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Table 1 to 3 are available in the Supplementary Files section.

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Abstract

Brain injury biomarkers may predict the treatment outcomes in adults with tuberculous (TB) meningitis, and their incremental value is unknown. We studied 115 HIV-negative adults with TB meningitis – 39 prospectively and 76 retrospectively. Concentrations of glial fibrillary acidic protein, S100B, neuron-specific enolase (NSE), phosphorylated neurofilament-heavy and total tau were estimated in lumbar cerebrospinal fluid specimens collected at diagnosis. In the prospective cohort, the 5-biomarker first principal component (PC1) had good discriminative accuracy for disability-free survival at hospital discharge (area under ROC curve [95% CI] 0.80[0.64–0.91]), and moderate accuracy for on-treatment neurological worsening (OTNW; 0.69[0.52–0.83]). In the retrospective cohort, the 4-biomarker PC1 (without NSE) had good discriminative accuracy for OTNW (0.81[0.70–0.89]) and disability-free survival at treatment completion (0.79[0.68–0.87]). Thao's clinical prediction model (CPM) had good discriminative accuracy for death (0.76[0.67–0.85]) in the combined cohort. However, risk calibration was weak. Combining PC1 with recalibrated CPM incrementally improved the prediction of death (category-free net reclassification improvement = +0.786; integrated discrimination improvement = +0.163). To conclude, brain injury biomarkers estimated at diagnosis were reproducibly predictive of OTNW and disability-free survival in HIV-negative adults with TB meningitis, and incrementally improved the prediction of death over and above a recalibrated CPM.

Key words: glial fibrillary acidic protein; neuron-specific enolase; phosphorylated neurofilament-heavy; S100B; total tau; tuberculous meningitis

Introduction

Tuberculous (TB) meningitis is the most lethal form of extrapulmonary tuberculosis. Despite early recognition and treatment with four-drug combination chemotherapy for 9-12 months and adjunctive dexamethasone for 6-8 weeks, TB meningitis is fatal in nearly 25%-40% of adults and leaves behind considerable neurological disability in about 10%-25% of survivors.[1] The prognostic factor that best predicts treatment outcome in TB meningitis is the modified Medical Research Council (MRC) stage of meningitis, which is largely based on the Glasgow coma scale (GCS) score.[2] Notwithstanding, the pathogenesis of impaired consciousness in TB meningitis remains poorly understood. Increase in blood-brain barrier (BBB) permeability leading to raised intracranial pressure, development of hydrocephalus and vasculitic infarcts, seizures and hyponatremia have been postulated as possible mechanisms.[3] Sometimes, patients without any of these complications do have impairment of consciousness.

In this context, Dittrich et al found in a mixed group of central nervous system infections that cerebrospinal fluid (CSF) concentrations of glial fibrillary acidic protein (GFAP), S100B, neuron-specific enolase (NSE) and total tau correlated with GCS scores, but not mortality.[4] Subsequently, Rohlwink et al found that persistently elevated levels of S100B, NSE and GFAP in lumbar CSF were associated with poor outcome at 6 months among children with TB meningitis and hydrocephalus.[5] However, the following knowledge gaps still exist — i) little information is available on adults with TB meningitis; and ii) incremental value of these biomarkers as compared to clinical prediction models (CPM) is unknown. We, therefore, aimed to answer the following questions: i) Do CSF biomarkers of brain injury correlate with the GCS score; ii) Do they predict on-treatment neurological worsening, death, and disability-free survival; iii) Do they maintain the prognostic accuracy in a historical cohort of patients with TB meningitis; and iv) Do they provide prognostic information over and above a validated CPM for 9-month mortality in adults with TB meningitis.

Methods

This study was conducted at the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), a tertiary care teaching hospital located in Puducherry, India. The study comprises of two parts. First, we prospectively studied consecutive hospitalized patients aged 18 years or more with TB meningitis diagnosed during the period September 2016 through March 2018. The study protocol was reviewed and approved (No. JIP/IEC/2016/29/967 & JIP/IEC/2017/0245) by the Institute Ethics Committee (Human Studies) at JIPMER, Puducherry. We adhered to the national ethical guidelines for biomedical and health research involving human participants issued by the Indian Council of Medical Research. We enrolled patients after obtaining informed written consent from the patient or their legally acceptable representative. We excluded patients if they had received TB treatment or steroids for more than 48 hours before enrollment, if they did not satisfy the consensus case definition for TB meningitis[6], or if they had human immunodeficiency virus (HIV) infection. We also excluded patients if the CSF specimen was blood-stained.

We collected the clinical information using a data collection form. GCS score was recorded at admission using standardized methods.[7] If there was any worsening of consciousness during hospital stay, the lowest GCS score was also noted. CSF analysis was done for leukocyte counts, glucose, Gram stain and culture, culture in mycobacterial growth indicator tube (MGIT), and nucleic acid amplification by Xpert MTB/RIF assay. Additional 2 mL of CSF was collected in a pre-chilled polypropylene tube, centrifuged at 3,000 rpm for 5 minutes, and the supernatant was aliquoted and stored frozen at −80°C.

A non-contrast/contrast-enhanced computed tomographic scan of brain was done for all patients at baseline, and subsequently as clinically indicated. Presence of hydrocephalus, infarcts, basal exudates and tuberculomas was noted. All patients were offered HIV testing and were also evaluated for evidence of TB elsewhere in the body. Patients were followed up to treatment completion or death, whichever was earlier. Two observers (SBG, TK) independently scored disability

using the modified Rankin scale (MRS),[8] and disagreement was resolved by consensus before the estimation of biomarkers.

We defined poor treatment outcome as death or survival with significant disability (MRS score >2).[9] Since the initial response to treatment could be compromised by events such as treatment default and paradoxical reactions, we assessed treatment outcome at two time-points — hospital discharge and treatment completion. We defined on-treatment neurological worsening (OTNW) as a sustained drop in GCS score any time during hospital stay by at least 1-point compared to the admission value.[10,11]

In the second part of the study, we identified HIV-negative patients with TB meningitis aged more than 12 years who had participated in research studies conducted at JIPMER between December 2012 and August 2016.[12-14] CSF specimens collected for these studies were stored frozen at −80°C. We included patients if at least 250 µL of residual CSF specimen was available for use. The Institute Ethics Committee granted a waiver of consent (No. JIP/IEC/2019/371) to use residual CSF specimens and anonymized clinical data for the present study. We abstracted necessary data points from previous study records.

Laboratory methods

We estimated the following CSF biomarkers — GFAP and S100B (astroglial injury), NSE (neuronal injury), phosphorylated neurofilament heavy (pNF-H; axonal injury), and total tau (injury to non-myelinated axons of cortical interneurons). We did not estimate NSE in the retrospective cohort since some specimens had been collected in EDTA[13], which interferes with the NSE assay. We used commercially available ELISA kits — human GFAP, S100B, and pNF-H (BioVendor, Czech Republic); NSE (Quantikine ELISA, Minneapolis); and human total tau (Invitrogen, Massachusetts). CSF specimens were diluted 2–10 fold for the assays. We set up all assays in duplicate and took the average of values. We estimated CSF albumin (by turbidimetry) and electrolytes (ion specific electrodes) using an autoanalyzer (Beckman Coulter AU400). Serum and CSF osmolality were

125 determined using a freezing point depression osmometer (Model 3250, Advanced Instruments Inc.,
126 Norwood, USA). We calculated albumin index as $albumin_{CSF}/albumin_{serum}$.

127 *Sample size estimation*

128 In the first part, we hypothesized that biomarkers would explain at least 25% of the variation in GCS
129 scores. To determine whether the expected correlation coefficient (r = 0.5) significantly differed (α =
130 0.05) from the null with 90% power, we required 38 patients. We powered the second part to
131 estimate the discriminative accuracy of biomarkers (combined into first principal component). Given
132 that about two-thirds of patients with TB meningitis have a good neurological outcome, we required
133 72 patients with TB meningitis for receiver-operating characteristic (ROC) curve analysis to estimate
134 an area under curve (AUC) of 0.7, assuming an alpha error of 0.05 and a beta error of 0.2.

135 *Statistical analysis*

136 We used a statistical software package (Stata/IC for Windows, version 12, College Station, Texas).
137 We summarized continuous variables with an approximate normal distribution as mean±SD and
138 those with a skewed distribution and ordinal variables as median with interquartile range. We
139 presented categorical variables as numbers with proportion, n(%). We assessed the strength of
140 linear relationship between GCS and MRS scores with the concentrations of biomarkers using
141 Pearson correlation. We applied the Wilcoxon rank sum test to compare the median levels of
142 biomarkers between two groups. We did not adjust for multiple comparisons. We used the chi-
143 squared test or Fisher's exact test, as appropriate, to compare categorical variables between two
144 groups.

145 We assessed the discriminative accuracy of individual biomarkers to predict disability-free
146 survival and OTNW using ROC curve analysis. After confirming sampling adequacy using the Kaiser-
147 Meyer-Olkin measure, we performed a principal component analysis and used the first principal
148 component (PC1) Z-score to evaluate the combined prognostic accuracy of CSF biomarkers. We

calculated predicted 9-month mortality using the CPM risk calculator developed by Thao et al.[15,16] This CPM includes age, MRC stage, previous TB, focal signs, dexamethasone use and CSF lymphocyte count as predictors. After combining the two cohorts, we evaluated the discriminative accuracy of the CPM using ROC curve analysis to predict death and assessed model calibration using the pmcalplot function.[17]

We converted the CPM-predicted probability to its underlying linear predictor (logit), and performed a logistic recalibration of the CPM.[18] We employed the postestimation command to obtain revised probability estimates using the recalibrated CPM with and without the PC1 Z-score in the models. From these estimates, we calculated category-free net reclassification improvement (cfNRI) and integrated discrimination improvement (IDI) indices to assess the incremental value of biomarkers.[19] We estimated bias-corrected and accelerated (BCa) confidence interval from 10,000 bootstrap resamples using R, version 4.6.0. Finally, we did a binary logistic regression to evaluate the association of PC1 Z-score with clinical outcomes adjusted for confounding. All tests were two-sided, and *P* value <0.05 was considered statistically significant.

Results

Description of patient cohorts

In the prospective cohort, we included 39 of 44 consecutive patients with a presumptive diagnosis of TB meningitis — an alternative diagnosis was made in 3 patients, and 2 patients did not fulfil the consensus case definition. Of the 103 patients identified in the retrospective cohort, residual CSF specimens were available for 76 patients. The two cohorts were comparable with respect to age, sex, time to treatment initiation, dexamethasone use, previous TB treatment, CSF findings, and overall mortality (Table 1). In the retrospective cohort, symptom duration was significantly longer, more patients had MRC stage I disease, and OTNW was more common. Although hydrocephalus was

more frequent in the prospective cohort, hydrocephalus requiring CSF shunt procedure was similar between the two cohorts. More patients in the retrospective cohort were classified as possible TB meningitis. CPM predicted 9-month mortality was slightly higher in the prospective cohort as compared to the retrospective cohort, although not statistically significant. Most (57%) deaths occurred after hospital discharge in the prospective cohort, while two-thirds of all deaths occurred during hospitalization in the retrospective cohort.

CSF biomarkers, GCS score and treatment outcomes in prospective cohort

Pretreatment concentrations of NSE had a weak but significant negative linear association with the admission GCS score, and concentrations of GFAP, NSE, and total tau had a moderately strong negative linear relationship with the lowest GCS score (Table 2). Pretreatment concentrations of all biomarkers were significantly higher in those with a poor outcome at hospital discharge (Table 3), and GFAP, S100B and total tau levels were significantly higher in those with poor outcome on follow-up. All CSF biomarkers except S100B had a moderately strong positive linear association with the MRS score at discharge (Table 2). All biomarkers had moderate discriminative accuracy to predict good functional status at hospital discharge; AUCs ranged between 0.71 for S100B and 0.79 for NSE. GFAP, S100B and total tau had moderate accuracy for final outcome (Suppl. Table 1), and NSE had good discriminative accuracy for OTNW (AUC = 0.78 [0.61–0.89]).

All CSF biomarkers had moderate to strong positive linear association with each other (Suppl. Table 2). The PC1 captured 65% of total variance, and all biomarkers had similar positive factor loadings on PC1. Biomarkers of astroglial and neuroaxonal injury had divergent factor loadings on the PC2 (Figure 1A). The 5-biomarker PC1 had a moderately strong negative linear association with the lowest GCS score and a strong negative linear association with the MRS score at hospital discharge (Table 2). PC1 was able to discriminate between good vs poor outcome at discharge, and the AUC was better than that of admission GCS score (Suppl. Table 1), although not statistically

significant (Figure 1B; *P* = 0.124). PC1 also had moderate discriminative accuracy for OTNW and final outcome.

CSF biomarkers and admission GCS scores did not differ significantly by the presence of hydrocephalus or infarcts (data not shown). Likewise, biomarkers of astroglial injury (GFAP and S100B) did not correlate with albumin index, CSF osmolality, and concentrations of CSF potassium, calcium, or sodium (data not shown). CSF osmolality and sodium concentration had moderately strong linear relationships with serum osmolality (r = 0.455; *P* = 0.004) and sodium concentration (r = 0.447; *P* = 0.004), respectively.

CSF biomarkers, GCS score and treatment outcomes in retrospective cohort

GFAP had a weak but significant negative linear association with the admission GCS score, and S100B and pNF-H had a moderately strong negative linear relationship with the admission GCS score (Table 2). Pretreatment concentrations of all 4 CSF biomarkers were significantly higher in those with poor outcome at discharge and on follow-up (Table 3). GFAP and S100B had a moderately strong positive linear association with the MRS score at discharge and on follow-up (Table 2). All 4 biomarkers had moderate to good discriminative accuracy for predicting OTNW, in-hospital as well as final functional outcomes (Suppl. Table 1).

All biomarkers had a moderate to strong positive linear association with each other (Suppl. Table 2). The 4-biomarker PC1 had a moderately strong negative linear association with the admission GCS score and a strong positive linear association with the MRS score at hospital discharge and on follow-up (Table 2). PC1 had a good discriminative accuracy for OTNW, in-hospital and final outcomes, with AUCs numerically higher than that of admission GCS score (Suppl. Table 1). In patients with hydrocephalus, all biomarkers particularly GFAP (8.68 [1.88- 44.00] vs. 0.80 [0.28-3.33] ng/mL; *P* < 0.001) and S100B (548 [294-2310] vs. 306 [143-530] pg/mL; *P* = 0.002) were significantly higher, and the admission GCS scores were significantly lower (10 [9-13 vs. 13 [11-15]; *P*

= 0.011). On the other hand, presence of infarcts was not associated with biomarkers and admission GCS scores.

Prognostic accuracy of CPM and incremental value of biomarkers

CPM-predicted probability of death had a good discriminative accuracy for the final functional outcome in both cohorts – AUCs were 0.74 (0.58–0.87) and 0.79 (0.68–0.87) respectively in the prospective and retrospective cohorts. The predicted mortality also had good accuracy for OTNW as well as in-hospital functional outcome (Suppl. Table 1). In the combined cohort of 115 patients, the AUC was 0.76 (0.67–0.85) for predicting death on follow-up (Figure 2A). However, calibration-in-the-large was weak with an overall predicted probability of 17.4% against the observed mortality of 38.3%. The CPM consistently underestimated risk in the three upper quartiles (Figure 2B). The CPM appeared to have a ceiling effect in this population, with the predicted probabilities seldom exceeding 60%. The predicted probability of death was more than 50% in only 4 of the 115 patients (Figure 2C). The median (IQR) predicted risk of death was 20.2% (9.3%–28.4%) among those who died vs 9.7% (5.9%–16.7%) among those who survived. Recalibration substantially improved the prediction (Figure 2D).

Most patients with positive PC1 Z-scores in the prospective cohort had a poor outcome irrespective of the CPM-predicted risk of death (Figure 3A). Likewise, in the retrospective cohort, almost all patients with positive PC1 Z-scores expired; three patients who survived despite a positive PC1 Z-score had severe disability (Figure 3B). On multivariable analysis, the odds of OTNW was independently associated with the PC1 (adjusted OR = 2.13 [1.15–3.92]; *P* = 0.016) and the admission GCS score (0.77 [0.61–0.97]; *P* = 0.023) in the retrospective cohort. Further, the PC1 was independently associated with death (2.60 [1.38–4.92]; *P* = 0.003) and functional outcome (4.13 [1.46–11.69]; *P* = 0.008) on follow-up, after adjusting for the recalibrated CPM prediction. Addition of PC1 to the recalibrated CPM incrementally improved the prediction of death (Figure 3C), as

indicated by positive values for cfNRI (+0.786 [95% BCa bootstrap CI 0.344–1.176]; event NRI 0.133; non-event NRI 0.652) and IDI (+0.163 [0.072–0.263]).

Discussion

We found that biomarkers of brain injury explained a considerable proportion of variation in the GCS scores of HIV-negative adults with TB meningitis. Further, pretreatment concentrations of biomarkers were predictive of OTNW and disability-free survival at hospital discharge and at treatment completion. More importantly, the CSF biomarkers provided incremental prognostic information when added to the recalibrated CPM, and the main findings were temporally validated in an independent cohort of TB meningitis patients. There are only a handful of studies on the prognostic accuracy of brain injury biomarkers in TB meningitis. The present study substantially extends the findings of previous studies.

The correlation of GCS scores with biomarker concentrations is intuitive, since these biomarkers are known to reflect the severity of brain damage in the context of head injury.[20] Concurrent elevation of multiple biomarkers and their intercorrelation indicate that TB meningitis does not preferentially affect any particular cellular component of the brain. A similar rise in multiple biomarkers could be expected in infarction of brain parenchyma, which is quite common in TB meningitis. However, we did not find an association between biomarker concentrations and the presence of infarcts on imaging, while a previous study found an association.[5] One reason could be that we did not routinely use magnetic resonance imaging, which is more sensitive to identify small-sized and early infarcts.[21] Another possibility could be that brain injury in TB meningitis is rather a diffuse process involving subpial regions of the cortex in the form of border zone encephalitis.[22]

'New neurological event' defined as "a fall in GCS of ≥2 points for ≥48 hours, new focal neurological sign, or new onset of seizures" has been proposed as an outcome measure for TB

meningitis studies.[23] OTNW is conceptually quite similar to this, but more specific since it did not include focal deficits and seizures, which may have other mechanisms; and more sensitive since 1-point drop in GCS was enough. We defined OTNW in line with the modified definition of 'neuroworsening' as used in traumatic brain injury.[10,11] Interestingly, positive PC1 Z-scores were associated with OTNW, suggesting that biomarker estimation at baseline could be used to identify a subset of patients for targeted interventions. In clinical practice, it might suffice to estimate just any one of these biomarkers in lieu of the full panel studied by us.[5]

The CPM had an AUC of 0.77 (95% CI 0.72–0.83) for predicting 9-month mortality in the original development cohort from Vietnam.[15] Upon external validation, the AUC was reported to be lower (0.60 [0.54–0.66]) among Indonesian patients.[24] While we could replicate the originally reported discriminative accuracy of the CPM in our study population, we found that the calibration was weak. It is well known that CPMs often retain their discriminative accuracy on external validation but exhibit poor risk calibration, due to various reasons such as differences in patient characteristics, varying baseline risk and overfitting.[25] Previous TB treatment and focal neurological deficits were less frequent, while stage III disease and dexamethasone use were more frequent in our cohorts as compared to the Vietnam cohort.[15] Poor calibration does not necessarily imply a lack of external validity. Rather, it indicates a need to update the model calibration, which we successfully did using logistic recalibration. Although the CPM was originally developed for predicting 9-month mortality, we found that it also had good discriminative accuracy for in-hospital mortality, OTNW and disability-free survival. This probably indicates that OTNW, disability and death in TB meningitis have shared underlying mechanisms.

The functional importance of astroglia in TB meningitis and the morphological changes in the form of reactive astrogliosis in the subpial zone and around the granulomatous meningeal exudate are known for quite some time now.[22,26,27] Dittrich et al found that GFAP concentrations correlated with albumin index, and patients with low GCS had higher levels of total tau in a mixed group of CNS

infections including 11 with TB meningitis in Laos.[4] Rohlwink et al reported that a combined increase in GFAP, S100B and NSE at week 2 from admission best predicted mortality despite a decrease in inflammatory markers in a group of 44 South African children with TB meningitis.[5] While these findings provided key insights into the mechanisms contributing to death and disability in TB meningitis, it remains important to know the prognostic utility of these biomarkers measured at admission, particularly in the lumbar CSF, so that it informs a potential clinical decision in the future to deliver neuroprotective interventions to prevent secondary brain injury. Subsequently, van Laarhoven et al reported that elevated CSF NSE levels were associated with mortality in 97 HIV-negative Indonesian adults with TB meningitis.[28] Barring these, no other study had evaluated the prognostic value of brain injury biomarkers in TB meningitis.

Compared to the earlier studies, ours had several strengths – i) We studied adults with TB meningitis, a population not included by Rohlwink et al.; ii) We enrolled patients irrespective of the presence of hydrocephalus, enabling us study the relationship with the GCS score; iii) We used a broader panel of biomarkers to characterize astroglial and neuroaxonal injury; iv) We studied clinically relevant outcomes such as death and disability-free survival; v) Additionally, we evaluated OTNW as a novel intermediate outcome measure; vi) We evaluated adding biomarkers to a recalibrated CPM to demonstrate incremental value; and vi) Most importantly, we externally validated our findings in another larger cohort of TB meningitis patients. Possible limitations of our study are, i) Data on lowest GCS scores was not available in the retrospective cohort; ii) We could not combine the biomarker concentrations across the two cohorts since the biomarkers were estimated on archived CSF samples in the historical cohort; and iii) NSE was not estimated in the historical cohort due to the use of EDTA. However, these issues will not affect the validity of our findings.

Conclusions

In two separate cohorts of HIV-negative adults with TB meningitis, pretreatment CSF biomarkers of brain injury were predictive of OTNW, death and disability-free survival at hospital discharge and at treatment completion. Inclusion of brain injury biomarkers incrementally improved the prognostic prediction over and above that is currently possible using a recalibrated CPM.

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Author contributions

TK conceived the study and designed the protocol in consultation with RS and NMJ. AT, SBG, SM, GVD and CN enrolled patients into the studies and collected clinical data. AT, SBG and RM did the biomarker assays, which were supervised by RS. SD validated the calculation of predicted risk. JM and NMJ did the microbiological assessments. TK did the statistical analyses, aided by AT, SBG, SM, GVD and CN. SD, RM, JM, NMJ and RS provided critical comments on the manuscript. TK drafted the manuscript, which was read and approved by all authors. TK is the guarantor.

Competing interests

The authors declare no competing interests.

Data availability

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

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Figure

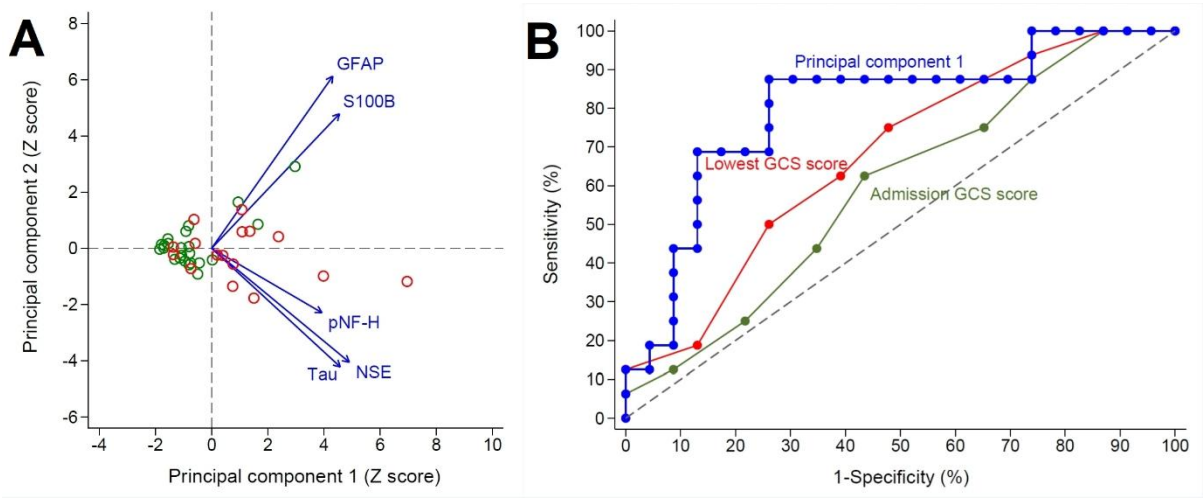
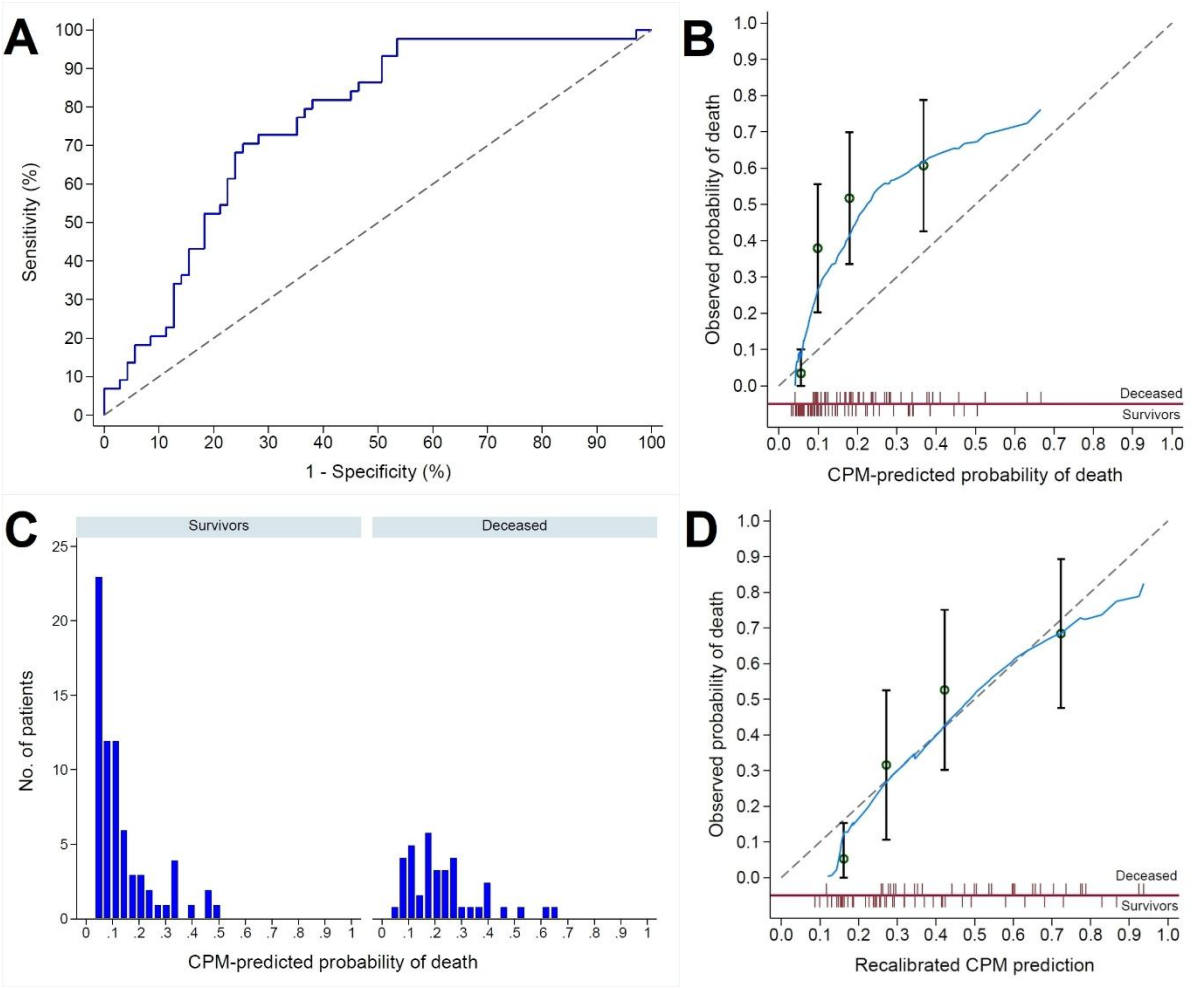


Figure 1. Prospective cohort: Principal component analysis biplot depicting scatter plot of Z-scores of individual patients for the first two principal components, and the factor loadings of individual biomarkers as vectors. Green circles indicate a good outcome at hospital discharge and red circles indicate a poor outcome (Panel A); ROC curves depicting the discriminative accuracies of the first principal component Z-score, admission GCS score and the lowest GCS score to predict good outcome at hospital discharge (Panel B). GCS = Glasgow coma scale; GFAP = glial fibrillary acidic protein; NSE = neuron-specific enolase; pNF-H = phosphorylated neurofilament-heavy

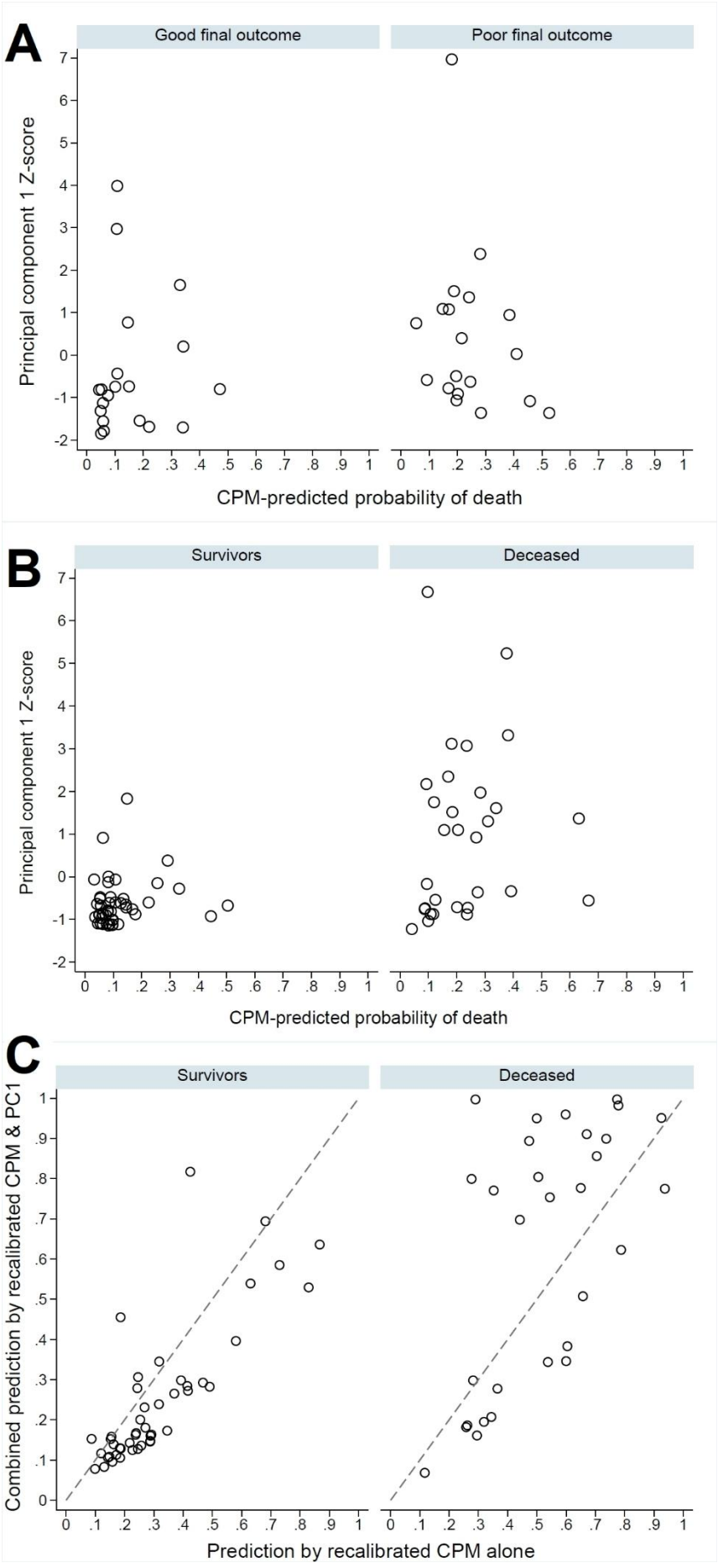


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422 **Figure 2.** Combined cohort of 115 patients: Receiver-operating characteristic (ROC) curve depicting
423 the discriminative accuracy of the clinical prediction model (CPM) to predict death on follow-up
424 (Panel A); Calibration plot of observed vs CPM-predicted probability of death across quartiles of risk
425 (Panel B). The cyan blue line indicates locally weighted scatterplot smoothing (LOWESS) to depict
426 calibration at the individual patient level. Dotted lines indicate perfect calibration and error bars
427 depict 95% CI. A spike plot of the distribution of events is included at the bottom (Panel B);
428 histograms depicting the distribution of CPM-predicted probabilities of death presented by survival
429 status (Panel C). Calibration plot as above for the retrospective cohort after logistic recalibration
430 (Panel D).

431

432


A
Good final outcome
Poor final outcome
Principal component 1 Z-score
CPM-predicted probability of death
B
Survivors
Deceased
Principal component 1 Z-score
CPM-predicted probability of death
C
Survivors
Deceased
Combined prediction by recalibrated CPM & PC1
Prediction by recalibrated CPM alone

433

Figure 3. Scatterplots of PC1 Z-scores against the clinical prediction model (CPM)-predicted probability of death presented by final outcome in the prospective cohort (Panel A) and survival status in the retrospective cohort (Panel B). PC1 Z-scores were based on 5 biomarkers in the prospective cohort and 4 biomarkers in the retrospective cohort. Reclassification plot depicting shift in probabilities when PC1 is combined with the recalibrated CPM compared to recalibrated CPM alone, presented by survival status in the retrospective cohort (Panel C). Points lying above the dotted lines indicate an increase while those lying below indicate a decrease in probability with the addition of biomarkers.

Supplementary Files

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