GFAP and UCH-L1 are not specific biomarkers for mild CT-negative traumatic brain injury

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ABSTRACT

Glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase-L1 (UCH-L1) have been studied as potential biomarkers of mild traumatic brain injury (mTBI). We report the levels of GFAP and UCH-L1 in patients with acute orthopedic injuries without central nervous system involvement and relate them to the type of extracranial injury, head magnetic resonance imaging (MRI) findings, and to the levels of GFAP and UCH-L1 in patients with computed tomography (CT) negative mTBI. Serum UCH-L1 and GFAP were longitudinally measured from 73 patients with acute orthopedic injury on arrival and on days 1, 2, 3, 7 after the admission, and on the follow-up visit 3-10 months after the injury. The injury types were recorded and 71% patients underwent also head MRI. The results were compared to those found in patients with CT-negative mTBI (n=93). The levels of GFAP were higher in patients with acute orthopedic trauma than in patients with CT-negative mTBI (p=0.026) on arrival, but no differences were found on the following days. The levels of UCH-L1 were not significantly different between these two groups at any measured point of time. Levels of GFAP and UCH-L1 were not able to distinguish patients with CT-negative mTBI from patients with orthopedic trauma. Patients with orthopedic trauma and high levels of UCH-L1 or GFAP values may be falsely diagnosed as having a concomitant mTBI, predisposing them to unwarranted diagnostics and unnecessary brain imaging. This casts a significant doubt on their diagnostic value in cases with mTBI.

INTRODUCTION

Mild traumatic brain injury (mTBI) with or without a short period of unconsciousness and amnesia represents a substantial healthcare burden at emergency departments. mTBI diagnosis is challenging in emergency departments practice, because neurological symptoms may be vague and the criteria for acute head computed tomography (CT) are often not fulfilled. Most patients who undergo head CT at emergency departments have a negative scan.[1] Many patients with a "mTBI" and normal CT imaging may still have a significant injury with prolonged or permanent sequelae[2]. Furthermore, intracranial mass lesions or aggressive swelling that develop after an initial normal CT scan are rare but recognized events.[1,3] MRI may allow detection of injury responsible for acute deterioration or late sequelae in CT-negative patients,[4] but the widespread use of MRI is both logistically difficult and probably cost-ineffective. Since the foundation of other specific organ based biomarkers there has been a quest for similar markers for TBI, which would help in assessing the true severity and prognosis of the eventual brain damage.[5]

Several groups have reported that central nervous system (CNS) derived blood-based biomarkers have been able to predict intracranial lesions and that they may help in diagnosis and in avoiding unnecessary CT scans.[6-10] The association of glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase-L1 (UCH-L1) with visible CT pathologies has raised a special interest.

GFAP is a cytoskeletal monomeric filament protein present in astrocytes located both in white and grey brain matter.[11] It has been suggested that GFAP has good sensitivity and specificity for intracranial findings of traumatic brain injury (TBI)[10] and it is considered to be a better marker for a focal than diffuse injury.[6,12] Two groups reported that serum levels of GFAP were increased in those patients with clinically diagnosed mTBI with abnormal CT compared to those patients with mTBI with normal CT.[7,13] They also found that GFAP levels were higher in patients with axonal injury on magnetic resonance imaging (MRI)[13] and in patients, who required neurosurgical

interventions[7]. It has earlier been reported that GFAP is not affected by extracranial fractures [14] and that it is able to distinguish both patients with mTBIs and moderate TBI (moTBI) from healthy controls and from patients with orthopedic injury without a TBI. [7]

UCH-L1 is involved in both adding and removing ubiquitin from proteins, which are set for internal metabolism within cells. [15,16] UCH-L1 is considered a suitable counterpart for GFAP in TBI diagnostics as it is produced by different cell types and UCH-L1 is found more abundant after diffuse than focal injury. [6] In patients with mTBI, serum UCH-L1 levels were increased in comparison to healthy controls and non-brain injured orthopedic patients [8] and UCH-L1 was able to discriminate between CT positive and CT negative mild TBI [8] and between healthy controls and full spectrum TBI patients. [9] However, there are also contradictory results where UCH-L1 levels were unable to distinguish healthy controls from patients with mTBI when analyzed with two different immunoassays. [17]

However, GFAP and UCH-L1 are also found outside the CNS [18,19] and variable serum levels of GFAP and UCH-L1 have been observed in non-brain injured patients in previous TBI studies [7,8,20,21]. Based on the present knowledge, the specificity of GFAP and UCH-L1 in mTBI diagnostics remains uncertain. Multiple studies have suggested that GFAP and UCH-L1 could be sensitive and specific markers of the existence and severity of brain injury in trauma, especially in case of moTBI, severe TBI, and complex mTBI. [9,14,22-24] However, the published studies include generally healthy individuals or small number of orthopedic trauma patients as controls and comparison studies with CT-negative mTBI patients are few in number. In order to further explore the performance of GFAP and UCH-L1 in discriminating patients with orthopedic trauma and CT-negative mTBI, we assessed GFAP and UCH-L1 in patients with acute orthopedic injuries without CNS involvement and related them to the type of extracranial injury, head magnetic resonance imaging (MRI) findings, and with GFAP and UCH-L1 levels in patients with CT-negative mTBI in relatively large precisely characterized populations.

MATERIALS AND METHODS

Study population

This prospective two-center study was part of the EU-funded TBIcare project (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries) project. In that project, we recruited patients with TBI of all severities as well as a control group of patients with acute orthopedic injuries at the Turku University Hospital (Finland) and at the Addenbrooke's Hospital Cambridge (United Kingdom). The analysis in this study included all 73 patients with acute orthopedic trauma and those 93 patients with mTBI who had no pathological brain parenchymal findings on head CT. Three patients with skull base fracture, but concomitant negative parenchymal findings were included in mTBI group.

Inclusion criteria for the orthopedic group were age $\geq$ 16 years, and acute orthopedic non-trivial injury or injuries without any signs of acute CNS involvement. Exclusion criteria were any suspicion of an acute TBI (injury signs in the head, any suspicion of TBI signs at the time of injury, symptoms suggesting a possible TBI), previous TBIs or brain diseases, polytrauma needing intensive care, or trivial injuries not needing acute measures or follow-up. mTBI was diagnosed if the patient had sustained a head injury, which fulfilled the ACRM criteria for TBI [26] and their lowest recorded GCS was $\geq$ 13. However, patients with GCS 13 and deteriorating or patients with GCS 13 and concomitant multitrauma needing intensive care were excluded from the mTBI group. If there was any suspicion that the signs of TBI could be caused by confounders (inebriation, medications, etc.) the patient was not included.

One patient with an orthopedic injury was sedated with propofol due to an orthopedic operation before the blood samples were obtained, while other patients did not receive anesthetics before the initial sample was obtained. None of those who had high GFAP and/or UCH-L1 levels underwent general anesthesia.

The study protocol was approved by the ethical review board of Hospital District of South-West Finland, the Cambridgeshire 2 Research Ethics and the Norfolk Research Ethics Committee.

All patients were given necessary information about the study in both oral and written form and written informed consent was obtained.

Analysis of GFAP and UCH-L1

Blood samples for GFAP and UCH-L1 were collected upon arrival and on days 1, 2, 3, 7 (when available) after the admission, and on the follow-up visit 3-10 months after the injury. We did not recruit patients during the night. In case a patient was recruited more than 24 hours after admission, samples were considered taken on day 1 after the injury. Longitudinal samples were possible to obtain from several patients with mTBI, because they were admitted due to prolonged symptoms. The samples were centrifuged for 10 minutes at 10 000 rpm at 4 ˚C and the serum was immediately frozen at -70 ˚C for further analysis.

Proteomic analyses were conducted at Randox Laboratories Ltd (Crumlin, County Antrim, United Kingdom) with Randox Biochip technology, which is a solid-state device containing an array of discrete test regions of immobilized antibodies specific to different cerebral immunoassays. The samples were prepared singly. Increased levels in a specimen led to increased binding of antibody labeled with horseradish peroxidase and thus an increase in the chemiluminescent signal emitted. The light signal generated from each of the test regions on a biochip was detected with digital imaging technology and compared with that from a stored calibration curve. The concentration of analyst present in the sample was calculated from the calibration curve. The Evidence Investigator Cerebral Custom Array IV (Randox Laboratories Ltd) was used to quantitatively test for UCH-L1 and GFAP simultaneously.

The lower limits of quantification were 0.3 ng/ml and 0.16 ng/ml and the upper limits 50 ng/ml and 100 ng/ml for UCH-L1 and GFAP, respectively. For the UCH-L1 assay coefficient of variation was 6-7% and for GFAP assay 3-4%. The samples with no detectable biomarker levels were assigned a value of zero.

MRI

Most patients with orthopedic trauma (n=52/73) underwent head magnetic resonance imaging (MRI) during the first four weeks after their injury as part of the study protocol as well as at the follow-up visit 3-10 months after the injury. The included 3T MRI sequences were T13D, T2, FLAIR, SWI, DWI and DTI.

Statistical analyses

The normality of UCH-L1, GFAP, age, and injury severity score were assessed using Kolmogorov-Smirnov test and by visually inspecting histograms. Non-parametric methods were used in the further analyses, because these variables were not normally distributed. Differences in background variables between patients with orthopedic injury and patients with mTBI were studied using Mann-Whitney U test for age and injury severity score and χ^2 test for gender. Spearman correlation coefficient was used to assess correlation between GFAP and UCH-L1 on different days in patients with orthopedic injury. Association of the biomarkers on arrival and background variables were assessed using Spearman correlation coefficients (age, injury severity score) and Mann-Whitney U test (gender).

Given that we wished to explore the ability of these protein biomarkers to distinguish CT-negative mTBI from extracranial injury, we chose not to define diagnostic thresholds based on values from healthy subjects, but on values obtained in patients with extracranial orthopedic injuries. The cutoff value for an individual biomarker was set at the 95th percentile, which is determined theoretically

based on a previous publication [28] in order to provide a basis to undertake more detailed exploratory analysis in subjects with high levels of UCH-L1 and GFAP in the orthopedic injury population.

MRI findings were also studied in the population of the orthopedic injury. MRI findings were categorized as MRI not done, normal MRI findings, and abnormal MRI finding. Differences in the biomarkers between these categories were studied with Kruskal-Wallis test. Mann-Whitney U test was used to study differences in GFAP and UCH-L1 values between patients with orthopedic injuries and patients with mTBI. Ability of the biomarkers to differentiate these two patient groups was evaluated using the receiver operating characteristics curve (ROC) and the area under the ROC curve (AUC). Levels of UCH-L1 and GFAP on arrival were compared to levels on day 1 and follow-up with Wilcoxon Signed-Rank test. Differences in biomarker levels between patients with orthopedic injuries, patients with CT-negative mTBI and concomitant orthopedic injuries, and patients with isolated CT-negative mTBI were studied using Kruskal-Wallis test. Data were analyzed using IBM SPSS Statistics 22 (IBM Corp, New York, USA) and Matlab R2012b (MathWorks, Natick, MA, USA).

RESULTS

The demographic features, mechanisms of injuries, injury severity scores [29], and injury types of the two study groups are shown in Table 1. The mean age of the patients with orthopedic injury was 46.7 ± 18.3 years and the majority was female (55%). In patients with orthopedic injury, the most common injuries were ankle fractures 29 % (n=21), upper or lower extremity soft tissue contusions and bruises 14% (n=10), and wrist fractures 10% (n=7). The mean age of the patients with CT-negative mTBI was 42.1 ± 18.6 years and the majority was male (63%). In the mTBI group, 43 patients (47%) had a concomitant orthopedic injury.

Levels of UCH-L1 and GFAP

Most of the patients with orthopedic injury had GFAP and UCH-L1 levels available upon arrival, on the following day and at the follow-up visit.

Figure 1 presents biomarker levels on different days in patients with orthopedic trauma. Figure 2 shows scatter plots of UCH-L1 and GFAP and Spearman correlation coefficients between the biomarkers in the same subjects. Multiple subjects had identical values of UCH-L1 and GFAP, thus, the number of plotted dots in the panels of Figure 2 seems to be smaller than the number of subjects. Significant correlations between UCH-L1 and GFAP levels were found upon arrival (Spearman rho 0.739, p<0.001), on day 1 (Spearman rho 0.544, p=0.004) and at the follow-up visit (Spearman rho 0.382, p=0.007). Supplemental Figures 1 and 2 show relations between the biomarkers and gender, age, and injury severity score in patients with orthopedic injury and patients with CT-negative mTBI on arrival day. Female patients with orthopedic injury had significantly higher levels of UCH-L1 than male patients with orthopedic injury (p=0.036). There were no other demographic associations with biomarker values.

Table 2 shows in more detail the biomarker levels of those patients with orthopedic injuries who presented with GFAP or UCH-L1 levels in the 95th percentile (n=6, 8%, UCH-L1 ≥2.74 ng/ml or GFAP ≥3.61 ng/ml) along with details of their demographics, comorbidities, current and previous injuries, and MRI findings. All patients were females and had injuries in the extremities. Four out of six subjects (67%) showed levels for both GFAP and UCH-L1 in the 95th percentile. Five out of six patients (83%) had fractures in the distal part of the extremities, while one patient had only superficial injuries of the extremities. Of those six patients who showed high levels during the first post-injury week, five (83%) showed an elevated level of either one or both biomarkers also at the follow-up visit (Table 2).

Within the CT-negative mTBI group, only one patient showed biomarker levels, which were in the 95th percentile defined in the orthopedic controls (UCH-L1 ≥2.74 ng/ml or GFAP ≥3.61 ng/ml).

This patient was a previously healthy 21-years-old male who did not have extracranial injuries. His UCH-L1 level was 3.50 ng/ml and GFAP 0 ng/ml on arrival day, while the corresponding levels were 0.80 ng/ml and 1.31 ng/ml on the follow-up visit. Those three patients who had a skull base fracture without intracranial abnormalities did not have biomarker values differing from other patients with mTBI.

MRI findings

In patients with orthopedic trauma, 52 patients (71%) underwent MRI imaging of the head. Thirty patients (58%) had normal findings, the rest showing non-specific ischemic-degenerative changes or other insignificant abnormalities, and in one case an old contusion was suspected. None was found to have any imaging changes compatible with an acute TBI. Four out of six patients (67%) with levels of UCH-L1 or GFAP in the 95th percentile underwent head MRI and their findings were normal. The only patient with mTBI, whose UCH-L1 level was in the 95th percentile on arrival had normal head MRI.

Table 3 presents the MRI findings in the patients with orthopedic injuries. Table 4 shows levels of UCH-L1 and GFAP in three MRI finding classes in the population of the orthopedic patients: MRI not done, normal MRI findings, and abnormal MRI findings. Levels of UCH-L1 and GFAP did not differ significantly between the three classes.

Comparison between patients with orthopedic trauma and CT-negative mTBI

The levels of GFAP were higher in patients with orthopedic trauma as compared to patients with CT-negative mTBI on arrival day (p=0.026), but there were no difference on the following days (Figure 3). The levels of UCH-L1 were not significantly different between patients with CT-negative mTBI and patients with orthopedic injury. As a result of this, GFAP levels on arrival day were able to modestly discriminate the patient groups in receiver operating characteristic analysis (AUC=0.629, 95% CI 0.514, 0.731) (Table 5).

Table 6 shows differences in UCH-L1 and GFAP levels over time. Levels of UCH-L1 were significantly lower on day 1 and follow-up than on arrival day in patients with CT-negative mTBI, while significant differences in the levels of GFAP were not observed. There were no significant differences over time in the levels of GFAP and UCH-L1 in patients with orthopedic injury, Figure 4 presents changes in levels of UCH-L1 and GFAP between the arrival and follow-up in CT-negative mTBI and orthopedic injury populations. There were no statistically significant differences in levels of GFAP and UCH-L1 between patients with orthopedic injuries, patients with CT-negative mTBI and concomitant orthopedic injuries, and patients with isolated CT-negative mTBI (Supplemental Figure 3).

DISCUSSION

This prospective, observational, two-center study assessed the serum levels of GFAP and UCH-L1 in patients with acute orthopedic injury and compared the results to patients with CT-negative mTBI. Our main finding was that the levels of these biomarkers were unable in a clinically relevant sense to separate these groups and thus provide any diagnostic benefit for the common problem if a patient with acute injury has a concomitant TBI or not. Additionally, the levels of the biomarkers were not able to discriminate patients with orthopedic injuries, patients with CT-negative mTBI and concomitant orthopedic injuries, and patients with isolated CT-negative mTBI. Another main finding was that in patients with orthopedic injury, high levels of these biomarkers tended to persist between the acute phase and follow-up visit several months later, suggesting that some people have clearly higher levels than others, irrespective of any injury. All the patients with orthopedic injury who had biomarker levels in the 95th percentile were females with injuries in the extremities. Of 52 patients with orthopedic injuries who underwent MRI imaging of the head, 30 patients had normal findings while the rest showed non-specific ischemic-degenerative changes or other insignificant abnormalities. None of these findings were suggestive of an acute TBI, which was carefully

excluded also clinically. Additionally, the only patient with CT-negative mTBI and high biomarker value had normal MRI findings.

There are many previous studies that have explored the correlation of GFAP and UCH-L1 levels with different severity classes of TBIs, while the validation in mTBI is incomplete as the controls have generally been healthy volunteers, and where non-CNS trauma controls have been used, numbers are small and patients poorly characterized, and the comparison has generally been with moTBI and severe TBI rather than with mTBI – which is the real diagnostic differentiation we are seeking in the current study. Several studies have explored the potential of GFAP and UCH-L1 in the diagnostics and outcome prediction of TBI with promising results. [7,8,24,30,31] In some studies, which have included orthopedic injury patients without TBI, low but measurable levels of GFAP [7,21,32,33] and UCH-L1 [8,21,22,31] have been detected in these patients. In two studies, the levels of GFAP [7] and UCH-L1 [8] were higher in patients with orthopedic trauma than in uninjured controls, and there were significant differences between the uninjured controls and all other groups, including orthopedic controls and different severities of TBI. [7,8] Papa et al. reported that GFAP breakdown product levels were significantly higher in patients with CT-positive findings than those with CT-negative finding irrespective of orthopedic injury, mTBI, or moTBI. Furthermore, they reported that patients with CT-negative mTBI and moTBI had significantly higher levels of GFAP breakdown product levels than patients with orthopedic injury, although they did not provide median and p values. [7] Another study by the same group investigating the performance of UCH-L1 in patients from the same centers, reported that UCH-L1 levels in patients with CT-negative TBI (mTBI and moTBI) had higher levels of UCH-L1 than trauma controls with a negative CT (p=0.057). [8] These findings are inconsistent with our current results. However, another earlier study reported that UCH-L1 levels could not distinguish uninjured controls from patients with mTBI when analyzed with two different immunoassays. [17] In a recent publication Papa et al. reported very promising results regarding performance of GFAP and UCH-L1 in discrimination of patients with

orthopedic injuries and patients with CT-negative and CT-positive mTBI and moTBI. [21] The study differs from our current study in terms of the TBI populations, but in some of their orthopedic trauma controls Papa and colleagues detected high UCH-L1 levels (range 0.045-4.241 ng/ml), which is parallel with our findings.

We used a different assay than that used by these authors, which may make cross study comparisons (e.g. our controls with those from other studies) difficult. However, the key findings of our analysis should not be affected, since it relies on within-study comparisons performed using a single analytic platform. We found that in patients with orthopedic trauma, there were significant correlations between GFAP and UCH-L1 levels on arrival day, day 1, and on the follow-up visit. The only significant difference in the biomarker levels over time was observed in patients with CT-negative mTBI: the levels of UCH-L1 were significantly lower on day 1 and follow-up than on arrival day. Our results suggest that the relatively high levels of GFAP and UCH-L1 found in some of the patients with orthopedic trauma are not related to their acute injury, since the injuries were very heterogeneous and the levels were still elevated during the outcome visit several months after the injury. Excluding the recent paper by Papa [21] the other aforementioned groups did not measure GFAP and UCH-L1 levels of patients with orthopedic trauma at different time points and they did not assess their correlations.

An interesting finding is that all the patients with orthopedic injury who had GFAP and UCH-L1 levels in the 95th percentile were females and most of them had persistently high biomarker values on the follow-up visit. On arrival, female patients with orthopedic injury had significantly higher levels of UCH-L1 than male patients. The reason for this difference remains unexplained. However, when both genders were analyzed together, patients with orthopedic injury had higher GFAP levels than patients with mTBI, while no statistical difference was observed in UCH-L1 levels on arrival.

Both GFAP and UCH-L1 were originally presumed to be specific to the CNS, but subsequently GFAP has been detected also in non-glial and non-CNS cells, such as Schwann cells [34], chondrocytes [35], fibroblasts [35], myoepithelial cells [36], lymphocytes [37] and liver stellate cells [38, 39], whereas expression of UCH-L1 outside the CNS has been reported in cells of testis, ovaries, and kidney [15,19]. Based on studies on knockout mice, UCH-L1 appears to have an integral role in the structure and function of the neuromuscular junction. [40] Despite this, GFAP and UCH-L1 have generally been considered specific for TBI in terms of TBI diagnostics. [7-9,33] The most studied astroglial TBI biomarker S100β is a Ca^{2+}-binding protein that regulates intracellular levels of calcium in glia and oligodendrocytes. [41] S100β remains a promising diagnostic tool for TBI as it has excellent negative predictive value for pathological intracranial CT findings [42], but its lack of specificity to intracranial injuries has caused concerns. [43-45] Like GFAP and UCH-L1, expression of S100β has been detected outside of CNS, such as in adipocytes and chondrocytes, [41] but it has been reported to be inferior in detecting intracranial injuries in comparison to GFAP in patients with mTBI[9] and sTBI[8,22] and in multitrauma patients with TBI[23].

Even though GFAP and UCH-L1 may mostly be of CNS origin, they are not TBI-specific. Elevated levels of GFAP and UCH-L1 have been reported after seizures [46,47] and stroke [48]. High plasma levels of GFAP have been associated with neuroepithelial tumors, such gliomas [49] and ependymomas [50]. However, in a recent study, Kiviniemi and coworkers investigated the association of GFAP with prognostic markers in patients with high-grade gliomas. In their healthy control population, all subjects but one had unmeasurable GFAP levels (detection limit 0.014 ng/ml). This subject had substantially higher level of GFAP than the median level of patients with high-grade glioma. [20]

We recently reported that in a TBI population of all severities, UCH-L1 and GFAP levels were able to discriminate patients with unfavorable outcome (Glasgow outcome scale 1-3) from patients with favorable outcome (Glasgow outcome scale 4-5). In that study, the cut-off value of UCH-L1 for

unfavorable outcome was 1.03 ng/ml, [27] while a cut-off value of 1.89 ng/ml has been reported to predict in-hospital mortality in another study. [6] Similarly, we reported a cut-off value of GFAP for unfavorable outcome at 1.26 ng/ml, [27] while values above 1.5 ng/ml were reported to be predictive of mortality by another group. [51] The median levels of UCH-L1 and GFAP in our orthopedic injury population are relatively comparable to those reported earlier by Papa et al. [7,8] Intriguingly the circulating levels obtained in our study are consistent with those from the aforementioned research. Thus, our results show that the patients with acute orthopedic injury may often show levels of GFAP and UCH-L1 that overlap with those obtained from patients with mTBI and, if used as a diagnostic tool, predispose these patients to unwarranted diagnostics and recurrent head imaging. This paper does not address the role of these biomarkers in assessing different subgroups of patients with mTBI or more severe TBI.

All patients with levels of UCH-L1 and GFAP in the 95th percentile, who had MRI available (67%), had normal imaging findings. None of these patients had history of epilepsy or seizures related to their current injuries and none them had intracranial tumors. Based on this finding, it seems obvious that elevated levels of these biomarkers do not reflect any acute brain condition in these patients. This raises three possible explanations and interferences.

1) A potential extracerebral origin for these elevations might be supported by the results from previous studies indicating that the levels of GFAP [7] and UCH-L1 [8] were higher in patients with orthopedic injuries than in healthy controls. Hainfeller and coworkers reported staining of chondrocytes and fibroblasts with anti-GFAP antibodies in epiglottis and ligamentum flavum *in vitro*. [35] Fellenberg et al reported that UCH-L1 was richly expressed in bone marrow-derived mesenchymal cell samples [52], and Hsu and coworkers were able to detect UCH-L1 in skin fibroblasts of patients with spinal muscular atrophy *in vitro* [53]. Thus, one potential explanation for our current results is the expression of GFAP and UCH-L1 in chondrocytes and fibroblasts of extremity joint cartilage and bone marrow, which results in serum elevation of GFAP and UCH-L1

in peripheral blood after traumatic fractures. This explanation can be brought further by a possibility of traumatic peripheral neuropathy, because GFAP and UCH-L1 may be expressed in Schwann cells and the neuromuscular junction. [34,40] Regrettably we have no clinical data available on possible development of symptoms of neuropathy and electrophysiological tests were not undertaken in these patients, why this explanation remains theoretical.

2) Some orthopedic patients might had a concomitant mTBI by current definitions that remained undetected. However, this is very unlikely as none of these patients had a history suggesting any TBI – i.e. all diagnostic criteria for a TBI were lacking, as was any suspicion for the presence of these criteria. In addition, none of the controls showed external signs of head or neck injury, or showed any symptoms suggesting a possible concussion, or were involved in a high-energy trauma. Also their MRI showed no acute changes, even in patients with elevated biomarkers. Furthermore, as the controls were recruited in the study they were carefully interviewed about the details of their injury and their patient history reviewed.

3) Some of the orthopedic patients had a non-traumatic CNS insult caused by the consequences of peripheral trauma – e.g. the cytokine and neurohumoral storm associated with their orthopedic injury. It has been previously reported that orthopedic injuries may result in elevated proinflammatory interleukin-1β and interleukin-8 production leading to CNS inflammation response. [54,55]

Since in both orthopedic injury and mTBI groups several subjects continued to show elevated levels several months after the injury – substantially later than could be explained with the half-life of these biomarkers in blood – it seems more likely that the individual high levels seen in our study are not related to their acute injuries.

Even though these data provides new insights concerning biochemical diagnostic challenges in diagnostics of CT-negative mTBI, and demonstrates for the first time that some patients seem to

exhibit high levels of GFAP and UCH-L1 irrespective of any injury that tend to persist, the authors recognize that there are limitations to this study. In this study, levels of biomarkers were not compared with healthy controls, which would have given more information about possible differences between uninjured patients and studied populations. Another limitation is that we have used custom arrays in assessments of biomarkers, and therefore, the manufacturer has not been able to provide values for the lower limit of detection. Furthermore, we did not recruit patients during the night, which is why some of the first samples are taken on day 1. On the other hand, many patients with milder injuries do not seek for medical attention immediately but only after a variable delay.

Our main finding was that GFAP and UCH-L1 are not specific biomarkers for mTBI and their levels are not clinically relevantly able to discriminate patients with CT-negative mTBI and patients with acute orthopedic trauma. Indeed, many patients with orthopedic injury had higher biomarker levels than patients with CT-negative mTBI. The source of high GFAP and UCH-L1 levels in these patients remains unknown, but persistent elevation several months after the injury suggests a source, which is not related to the injury. Our results cast a significant doubt on the diagnostic value of these biomarkers in the differential diagnostics of CT-negative mTBI.

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AUTHOR DISCLOSURE STATEMENT

REFERENCES

1. Isokuortti, H., Luoto, T., Kataja, A., Brander, A., Siironen, J., Liimatainen, S., Iverson, G., Ylinen, A., and Ohman, J. (2014). Necessity of monitoring after negative head CT in acute head injury. Injury 45, 1340-4.

2. Sigurdardottir, S., Andelic, N., Roe, C., Jerstad, T., and Schanke, A. (2009). Post-concussion symptoms after traumatic brain injury at 3 and 12 months post-injury: a prospective study. Brain Inj 23, 489-97.

3. Carlson, A. P., Ramirez, P., Kennedy, G., McLean, A. R., Murray-Krezan, C., and Stippler, M. (2010). Low rate of delayed deterioration requiring surgical treatment in patients transferred to a tertiary care center for mild traumatic brain injury. Neurosurg Focus 29, E3.

4. Yuh, E. L., Mukherjee, P., Lingsma, H. F., Yue, J. K., Ferguson, A. R., Gordon, W. A., Valadka, A. B., Schnyer, D. M., Okonkwo, D. O., Maas, A. I., Manley, G. T., and TRACK-TBI Investigators. (2013). Magnetic resonance imaging improves 3-month outcome prediction in mild traumatic brain injury. Ann Neurol 73, 224-235.

5. Bakay, R. A., Sweeney, K. M., and Wood, J. H. (1986). Pathophysiology of cerebrospinal fluid in head injury: Part 2. Biochemical markers for central nervous system trauma. Neurosurgery 18, 376-382.

6. Mondello, S., Papa, L., Buki, A., Bullock, M. R., Czeiter, E., Tortella, F. C., Wang, K. K., and Hayes, R. L. (2011). Neuronal and glial markers are differently associated with computed tomography findings and outcome in patients with severe traumatic brain injury: a case control study. Crit Care 15, R156.

7. Papa, L., Lewis, L. M., Falk, J. L., Zhang, Z., Silvestri, S., Giordano, P., Brophy, G. M., Demery, J. A., Dixit, N. K., Ferguson, I., Liu, M. C., Mo, J., Akinyi, L., Schmid, K., Mondello, S., Robertson, C. S., Tortella, F. C., Hayes, R. L., and Wang, K. K. (2012). Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. Ann Emerg Med 59, 471-483.

8. Papa, L., Lewis, L. M., Silvestri, S., Falk, J. L., Giordano, P., Brophy, G. M., Demery, J. A., Liu, M. C., Mo, J., Akinyi, L., Mondello, S., Schmid, K., Robertson, C. S., Tortella, F. C., Hayes, R. L., and Wang, K.

K. (2012). Serum levels of ubiquitin C-terminal hydrolase distinguish mild traumatic brain injury from trauma controls and are elevated in mild and moderate traumatic brain injury patients with intracranial lesions and neurosurgical intervention. J Trauma Acute Care Surg 72, 1335-1344.

9. Diaz-Arrastia, R., Wang, K. K., Papa, L., Sorani, M. D., Yue, J. K., Puccio, A. M., McMahon, P. J., Inoue, T., Yuh, E. L., Lingsma, H. F., Maas, A. I., Valadka, A. B., Okonkwo, D. O., Manley, G. T., and TRACK-TBI Investigators. (2014). Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. J Neurotrauma 31, 19-25.

10. Lumpkins, K. M., Bochicchio, G. V., Keledjian, K., Simard, J. M., McCunn, M., and Scalea, T. (2008). Glial fibrillary acidic protein is highly correlated with brain injury. J Trauma 65, 778-82; discussion 782-4.

11. Eng, L. F., Vanderhaeghen, J. J., Bignami, A., and Gerstl, B. (1971). An acidic protein isolated from fibrous astrocytes. Brain Res 28, 351-354.

12. Pelinka, L. E., Kroepfl, A., Leixnering, M., Buchinger, W., Raabe, A., and Redl, H. (2004). GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome. J Neurotrauma 21, 1553-1561.

13. Metting, Z., Wilczak, N., Rodiger, L. A., Schaaf, J. M., and van der Naalt, J. (2012). GFAP and S100B in the acute phase of mild traumatic brain injury. Neurology 78, 1428-33.

14. Pelinka, L. E., Kroepfl, A., Schmidhammer, R., Krenn, M., Buchinger, W., Redl, H., and Raabe, A. (2004). Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. J Trauma 57, 1006-1012.

15. Wilkinson, K. D., Lee, K. M., Deshpande, S., Duerksen Hughes, P., Boss, J. M., and Pohl, J. (1989). The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science 246, 670-3.

16. Liu, Y., Fallon, L., Lashuel, H. A., Liu, Z., and Lansbury, P. T.,Jr. (2002). The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. Cell 111, 209-218.

17. Puvenna, V., Brennan, C., Shaw, G., Yang, C., Marchi, N., Bazarian, J. J., Merchant-Borna, K., and Janigro, D. (2014). Significance of ubiquitin carboxy-terminal hydrolase L1 elevations in athletes after sub-concussive head hits. PLoS One 9, e96296.

18. Viale, G., Doglioni, C., Dell'Orto, P., Zanetti, G., Iuzzolino, P., Bontempini, L., and Coggi, G. (1988). Glial fibrillary acidic protein immunoreactivity in human respiratory tract cartilages and pulmonary chondromatous hamartomas. Am J Pathol 133, 363-373.

19. Meyer Schwesinger, C., Meyer, T. N., Münster, S., Klug, P., Saleem, M., Helmchen, U., and Stahl, R. A. K. (2009). A new role for the neuronal ubiquitin C-terminal hydrolase-L1 (UCH-L1) in podocyte process formation and podocyte injury in human glomerulopathies. J Pathol 217, 452-64.

20. Kiviniemi, A., Gardberg, M., Frantzén, J., Parkkola, R., Vuorinen, V., Pesola, M., and Minn, H. (2015). Serum levels of GFAP and EGFR in primary and recurrent high-grade gliomas: correlation to tumor volume, molecular markers, and progression-free survival. J Neurooncol 124, 237-45.

21. Papa, L., Brophy, G., Welch, R., Lewis, L., Braga, C., Tan, C., Ameli, N., Lopez, M., Haeussler, C., Mendez Giordano, D., Silvestri, S., Giordano, P., Weber, K., Hill Pryor, C., and Hack, D. (2016). Time Course and Diagnostic Accuracy of Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 in a Large Cohort of Trauma Patients With and Without Mild Traumatic Brain Injury. JAMA Neurol 73, 551-560.

22. Papa, L., Akinyi, L., Liu, M. C., Pineda, J. A., Tepas, J. J.,3rd, Oli, M. W., Zheng, W., Robinson, G., Robicsek, S. A., Gabrielli, A., Heaton, S. C., Hannay, H. J., Demery, J. A., Brophy, G. M., Layon, J., Robertson, C. S., Hayes, R. L., and Wang, K. K. (2010). Ubiquitin C-terminal hydrolase is a novel biomarker in humans for severe traumatic brain injury. Crit Care Med 38, 138-144.

23. Brophy, G. M., Mondello, S., Papa, L., Robicsek, S. A., Gabrielli, A., Tepas, J.,3rd, Buki, A., Robertson, C., Tortella, F. C., Hayes, R. L., and Wang, K. K. (2011). Biokinetic analysis of ubiquitin C-terminal hydrolase-L1 (UCH-L1) in severe traumatic brain injury patient biofluids. J Neurotrauma 28, 861-870.

24. Okonkwo, D. O., Yue, J. K., Puccio, A. M., Panczykowski, D. M., Inoue, T., McMahon, P. J., Sorani, M. D., Yuh, E. L., Lingsma, H. F., Maas, A. I., Valadka, A. B., Manley, G. T., and Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Investigators. (2013). GFAP-BDP as an acute diagnostic marker in traumatic brain injury: results from the prospective transforming research and clinical knowledge in traumatic brain injury study. J Neurotrauma 30, 1490-1497.

25. Marshall, L. F., Marshall, S.B., Klauber, M.R., van Berkum Clark, M., Eisenberg, H. M., Jane, J. A., Luerssen, T. G., Marmarou, A., and Foulkes, M. A. (1991). **A new classification of head injury based on computerized tomography**. J Neurosurg 75, S14-S20.

26. The American Congress of Rehabilitation Medicine (ACRM). (1993). Definition of mild traumatic brain injury. J Head Trauma Rehabil 8, 86-87.

27. Takala, R. S., Posti, J. P., Runtti, H., Newcombe, V. F., Outtrim, J., Katila, A. J., Frantzen, J., Ala-Seppala, H., Kyllonen, A., Maanpaa, H. R., Tallus, J., Hossain, M. I., Coles, J. P., Hutchinson, P., van Gils, M., Menon, D. K., and Tenovuo, O. (2015). GFAP and UCH-L1 as outcome predictors in traumatic brain injury. World Neurosurg

28. Biberthaler, P., Linsenmeier, U., Pfeifer, K., Kroetz, M., Mussack, T., Kanz, K., Hoecherl, E. F. J., Jonas, F., Marzi, I., Leucht, P., Jochum, M., and Mutschler, W. (2006). Serum S-100B concentration provides additional information fot the indication of computed tomography in patients after minor head injury: a prospective multicenter study. Shock 25, 446-453.

29. Baker, S. P., O'Neill, B., Haddon, W.,Jr, and Long, W. B. (1974). The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. J Trauma 14, 187-196.

30. Nylén, K., Ost, M., Csajbok, L. Z., Nilsson, I., Blennow, K., Nellgård, B., and Rosengren, L. (2006). Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. J Neurol Sci 240, 85-91.

31. Mondello, S., Linnet, A., Buki, A., Robicsek, S., Gabrielli, A., Tepas, J., Papa, L., Brophy, G. M., Tortella, F., Hayes, R. L., and Wang, K. K. (2012). Clinical utility of serum levels of ubiquitin C-terminal hydrolase as a biomarker for severe traumatic brain injury. Neurosurgery 70, 666-675.

32. Honda, M., Tsuruta, R., Kaneko, T., Kasaoka, S., Yagi, T., Todani, M., Fujita, M., Izumi, T., and Maekawa, T. (2010). Serum glial fibrillary acidic protein is a highly specific biomarker for traumatic brain injury in humans compared with S-100B and neuron-specific enolase. J Trauma 69, 104-109.

33. Papa, L., Silvestri, S., Brophy, G. M., Giordano, P., Falk, J. L., Braga, C. F., Tan, C. N., Ameli, N. J., Demery, J. A., Dixit, N. K., Mendes, M. E., Hayes, R. L., Wang, K. K., and Robertson, C. S. (2014). GFAP Out-Performs S100beta in Detecting Traumatic Intracranial Lesions on Computed Tomography in Trauma Patients with Mild Traumatic Brain Injury and Those with Extracranial Lesions. J Neurotrauma 31, 1815-1822.

34. Jessen, K. R., Thorpe, R., and Mirsky, R. (1984). Molecular identity, distribution and heterogeneity of glial fibrillary acidic protein: an immunoblotting and immunohistochemical study of Schwann cells, satellite cells, enteric glia and astrocytes. J Neurocytol 13, 187-200.

35. Hainfellner, J. A., Voigtlander, T., Strobel, T., Mazal, P. R., Maddalena, A. S., Aguzzi, A., and Budka, H. (2001). Fibroblasts can express glial fibrillary acidic protein (GFAP) in vivo. J Neuropathol Exp Neurol 60, 449-461.

36. Viale, G., Gambacorta, M., Coggi, G., Dell'Orto, P., Milani, M., and Doglioni, C. (1991). Glial fibrillary acidic protein immunoreactivity in normal and diseased human breast. Virchows Arch A Pathol Anat Histopathol 418, 339-348.

37. Riol, H., Tardy, M., Rolland, B., Levesque, G., and Murthy, M. R. (1997). Detection of the peripheral nervous system (PNS)-type glial fibrillary acidic protein (GFAP) and its mRNA in human lymphocytes. J Neurosci Res 48, 53-62.

38. Carotti, S., Morini, S., Corradini, S. G., Burza, M. A., Molinaro, A., Carpino, G., Merli, M., De Santis, A., Muda, A. O., Rossi, M., Attili, A. F., and Gaudio, E. (2008). Glial fibrillary acidic protein as an early marker of hepatic stellate cell activation in chronic and posttransplant recurrent hepatitis C. Liver Transpl 14, 806-814.

39. Middeldorp, J., and Hol, E. M. (2011). GFAP in health and disease. Prog Neurobiol 93, 421-443.

40. Chen, F., Sugiura, Y., Myers, K., Liu, Y., and Lin, W. (2010). Ubiquitin carboxyl-terminal hydrolase L1 is required for maintaining the structure and function of the neuromuscular junction. Proc Natl Acad Sci U S A 107, 1636-1641.

41. Olsson, B., Zetterberg, H., Hampel, H., and Blennow, K. (2011). Biomarker-based dissection of neurodegenerative diseases. Prog Neurobiol 95, 520-534.

42. Romner, B., Ingebrigtsen, T., Kongstad, P., and Borgesen, S. E. (2000). Traumatic brain damage: serum S-100 protein measurements related to neuroradiological findings. J Neurotrauma 17, 641-647.

43. Rothoerl, R. D., Woertgen, C., Holzschuh, M., Metz, C., and Brawanski, A. (1998). S-100 serum levels after minor and major head injury. J Trauma 45, 765-767.

44. Romner, B., and Ingebrigtsen, T. (2001). High serum S100B levels for trauma patients without head injuries. Neurosurgery 49, 1490; author reply 1492-3.

45. Savola, O., Pyhtinen, J., Leino, T. K., Siitonen, S., Niemela, O., and Hillbom, M. (2004). Effects of head and extracranial injuries on serum protein S100B levels in trauma patients. J Trauma 56, 1229-34; discussion 1234.

46. Gurnett, C., Landt, M., and Wong, M. (2003). Analysis of cerebrospinal fluid glial fibrillary acidic protein after seizures in children. Epilepsia 44, 1455-1458.

47. Mondello, S., Palmio, J., Streeter, J., Hayes, R., Peltola, J., and Jeromin, A. (2012). Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is increased in cerebrospinal fluid and plasma of patients after epileptic seizure. BMC Neurol 12, 85-85.

48. Ren, C., Kobeissy, F., Alawieh, A., Li, N., Zibara, K., Zoltewicz, S., Guingab Cagmat, J., Larner, S., Ding, Y., Hayes, R., Ji, X., and Mondello, S. (2016). Assessment of Serum UCH-L1 and GFAP in Acute Stroke Patients. Sci Rep 6, 24588-24588.

49. Jung, C. S., Foerch, C., Schänzer, A., Heck, A., Plate, K. H., Seifert, V., Steinmetz, H., Raabe, A., and Sitzer, M. (2007). Serum GFAP is a diagnostic marker for glioblastoma multiforme. Brain 130, 3336-3341.

50. Ilhan, A., Furtner, J., Birner, P., Rössler, K., Marosi, C., and Preusser, M. (2011). Myxopapillary ependymoma with pleuropulmonary metastases and high plasma glial fibrillary acidic protein levels. J Clin Oncol 29, e756-e757.

51. Vos, P. E., Lamers, K. J., Hendriks, J. C., van Haaren, M., Beems, T., Zimmerman, C., van Geel, W., de Reus, H., Biert, J., and Verbeek, M. M. (2004). Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. Neurology 62, 1303-1310.

52. Fellenberg, J., Lehner, B., and Witte, D. (2010). Silencing of the UCHL1 gene in giant cell tumors of bone. Int J Cancer 127, 1804-1812.

53. Hsu, S., Lai, M., Er, T., Yang, S., Hung, C., Tsai, H., Lin, Y., Chang, J., Lo, Y., and Jong, Y. (2010). Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) regulates the level of SMN expression through ubiquitination in primary spinal muscular atrophy fibroblasts. Clin Chim Acta 411, 1920-8.

54. Chuang, D., Power, S. E., Dunbar, P. R., and Hill, A. G. (2005). Central nervous system interleukin-8 production following neck of femur fracture. ANZ J Surg 75, 813-816.

55. Cape, E., Hall, R. J., van Munster, B. C., de Vries, A., Howie, S. E., Pearson, A., Middleton, S. D., Gillies, F., Armstrong, I. R., White, T. O., Cunningham, C., de Rooij, S. E., and MacLullich, A. M. (2014).

Cerebrospinal fluid markers of neuroinflammation in delirium: a role for interleukin-1beta in delirium after hip fracture. J Psychosom Res 77, 219-225.