HENRI SALO

Human serum metabolites as outcome predictors in moderate and severe traumatic brain injury

Turun yliopisto, kliininen laitos, kliiniset neurotieteet

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Traumatic brain injury (TBI) is major global health problem. Outcome of TBI varies between death and good neurological recovery. Several clinical characteristics are known to affect outcome, still prognostication after TBI has proven difficult. Novel biomarkers have been studied, but none have performed well enough to be useful in clinical work. Metabolic profiling has shown promise in many disease entities including TBI. In this study metabolic profile is compared between unfavorable outcome patients and favorable outcome patients during first week after the injury. Also, between fatalities and survivors. Prognostic model using logistic regression was generated and its performance was compared to existing IMPACT and CRASH models.

Metabolic profile was found to differ between unfavorable and favorable outcome patients. It was observed that metabolic difference is likely to change in unfavorable and favorable outcome patients during the first week after injury. Change in metabolic profile is even greater between fatalities and survivors than between unfavorable and favorable outcome patients in first days after injury. Metabolic modeling shows promise as outcome predictor in moderate and severe TBI and performs with good accuracy in single center setup but validates poorly. Existing clinical models perform with poor accuracy in this cohort.

Larger studies are needed in future to validate these findings. The changing difference in metabolic profiles between unfavorable and favorable outcome patients during the first week after injury is interesting finding, which have not been reported before.

Keywords: Traumatic brain injury, TBI, biomarker, metabolomics

Introduction

Traumatic brain injury (TBI) is traditionally classified as mild, moderate, or severe based on acute clinical findings using the Glasgow Coma Scale (GCS). In mild TBI the lowest GCS is recorded as 13 or higher, in moderate between 9-12 and in severe 8 or less. [1,2] Mild TBI (mTBI) is more common than moderate TBI (moTBI) or severe TBI (sTBI)[3], but most of the morbidity and mortality related to TBI is seen on individuals with moTBI or sTBI.[4]

Despite being major health and economic problem worldwide, the actual incidence of TBI is not well defined in the current literature. This is primarily due to the heterogeneity of studies and how TBI is defined in these studies.[3] However, it is estimated that TBI is still the leading cause of neurological disability worldwide[5].

TBI is complex and heterogeneous disease in which prognosis has proven difficult. Though continued research early prognostication after TBI has evolved. Multiple factors have been associated with outcome after TBI. These can be divided into patient characteristics, admission details, imaging studies, laboratory values, and novel blood-based biomarkers.[6]

Two different prognostic models based on a large cohort of patients have been published. These are available in tabular form and as online calculators. The CRASH model was published by the Corticosteroid Randomisation After Significant Head Injury investigators. The model is based on data from 10 008 patients with all severities of TBI.[7] The IMPACT model is based on data from 8509 patients in the IMPACT database. It only includes patients who have suffered moTBI or sTBI and had GCS equal to or less than 12.[8,9]

A systematic review published in 2019 reported that both models showed moderate to good discrimination but with significant variation and highly variable calibration in external validation studies. The same study also reported multiple extensions of these two models using different prognostic factors. However, only one study was identified in which novel biomarkers from blood or cerebrospinal fluid were added to the models.[10] In this study it was found that the performance of the IMPACT core model can be improved by addition of biomarker levels. This was particularly true for performance regarding mortality, but for unfavorable outcome, the improvement of performance was modest.[11]

Biomarkers measured from blood or cerebrospinal fluid may be useful in various aspects of TBI.[12] S100beta has already been included in the Scandinavian guidelines for triaging patient with mTBI for a CT scan[13]. Despite promising studies there are still many unknowns and problems with biomarkers in TBI. Many of the proposed markers are not brain-specific and can be elevated due to multiple reasons—such extracranial trauma or neurodegenerative diseases. Therefore, interest is shifting to biomarker panels and studying the evolution of biomarker levels, particularly in sTBI.[12,14]
Metabolic markers and metabolomic profiling have been less studied in TBI. There are significant changes in brain metabolism after TBI. These changes can lead to worsening of secondary injury that can have effect on brains ability

to recover from the initial injury.[15–18] Metabolic markers can be measured either from cerebrospinal fluids, brain microdialysate, or serum samples. Currently, brain microdialysate analysis is the only application in the clinical use.[19]

Metabolic markers also have other advantages over protein biomarkers as they can cross blood-brain barrier (BBB) more easily than protein biomarkers due to their similarity in structures in the case of lipids and transporters in the case of polar metabolites. This makes them less dependent on fluctuations caused by disruption of BBB. For this reason, metabolomics measured from serum could provide a better and more immediate picture of the state of the brain when BBB is intact. Although changes in metabolic concentrations could be partly from extracranial sources.[20,21] TBI has also been shown to alter the metabolic profile of serum or plasma. Various metabolites have also been associated with outcome after TBI.[15,16,19,22]

Herein, we conducted a metabolite discovery study in patients with moderate to severe TBI. The hypothesis for this study was that favorable functional outcome in moderate and severe TBI is associated with a specific metabolic profile and that adding these metabolic markers to the already existing prognostic calculators (IMPACT and CRASH) would improve prognostic performance. Both existing clinical prognostic models also predict mortality in TBI. We hypothesized that the metabolic profile of dying from TBI would differ from that of survivors. A metabolic model to predict for TBI mortality was also created, and compared and tested with existing clinical models. We also report the change in first 7 days after injury in favorable and unfavorable outcome groups.

Materials and methods

Ethics statement

Study protocol was approved by South-West Finland hospital district ethics committee, the Cambridge 2 research ethics committee, and the Norfolk research ethics committee. Oral and written information about the study was given to patient or their next of kin. Oral and written consent was also obtained from patient or their next of kin. Patients were treated according to local guidelines that were based on international guidelines and recommendations at the time.[23]

Sample selection

This study involves subset of patients from a previously published cohort.[24] Patient were recruited to this study as a part of EU funded TBIcare (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries). Arrival day blood samples were collected within 12 hours of hospital admission. Handling of samples have been previously described[24]. During hospital stay blood samples were collected also on days 1, 2, 3 and 7. These were analyzed in same manner as the arrival day samples.

Patient demographic data and general injury characteristics are summarized in Table 1. We included all patients with moTBI or sTBI and whose outcome was available. Data was collected in Turku university hospital, Turku, Finland (Turku cohort) and in Addenbrook`s Hospital, Cambridge, the UK (Cambridge cohort). Turku cohort includes 33 patients with arrival day samples and Cambridge cohort includes 23 patients with arrival day samples. In later timepoints there are less patients, the number of these are described later and can be found in Table 1.

MoTBI was defined as GCS of 9–12 and sTBI as GCS of 3–8. The lowest recorded GCS before sedation and intubation was used.

Outcome was assessed 6–12 months after the injury using Glasgow outcome scale extended (GOSe).[25] GOSe of 1-4 was classified as unfavorable outcome and GOSe of 5-8 was classified as favorable outcome.

Metabolomic analyses

Metabolomic analyses were done using comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GCxGC-TOFMS). This is described in previous publication.[24] Unknown metabolites were characterized further using a GC coupled to an orbitrap high resolution MS

system and electron and chemical ionization. If metabolite appeared in less than 70% of the samples, it was excluded from subsequent analysis. Using these criteria 465 metabolites were detected. As previously described any metabolites identified as drugs were excluded. Also, downstream metabolites of drugs were excluded by excluding metabolites that highly correlated to the drugs. After these exclusions there were 455 metabolites that was used for analysis. [24]

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 26 for Mac (IBM Corporation, Armonk, New York, USA).

For any missing metabolite data, we imputed the value using the ½ minimum for that metabolite across the whole dataset. This is because a missing value means that the metabolite fell below the limit of detection in the MS.

First, we identified metabolites that differed significantly between the favorable and unfavorable groups in the Turku cohort. Normal distribution was tested using the Shapiro-Wilk test. This was done separately for each metabolite, group, and time point. If it was deemed that the data were normally distributed, then means were compared using a student`s t-test unequal variances. If the data were not normally distributed, the distribution between groups were tested using nonparametric Mann-Whitney U-test. Metabolites that differed significantly between unfavorable and favorable groups in Turku cohort were then tested in Cambridge cohort using same statistical tests.

With arrival day and day 1 samples metabolite levels were also tested between the mortality and survivor groups in the Turku cohort. This was not done in the Cambridge cohort as there were only 2 cases of death in the arrival day group and none in the day 1 or subsequent day group. Same statistical tests were used as with favorable and unfavorable group in same manner.

For each subject, the probability of death and unfavorable outcome was calculated using the online IMPACT and CRASH calculators. IMPACT model that included clinical and CT data was used. Laboratory results needed for the IMPACT model with laboratory values were not used because these values were not available in many subjects. These probabilities were used in subsequent analyses.[8,26–28] Performance of these models was tested in both Turku and Cambridge cohorts by calculating area under curve (AUC) -values on receiver operating characteristics (ROC) curves.

A logistic regression model for unfavorable outcome was then generated using Turku cohort. Starting with the 6 most significant metabolites a base model was generated. After iterations, metabolites were removed from the model if they did not contribute to model performance. All metabolites that did significantly differ between the unfavorable and favorable groups in the Turku cohort were tested,

and if they contributed to the model they remained in the model; if not, they were removed.

A logistic regression model was generated for mortality in same manner as for unfavorable outcome using Turku cohort. For this model metabolites that differed significantly between mortality and survivor groups was used. The performance of the logistic regression models was tested by calculating AUC-values ROC-curves for both the Turku and the Cambridge cohorts separately.

To test whether the generated logistic regression model improved the performance of the CRASH and IMPACT models, the results of these models were added to the model after iterations. The combined models were tested in same manner as other models on Turku cohort and then on Cambridge cohort for validation.

Results

Metabolomic profile differs between unfavorable and favorable outcome groups

In the analyses there were total of 57 patients with available samples and outcome data. From these 33 were in the Turku cohort and 24 in the Cambridge cohort. All but 1 patient from Cambridge cohort had arrival day samples available for analysis, from this 1 patient first sample was available on day 2. From 24 patients that had only arrival day samples available 13 were in the Turku cohort and 11 in the Cambridge cohort. From these 16 were classified as having sTBI, 7 in the Turku cohort and 9 in the Cambridge cohort. There were total of 4 fatalities in patients that had only arrival day samples available, 3 in the Turku cohort and 1 in the Cambridge cohort.

On timepoint day 1 there was total of 24 patients with available samples from day 1 after injury and outcome data. From these 18 were in the Turku cohort and 6 in the Cambridge cohort. All patients also had arrival day samples available. There was total of 9 patients, 2 in the Turku cohort and 7 in the Cambridge cohort, that did not have day 1 samples available but had available samples on later timepoints. All but one of these, in the Cambridge cohort, had however arrival day samples available. There were 7 patients that had arrival day and day 1 samples available but no samples on later timepoints, all were in the Turku cohort. 5 of these were classified as having sTBI. From these 7 patients that had no samples on later timepoints 5 patients died because of TBI.

On timepoint day 2 there was total of 26 patients with available samples from day 2 after injury and outcome data. From these 13 were in the Turku cohort and 13 in the Cambridge cohort. In the Turku cohort 2 patients did not have samples from day 1. In the Cambridge cohort there were 7 patients that did not have samples from day 1, including 1 patient that did not have sample from arrival day. There was 1 patient, in the Turku cohort, that had samples from arrival day, day 1 and 2, but no samples from later timepoints. This patient was classified as having sTBI and died because of TBI.

On timepoint day 3 there was total of 24 patients with available samples from day 3 after injury and outcome data. From these 12 were in the Turku cohort and 12 in the Cambridge cohort. All patients had samples from day 2 also. There were 5 patients that had samples from day 3, but not on day 7, 3 in the Turku cohort and 2 in the Cambridge cohort. From these patients 2 were classified as having sTBI, 1 in the Turku cohort and 1 in the Cambridge cohort. This 1 patient from Turku cohort died because of TBI.

On timepoint day 7 there was total of 20 patients with available samples from day 7 after the injury and outcome data. From these 9 were in the Turku cohort and 11 in the Cambridge cohort. 1 patient from Cambridge cohort did not have sample from day 3. From these 20 patients 12 had samples taken from all timepoints, arrival day, day 1, day 2, day 3 and day 7. 8 were in the Turku cohort and 4 in the Cambridge cohort.

On arrival day there were 19 patients with sTBI and 14 patients with moTBI in the Turku cohort. In the Cambridge cohort 16 patients had sTBI and patients had 7moTBI. Mean worst recorded GCS was 6.79 in the Turku cohort compared to 7.26 in the Cambridge cohort. Mean age was higher in the Turku cohort 56.1 years compared to 45.1 years in the Cambridge cohort. This difference was also statistically significant (two-sided t-test unequal variances p=0.029). In the Turku cohort there was 24 patients with mass lesions present on CT-scans (Marshall CT class 5-6) and 9 patients with diffuse injuries (Marshall CT class 1-3)[29]. In the Cambridge cohort there was 11 patients with mass lesions and 12 with diffuse injuries. The difference in distribution between the Turku and Cambridge cohorts was not statistically significant (Fischer`s exact test p >0.05). Mortality was 30.3% in the Turku cohort and 8.7% in the Cambridge cohort. 17 patients in the Turku cohort had favorable functional outcome defined as GOSe of 5-8 and 16 had unfavorable outcome defined as GOSe of 1-4. In the Cambridge cohort there was 11 patients with favorable functional outcome and 12 with unfavorable. Mean GOSe was 4.03 in the Turku cohort and 4.60 in Cambridge cohort.

On day 1 there were 12 patients with sTBI and 6 patients with moTBI in the Turku cohort. In the Cambridge cohort 2 patients had sTBI and 4 patients had moTBI. Mean worst recorded GCS was 6.22 in the Turku cohort compared to 9.50 in the Cambridge cohort, difference was not statistically significant (two-sided t-test unequal variances p=0.063). Mean age was 50.0 in the Turku cohort and 43.3 in the Cambridge cohort, difference was not statistically significant (two-sided t-test unequal variances p=0.530). In the Turku cohort 13 patients had mass lesions present in CT-scan and 5 had diffuse injury. In the Cambridge cohort 4 patient had mass lesions and 2 patients had a diffuse injury. Difference between the Turku cohort and the Cambridge cohort was not statistically significant (Fischer`s exact test p=1.00). Mortality was 33.3% in the Turku cohort and 0% in the Cambridge cohort. 8 patients had favorable functional outcome in the Turku cohort and 10 had unfavorable outcome. In the Cambridge cohort 3 patients had favorable functional outcome and 3 had unfavorable outcome. Mean GOSe was 3.72 in the Turku cohort and 5.17 in the Cambridge cohort, difference was not statistically significant (two-sided t-test unequal variances p=0.191).

On day 2 there were 7 patients with sTBI and 6 patients with moTBI in the Turku cohort. In the Cambridge cohort 8 patients had sTBI and 5 patients had moTBI. Mean worst recorded GCS was 6.92 in the Turku cohort and 8.31 in the Cambridge cohort, difference was not statistically significant (two-sided t-test unequal variances p=0.318). Mean age was 50.1 in the Turku cohort and 40.5 in the Cambridge cohort, difference was not statistically significant (two-sided t-test unequal variances p=0.224). In the Turku cohort 10 patients had mass lesions present in CT-scan and 3 had diffuse injury. In the Cambridge cohort 6 patients had mass lesions and 7 had diffuse injury. The difference was not statistically significant (Fischer`s exact test p=0.226). Mortality was 15.4% in the Turku cohort and 7.7% in the Cambridge cohort.

On day 3 there were 6 patients with sTBI and 6 patients with moTBI in the Turku cohort. In the Cambridge cohort 7 patients had sTBI and 5 moTBI. Mean worst recorded GCS was 7.25 in the Turku cohort and 8.33 in the Cambridge cohort, difference was not statistically significant (two-sided t-test unequal variances p=0.457). Mean age was 50.6 in the Turku cohort and 41.9 in the Cambridge cohort, difference was not statistically significant (two-sided t-test unequal variances p=0.299). In the Turku cohort 10 patients had mass lesions in CT-scan and 2 had diffuse injury. In the Cambridge cohort 6 patients had mass lesions and 6 had diffuse injury. Difference was not statistically significant (Fischer`s exact test p=0.193). Mortality was 8.3% in both Turku and Cambridge cohorts.

On day 7 there were 5 patients with sTBI and 4 with moTBI in the Turku cohort. In the Cambridge cohort 7 patients had sTBI and 4 moTBI. Mean worst recorded GCS was 7.00 in the Turku cohort and 8.45 in the Cambridge cohort. Difference was not statistically significant (two-sided t-test unequal variances p=0.389). Mean age was 44.2 in the Turku cohort and 43.4 in the Cambridge cohort. In the Turku cohort 7 patients had mass lesions in CT-scan and 2 had diffuse injury. In the Cambridge cohort 6 had mass lesions and 6 had diffuse injury. The difference was not statistically significant (Fischer`s exact test p=0.197). Mortality was 0% in the Turku cohort and 9.1% in the Cambridge cohort.

Clinical characteristics are summarized in Table 1.

In the arrival day samples 31 metabolites differed significantly between favorable and unfavorable groups when tested in the Turku cohort. Most of the metabolites were upregulated in the unfavorable outcome group in the Turku cohort (Table 2), but when tested then in the Cambridge cohort, only glycerol (p=0.025 in the Turku cohort and p=0.0014 in the Cambridge cohort) and decanoic acid (p=0.025 in the Turku cohort and p=0.011 in the Cambridge cohort) were also upregulated in the unfavorable group. Ethanolamine was significantly upregulated in the unfavorable group in the Turku cohort, but significantly downregulated in the Cambridge cohort. No other metabolites that differed significantly between groups in the Turku cohort did so in the Cambridge cohort.

In the day 1 samples, the metabolomic profile differed between the unfavorable and favorable groups when tested in the Turku cohort. The metabolites that differed significantly between groups were mostly upregulated in the unfavorable group. The number of metabolites that differed significantly was lower compared with the arrival day samples: 31 in the arrival day samples and 14 in the day 1 samples. The number of subjects was considerably lower in the day 1 samples. None of the metabolites differed significantly in the Cambridge cohort. This was probably due to the even smaller sample size in the Cambridge cohort on day 1 (n=6). Few metabolites that were significantly upregulated in the arrival day samples did so in day 1 samples. Decanoic acid, adipic acid and 1,4-benzenedicarboxylic acid were significantly elevated in the unfavorable groups at arrival day and day 1 samples.

In the day 2 samples, there were 22 metabolites that differed significantly between the unfavorable and favorable groups in the Turku cohort. None of these differed significantly in the Cambridge cohort. Most of these metabolites were upregulated in the unfavorable outcome group. Of the metabolites that differed significantly on day 1, decanoic acid, octanoic acid and one unknown compound were also significantly elevated in the day 2 samples.

There was a noticeable change in day 3 samples, and at this time, metabolites that were significantly different were mostly upregulated in the favorable group. Of 21 metabolites 4 were upregulated in the unfavorable group and the rest in the favorable group. Octanoic acid remained upregulated in the unfavorable group and an unknown compound that was also upregulated on day 2. There were no significant differences when tested in Cambridge cohort.

In day 7 samples almost all metabolites that had significantly different concentrations were upregulated in the favorable group. Of 20 metabolites 19 were upregulated in favorable group and only 1 in unfavorable group in the Turku cohort. Most compounds were different from those in the day 3 samples. 3-Methyl-2-oxovaleric acid and one unknown compound were significantly upregulated in the favorable group in the day 3 and 7 samples. When tested in Cambridge cohort, an unknown compound was significantly upregulated in the unfavorable group was also upregulated in the unfavorable group of the Turku cohort, and an unknown compound that was upregulated significantly in favorable group on Turku cohort was significantly upregulated in the unfavorable group of the Cambridge cohort.

Findings are summarized in Table 2. Means and standard deviations are reported for metabolites that were found to be normally distributed. Medians and interquartile ranges are given for metabolites that were classified as not normally distributed. For day 7 samples of the Turku cohort with unfavorable outcome group ranges are reported as interquartile ranges could not be calculated due to the low number of subjects. The ranges are also reported in day 1 samples of the unfavorable group of the Cambridge cohort also due to low number of subjects.

Metabolomic profile differs between fatalities and survivors.

Differences in metabolite concentrations between the group of fatalities due to TBI and the group of survivors in the Turku cohort were examined using samples from arrival and day 1. In the Cambridge cohort, there were only 2 deaths in the arrival day samples and none in day 1. For this reason, we were unable to validate the findings in the Cambridge cohort.

The metabolites that differed significantly between groups are shown in Table 3. There were total of 52 metabolites that differed significantly between the mortality group and the survivor group. Of these 52 metabolites 48 were upregulated in the mortality group. Of the metabolites that differed significantly between the unfavorable and favorable groups in both the Turku and

Cambridge cohorts in the arrival day samples, glycerol and decanoic acid were also upregulated in the group of fatalities due to TBI compared with survivors in the arrival day samples.

On day 1, the metabolomic profile of the samples remained quite similar to that of the arrival day. Most of the metabolites that differed significantly between groups were upregulated in the mortality group; a total of 46 metabolites differed significantly between groups and of these 42 were upregulated in the mortality group. The metabolites that were upregulated in the mortality group on the arrival day remained significantly upregulated on day 1: a few unknown compounds, 3,4-dihydroxybutanoic acid, 4-hydroxyphenyllactic acid, ethanolamine, arabinofuranose, malic acid, adipic acid, pentitol 3-desoxy, 3-aminoisobutyric acid, 2-butenedioic acid and 1,4-benzenedicarboxylic acid.

On subsequent days, there was not enough cases of death for analyses.

Metabolite model predicts outcome with good accuracy in single center but validates poorly

The logistic regression model generated using the Turku cohort was tested with the Cambridge cohort. The CRASH and IMPACT models were also tested, and the results of these models were added to the logistic regression model. Only arrival day samples were used for these analyses.

The model created using the Turku cohort used four metabolites: pentitol-3-desoxy, nonanoic acid and two unknown compounds. In the Turku cohort, the predictive power was very good (AUC = 0.956; 95% CI 0.888-1.024). When tested in the Turku cohort, the CRASH model had poor prognostic accuracy (AUC = 0.695; 95% CI 0.515-0.875). The IMPACT model performed similarly to the CRASH model with poor prognostic accuracy (AUC = 0.676; 95% CI 0.492-0.861). Figures 1,2 and 3.

We then combined the metabolite model with the CRASH and IMPACT models. The predictive power in the Turku cohort was quite similar to that of the metabolite-only model. The combined CRASH and metabolite model showed very good accuracy (AUC = 0.956; 95% CI 0.889-1.022). The IMPACT and metabolite model also showed very good accuracy (AUC = 0.963; 95% CI 0.905-1.022). Figures 4 and 5.

These metabolite models generated in the Turku cohort were then tested in the Cambridge cohort as independent validation. The metabolite model had poor prognostic accuracy (AUC = 0.644; 95% CI 0.410-0.878). The combined metabolite and clinical model had similar accuracy. The combined CRASH and the metabolite model had poor prognostic accuracy (AUC = 0.644; 95% CI 0.408-0.812). IMPACT and metabolite model also had poor prognostic accuracy (AUC = 0.644; 95% CI 0.408-0.879). Figures 8, 9 and 10.

The CRASH and IMPACT models without metabolites also performed poorly when tested in the Cambridge cohort. The CRASH model had poor prognostic accuracy (AUC = 0.568; 95% CI 0.324-0.812). The IMPACT model also had poor prognostic accuracy (AUC = 0.670; 95% CI 0.434-0.907). Figures 6 and 7.

Metabolite model predicts mortality with very good accuracy in a single center setup, clinical models predict mortality with good accuracy in Turku cohort

The logistic regression model was built using the metabolites that were significantly different between the mortality group and the survivor group. This was done in the same way as for the unfavorable outcome described above using the Turku cohort.

The model used 5 different metabolites: pentitol 3-desoxy, 2,3,4-trihydroxybutyric acid, two unknown compounds and decanoic acid. The unknown compounds were different from those used in model for unfavorable outcome. The generated model was then tested on the Turku cohort. Data from the CRASH and IMPACT models were added to the generated metabolite model, which was then also tested also on the Turku cohort. The performance of both the CRASH and IMPACT models were also tested separately.

The metabolite model predicted mortality due to TBI with very good accuracy when tested in the Turku cohort (AUC = 0.930; 95% CI 0.799-1.062). In combination with the existing clinical models, performance was roughly same or even slightly better. The metabolite model combined with CRASH model showed with very good accuracy (AUC = 0.996; 95% CI 0.982-1.010). The combination of metabolite and the IMPACT model also performed with very good accuracy (AUC = 0.983; 95% CI 0.944-1.021). Figures 3, 4 and 5.

The CRASH and IMPACT models without metabolite model showed good accuracy. CRASH model (AUC = 0.796; 95% CI 0.624-0.967) and IMPACT model (AUC = 0.791; 95% CI 0.622-0.960). Figures 1 and 2.

The mortality models were then tested on the Cambridge cohort for validation, although the number of deaths in the Cambridge cohort on the arrival day was low (n=2). The models performed with varying accuracy. CRASH clinical model performed with very good accuracy (AUC = 0.976; 95% CI 0.911-1.041). IMPACT clinical model performed with adequate accuracy (AUC = 0.786; 95% CI 0.514-1.058). The metabolite model for mortality performed with poor accuracy (AUC = 0.571; 95% CI -0.030-1.172). Combined models showed poor accuracy; CRASH and metabolite model (AUC 0.690; 95% CI 0.300-1.081) and IMPACT and metabolite model (AUC = 0.548; 95% CI -0.024-1.119). Confidence intervals were wide because of the low number of deaths. Figures 6, 7, 8, 9 and 10

Discussion

In this study, we show that the metabolite profile significantly differs between unfavorable and favorable outcomes, and between fatalities and survivors in moTBI and sTBI. We also show that this metabolic profile appears to change in the first week after the TBI. This study is the first to report this change in metabolic profile during the first week of initial injury. Only arrival day samples have previously been published. Samples from later days are from same patient cohort as published previously but have not been published before. The metabolic profile also shows promise as outcome predictor in moderate and severe TBI.

The changing metabolic profile between unfavorable and favorable outcome during the first week after injury has not been reported before. Previously have been published studies, which suggests that some metabolites stay elevated during the first week after injury in TBI patients when compared to controls without TBI.[24,30] There was obvious shift between the first 2 days after the injury, when most metabolites that differed significantly between groups were upregulated in unfavorable outcome group and the later 3 and 7 days after injury when most of the significant differences were on metabolites that were upregulated in favorable outcome group. This finding is interesting, and it may suggest that there are changes happening in brain metabolism in the first week after the injury that have effect on patient outcome. There was a significant difference between these earlier and later time points in that there were not many fatalities in day 3 and day 7 cohorts. This might have effect on observed metabolite differences. In a previous study, there was not significant difference in metabolomic concentrations between unfavorable and favorable outcome groups. In that study, samples were collected on median of 4.5 days after the injury. It is possible that the reason, why there was not difference between unfavorable and favorable outcome groups, is because of this change in metabolomic profile during the first week after injury that was observed in our study.[31]

It is well known that branched-chain amino acids are important for brain metabolism.[32]. We did not find statistically significant differences in the concentrations of amino acids in the first 3 days post injury, but at day 7 most of the significantly elevated metabolites in the favorable group, which could be identified, were amino acids: serine, tryptophan, phenylalanine, leucine, isoleucine and hydroxyproline. The nutritional status of the subjects was not controlled, and this change may be due to the fact that more patients in the favorable group were able to consume food or received enteral nutrition. In a previous study levels of plasma amino acids were significantly elevated in patients receiving enteral nutrition, except phenylalanine. In the same study increased plasma levels of phenylalanine was associated with decreased ICP and increased $SjvO_2$. Whereas increased plasma levels of isoleucine and leucine were associated with an increase in ICP.[22] ICP values are shown to have effect on outcome after TBI and higher ICP levels have been associated with worse outcome[33]. It is interesting to note that we found phenylalanine, isoleucine and leucine levels elevated in the favorable outcome group. For phenylalanine, this is expected as it was associated with decreased ICP, but it

is not expected that isoleucine and leucine that were associated with increased ICP to be elevated in the favorable outcome group. The observed effect of enteral nutrition on isoleucine and leucine levels but not on phenylalanine levels, and the lack of controlling for nutrition status in our cohort might partly explain these findings. There is also a previous study in which isoleucine and leucine levels were not associated with changes in ICP, although the study only included samples taken 24 hours post injury[34].

When metabolites were tested between fatalities due to TBI and survivors, there were more metabolites that differed significantly between groups, than between unfavorable and favorable groups. This shows that the change in metabolic profile is even greater between fatalities and survivors than that between unfavorable and favorable outcome. It suggests that the disruption in brain metabolism increases with more severe injury. It would have been interesting to see how these changes would evolve during the first week after the injury and if there would have been same kind of change in metabolic profile as seen in between the unfavorable and favorable outcome groups.

The generated metabolite models for unfavorable outcome performed with very good accuracy in Turku cohort, in which these were generated, but disappointingly the performance was poor in the Cambridge cohort used for validation. This was mostly likely due to small number of subjects in the Turku cohort at arrival day (n=16 in unfavorable group and n=17 in favorable group). Small sample size used in logistic regression probably resulted in overfitting the model to Turku cohort, which caused the model to perform with very good accuracy in Turku cohort but to perform poorly in the Cambridge cohort. Also, only decanoic acid and glycerol did differ significantly in both the Turku and the Cambridge cohort from arrival day samples and neither of these were included in generated logistic regression model. This might be because they don`t contribute to model accuracy in multivariate setting as much as they do in univariate setting.

The model generated to predict TBI deaths showed very good accuracy in the Turku cohort. However, the model was not statistically significantly different from random prediction in the Cambridge cohort, but this was most likely due to the low mortality in the Cambridge cohort, as all mortality models tested had very wide confidence intervals. Larger studies would be needed to investigate whether metabolite profiling could be key factor in predicting mortality after TBI.

Existing clinical models CRASH, and IMPACT had poor prognostic accuracy in both our cohorts. Both models have performed considerably better in external validations studies previously, especially in larger cohorts[35,36]. This might be due to small number of subjects in our study. However, it highlights the importance that even these widely tested and validated prognostic models should not be used alone to make decisions on patient care. There is always a certain amount of uncertainty, and the models provide only statistical probability.[37]

Of all metabolites tested, only pentitol 3-desoxy was present in both the unfavorable outcome and mortality models. It was significantly elevated in the

unfavorable outcome group compared to the favorable outcome group (p=0.001) and with even greater degree in the mortality compared to the survivor group (p <0.0001). In the Cambridge cohort, the difference between unfavorable and favorable group was not statistically significant (p=0.091), but the trend was similar to that in the Turku cohort (median 3.43-fold greater in unfavorable group). Pentitol 3-desoxy is sugar derivate but searches from databases yielded no results. It is not mentioned in the Human metabolome database or in the Blood exposome database.[38,39] Therefore, the origin of it is not known and not many conclusions can be made about its contribution in pathophysiology of TBI. It still appears to be an important metabolite to be tracked in future studies and may play a key role in TBI. It is also interesting that it was not elevated in either group in later timepoints, so it seems to be only having a role in acute phase of TBI. TBI is already known to alter brains glucose metabolism and that can explain finding of abnormal sugar derivates in TBI.[40] Upregulation of sugar derivatives in patients with worse outcome might reflect that glucose metabolism is altered to even greater degree in patients with worse outcome.

This study has several limitations. The most obvious drawback is that we were unable to validate most of the findings in our Cambridge validation cohort. Also, the generated regression models performed with much worse accuracy in the Cambridge cohort. The reason for this is not clear. There seems not to be any obvious differences between the cohorts in clinical characteristics, but at one time point, day 1, the Cambridge cohort was quite small with only 6 patients, which may reduce the statistically significant differences. At other time points, the cohorts had similar number of patients. In the Cambridge cohort, there were fewer cases of death and only 2 in the arrival day samples. Because of that we were unable to test for differences between fatalities and survivors in metabolite concentrations. Mortality model testing was done in the Cambridge cohort but had wide confidence intervals. We chose to use our cohorts separately as discovery and validation cohorts to increase the external validity of our study. Unfortunately, most of the findings could not be validated, which significantly limits the external validity of our study.

Another limitation is that large number of metabolites could not be identified. However, with spectra and chromatographic information, including retention indices, these metabolites could be followed up in future studies and when better analytical tools are developed, these might be identified.

Conclusions

Overall, we show here that the metabolic profile differs significantly between the unfavorable and favorable outcome groups as well as between fatality and survivor groups. This metabolomic profile seems to change during the first week after injury between the unfavorable and favorable outcome groups. Metabolic profile also shows promise in prognostication between unfavorable and favorable outcome as well as between mortality and survival. These findings should be considered hypothesis generating as we were unable to validate most of the findings in our validation cohort probably due to lack of subjects and therefore statistical power. Despite promising initial results, metabolomics is still

a largely undiscovered field in TBI outcome prediction. Larger trials should be done in future to better characterize this phenomenon.

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Tables and figures

	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
Turku Arrival Day	Moderate	14	59.6(17.3)	8/6	0	3	1	6	3	8	0	0	3	0	0	0	6	5	11.00(0.96)	4.79(2.01)	2(13.3%)
	Severe	19	53.5(15.9)	17/2	1	5	0	8	6	11	1	1	2	2	2	0	7	6	3.68(1.34)	3.47(2.41)	8(42.1%)
	Total	33	56.1(16.5)	25/8	1	8	1	14	9	19	1	1	5	2	2	0	13	11	6.79(3.86)	4.03(2.31)	10(30.3%)
	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
Cambridge Arrival day	Moderate	7	41.6(20.5)	7/0	0	2	0	1	3	0	1	5	1	1	0	0	4	1	11.29(0.95)	5.67(2.25)	0(0%)
	Severe	16	46.7(18.4)	11/5	4	7	0	0	4	1	0	0	1	9	0	0	4	2	5.50(2.91)	4.14(2.14)	2(12.5%)
	Total	23	45.1(18.7)	18/5	4	9	0	1	7	1	1	5	2	10	0	0	8	3	7.26(3.31)	4.60(2.23)	2(8.7%)

	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
Turku day 1	Moderate	6	54.3(18.4)	4/2	0	1	1	1	3	2	0	0	2	0	0	0	1	2	10.83(0.75)	5.17(1.17)	0(0%)
	Severe	12	47.8(16.2)	10/2	0	4	0	3	6	6	1	1	1	0	2	0	8	2	3.92(1.56)	3.00(2.37)	6(50%)
	Total	18	50.0(16.7)	14/4	0	5	1	4	9	8	1	1	3	0	2	0	9	4	6.22(3.61)	3.72(2.27)	6(33.3%)
	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
Cambridge day 1	Moderate	4	44.5(23.7)	4/0	0	0	0	1	2	2	1	0	1	0	0	0	2	1	11.50(1.00)	6.25(1.71)	0(0%)
	Severe	2	41(29.7)	2/0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	5.50(0.71)	3.00(0)	0(0%)
	Total	6	43.3(22.7)	6/0	1	0	0	1	3	2	1	0	1	1	0	0	3	1	9.50(3.21)	5.17(2.14)	0(0%)

	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
Turku day 2	Moderate	6	60.2(17.2)	3/3	0	1	0	3	2	3	0	0	0	0	0	0	2	4	11.17(0.75)	4.50(2.26)	1(16.7%)
	Severe	7	41.4(17.1)	6/1	0	4	0	1	4	6	0	0	1	0	2	0	4	0	3.29(0.49)	4.43(2.15)	1(14.3%)
	Total	13	50.1(19.1)	9/4	0	5	0	4	6	9	0	0	1	0	2	0	6	4	6.92(4.13)	4.46(2.11)	2(15.4%)
	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
Cambridge day 2	Moderate	5	39.8(23.1)	5/0	0	1	0	1	2	2	1	0	1	1	0	0	2	1	11.20(1.10)	5.60(2.07)	0(0%)
	Severe	8	41.0(19.3)	6/2	3	3	0	0	1	1	0	1	0	5	0	0	2	1	6.50(1.07)	4.13(2.30)	1(12.5%)
	Total	13	40.5(19.9)	11/2	3	4	0	1	3	3	1	1	1	6	0	0	4	2	8.31(2.59)	4.69(2.25)	1(7.7%)

	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
Turku day 3	Moderate	6	60.2(17.2)	3/3	0	1	0	3	2	3	0	0	0	0	0	0	2	4	11.17(0.75)	4.50(2.26)	1(16.7%)
	Severe	6	41.0(18.7)	5/1	0	4	0	1	3	5	0	0	0	0	2	0	4	0	3.33(0.52)	5.00(1.67)	0(0%)
	Total	12	50.6(19.8)	8/4	0	5	0	4	5	8	0	0	0	0	2	0	6	4	7.25(4.14)	4.75(1.91)	1(8.3%)
	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
Cambridge day 3	Moderate	5	39.8(23.1)	5/0	0	1	0	1	2	2	1	0	1	1	0	0	2	1	11.20(1.10)	5.60(2.07)	0(0%)
	Severe	7	43.4(19.5)	5/2	3	2	0	0	1	1	0	1	0	4	0	0	2	1	6.29(0.95)	3.86(2.34)	1(14.3%)
	Total	12	41.9(20.1)	10/2	3	3	0	1	3	3	1	1	1	5	0	0	4	2	8.33(2.71)	4.58(2.31)	1(8.3%)

	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
	Moderate	4	53.8(18.0)	2/2	0	0	0	2	2	2	0	0	0	0	0	0	1	3	11.50(0.58)	5.50(1.73)	0(0%)
Turku day 7	Severe	5	36.6(17.1)	4/1	0	4	0	0	3	4	0	0	0	0	2	0	3	0	3.40(0.55)	4.80(1.79)	0(0%)
	Total	9	44.2(18.7)	6/3	0	4	0	2	5	6	0	0	0	0	2	0	4	3	7.00(4.30)	5.11(1.69)	0(0%)
	TBI Severity	Number of subjects	Age(SD)	Sex M/F	Injury mechanism								Marshall CT Class						Worst GCS (SD)	GOSE (SD)	Mortality (%)
					BTH	A/C	V	GLF	FFH	HAO	O	N/A	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6			
	Moderate	4	42.3(25.9)	4/0	0	1	0	0	2	1	1	0	1	1	0	0	2	0	11.50(1.00)	6.00(2.16)	0(0%)
Cambridge day 7	Severe	7	44.0(18.8)	5/2	2	3	0	0	1	1	0	1	0	4	0	0	2	1	6.71(0.95)	4.29(2.43)	1(14.3%)
	Total	11	43.4(20.3)	9/2	2	4	0	0	3	2	1	1	1	5	0	0	4	1	8.45(2.58)	4.91(2.39)	1(9.1%)

Supplemental Table 1. Clinical characteristics of patients included in study. Injury mechanism abbreviation: BTH = blow to the head, A/C = acceleration or deceleration, V = violence, GLF = ground level fall, FFH = fall from height, HAO = head against an object, O = other, N/A = not applicable or unknown.

			Turku Arrival day					**Cambridge Arrival day**		
ID	Metabolite	RI	Favorable Turku	Unfavorable Turku	Fold Turku	p (Turku)	Favorable Cambridge	Unfavorable Cambridge	Fold Cambridge	p (Cambridge)
			n=17	n=16			n=11	n=12		
106	**Ethanolamine**	**1278.73**	**62.27(26.31IQR)**	**117.27(72.23IQR)**	**1.88**	**0.0002**	**58.47(24.42IQR)**	**39.58(33.02IQR)**	**0.68**	**0.032**
53	Myo-inositol	2132.72	2360.62(1150.53IQR)	3485.87(2008.54IQR)	1.48	0.031	2388.14(2719.34IQR)	3249.17(2790.18IQR)	1.36	0.880
65	Arabinofuranose	1648.13	12.84(16.79IQR)	21.38(16.52IQR)	1.67	0.031	12.20(5.10SD)	12.74(5.19SD)	1.04	0.802
68	**Glycerol**	**1297.70**	**4939.85(2195.64IQR)**	**6681.64(4878.49IQR)**	**1.35**	**0.025**	**4946.52(1055.85SD)**	**6790.08(2039.63SD)**	**1.37**	**0.014**
93	Nonanoic acid	1369.61	73.49(95.52IQR)	174.52(132.42IQR)	2.37	0.015	57.99(196.79IQR)	153.62(231.22IQR)	2.65	0.316
99	**Decanoic acid**	**1464.27**	**42.31(582.85IQR)**	**614.51(944.82IQR)**	**14.52**	**0.025**	**170.51(242.42IQR)**	**440.67(171.04IQR)**	**2.58**	**0.011**
187	Adipic acid	1519.64	6.58(7.10IQR)	11.50(25.70IQR)	1.75	0.019	8.29(12.52IQR)	13.46(7.08IQR)	1.62	0.059
253	Pentitol 3-desoxy	1659.63	3.62(1.74IQR)	6.07(9.42IQR)	1.68	0.001	0.70(0.00IQR)	2.40(4.37IQR)	3.43	0.091
1321	1,4-Benzenedicarboxylic acid	1807.41	0.47(0.38IQR)	2.54(4.50IQR)	5.40	0.034	0.47(0.00IQR)	0.47(0.00IQR)	1.00	0.740
140	Unknown compound	1207.57	39.32(15.30SD)	59.25(28.56SD)	1.51	0.021	59.02(16.44SD)	51.15(24.06SD)	0.87	0.368
142	Unknown compound	1189.57	753.86(159.06SD)	971.45(205.70SD)	1.29	0.002	1249.87(1110.16IQR)	369.59(1184.14IQR)	0.30	0.379
96	Unknown compound	2499.67	46.30(34.81IQR)	13.85(31.57IQR)	0.30	0.012	12.57(16.89IQR)	22.10(40.41IQR)	1.76	0.379
113	Unknown sugar derivate	2075.09	69.46(68.50IQR)	131.55(143.24IQR)	1.89	0.028	84.80(256.14IQR)	105.15(345.88IQR)	1.24	0.651
168	Unknown compound	1269.37	1062.97(1684.70IQR)	2726.75(1332.48IQR)	2.57	0.006	5395.84(17002.47IQR)	10105.70(13530.53IQR)	1.87	0.880
287	Unknown compound	2494.44	18.72(21.83IQR)	6.84(17.20IQR)	0.37	0.034	1.82(6.27IQR)	3.41(25.29IQR)	1.87	0.379
293	Unknown carboxylic acid	2518.04	4.94(2.21IQR)	6.72(4.60IQR)	1.36	0.009	1.15(0.00IQR)	3.14(5.04IQR)	2.73	0.235

307	Unknown compound	1829.43	18.12(26.32IQR)	45.44(44.38IQR)	2.51	0.014	3.52(15.21IQR)	3.52(36.97IQR)	1.00	0.928
347	Unknown compound	1816.50	3.42(13.60IQR)	16.94(19.79IQR)	4.95	0.025	3.42(20.02IQR)	17.55(19.91IQR)	5.13	0.608
389	Unknown compound	2264.40	103.47(68.07IQR)	19.27(77.68IQR)	0.19	0.001	1.81(0.00IQR)	1.81(46.70IQR)	1.00	0.347
551	Unknown carboxylic acid	1190.36	3.11(2.58IQR)	15.03(26.42IQR)	4.83	0.015	3.11(0.00IQR)	3.11(0.00IQR)	1.00	0.525
1151	Sorbopyranose	1862.37	563.44(433.91IQR)	315.37(695.69IQR)	0.56	0.028	7.97(560.20IQR)	406.72(810.31IQR)	51.03	0.288
1163	Unknown sugar derivate	1214.91	63.98(104.75IQR)	5.26(32.33IQR)	0.08	0.014	155.49(187.39IQR)	27.38(111.55IQR)	0.18	0.413
1167	Unknown compound	2512.32	8.58(7.51IQR)	0.78(5.31IQR)	0.09	0.012	0.78(2.34IQR)	2.41(7.18IQR)	3.09	0.316
1179	Unknown compound	2563.09	7.34(24.51IQR)	50.42(94.17IQR)	6.87	0.049	7.34(17.58IQR)	12.02(32.27IQR)	1.64	1.000
1270	Unknown compound	1718.26	13.10(14.04IQR)	27.61(21.17IQR)	2.11	0.019	24.94(14.67SD)	29.38(14.16SD)	1.18	0.454
1351	Unknown sugar derivate	2628.83	1.55(4.30IQR)	5.55(7.74IQR)	3.58	0.011	1.55(0.00IQR)	1.55(3.19IQR)	1.00	0.487
146	I-Threonine	1311.13	639.17(179.40SD)	859.59(347.15SD)	1.34	0.033	657.80(330.93SD)	621.61(420.48SD)	0.94	0.820
1156	4-Methyl-2-oxovaleric acid	1235.16	434.19(149.79SD)	310.52(159.90SD)	0.72	0.029	6.56(0.00IQR)	13.46(181.40IQR)	2.05	0.235
1272	Unknown compound	1774.57	10.38(7.37SD)	18.26(13.21SD)	1.76	0.047	2.03(14.73IQR)	6.03(44.03IQR)	2.97	0.347
52	A203003 (sugar)	2084.20	1310.01(1077.27IQR)	2140.88(1526.18IQR)	1.63	0.049	1495.70(401.34SD)	1630.12(501.31SD)	1.09	0.651
108	Unknown compound	2051.79	99.00(70.61IQR)	184.33(366.68IQR)	1.86	0.014	198.16(59.45IQR)	266.46(312.93IQR)	1.34	0.211
			Turku Day 1					**Cambridge Day 1**		
ID	Metabolite	RI	Favorable Turku	Unfavorable Turku	Fold Turku	p (Turku)	Favorable Cambridge	Unfavorable Cambridge	Fold Cambridge	p (Cambridge)
			n=8	n=10			n=3	n=3		

99	Decanoic acid	1464.27	40.80(438.27IQR)	736.88(778.51IQR)	18.06	0.027	357.52(14.85SD)	237.94(219.44SD)	0.67	0.445
165	Octanoic acid	1273.01	106.47(951.02IQR)	1362.73(1677.07IQR)	12.80	0.034	745.53(46.20SD)	527.46(415.42SD)	0.71	0.460
187	Adipic acid	1519.64	5.85(2.80IQR)	17.11(28.35IQR)	2.92	0.012	13.88(10.64SD)	8.94(4.64SD)	0.64	0.520
1321	1,4-Benzenedicarboxylic acid	1807.41	0.47(1.02IQR)	5.69(5.50IQR)	12.11	0.012	0.47(0.00Range)	0.47(0.00Range)	1.00	1.000
185	Unknown compound	2768.03	132.85(63.88SD)	262.91(140.02SD)	1.98	0.021	49.37(44.14SD)	67.57(10.51SD)	1.37	0.553
220	Unknown compound	1197.82	404.44(74.58SD)	518.73(129.10SD)	1.28	0.041	243.36(233.39Range)	316.27(108.25Range)	1.30	0.400
461	Unknown compound	1369.14	10.55(17.92IQR)	1.25(2.24IQR)	0.12	0.034	1.25(2.46Range)	1.25(3.05Range)	1.00	1.000
497	Unknown compound	2851.95	1.74(3.71IQR)	35.60(56.89IQR)	20.46	0.009	1.74(0.00Range)	1.74(9.73Range)	1.00	0.700
1145	Hydroxy acid	1201.03	292.13(157.92SD)	113.96(106.81SD)	0.39	0.011	4.94(79.92Range)	171.04(406.46Range)	34.62	0.400
1180	Unknown carboxylic acid	1405.37	16.09(23.14IQR)	3.07(1.49IQR)	0.19	0.021	3.07(0.00Range)	3.07(11.41Range)	1.00	0.700
3258	Phenolic metabolite	1652.74	6.62(36.05IQR)	69.37(107.80IQR)	10.48	0.003	86.70(49.76SD)	113.28(67.94SD)	1.31	0.616
69	Lauric Acid	1658.95	49.03(25.09SD)	89.21(44.10SD)	1.82	0.028	55.11(16.58SD)	60.96(51.45SD)	1.11	0.866
74	Glycerol-2-phosphate	1768.61	25.72(5.47SD)	38.81(16.21SD)	1.51	0.035	34.43(18.34SD)	31.27(5.79SD)	0.91	0.799
104	Glutamic acid	1547.38	155.29(48.57SD)	243.26(102.70SD)	1.57	0.032	210.29(22.93SD)	112.48(68.06SD)	0.53	0.119
			Turku Day 2					**Cambridge Day 2**		
ID	Metabolite	RI	Favorable Turku	Unfavorable Turku	Fold Turku	p (Turku)	Favorable Cambridge	Unfavorable Cambridge	Fold Cambridge	p (Cambridge)
			n=7	n=6			n=6	n=7		
17	Lactic acid	1093.01	927.41(210.47SD)	1314.42(306.55SD)	1.42	0.021	1087.63(756.21SD)	976.00(572.02SD)	0.90	0.774
68	Glycerol	1297.70	4397.50(1506.28D)	6684.96(1652.31SD)	1.52	0.024	3324.21(6419.42IQR)	3868.04(4583.45IQR)	1.16	0.628

150	Hexadecanoic acid, 2,3bishy	2580.61	84.90(33.11SD)	122.69(25.59SD)	1.45	0.044	24.78(53.74IQR)	22.71(65.24IQR)	0.92	0.836
335	Methyl succinic acid	1361.29	1.72(5.12IQR)	14.62(4.49IQR)	8.5	0.005	13.87(26.59IQR)	1.72(6.62IQR)	0.12	0.181
65	Arabinofuranose	1648.13	15.63(8.17IQR)	25.80(9.83IQR)	1.65	0.035	22.23(17.46IQR)	16.60(14.82IQR)	0.75	0.534
99	Decanoic acid	1464.27	21.84(459.04IQR)	778.63(663.48IQR)	35.65	0.014	265.58(138.87SD)	226.17(184.27SD)	0.85	0.669
165	Octanoic acid	1273.01	57.75(1010.84IQR)	1911.03(725.52IQR)	33.09	0.005	569.84(262.46SD)	412.46(332.68SD)	0.72	0.361
280	Cholesterol	2823.07	152.96(206.66IQR)	376.55(203.02IQR)	2.46	0.035	26.43(76.48IQR)	26.43(413.50IQR)	1.00	0.366
357	Pyruvic acid	1086.71	550.39(262.88SD)	172.05(163.70SD)	0.31	0.011	3.24(0.00IQR)	3.24(0.00IQR)	1.00	0.731
1184	Phosphoric acid	1302.02	3395.43(4061.23IQR)	0.16(2915.12IQR)	<0.01	0.022	0.16(1122.63IQR)	0.16(8082.41IQR)	1.00	0.445
59	Unknown compound	1345.61	604.19(235.25SD)	1069.69(317.25SD)	1.77	0.011	1277.54(315.11SD)	1243.76(320.07SD)	0.97	0.852
62	Unknown compound	1513.73	52.32(20.66SD)	95.47(35.16SD)	1.82	0.019	32.77(9.85SD)	35.71(12.59SD)	1.09	0.647
75	Unknown compound	2266.48	226.54(85.12SD)	113.49(52.69SD)	0.50	0.017	139.71(71.15SD)	169.47(53.25SD)	1.21	0.421
199	Unknown compound	1397.22	5.32(0.75SD)	8.54(2.09SD)	1.61	0.011	1.35(5.06IQR)	5.34(7.16IQR)	3.96	0.534
235	Unknown compound	1742.49	70.09(85.23IQR)	7.88(26.90IQR)	0.11	0.007	10.52(160.53IQR)	24.46(55.91IQR)	2.33	0.945
196	Unknown compound	2548.50	12.45(21.25IQR)	34.77(13.02IQR)	2.79	0.022	2.25(26.48IQR)	9.27(29.69IQR)	4.12	0.366
297	Unknown compound	2236.43	3.95(6.20IQR)	12.32(9.85IQR)	3.12	0.022	0.59(3.07IQR)	0.59(4.25IQR)	1.00	0.731
347	Unknown compound	1816.50	3.42(11.95IQR)	17.46(13.84IQR)	5.11	0.035	17.95(18.32SD)	18.12(12.36SD)	1.01	0.985
380	Unknown compound	2591.22	18.84(36.33IQR)	1.84(2.42IQR)	0.10	0.001	0.83(0.00IQR)	0.83(0.00IQR)	1.00	0.731
423	Unknown compound	1684.10	5.15(6.46IQR)	0.76(2.33IQR)	0.15	0.022	0.76(0.00IQR)	0.76(0.00IQR)	1.00	0.731
2650	Unknown compound	2606.08	2.09(2.63IQR)	7.64(6.50IQR)	3.66	0.014	2.09(0.00IQR)	2.09(0.00IQR)	1.00	1.00

3258	Phenolic metabolite	1652.74	0.94(28.39IQR)	116.53(156.68IQR)	123.97	0.005	43.34(79.24IQR)	31.13(11.91IQR)	0.72	0.295
3279	Unknown amino acid	2556.36	2.19(18.88IQR)	36.30(20.57IQR)	16.58	0.002	7.21(8.38IQR)	2.19(5.20IQR)	0.30	0.181
			Turku Day 3					**Cambridge Day 3**		
ID	Metabolite	RI	Favorable Turku	Unfavorable Turku	Fold Turku	p (Turku)	Favorable Cambridge	Unfavorable Cambridge	Fold Cambridge	p (Cambridge)
			n=7	n=5			n=5	n=7		
4	Stearic acid	2256.21	442.34(46.47SD)	369.57(47.523SD)	0.84	0.024	401.87(118.93IQR)	336.54(263.10IQR)	0.84	0.755
8	Oleic acid	2229.29	1169.58(378.62SD)	702.99(297.34SD)	0.60	0.045	550.10(773.15IQR)	666.39(1191.55IQR)	1.21	0.876
6	Linoleic acid	2223.96	557.69(142.67SD)	371.26(65.95SD)	0.67	0.022	404.12(372.69IQR)	397.87(560.35IQR)	0.98	1.000
69	Lauric acid	1658.95	43.68(18.19SD)	21.38(5.22SD)	0.49	0.017	42.33(20.15IQR)	47.00(39.35IQR)	1.11	0.530
103	Diethylene glycol	1260.24	72.80(17.97SD)	49.54(12.90SD)	0.68	0.034	54.72(13.58IQR)	59.38(16.69IQR)	1.09	0.639
165	Octanoic acid	1273.01	71.62(436.80IQR)	1018.50(1234.86IQR)	14.22	0.048	673.42(216.37SD)	414.12(407.25SD)	0.61	0.186
111	3-Methyl-2-oxovaleric acid	1222.75	94.92(47.80SD)	32.62(23.89SD)	0.34	0.015	172.91(156.50SD)	140.14(129.11SD)	0.81	0.711
138	Linolenic acid	2229.41	185.87(180.64IQR)	69.78(90.67IQR)	0.38	0.048	69.95(109.19IQR)	54.74(108.24IQR)	0.78	0.343
371	3-Aminoisobutyric acid	1470.78	4.92(10.77IQR)	0.86(1.80IQR)	0.17	0.048	0.86(1.90IQR)	0.86(10.62IQR)	1.00	0.755
97	Unknown compound	2117.94	24.11(7.92SD)	15.50(3.27SD)	0.64	0.031	12.56(6.19SD)	19.64(13.35SD)	1.56	0.250
136	Unknown compound	2274.69	132.41(27.06SD)	55.92(35.24SD)	0.42	0.002	121.99(61.78SD)	108.33(54.00SD)	0.89	0.701
148	Unknown compound	2126.16	32.71(17.51SD)	12.46(4.78SD)	0.38	0.022	4.78(20.79IQR)	10.83(46.02IQR)	2.27	0.639
237	Unknown compound	1612.09	16.58(10.59SD)	3.68(2.05SD)	0.22	0.018	0.92(1.58IQR)	0.92(0.00IQR)	1.00	0.639
3258	Phenolic metabolite	1652.74	3.28(48.69IQR)	80.04(108.29IQR)	24.40	0.048	98.38(101.39SD)	57.24(47.92SD)	0.58	0.436
139	Unknown compound	2318.42	115.09(29.96SD)	66.98(36.40SD)	0.58	0.031	76.37(123.40IQR)	79.32(114.97IQR)	1.04	0.755

164	Unknown carboxylic acid	1590.49	10.89(24.87IQR)	1.11(2.64IQR)	0.10	0.018	39.27(36.38SD)	23.31(20.97SD)	0.59	0.412
179	Unknown amino acid	1380.72	40.53(41.84IQR)	49.85(14.43IQR)	1.23	0.030	42.75(41.84IQR)	34.00(38.96IQR)	0.80	0.149
423	Unknown compound	1684.10	9.67(11.35IQR)	0.76(5.18IQR)	0.08	0.048	0.76(0.00IQR)	0.76(0.00IQR	1.00	1.000
1162	Unknown amino acid	1283.01	112.50(144.55IQR)	14.50(42.28IQR)	0.13	0.048	39.72(1621.13IQR)	710.05(3932.91IQR)	17.88	0.268
1186	Unknown compound	1641.52	0.31(1.70IQR)	2.27(3.08IQR)	7.32	0.048	0.31(0.50IQR)	0.31(12.28IQR)	1.00	0.432
1269	Unknown compound	2450.87	8.25(2.83IQR)	2.51(4.10IQR)	0.30	0.018	9.17(1.64SD)	9.47(4.15SD)	1.03	0.868
1145	Hydroxy acid	1201.03	256.42(156.35SD)	70.03(35.00SD)	0.27	0.019	60.26(175.16IQR)	77.05(227.88IQR)	1.28	0.876
			Turku Day 7					**Cambridge day 7**		
ID	Metabolite	RI	Favorable Turku	Unfavorable Turku	Fold Turku	p (Turku)	Favorable Cambridge	Unfavorable Cambridge	Fold Cambridge	p (Cambridge)
			n=6	n=3			n=6	n=5		
1	Citric acid	1862.14	104.43(51.11SD)	43.33(8.22SD)	0.41	0.032	52.29(22.02SD)	67.05(35.30SD)	1,28	0.446
21	Serine	1379.20	264.95(155.45SD)	66.08(26.33SD)	0.25	0.025	138.68(29.27SD)	132.32(27.86SD)	0.95	0.721
156	Tryptophan	2242.37	61.02(19.00SD)	18.44(7.75SD)	0.30	0.008	33.13(18.13SD)	35.97(11.45SD)	1.08	0.760
20	Phenylalanine	1636.49	115.44(176.43IQR)	69.61(61.45Range)	0.60	0.024	161.05(45.55SD)	179.18(47.93SD)	1.11	0.540
77	Malic acid	1510.75	59.27(35.46IQR)	13.51(10.88Range)	0.23	0.024	31.86(14.23SD)	33.26(17.21SD)	1.04	0.888
111	3-Methyl-2-oxovaleric acid	1222.75	65.33(67.99IQR)	38.61(8.16Range)	0.59	0.024	236.74(191.96SD)	337.04(290.30SD)	1.42	0.530
75	**Unknown compound**	**2266.48**	**255.25(76.66SD)**	**142.57(15.93SD)**	**0.56**	**0.014**	**174.57(41.35SD)**	**284.61(54.91SD)**	**1.63**	**0.007**
214	Unknown compound	2342.49	97.04(33.29SD)	42.74(5.25SD)	0.44	0.030	14.79(28.93IQR)	1.12(26.31IQR)	0.08	0.177
349	Unknown compound	2382.78	116.43(42.18SD)	28.81(4.15SD)	0.25	0.010	74.91(269.70IQR)	2.45(81.56IQR)	0.03	0.247

359	Unknown phenolic compound	2645.10	36.03(24.31IQR)	2.22(12.29range)	0.06	0.024	7.15(62.33IQR)	2.22(25.54IQR)	0.31	0.537
467	Unknown sugar derivate	2445.38	39.65(21.66SD)	8.92(7.01SD)	0.22	0.017	7.07(48.92IQR)	1.86(18.52IQR)	0.26	0.537
495	Unknown compound	2148.25	5.42(4.38IQR)	0.89(1.94range)	0.16	0.048	2.98(15.50IQR)	0.89(14.95IQR)	0.30	0.537
560	Unknown compound	2363.78	23.15(20.23IQR)	2.09(7.04range)	0.09	0.024	2.09(0.00IQR)	2.09(22.09IQR)	1.00	0.329
22	Isoleucine	1305.71	637.91(346.83SD)	188.03(100.55SD)	0.29	0.024	156.57(356.34IQR)	170.39(372.88)	1.09	1.000
23	Leucine	1283.81	881.53(511.90SD)	224.80(111.55SD)	0.26	0.025	333.52(467.08IQR)	348.85(622.16IQR)	1.05	0.792
107	Hydroxyproline	1546.59	18.10(8.49SD)	2.66(1.40SD)	0.15	0.019	37.40(30.65SD)	55.24(23.60SD)	1.48	0.304
203	Unknown compound	2223.37	165.49(56.96SD)	60.00(27.32SD)	0.36	0.007	31.02(35.78IQR)	1.21(25.34IQR)	0.04	0.177
237	Unknown compound	1612.09	12.29(4.31SD)	4.19(4.30SD)	0.34	0.033	0.92(0.00IQR)	0.92(0.00IQR)	1.00	1.000
1177	Unknown compound	1385.08	212.18(113.25SD)	58.74(47.73SD)	0.28	0.025	12.79(13.99IQR)	12.79(0.00IQR)	1.00	0.662
123	**Unknown compound**	**2567.78**	**4389.60(1199.39IQR)**	**4822.38(615.48Range)**	**1.10**	**0.024**	**2304.69(736.98SD)**	**3867.63(1170.43SD)**	**1.68**	**0.038**

Supplemental Table 2. Levels of metabolites that differed significantly between unfavorable and favorable groups in Turku cohort. Level of these metabolites were also tested in Cambridge cohort and presented in table. Bolded lines differed significantly in both Turku and Cambridge cohorts. For metabolites, that were deemed normally distributed using Shapiro-Wilks's test, means and standard deviations are presented. For non-normally distributed metabolites medians and interquartile ranges are presented, except in Turku day 7 and Cambridge day 1 unfavorable group where range is presented as interquartile ranges could not be determined due to low number of subjects. For normally distributed metabolites p-values are calculated with two-sided t-test unequal variances and for non-normally distributed Mann-Whitney U-test.

			Turku Arrival day			
ID	RI	Metabolite	Survivor group	Mortality group	Fold	p
			n = 23	n = 10		
68	1297.70	Glycerol	5397.02(2227.57SD)	7873.07(3562.68SD)	1.46	0.021
74	1768.61	Glycerol-2-phosphate	33.69(10.36SD)	45.09(13.02SD)	1.34	0.011
142	1189.57	Unknown compound	787.18(172.79SD)	1025.37(204.79SD)	1.30	0.002
149	1616.41	Alcohol	47.15(33.87SD)	99.08(44.59SD)	2.10	0.001
191	2001.32	Unknown compound	144.47(91.82SD)	77.74(57.79SD)	0.54	0.043
199	1397.22	Unknown compound	6.74(2.11SD)	8.60(2.50SD)	1.28	0.035
220	1197.82	Unknown compound	429.46(134.04SD)	551.84(176.80SD)	1.28	0.036
293	2518.04	Unknown carboxylic acid	4.98(2.01SD)	8.50(3.97SD)	1.71	0.022
10	1455.71	3,4-Dihydroxybutanoic acid	8.95(5.05IQR)	16.24(10.32IQR)	1.81	0.009
14	1177.70	3-Hydroxybutyric acid	779.95(1285.83IQR)	2981.14(3743.88IQR)	3.82	0.042
335	1361.29	Methyl succinic acid	1.72(9.43IQR)	12.37(22.88IQR)	7.19	0.016
78	1931.96	4-Hydroxyphenyllactic acid	22.81(7.21IQR)	34.15(30.81IQR)	1.50	0.0004
106	1278.73	Ethanolamine	72.89(31.20IQR)	134.75(88.88IQR)	1.85	0.004
48	1578.52	2,3,4-Trihydroxybutyric acid	132.42(41.55IQR)	231.29(149.76IQR)	1.75	0.004
59	1345.61	Unknown compound	734.36(764.88IQR)	1094.83(435.43IQR)	1.49	0.042
65	1648.13	Arabinofuranose	15.79(15.26IQR)	23.29(29.06IQR)	1.47	0.018
77	1510.75	Malic acid	70.26(33.88IQR)	135.98(325.54IQR)	1.94	0.031
84	1703.11	Unknown compound	104.50(52.12IQR)	143.34(111.04IQR)	1.37	0.025
93	1369.61	Nonanoic acid	74.22(98.84IQR)	181.32(88.79IQR)	2.44	0.002
99	1464.27	Decanoic acid	122.46(597.49IQR)	634.49(1330.26IQR)	5.18	0.034
108	2051.79	Unknown compound	109.54(68.48IQR)	242.14(489.03IQR)	2.21	0.034

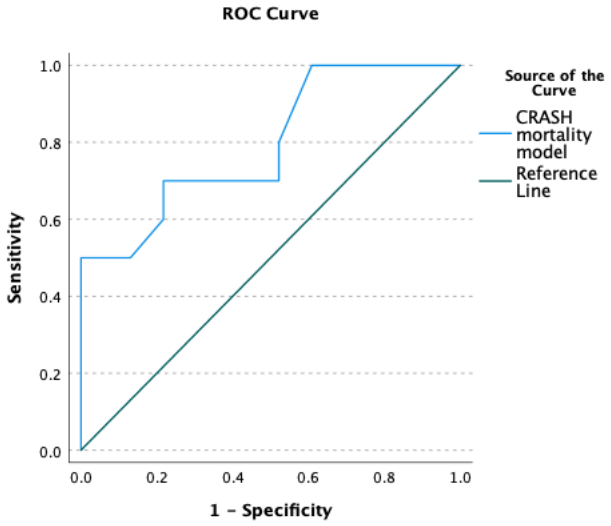
150	2580.61	Hexadecanoic acid, 2,3-bishydroxypropyl ester	82.87(30.34IQR)	101.39(42.24IQR)	1.22	0.025
176	1811.13	similar to fructose derivate	16.17(9.22IQR)	23.74(45.71IQR)	1.47	0.010
187	1519.64	Adipic acid	6.58(5.24IQR)	17.24(59.82IQR)	2.62	0.0004
189	2419.47	Unknown compound	34.37(41.77IQR)	54.07(18.61IQR)	1.57	0.008
198	1558.95	Hydroxy acid	2.41(3.09IQR)	6.56(10.28IQR)	2.72	0.014
208	2364.79	Unknown sugar derivate	37.24(84.88IQR)	126.28(268.36IQR)	3.39	0.013
253	1659.63	Pentitol, 3-desoxy	3.68(1.63IQR)	11.08(16.69IQR)	3.01	0.00005
282	2107.21	Unknown compound	12.46(4.08IQR)	13.94(6.01IQR)	1.12	0.011
297	2236.43	Unknown compound	4.71(5.29IQR)	9.52(11.51IQR)	2.02	0.042
309	1936.87	Unknown carboxylic acid	19.63(209.66IQR)	310.37(1130.35IQR)	15.81	0.042
312	1538.66	Unknown compound	15.77(69.10IQR)	412.57(684.70IQR)	26.16	0.020
340	2484.34	Serotonin	3.80(2.32IQR)	1.33(1.97IQR)	0.35	0.011
347	1816.50	Unknown compound	3.42(13.68IQR)	20.62(14.16IQR)	6.03	0.007
371	1470.78	3-Aminoisobutyric acid	4.68(8.22IQR)	11.10(16.12IQR)	2.37	0.022
382	2354.25	Nonadecanoic acid	7.20(9.14IQR)	10.62(2.84IQR)	1.48	0.005
389	2264.40	Unknown compound	79.87(83.24IQR)	21.75(91.84IQR)	0.27	0.028
392	1485.27	Erythrose	2.33(6.13IQR)	9.69(20.88IQR)	4.16	0.042
424	1360.49	2-Butenedioic acid	13.32(32.83IQR)	43.94(69.99IQR)	3.30	0.031
437	2171.81	d-Mannose	3.20(4.38IQR)	6.94(19.56IQR)	2.17	0.047
486	1420.27	Pentanedioic acid	2.29(5.95IQR)	12.61(41.36IQR)	5.51	0.022
551	1190.36	Unknown carboxylic acid	3.11(7.13IQR)	25.72(29.35IQR)	8.27	0.020
1147	2387.37	Arachidonic acid	55.70(53.85IQR)	92.31(57.00IQR)	1.66	0.025
1163	1214.91	Unknown sugar derivate	44.12(103.37IQR)	5.26(3.18IQR)	0.12	0.014
1179	2563.09	Unknown compound	7.34(37.60IQR)	82.05(87.33IQR)	11.18	0.006
1269	2450.87	Unknown compound	7.42(8.06IQR)	12.04(17.56IQR)	1.62	0.034

1270	1718.26	Unknown compound	13.10(16.24IQR)	29.42(13.80IQR)	2.25	0.0004
1271	2397.39	Unknown sugar (deoxyaldose)	46.40(97.68IQR)	144.57(233.23IQR)	3.12	0.010
1321	1807.41	1,4-Benzenedicarboxylic acid	0.47(1.50IQR)	2.85(4.18IQR)	6.06	0.028
1994	2408.19	Unknown sugar derivate	12.74(36.67IQR)	63.68(123.34IQR)	5.00	0.038
3258	1652.74	Phenolic metabolite	13.33(48.19IQR)	57.39(56.57IQR)	4.31	0.034
			Turku day 1			
ID	RI	Metabolite:	Survivor group	Mortality group		
			n = 12	n = 6		
3	1436.78	2,4-Dihydroxybutanoic acid	5.74(1.53SD)	11.02(4.85SD)	1.92	0.044
33	1109.77	Alanine	1442.75(687.90SD)	716.08(583.45SD)	0.50	0.042
78	1931.96	4-Hydroxyphenyllactic acid	18.29(7.92SD)	64.34(38.59SD)	3.52	0.032
42	1533.06	Pyroglutamic acid	2124.81(341.79SD)	3462.31(901.70SD)	1.63	0.00028
106	1278.73	Ethanolamine	57.73(27.42SD)	115.57(32.03SD)	2.00	0.001
47	1862.79	Myristic acid	278.65(129.42SD)	456.12(121.74SD)	1.64	0.013
53	2132.72	Myo-Inositol	1916.43(698.50SD)	4411.26(2334.53SD)	2.30	0.047
65	1648.13	Arabinofuranose	15.53(7.57SD)	34.06(12.32SD)	2.19	0.001
80	1523.39	Unknown compound	63.68(10.09SD)	82.80(17.67SD)	1.30	0.045
100	1551.67	Alanine, phenyl-	262.59(100.08SD)	505.43(156.81SD)	1.92	0.001
142	1189.57	Unknown compound	688.03(158.69SD)	1073.04(139.24SD)	1.56	0.00012
148	2126.16	Unknown compound	31.07(18.57SD)	51.21(16.94SD)	1.65	0.041
170	2402.22	Unknown compound	14.26(8.66SD)	29.77(17.06SD)	2.09	0.019
222	1708.99	Arabinofuranose	4.58(2.89SD)	8.33(2.60SD)	1.82	0.017

224	1639.34	Alanine, phenyl-	39.26(14.02SD)	76.85(34.84SD)	1.96	0.045
252	1117.12	Unknown compound	269.06(100.38SD)	379.97(97.84SD)	1.41	0.041
1166	1855.74	Unknown amino acid	50.98(34.34SD)	18.54(17.66SD)	0.36	0.047
10	1455.71	3,4-Dihydroxybutanoic acid	7.47(3.25IQR)	10.47(14.96IQR)	1.40	0.041
58	1596.21	Proline [+CO2]	90.60(98.20IQR)	149.37(119.89IQR)	1.65	0.041
69	1658.95	Lauric acid	41.72(29.42IQR)	110.76(54.39IQR)	2.65	0.003
77	1510.75	Malic acid	42.33(24.99IQR)	75.03(158.29IQR)	1.77	0.007
104	1547.38	Glutamic acid	169.49(66.79IQR)	307.50(163.77IQR)	1.81	0.018
112	1272.83	Unknown amino acid	408.22(269.87IQR)	697.79(385.34IQR)	1.71	0.024
114	1587.53	Unknown compound	13.69(11.61IQR)	34.59(60.19IQR)	2.53	0.018
141	1750.72	Unknown compound	11.87(24.24IQR)	248.28(668.64IQR)	20.92	0.024
146	1311.13	l-Threonine	503.08(280.20IQR)	793.85(599.06IQR)	1.58	0.032
171	1599.22	Alpha-ketoglutaric acid	23.21(45.96IQR)	92.89(94.63IQR)	4.00	0.010
172	1416.80	Alanine	16.39(25.41IQR)	46.52(32.16IQR)	2.84	0.024
187	1519.64	Adipic acid	5.86(3.66IQR)	28.60(48.00IQR)	4.88	0.002
199	1397.22	Unknown compound	5.56(2.64IQR)	9.85(2.63IQR)	1.77	0.024
223	1600.05	Pentanedioic acid 2-hydroxy	14.65(15.30IQR)	31.69(101.29IQR)	2.16	0.013
253	1659.63	Pentitol, 3-desoxy	2.39(1.37IQR)	13.72(35.92IQR)	5.74	0.001
282	2107.21	Unknown compound	11.34(2.64IQR)	19.00(8.71IQR)	1.68	0.032
284	2430.85	Unknown compound	3.76(5.58IQR)	10.69(7.70IQR)	2.84	0.002
312	1538.66	Unknown compound	2.70(31.90IQR)	265.86(344.72IQR)	98.47	0.024
347	1816.50	Unknown compound	3.42(9.80IQR)	23.46(19.19IQR)	6.86	0.013
371	1470.78	3-Aminoisobutyric acid	0.86(4.53IQR)	13.32(47.40IQR)	15.49	0.013
416	1597.94	2-Phenyl-2-hydroxypropanoic acid	1.72(1.56IQR)	15.74(15.30IQR)	9.15	0.007
424	1360.49	2-Butenedioic acid	2.18(1.63IQR)	40.97(32.11IQR)	18.79	0.005

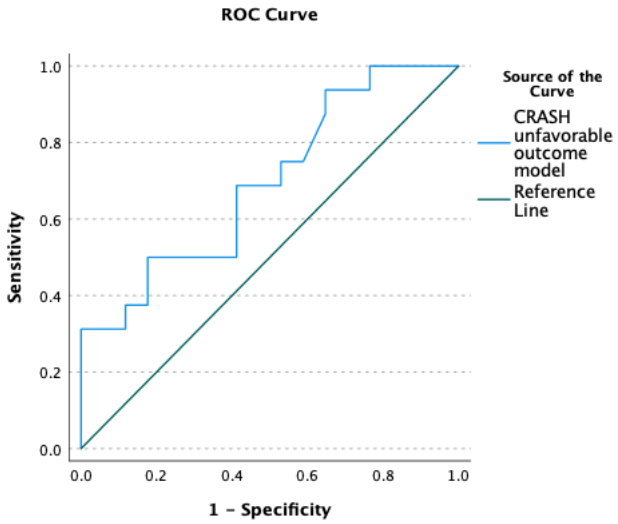
437	2171.81	d-Mannose	2.50(6.12IQR)	13.95(15.09IQR)	5.58	0.041
573	1229.96	Unknown compound	13.10(27.60IQR)	130.08(235.25IQR)	9.93	0.024
1284	1849.35	9-Tetradecenoic acid, trimethylsilyl ester	2.80(19.31IQR)	43.88(49.68IQR)	15.67	0.002
1298	1932.25	Unknown carboxylic acid	32.00(40.37IQR)	4.05(5.18IQR)	0.13	0.002
1320	1266.16	Unknown compound	47.72(57.95IQR)	2.63(10.57IQR)	0.06	0.032
1321	1807.41	1,4-Benzenedicarboxylic acid	0.47(1.53IQR)	6.05(2.32IQR)	12.87	0.005
3258	1652.74	Phenolic metabolite	18.52(42.32IQR)	115.26(166.84IQR)	6.22	0.001

Supplemental Table 3. Levels of metabolites that differed significantly between survivor and mortality groups as measured in Turku cohort. Mean values and standard deviations are presented from metabolites that were deemed normally distributed using Shapiro-Wilk`s test. For non-normally distributed metabolites medians and interquartile ranges are presented. Presented p-values are determined using two sided t-test unequal variances for normally distributed metabolites and with Mann-Whitney U-test on non-normally distributed metabolites.



CRASH mortality AUC on ROC-curve with 95% confidence interval.

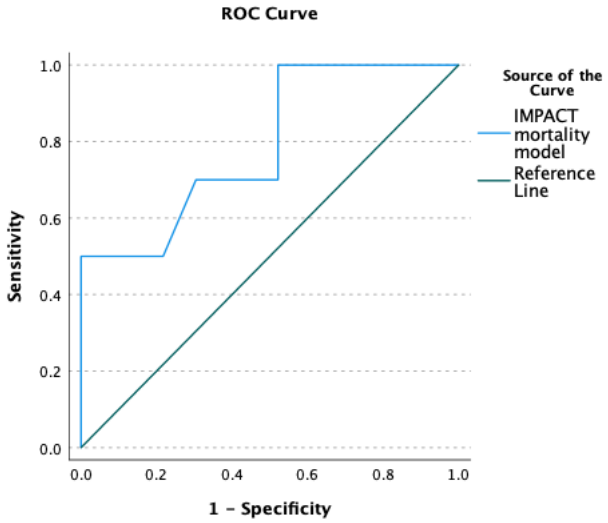
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.796	0.087	0.001	0.624	0.967



CRASH unfavorable outcome model AUC on ROC-curve with 95% confidence interval.

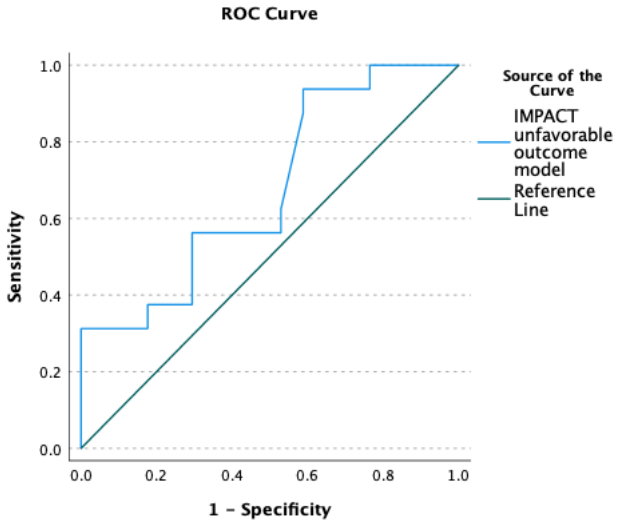
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.695	0.092	0.034	0.515	0.875

Figure 1. CRASH model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Turku cohort.



IMPACT mortality model AUC on ROC-curve with 95% confidence interval.

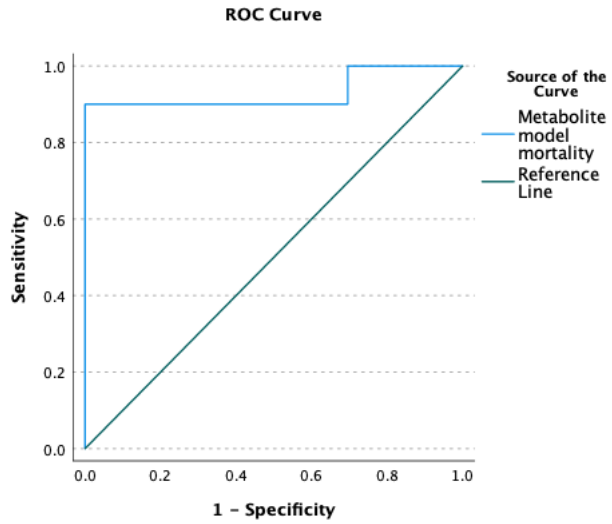
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.791	0.086	0.001	0.622	0.960



IMPACT unfavorable outcome model AUC on ROC-curve with 95% confidence interval.

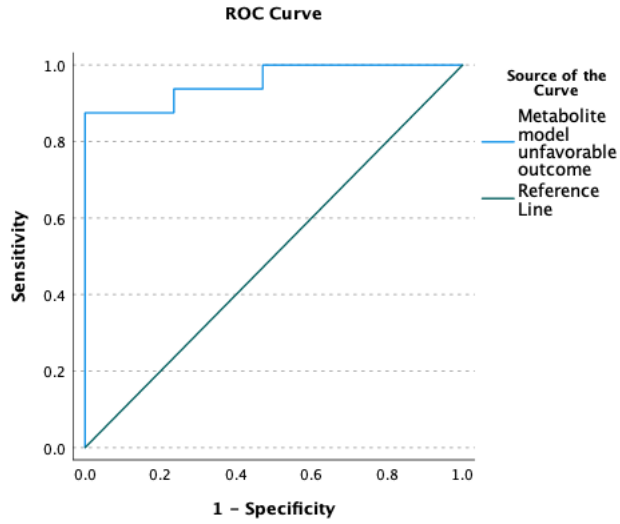
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.676	0.094	0.061	0.492	0.861

Figure 2. IMPACT model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Turku cohort.



Metabolite model for mortality AUC on ROC-curve with 95% confidence intervals.

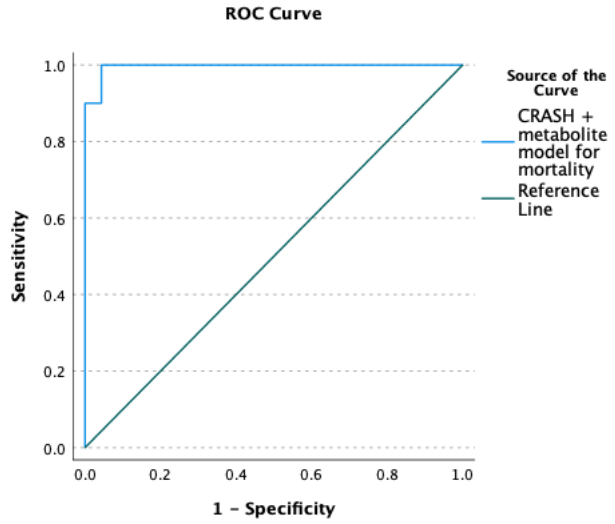
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.930	0.067	<0.001	0.799	1.062



Metabolite model for unfavorable outcome AUC on ROC-curve with 95% confidence intervals.

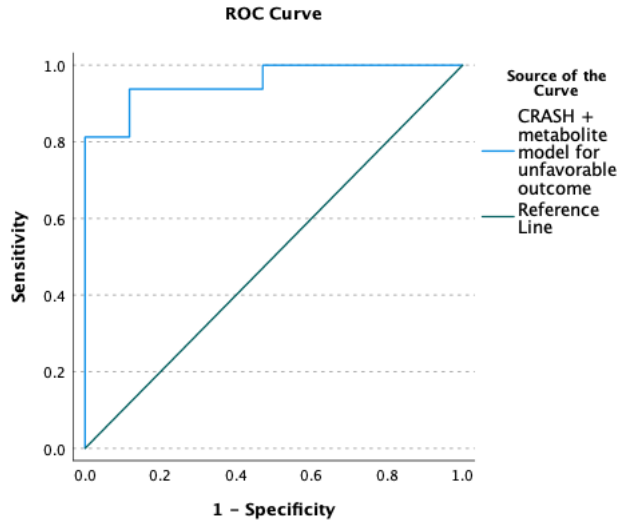
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.956	0.035	<0.001	0.888	1.024

Figure 3. Metabolite model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Turku cohort.



Combined CRASH and metabolite model for mortality AUC on ROC-curve with 95% confidence interval.

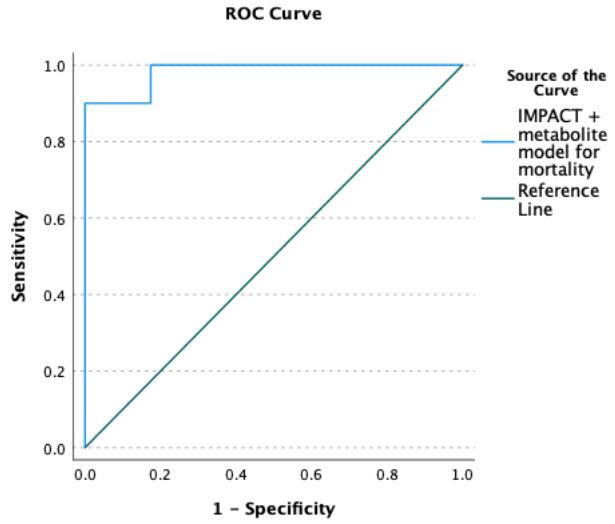
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.996	0.07	<0.001	0.982	1.010



Combined CRASH and metabolite model for unfavorable outcome AUC on ROC-curve with 95% confidence interval.

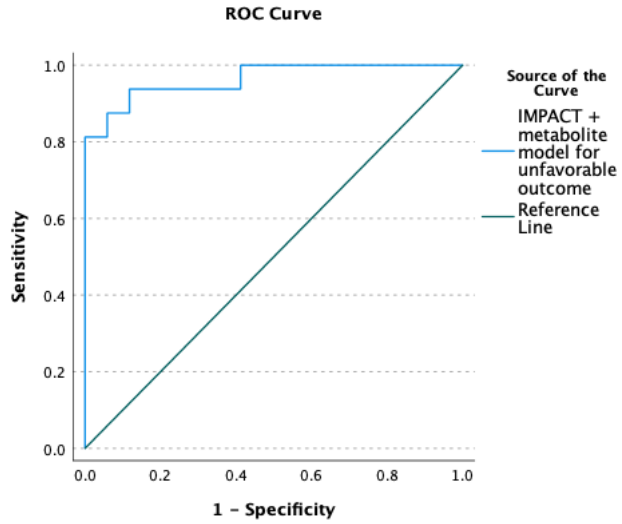
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.956	0.034	<0.001	0.889	1.022

Figure 4. Combined CRASH and metabolite model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Turku cohort.



Combined IMPACT and metabolite model for mortality AUC on ROC-curve with 95% confidence interval.

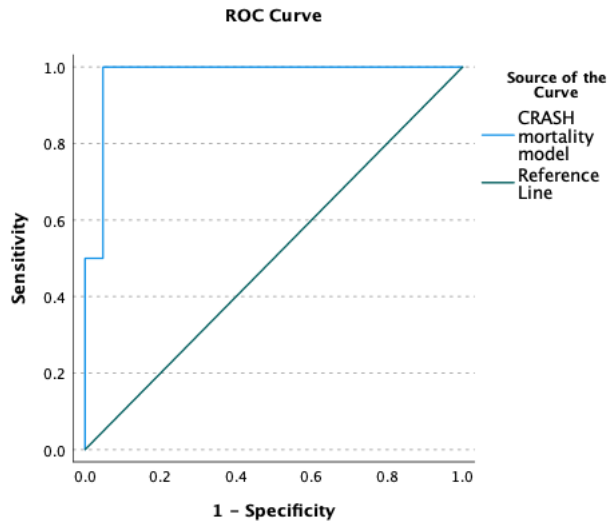
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.983	0.20	<0.001	0.944	1.021



Combined IMPACT and metabolite model for unfavorable outcome AUC on ROC-curve with 95% confidence interval.

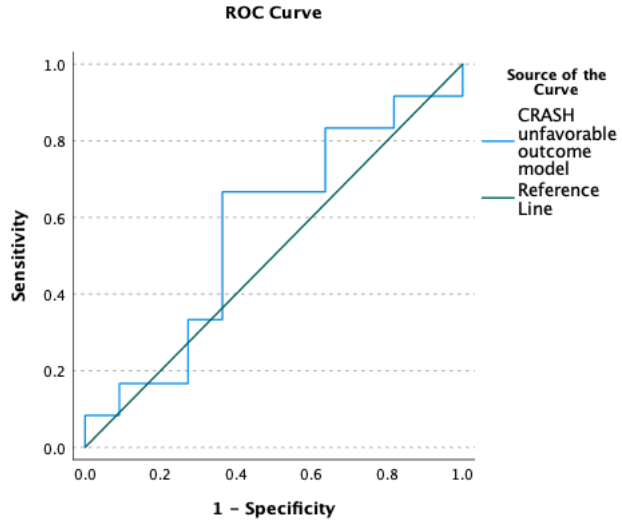
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.963	0.30	<0.001	0.905	1.022

Figure 5. Combined IMPACT and metabolite model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Turku cohort.



CRASH mortality AUC on ROC-curve with 95% confidence interval.

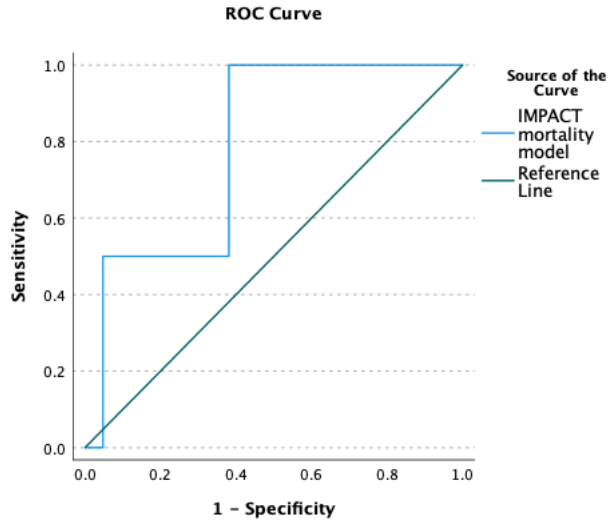
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.976	0.033	<0.001	0.911	1.041



CRASH unfavorable outcome model AUC on ROC-curve with 95% confidence interval.

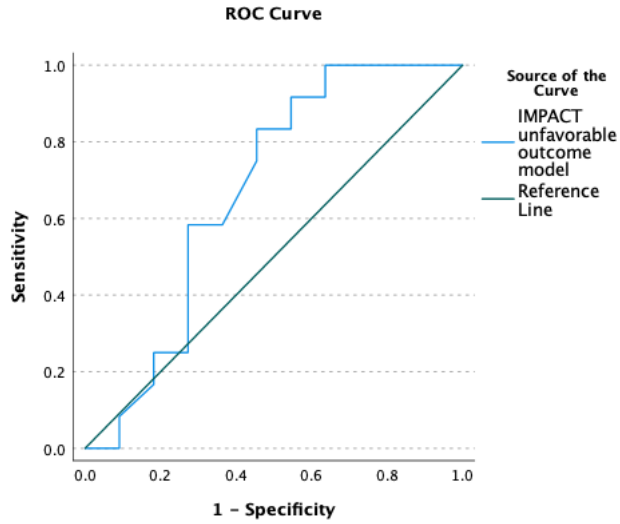
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.568	0.124	0.584	0.324	0.812

Figure 6. CRASH model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Cambridge cohort.



IMPACT mortality model AUC on ROC-curve with 95% confidence interval.

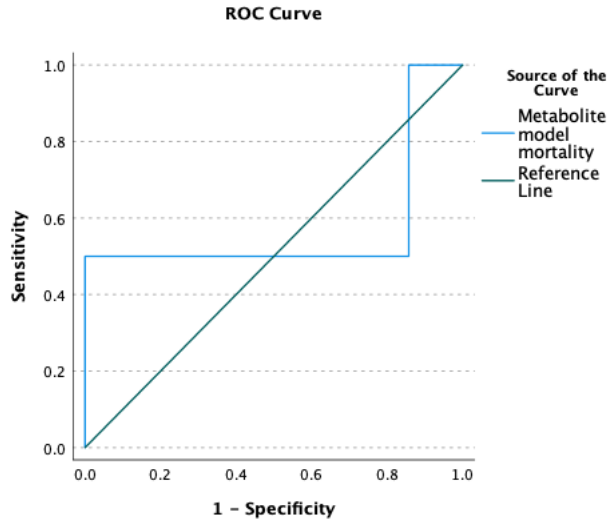
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.786	0.139	0.040	0.514	1.058



IMPACT unfavorable outcome model AUC on ROC-curve with 95% confidence interval.

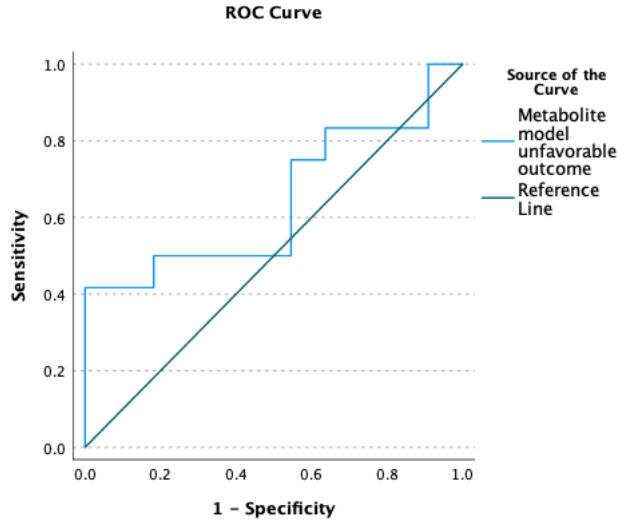
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.670	0.121	0.158	0.434	0.907

Figure 7. IMPACT model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Cambridge cohort.



Metabolite model for mortality AUC on ROC-curve with 95% confidence intervals.

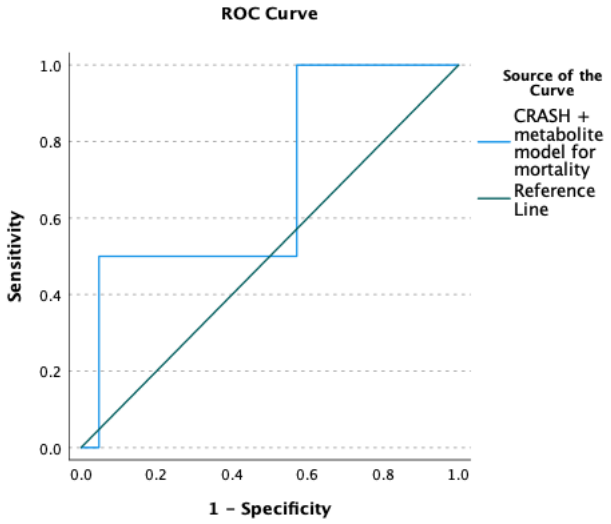
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.571	0.307	0.816	-0.030	1.172



Metabolite model for unfavorable outcome AUC on ROC-curve with 95% confidence intervals.

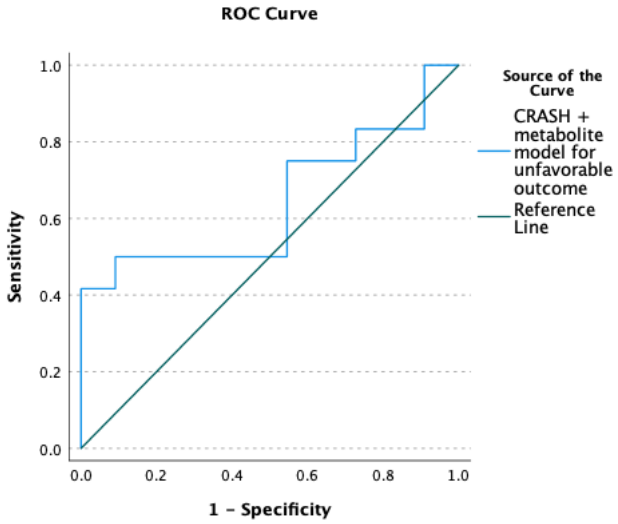
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.644	0.119	0.228	0.410	0.878

Figure 8. Metabolite model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Cambridge cohort.



Combined CRASH and metabolite model for mortality AUC on ROC-curve with 95% confidence interval.

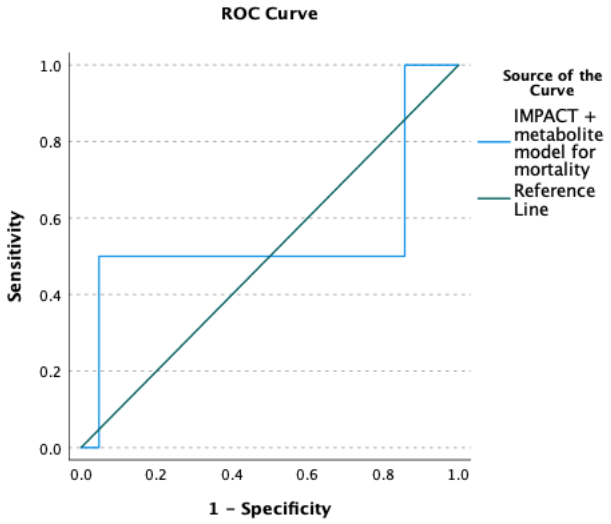
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.690	0.199	0.339	0.300	1.081



Combined CRASH and metabolite model for unfavorable outcome AUC on ROC-curve with 95% confidence interval.

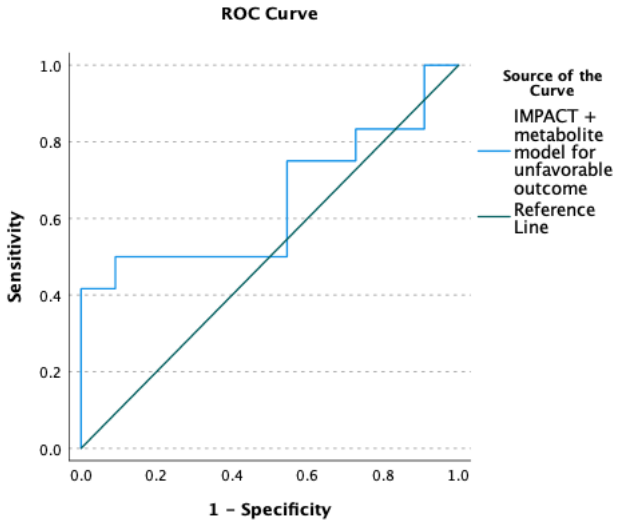
AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.644	0.120	0.231	0.408	0.879

Figure 9. Combined CRASH and metabolite model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Cambridge cohort.



Combined IMPACT and metabolite model for mortality AUC on ROC-curve with 95% confidence interval.

AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.548	0.292	0.870	-0.024	1.119



Combined IMPACT and metabolite model for unfavorable outcome AUC on ROC-curve with 95% confidence interval.

AUC	*Standard error*	*p-value*	*Lower 95% CI*	*Upper 95% CI*
0.644	0.120	0.231	0.408	0.879

Figure 10. Combined IMPACT and metabolite model receiver operating characteristics (ROC) curves with area under curve (AUC) values for mortality and unfavorable outcome as measured in Cambridge cohort.