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# CSF and Plasma Amyloid- $\beta$ Temporal Profiles and Relationships with Neurological Status and Mortality after Severe Traumatic Brain Injury

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The role of amyloid- $\beta$  (A $\beta$ ) neuropathology and its significant changes in biofluids after traumatic brain injury (TBI) is still debated. We used ultrasensitive digital ELISA approach to assess amyloid- $\beta_{1-42}$  (A $\beta$ 42) concentrations and time-course in cerebrospinal fluid (CSF) and in plasma of patients with severe TBI and investigated their relationship to injury characteristics, neurological status and clinical outcome. We found decreased CSF A $\beta$ 42 levels in TBI patients acutely after injury with lower levels in patients who died 6 months post-injury than in survivors. Conversely, plasma A $\beta$ 42 levels were significantly increased in TBI with lower levels in patients who survived. A trend analysis showed that both CSF and plasma A $\beta$ 42 levels strongly correlated with mortality. A positive correlation between changes in CSF A $\beta$ 42 concentrations and neurological status as assessed by Glasgow Coma Scale (GCS) was identified. Our results suggest that determination of A $\beta$ 42 may be valuable to obtain prognostic information in patients with severe TBI as well as in monitoring the response of the brain to injury.

Studies in Alzheimer disease (AD) have highlighted the utility of CSF amyloid- $\beta$  peptide (A $\beta$ ) as a 'state marker' of the disease, reliably reflecting AD pathology<sup>1,2</sup>. Recently, an increasing body of literature has shown potential links between traumatic brain injury (TBI) and forms of neurodegeneration such as Alzheimer disease<sup>3-5</sup>. Recent studies have also shown significant changes in brain extracellular amyloid- $\beta$  dynamics in patients with severe brain injury, either in fluids or tissue<sup>6-8</sup>. The 42-amino acid form of amyloid- $\beta_{1-42}$  (A $\beta$ 42) is of special interest; this form appears to have the greatest propensity to deposit into insoluble plaques, one of the pathological hallmarks of Alzheimer's disease, as well as to aggregate into oligomeric A $\beta$  species and is deemed to underlie the neurodegeneration/neurotoxicity observed in AD in combination with other molecular targets and biomarkers such as tau<sup>9</sup>. In this study we have used a novel ultrasensitive digital ELISA (Single Molecule Arrays, SiMoA) to assess amyloid- $\beta_{1-42}$  (A $\beta$ 42) concentrations in CSF and matching plasma samples of patients with severe traumatic brain injury (TBI) and correlated results with injury characteristics, neurological status and clinical outcome. The developed ELISA for A $\beta$ 42 has been analytically qualified and validated and shows no matrix interference and good precision and accuracy.

## Results

**Study population.** A total of 12 patients with severe TBI and 20 controls were included for analyses. The clinical and demographic characteristics of the patients are summarized in Table 1. Patients with severe TBI had similar percentage of diffuse injury and focal mass lesion as well as survival/mortality rate (Table 1). In the control population (n = 20), 100% were men, and the average age was 26  $\pm$  4 years. Except for the age (p = 0.001), there were no significant differences in the characteristics between control subjects and TBI patients.

**CSF and plasma concentrations of A $\beta$ 42 acutely after injury.** The median CSF and plasma concentrations of A $\beta$ 42 acutely after injury for patients with severe TBI and for controls are shown in Table 2. A $\beta$ 42 concentrations



**Table 1 | Summary of Demographic and Clinical Data for Severe Traumatic Brain Injury cases included in the study**

	sTBI (n = 12)
<b>Age</b> , years, mean (SD)	49 (17.6)
<b>F/M</b> , n (%)	1/11 (8/92)
<b>GCS</b> , median (range)*	7 (3–8)
<b>Time to first sample withdrawal</b> , h, median (range)	15 (5–24)
<b>Mechanism of injury</b> , n (%)	
Motor vehicle	4 (33)
Motor cycle	1 (8)
Fall	5 (42)
Other	2 (17)
<b>CT classification</b> , n (%)*	
<b>Diffuse injury</b>	6 (50)
I	-
II	5 (42)
III	-
IV	1 (8)
<b>Focal Mass Lesion</b>	6 (50)
V	1 (8)
VI	5 (42)
<b>Outcome GOSE 6 mo</b> , n (%)	
<b>Poor outcome</b>	6 (50)
1	6 (50)
<b>Good outcome</b>	6 (50)
5	2 (17)
7	1 (8)
8	3 (25)

Abbreviations: GCS = Glasgow Coma Scale; CT = Computed Tomography; GOSE = Glasgow Outcome Score Extended.  
\*At the time of admission.

in CSF were significantly lower in TBI patients than in controls ( $p < 0.0001$ ); in contrast, plasma concentrations of A $\beta$ 42 were significantly lower in controls than in TBI patients ( $p < 0.0001$ ) (Table 2, Fig. 1). CSF and plasma levels of A $\beta$ 42 did not correlate with age, time to sample withdrawal and severity of injury as assessed by GCS and motor GCS. There was no correlation between CSF and plasma levels of A $\beta$ 42 (Spearman correlation coefficients =  $-0.02$ ,  $p = 0.946$ ). CSF and plasma A $\beta$ 42 concentrations did not differ between patients with diffuse injury and focal mass lesion ( $p = 0.93$  and  $p = 0.49$ , respectively). CSF A $\beta$ 42 concentrations were lower in patients who died compared to patients who survived; conversely, plasma concentrations were lower in survivors than non-survivors (Table 2). CSF A $\beta$ 42 significantly decreased from normal through TBI survivors to TBI non-survivors (P for trend =  $p < 0.0001$ ; Jonckheere-Terpstra test), while plasma A $\beta$ 42 significantly increased from normal through TBI survivors to TBI non-survivors (P for trend =  $p < 0.0001$ ; Jonckheere-Terpstra test) (Fig. 2).

**Description of Longitudinal CSF and plasma A $\beta$ 42 Levels.** CSF A $\beta$ 42 levels were decreased in TBI patients compared with

controls over the study period (Fig. 3). CSF A $\beta$ 42 concentrations were significantly lower on day 1 and 3 and from day 5 to day 7 (Fig. 3). CSF A $\beta$ 42 nadir level was on day 6 after injury (62.62 pg/mL [42.73–233.4]). Figure 3B shows daily plasma A $\beta$ 42 concentrations that, in contrast, were significantly and persistently elevated in TBI subjects compared to controls. Plasma A $\beta$ 42 levels peaked 6 days after injury (25.03 pg/mL [16.01–31.11]). Within-subjects comparison in the temporal analysis window showed that CSF and plasma A $\beta$ 42 levels did not vary significantly over the study period ( $p = 0.09$  and  $p = 0.55$ , respectively, Friedman test). Plasma A $\beta$ 42 concentrations did not correlate with CSF A $\beta$ 42 concentrations at any of the time points examined.

**CSF A $\beta$ 42 Levels in Relation to Neurological Status.** In several patients, CSF A $\beta$ 42 was associated with the global neurological status, as assessed with the Glasgow Coma Score (GCS); higher concentrations of CSF A $\beta$ 42 were associated with patient neurological status improvement, whereas reduced A $\beta$ 42 levels correlated with patient deterioration/worsening. CSF A $\beta$ 42 changes appeared to track, and in some cases even precede neurological status changes (Fig. 4).

## Discussion

In this investigation, we assessed and monitored CSF and plasma A $\beta$ 42 values in matched longitudinal samples of patients with severe TBI using the SiMoA A $\beta$ 42 assay. This breakthrough technology allowed highly sensitive and precise quantification of A $\beta$ 42, improving overall diagnostic accuracy, in a small but clinically well-characterized TBI cohort.

We found that initial A $\beta$ 42 levels presented opposite dynamics in CSF and plasma of patients with severe TBI, with significant reductions in CSF A $\beta$ 42 concentrations and increases in plasma A $\beta$ 42 levels early after injury compared to controls. The reduction of CSF A $\beta$ 42 concentrations in TBI patients might suggest deposition of aggregated A $\beta$ 42 and plaque formation<sup>10</sup> in the brain early after injury, as reported in previous neuropathological studies<sup>10,11</sup>. Nonetheless, it might also result from A $\beta$ 42 leakage across an impaired blood-brain barrier (BBB) into the blood. This later hypothesis is supported by a substantial rise of A $\beta$ 42 in plasma.

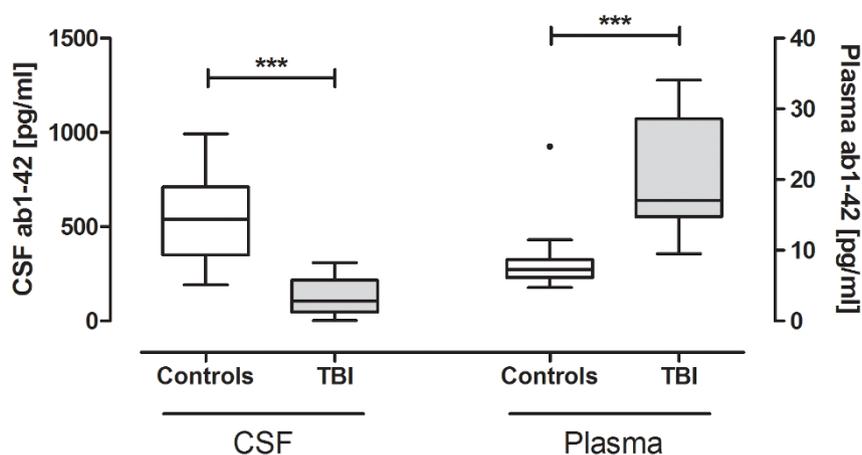
Interestingly, TBI patients who died were characterized by more marked decrease in CSF A $\beta$ 42 and highly elevated levels of plasma A $\beta$ 42 compared to controls, while CSF and plasma A $\beta$ 42 values in survivors were intermediate between these 2 groups (Fig. 2). This suggests that the magnitude of both CSF reduction and plasma elevation in A $\beta$ 42 increases with increasing brain injury severity. These findings may be explained by the fact that severely injured patients doomed to die had a more extensive BBB damage/breakdown and consequently higher A $\beta$ 42 levels entering the peripheral circulation as compared to individuals with mild injuries. Supporting these observations, substantial evidence has now accumulated showing the direct influence of BBB disruption on the clinical outcome after TBI<sup>12</sup>. A sensitive marker that can predict outcome, and capture TBI-

**Table 2 | CSF and plasma A $\beta$ 42 levels in patients with severe TBI acutely after injury and in controls. Data are given as median (interquartile range)**

	N	CSF A $\beta$ 42 (pg/mL)	N	Plasma A $\beta$ 42 (pg/mL)
<b>TBI Admission</b>	12	105.9 (46.02–216.2)*	12	17.02 (14.75–28.59)*
<b>Diffuse Injury (Admission)</b>	6	105.9 (52.45–227.6)	6	19.65 (14.91–32.90)
<b>Focal Mass Lesion (Admission)</b>	6	118.7(16.59–239.3)	6	16.61 (13.21–27.35)
<b>Survivors (Admission)</b>	6	161.1 (65.67–286.9)	6	16.29 (14.13–18.88)*
<b>Non-Survivors (Admission)</b>	6	46.02 (11.55–172.4)*	6	27.97 (13.66–32.90)*
<b>Controls</b>	15	537.6 (350.8–710.0)	20	7.289 (6.126–8.668)

\* $p < .0001$  ( $p$  values of the Mann-Whitney test for differences between the groups [TBI versus Controls]).

$p < .05$ , or  $^{\dagger} p < .0001$  ( $p$  values of the posthoc test for differences between Survivors and Non-survivors versus Controls).



**Figure 1** | CSF and plasma A $\beta$ 42 concentrations acutely after injury in patients and controls. A $\beta$ 42 concentrations acutely after injury (24 hrs) in CSF (A) and in plasma (B) of patients with severe TBI and controls. The horizontal line in each box represents the median and the boxes representing 25th to 75th quartile. Significant differences are indicated with \*\*\* ( $P < 0.0001$ ) (Mann–Whitney U-test).

induced BBB disruption could be extremely useful; further studies assessing the relationships between TBI, BBB disruption and levels of A $\beta$ 42 are warranted.

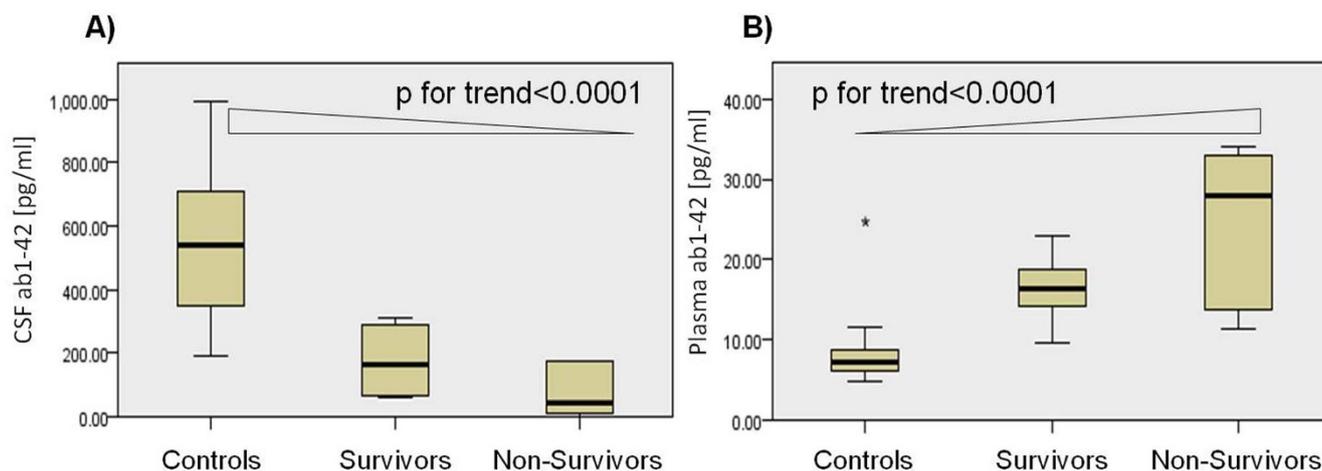
Overall, our findings complement and extend previous results on the topic of amyloid peptides in biofluids following TBI<sup>13</sup>. Consistently with our observations, several studies have shown decreased CSF A $\beta$ 42 concentrations after TBI<sup>14,15</sup> and an association with poor clinical outcome<sup>14</sup>. On the other hand, in a study by Olsson and colleagues<sup>16</sup>, marked increase in ventricular CSF A $\beta$ 42 and unchanged level of plasma A $\beta$ 42 were observed in patients after severe TBI. However, this discrepancy may be due to different collection protocols, lack of control group, patient characteristics and outcome, or different determination techniques. In particular, in our study the ultrasensitive digital immunoassay for quantification of the A $\beta$ 42 in plasma enables measurement of this marker at concentrations not reliably detected with prior generations of commercial assay and might explain the conflicting results with the earlier report.

Within this study, we did not find differences between the A $\beta$  levels in patients with diffuse brain injury compared with focal TBI. This finding stands in contrast to previous microdialysis (MD) studies, which found increased interstitial fluid (ISF) A $\beta$ 42 levels in patients who sustained diffuse brain injury<sup>8,17</sup>. However, MD data are not directly comparable to CSF and plasma owing to the fact that

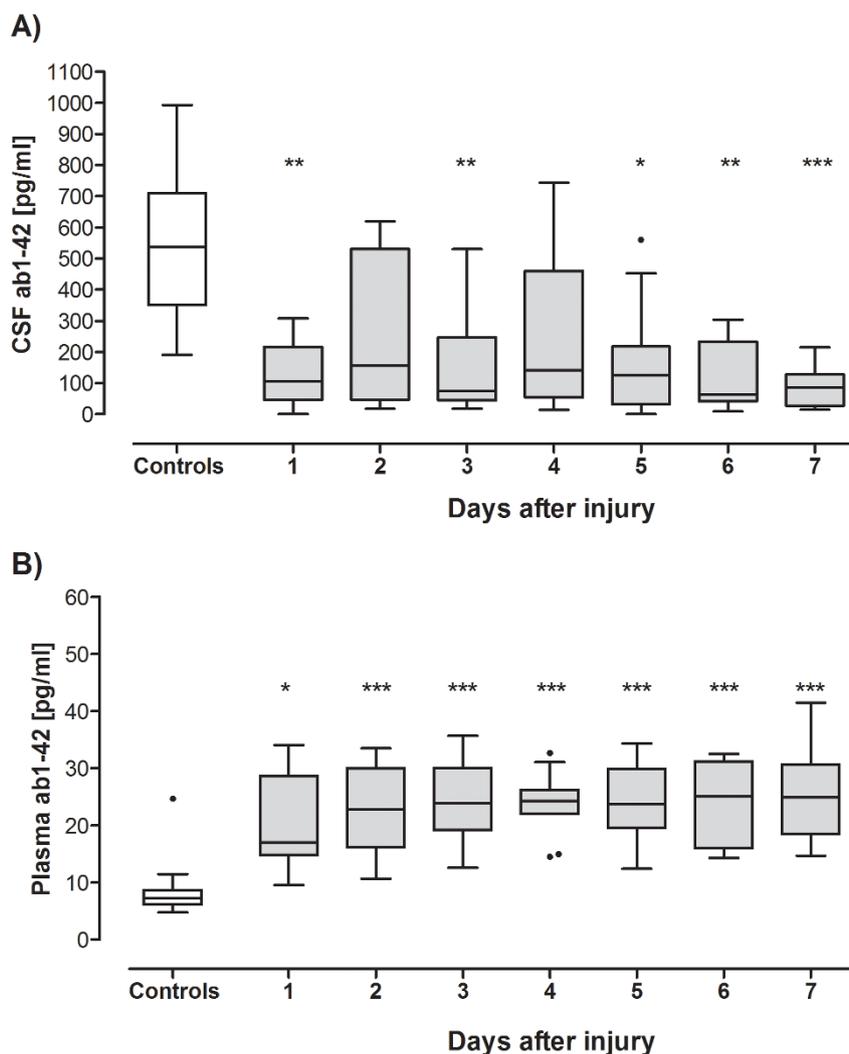
interstitial fluid comes from a relatively restricted area of the brain and its composition depends on the microdialysis catheter location. An alternative explanation is that CT and the broad distinction between focal TBI and diffuse axonal injury (DAI) based on the Marshall classification<sup>18</sup> may underestimate the extent of components of axonal injury in patients with predominately focal TBI<sup>19</sup>. Advanced neuroradiological tools such as MRI and ideally diffusion tensor imaging (DTI) could be appropriate approach to solve this question.

In line with previous investigations in patients with mild cognitive impairment or AD<sup>20,21</sup>, no significant correlation between the levels of A $\beta$ 42 in CSF and plasma was identified in our study. These findings might indicate a delayed release of A $\beta$ 42 into the blood after TBI. However, the relation between brain ISFA $\beta$  CSFA $\beta$ 42 and plasma A $\beta$ 42 is fairly complex<sup>21</sup> possibly involving BBB, paravascular pathways and astrocytic water transport (aquaporin4-dependent bulk flow). However, the role of such structures in the clearance of biomarkers has only recently been explored<sup>22</sup> and will be a critical area for future investigation.

The longitudinal study showed persistent reduced levels of CSF A $\beta$ 42 and elevated levels of plasma A $\beta$ 42 over time suggesting that levels of this marker reflect and characterize pathophysiological processes that start with the primary injury, evolve over the acute period



**Figure 2** | Box-and-whisker plots demonstrating median CSF (A) and plasma (B) A $\beta$ 42 concentrations acutely after injury in TBI patients who died and in TBI patients who survived, and in controls. Jonckheere–Terpstra test demonstrates an increase in CSF (A) A $\beta$ 42 in patients across the groups and a decrease in plasma (B) A $\beta$ 42 in patients across the groups. The black horizontal line in each box represents the median, with the boxes representing the interquartile range.



**Figure 3 | Longitudinal CSF and plasma Aβ42 Levels in TBI patients and controls.** The concentration of CSF Aβ42 (A) was significantly decreased in TBI patients on day 1 and 3 and from day 5 to day 7 after injury compared to controls. Plasma Aβ42 (B) was significantly elevated over the study period, compared to controls. The horizontal bar represents median concentration. Significant differences are indicated with \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) or \*\*\* ( $P < 0.001$ ) ( $p$  values of the post-hoc Dunn's Test for differences between the groups [TBI versus Controls]).

and encompass the subacute and chronic phases. It is of note that, the pattern of changes in CSF levels of this protein observed over time correlated with neurological outcome, as reported by others<sup>6</sup>.

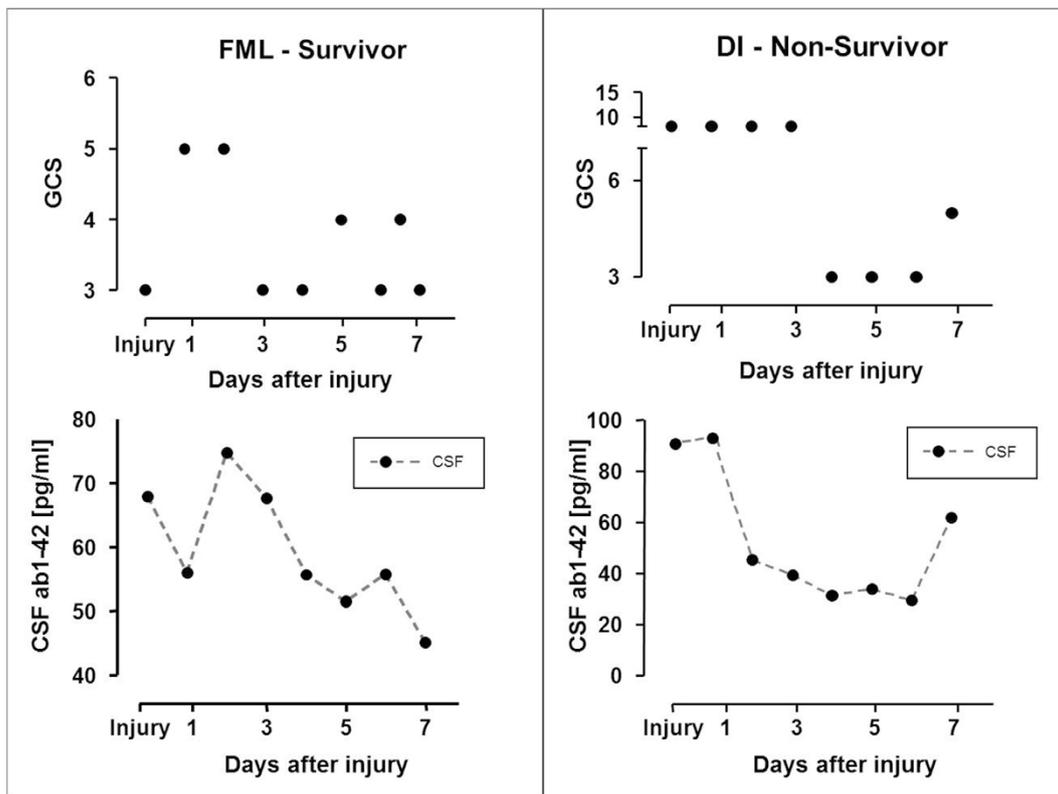
Our study has several limitations. First, it consists of a relatively small sample size that did not allow multivariate analyses including significant clinical and demographic variables. Second, Aβ is produced by many different cells in the body<sup>20,23</sup>, therefore, increased release of Aβ42 from extracerebral origin cannot be excluded. Nonetheless, the exclusion of multiple injuries makes it likely that the changes in plasma levels of this marker fundamentally reflect the brain injury and associated BBB disruption. Third, there was a significant age difference between TBI patients and controls. However, we did not find any significant correlation between age and Aβ42 concentrations in CSF and plasma. In addition, the prognostic value of Aβ42 levels was unrelated to age. Furthermore, these findings are in agreement with those of 2 recent studies demonstrating a diagnostic and prognostic value of the Aβ42 levels in patients with acute and chronic intracerebral hemorrhage that was independent of age<sup>24,25</sup>. Finally, in future studies it might be worthwhile to analyze the influence of other relevant clinical variables, such as polytrauma, renal function and APOE ε4 status, on plasma Aβ42 concentrations.

In conclusion, our data show an opposite dynamics of Aβ42 in CSF and plasma and a stepwise decrease and increase in CSF and

plasma Aβ42 concentrations, respectively, occurring with increasing severity of injury. Importantly, our work indicates for the first time a potential clinical relevance in TBI of plasma Aβ42 as detected by novel ultrasensitive digital immunoassay. Future studies that include a larger sample size will be required to validate these findings and to determine whether combined information from CSF and plasma Aβ42 levels might be effective predictors of outcome and BBB disruption after severe TBI.

## Methods

**Patients.** This study is part of the BANDITS (Biomarker Assessment for Neurotrauma Diagnosis and Improved Triage System) Feasibility Study, an observational study on the association between brain damage markers and demographic and clinical variables, neuroimaging, and clinical outcome of patients with severe TBI. Other biomarker analyses from this project have previously been reported elsewhere<sup>26,27</sup>. In the current study we focused on a pilot cohort of 12 patients in whom paired CSF and plasma samples were available. Severe traumatic brain injury was defined as a Glasgow Coma Score (GCS) of 8 or less on the hospital admission. Exclusion criteria were no informed consent, age < 18 years, known history of neurological and/or autoimmune disease, multiple injuries and pregnancy. All patients underwent insertion of an intracranial pressure (ICP) monitor using a ventriculostomy catheter that was placed as part of the routine medical care for patients with severe TBI. The study protocol was approved by the local ethics committee of the two sites involved (Pecs, Szeged) and by the Western Institutional Review Board (WIRB) and Human Research Protection Office (HRPO). Next of kin or legal representatives provided written informed consent for study participation.



**Figure 4** | CSF A $\beta$ 42 levels and neurological status. Graph showing the time course of CSF A $\beta$ 42 concentrations and changes in neurological status, as reflected by GCS, in 2 severely brain injured patients (FML, Focal Mass Lesion; DI, Diffuse Injury).

The study was conducted in accordance with the approved guidelines and regulations, in line with the tenets of the Declaration of Helsinki.

Initial computed tomography (CT) scans obtained on admission were classified according to the classification of Marshall et al. For the purpose of our analysis, Marshall score was further categorized into two groups (diffuse injury versus focal mass lesion), as previously described<sup>28</sup>. Outcome was assessed at 6 months post-injury using the Glasgow Outcome Score Extended (GOSE). Patient characteristics are shown in Table 1.

Because there are no certified reference standards for A $\beta$ 42 and values vary depending on the assay used, we included a control population consisting of 20 individuals who underwent lumbar puncture (LP) to exclude possible peripheral nervous system disorders, suspicion of subarachnoid hemorrhage or meningitis and with proven negative results. Exclusion criteria were antecedents of neurologic disease and any contraindication for lumbar puncture.

**Sample Collection and Handling.** In patients with severe TBI, CSF for biomarker analysis was collected on admission after the insertion of an intracranial pressure monitoring device (median 12.5 hrs, range 5–24 hrs) and daily up to 7 days. In control subjects CSF was collected by LP. Blood and CSF samples were drawn at the same time. Approximately 4–5 mL of CSF and plasma were collected from each subject at each sample point. The samples were immediately centrifuged for 10 min at 4000 rpm, frozen and stored at  $-80^{\circ}\text{C}$  until assayed.

**Measurement of A $\beta$ 42.** Quantification of CSF and plasma A $\beta$ 42 concentrations was performed at Quanterix Corporation, Cambridge, Massachusetts, USA. All samples were blinded to case identity and assayed in triplicate. Samples from individual patients were tested within a single plate. A $\beta$ 42 was measured using SiMoA technology. This method involves performing a paramagnetic bead-based ELISA, followed by isolation of individual capture beads in arrays of femtoliter-sized reaction wells<sup>29</sup>. Singulation of capture beads within microwells permits buildup of fluorescent product from an enzyme label, so that signal from a single immunocomplex can be readily detected with a CCD camera. At very low A $\beta$ 42 concentrations, Poisson statistics predict that bead containing microwells in the array will contain either a single labeled A $\beta$ 42 molecule or no A $\beta$ 42 molecules, resulting in a digital signal of either “active” or “inactive” wells. At higher A $\beta$ 42 concentrations, when all wells become occupied by at least 1 labeled A $\beta$ 42 molecule, digital measurements transition to non-digital (analog) measurements of total fluorescence intensity. With single molecule sensitivity, concentrations of labeling reagents can be lowered, resulting in reduced nonspecific background. This effect enables high signal- to-background ratios at extremely low analyte concentrations.

Arrays of femtoliter-volume wells were prepared as described. In brief, the ends of bundles of 50,000 optical fibers were polished with diamond lapping films and etched

one end of each bundle in mild acid solution. Differential etch rates of the optical fiber core and cladding glass of the bundles causes 4.5  $\mu\text{m}$ -diameter, 3.5  $\mu\text{m}$ -deep wells to be formed, giving an array of 50 000 microwells across the bundle. Optical fiber arrays were mounted in linear groups of 8 within glass holders for bead loading and imaging, to correspond with microtiter plate columns of 8 wells, which were used as rinse troughs. Paramagnetic capture beads were comprised of a monoclonal anti-A $\beta$ 42 antibody (Covance, 6E10) directed to the N-terminus. Biotinylated detector reagent was comprised of a monoclonal anti A $\beta$ 42 antibody (Invitrogen H31L21) directed to the C-terminus. Streptavidin:  $\beta$ -galactosidase ( $\beta$ G) was prepared by covalent conjugation of purified streptavidin (Thermo Scientific) and  $\beta$ G (Sigma) using standard coupling chemistry. Bead-sample incubations and labeling of immunocomplexes in conical 96 well plates (Axygen) were conducted. The assay was performed in three steps, starting with analyte capture, incubation with biotinylated detector, and labeling of the immunocomplexes with  $\beta$ G. Following assay and bead collection with a magnet, beads were loaded onto the arrays for imaging in a loading buffer comprised of PBS and 0.01% Tween-20,  $\text{MgCl}_2$ , and sucrose. Wells containing beads with labeled A $\beta$ 42 were visualized by the hydrolysis of enzyme substrate (resorufin  $\beta$ -D-galactopyranoside, RGP, Invitrogen) by  $\beta$ G into fluorescent product. RGP was introduced to the wells during sealing of the arrays with a silicon gasket. Enzyme-containing wells were imaged by fluorescence microscope fitted with a CCD camera. The images were analyzed to determine the average number of label enzymes/bead (AEB) as described. At  $<70\%$  active beads relative to total beads (low A $\beta$ 42), the signal output is a count of active beads corrected for a low statistical probability of multiple enzymes/bead. At  $>70\%$  active beads (higher A $\beta$ 42), the probability of multiple enzymes/bead increases, and average fluorescence of the wells is converted to AEB based on the average intensities of wells containing single enzymes determined at lower concentrations.

The assay was calibrated with A $\beta$ 42 obtained from Covance and a stock solution prepared by dilution to 3.5 ng/mL in PBS/Tween-20. Assay calibrators and controls were prepared by dilution of the stock solution in PBS diluent containing a surfactant and BSA (PBS/BSA). Calibrators were prepared by serial 3-fold dilution to give a calibration range of 0–250 pg/mL. Limits of detection (LoD) were estimated as three standard deviations above the zero calibrator across calibration curves on six separate days ( $n = 3$  replicates per curve). LoDs ranged from 0.014 to 0.032 pg/mL, with an average of 0.020 pg/mL. The limit of quantification (LoQ) was estimated to be 0.038 pg/mL from repeated measurement of immunodepleted plasma, as previously described<sup>30</sup>. Assay imprecision was estimated at low levels of A $\beta$ 42 as total coefficients of variation (CV) from six days of repeated testing of three plasma samples. CVs ranged from 6.1 to 10.0% at A $\beta$ 42 concentrations of 0.5 to 4.8 pg/mL. Specificity for A $\beta$ 42 was evaluated by assaying 0.5, 1.0, 5.0, 10 and 50 pg/mL of the peptide variants A $\beta$ 38, A $\beta$ 40, and A $\beta$ 43 in PBS/BSA (Merck). No detectable cross reactivity was noted



for the shorter A $\beta$ 38 and A $\beta$ 40 peptides, while the longer A $\beta$ 43 variant exhibited a cross reactivity of 11–16%. Linearity of the assay has been described previously<sup>20</sup>.

**Statistical analyses.** Statistical analyses were carried out using the SPSS 20.0 software package (SPSS Inc, Chicago, Illinois, USA) and JMP version 10.0 (SAS Institute, Inc, Cary, NC). Data normality was assessed. For descriptive analyses, continuous variables are presented as median and interquartile range; differences were tested using the Mann–Whitney U test. Distributions of categorical variables are presented as frequencies and percentages. The significance of differences in proportions was assessed using chi-square or Fisher's exact test where appropriate. To test for significant trends in biomarker concentrations across groups, the Jonckheere–Terpstra test for non-parametric trend analysis was used. When trends were significant ( $p < 0.05$ ), pairwise between-group comparisons was applied (post-hoc Wilcoxon signed-rank test). The statistical significance of within-subject longitudinal change in CSF and plasma A $\beta$ 42 concentration was analyzed using the non-parametric Friedman test followed by *post-hoc* comparisons applying Dunn's test. The relation between quantitative variables was assessed by bivariate correlations (Spearman rank correlation test). All statistical tests were two-tailed. P values less than 0.05 were considered significant.

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## Author contributions

S.M. performed data analysis and interpretation and drafted the manuscript. A.B. and P.B. performed clinical work and contributed to the interpretation of the results. J.R., G.P., D.H., D.W. and F.K. participated in the laboratory work. A.J. contributed to the design of the study and participated in the interpretation of the analytical results. All authors contributed substantially to the revision of the manuscript and have approved the article for publication.

## Additional information

**Competing financial interests:** Drs. Mondello, Buki, Barzo and Kobeissy declare no competing financial interests. Drs. Randall, Provncher, Hanlon, Wilson and Jeromin are employees and receive salaries from Quanterix Corporation.

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