

GFAP and UCH-L1 as outcome predictors in traumatic brain injury

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Abbreviations:

AUC	Area under the receiver operating characteristic curve
CSF	cerebrospinal fluid
CT	Computed tomography
GCS	Glasgow Coma Scale
GFAP	Glial fibrillary acidic protein (GFAP)
GFAP-BDP	Glial fibrillary acidic protein breakdown products
GOS	Glasgow Outcome Scale
GOSe	Glasgow Outcome Scale extended
ISS	Injury Severity Score
ROC	Receiver operating characteristic
TBI	Traumatic brain injury
UCH-L1	Ubiquitin C-terminal hydrolase -L1

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ABSTRACT

Object: Biomarkers ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) may help detect brain injury, assess its severity and improve outcome prediction. This study aimed to evaluate their prognostic value during the first post-injury days.

Methods: Serum UCH-L1 and GFAP were measured from 324 patients with traumatic brain injury (TBI) enrolled in a prospective study. The outcome was assessed using Glasgow Outcome Scale (GOS) or its extended version (GOSe).

Results: Patients with full recovery had lower UCH-L1 concentrations on second day and patients with favourable outcome during the first two days than patients with incomplete recovery and unfavourable outcome. Patients with full recovery and favourable outcome had significantly lower GFAP concentrations on the first two days than patients with incomplete recovery or unfavourable outcome. There was a strong negative correlation with the outcome and UCH-L1 on the first three days and GFAP levels on the first two days. Both UCH-L1 and GFAP upon arrival distinguished patients with GOS 1-3 from GOS 4-5 patients but not patients with GOSe8 from GOSe 1-7. For UCH-L1 and GFAP to predict unfavourable outcome ($GOS \leq 3$), the AUC was 0.727, and 0.723, respectively. Neither UCH-L1 nor GFAP was independently able to predict the outcome, when age, worst GCS, pupil reactivity, Injury Severity Score, and Marshall score were added into multivariate logistic regression model.

Conclusion: GFAP and UCH-L1 are significantly associated with outcome but they do not add predictive power to commonly used prognostic variables in a TBI population of varying severities.

INTRODUCTION

Traumatic brain injury (TBI) is a heterogeneous disease (15) with limited diagnostic tools. Imaging studies do not reveal all injuries (35) and some patients with mild TBI and no visible lesions are left with permanent symptoms. Consequently, specific biochemical markers which would reveal even the mildest brain injury, help assessing its severity (7, 29), improve existing outcome prediction models (4) and monitor treatment efficacy are needed. S-100 β has been a candidate biomarker (16) but it has low sensitivity (1, 16) and lacks brain-specificity (9, 36, 22).

Both ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) are new promising TBI biomarkers. GFAP is an intermediate filament protein of astroglial skeleton (1) and represents glial injury (12). Neuronal UCH-L1 is involved in either adding or removing ubiquitin from proteins (19) and represents neuronal injury (5, 7).

Increased UCH-L1 concentrations have been linked to injury severity and worse outcome after TBI (6, 20, 24). GFAP has been found to correlate with axonal injury, elevated intracranial pressure and mortality (27, 31, 17) and has outperformed S-100 β in detecting intracranial injuries in head computed tomography (CTs) scans of patients with extracranial injuries (10, 26). Biomarkers may also help identifying the injury types, as UCH-L1 seems to increase more in diffuse injuries and GFAP in mass lesions (20).

The aim of our study was to assess whether UCH-L1 and GFAP concentrations during the first post-injury days correlate with the outcome of patients with TBI.

MATERIAL AND METHODS

Patient population

This prospective multicentre study was part of the EU funded TBicare (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries) project. The study population consisted of 389 patients with TBI (Turku 201, Cambridge 188), of these 324 had proteomics data available and were included into the analysis. Patients were recruited in Turku University Hospital (Finland) and in Addenbrooke's Hospital Cambridge (United Kingdom).

Inclusion criteria were as follows: age ≥ 16 years, clinical diagnosis of TBI and indications for acute head CT according to NICE criteria (<http://www.nice.org.uk/guidance/cg176>). Exclusion criteria were: age < 16 at study entry, blast-induced or penetrating injury, chronic subdural haematoma, inability to live independently due to pre-existing brain disease, TBI or suspected TBI not needing head CT, more than 2 weeks from the injury, not living in the district thus preventing follow-up visits (Turku), not speaking native language, and no consent received.

The ethical review board of Hospital District of South-West Finland and the Cambridgeshire 2 Research Ethics Committee and the Norfolk Research Ethics Committee approved the study. All patients or their proxies were given both oral and written information about the study and a written informed consent was obtained.

All patients were treated according to local guidelines based on current international guidelines and recommendations (2).

Measures

Initial severity of the TBI was based on the lowest Glasgow Coma Scale (GCS) before intubation. GCS of 13-15 was considered mild, 9-12 moderate and 3-8 severe. The mechanism of injury was recorded upon arrival. Injury Severity Score (ISS) was used to describe the overall injury severity of the patients.

Blood samples for protein biomarkers were collected at admission and on days 1, 2, 3 and 7 and were centrifuged for 10 minutes at 10 000 rpm at 4 °C. The plasma was immediately frozen at -70 °C for further analysis. Levels of UCH-L1 and GFAP were analysed at Randox Laboratories Ltd (Crumlin, County Antrim, BT29 4QY, United Kingdom) with The Evidence Investigator™ Cerebral Custom Array IV using a sandwich chemiluminescent immunoassay.

Outcome

Outcomes were assessed at 3 months in patients with mild TBI (Cambridge) and at 6-12 months (all other patients) after the injury using both Glasgow Outcome Scale (GOS) (11) and Glasgow Outcome Scale extended (GOSe) (34). GOS 4-5 was classified as favourable and GOS 1-3 as unfavourable outcome. GOSe 8 was classified as complete recovery whereas GOSe 1-7 as incomplete recovery.

Statistical analysis

Data were analysed using IBM SPSS Statistics 22 (IBM Corp, New York, USA) and Matlab R2012b (MathWorks, Natick, MA, USA). Demographic data are presented as mean \pm SD. Normality of the biomarkers was assessed using the Kolmogorov-Smirnov test and histograms. As levels of UCH-L1 and GFAP were not normally distributed, non-parametric tests were used and data are presented as medians and 25th and 75th percentiles. Correlations between the biomarkers and the outcomes were studied with Spearman's rank correlation coefficient. Levels

of the biomarkers between two groups were compared using the Mann-Whitney U test. Prognostic ability of the biomarkers to predict the dichotomised outcomes was evaluated with univariate logistic regression. In addition, multivariate logistic regression analysis was performed including the worst GCS before intubation, age, Marshall, ISS, pupil reactivity and UCH-L1 or GFAP as predictors. Prognostic ability of the biomarkers was also evaluated with the area under the receiver operating characteristic (ROC) curve (AUC). Cut-off values for the prediction of dichotomised outcomes were defined using the ROC curve, Youden index (28), and 10 iterations of 3-fold cross-validation stratified according to the classes of GOS or GOSe. A p-value < 0.05 was considered statistically significant. AUC of 0.8-1.0 was considered very good, AUC of 0.7-0.8 adequate, and AUC < 0.7 poor (6).

RESULTS

Biomarkers were evaluated from 324 patients with mean age of 45.3 ± 19.2 and most being male (73.8%). The majority of patients had mild TBI and one-third had severe TBI. The patient characteristics are presented in Table 1. Injuries were mostly related to falls and traffic accidents. The majority of patients (88.9%) had available GOS_e and slightly more patients (92.3%) had available GOS. Numbers of available GOS_e and GOS differ because general practitioners assessed some of the patients using only GOS. Table 1 presents the outcome distribution: 23.8% had complete recovery (GOS_e 8), 63% had good outcome (GOS 4-5), and mortality rate was 9.6%.

Table 2 presents number of subjects having outcomes and UCH-L1 and GFAP measurements on different days. Less than half of the patients had samples taken on the arrival day. Only 21 patients had samples taken during all five time-points. This is mainly due to early discharge, usually within one to three days, of patients with mild TBI.

UCHL-1 and outcome

Biomarker levels were compared between complete recovery and incomplete recovery (GOS_e 8 vs. GOS_e 1-7), between favourable and unfavourable outcomes (GOS 4-5 vs. GOS 1-3), and between dead (GOS 1) and survived (GOS 2-5) patients. Levels of UCH-L1 in these groups are shown in Figures 1-3. Patients with complete recovery had significantly lower levels of UCH-L1 than patients with incomplete recovery only on day 2. Patients with favourable outcome had significantly lower UCH-L1 levels than patients with unfavourable outcome on arrival day, and days 1-2. Patients who died had significantly higher median UCH-L1 levels on days 1 and 3 when compared to survived patients.

There was a significant negative correlation between UCH-L1 and GOS upon arrival and days 1-3, Spearman rho -0.273 $p < 0.002$, -0.304 $p < 0.001$, -0.269 $p < 0.001$ and -0.185 $p < 0.026$, respectively. A significant negative correlation between UCH-L1 and GOS_e was also found upon arrival and days 1-3, Spearman rho -0.222 $p < 0.012$, -0.258 $p < 0.001$, -0.283 $p < 0.001$ and -0.177 $p < 0.036$, respectively. On the basis of univariate logistic regression, UCH-L1 on the arrival day was a significant predictor of unfavourable outcome (GOS 4-5), OR 2.865, 95% CI 1.344-6.112, In addition, UCH-L1 on days 2 and 3 was a significant predictor of death (GOS1), OR 1.302, 95% CI 1.008-1.681 and OR 1.227, 95% CI 1.018-1.479, respectively (Table 3). However, in multivariate regression model UCH-L1 was no longer able to predict outcome. Age, worst GCS and Marshall score had statistically significant ORs for predicting death and unfavourable outcome, whereas ORs of UCH-L1 were not significant. Worst GCS was also able to predict incomplete recovery

GFAP and outcome

Levels of GFAP were significantly lower in patients with complete recovery and favourable outcome on the day of arrival and days 1-2 compared to patients with incomplete recovery and unfavourable outcome (Figures 1-3). Patients who died had significantly higher median GFAP levels on arrival and days 1-3 when compared to survived patients (GOS 2-5).

There was a significant negative correlation between GFAP levels and GOS upon arrival and days 1-2, Spearman rho -0.349 $p < 0.000$, -0.433 $p < 0.001$ and -0.311 $p < 0.001$, respectively. A significant negative correlation between GFAP and GOS_e was also found upon arrival and days 1-2, Spearman rho -0.335 $p < 0.001$, -0.417 $p < 0.001$ and -0.305 $p < 0.001$, respectively. On the basis of univariate logistic regression, GFAP measured on the arrival day was a significant

predictor of death, OR 1.110 (95% CI 1.006-1.225). GFAP on the arrival and on day one was a significant predictor of unfavourable outcome, OR 1.537 (95% CI 1.194-1.979) and OR 1.222 (95% CI 1.005-1.485), respectively (Table 4). In multivariate regression model GFAP was not able to predict outcome, whereas age, worst GCS and Marshall score had significant ORs for predicting death and unfavourable outcome. Worst GCS was also able to predict incomplete recovery,

ROC analysis

ROC analysis was done only for arrival samples. AUC for UCH-L1 to help distinguish GOS 1-3 vs. GOS 4-5 was 0.727 (95% CI 0.609-0.820), GOSe 1-7 vs. GOSe 8 was 0.538 (95% CI 0.433-0.631) and for GOS 1 vs. GOS 2-5 was 0.655 (95% CI 0.423-0.809). AUC for GFAP to help distinguish GOS 1-3 vs. GOS 4-5 was 0.723 (95% CI 0.602-0.814), GOSe 1-7 vs. GOSe 8 was 0.628 (95% CI 0.523-0.712) and for GOS 1 vs. GOS 2-5 was 0.716 (95% CI 0.437-0.893). The ROC curves are displayed in Figure 4.

Cut-off value

Cut-off values for UCH-L1 and GFAP were defined using the arrival samples For UCH-L1 the cut-off value (mean \pm SD) for unfavourable outcome was 1.03 ± 0.30 with a sensitivity of 0.43 ± 0.17 and specificity of 0.83 ± 0.12 . For incomplete recovery the cut-off value was 0.95 ± 0.26 with a sensitivity of 0.31 ± 0.10 and specificity of 0.82 ± 0.14 . Accuracies were 0.73 ± 0.08 and 0.50 ± 0.04 , respectively.

For GFAP the cut-off value for unfavourable outcome was 1.26 ± 1.52 with a sensitivity of 0.51 ± 0.15 and specificity of 0.82 ± 0.09 . For incomplete recovery the cut-off value was 0.67 ± 0.27

with a sensitivity of 0.38 ± 0.11 and specificity of 0.85 ± 0.11 . Accuracies were 0.74 ± 0.06 and 0.55 ± 0.06 , respectively.

DISCUSSION

Patients with incomplete recovery and unfavourable outcome showed higher levels of UCH-L1 and GFAP during the acute phase and there was a strong negative correlation between the outcome and admission levels of UCH-L1 and GFAP. Furthermore, both UCH-L1 and GFAP obtained upon arrival were able to distinguish GOS 1-3 patients from GOS 4-5 patients. In addition, GFAP distinguished death from survival. However, neither of them was independently able to predict the outcome of patients with TBI in a multivariate model, which included also age, worst GCS, pupil reactivity, ISS and Marshall score as outcome predictors.

Previous studies have already demonstrated that both serum UCH-L1 and GFAP are promising biomarkers of TBI (10, 14, 23, 25, 26). As the course of TBI is difficult to predict, there is interest to assess if these biomarkers could also detect secondary injuries (30) and contribute in outcome prediction.

Earlier studies in patients with severe TBI observed that UCH-L1 level in cerebrospinal fluid (CSF) 24 hours after injury was significantly higher in those patients who died within 6 weeks of injury, and UCH-L1 levels were significantly higher in patients with unfavourable outcome 4 and 8 days after the injury (24). Another study found that the maximal serum UCH-L1 during the first seven days predicted independently three month mortality (3). Similarly, UCH-L1 has been an independent predictor of in-hospital mortality whereas both UCH-L1 and GFAP have been able to predict mortality at 6 months after TBI (20). UCH-L1 has been poor in predicting

complete recovery (GOSe 8) but fairly predictive of unfavourable outcome (GOSe ≤ 4) and it seems that UCH-L1 perhaps outperforms GFAP in this regard (6). In contrast to these, Czeiter et al. were unable to show that UCH-L1 alone would be capable to predict outcome at 6 months, albeit adding biomarkers into IMPACT calculator may strengthen its predictive value (4). Our results of lower UCH-L1 levels in patients with GOS 4-5 on the day of arrival and days 1 and 2 compared to those with unfavourable outcome is well in line with earlier studies. In our study, those who died had higher UCH-L1 levels on days one and three than those who survived, which is also in accordance with other studies. The observation that in patients with full recovery the UCH-L1 levels were significantly lower on the second day after the injury is a novel finding, suggesting that the first two-three days may be critical in determining the course of injury. However, the time of admission may be too early to estimate full recovery since UCH-L1 obtained upon arrival was able to discriminate GOS 1-3 patients from GOS 4-5 patients but not GOSe 8 patients from GOSe 1-7. In addition, it is possible that the number of patients upon admission and first day were too small for reaching statistical significance. Despite of these findings, in multivariate analysis UCH-L1 had no independent significance in outcome prediction.

Earlier studies demonstrated that GFAP levels less than 12 hours after the injury were significantly higher in patients with unfavourable outcome than in patients with favourable outcome and GFAP was able to predict mortality at 3 and 6 months (27, 32). However, neither of these studies included other prognostic factors into their analysis. In a later study, GFAP levels less than 6 hours after TBI also correlated with outcome but not anymore when taken 24 hours after the injury (33). On the other hand, in some studies increased GFAP levels at 24 hours and 2 days have been found to predict mortality and unfavourable outcome (4, 14). Similar results have been observed also in paediatric patients (37). Increased GFAP and breakdown products (GFAP-

BDP) have been associated with incomplete recovery ($\text{GOSe} \leq 7$) and they seem fairly predictive of unfavourable outcome ($\text{GOSe} \leq 4$) but poor in predicting complete recovery (23). GFAP levels have also been higher during the first few days in patients with unfavourable outcome (21). Our results with significantly higher GFAP levels in patients with unfavourable outcome (GOS 1-3) for the first two days and higher GFAP levels in those who died during the first three days are in agreement with earlier studies. The GFAP levels on the arrival day were able to discriminate poor outcome (GOS 1-3) patients but not those with incomplete recovery ($\text{GOSe} 1-7$) from those with complete recovery ($\text{GOSe} 8$). This extends earlier findings and is in line with our results with UCH-L1 discussed above, suggesting that the events during the two first days after the injury may be critical in respect to full recovery. However, as with UCH-L1, GFAP had no independent significance in outcome prediction when GCS, age, pupil reactivity, ISS and CT findings were taken into account.

The ability of UCH-L1 and GFAP obtained within 24 hours after TBI has been assessed to screen those TBI patients who have incomplete recovery. Both biomarkers have been able to predict poor outcome ($\text{GOSe} \leq 4$) but not incomplete recovery ($\text{GOSe} < 8$) and their combination has slightly improved the ability to predict poor outcome (6). Similarly, GFAP-BDP is unable to predict complete recovery (23), whereas GFAP at admission has been able to predict unfavourable outcome (GOS 1-3) (4, 32). Our study is in agreement with the earlier findings that both admission UCH-L1 and GFAP are able to distinguish patients with favourable vs. unfavourable outcome with the AUC 0.727 and 0.723, respectively, but not complete recovery. However, our study was not able to confirm previous observations in severe TBI patients (3,14) that UCH-L1 and GFAP could have independent significance and add predictive power to commonly used prognostic clinical and imaging variables

The cut-off value of UCH-L1 for poor outcome was 1.03 with a sensitivity and specificity of 0.43 and 0.83. Previously, a cut-off value of 1.89 for UCH-L1 to predict in hospital mortality with a sensitivity of 0.52 and specificity of 0.96 has been found (20). For incomplete recovery the cut-off value was slightly lower, 0.95 with a sensitivity and specificity of 0.31 and 0.82. For GFAP the cut-off value for poor outcome was 1.26 with a sensitivity of 0.51 and specificity of 0.82. Vos et al. received comparable cut-off value of 1.5 for poor outcome although with higher sensitivity and lower specificity (32). For incomplete recovery the cut-off value was 0.67 with a sensitivity of 0.38 and specificity of 0.85. Similar cut-off value 0.68 was found by Okonkwo et al. for incomplete recovery with higher sensitivity and specificity (23). There are slight differences between our results and the others, especially in the sensitivities and specificities. As the methods of cut-off selections in the other studies have not been clarified, it is difficult to compare our results with previous findings. Differences in patient populations and outcome assessments may easily cause some discrepancy and the results from different laboratories may not be identical either.

The biomarkers in our study were measured only from serum. CSF concentrations of UCH-L1 and GFAP are higher and remain longer than in the serum (8, 19). However, serum samples are practical as CSF and serum concentrations and kinetics of UCH-L1 correlate well with each other during the acute phase and serum concentration seems to correlate better with the outcome (3, 8). Besides, in clinical practice, biomarkers that are obtainable from serum are much more feasible, especially at the emergency department. Maximal serum concentration of UCH-L1 is achieved approximately 9 hours post injury and its half-life is less than 10 hours (3), resulting in rapid decline of UCH-L1. GFAP has a half-life of < 2 days (6) and it is rapidly declined during the acute phase (18). Therefore only samples obtained during the acute phase seem usable for outcome prediction, especially in those patients who do not suffer secondary injuries.

There are limitations in our study. First, only 21 patients had samples taken at all five time points, mainly because merely patients with more severe injuries stayed seven days or longer in the hospital. Second, we did not analyse operated patients separately but it seems that surgery has no effect on GFAP levels (21). Third, we would have wished to examine the impact of including prognostic information from the biomarker assay on the predictive power of a standard prognostic calculator such as the IMPACT scheme as reported by Czeiter et al (4). However, our study covered the whole spectrum of TBI severity, with a large proportion of patients with mild TBI, though their proportion was less than usually found (13). The IMPACT prognostic scheme is not validated in mild TBI, and although there are schemes that address prognostication in mild TBI, there is no prognostic scheme that is validated across the entire TBI severity range. Therefore, we performed a multivariate prognostic evaluation using variables that have shown predictive value in earlier studies. Our results do not exclude the possibility that these biomarkers may have independent predictive value in subpopulations of TBI, which will be studied in our more detailed future analyses. Further, our assessment of outcome with the GOSe, probably does not provide the optimal sensitivity needed to address outcomes for mild TBI. In addition, the use of GOS or GOSe as an outcome measure is not devoid of problems. Especially pre-existing disabilities as well as the role of other injury-related or indirect consequences are not straightforward to interpret. These issues should be taken into account when evaluating the results and comparing different studies. The strength of this study is that samples were collected thus far from the largest population of largely unselected patients with TBI and samples were collected up to seventh day post injury whereas most of the previous studies collected samples only at the arrival or within 24 hours after the injury. This paper does not handle the role of these biomarkers in assessing the diagnosis and acute severity, which will be published in separate reports.

CONCLUSION

In conclusion, our results originating from entire TBI severity spectrum suggests that both GFAP and UCH-L1 are significantly associated with outcome. However, they do not add predictive power to commonly used prognostic clinical and imaging variables, at least not in a TBI population of varying severities. Further studies are also warranted to find optimal cut-off values with higher sensitivity and specificity. Even after this, the true value of these biomarkers in clinical practice and decision-making remains to be seen.

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Figure 1. Boxplots of A) UCH-L1 and B) GFAP in GOSe 1-7 vs. GOSe 8 patients.

Figure 2. Box plots of A) UCH-L1 and B) GFAP in GOS 1-3 vs. GOS 4-5 patients.

Figure 3. Boxplots of A) UCH-L1 and B) GFAP in GOS 1 vs. GOS 2-5 patients.

Figure 4. Receiver operating characteristics curve of UCH-L1 and GFAP on arrival day for A) GOSe 1-7 vs. GOSe 8, B) GOS 1-3 vs. GOS 4-5 and C) GOS 1 vs. GOS 2-5.