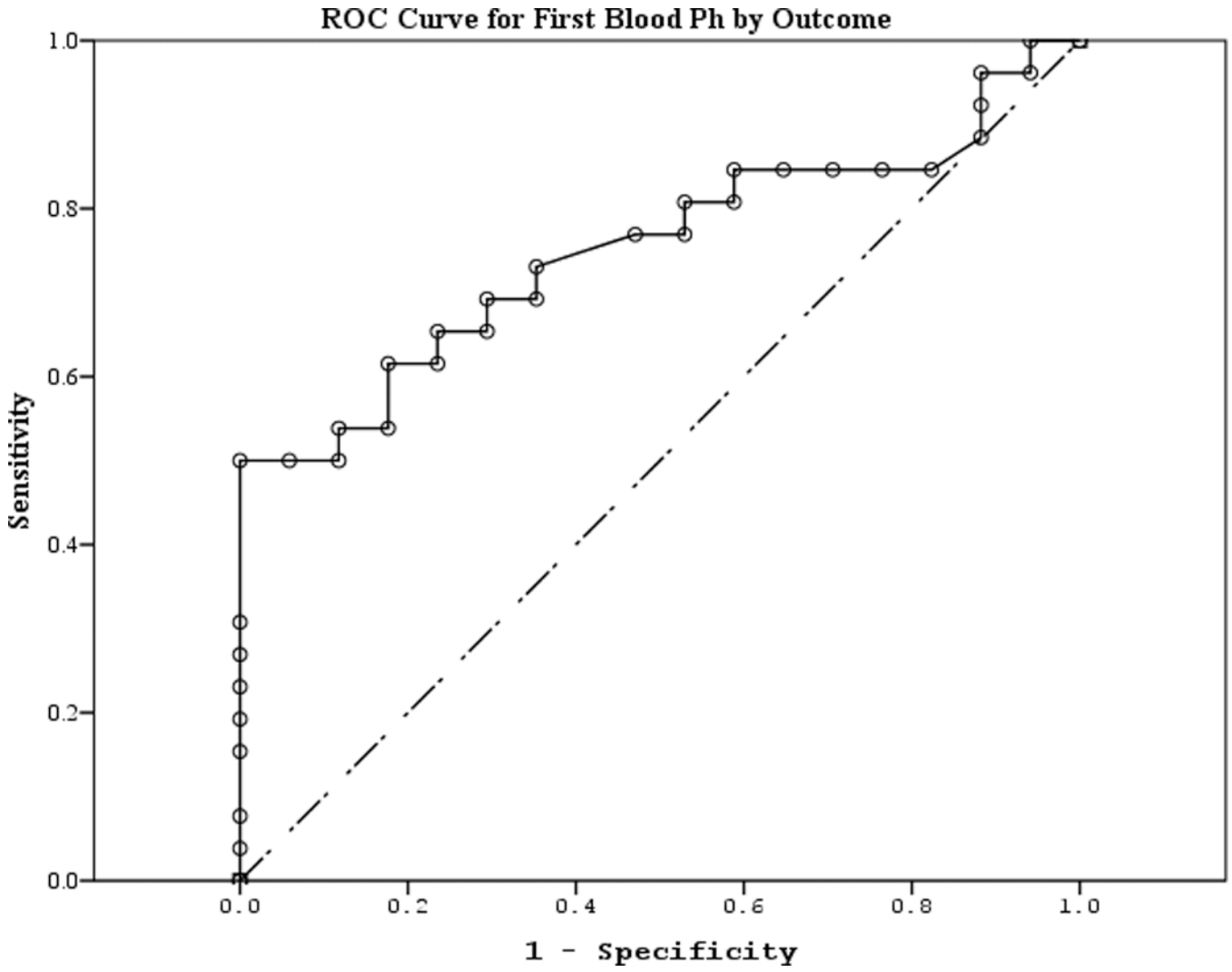












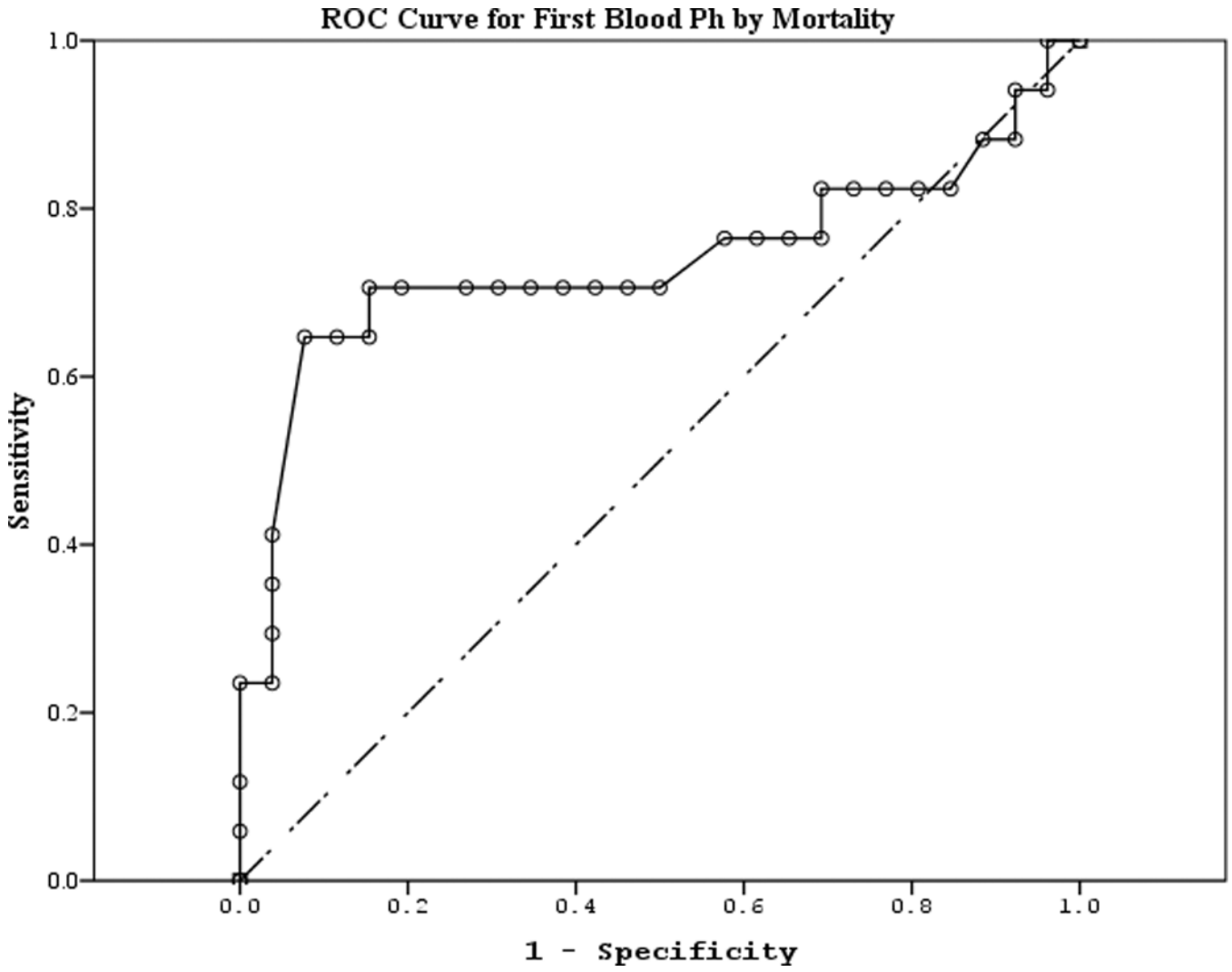


Figure 4.

a–l. Receiver operating characteristic curves for serum and clinical variables by good vs. poor outcome and mortality at 6 months.

Table 1

Subject demographics and details of cardiac arrest overall and by 6 month outcome post-arrest.

Mean±SD [25th, 75th %] or n (%)	All (n=43)	Good outcome (n=17)	Poor outcome (n=26)	p-value
Age, (y)	5.87±6.30 [0.33,11.52]	6.53±6.25 [0.32,13.14]	5.43±6.42 [0.48,10.21]	0.785
Sex, n (% male)	22 (51)	9 (53)	13 (50)	0.549
Race/ethnicity				0.929
White	34 (79)	13 (77)	21 (81)	
Black	7 (16)	3 (18)	4 (15)	
Other	2 (5)	1 (6)	1 (4)	
History of chronic illness	18 (42)	8 (47)	10 (39)	0.403
Primary etiology				0.155
Asphyxia/shock	37 (86)	13 (76)	24 (92)	
Cardiac	6 (14)	4 (24)	2 (8)	
Location				0.205
Out-of-hospital	32 (74)	11 (65)	21 (81)	
In-hospital	11 (26)	6 (35)	5 (19)	
Interval of CPR to ROSC (min)	26.6±28.3 [9,30]	18.0±16.3 [7.5,25]	32.4±33.2 [11,40]	0.120
Epinephrine boluses	2.9±2.9 [1,3]	2.8±2.8 [0,4.5]	2.9±3.0 [1,3]	0.811
Defibrillated	7 (16)	5 (29)	2 (8)	0.073
1st rhythm				0.066
Pulseless electrical activity	21 (49)	10 (59)	11 (42)	
Asystole	16 (37)	3 (18)	13 (50)	
Sinus	3 (7)	3 (18)	1 (4)	
VT/VF	1 (2)	1 (6)	1 (4)	
Witnessed event	20 (47)	14 (82)	6 (23)	<0.001
Bystander CPR	35 (81)	13 (77)	22 (85)	0.388

SD, standard deviation; ROSC, return of spontaneous circulation; CPR, cardiopulmonary resuscitation; VT/VF, ventricular tachycardia/ventricular fibrillation

Table 2

Clinical, laboratory, radiological results, and outcomes.

Mean±SD [25th,75th %] or n (%)	All (n=43)	Good outcome (n=17)	Poor outcome (n=26)	p-value
Pupillary reaction d1 (% reactive bilaterally)	24 (56)	15 (88)	9 (35)	<0.001*
Pupillary reaction d3 (% reactive bilaterally)[1]	31 (72)	17 (100)	14 (54)	0.021*
Motor GCS d1[2]	2.6±1.7 [1,4]	3.8±1.4 [3,5]	1.8±1.4 [1,3]	0.001**
Motor GCS d3[3]	3.1±2.1 [1,5]	5.1±0.9 [4,6]	1.7±1.4 [1,2]	<0.001**
GCS d1[4]	5.2±2.7 [3,6.5]	6.6±2.4 [5,8]	4.4±2.5 [3,5.5]	0.001**
GCS d3[4]	6.6±3.9 [3,9]	10.4±2.6 [9,11]	4.2±2.2 [3,4.5]	<0.001**
First lactate (mMol/L)	7.7±6.2 [2.9,12.7]	4.4±3.0 [2.0,5.8]	9.9±6.9 [4.5,15.1]	0.006*
First metered glucose (mg/dL)	271±237 [157,321]	315±355 [175,340]	242±108 [150,309]	0.882
First blood pH	7.02±0.28 [6.8,7.2]	7.16±0.22 [7.02,7.27]	6.92±0.27 [6.7,7.1]	0.006*
Inotrope score 1st 24h	31.4±342.6 [0,48]	10.1±20.2 [0,15]	45.3±47.8 [3,100]	0.003*
First brain CT				0.330
No acute lesion	20 (47)	10 (59)	10 (38)	
Acute lesion mild-moderate	3 (7)	1 (6)	2 (8)	
Acute lesion severe	8 (19)	1 (6)	7 (27)	
Not performed	12 (28)	5 (29)	7 (27)	
Day CT performed after CA[5]	1.8±1.4 [1,2]	2.1±1.7 [1,3.5]	1.6±1.2 [1,2]	0.765
EEG pattern				0.005*
Continuous	5 (12)	5 (29)	0 (0)	
Diffuse slowing or discontinuous	33 (77)	9 (53)	24 (92)	
Not performed	5 (12)	3 (18)	2 (8)	
Day EEG performed after CA	1.5±0.5[1,2]	1.7±0.5 [1,2]	1.5±0.6 [1,2]	0.121
Hospital LOS (d)	20.3±26.4 [3,30]	26.2±36.1 [7.5,28]	16.5±17.3 [2,33]	0.268
ICU LOS (d)	17.5±24.7 [2,25]	18.6±32.8 [4,17.5]	16.9±18.3 [2,30]	0.823
Survival at HD	26 (60)	17 (100)	9 (35)	<0.001**
Good outcome HD	17 (40)	15 (100)	0 (0)	NA

* $p < 0.05$

[1] N = 42, 17, 25 for all, good outcome, and poor outcome columns

[2] N = 41, 16, 25 for all, good outcome, and poor outcome columns

[3] N = 39, 16, 23 for all, good outcome, and poor outcome columns

[4] N = 41, 16, 25 for all, good outcome, and poor outcome columns

[5] N = 31, 12, 19 for all, good outcome, and poor outcome columns

HD, hospital discharge; LOS, length of stay; ICU, intensive care unit; GCS, Glasgow coma scale; CT, computed tomography; CA, cardiac arrest; EEG, electroencephalography

Table 3

Serum NSE, S100b, MBP concentrations by good vs. poor outcome and mortality at 6 months.

Mean±SD [25th,75th %]	Median	All (n=43)	Good outcome (n=17)	Poor outcome (n=26)	p-value Good vs. Poor	Survived (n=26)	Died (n=17)	p-value Mortality
NSE (ng/ml)								
Initial	15.40	26.9±33.4	16.1±20.6	33.9±38.4	**0.047**	14.5±16.9	45.7±43.1	**0.003**
		[5.8,34.0]	[3.0,25.2]	[10.8,46.3]		[3.5,17.7]	[14.3, 71.5]	
Mean	29.92	38.2±38.2	14.6±18.9	53.6±39.9	**<0.001**	23.4±23.8	60.8±45.2	**0.001**
		[8.8,59.0]	[2.5,17.6]	[32.4,66.2]		[3.7,49.8]	[35.1, 75.1]	
Median	28.00	34.4±37.3	13.7±19.4	47.8±40.3	**<0.001**	20.4±23.8	55.6±44.4	**0.001**
		[6.1,52.0]	[2.1,14.3]	2[29.3,55.2]		[3.1, 35.3]	[30.7, 60.4]	
Peak	49.74	67.4±60.1	32.6±37.8	90.1±61.5	**<0.001**	49.4±45.6	94.8±70.0	**0.015**
		[16.7,101.0]	[5.8,38.6]	[48.2,117.5]		[9.5, 91.7]	[51.5, 52.1]	
24 h	16.70	27.9±32.2	15.1±14.7	36.3±37.6	**0.019**	15.6±13.9	46.8±42.3	**0.003**
		[5.8,38.0]	[3.8,24.2]	[9.7,50.6]		[3.9, 22.3]	[15.1, 59.2]	
48 h	34.18	44.4±52.7	20.4±24.6	60.8±60.5	**0.011**	25.2±28.6	75.8±67.4	**0.007**
		[4.0,60.0]	[2.9,38.4]	[22.9,74.8]		[3.2, 39.0]	[31.9, 115.7]	
72 h	30.59	46.5±53.6	17.2±38.8	67.0±53.6	**0.001**	29.8±42.8	81.5±58.7	**0.004**
		[5.4,55.0]	[0.0,11.6]	[28.4,120.0]		[0.0, 49.6]	[42.8, 142.0]	
S100b (ng/ml)								
Initial	0.031	0.150±0.250	0.041±0.075	0.221±0.297	**0.001**	0.035±0.061	0.325±0.324	**<0.001**
		[0.009,0.190]	[0.005, 0.380]	[0.020, 0.290]		[0.007, 0.030]	[0.060, 0.660]	
Mean	0.020	0.109±0.159	0.027±0.059	0.163±0.180	**<0.001**	0.024±0.047	0.239±0.181	**<0.001**
		[0.010,0.170]	[0.001, 0.019]	[0.010, 0.310]		[0.003, 0.020]	[0.070, 0.370]	
Median	0.018	0.094±0.140	0.024±0.064	0.140±0.157	**<0.001**	0.021±0.052	0.260±0.158	**<0.001**
		[0.007,0.130]	[0.000, 0.010]	[0.010, 0.270]		[0.001, 0.010]	[0.060, 0.300]	
Peak	0.048	0.196±0.277	0.064±0.099	0.283±0.321	**0.001**	0.055±0.081	0.412±0.331	**<0.001**
		[0.020,0.250]	[0.006, 0.060]	[0.030, 0.590]		[0.008, 0.050]	[0.110, 0.750]	
24 h	0.021	0.107±0.181	0.023±0.038	0.162±0.215	**<0.001**	0.021±0.031	0.239±0.232	**<0.001**
		[0.008, 0.120]	[0.000, 0.020]	[0.010, 0.270]		[0.001, 0.210]	[0.040, 0.310]	
48 h	0.019	0.113±0.191	0.034±0.075	0.167±0.227	**0.001**	0.029±0.061	0.251±0.248	**<0.001**
		[0.006, 0.200]	[0.001, 0.018]	[0.010, 0.210]		[0.002, 0.210]	[0.030, 0.430]	

Mean±SD [25th,75th %]	Median	All (n=43)	Good outcome (n=17)	Poor outcome (n=26)	p-value Good vs. Poor	Survived (n=26)	Died (n=17)	p-value Mortality
72 h	0.014	0.070±0.125	0.028±0.074	0.099±0.146	**0.002**	0.024±0.058	0.167±0.171	**<0.001**
		[0.003, 0.060]	[0.000, 0.010]	[0.010, 0.150]		[0.000, 0.020]	[0.010, 0.250]	
MBP(ng/ml)								
Initial	0.20	0.75±1.48	0.74±1.54	0.76±1.47	0.153	0.81±1.82	0.65±0.75	0.094
		[0.09,.44.00]	[0.07, 0.40]	[0.15, 0.72]		[0.08, 0.28]	[0.15, 1.11]	
Mean	0.36	1.11±1.78	0.61±1.18	1.44±2.03	**0.010**	0.83±1.57	1.54±2.03	**0.021**
		[0.15,1.37]	[0.09,.40.00]	[0.22,1.79]		[0.11, 0.49]	[0.25, 1.82]	
Median	0.29	0.97±1.75	0.62±1.28	1.20±2.00	**0.030**	0.71±1.51	1.36±2.06	**0.006**
		[0.11,68.00]	[0.09,.31.00]	[0.19,1.41]		[0.09, 0.33]	[0.22, 1.79]	
Peak	0.69	2.46±3.85	0.92±1.50	3.46±4.6	**0.007**	1.86±3.17	3.36±4.67	0.074
		[0.29,2.61]	[0.16, 0.95]	[0.42,4.38]		[0.19, 1.68]	[0.47, 3.56]	
24 h	0.20	0.82±1.47	0.59±1.13	0.98±1.65	0.053	0.73±1.67	0.97±1.11	**0.017**
		[0.09, 0.68]	[0.08,.36.00]	[0.15,1.12]		[0.09, 0.36]	[0.19, 1.67]	
48 h	0.27	1.17±2.23	0.62±1.23	1.55±2.67	0.055	0.72±1.54	1.91±2.96	0.060
		[0.11, 0.74]	[0.09,.32.00]	[0.16,1.38]		[0.09, 0.31]	[0.24, 2.30]	
72 h	0.27	1.37±2.90	0.54±1.24	1.95±3.57	0.080	0.70±1.52	2.78±4.42	**0.020**
		[0.11,1.08]	[0.09,.36.00]	[0.13,1.56]		[0.10, 0.38]	[0.26, 2.31]	

IQR, interquartile range; SD, standard deviation; NSE, neuron specific enolase; MBP, myelin basic protein

Table 4

Area under the curve for serum and clinical biomarkers to predict good vs. poor outcome and mortality at 6 months.

				95% Confidence Interval	
Biomarker (outcome)	**Area**	**Standard Error**	**p-value**	**Lower**	**Upper**
NSE (good vs. poor)	0.859	0.032	<**0.001**	0.796	0.922
NSE (mortality)	0.787	0.033	<**0.001**	0.721	0.852
S100b (good vs. poor)	0.955	0.017	<**0.001**	0.922	0.987
S100b (mortality)	0.908	0.021	<**0.001**	0.866	0.950
MBP (good vs. poor)	0.732	0.043	<**0.001**	0.647	0.817
MBP (mortality)	0.727	0.037	<**0.001**	0.654	0.799
CPR-ROSC (good vs. poor)	0.642	0.086	0.121	0.474	0.811
CPR-ROSC (mortality)	0.696	0.086	**0.032**	0.528	0.865
First lactate (good vs. poor)	0.749	0.074	**0.006**	0.604	0.893
First lactate (mortality)	0.809	0.079	**0.001**	0.654	0.964
First blood pH (good vs. poor)	0.752	0.074	**0.006**	0.608	0.896
First blood pH (mortality)	0.736	0.091	**0.009**	0.558	0.915

NSE, neuron specific enolase; MBP, myelin basic protein; CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation

Table 5

a. Best sensitivity and specificity for serum biomarkers to predict good vs. poor outcome at 6 months.

	Concentration (ng/ml) Good/poor outcome	Sensitivity	Specificity	Positive predictive value	Negative predictive value
S100b					
24 h (best sensitivity)	0.001	0.96	0.29	67.6	83.3
24 h (best specificity)	0.128	0.40	1.00	100.0	53.1
48 h (best sensitivity)	0.008	0.96	0.59	77.4	90.9
48 h (best specificity)	0.283	0.20	1.00	100.0	45.9
NSE					
24 h (best sensitivity)	2.15	0.96	0.12	62.5	66.7
24 h (best specificity)	53.10	0.20	1.00	100.0	45.9
48 h (best sensitivity)	0.48	0.96	0.18	63.2	75.0
48 h (best specificity)	76.71	0.24	1.00	100.0	47.2
MBP					
24 h (best sensitivity)	0.08	1.00	0.18	65.0	100.0
24 h (best specificity)	5.83	0.04	1.00	100.0	40.5
48 h (best sensitivity)	0.05	1.00	0.06	61.0	100.0
48 h (best specificity)	5.43	0.12	1.00	100.0	43.6

b. Best sensitivity and specificity for serum biomarkers to predict mortality at 6 months.

	Concentration (ng/ml) Good/poor outcome	Sensitivity	Specificity	Positive predictive value	Negative predictive value
S100b					
24 h (best sensitivity)	0.001	0.96	0.29	45.9	100.0
24 h (best specificity)	0.128	0.40	1.00	100.0	81.3
48 h (best sensitivity)	0.008	0.96	0.59	81.8	77.4
48 h (best specificity)	0.283	0.20	1.00	100.0	70.3
NSE					
24 h (best sensitivity)	2.15	0.96	0.12	48.5	90.0
24 h (best specificity)	53.10	0.20	1.00	100.0	73.3
48 h (best sensitivity)	0.48	0.96	0.18	53.6	92.9

b. Best sensitivity and specificity for serum biomarkers to predict mortality at 6 months.

	Concentration (ng/ml) Good/poor outcome	Sensitivity	Specificity	Positive predictive value	Negative predictive value
48 h (best specificity)	76.71	0.24	1.00	80.0	67.6
MBP					
24 h (best sensitivity)	0.08	1.00	0.18	42.5	100.0
24 h (best specificity)	5.83	0.04	1.00	39.5	60.5
48 h (best sensitivity)	0.05	1.00	0.06	70.0	71.9
48 h (best specificity)	5.43	0.12	1.00	100.0	63.4

NSE, neuron specific enolase; MBP, myelin basic protein