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Cerebral metabolic dysfunction at the acute phase of traumatic brain injury correlates with long-term tissue atrophy.

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Abstract:

Cerebral metabolic dysfunction following traumatic brain injury (TBI) correlates with poor patient outcome, however the exact pathophysiological mechanisms underlying this association are not entirely established.

This was a pre-planned analysis of the BIOmarkers of AXonal injury after Traumatic Brain Injury (BIO-AX-TBI) study, including subjects (n=14) who underwent acute phase (0-96 hours post-TBI) cerebral microdialysis (CMD) monitoring and had longitudinal magnetic resonance imaging (MRI) quantification of annualized brain volume loss (subacute phase and 12-month post-TBI), computed with the MorphoBox prototype. Spearman's correlations were calculated to examine the relationship of CMD lactate/pyruvate (LP) ratio, to assess the degree of cerebral metabolic dysfunction, with long-term brain tissue atrophy.

On average, CMD showed elevated LP ratio (31 [IQR 24-34]), indicating acute cerebral metabolic dysfunction, while MRI-computed annualized whole brain and total grey matter (GM) atrophy rates were -3.2% [-9.3 – -2.2] and -1.9% [-4.4 – 1.7], respectively. Cerebral extracellular LP ratio correlated negatively with annualized total GM atrophy rate (Spearman $\rho = -0.75$, p-value = 0.003). Cerebral glucose also correlated with annualized total GM atrophy rate (Spearman $\rho = 0.61$, p-value = 0.027). After adjusting for age, admission GCS and Marshall score, CMD LP ratio remained strongly associated with 12-month total GM atrophy rate ($p<0.001$; multivariate analysis).

This clinical TBI study using MRI for the quantification of annualized brain volume loss, demonstrates a strong association between secondary acute cerebral metabolic dysfunction and 1-year grey matter atrophy rate and reinforce the role of CMD LP ratio as an early marker of poor long-term recovery after TBI.

Key words: metabolic dysfunction – traumatic brain injury – cerebral microdialysis – brain atrophy – lactate/pyruvate ratio

Introduction:

Traumatic brain injury (TBI) causes significant morbidity worldwide[1] and major burdens due to long-term behavioural and cognitive disability[2-4]. In the aftermath of TBI, a cascade of pathological molecular and cellular processes induce further secondary damage [1-3, 5, 6]. Among them, the so-called cerebral metabolic dysfunction, defined by an elevated brain extracellular lactate/pyruvate (LP) ratio is a major recognized early determinant of TBI pathogenesis and outcome[7-10]. Despite the relationship between elevated LP ratio and poor patient outcome appears established, the exact pathophysiological mechanisms underlying this association have not been fully characterized[11].

Chronic brain atrophy following a TBI is a hallmark and a major determinant of long-term prognosis and quality of life after TBI[6, 12-19]. Patterns of progressive brain atrophy, its duration and evolution still need to be elucidated[20]. Previous studies investigated the longitudinal volumetric changes in the brain post TBI with magnetic resonance imaging (MRI)[21-25]. However, they were relatively heterogeneous with respect to TBI severity (e.g., including both moderate and severe TBI patients)[24] and MRI long-term follow-up (ranging from 2 to 12 months post-injury)[21, 23]. In addition, they were partly limited by the lack of automated MRI computation of brain volumes[26].

Therefore, in a homogeneous cohort of subjects with severe TBI, we focus our investigation on 1) the acute phase of cerebral microdialysis monitoring, 2) longitudinal MRI follow-up (subacute vs. 12-month post-TBI) using automated quantitative volumetric analysis of brain structures (MorphoBox prototype)[27], 3) the association between cerebral metabolic markers (LP ratio, glucose) and 12-month brain atrophy rate. This was a pre-planned analysis of the BIOmarkers of AXonal injury after Traumatic Brain Injury (BIO-AX-TBI) study (NCT03534154).

Materials and methods:

Study design and population

Patients with severe TBI admitted between March 2018 and April 2021 to the Department of Adult Intensive Care Medicine, Lausanne University Hospital (Centre Hospitalier Universitaire Vaudois, CHUV), Switzerland, were prospectively recruited for the current study. All adult patients underwent cerebral multimodal monitoring with cerebral microdialysis (CMD) in combination with intracranial pressure (ICP) and brain tissue oxygen tension ($PbtO_2$) and had two MRI scans (subacute phase, i.e., baseline, and 12-month). More details about inclusion criteria have been previously described in the BIO-AX-TBI protocol[28].

All patients, next of kin or legally authorized representatives provided signed informed consent to the study approved by the local Ethical Committee of the University of Lausanne, Switzerland (no. 2017-01757).

General patient management

Patients were treated according to our standard protocols[29]. All patients underwent mechanical ventilation (aiming to keep PaO_2 and $PaCO_2$ at 90-100 mmHg and 35-40 mmHg, respectively) and sedation-analgesia (with propofol infusion, at a maximal dose of 4 mg/kg/h, and sufentanil infusion, at a maximal dose of 20 µg/h). Cerebral perfusion pressure was maintained at 60-70 mmHg, with the use of vasopressors (norepinephrine) and isotonic fluids (aiming for euvolemia). Normoglycemia (arterial blood glucose 6-8 mmol/L, with the use of continuous insulin infusion if needed) and normothermia (core body temperature < 37.5°C) were part of standard care. Management of elevated ICP followed a stepwise management algorithm, as described previously[30].

Cerebral microdialysis monitoring

CMD catheters (CMA 70 or CMA71®, CMA Microdialysis AB, Solna, Sweden) were inserted into the frontal brain parenchyma (in normal-appearing subcortical white matter). Catheters were perfused with artificial cerebrospinal fluid or dextran via a pump (CMA 106, CMA Microdialysis) at a constant rate of 0.3μL/min. CMD samples were collected hourly and analysed immediately at the bedside using a kinetic enzymatic analyser (ISCUS Flex®, CMA Microdialysis AB) for extracellular concentrations of glucose, glutamate, glycerol, lactate and pyruvate. Previous work showed that the two CMA catheters perform similarly with comparable recovery rates for brain metabolic markers[31]. The first two hours of CMD monitoring were discarded due to the stabilization time of the ISCUS machine.

Concomitant to the insertion of CMD catheter, the ICP probe (Codman®, Raynham, MA, USA) and $PbtO_2$ probe (Licox®, Integra Neurosciences, Plainsboro, NJ, USA) were placed by experienced neurosurgeons. A CT scan was used to confirm the placement of the CMD catheter.

Image acquisition and analysis

Patients underwent subacute and 12-month MRI scanning sessions including structural and functional imaging sequences. Both sessions were carried out on the same 3T scanner (MAGNETOM Skyra Fit, Siemens Healthcare, Erlangen, Germany) with a 64-channel head coil. MR acquisition protocol included a 3D T1 magnetization prepared rapid gradient echo (T1 MP-RAGE) sequence consistent with the 3T Alzheimer's disease neuroimaging initiative (ADNI) protocol as described in the BIO-AX-TBI study protocol[28].

The T1 MP-RAGE sequence of each patient was processed with the automated MorphoBox[27] prototype software, which provides an individual estimation of the total intracranial volume (TIV), as well as total and regional grey matter (GM) and white matter (WM) volumes in millilitres. The annualized atrophy rate of each region at 12 months was calculated as follows:

([V_{12m} - V_b]/V_b) * (1/Δt), where V_{12m} stands for the structure volume at 12 months, V_b the structure volume at baseline, and Δt the timespan between baseline and follow-up in years). For the corpus callosum, the mid-sagittal area was used instead of the volume, as provided by the software. All segmentations were reviewed by a neuroradiologist for errors.

Statistical analysis

Data processing and statistical analyses were conducted using the MATLAB R2021a (MATLAB R2021a, The MathWorks, Inc., Natick, Massachusetts, United States) and JMP 15 (JMP®, Cary, NC, USA). Results are expressed as mean ± standard deviation or median and interquartile range [25-75], unless stated otherwise. Normality of data distribution was tested for each variable with the Shapiro-Wilk test. ANOVA model for repeated measures, adjusting for patient and timespan were performed for univariate comparisons. Linear correlations were assessed with the non-parametric Spearman's rho coefficient test. A multivariate analysis was applied to identify independent predictors of whole-brain atrophy rate adjusted for age, Glasgow Coma Scale (GCS) and Marshall Score. Statistical significance was set at $p<0.05$.

Results:

Patient characteristics

Fourteen severe TBI patients (1 female, 13 males) had complete CMD datasets and underwent baseline and 12-month MRI post-injury. Injury was secondary to fall (50%), road traffic accidents (43%) and undetermined origin (7%). The mean age was 43.5 ± 14.2 years with a mean initial GCS of 5 [25-75% quantile: 3-8]. At 12 months, 36% of patients had a good outcome as defined by a score of 7 or 8 at the Glasgow Outcome Scale Extended (GOSE) (Table 1).

Neuro-monitoring and cerebral microdialysis dynamics

CMD monitoring started at a median time of 9.5 hours [5.9-14.1 hours] post-injury. Overall, as shown in Table 2, LP ratio remained constantly elevated (> 25) during the acute phase (first 96 hours) following TBI, as was CMD lactate (> 4 mmol/L). Brain glutamate peaked at 24-hour post-TBI and decreased thereafter (Supplementary Table S1, all $p<0.001$ when compared to 0-24h concentrations, ANOVA for repeated measures). Brain glucose remained relatively stable over the 96-hour of monitoring (Table 2, Table S1). ICP and $PbtO_2$ were within normal ranges (Table 2, Table S1)

MRI-based brain morphometry

Annualized atrophy rates of lobes and regions of interest are reported in Table 3. Segmentation quality control was overall very satisfactory. Brain volume loss between baseline and 12-month was statistically significant ($p <0.001$, ANOVA for repeated measures). The entire cohort resulted in a -3.21% annualized whole brain atrophy rate over 12 months, essentially due to WM loss (-9.18%).

Correlation between brain energy metabolic biomarkers and longitudinal volume changes over 12 months

Analysis of annualized atrophy rate revealed that brain GM appeared to be significantly associated with the cumulative effect of elevated 96-hour LP ratio, in particular the frontal GM lobe, the parietal GM, the temporal GM lobe, the cortical GM and the insula (frontal GM: Spearman ρ = -0.57, p-value = 0.041; parietal GM: Spearman ρ = -0.58, p-value = 0.048; temporal GM: Spearman ρ = -0.59, p-value = 0.033; cortical GM: Spearman ρ = -0.68, p-value = 0.011; bilateral insula: Spearman ρ = -0.58, p-value = 0.037, Figure 1A).

Overall, average 96-hour LP ratio total was strongly correlated with the total GM atrophy rate (Spearman ρ = -0.75, p-value = 0.003, Figures 1A and 2). After adjusting for age, GCS and Marshall Score multivariate analysis confirmed the robust association between acute phase LP ratio and 12-month total brain GM atrophy rate ($p<0.001$). There was no significant association between WM regions and CMD LP ratio over the first 96-hour (Figure 1B).

Total annualized GM and cortical GM atrophy rates were also correlated with average 0-96-hour CMD glucose (total GM: Spearman ρ = 0.61, p-value = 0.027; Cortical GM: Spearman ρ = 0.61, p-value = 0.027, Supplementary Figure S1A). Similarly to LP ratio, there was no significant association between WM regions and glucose over the first 96-hour (Figure S1B).

We found no relationship of other CMD biomarkers (glutamate, pyruvate, lactate), as well as ICP and $PbtO_2$ with any annualized brain structures atrophy rates.

Discussion:

To the best of our knowledge, this is the first clinical study in severe TBI investigating associations between early metabolic dysfunction biomarkers (within the first four days post-injury) and annualized brain volume changes. The main finding of our study is that the LP ratio is a strong predictor of chronic GM loss, which is a valid marker of poor recovery after TBI.

Early tissue biochemistry

Cerebral microdialysis provides an online assessment of tissue biochemistry following trauma. Our results showed that within the first 96 hours after injury, brain patient metabolism was altered as measured by sustained elevated LP ratio (>25) with no signs of frank ischemia/hypoxia. These results were in line with previous studies that demonstrated brain energy crisis after TBI was present and independent from brain ischemia[32, 33]. The LP ratio is a marker of cellular metabolic state, and elevated LP ratio has been attributed to energy or mitochondrial dysfunction[34] rather than a lack of brain oxygen supply[35]. Brain metabolic dysfunction is reflected as well with the high levels of extracellular glutamate concentrations, which would ensue in neuro-excitotoxicity damage and finally result in neuronal death[36]. To note, in our cohort, glucose concentration, which is the principal substrate for the brain but also important for several pathways and crucial for brain cell survival[37], was within the normal ranges. This may be likely due to a metabolic switch to lactate, reflected by concomitant elevated CMD lactate (> 4 mmol/L), and potentially indicating endogenous extracellular lactate use[38-40].

Longitudinal brain morphometry changes

The MorphoBox prototype, a volume-based approach, was the method used to analyse brain morphometry[27]. To date, this is the first study to use this tool for the quantification of brain atrophy in a severe TBI population. Our cohort experienced a cerebral volume loss of -3.21% at 12 months, which follows the atrophy pattern presented in other longitudinal studies. For

instance, Marcoux et al. reported a -8.5% brain volume loss at 6 months[41] whereas Warner et al.[25] or Sidaros et al.[23] reported a -4.5% loss at 8 months or -4% loss at 10 months respectively. Interestingly, atrophy seems to persist beyond a year as demonstrated by Cole et al., who reported a -1.51% cerebral volume loss between their baseline (1-year post-injury) and their follow-up at 12.7 months interval from baseline[24]. Considering the different timeline of each study, our results follow a comparable decreasing trend.

In addition to whole-brain quantification, the algorithm successfully quantified brain morphometry. Several distinct structures showed some non-negligible atrophy rates such as each WM lobes (frontal: -9.8%, parietal: -10.4%; temporal: -14.1%; occipital -7.1%) with a total annualized WM atrophy rate of approximately -9%. Our results for total WM could be compared to the recent study from Simeone et al. (WM volume decreased -11.4% [IQR -5.8; -14.6]) but again the period of observation is substantially different (1 vs. 5 years) [42]. However, Warner et al.[25] reported a -5.8% shrinking of WM in the first 8 months post-injury making our results within the expected range.

Comparison of selected quantified brain structures atrophy rates at one year with already published studies was not trivial as the time study periods varied between studies. Nevertheless, we observed atrophies in brain structures that were already reported in previous studies such as bilateral pallidum[12, 43], bilateral hippocampus[24, 44, 45], bilateral putamen[23, 43, 46], bilateral insula[24, 44], bilateral amygdala[47] or area reduction in the corpus callosum[12, 23, 24]. Altogether, our results are within the ranges of already accepted volume changes post-TBI and thus demonstrate the potential of the MorphoBox prototype to assist TBI patients' follow-up.

Association between metabolic biomarkers and brain atrophy

Due to the complex design setup, the association between LP ratio, glucose and brain atrophy has never been reported together in a single study. The study from Marcoux is to date the only one to report a correlation (Pearson correlation, $r=-0.56$, $p < 0.01$) between the extent of frontal

lobe atrophy at 6 months after injury with the mean percentage of time of elevated LP ratio within the initial 96 hours[41]. Whereas Xu et al. is the only study that observed an association between frontal lobe atrophy and glucose metabolism[48].

In the current study, we found a strong associations with both metabolic biomarkers: an inverse correlation between GM atrophy and mean LP ratio and a positive one with mean cerebral glucose. Although we did not look at the time percentage with a pathological LP ratio but rather at the mean LP ratio over 96-hour, our findings confirm LP ratio as a strong predictor of chronic brain loss. The MorphoBox prototype software enabled to perform a more refined analysis and enabled the attribution of frontal lobe loss predominantly to GM loss, which has never been reported so far in previous studies. In addition, LP ratio was negatively correlated with the parietal lobe, the temporal lobe and the insula resulting altogether in total GM atrophy. Insula functions are essential[49] and atrophy in this region could be linked to the long-term deficits observed in TBI patients. Similarly, glucose brain concentration correlated with total brain GM, meaning that the lower the concentration the higher the GM volume loss is. This is even more striking, if we consider that the average brain glucose levels, in contrast to the LP ratio, were in ranges that are considered “normal”. Apart from being the principal energetic substrate, glucose may be tightly linked to the regulation of apoptotic pathways as well[50]. Thus, impairments in its metabolism could cause chronic brain atrophy.

In summary, the results demonstrated a strong correlation between LP ratio and GM volume loss. In one hand, our data support the concept that interventions aiming at supplementing energy metabolic function at the early phase of TBI[51] may have potentially beneficial effects on long-term patient recovery. On the other hand, early CMD monitoring of LP ratio may be used to identify TBI subjects who would be more likely to benefit from other acute therapeutic interventions aimed at reducing chronic brain loss.

Study limitations

Our study has several limitations. First, our results are derived from a limited patient sample size due to the complex patient setup and design (cerebral microdialysis availability and 12-month follow-up with MRI scans). Therefore, additional larger studies are required. However, our size cohort was homogeneous and comparable to that reported by Marcoux and colleagues[41]. Second, our cohort was mostly composed of male patients and thus may introduce some bias. Third, brain morphometry data was computed with the MorphoBox prototype, optimized for brain morphometry analysis in the context of structural analysis of neurodegenerative diseases. Although our atrophy rate results were in line with previous reports, additional validating studies are needed.

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Author's contributions:

AB contributed to data acquisition, performed all data analysis and drafted the manuscript; JPM contributed to data acquisition and revised the manuscript. RCJ contributed to data processing and revised the manuscript. SM, DS, NG, GB, HZ critically revised the manuscript. MO and VD were responsible for the study concept and design, supervised data analysis and interpretation, and revised the manuscript.

Data availability:

The data underlying this study are available from the corresponding author on reasonable request.

Disclosures and conflicts of interest:

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). The other authors have no conflicts of interest to declare.

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TABLES

Table 1. Patient clinical characteristics and outcome

Case number	Age (years) M/F	Initial GCS	GOS-E at 12-month	Marshall CT classification	Type of surgery	Microdialysis catheter position
1	52 M	6	7	Evacuated mass lesion	Craniotomy	R Frontal, perilesional
2	47 M	3	5	Diffuse injury II	NA	R Frontal, normal appearing
3	37 M	10	8	Diffuse injury II	NA	R Frontal, normal appearing
4	51 M	5	6	Non evacuated mass lesion	NA	L Frontal, normal appearing
5	24 M	3	6	Diffuse injury II	NA	R Frontal, normal appearing
6	64 M	3	5	Evacuated mass lesion	Decompressive Craniectomy	R Frontal, normal appearing
7	68 F	8	3	Diffuse injury II	NA	R Frontal, normal appearing
8	38 M	4	6	Diffuse injury II	NA	L Frontal, normal appearing
9	30 M	7	6	Diffuse injury II	NA	L Frontal, perilesional
10	19 M	3	6	Diffuse injury III	Decompressive Craniectomy	R Frontal, normal appearing
11	31 M	7	8	Diffuse injury IV	Craniotomy + decompressive craniectomy	L Frontal, perilesional
12	31 M	8	8	Diffuse injury II	NA	R Frontal, normal appearing
13	53 M	3	6	Diffuse injury II	NA	R Frontal, normal appearing
14	34 M	7	7	Diffuse injury IV	Decompressive craniectomy	R Frontal, normal appearing

Table 2: Brain monitoring data

	0-96 hours
Glucose, mmol/L	1.47 [1.01-2.24]
Glutamate, µmol/L	11.65 [7.44-34.73]
Lactate, mmol/L	4.56 [3.75-5.98]
Pyruvate, µmol/L	169.98 [131.52-206.87]
LP ratio	30.84 [23.49-33.49]
ICP, mmHg	11 [5.5-15.7]
$PbtO_2$, mmHg	27.3 [20.7-31.3]

Data are presented as median and 25-75 interquartile range

Table 3: MRI-based brain atrophy

Annualized brain atrophy rate, %	12-month
Whole brain	-3.21 [-9.3 – -2.17]
Grey Matter:	
Total	-1.9 [-4.35 – 1.74]
Frontal	0.39 [-11.16 – 5.17]
Parietal	-1.66 [-10.4 – 4.45]
Temporal	-2.13 [-9.08 – 3.62]
Occipital	1.43 [-3.76 – 6.87]
Cortical	-2.11 [-5.66 – 3.27]
Bilateral insula	-2.27 [-6.62 – -0.23]
Bilateral thalamus	-1.78 [-2.77 – 0.68]
Bilateral putamen	-5.59 [-6.7 – 0.21]
Bilateral caudate	0.72 [-3.91 – 1.8]
Bilateral pallidum	-4.16 [-7.85 – -0.42]
Bilateral hippocampus	-4.15 [-6.42 – 2.63]
Bilateral amygdala	-6.15 [-12.1 – -0.36]
White Matter:	
Total	-9.18 [-16.12 – -4.38]
Frontal	-9.81 [-18.89 – -3.57]
Parietal	-10.4 [-17.59 – -6.12]
Temporal	-14.11 [-18.31 – -6.67]
Occipital	-7.11 [-15.08 – 6.97]
Corpus callosum*	-7.9 [-11.82 – -2.12]
Brainstem	-4.5 [-7.9 – -2.73]
Pons	-5.16 [-8.88 – -3.09]
Medulla	-0.68 [-6.09 – 1.76]
Mesencephalon	-7.55 [-10.47 – -3.21]

Data are presented as median and 25-75 interquartile range. * Surface measured on the sagittal plane was used instead of the volume.

Figure legends:

Figure 1: *Matrix correlation between brain structures atrophy rates and 0-96h cerebral LP ratio*

Matrix correlations of brain structures computed with the MorphoBox prototype and cerebral microdialysis monitoring of lactate/pyruvate (LP) ratio within the first 96-hour post injury. Figure 1A illustrates the correlation between grey matter structures and cerebral LP ratio. Figure 1B illustrates the correlation between white matter structures and cerebral LP ratio.

Abbreviation: Bil – bilateral; GM – grey matter; h – hour; LP – lactate/pyruvate; WM – white matter.

Figure 2: *Spearman correlation between total annualized GM atrophy rate and 0-96h cerebral LP ratio*

Figure illustrates the negative linear correlation between average 0-96 hours lactate/pyruvate (LP) ratio and total annualized grey matter (GM) atrophy rate; Spearman's rho linear correlation coefficient. Blue crosses are patients' data, the red line is the linear fit and the red dots show the 95% confidence bounds.

Figure S1: *Matrix correlation between brain structures atrophy rates and 0-96h cerebral glucose concentration*

Matrix correlations of brain structures computed with the MorphoBox prototype and cerebral microdialysis monitoring of glucose concentration within the first 96-hour post injury. Figure 1A illustrates the correlation between grey matter structures and cerebral glucose concentation. Figure 1B illustrates the correlation between white matter structures and cerebral glucose concentration.

Abbreviation: Bil – bilateral; GM – grey matter; h – hour; WM – white matter.